Davis, Jayne L. (2012) *The relationship between potassium deficiency and fungal pathogens in barley*. PhD thesis.

http://theses.gla.ac.uk/3226/

The relationship between potassium deficiency and fungal pathogens in barley

Jayne L. Davis

B.Sc. (Hons)

Thesis submitted for the degree of Doctor of Philosophy

Institute of Molecular, Cellular and Systems Biology

College of Medical, Veterinary and Life Sciences

University of Glasgow

July 2011

Abstract

In the field crops are subject to a wide variety of biotic and abiotic stresses. In order to manage crop protection effectively it is important that we understand not only the way plants respond to these stresses, but the way in which these responses interact. High levels of fertilisers and pesticides are often applied to maintain soil nutritional status and prevent disease in modern intensive farming systems. Potassium (K) is an essential element for plant growth and development, and is required for a wide variety of processes within the plant. These processes can be broadly divided into biophysical processes such as stomatal opening and cell extension and biochemical processes such as protein synthesis and enzyme activation. K starvation has been shown to lead to increased levels of the stress hormone jasmonate (JA) and related compounds in *Arabidopsis thaliana* plants which in turn modulates the plant's defence against herbivorous insects and probably other pests or pathogens (Armengaud *et al.*, 2004; Troufflard *et al.*, 2010). In order for these results to be applicable to agriculture it is important to assess whether crop plants respond to K in a similar manner as the model plant. In this project the effect of K-deficiency on growth, metabolite concentrations, transcript levels and pathogen susceptibility of barley were investigated. Plants were grown in full-nutrient (control) or K-free hydroponic culture. The physiological, biochemical and transcriptional effects of K-deprivation were accessed over a time course of 20 days. Roots and shoots from plants grown in K-free nutrient solution had significantly lower K concentration than those grown in the control solution after 3 and 6 days respectively. A significant reduction in growth was seen as early as 6 days after K withdrawal. K-starvation led to a slight decrease in nitrogen metabolism, while hexose sugars strongly accumulated. By day 9 a significant increase in the expression of JA marker genes was seen in plants grown in K-free nutrient solution. Thus, despite possible differences in downstream events an induction of JA biosynthesis in response to K-deficiency occurs in both Arabidopsis and barley. Detached leaf segments were used to assess the effect of K-deficiency on infection of barley by two fungal pathogens with different strategies for nutrient acquisition. K-deficient barley plants were less susceptible to the biotroph *Blumeria graminis f.* f. sp. *hordei* (powdery mildew) and more susceptible to the hemi-biotroph *Rhynchosporium secalis.* Treatment of detached leaves with methyl-jasmonate (Me-JA) also led to less *B. graminis* infection, but had no effect on the *R. secalis* infection, indicating that JA increase in response to K-deficiency influences *B. graminis* but not *R. secalis* infection. The study therefore provides strong evidence that the effect of K-deficiency on pathogen susceptibility is determined by the JA-sensitivity of the pathogen.

Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 6

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Acknowledgments

I would like to thank my supervisors Anna Amtmann, Adrian Newton and Philip White for all their guidance and support. I would also like to thank everyone at Glasgow University and the SCRI who helped me during my project, especially my lab and office mates past and present for all the advice and instruction. A special thank you to Pete Hedley for making it in to work through the snow to help with analysing my microarray data, and Jenny for helping with the arrays. I am also grateful to Hannah Florance of Exeter University for measuring the hormone levels in my samples.

Declaration

I hereby declare that all the work presented in this thesis is my own, except where explicitly stated otherwise. This thesis has not in whole or any part been previously presented for a higher degree.

Jayne Davis

20th July 2011

Chapter 1. Introduction

1.1. Importance of crops

In recent decades increasing world population, reduced availability of agricultural land, and demand for cheaper food has led to the use of more intensive farming practices. Increased cropping intensity leads to more nutrients being removed from the soil each season and higher likelihood of spread of disease. To maintain soil nutritional status and prevent disease in these systems high levels of fertilisers and pesticides are often used. Increasing prices and environmental concerns are putting pressure on farmers to reduce application of these chemicals. To accomplish this, it is important to understand the effect of nutrient deficiency on plants and how this interacts with susceptibility to pathogens.

The human population is expanding rapidly, the world population was predicted to rise from around 5,900 million between 1997 and 1999 to around 7,200 million by 2015, an increase of around 1.2 % each year, and is predicted to reach 10 billion by 2050 (FAO, 2002). This increase in population will lead to increased demand for food and other agricultural products. It is estimated that demand for agricultural products will raise by 1.6 % per annum between 1999 and 2015 (FAO, 2002). A number of other factors are putting pressures on food production. Reduced availability of agricultural land due to loss of fertility and changes in environmental conditions and increased use of land for non-food crop production such as biofuels, as well as increased consumption of animal products are all contributing to increased pressure on the available land.

The increased demand and reduced availability of land for food production is expected to lead to more intensive farming methods being employed to increase the yield for crop plants. At present a number of factors impact yield; two key factors are mineral nutrient availability and disease. Nutrient status can have a direct negative effect on yield, but can also affect yield indirectly as it can alter other important factors such as water uptake, abiotic stress resistance, or susceptibility to disease. To meet the increased demand for food production using available agricultural land it is important that we understand the factors affecting crop production and the ways in which these interact with each other.

1.1.1. Barley

Barley (*Hordeum vulgare* L.) is a versatile crop able to adapt to a number of stress conditions and it is therefore suitable for cultivation in a wide variety of environments throughout the world (Newton *et al.*, 2011). Although up to two thirds of the world's barley crop is used for animal feed, it is also essential for the brewing and distillation industries. In addition to production of alcoholic beverages, barley is used in non-alcoholic drinks, bread, health foods and soups or stews. Barley is ranked fourth world wide for both quantity produced and area of cultivation amongst the cereals after wheat, rice and maize (FAO), and it is second to wheat in the UK. In 2009, over 150 million tonnes of barley were produced worldwide, Russia being biggest producer with just under 18 million tonnes of grain while the UK was the ninth highest producer with just over 7 million tonnes (FAO).

The barley genome is very large, twice the size of the human genome, at around 5,500 MB, 80 % of which is repetitive DNA (Sreenivasulu *et al.*, 2008). Barley has seven chromosomes, which are representative of the other Poaceae, such as rye and wheat. The barley genome is diploid making it more amenable to genetic analysis and manipulation than the polyploid wheat; it is therefore a good genetic model for the family. Although there are a variety of genetic tools available for barley, such as ESTs, TILLING populations and RFLP and SNP linkage maps (Sreenivasulu *et al.*, 2008), the genome has not yet been fully sequenced, although, the International Barley Sequencing Consortium are currently working towards this goal (Mayer *et al.*, 2011; Schulte *et al.*, 2009).

1.2. Nutrients in plants

When land is heavily cultivated essential mineral nutrients are quickly depleted and this occurs particularly quickly where high density monoculture crops are grown. In order for plants to complete successfully their life cycle they must obtain 14 essential elements from the soil in addition to carbon, hydrogen and oxygen from the atmosphere (Marschner, 1995; Taiz and Zeiger, 1998; White and Brown, 2010). The three most commonly deficient elements in agricultural soil are potassium, nitrogen and phosphorus (Amtmann *et al.*, 2006). To correct these deficiencies heavy fertilization is used to maintain quality and yield of crops in the western world. However, with concerns about environmental impact of chemical fertilizers and increasing fertilizer costs, farmers are looking to reduce fertilizer input while maintaining productivity and quality. In poorer countries the importance of K for crop production is often neglected and many soils have become seriously K-deficient due to years of fertilisation with nitrogen and phosphorus alone.

1.2.1. Potassium in plants

The macronutrient potassium (K) is required in high quantities in plants. It is the most abundant inorganic cation in plants, and can account for up to 10 % of the dry weight (Broadley *et al.*, 2004; Leigh and Wyn Jones, 1984; White and Karley, 2010). K is important in a variety of physiological processes involved in growth and development and it is therefore found in the highest concentrations in growing and reproductive tissues. Unlike other macronutrients K is not metabolized or incorporated into other macromolecules, instead it acts as a co-factor, osmoticum or charge balancer in a number of biophysical and biochemical processes (Amtmann *et al.*, 2006; Marschner, 1995; White and Karley, 2010).

1.2.2. Physiological use and deficiency symptoms

1.2.2.1. Biophysical functions

K plays an important role as an osmoticum in growth, stomatal movement, and light driven and seismonastic movements of organs and phloem transport (Mengel and Arneke, 1982; White and Karley, 2010). During growth, accumulation of K in the vacuole, along with an anion, creates the osmotic potential required for water uptake leading to cell expansion (Dolan and Davies, 2004; Mengel *et al.*, 2001), allowing leaf expansion, extension of the roots and root hairs (Høgh-Jensen and Pedersen, 2003; Mouline *et al.*, 2002), elongation of pollen tubes (Fischer and Hsiao, 1968), and growth of fruits and tubers. K movement also delivers a charge-balancing counter flux, which is critical for the movement of other ions across membranes. Transport of sugars, amino acids and nitrate all require counter K fluxes (Marschner, 1995). Rapid movement of K across membranes is also required during stomatal opening for accumulation of K in the guard cells decreasing the water potential within the cell (Blatt, 2000; Schroeder *et al.*, 2001).

A high level of mobility is required to carry out these roles, and although K may be replaced by some other inorganic cations, sodium (Na) is toxic in high concentrations in the cytoplasm and can compete with K for enzyme binding sites (Horie *et al.*, 2007). Once expansion has taken place the osmotic potential can be maintained with sugars, organic acids and compatible solutes, and K can be partially recovered for use in other processes (Amtmann *et al.*, 2004; White and Karley, 2010).

1.2.2.2. Biochemical functions of K in plants

A number of biochemical processes in plants rely on a high, stable K concentration in the metabolically active components of the cell such as the cytoplasm, nucleus, stroma of the chloroplasts and the mitochondrial matrix (Evans, 1963; White and Karley, 2010). The optimal concentration for the reactions is around 100 mM (Evans and Sorger, 1966), however when K supply is ample the K concentration in these cellular compartments can be as high as 200 mM (Leigh and Wyn Jones, 1984).

One of the key biochemical roles of K is that of a co-factor for enzymes. More than 60 enzymes require K to function (Evans and Sorger, 1966). These enzymes have a wide variety of functions (Di Cera, 2006) and include pyruvate kinase (Peoples and Koch, 1979), ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) (Nitsos and Evans, 1969), and starch synthase (Leigh and Wyn Jones, 1984).

1.2.3. Effect of K-deficiency in plants

Plants are able to adapt well to a wide range of K conditions and grow well in external K concentrations between 10 µM to 100 mM (Marschner, 1995). K concentrations of around 2 % of the dry weight are required for near maximal growth (Leigh and Wyn Jones, 1984), however the critical tissue K concentration required for optimal plant growth depends on the presence of other cations such as magnesium (Mg) and sodium (Na) (White and Karley, 2010). Plants have a number of coping mechanisms when K is scarce; these include, the redistribution of K during mild and early K-deficiency from mature tissue to developing tissue (Gierth *et al.*, 2005; Schachtman and Shin, 2007; Wang *et al.*, 1998) and increased activity of high affinity K influx systems (Høgh-Jensen and Pedersen, 2003; White, 1993). During early K starvation, the available K is relocated from the vacuole to the cytoplasm and metabolically active organelles resulting in disruption of biophysical processes before the disruption of biochemical processes (Leigh and Wyn Jones, 1984).

The wide variety of biochemical and physiological processes that rely on K are reflected in the deficiency symptoms resulting from insufficient K. The processes in plants that are K-dependent are often interrelated, which makes determination of the critical process in development of the K-deficiency symptoms difficult (Amtmann *et al.*, 2006). For example the changes in transpiration, sugar metabolism, synthesis of photosynthetic enzymes and chlorophyll levels all interact with each other (Amtmann *et al.*, 2006).

1.2.4. Physiological effects of K-deficiency

A reduction in growth is one of the best documented effects of K-deficiency in plants. A reduction in shoot growth (Ashraf *et al.*, 2001; Høgh-Jensen and Pedersen, 2003; Jordan-Meille and Pellerin, 2008; Tewari *et al.*, 2007; White, 1993) and a reduction in the size of cells from young leaves of plants grown in low K media has been reported (Mengel and Arneke, 1982). A number of studies have shown that there is a reduction in root dry weight in K-starved plants (El Dessougi *et al.*, 2002; Stamp and Geisler, 1980). The effect of K-deficiency on root elongation appears to be more complicated, with different studies reporting contradictory results. Jordan-Meille & Pellerin (2008) found that root elongation was slightly increased in maize while other studies have shown a decrease in root elongation in barley, wheat and sugar beet (El Dessougi *et al.*, 2002; Shin and Schachtman, 2004; Walker *et al.*, 1998).

The physiological effects of K-deficiency on plants are not limited to changes in growth. K-starved plants often develop necrotic and chlorotic lesions on the leaves. Due to relocation of K from older leaves to younger leaves in low K conditions, lesions first occur on the older leaves. It has been suggested these lesions may be due to production of reactive oxygen species (ROS), such as hydrogen peroxide, in K-deficient tissue (Tewari *et al.*, 2007). K-deficiency also leads to changes in water relations within the plant, due to reduced availability of K as an osmoticum. This has a knock-on effect on the stomatal aperture, transpiration rate, turgor and vascular transport (Blatt, 1988; Deeken *et al.*, 2002; Mengel and Haeder, 1977; Zhao *et al.*, 2001).

1.2.5. Metabolic effects of K-deficiency

In addition to the physiological changes brought about by K-deficiency a number of biochemical changes occur. A reduction in photosynthesis has been widely reported in K-deficient plants including cotton (Bednarz *et al.*, 1998; Zhao *et al.*, 2001), *Medicago sativa* (Peoples and Koch, 1979) and young spruce trees (Kanai *et al.*, 2007; Lütz *et al.*, 1992). There are many possible contributing factors to this decline. The inhibition of K-dependent allosteric enzymes, such as RuBisCo, is seen in plants with low K (Peoples and Koch, 1979). It has also been suggested that a build-up of sugars in the leaves during K starvation, may lead to a reduction in photosynthesis through feedback inhibition (Hermans *et al.*, 2006). A further limiting factor to photosynthesis during K-starvation is a reduction in carbon up-take due to reduced stomatal aperture and reduced carbon dioxide fixation (Kanai *et al.*, 2007).

K-starved plants have been shown to have reduced levels of chlorophyll (Cakmak, 1994; Stamp and Geisler, 1980; Zhao *et al.*, 2001). Stamp and Geisler (1980) demonstrated a 40-50% reduction in chlorophyll in K-starved cereals compared with those supplied with sufficient K. Chlorophyll concentration returned to normal after K resupply. Insufficient K can also affect the ultrastructure of chloroplasts, resulting in fewer, less well defined grana stacks, reduced lamellae, large starch granules and greater plastoglobuli (Zhao *et al.*, 2001). Despite the reduction in photosynthetic rate, sugar concentrations are often increased in the leaves during periods of K-starvation (Armengaud *et al.*, 2009; Cakmak, 2005; Huber, 1984), possibly due to a reduction of long-distance transport of sugars in K-deficient plants (Cakmak, 2005; Marschner, 1995; Mengel and Haeder, 1977; Richards and Coleman, 1952). This increase in sugar concentration in the leaves may affect the photosynthetic rate through feedback inhibition (Hermans *et al.*, 2006; Mengel and Haeder, 1977).

Nitrogen metabolism is also affected by K-deficiency. This may be due to inhibition of K dependent N-metabolising enzymes or disruption of amino acid and nitrate transport due to a requirement for K for it to function fully (Bednarz and Oosterhuis, 1999; Lütz *et al.*, 1992; Peoples and Koch, 1979). In barley, low K availability can lead to low total nitrogen in roots and shoots and changes in amino acid content within the plant (Helal and Mengel, 1979; Høgh-Jensen and Pedersen, 2003; Richards and Berner, 1954). Putrescine, a product of the breakdown of amino acids, has also been shown to increase in K-deficient plants (Altman and Levin, 1993). Polyamines such a putrescine are known to be involved in regulation of growth and stress, by binding macromolecules with negative charges (Kang *et al.*, 2004).

Based on comprehensive analysis of metabolic, transcript and enzyme activities in K-deficiency and K resupply in *A. thaliana* plants, Armengaud *et al.* (2009) proposed that changes in carbon and nitrogen compounds during K-deficiency are ultimately the result of decreased glycolytic flux in the roots due to decrease in pyruvate kinase activity at low cytoplasmic K (Figure 1-1).

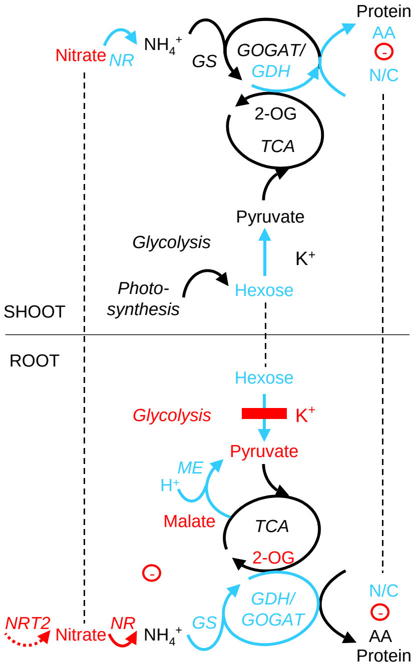


Figure 1-1 Effect of low K on primary metabolism in *A thaliana* (Armengaud *et al.*, 2009) Biochemical and transport pathways are shown with solid and dashed arrows respectively. Metabolite concentrations and enzyme activities increased in response to K-deficiency are in blue and decreased in red. The red bar represents putative direct inhibition of PK by low K. Abbreviations: NRT2: nitrate transporter 2, NR: nitrate reductase, GS: glutamine synthase, GDH: glutamate dehydrogenase, GOGAT: glutamine-2-oxoglutarate aminotransferase, AA: amino acids, 2-OG: 2-oxoglutarate, TCA: tricarboxylic acid (Krebs) cycle, ME: malic enzyme.

1.2.6. Influence of K-deficiency on response to stress

K-deficiency generally decreases tolerance to abiotic stresses, such as frost (Grewal and Singh, 1980), drought (Egilla *et al.*, 2005; Egilla *et al.*, 2001) and salt (Cakmak, 2005). Plant susceptibility to biotic stresses is, therefore, affected by K-supply. A common perception is that high K availability leads to a decrease in disease, and provision of sufficient K is recommended to reduce disease (Imas and Magen, 2000). The effect of K-supply on disease however does not seem to be that simple; studies looking at the effect of K-availability on disease show contrasting results (Perrenoud, 1990). For example, specific host-pathogen interactions can vary in response to K supply, Perronould (1990) reported an decrease in powdery mildew on barley in 17 out of 21 studies, however, the remaining studies showed an increase in disease occurrence (9 studies) or no change (5 studies). This may be due to differences in K availability between trials, but variety of barley and other growth conditions might also have influenced the outcome.

1.2.7. Molecular responses to K-deficiency

The physiological effects of K-deficiency on plants have been widely studied, however until recently little was known about the effects of K-deficiency at a molecular level. Kang *et al.* (2004) carried out a proteomics study comparing K-deficient (3 h and 7 d in 10 µM K) and K-sufficient (2 mM K) *A. thaliana* seedlings. They identified over 100 proteins which showed a change in expression in response to K- deficiency and assigned putative functions to many of them, including 29 related to metabolism, 17 to signal transduction, 14 to transcription and translation, 6 to defence and 2 storage related proteins (Kang *et al.*, 2004). Several microarray studies have been carried out with the aim to identify responses to K-deficiency at the level of transcripts (Armengaud *et al.*, 2004; Gierth *et al.*, 2005; Hammond *et al.*, 2003; Hampton *et al.*, 2004; Maathuis *et al.*, 2003). Maahuis *et al*. (2003) concentrated on effects of nutrient deficiency on root transporters, and Girth *et al.* (2005) investigated the effect of short term K-deprivation on *A. thaliana*. Microarray studies were used to investigate the interactions between cesium (Cs) toxicity and K starvation in *A. thaliana* (Hampton *et al.*, 2004), different sets of genes had altered expression in response to each stress, indicating that Cs toxicity is not perceived as K-deficiency.

Armengaud *et al*. (2004) used microarray technology to investigate transcriptional changes of *A. thaliana* plants during K-starvation (2 weeks in micro molar K) and K-resupply (2-24 h) experiments. They used iterative group analysis (Breitling *et al.*, 2004b) to assign differentially regulated genes to functional groups, allowing the identification of four main functional categories as being dependent on external K-supply. These were 1) genes related to jasmonic acid (JA) biosynthesis and signalling pathway, 2) genes encoding cell wall proteins, 3) genes with a function in transport and 4) genes encoding calcium binding proteins (Armengaud *et al.*, 2004). Based on known and putative functions of the regulated genes the authors produced a schematic overview of processes that altered during K-deficiency (Figure 1-2).

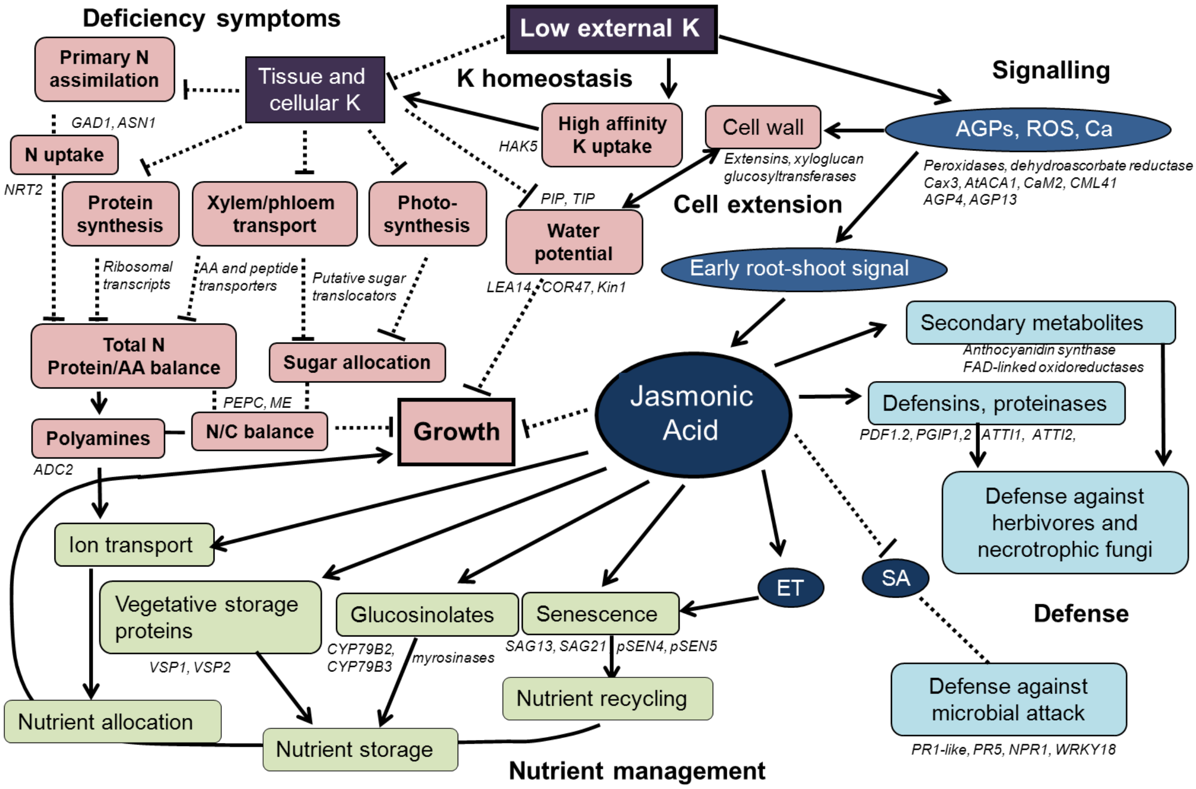


Figure 1-2 Plant adaptation to K-deficiency (adapted from Amtmann *et al*., 2006). Black arrows indicate stimulation, dashed lines inhibition. Known K-deficiency symptoms are shown in pink boxes, putative components of signalling events are indicated in dark blue, JA-dependent processes potentially leading to adaptive nutrient management in green and defence responses in pale blue.

1.2.8. Hormonal responses to K-deficiency

The largest category of genes up-regulated during K-starvation contained genes involved in signalling, including JA-biosynthesis genes such as lipoxygenase 2 (*LOX2*), allene oxide synthase (*AOS*) and allene oxide cyclase (*AOC*) as well as well-known JA-responsive genes such as vegetative storage protein 1 (*VSP1*) and JA inducible thionin (*Thi2.1*). An increase in JA concentration during K-deficiency in *A. thaliana* was confirmed by Cao *et al.* (2006) and Troufflard *et al.* (2010), who also showed that induction of JA marker genes was specific for K, i.e. it did not occur in response to deficiencies in N, S or Ca. K-deficient *A. thaliana* plants also show increased ethylene (ET) concentration and ET-related gene expression (Hampton *et al.*, 2004; Shin and Schachtman, 2004). ET and JA have been shown to act synergistically during defence responses (Figures 1-2 and 1-6) (Ellis and Turner, 2001). Hampton *et al.* (2004) also identified a number of genes related to auxin that were up-regulated during K-starvation.

1.3. Jasmonate

Phytohormones play an important role in controlling a number of plant processes including growth, reproduction and defence (Taiz and Zeiger, 1998). The phytohormone jasmonic acid (JA) is involved in processes related to growth, development and response to stress. A number of comprehensive reviews on JA signalling have been published in recent years (Avanci *et al.*, 2010; Browse, 2009; Chico *et al.*, 2008; Kazan and Manners, 2008; Memelink, 2009; Reinbothe *et al.*, 2009; Staswick, 2008; Wasternack, 2007). JA forms part of a complex network of signals that respond to abiotic and biotic stresses (discussed further in Section 1.3.6.). One of the best known abiotic stress that elicits a JA response is wounding (Li *et al.*, 2005; Wasternack *et al.*, 2006), but JA has also been shown to be increased in response to drought, cold, and nutrient deficiencies including K (Armengaud *et al.*, 2004; Jung *et al.*, 2007).

1.3.1. Jasmonate biosynthesis

JA is one of a group of compounds derived from oxygenated polyunsaturated fatty acids known as oxylipins (Howe and Schilmiller, 2002; Mosblech *et al.*, 2009; Reinbothe *et al.*, 2009). Work by Vick and Zimmerman (1984) first described the biosynthesis of JA, one of the first oxylipins discovered in plants. Since then JA-biosynthesis has been extensively studied. Most of this research has been carried out in dicots such as *A. thaliana*, tomato and tobacco, while monocots such as wheat and barley have been studied to a lesser extent (Kazan and Manners, 2008).

The synthesis of oxylipins in plants starts in the chloroplast where linolenic acid (LA) is released from the chloroplast membrane by phospholipases (Figure 1-3). Phospholipase 1A (Defective in Anther Dehiscence - DAD) has been shown to be involved in this step of JA-biosynthesis during anther dehiscence, pollen maturation, filament elongation and flower opening, but not in response to wounding in *A. thaliana* (Ishiguro *et al.*, 2001). A homolog of DAD1, Dongal (DGL) has also been identified in leaf chloroplasts. DGL has a weak phospholipase A activity and appears to play a role in growth regulation and the rapid JA burst after wounding (Hyun *et al.*, 2008). A phospholipase D (PLD) has been shown to be induced by wounding and this response was abolished in an *A. thaliana pld* knockout mutant (Zien *et al.*, 2001). After release from the membrane, the next step is the oxygenation of LA by lipoxygenase 2 (LOX2 also referred to as 13-LOX) to form 13-hydroperoxy linolenic acid (13-HPOT) (Bell *et al.*, 1995); this is the first step of the LOX pathway by which JA is formed.

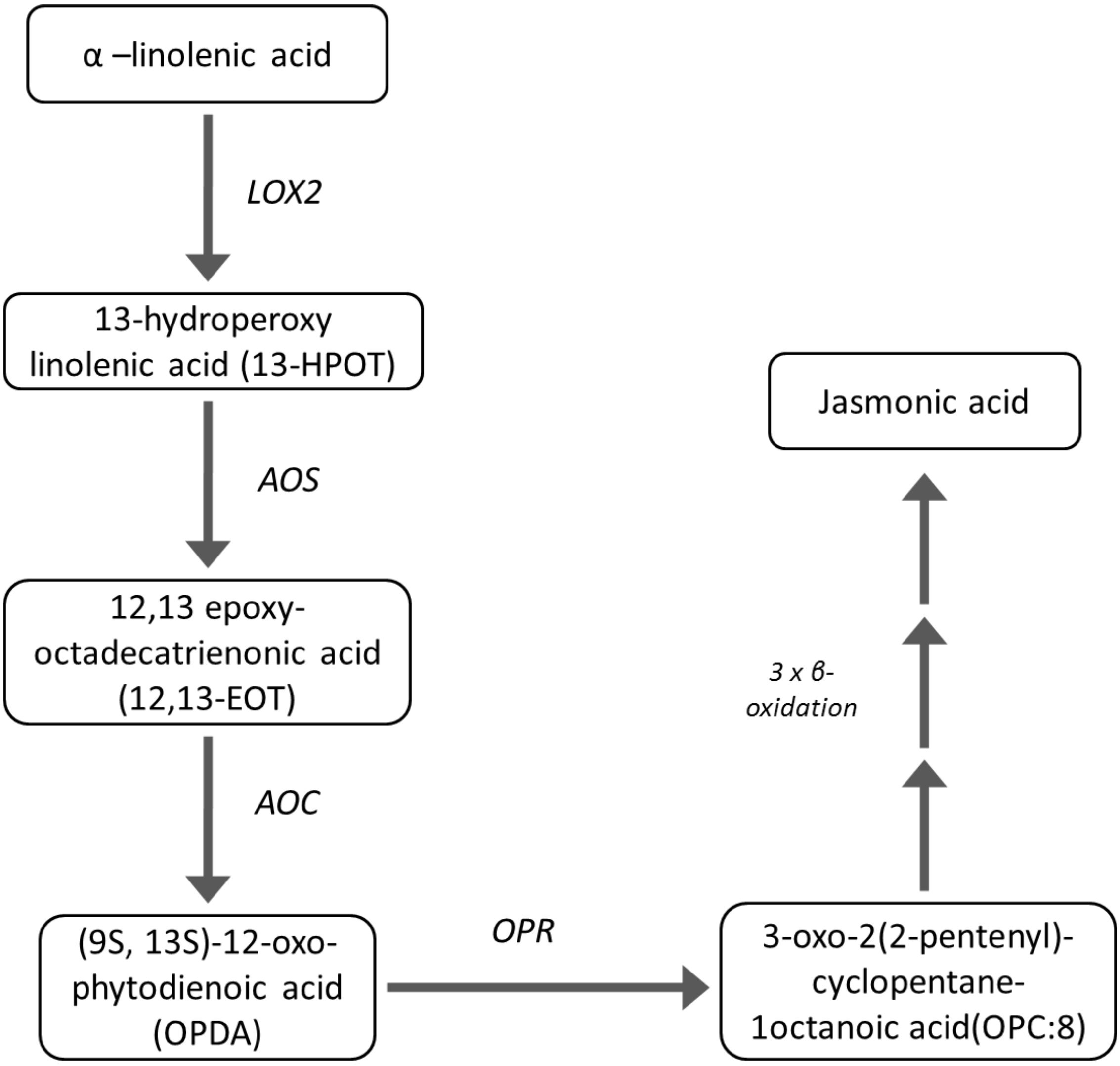


Figure 1-3 The Jasmonate biosynthesis pathway (adapted from Browes *et al.*, 2009).
A flow diagram showing the formation of Jasmonic acid from linolenic acid, biosynthesis intermediates are in boxes and enzymes are in italics. Abbreviation: LOX2: lipoxygenase 2, AOS: allene oxide synthase, AOC: allene oxide cyclase and OPR: OPDA reductase.

The first committed step in JA-biosynthesis is the dehydration of 13-HPOT by allene oxide synthase (Laudert *et al.*, 2000; Laudert and Weiler, 1998; Song *et al.*, 1993), forming the chemically unstable allene oxide intermediate 12,13 epoxy-octadecatrienonic (12,13 EOT), which naturally decomposes to form α-ketones and β-ketones. AOS is a cytochrome P450 which uses oxygenated fatty acid hydroperoxide substrates as oxygen donors and as source for reducing equivalents (Howe and Schilmiller, 2002). A cyclopentone ring (9S,13S)-12-oxo-phytodienoic acid (OPDA) is formed from 12, 13 EOT, catalysed by allene oxide cyclase (Hamberg and Fahlstadius, 1990; Ziegler *et al.*, 2000). Coupling of AOS and AOC is required for a successful formation of OPDA, however formation of a complex is not necessary (Zerbe *et al.*, 2007). This is the last step to take place in the chloroplast.

It is still unclear how OPDA is exported from the chloroplast; no transporters have been identified to date. The ABC transporter COMATOSE peroxisomes (CTS) have been shown to be at least partially responsible for the uptake of OPDA into the protosome in an ATP dependent process (Delker *et al.*, 2006; Theodoulou *et al.*, 2005), however, residual JA implies that other modes of transport also exist. An ion trapping mechanism, taking advantage of the pH difference between the peroxisomes and cytoplasm could possibly be a second mechanism for OPDA transport through peroxisome membranes (Theodoulou *et al.*, 2005).

OPDA is reduced by 12-oxo-phytodienoic acid reductase (OPR3), which has been shown to contain a peroxisome target sequence (Strassner *et al.*, 2002), to form 3-oxo-2(2'-pentenyl)-cyclopentane-1-octanoic acid (OPC:8). *A. thaliana opr3* mutants have been identified and have been shown to be deficient in JA signalling and to accumulate OPDA after wounding (Stintzi and Browse, 2000). JA is formed from OPC:8 by a series of 3 β-oxidation steps, shortening the β-oxidative side chain (Wasternack, 2007). A number of enzymes have been identified as being involved in these reactions, including acyl-CoA oxidase (ACX) in tomato (Li *et al.*, 2005) and multifunctional protein (MFP) and 3-ketoacyl-CoA thiolase (KAT) in *A. thaliana*. In *A. thaliana* knocking out *acx1* alone did not prevent JA synthesis, however, the *acx1/5* double mutant did. Further experiments showed that *ACX1/5* are essential for male reproductive development and defence against chewing insects such as *Trichoplusia ni* (Schilmiller *et al.*, 2007). The *aim1* mutant, which is disrupted in one of the two *MFP* genes, has impaired JA response to wounding and expression of JA-related genes (Delker *et al.*, 2007). When *KAT2* in *A. thaliana* is silenced JA accumulation in response to wounding and expression of JA marker gene JR2 were reduced (Cruz Castillo *et al.*, 2004).

1.3.2. JA conjugates

Once JA is produced it can be metabolised by a variety of enzymes to produce a number of compounds known collectively as jasmonates (Figure 1-4) (Wasternack, 2007). Few of the genes encoding enzymes involved in their production have been cloned so far. Some JA precursors and JA conjugates are signalling molecules in their own right and often induce a specific subset of JA-responsive genes (Kramell *et al.*, 2000).

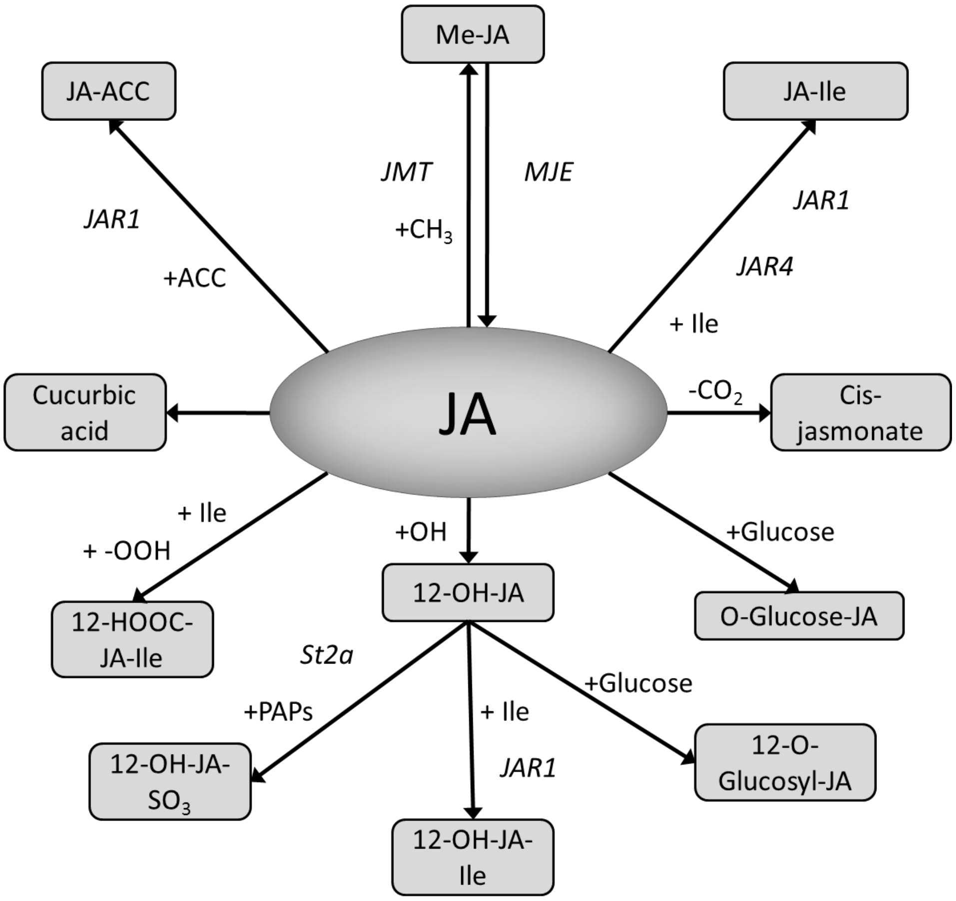


Figure 1-4 Metabolic fate of Jasmonic acid (adapted from Wasternack, 2007 and Wasternack & Kombrick 2010). After biosynthesis JA can undergo a variety of modifications, JA conjugates are shown in grey boxes and enzymes are in italics. Abbreviations: Me-JA: Methyl jasmonate, JA:Ile: jasmonoyl-l-isoleucine, 12-OH-JA: 12-hydroxyjasmonic acid, 12-HO-JA–Ile: 12-hydroxyjasmonoyl Isoleucine, 12-HOOC-JA–Ile: 12-carboxyjasmonoyl isoleucine, JA-ACC: jasmonoyl 1 aminocyclopropane-1-carboxylic acid, JMT: JA methyl transferase, MJE: methyl jasmonate esterase, JAR1/4: jasmonic acid resistance 1/4, St2A: 12-OHJA-sulphotransferase.

1.3.3. JA signal recognition

A number of JA-responsive promoter elements have been identified in plants, the most common of which are the GCC and G-box promoter sequences (Memelink, 2009). A jasmonate- and elicitor- responsive element (JERE) containing a GCC-box-like sequence has been identified in the promoter of the terpenoid indole alkaloid biosynthesis gene strictosideine synthesis (STR) from *Catharanthus roseus* (Menke *et al.*, 1999). When a JERE tetramer is fused to a minimal promoter, Me-JA-responsive gene expression is conferred, showing JERE is an autonomous Me-JA-responsive sequence. Some GCC reporter elements have been shown to act as ethylene reporter elements (Fujimoto *et al.*, 2000; Ohme-Takagi and Shinshi, 1995). The *PDR1.2* gene is expressed in response to a combination of JA and ET it has been suggested that this may be due to the convergence of the two hormones on the GCC-box within the PDF1.2 promoter (Memelink, 2009). G-box elements are highly represented in the promoters of JA-related genes in *A. thaliana,* and have been identified in promoters for a variety of JA-responsive genes, including Protease inhibitor 2 in potato (Kim *et al.*, 1992), vegetative storage protein B (*VSPB*) in soybean (Mason *et al.*, 1993) and *VSP1* in *A. thaliana* (Guerineau *et al.*, 2003). Other JA-responsive TGACG (as-1-type) sequences have been found in the promoters of the *Agrobacterium tumefaciens* T-DNA *nopaline synthase* (*nos*) gene in tobacco (Kim *et al.*, 1993) and the barley *LOX1* gene (Rouster *et al.*, 1997). Two jasmonate-responsive elements JASE1 and JASE2 have been identified in the promoter of *OPR1* gene in *A. thaliana* (He and Gan, 2001).

Despite this knowledge of the promoter elements required for JA responsiveness, until recently the mechanism of JA detection remained elusive. However, the discovery of the Jasmonate ZIM domain (JAZ) repressor protein in 2007 (Chini *et al.*, 2007; Thines *et al.*, 2007) led to the identification of the SCF^{Coi1} complex (Figure 1-5). The *coi1* mutant was first identified in 1994 during screens of coronatine insensitive mutants and has been shown to be insensitive to Me-JA (Feys *et al.*, 1994; Staswick, 2008). The *coi1* mutant has been shown to be insensitive to JAs, more susceptible to pathogens (Feys *et al.*, 1994) and lacking JA-induced gene expression (Turner *et al.*, 2002). Mutants of *COI1* have also been identified in tomato (Li *et al.*, 2004b), soybean (Wang *et al.*, 2005) and *Nicotiana attenuata* (Paschold *et al.*, 2007) and *COI1* has been suppressed in tobacco (Shoji *et al.*, 2008), and potatoes (Halim *et al.*, 2009). All of these mutants are impaired in JA signalling. COI1 has been identified as an F-box protein (Xie *et al.*, 1998) and has been shown to associate with Rbx1, a ring box protein, cullin and S-phase kinase-associated protein (SKP1) to form an E3 ubiquitin ligase known as a SCF complex (Devoto *et al.*, 2002; Xu *et al.*, 2002). SCF E3 complexes are found in a variety of signalling pathways including auxin signalling (Memelink, 2009) and are involved ubiquitination of proteins in order to mark them for degradation by the 26S proteasome (Stone and Callis, 2007). The F-box region is responsible for the specification of SCF E3 to its targets and COI1 is specific to the JA pathway. However, the other components maybe involved in several pathways, *axr* mutants are also deficient in auxin signalling.

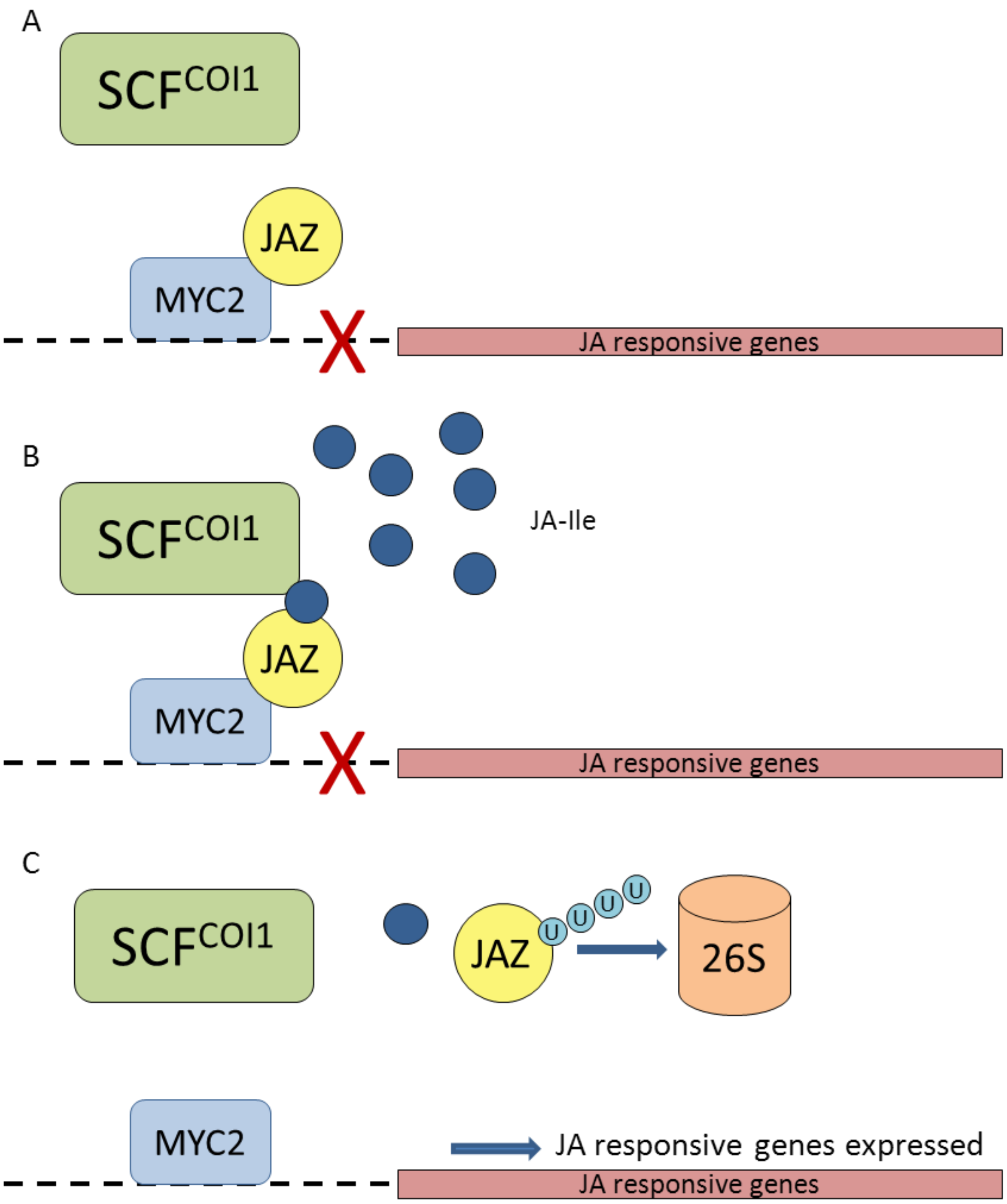


Figure 1-5 SCF^{COI1}/ JAZ signalling complex (Browse, 2009; Staswick, 2008). A: JAZ proteins suppress JA-related gene expression by the transcription factor MYC2. B: In the presence of jasmonoyl-l-isoleucine (Ile-JA, shown as a blue circle) the JAZ proteins interact with the SCF^{coi1} complex. C: The interaction with the SCF^{coi1} complex leads to the ubiquitination of JAZ marking it for destruction by the 26S proteasome and the removal of suppression of MYC2.

In the yeast-2-hybrid system and pull down assays, JAZ1 has been shown to interact with the SCF^{COI1} complex in the presence of JA-Ile and to a lesser extent JA-Leu but not in the presence of other JA or JA conjugates such as OPDA, Me-JA, JA-Trp and

JA-Phe (Thines *et al.*, 2007), indicating that JA-Ile and JA-Leu facilitate the binding of the SCF^{COI1} complex to the JAZ1 protein. The JAS region of the JAZ protein is necessary for this interaction to occur as it binds the JA-Ile (Melotto *et al.*, 2008). The SCF^{COI1}/ JAZ interaction is believed to lead to the removal of suppression of the MYC2 by JAZ3 leading to the expression of the early JA-responsive genes (Chico *et al.*, 2008; Staswick, 2008). Once released from the complex the JAZ protein is ubiquitinated in order to mark it for degradation by the 26S proteasomes.

MYC2 has been shown to bind to G-box and other related sequences in the promoter regions of different JA-related genes (Boter *et al.*, 2004; Chini *et al.*, 2007; Dombrecht *et al.*, 2007). MYC2 has been shown to induce genes related to wounding, JA biosynthesis, and drought (Abe *et al.*, 1997; Chini *et al.*, 2007; Kazan and Manners, 2008; Lorenzo *et al.*, 2004). Under low JA conditions, JAZ3 represses MYC2 activity at a protein level (Memelink, 2009) preventing transcription of JA-related genes. JAZ proteins were identified during genetic screens and microarray studies (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007) and form part of the Zim or TIFY family of proteins. JAZ proteins have been shown to contain a highly conserved Zim motif (Chini *et al.*, 2007), a C-terminal JAS domain and a less conserved N-terminal region (Memelink, 2009). The expression of the JAZ3 protein is controlled by MYC2, hence expression is increased in a positive feedback response to JA (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007).

1.3.4. JA target genes in barley

A number of JA-responsive genes have been identified in *A. thaliana* and are often used as JA-marker genes, such as *VSP* and *LOX2*. Few genes induced by JA in barley have been fully characterised although a number have been identified. Weidhase *et al.* (1987) reported the expression of a number of proteins in response to JA and methyl jasmonate (JA-Me) in senescing barley leaf tissue, including peptides with molecular weights of 66, 37 and 23 kDa, in addition to a number of minor proteins. In the absence of a known function, Jasmonate Induced Proteins (JIPs) were labelled with their molecular weight, for example a Mr 23,000 peptide is referred to as JIP23. In 1992, an attempt was made to characterise JIP23 and JIP15 (Andresen *et al.*, 1992). *JIP15* was only expressed *in vitro*, hence it had not been observed previously, but was strongly expressed when translated in a cell free protein-synthesising wheat germ extract. JIP15 was shown to be homologous to a precursor of a leaf thionin, which contains a signal peptide, a thionin domain and an acid peptide domain. JIP6, a possible thionin, was identified in mature leaf tissue. No protein sequences similar to JIP23 were identified (Andresen *et al.*, 1992). A JIP23 promoter and β-glucuronidase (GUS) fusion was used to demonstrate that JIP23 expression was regulated in response to JA at a transcriptional level. After introducing the construct by particle bombardment, a 3-fold increase in GUS expression was observed in response to JA (Müller-Uri *et al.*, 2002). Over-expression of barley *JIP23* in tobacco plants led to repression of several proteins, but no change in abundance of transcripts, indicating a role in translation (Görschen *et al.*, 1997).

JIP60 was identified as a ribosome inactivating protein (RIP), able to cleave animal and plant polysomes into their ribosomal subunits (Chaudhry *et al.*, 1994; Reinbothe *et al.*, 1994). Therefore, it may have functions both in defence and in the regulation of protein synthesis in stressed plants (Chaudhry *et al.*, 1994; Reinbothe *et al.*, 1994). JIP60 has been shown to exhibit N-glycosidase activity *in vivo* resulting in accumulation of inactive polysomes (Dunaeva *et al.*, 1999). One further JIP has been shown to be induced by JA, ABA, osmotic stress and wounding but not salt stress; *JIP37* was shown to be located on chromosome 3 and closely related to *JIP23*. JIP37 is located in the nucleus, cytoplasm and vacuoles (Leopold *et al.*, 1996)

A 40 % increase in trypsin inhibitor activity was observed in barley in response to exogenous application of JA, suggesting that formation of proteinase inhibitors may also occur in response to JA, but no change in chymotrypsin inhibitor activity was seen (Casaretto *et al.*, 2004).

1.3.5. Role of JA in defence

Plant defence against pathogens involves a number of signalling pathway, JA is a key hormone in plant defence responses. It is well established that JA is important in plants responses to insect attack (Howe and Jander, 2008); tomato (Howe *et al.*, 1996) and *A. thaliana* (Armengaud *et al.*, 2010; McConn *et al.*, 1997) plants with defective JA signalling have been shown to be more susceptible to herbivores. A large number of JA responsive genes are induced by wounding (Reymond *et al.*, 2000); these include antifeedants and poisonous compounds such as proteinase inhibitors (PI) of tomato and potato (Farmer *et al.*, 1992; Farmer and Ryan, 1992; Peña-Cortés *et al.*, 1992) and trypsin inhibitor in alfalfa (Brown and Ryan, 1984).

JA is also involved in defence against microbial pathogens. Plants responses to pathogens are dependent on the way in which they obtain nutrients. Biotrophs obtain nutrients from living cells and defence against these centres around the SA signalling pathway and hypersensitive response leading to programmed cell death preventing further spread of the pathogen throughout the plant. However, for Necrotrophs, which obtain nutrients from dead tissue, this strategy would be counterproductive, therefore the JA and ET pathways are triggered, leading to the activation of defence responses.

1.3.6. Hormone cross talk

Jasmonic acid, salicylic acid (SA), ethylene (ET), and to a lesser extent, ABA, are the main hormones that make up a complex signalling network controlling the response of plants to biotic stresses (Figure 1-6). A simplistic conceptualisation of this network is that JA and ET signalling act synergistically in defence against necrotrophs and herbivorous insects, while SA is involved in responses to biotrophic fungi and viruses (Bari and Jones, 2009; Berrocal-Lobo *et al.*, 2002; Dewdney *et al.*, 2000; Pieterse *et al.*, 2009). However, the reality is more complex due to the fact that JA and SA interact both as synergists and as antagonists (Balbi and Devoto, 2008; Devoto and Turner, 2005; Loake and Grant, 2007; Lorenzo and Solano, 2005; Rojo *et al.*, 2003).

Cross-talk is also seen between the JA and SA pathways, most of which is antagonistic, indicating that there is a trade-off between the two responses to pathogen attack. There are examples of SA blocking the JA signalling pathway in tomato (Pena-Cortés *et al.*, 1993), flax (Harms *et al.*, 1998), tobacco (Niki *et al.*, 1998) and *A. thaliana* (Cipollini *et al.*, 2004). The inhibition of JA synthesis by SA appears to be due to the inhibition of AOS synthesis (Harms *et al.*, 1998; Norton *et al.*, 2007), however, activation of AOS by SA has also been shown (Laudert and Weiler, 1998). There is also evidence of the reverse antagonistic interaction, the inhibition of SA by JA. JA has been shown to inhibit expression of PR genes (Niki *et al.*, 1998) and JAI1 has been shown to suppress SA signalling during *Pseudomonas syringae* infection in *A. thaliana* (Laurie-Berry *et al.*, 2006).

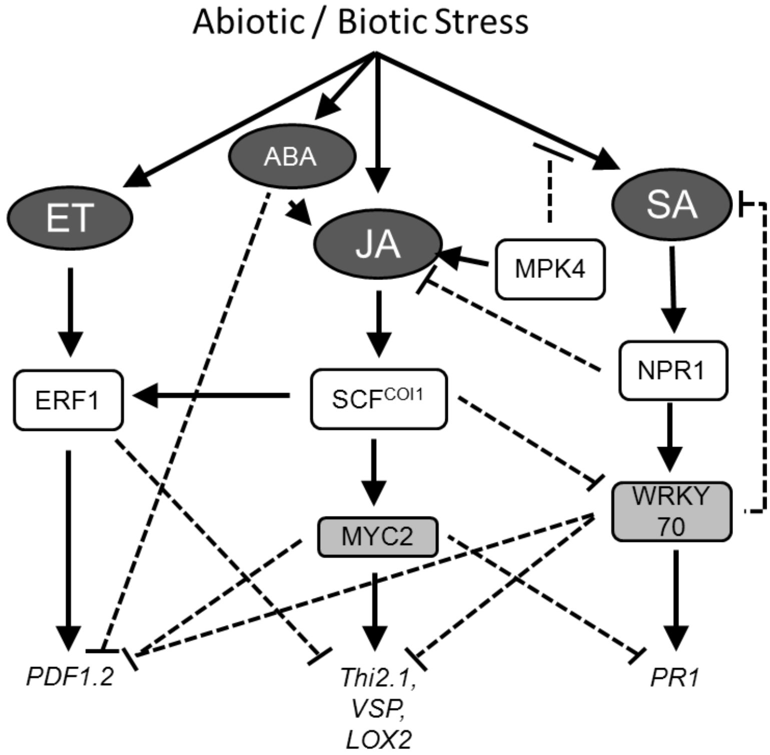


Figure 1-6. Stress-responsive network involving the JA, ET, SA and ABA signalling pathways. Different types of biotic or abiotic stress, such as pathogen infection or wounding, induce the synthesis and subsequent activation of several hormonal pathways (i.e. JA, ET, SA and ABA, shown in dark grey circles). (modified from Lorenzo and Solano, 2005). These hormones interact with one another via a variety of different signal molecules (shown as white squares) and transcription factors (shown in pale grey) in either a synergistic or antagonistic manner ultimately leading to the induction of genes involved in a variety of stress responses. Abbreviations: ET: ethylene, ABA: abscisic acid, JA: Jasmonate, SA: salicylic acid, ERF1: ethylene responsive factor 1, MPK4: MAP kinase 4, NPR1: Natriuretic peptide receptor 1, MYC2: bHLHzip transcription factor, PDF1.2: plant defensin, Thi2.1: JA-related gene expression, VSP: vegetative storage protein, LOX2: Lipoxygenase 2 and PR1: Pathogen related 1.

Positive interactions between SA and JA have also been demonstrated, e.g. simultaneous activation of the SA and JA pathways were observed in response to *Pseudomonas syringae* (van Wees *et al.*, 2000) and *Xanthomonas campestris* (Ton *et al.*, 2002). Four *A. thaliana cet* mutants show constitutive activation of both pathways, implying that they act in a signal cascade prior to the JA and SA pathways (Nibbe *et al.*, 2002). Mur *et al.* (2006) demonstrated that at low concentration JA and SA work synergistically, to increase the expression of PR1 and other JA markers, but at higher concentrations an antagonistic interaction occurs and apoplastic ROS is produced. Two possible points of convergence of these signals have been identified. The transcription factor WRKY70 and the MAP kinase 4 seem to play key roles in interactions between SA and JA (See Figure 1-6). WRKY70 is activated by SA and leads to the activation of SA-target genes (e.g. *PR1*), but is suppressed by JA and leads to inhibition of JA-related gene expression (*Thi2.1*) (Li *et al.*, 2004a). MAP-kinase 4 (MPK4) suppresses the SA pathway while activating the JA pathway (Petersen *et al.*, 2000).

1.4. The plant immune system

Plants are continuously attacked by an assortment of pathogens, including microbes such as fungi and bacteria as well as herbivores such as insects. However, in the majority of cases the pathogen is prevented from colonising by the plant's complex defence mechanisms. Although plant pathogens have diverse infection strategies they can be broadly categorized into two groups: biotrophs and necrotrophs (Glazebrook, 2005). Some pathogens utilize both strategies during their life cycle and are referred to as hemi-biotrophs. Biotrophic pathogens obtain the nutrients they require from living tissue and therefore it is beneficial for them to keep the plant alive. Necrotrophic pathogens obtain their nutrients from dead or dying tissue. These different infection strategies have led to a number of different plant responses to pathogens including hormone signalling (Discussed in Section 1.3.), *R*-gene mediated resistance and induced resistance.

1.4.1. *R*-gene mediated resistance

R-gene mediated resistance is the recognition of a pathogen product of the avirulence (*avr*) genes by the corresponding resistance (*R*) gene product in plants to initiate plant defences (Dangl and Jones, 2001; De Wit *et al.*, 1995; Glazebrook, 2005; Jones and Dangl, 2006). R genes have been shown to mediate plant responses to a wide range of pathogens including bacteria, fungi, oomycetes, nematodes, viruses and insects (Dangl and Jones, 2001). When a plant possesses the *R*-gene conferring resistance to the pathogen avr gene an incompatible interaction occurs and highly efficient defence mechanisms are triggered preventing pathogen growth. In the absence of the compatible *R*-gene, defences are not triggered, leading to a compatible interaction between the plant and pathogen. The triggering of *R*-gene mediated resistance often leads to the hyper-activation of basal defence responses and triggers the hypersensitive response (HR) leading to programmed cell death (Nimchuk *et al.*, 2003). The *avr* genes can be virulence factors and therefore are maintained within the pathogen population despite triggering *R*-gene mediated responses in some plants to allow full levels of pathogen growth when a susceptible host is found (Kjemtrup *et al.*, 2000). This interplay of *avr*-genes and *R*-genes means that the plants and the pathogens are constantly evolving, plants to recognise invaders and the pathogens to avoid recognition and maintain virulence. In the absence of the appropriate *R*-gene to allow specific recognition of the pathogen other non-specific defence mechanisms will be triggered restricting growth and spread of the pathogen.

1.4.2. Induced resistance

A primary infection by a pathogen or environmental stress my lead to enhanced resistance to any secondary attack, this in known as induced resistance and was fist reported by Ross *et al.* (1961) who observed an induceable response to tomacco mosaic virus (TMV) in tabacco. The best studied forms of induced resistance are systemic acquired resistance (SAR) and induced systemic resistance (ISR).

1.4.2.1. Systemic acquired resistance

Systemic aquired resistance (SAR) can be triggered by exposure to virulent, avirulent and nonpathogenic organisums or by chemical stimuli such as SA, 2,6-dichloro-isonicotinic acis (INA) or benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) and leads to heightened resistance both locally and distinct to stimulation (Vallad and Goodman, 2004) and can least a significant length of time. The increased resistance is not limited to the stimulus that triggered the resposne, SAR is effective against a wide variety of plant pathogens including fungi, bacteria and viruses (Kuć, 1982; Walters *et al.*, 2005). When the SAR is triggered an accumulation of SA occurs both locally and systemically leading to increased expression of a specific set of Pathogen Related (*PR*) genes (Van Loon, 1997) which required the ‘non-expressor of PR genes’ NPR1 (Shah *et al.*, 1997) . This response can be mimiced by treating plants with SA or one of its functional analogs which leads to the expression of the same set of *PR* genes (Ryals *et al.*, 1996; White, 1979). The SA pathway is essential for SAR and the activation of *PR* genes; plants unable to accumulate SA do not develop SAR or express the *PR* genes after infection (Delaney *et al.*, 1994; Gaffney *et al.*, 1993; Lawton *et al.*, 1995). In turn the SA signal transduction is dependant on the regulatory protein ‘non-expressor of PR genes’ NPR1 (Cao *et al.*, 1997; Shah *et al.*, 1997).

1.4.2.2. Induced systemic resistance

Non-pathogenic microbes are also able to elicit an increased disease resistance in plants. During ISR, beneficial rhizobacteria and fungi can trigger priming of defence mechanisms in the above ground plant tissues (Pieterse *et al.*, 2009; Van Loon, 1997; Walters and Heil, 2007). ISR is independent of SA, but instead requires JA and ET signalling (Léon-Kloosterziel *et al.*, 2005; Shoresh *et al.*, 2005), and has been shown to be effective against pathogens that are responsive to the JA- and ET-pathways (Ton *et al.*, 2002; Van Oosten *et al.*, 2008).

1.5. Pathogens in crops

1.5.1. Consequences of pathogen infection in crops

Plant disease can have a large impact on crop yield with losses of between 10 and 16 % worldwide (Oerke, 2006; Strange and Scott, 2005) costing up to 200 billion US dollars (Chakraborty and Newton, 2011). These losses are even more substantial when other biological stresses are included, such as weeds and herbivores and could reach up to 30 % (Christou and Twyman, 2004). Postharvest losses of between 6 and 12 % further increase the problem (Agrios, 2005). Major out-breaks of disease can lead to major food shortages and starvation especially when one crop is relied upon heavily. For example the Irish potato famine caused by *Phytophthora infestans* during the 1840s led the starvation of many and drove still more to emigrate (Fry, 2008; Large, 1940). The Great Bengal famine of 1943 was caused by devastation of the rice crop by the fungus *Cochliobolus miyabeanus* (Padmanabhan, 1973). A wide range of organisms cause disease in all crops, the major groups consisting of viruses, oomycetes, bacteria, fungi, nematodes and parasitic plants using a wide variety of infection strategies.

1.5.2. Barley pathogens

Barley plants are subject to attack by a wide range of pathogens. Oerke and Dehne (2004) estimated that without any treatment over 50 % of the world's attainable barley yield would be lost to pests. They estimated that weeds would account for 23 % of this loss and fungal pathogens (mostly *Pyrenophora teres*, *Rhynchosporium secalis*, *Puccinia hordei* and *Blumeria graminis* f. sp. *hordei and Cochliobolus sativus*) for 15 %, animal pests for 7 % and viruses for 3 %. In reality, the losses due to weeds and pathogenic microorganisms were much lower at just 8 % and 9 % respectively (Oerke and Dehne, 2004). Two major pathogens of barley are *R. secalis* and *B. graminis.*

1.5.3. ***Rhynchosporium secalis***

Rhynchosporium or leaf blotch or scald is caused by the fungal pathogen *Rhynchosporium secalis* which is able to infect barley, wheat, rye, and triticale as well some wild grass species (HGCA, 2008; Zaffarano *et al.*, 2006; Zhan *et al.*, 2008). The fungus causes scald-like lesions on the leaves, leaf sheaths and ears, however, infection occurs most often in leaf axils (HGCA, 2008). The lesions appear initially as pale green areas on the leaf surface but as the infection progresses the margins become dark brown, while the centre of the lesion remains pale green or brown (HGCA, 2008).

Infection with *R. secalis* leads to a large reduction of yield in barley crops, up to 40%, as well as poor grain quality leading to reduced value (Shipton *et al.*, 1974). Resistant cultivars, cultural practice or fungicide usages have not proved to be sustainable ways to protect against *R. secalis* (Zaffarano *et al.*, 2006).

1.5.3.1. Life cycle

Rhynchosporium is a polycyclic disease, the fungus completes a number of generations during a barley growing season (Figure 1-7) (Zaffarano *et al.*, 2006). Primary infection can occur when conidia of *R. secalis* from infected crop debris or other plant species (weeds) land on barley leaves (Davis and Fitt, 1992) or to a lesser extent through infected seed (Habgood, 1971). Secondary infection occurs when *R. secalis* conidia are dispersed within water droplets. The success of these infections often depends on the developmental stage of the plants and environmental conditions. After landing on the surface of the leaf, if conditions are favourable, the *R. secalis* conidia germinate and form one or two germ tubes (Linsell *et al.*, 2010; Zhan *et al.*, 2008) as early as one hour post inoculation (Linsell *et al.*, 2010). It is unclear if these germ tubes penetrate the cuticle directly or if an apposorium is formed, both possibilities have been reported (Linsell *et al.*, 2010; Thirugnanasambandam *et al.*, 2011; Zhan *et al.*, 2008).

Once the cuticle has been penetrated, hyphae grow sub-cuticularly between the epidermal cells above adjacent anticlinal walls (Jones and Ayres, 1974; Linsell *et al.*, 2010; Lyngs Jørgensen *et al.*, 1993) in the direction of the base of the leaf (Lehnackers and Knogge, 1989). Degradation of the epidermal cells occurs between 3 and 10 days post inoculation (Jones and Ayres, 1974; Linsell *et al.*, 2010; Lyngs Jørgensen *et al.*, 1993), leading to collapse of the cell which can be observed as pale green lesions on the leaf. As the infection progresses the hyphae invade the mesophyll cells. Ayesu-Offei and Clare (1970) suggested that hyphae might enter the mesophyll cells through stomata, however this was not observed by Linsell *et al.* (2010) although the hyphae circled the stomata. Instead Linsell *et al.*(2010) suggested that penetration may occur between the guard cells and the neighbouring epidermal cells. Penetration leads to the collapse of the mesophyll cells between 7 and 14 days post inoculation (Linsell *et al.*, 2010; Lyngs Jørgensen *et al.*, 1993). Directly after mesophyll collapse an increase in hyphal proliferation and branching is observed (Linsell *et al.*, 2010), possibly due to increased nutrient availability. Sporulation occurs 12 to 14 days post inoculation when conidia protrude through the cuticle layer onto the surface of the leaf (Linsell *et al.*, 2010; Lyngs Jørgensen *et al.*, 1993).

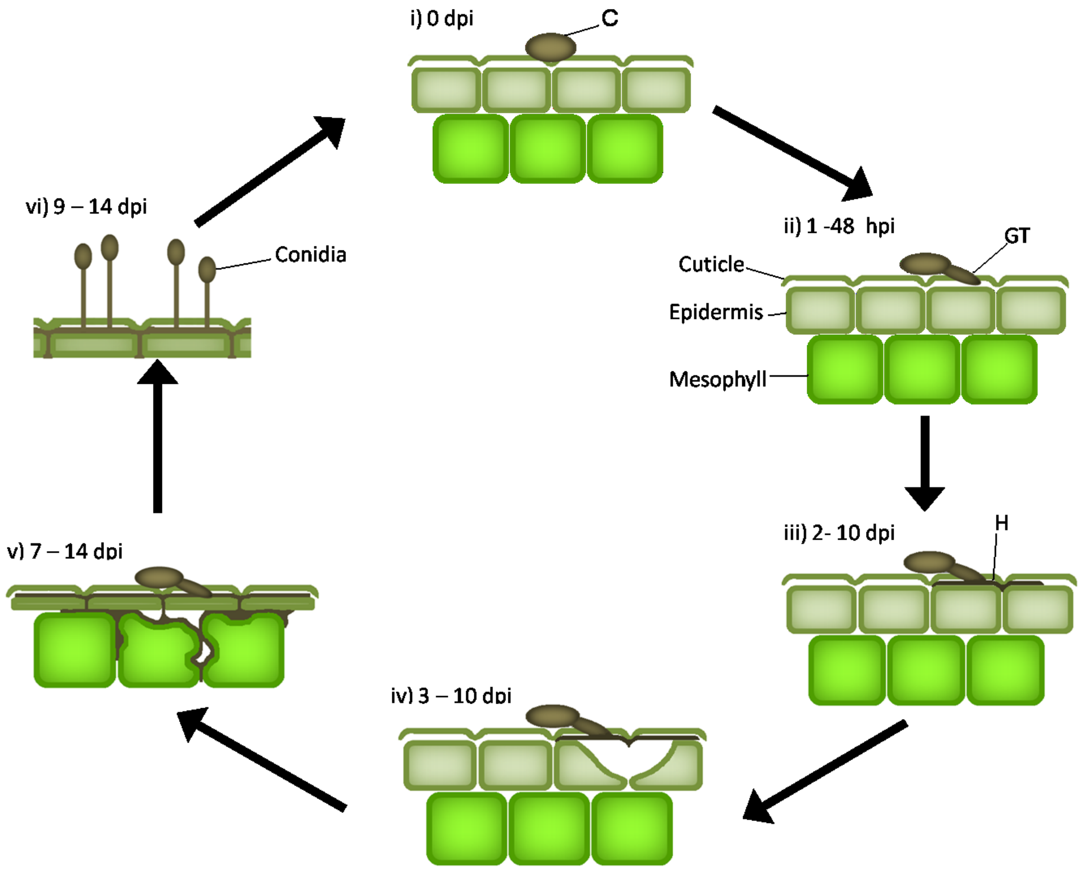


Figure 1-7 *Rhynchosporium secalis* life cycle (adapted from Zhan *et al.*, 2008)
After conidia land on the surface of the leaf (i) germination occurs and the cuticle is penetrated (ii) by the germ tube. Once the cuticle is penetrated sub-cuticlar hyphae form along the anticlinal walls of the host cells (iii), leading to the collapse of the epidermal cells (iv) and the spread to the mesophyll layer (v). Sporulation occurs (vi). Abbreviation: dpi: days post inoculation, C: conidia, GT: germ tube and H: hyphae

1.5.3.2. Defence against *R. secalis*

One of the best studied ways in which defence mechanisms are triggered by *R. secalis* infection is gene-for-gene interaction, during which interactions between the resistance genes (*R*-genes) and corresponding *R. secalis* genes (*avr*-genes) determine the outcome of the infection. A number of genes conveying resistance to *R. secalis* in barley have been identified and a single barley cultivar may contain several of these (Lehnackers and Knogge, 1989; Zhan *et al.*, 2008). Different races or pathotypes of *R. secalis* exist, with different avirulent and virulent alleles (Xi *et al.*, 2000). The presence of the correct *R*-gene leads to the collapse of single epidermal cells after cuticle penetration preventing further spread of the pathogen (Steiner-Lange *et al.*, 2003). During a susceptible interaction this occurs later after the *R. secalis* has spread to neighbouring cells (Jones and Ayres, 1974).

A number of *R*-genes have been identified in barley that confer resistance to *R. secalis*. Many experiments looking at *R. secalis* resistance use the barley near isogenic cultivars Atlas and Atlas 46 which differ in the *Rrs1* resistance locus (Rohe *et al.*, 1995; Steiner-Lange *et al.*, 2003; Thirugnanasambandam *et al.*, 2011; van't Slot *et al.*, 2007). The corresponding *avr*-gene *AvrRrs1* is one of a small number of *avr*-genes to be characterised in plant pathogens. The gene product of *AvrRrs1* is referred to as necrosis-inducing protein 1 (NIP1) and initiates defence responses in barley plants carrying the *Rrs1* gene (Hahn *et al.*, 1993; Rohe *et al.*, 1995; Steiner-Lange *et al.*, 2003). As *R. secalis* behaves as a necrotrophic pathogen for at least part of its infection cycle, HR may not be the best defence mechanism to defend against it. However, a build-up of reactive oxygen species has been observed in the epidermal layer of both resistant and susceptible barley plants after *R. secalis* infection (Able, 2003).

Increased cuticle thickness, formation of papillae, cell wall appositions and halo formation can reduce *R. secalis* penetration (Carisse *et al.*, 2000; Humphry *et al.*, 2006; Lyngs Jørgensen *et al.*, 1993).

1.5.4. Powdery mildew

Powdery mildews (*Ascomycota, Erysiphales*) are some of the most common fungal pathogens of plants, infecting the leaves, stems, flowers and fruits of almost 10000 species of angiosperms (Glawe, 2008; Panstruga and Schulze-Lefert, 2002). In moderately temperate humid climates, losses due to powdery mildew can have a big impact on the farming of a variety of crops including grapes, fruit trees, small grains and hops (Glawe, 2008). Powdery mildews are easily identifiable due to the distinctive colonies of white conidia with a fluffy appearance that form on the surface of the plant (Glawe, 2008). Under favourable conditions the pustules grow and can merge to cover a larger area of the leaf, resulting not only in loss of nutrients but also reduction of photosynthesis. Powdery mildews are obligate biotrophs and therefore must acquire all their nutrients from intact host cells, using specialised feeding organs called haustoria.

High level of host-species specificity means that different formae species of the disease infect individual crop species and cross infection does not occur (Johnson *et al.*, 1981; Tosa *et al.*, 1989). *Blumeria graminis* f. sp. *hordei* (*Bgh*) infects barley plants (HGCA, 2008). In susceptible barley varieties the reduction of yield caused by *Bgh* can be up to 20% during a severe infection (HGCA, 2008).

1.5.4.1. Life cycle

Infection is initiated when an ascospore or a conidium lands on a susceptible host plant (Figure 1-8). These are then able to form germ tubes. Germination of spores can happen as soon as 60 seconds after landing on a host plant (Carver *et al.*, 1999) and 30-60 minutes later a cuticular peg is formed which appears to have a role in attaching the spore to the host (Zhang *et al.*, 2005) and the primary germ tube penetrates the cuticle (Edwards, 2002). The germ tube then elongates forming appressoria around 10 hours after infection which in turn produce penetration pegs to infect the host cells (Zhang *et al.*, 2005). The penetration pegs are able to penetrate the host cell walls using turgor pressure and enzyme activity. If penetration is successful, haustoria are then able to form within the host cell (Glawe, 2008). The haustoria allow the uptake of nutrients and mediate molecular signalling events between the host and fungus. This structure is surrounded by an extrahaustorial matrix that may protect the haustoria from host defence mechanisms. It has also been suggested that the fungus is able to bring about changes within the plant cell other than aiding the uptake of resources and preventing the senescence of infected tissue (Coghlan and Walters, 1990; Fotopoulos *et al.*, 2003). After infection the hyphae expand and branch to form oval colonies. Finally, the hyphae produce the reproductive structures, the conidiophores, several days after infection of the host plant. *Bgh* is also able to reproduce sexually by producing ascospores.

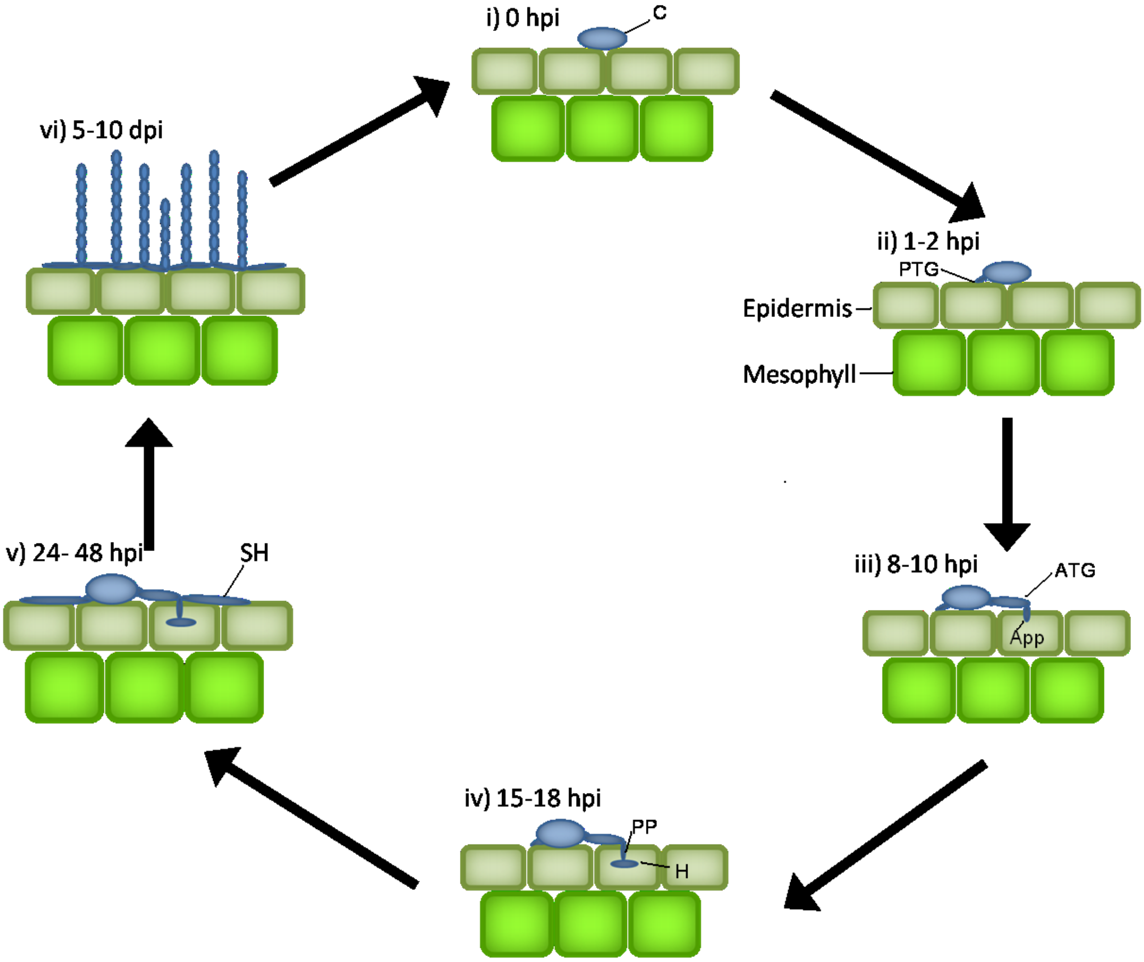


Figure 1-8 The asexual life cycle of *Bgh* (adapted from Zhang *et al.*, 2005)
Conidia on the leaf surface (i) germinate after one hour and the primary germ tube (PGT) is formed (ii). The appressorial germ tube and appressium are formed between 8 and 10 hours after inoculation (iii) leading to the formation of the penetration peg and the haustoria 15-18 hours after inoculation (iv). After 24 hours secondary hyphae are formed and further haustoria are produced (v). Once the colony has matured the conidiophores form conidia (vi). Abbreviations: dpi: days post inoculation, C: conidia, PGT: appressorial germ tube, App: appressorium, PP: penetration peg, H: haustoria and SH: secondary hyphae.

1.5.4.2. Defence against Powdery mildew

For a successful powdery mildew infection to take place the host plant must have the mildew resistance locus (MLO) protein (Büschges *et al.*, 1997; Jørgensen, 1992), and this protein is likely to be involved in the regulation of cell wall repair (Mejlhede *et al.*, 2006). Plants lacking a functional copy of this gene exhibit excessive papilla growth (Mejlhede *et al.*, 2006; Wolter *et al.*, 1993) and are not susceptible to powdery mildew as penetration of the host epidermal cells is prevented (Humphry *et al.*, 2006). The Mlo resistance is broad-spectrum and prevents infection by all known isolates of *Bgh* (Jørgensen, 1977). It was believed that *Mlo*-dependent resistance was unique, however, Humphry *et al.* (2006) suggested that Mlo resistance is a form of non-host resistance

(Humphry *et al.*, 2006). Thirty-two independent mutant alleles of *Mlo* have been identified in barley and have been assigned resistance gene symbols *mlo1* to *mlo32* (Büschges *et al.*, 1997; Molina-Cano *et al.*, 2003; Piffanelli *et al.*, 2002).

R-gene-mediated resistance is seen in barley in response to powdery mildew infection, possibly the best studied example of this is the *Mla* genes (Jørgensen and Wolfe, 1994). The *Mla* loci contain multiple classes of genes involved in resistance. The resistance phenotypes conferred by different *Mla* specificities are diverse, ranging from almost complete immunity to allowing some fungal mycelium development. The most effective resistance alleles are associated with the HR response and early growth arrest.

1.6. Open questions and aims of study

The main aim of this thesis is to investigate the way in which K-deficiency affects barley and how this influences the plant's susceptibility to pathogens. Although many studies have been carried out looking at the effect of K-deficiency in plants, and there is broad knowledge of the ways in which K-starvation affects barley plants, these studies were carried out by different groups using different growth conditions and varieties of barley. During this thesis I aim to examine the effect of K-deficiency on growth, metabolism, hormone signalling and pathogen susceptibility in the same system allowing results to be compared and contrasted in a meaningful way.

It has previously been demonstrated that *A. thaliana* has increased concentration of JA and related compounds during K-starvation (Armengaud *et al.*, 2004; Troufflard *et al.*, 2010). In order to make this knowledge applicable to agriculture I have investigated whether this increase in oxylipins also occurs in monocot crop plants, such as barley (Chapters 4 and 6). After confirming the increase of JA-related compound in K-starved plants I address the hypothesis that the increase in this hormone involved in stress signalling will affect susceptibility to pathogen infection in the K-deficient plants (Chapters 5). I investigate this by assessing the effect of K-deficiency and Me-JA treatment on the susceptibility of barley to two fungal pathogens; the biotroph *Blumeria graminis* f. sp. *hordei* and hemi-biotroph *Rhynchosporium secalis.*

Chapter 2: Materials and Methods

2.1. Plant growth and treatments

Barley (*Hordeum vulgare* L. cv Optic) seeds were sown on water saturated paper towels and germinated in an environment-controlled growth chamber with 9 h light (270 µmol m^{-2} sec^{-1} at 22 °C and 15 h dark at 18 °C and constant 70 % humidity). After 4 days, seedlings were transferred to a hydroponics system (10 l containers with 60 plants each) containing either full nutrient (control) or K-free nutrient solution (see Table 2-1 for composition).

Table 2-1 Composition of control and –K nutrient solutions

Values are final concentrations. Taken from (Armengaud *et al.*, 2004)

Stock solution	**control**	**-K**	**-Ca**	**-N**	**-P**	**-S**
KNO_3	1.25 mM		1.25 mM		1.25 mM	1.25 mM
$Ca(NO_3)_2 . 4H_2O$	0.5 mM	1 mM	0.1 mM		0.5 mM	0.5 mM
$MgSO_4 . 7H_2O$	0.5 mM	0.5 mM	0.5 mM	0.5 mM	0.5 mM	
FeNa EDTA	42.5 µM	42.5 µM	42.5 µM	42.5 µM	42.5 µM	42.5 µM
KH_2PO_4	625 µM		625 µM	625 µM		625 µM
NaCl	2 mM	1.38 mM	2 mM	2 mM	2 mM	2 mM
NaH_2PO_4		625 µM				
$NaNO_3$			0.8 mM			
KCl				1.25 mM	0.63 mM	
$MgCl_2$						0.5 mM
$CaCl_2$				0.5 mM		
Micronutrients						
$CuSO_4 \bullet 5H_2O$	160 nM	160 nM	160 nM	160 nM	160 nM	160 nM
$ZnSO_4 \bullet 7H_2O$	380 nM	380 nM	380 nM	380 nM	380 nM	380 nM
$MnSO_4 \bullet H_2O$	1.8 µM	1.8 µM	1.8 µM	1.8 µM	1.8 µM	1.8 µM
H_3BO_3	45 µM	45 µM	45 µM	45 µM	45 µM	45 µM
$(NH_4)_6Mo_7O_{24} \bullet 4H_2O$	15 nM	15 nM	15 nM	15 nM	15 nM	15 nM
$CoCl_2 \bullet 6H_2O$	10 nM	10 nM	10 nM	10 nM	10 nM	10 nM

The seedlings were wrapped in 15 mm lengths of 10 mm diameter rubber tubing and inserted in to holes in 5 mm thick corrugated plastic sheets, each holding 60 plants. The assembly was floated on top of 10 l of nutrient solution in plastic troughs (Figure 2-1). The nutrient solution was replaced every 7 days.

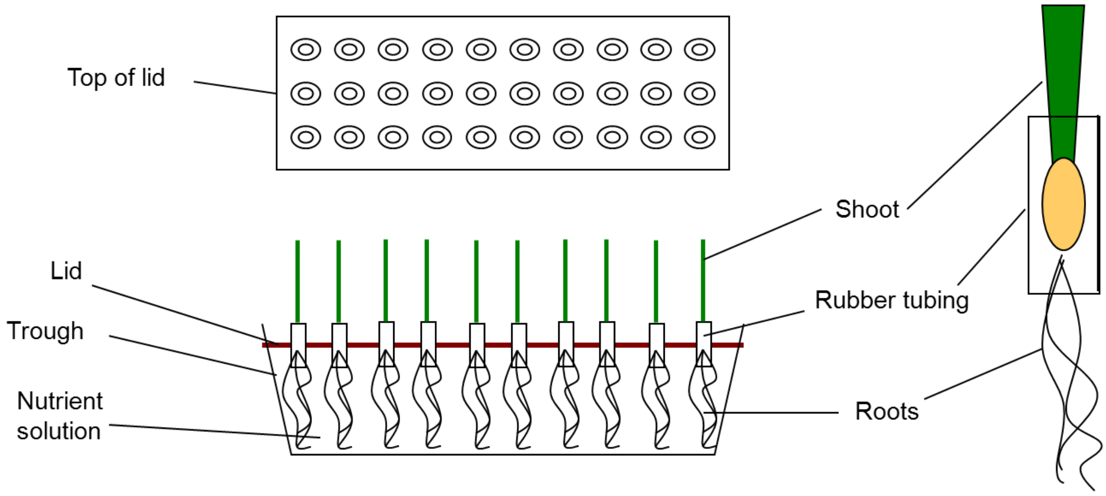


Figure 2-1 Hydroponics system for plant growth.

The seeds of the barley seedlings were wrapped in rubber tubing and inserted into holes in corrugated plastic sheets. The corrugated plastic sheets were floated on top of 10 l of nutrient solution in plastic troughs.

2.1.1. Plant growth in solution deficient in other nutrients

Barley seeds were germinated on paper towels as described above (Section 2.1). Four days after germination 10 seedlings were transferred to ceramic pots containing 2 l of control, -K, low calcium (-Ca), nitrogen free (-N), phosphorus free (-P) or sulfur free (-S) nutrient solution as described above (see Table 2-1 for composition). The plants were grown for 10 days after which 8 plants were sampled. The shoot tissue and root tissue were excised using a scalpel and immediately frozen in liquid nitrogen. The samples were stored at -80 °C.

2.1.2. Time course sampling

Barley seedlings were grown in control or –K hydroponics solution as previously described (Section 2.1). Five plants were taken from each condition at 3 pm (after 6 h of light) every 3 days up to 18 days after transfer to hydroponics The shoot tissue and root tissue were excised using a scalpel and immediately frozen in liquid nitrogen. The samples were stored at -80 °C.

2.1.3. Preparation of detached leaf segments

Barley seedlings were grown for 14 days in control or –K hydroponics solution as previously described (Section 2.1). 25 ml of 0.5 % Agar/120 mg/l benzimidazole was dispensed into 45 mm x 80 mm push top boxes and allowed to set (Newton *et al.*, 1998). Plants were randomly selected and the 2nd leaf removed. 40 mm long segments were cut from the tip, middle and base of the leaf as shown in Figure 2-2 and placed on the plates. The specimens were placed in a lit incubator (LEEC) with continuous light (light intensity 200 µmol $m^{-2}s^{-1}$ at 17°C) and left for 24 h to recover.

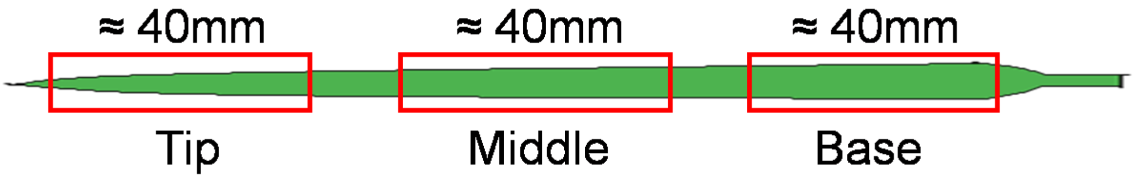


Figure 2-2 Barley leaf segments used in pathogen experiments

Leaf segments were prepared from the second leaf of 14 day old barley plants, 40 mm sections of leaf were cut from the tip, middle and base segments as shown.

2.1.4. Sampling of first and second leaves

Barley seedlings were grown for 14 days in control or –K hydroponics solution as previously described (Section 2.1). The first and second leaves were excised using a scalpel and immediately frozen in liquid nitrogen. The samples were stored at -80 °C.

2.1.5. Sampling of leaf regions

Leaf segments were prepared as described in Section 2.1.3. After the 24 hour recovery period six leaf segments were sampled from each treatment and leaf region at 24 hour intervals (around 3pm) for 5 days and flash frozen in liquid nitrogen, and stored at -80 °C.

2.1.6. Determination of plant size and weight

Every two days, five plants were randomly selected from each treatment. The length of each leaf (from base of the plant to tip of leaf), the shoot and longest root was measured using a ruler. The fresh weight was recorded for the whole plant, shoot and root for each plant.

2.1.7. Wounding of barley leaves

In order to mimic the wounding caused by herbivores feeding on the barley plants, a wounding experiment was carried out. The barley seedlings were grown for 14 days in control or K-free nutrient solution (section 2.1). The second leaf was wounded, approximately 1 cm from its tip by squeezing with a pair of fine tipped forceps. The first and second leaves were sampled at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after wounding.

2.1.8. K resupply experiments

After 10 days growth in hydroponics half of the plants grown in K-free solution were resupplied with K by replacing the -K nutrient solution with control solution. All other plants were given fresh control or –K solution (same solution as before). Five plants sampled from each treatment 0, 1, 2, 3, 4, 5, 6, and 7 days after solution change. The shoot tissue and root tissue were excised using a scalpel and immediately frozen in liquid nitrogen. The samples were stored at -80 °C.

2.1.9. Water content determination

Approximately 100 mg of frozen shoot, root, leaf or leaf region tissue was weighted out and freeze dried overnight (Prepared in sections 2.1.2, 2.1.4 and 2.1.5), the sample was reweighted and the weight of water removed during drying calculated. The percentage of the original tissue weight accounted for by water was calculated.

2.2. Measurement of tissue ion concentrations

100 µl of 2 M HCl were added to 1 mg of freeze dried tissue and mixed well. The samples were incubated at room temperature for 48 hours. Following the incubation period the samples were diluted 1:500 in water. Standards were prepared at 250, 125, 62.5, 31.25 and 15.625 µM KCl and measured using the flame photometer (Sherwood flame photometer 410). A standard curve was produced and used to calculate the concentration of K in each sample. The % K was calculated as follows:

$$\% \text{ DW} = \frac{\text{Conc. x } 39}{200} \qquad \text{[Equation 2.1]}$$

Conc. = Concentration (μmol/ml) from standard curve

39 = Molecular weight of K

2.3. Determination of metabolite concentrations in barley tissues

2.3.1. Preparation of Ethanolic extract for metabolite analysis

Ethanol extractions were carried out as per Cross *et al*. (2006), 20 mg of frozen barley tissue (shoot, root, first and second leaf and tip, middle and base segments, prepared as per 2.1.2, 2.1.4 and 2.1.5) was mixed with 250 µl of 80 % ethanol, incubated for 20 min at 30 °C and centrifuged at 13,000 rpm for 5 min at 4 °C (in the Eppendorf centrifuge 5415R). The supernatant was transferred into a new tube. 150 µl of 80 % ethanol was added to the remaining pellet, mixed, incubated for a further 20 min at 80 °C, centrifuged at 13,000 rpm for 5 min at 4 °C, the supernatant was added to the supernatant obtained in the previous centrifugation. 250 µl of 50 % ethanol was added to the remaining pellet, incubated for 20 min at 80 °C, centrifuged at 13,000 rpm for 5 min at 4 °C, the supernatant was added to the supernatant from the previous steps. The pellet was retained for protein level analysis. All samples were stored at -20 °C.

2.3.2. Analysis of Chlorophyll levels

The chlorophyll A and B levels were measured immediately after extraction. 50 µl of the extract was mixed with 120 µl of 98% ethanol. The absorbance at 645 nm and 665 nm was measured using the uv/vis spec (Arnon, 1949).

The Chlorophyll levels were calculated as follows (Equation 2.2).

$$\text{Chlorophyll A } (\mu\text{g/mg}) = \frac{(5.45 \text{ x } A_{665} - 2.16 \text{ x } A_{645})}{\text{Sample weight}} \text{ x } 650 \qquad \text{[Equation 2.2]}$$

$$\text{Chlorophyll B } (\mu\text{g/mg}) = \frac{(9.67 \text{ x } A_{665} - 3.04\text{x } A_{645})}{\text{Sample weight}} \text{ x } 650$$

2.3.3. Analysis of hexose sugar concentration

The concentration of the three hexose sugars, glucose, sucrose and fructose was measured. The absorbance at 340 nm was measured to follow the synthesis of NADPH in the samples during break-down of phosphorylated glucose by glucose-6-phosphate dehydrogenase (G6PDH, See Equations 2.3-2.5).

Glucose [Equation 2.3]

i) $\text{D- glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$

ii) $\text{glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}$

Fructose [Equation 2.4]

i) $\text{D-fructose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{fructose-6-phosphate} + \text{ADP}$

ii) $\text{fructose-6-phosphate} \xrightarrow{\text{Phosphoglucose isomerase}} \text{glucose-6-phosphate}$

iii) $\text{glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}$

Sucrose [Equation 2.5]

i) $\text{Sucrose} + H_2O \xrightarrow{\text{Invertase}} \text{Glucose} + \text{fructose}$

ii) $\text{glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$

iii) $\text{glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}$

A master mix was prepared containing 15.5 ml 0.1 M HEPES/ 3 mM $MgCl_2$ buffer (pH 7), 480 µl 60 mg/ml ATP, 480 µl 36 mg/ml NADP and 80 µl G6PDH grade II (with $(NH_4)_2SO_4$, removed). 50µl ethanolic extract and 160 µl of the master mix were dispensed into each well of a 96-well flat bottomed plate. The plate was placed in a plate reading spectrophotometer (Biotek ELX808), the absorbance at 340 nm was measured to establish a base line.

The following enzyme solutions were added in succession, interrupted by periods of absorbance reading: 1) 1µl Hexokinase (120 µl of suspension, centrifuged for 2 min and supernatant removed, pellet resuspended in 200 µl 0.1M HEPES/3mM $MgCl_2$ buffer). This enzyme transforms glucose into glucose-6-phopshate which is broken down by G6PDH to give Gluconate-6-phosphate + NADPH + H. The subsequent change in NADPH is a measure of the amount of glucose. 2) 1 µl of phosphoglucose isomerase (60 µL suspension centrifuged for 2 min and supernatant removed, pellet resuspended in 200 µL 0.1M HEPES/3mM $MgCl_2$ buffer). This enzyme transforms fructose into glucose, which is phosphorylated and broken down as before. The subsequent change in NADPH is a measure of the amount of fructose. 3) 1 µl Invertase (saturated solution). This enzyme transforms sucrose into fructose and glucose, which are isomerised, phosphorylated and broken down as before. The subsequent change in NADPH is a measure of the amount of sucrose. All enzymes were purchased from Roche.

A summary of the experiment is given below:

Insert plate with master mix into spectrophotometer.

- read every minute for 15 min
    → Add 1 µl Hexokinase to each well when prompted
- read every min for 25 min
    → Add 1 µl Phosphoglucose isomerase to each well when prompted
- read every minute for 30 min
    → Add 1 µL Invertase to each well
- read every minute for 35 min.

The concentration of each sugar was calculated using the following equation (Equation 2.6).

$$\mu\text{mol NADPH/mg FW} = \frac{\Delta OD/(2.85*6.22)}{W} \times V \qquad \text{[Equation 2.6]}$$

When:

$\Delta OD = A_{\text{end point}} - A_{\text{start point}}$

W = sample weight

V = total volume of ethanol extract prepared

µmol NADPH/mg FW is equivalent to µmol sugar/mg FW.

2.3.4. Analysis of malate and fumarate concentration

Malate and fumarate levels in barley tissue was measured as described by Nunes *et al.* (2007) based on absorbance of NADH produced in the following reactions (Equation 2.7).

Malate [Equation 2.7]

$$\text{Malate} + \text{NAD}^+ \xrightarrow{\text{Malate dehydrogenase}} \text{Oxaloacetate} + \text{NADH} + \text{H}^+$$

Fumarate

$$\text{Fumarate} + \text{H}_2\text{O} \xrightarrow{\text{Fumarase}} \text{Malate}$$

Standards were prepared 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL malic acid and fumaric acid. A master mix was prepared containing 50 µl 0.2M Tricine (pH 9.0), 10 µl 10 mM MTT, 2 µl 20 mM Phenazine ethosulphate, 10 µl 30 mM NAD^+, 5 µl 10% Triton X100, 3 µl water. 80 µl of the master mix was dispensed into each of the wells in a flat bottomed 96-well plate. 10 µl of the either ethanolic extract or standard was dispensed in to each well. The plate was placed in the plate reading spectrophotometer and the absorbance read at 570 nm as described below

- read every minute for 5 min
    → 1 µL 1000 U/mL Malate dehydrogenase added to each well
- read every min for 40 min
    → 1 µL 100 U/mL Fumarase added to each well
- read every minute for 50 min

Standard curves were produced for malate and fumarate using the change in the absorbance at 570 between the start point and end point of the reaction (ΔOD). Malate and fumarate concentrations in the tissue samples were determined by comparison with the standard curve and transformed into µg/mg FW using the following equation (Equation 2.8).

$$\text{Conc. } \mu\text{g/mg FW} = \frac{C}{W} \times V \qquad \text{[Equation 2.8]}$$

With:

C = Concentration µg/well from standard curves
W = sample weight
V = total volume of ethanol extract prepared

2.3.5. Analysis of glutamate concentration

Glutamate standard sets were prepared containing 0, 0.1, 0.25, 0.5 mM glutamate in 70% ethanol. A master mix was prepared containing 10 µl 2M Tricine buffer (pH 8.5), 10 µl 10 mM MTT, 10 µl 30mM NAD, 5 µl 10% Triton X100, 2 µl 50mM ADP, 1 µl 20U/ml Diaphorase and 147 µl water. 185 µl of the master mix was dispensed into each well of a 96 well flat bottomed plate. 15 µl of either the ethanolic extract or standard was dispensed into each well.

[Equation 2.9]

$$\text{2-Oxoglutarate} + \text{NADH} \xleftrightarrow{\text{Glutamate dehydrogenase}} \text{Glutamate} + \text{NAD}$$

The plate was placed in the plate reading spectrophotometer and the absorbance at 570 nm was measured every minute for 10 min, 1 µl of 500 U/ml Glutamate dehydrogenase was added to each well and reading continued every 1 min for a further 30 min.

Standard curve was produced using the ΔOD of the standards. This was used to determine the concentration in each of the experimental samples, which was subsequently transformed to µmol/mg FW using Equation 2.8.

2.3.6. Analysis of nitrate concentration

The level of nitrate in the samples was determined according to Mori (2000). The ethanolic extracts described above (Section 2.3.1) were diluted 1:2. Nitrate standards of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mM were prepared. 5 µl 1:2 ethanolic extract or standard, 10 µl 1 M potassium phosphate buffer (pH 7.5), 0.5 µl 50 mM NADPH in 5 mM NaOH, 1 µl 5 U/ml Nitrate reductase in potassium phosphate buffer and 83.5 µl water was added to each well of a 96 well flat bottomed plate and mixed. Plates were incubated in the dark at 25 °C for 30 min, 15 µl 0.25 mM PMS was added to each well and mixed. Plates were then incubated in the dark for a further 20 min. 60 µl of 1 % w/v sulphanilamide in 3 M phosphoric acid and 60 µl 0.02 % w/v NNEDA were added to each well, mixed and incubated in the dark, at 25 °C for 10 min. The plate was then placed in the spectrophotometer and the absorbance measured at 540 nm.

A standard curve was used to determine the concentration in each well, which was subsequently transformed into µmol/mg FW using Equation 2.8.

2.3.7. Analysis of protein concentration

The pellet produced from 20 mg of frozen barley tissue during the extraction process described above (Section 2.3.1) was re-suspended in 400 µl of 0.1 M NaOH, and incubated for 30 min at 95 °C. The samples were allowed to equilibrate to room temperature before mixing and centrifuging at 13,200 rpm for 5 min. 0, 80, 160, 240, 320 and 400 µg/ml BSA standards were prepared in 0.1M NaOH. 3 µl of sample or standard and 180 µl of 1:5 Bio-Rad protein assay dye reagent were added to each well in a 96 well flat bottomed plate, mixed, and incubated at room temperature for 5 min. The plate was placed in the spectrophotometer and the absorbance measured at 595 nm.

A standard curve was used to determine the concentration (in mM) in each well and Equation 2.8 was used to transform the concentration into µmol/mg FW.

2.4. Measurement of transcript levels using quantitative PCRs

2.4.1. RNA extraction

RNA extraction from plant tissue was carried out using Trizol® Reagent (Invitrogen, Cat. 15596-026). Tissue samples were ground in liquid nitrogen, using a pestle and mortar. 500 µl of Trizol was added to 50 mg of tissue, mixed well and incubated at room temperature for 5 min. The samples were centrifuged for 10 min at 10,000 rpm (12,000 x g) at 4 °C before the supernatant was transferred to a fresh tube and 100 µl chloroform (BDH, No 100776B) added and mixed. The samples were incubated at room temperature for 10 min before further centrifugation for 10 min at 10,000 rpm (12,000 x g) at 4 °C. The aqueous layer was transferred to a fresh tube and the chloroform extraction repeated. The aqueous layer was transferred to a fresh tube and equal volume of isopropanol was added, mixed well, incubated at room temperature for 10 min. The samples were centrifuged for 10 min at full speed at 4 °C, the supernatant was removed and two 500 µl washes with 70 % ethanol were carried out. The final pellet was resuspended in RNase free water.

2.4.2. RNA quantification

The RNA was quantified using a NanoDrop ND-1000 UV-VIS spectrophotometer. The RNA was calculated based on the absorbance at 260 nm (A_{260}). Absorbance at 230 nm (A_{230}) and 280 nm (A_{280}) was also measured and the A_{260}/A_{230} and the A_{260}/A_{280} ratios used to assess the purity of the RNA. If either of the ratios was less than 1.8, the RNA was subjected to further clean-up (see below Section 2.4.3).

2.4.3. RNA clean up

The RNA was made up to 100 µl with RNAse-free water and heated for 10 min at 55 °C. 100 µl of phenol:chloroform (1:1 at pH 4.3) was added to each sample, mixed well, and incubated on ice before centrifuging for 5 min at 10,000 rpm (12,000 x g) at 4 °C. The aqueous phase was transferred to a fresh tube, 250 µl 100 % ethanol and 10 µl 3 M Sodium Acetate (pH 5.5) added and mixed. The samples were incubated at -20 °C for 1 h before being centrifuged at full speed (13,000 rpm) at 4° C for 30 min. The pellets were washed twice with 500 µl of 70 % ethanol. The final pellet was dried and resuspended in 30 µl RNAse free water.

The samples were quantified with the spectrophotometer, and 1 ng RNA was run on a 1 % agrose/TAE gel to check the integrity of the RNA.

2.4.4. cDNA preparation

Superscript III™ Reverse Transcriptase kit (Invitrogen, Cat. 18080-044) was used to prepare cDNA. A reaction mix containing 1 µg template RNA, 1 µl 10 mM dNTPs, 0.5 µl Oligo dT 20 primer (Invitrogen, Cat. 18418020) and made up to 13 µl with RNAse free water was prepared and incubated for 5 min at 65°C.

The reaction was chilled on ice for 1 min before adding 4 µl 5 x First strand buffer, 1 µl 0.1 M DTT, 1 µl RNaseOUT RNAse inhibitor (Invitrogen Cat. 100000840) and 1 µl Superscript III RT and mixed by pipetting.

The samples were then incubated at 25° C for 5 min, 50 °C for 60 min and, 70 °C for 15 min to stop the reaction.

2.4.5. Primers

Genes encoding enzymes for JA biosynthesis were selected for gene expression analysis using quantitative PCR (qPCR). Lipoxygenase 2 (LOX2) catalyses the first committed step in JA biosynthesis (Bachmann *et al.*, 2002) and expression of the *LOX2* gene in *Arabidopsis thaliana* is further induced by JA in a positive feedback loop. Three possible *LOX2* genes were selected for qPCRs *LOX2Hv2* (*LOX2.2*), and *LOX2Hv3* (*LOX2.3*) were identified as *LOX2* genes by Bachmann *et al*. (2002), while *LOX2.A* was selected from the microarray results as having the largest increase in expression after K-deficiency of the *LOX* genes. A Blast search showed its closest homolog to be a rice *LOX2* gene and its closest homolog in Arabidopsis was also *LOX2*. Allene oxide synthase (AOS) (Maucher *et al.*, 2000) catalyses the transformation of 13-hydroperoxy linolenic acid (13-HOT) into 12,13 epoxy-octadecatrienonic acid (12,13-EOT) and Allene oxide cyclase (AOC) (Maucher *et al.*, 2004) which catalyses the transformation of 12,13-EOT into (9S, 13S)-12-oxo-phytodienoic acid (OPDA) expression in *Arabidopsis thaliana* is induced after stimuli triggering the JA pathway for both enzymes.

Three genes reported to be induced by JA in barley (Jasmonate induced proteins, JIPs, (Leopold *et al.*, 1996; Ozturk *et al.*, 2002), were also selected for expression analysis. *JIP23, JIP37* and *JIP60* were chosen for initial experiments, however for later experiments only *JIP60* was used.

Based on experimental evidence from barley phenylalanine ammonia-lyase (Kervinen *et al.*, 1997) expression was chosen as a pathogen-induced gene. PAL is an enzyme that catalyses the first reaction in the general phenylpropanoid synthesis pathway, which provides the precursors for a variety of secondary metabolites including SA. Tobacco plants with *PAL* suppressed are unable to establish SAR, and increase susceptibility to fungal pathogens (Bate *et al.*, 1994; Dixon and Paiva, 1995). Glyceraldehyde 3-phosphate dehydrogenase (Chojecki, 1986) and alpha tubulin (TUBA) were selected as reference genes based on analysis of raw data from qPCR experiments confirming constitutive expression in all samples (See Chapter 4, Section 4.2 and Figure 4-1 for more detail). They were used to normalise the transcript levels of the other genes. Primers for qPCR were designed using Primer 3 software (Rozen and Skaletsky, 2000). Primer sequences are given in Table 2-2, See Appendix 1 for full gene sequences.

Table 2-2 Genes and primers used in qPCR

Gene	Accession	Category	Forward primer	Reverse primer	Frag. length
α-TUB	U40042	Constitutive	AGTGTCCTGTCCACCCACTC	AGCATGAAGTGGATCCTTGG	248 bp
GAPDH	M36650	Constitutive	GGAGGAGTCTGAGGGAAACC	GCTGTATCCCCACTCGTTGT	175 bp
LOX2.2	AJ507212	JA biosynthesis	CGACAAGCGTACCTTCTTCC	GTTTGCCGAGGTCGTTGTAT	173 bp
LOX2.3	AJ507213	JA biosynthesis	GAAGGGAGAGGGAGAGAGT	GTGATGAGCTTGATGTCCTT	163 bp
LOX2.A	AK362687	JA biosynthesis	AGTACCTGGGAGGGATGGAG	TGGTTTCATGAGCTGGTACG	179 bp
AOS	AJ250864	JA biosynthesis	ACCGTCTTCAACAGCTACGG	TCTTCTCCAGCGCCTCTATC	160 bp
AOC	AJ308488	JA biosynthesis	GCTACGAGGCCATCTACAGC	AAGGGGAAGACGATCTGGTT	163 bp
JIP23	BM816519	JA induced	ATCACAGTGTGTGTGCAAAG	ACTTTTGCGCGTTAACATCC	151 bp
JIP37	X82937	JA induced	GATCCATCGACAAGAAGTCC	ACTGTGGGTCTTGAGCTTGT	286 bp
JIP60	BM815987	JA induced	CAGCAGCGACTTCATTTACA	ATGGTGTCGCAGACTATCCT	201 bp
PAL	Z49147	Pathogen induced	CTGTTCCGAGCTTCAGTTCC	CGACCAAAAATGTCGAGGAC	237 bp

2.4.6. Standard preparation

DNA of all genes listed in Table 2-2 was amplified from barley cDNA by PCR for use as template for standard curves during qPCR experiments. A 25 µl reaction was prepared for each primer set shown in Table 2-2 containing 1 µl barley cDNA template, 1 µl 20 µM forward primer, 1 µl 20 µM reverse primer, 2.5 µl 10 x PCR reaction buffer, 0.5 µl 10 mM dNTPs, 0.1 µl Taq DNA polymerase (Roche cat. 11146165001) and 19.9 µl RNAse free water. After a 5 minute activation step at 95 °C the DNA was amplified with 35 cycles of 95 °C for 30 sec, 52 °C for 30 sec and 72 °C for 30 sec, followed by a 5 minute elongation step.

The obtained DNA samples were run on a 1 % agrose/TAE gel and the resulting band excised. The DNA was extracted from the gel using the Qiagen Qiaquick gel extraction kit (Qiagen, cat. 28704).

The samples were sequenced by the Dundee University sequencing service to check the correct target had been amplified.

2.4.7. qPCR

A light cycler (Bio-Rad Chromo 4 with Opticon Monitor 3 software) was used to carry out qPCR. 10 pg/µl of 1:10 serial dilutions of standards were prepared for each of the genes.

A reaction was prepared per sample using the QuantiTech®SYBR® Green PCR Kit (Qiagen, cat 2041453) containing 5 µl cDNA template/standard, 0.25 µl 20 µM forward primer, 0.25 µl reverse primer, 6.25 µl 2 x Sybr Green, 0.75 µl RNAse free water. After a 15 minute activation step at 95 ˚C the DNA was amplified with 40 cycles of 95 °C for 15 sec, 58 °C for 30 sec and 72 °C for 30 sec. The reaction was followed by a melting curve between 60 °C and 95 °C, fluorescence intensity was measured every 1 °C.

The Ct values were determined from two technical replicates of the gene of interest and reference gene. The Ct was transformed by comparison with the standard curve to give concentration (pg) of transcript for each gene in each technical replicate. A cross wise comparison was carried out between the gene of interest and reference gene, resulting in four values per sample. The mean of these values were used to calculate the standard error for replicate experiments and analyzed using an ANOVA (For ANOVA results see Appendix 2).

2.5. Hormone quantification

Tissue collected as per Section 2.1.2 was freeze dried overnight, and 10 mg samples were analyzed by LC-MS by the Metabolomics and Proteomics Mass Spectrometry Facility at Exeter University. Samples were extracted using 400 µl of 10 % ethanol/1 % acetic acid. 20 µl of sample was analyzed on 1200 series HPLC from Agilent technologies, using a 3.5 2.1 x 150 mm Elipse Plus C18 column from Agilent.

2.6. Pathogen infection

2.6.1. Inoculation of detached leaves with *Rhynchosporium secalis*

2.6.1.1. Preparation of Rhynchosporium spore solution

Rhynchosporium secalis (13-13) was isolated from infected leaves. Infected tissue was selected and excess tissue removed. The tissue was washed with a bleach/tween 80 solution for 10 min, dried on tissue and placed on CZV8CM plates (see Table 2-3 for ingredients). The plates were incubated at 17 °C for 7 days or until colonies formed around the lesion margins. Colonies were then transferred to fresh CZV8CM plates using a sterile scalpel. The plates were incubated at 17 °C for 2 weeks until a large colony had established. Colonies were maintained by cutting areas of the colony from the plate and transferring to a fresh CZV8CM plate.

Table 2-3 CZV8CM medium

CZV8CM ingredients	**Quantity per liter**
Oxoid Czapek Dox	56.0 g
Agar	10 g
V8 juice	200 ml
Calcium carbonate	4.0 g
Complete supplement	50 ml
Complete supplement	**Quantity per liter**
Casein Hydrolysate	20 g
Myc peptone	20 g
Yeast extract	20 g
Adenine (in 1 M HCl)	3 g
Biotin	0.02 g
Pyridoxine	0.02 g
Thiamine HCl	0.02 g
Para amino benzoic acid (PABA)	0.02 g
Nicotinic acid	0.02 g

So-called spread plates were prepared to produce spores for a spore solution. CZV8CM plates were prepared and 400 µl sterile water was dispensed on to each plate. A 1 cm^2 piece of Rhynchosporium culture prepared as above was cut and placed upside down on the new plate, then moved around the plate to spread the spores. The plates were placed in the 17 °C incubator for 2 weeks.

Once the spores had formed on the spread plates, the mycelia were scraped from the plate surface using a sterile spatula and transferred to a homogenizer containing sterile water and homogenized for approximately 30 sec. The spore solution was centrifuged at 4000 rpm at 10 °C for 4 min, the pellet was washed in 1 ml sterile water and spun down at 4000 rpm at 10 °C for 4 min, and resuspended in 500 µl sterile water. The concentration of the spore solution was determined using a haemocytometer and calculated using the following equation (Newton *et al.*, 2001).

$$\text{Conc.(spores/ml)} = (n/0.02) \times 1000 \qquad \text{[Equation 2.10]}$$

With

n = mean number of spores per 200 µm x 200 µm haemocytometer square

The spore solution was diluted with sterile water to give a 10^6 spore/ml solution.

2.6.1.2. Inoculation with *R. secalis*

Leaf segments were prepared as described previously (Section 2.1.3). The area to be inoculated was brushed gently with a trimmed-down paint brush to disrupt the cuticle allowing more efficient infection and preventing the spore solution from rolling off the leaf segment (Newton *et al.*, 2001). 10 µl of 10^6 spores/ml solution was dispensed on to each leaf segment. The plates were returned to the 17°C lit incubator. The severity of infection was assessed by measuring the length of the lesions from top to bottom.

For qPCR and Microarray analysis samples were inoculated with three 10 µl drops of 10^6 spores/ml. After inoculation five leaf segments were sampled from each treatment and leaf region at 24 hour intervals (around 3pm) for 5 days and flash frozen in liquid nitrogen, and stored at -20 °C (for a more detailed description of samples preparation see Section 2.7.1).

2.6.2. Inoculation of detached leaves with *B. graminis*

B. graminis was isolated from infected barley leaves. The spores from individual colonies were used to inoculate detached leaf segments (prepared as per Section 2.1.3) with a paint brush and the fungus was allowed to grow for approximately 2 weeks. To ensure a pure culture individual colonies where selected twice more. The spores from a single colony were used to inoculate multiple segments to bulk up the powdery mildew spores.

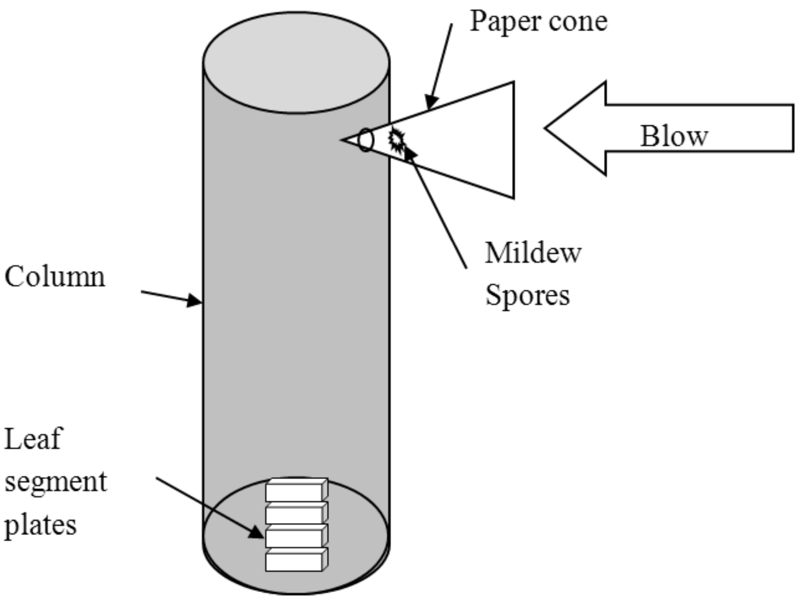


Figure 2-3 Inoculation of detached leaf segments with Powdery mildew.
To ensure even spore distribution across the leaf segments an inoculation column was used to disperse *Bgh* spores. Plates containing the detached leaf segments were placed in the bottom of the inoculation column. Spores were transferred to a paper cone, blown into the inoculation column and allowed to settle on the leaf segments.

In order to allow qPCR analysis to be carried out after inoculation six leaf segments were sampled from each treatment and leaf region at 24 hour intervals (around 3pm) for 5 days and flash frozen in liquid nitrogen, and stored at -20 °C.
To inoculate the leaf segments (prepared as per Section 2.1.3) in a plate uniformly plates were placed inside an inoculation column (Figure 2-3). The plate containing the spores was inverted over a sheet of paper and tapped to dislodge the spores. A cone was formed from the paper and was used to blow the spores into the inoculation column. The spores were allowed to settle on the leaf segments for 5 min, before the lids were replaced on the boxes, and plates were returned to the lit incubator at 17 °C.

The level of infection was assessed by counting the number of visible powdery mildew colonies on each leaf segment. In order to allow for differences in leaf size between plants grown in control and K-free media the number of colonies were divided by the leaf area (measured using image J from photographs) to give colonies/cm^2.

2.6.3. Treatment of detached leaves with Methyl Jasmonate

Barley seedlings as were grown for 14 days in full nutrient control hydroponics. The middle segments from the second leaf of each seedling were floated on 50 ml, 45 µM Methyl Jasmonate (Me-JA) (0.1 M solution prepared in ethanol) in water or 225 µl ethanol in 500 µl water, for 24 h in lit incubator (LEEC) at 17 °C.

The detached leaf segments were dried on paper towel and transferred to 0.5% Agar/120mg/l benzimidazole 45 mm x 80 mm push top boxes. The segments were inoculated with *Rhynchosporium secalis* or *Blumeria graminis* f. Sp. *hordei*. Infection severity for each pathogen was determined as described in Sections 2.6.1.2 and 2.6.2.

2.7. Microarray Analysis

2.7.1. Sampling for Microarray analysis

Leaf segments were prepared as previously described (Section 2.1.3). 10 boxes of 8 leaf middle segments were prepared from detached leaves grown in Control and K-free nutrient solution. Five boxes of each control and –K segments were inoculated with three 10 µl 10^6 spores/ml Rhynchosporium and the remaining five were inoculated with water. 1 segment was sampled from each box at 0, 1, 2, 3, 4, 5 and 10 days after inoculation, segments from each treatment were pooled. Four replicate experiments were carried out. Two time points (2 & 4 days after infection) were subjected to microarray analysis.

Table 2-4 Design of sample hybridization to microarrays.

Sample	Treatment	Infection	Time	Rep	Slide	Array	Dye
1	control	mock	2	1	1	1	cy3
2	-K	mock	2	1	1	1	cy5
3	control	mock	4	1	1	2	cy3
4	-K	mock	4	1	1	2	cy5
5	control	Rhyncho	2	1	1	3	cy3
6	-K	Rhyncho	2	1	1	3	cy5
7	control	Rhyncho	4	1	1	4	cy3
8	-K	Rhyncho	4	1	1	4	cy5
9	-K	Rhyncho	4	2	2	1	cy3
10	control	Rhyncho	4	2	2	1	cy5

11	-K	mock	2	2	2	2	cy3
12	control	mock	2	2	2	2	cy5
13	-K	mock	4	2	2	3	cy3
14	control	mock	4	2	2	3	cy5
15	-K	Rhyncho	2	2	2	4	cy3
16	control	Rhyncho	2	2	2	4	cy5
17	control	Rhyncho	2	3	3	1	cy3
18	-K	Rhyncho	2	3	3	1	cy5
19	control	Rhyncho	4	3	3	2	cy3
20	-K	Rhyncho	4	3	3	2	cy5
21	control	mock	2	3	3	3	cy3
22	-K	mock	2	3	3	3	cy5
23	control	mock	4	3	3	4	cy3
24	-K	mock	4	3	3	4	cy5
25	-K	mock	2	4	4	1	cy3
26	control	mock	2	4	4	1	cy5
27	-K	Rhyncho	4	4	4	2	cy3
28	control	Rhyncho	4	4	4	2	cy5
29	-K	Rhyncho	2	4	4	3	cy3
30	control	Rhyncho	2	4	4	3	cy5
31	-K	mock	4	4	4	4	cy3
32	control	mock	4	4	4	4	cy5

RNA was extracted as described previously and its quality checked using the Bioanalyzer (Agilent, Model 2100). The samples were labelled with Cy3 or Cy5 dye (2 replicates each) as described below and hybridized to Agilent barley microarrays (For further information on microarray design see Section 276) according to the design shown in Table 2-4.

2.7.2. Preparation of labeling reaction

The samples were labeled using the Agilent Quick AMP labeling kit (Agilent, p/n 5190.0424) and RNA Spike-In Kit, Two-Color (Agilent p/n 5188-5279). 4.15 µl of 250 ng/µl RNA was used per samples, 0.6 µl T7 promoter primer and 1 µl cyanine 3 CTP or cyanine 5 CTP (as per Table 2-4) was mixed with each sample in an amber micro centrifuge tube to protect the samples from the light, incubated at 65 °C for 10 min and chilled on ice for 5 min. 2 µl 5 x first strand buffer, 1 µl 0.1 M DTT, 0.5 µl 10 mM dNTP mix, 0.5 µl MMLV-RT and 0.25 µl RNaseOUT were added to each tube and mixed by pipetting. The samples were inoculated at 40 °C for 2 h, 65 °C for 15 min and chilled on ice for 5 min. 7.65 µl nuclease free water, 10 µl 4 x transcription buffer, 4 µl 0.1 M DTT, 4 µl NTP mix, 3.2 µl PEG, 0.25 µl RNaseOUT, 0.3 µl inorganic pyrophosphatase, 0.4 µl RNA polymerase and 1.2 µl of Cy-day 3 or Cy-dye

5T7 were added to each sample and mixed by pipetting. The samples were incubated at 40 °C for 2h.

2.7.3. Purification of labelled/amplified RNA

The labelled and amplified RNA was purified using the RNeasy Plant mini kit (Qiagen, Cat no. 74904) as per protocol. All the samples were made up to 100 µl by adding 40 µl of water, 350 µl Buffer RLT and 250 µl 100% Ethanol were added and mixed by pipetting. The samples was transferred to a RNeasy mini column and centrifuged for 30 sec at 13, 000 rpm (in the Eppendorf centrifuge 5415R), then washed twice with 500 µl RPE buffer. The cRNA was eluted in 30 µl of RNAse free water.

2.7.4. cRNA quantification

The cDNA was quantified using a NanoDrop ND-1000 UV-VIS spectrophotometer. The yield and specific activity where calculated using the following equation (Equation 2.10).

$$\text{Yield (µg cDNA)} = \frac{\text{Conc. cRNA x elution volume}}{1000} \qquad \text{[Equation 2.11]}$$

$$\begin{array}{l}\text{Specific activity (pmol} \\ \text{cy3/5 per µg cRNA)}\end{array} = \frac{\text{Conc. Cy 3/5}}{\text{Conc. cRNA (ng/ µl)}} \text{ x } 1000$$

All the samples had a yield less than 825 ng and specific activity < 8.0 pmol cy3/5 per µg cRNA.

2.7.5. Hybridization of probes

Hybridisation was carried out using Gene Expression Hybridization Kit (Agilent p/n 5188-5242). The samples labelled with the different dyes were combined as per table 2-4. A mix was prepared containing 900 ng Cyanine 3 – labelled cRNA, 900 ng Cyanine 5 – labelled cRNA, 11 µl 10 blocking agent, 2.2 µl fragmentation buffer and made up to 55 µl with nuclease free water for each array. The samples were incubated in the dark at 60 °C for 30 min. 55 µl 2x GEx Hybridization Buffer HI-RPM was added to each tube and mixed by pipetting to stop the reaction. 100 µl of each sample was loaded on the arrays. Arrays were inserted into hybridization chambers and placed in the hybridization oven at 65 °C rotating at 10 rpm for 17 h.

2.7.6. The SCRI_Hv35_44K_v1 microarray chip

The SCRI_Hv35_44K_v1 (Agilent design 020599) microarray was used for the microarray experiments. The chip represents 42,302 barley sequences and was designed based on a total of 50,938 unigenes from the HarvEST assembly 35 (http://www.harvest-web.org/) representing around 450,000 Expressed Sequence Tags (ESTs). Selection criteria were based upon the ability to define orientation derived from:

(i) homology to members of the non-redundant protein database (NCBI nr);
(ii) homology to ESTs known to originate from directional cDNA libraries;
(iii) presence of a significant polyA tract.

The microarray was designed with one 60mer probe per selected unigene (42,302 in total) in 4x 44k format using default parameters in the web-based Agilent Array software.

2.7.7. Microarray wash

The slides were removed from the chambers under Gene Expression Wash Buffer 1 (Agilent p/n 5188-5325), washed for 1 min in Gene Expression Wash Buffer 1 and 1 min in Gene Expression Wash Buffer 2 (Agilent p/n 5188-5326) at 37 °C. The slides were then spun dry.

2.7.8. Quantification of hybridization signal

Quantification of the Hybridisation signal was carried out as per Stushnoff *et al.* (2010). After hybridisation the slides were scanned with an Agilent G2505B scanner at resolution of 5 lm at 532 nm (Cy3) and 633 nm (Cy5) wavelengths with extended dynamic range (laser settings at 100% and 10%). The Microarray images were imported into Agilent Feature Extraction (v. 10.7.3.1) software and aligned with the array grid template file (020599_D_F_20080612). Intensity data and QC metrics were extracted using the FE protocol. The FE datasets the arrays were loaded into GeneSpring (v.7.3) software for further analysis.

The data was normalized using default settings for two-channel arrays, data was transformed to account for dye-swaps and normalized using the Lowess algorithm to minimize differences in dye incorporation efficiency.

2.8. Experimental design and statistical analysis

2.8.1. Growth experiment

Time course experiments were carried out in order to establish how long after germination physiological changes occurred when K was withheld (Section 2.1.6). The length of shoots, roots and individual leaves and weight of shoots, roots and whole plants were measured 2, 4, 6, 10, 12, 14, 16, 18 and 20 days after seedlings were transferred to nutrient solutions. Samples were measured for three replicates, Table 2-6 gives a summary of the experimental design for growth over time experiments, data obtained during this analysis is shown in Chapter 3, Figures 3-2, 3-3 and 3-4.

Table 2-6 Summary of experimental design for growth over time

Factor		Level
Treatment	Control and -K	2
Time point	0, 2, 4, 6, 10, 12, 14, 16, 18 and 20 days after transfer to nutrient solution	10
Replicate	1, 2 and 3	3
		n=60
Parameters	Length: total, shoot, root, 1st, 2nd and 3rd leaf Weight: total, shoot, root	9

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and time on length and weight of the whole plant, shoots and roots, and the length of the individual leaves (from the intercalary meristem to the tip). The effect of K was then compared at each time point using a T-test in order to determine at which time point any effects were seen (Appendix 2, Table S2-1). The statistical package Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate tub samples were taken.

2.8.2. Time course experiments

Time course experiments were carried out in order to establish how long after germination biochemical and transcriptional changes occurred when K was withheld. The shoot and root tissue from five plants were pooled 3, 6, 9, 12, and 15 days after transfer to control and –K nutrient solution. Samples were analysed from three or four replicates of this experiment depending on parameters being measured, Table 2-7 gives a summary of the experimental design for time course experiments. The changes in metabolite levels in the root and shoot samples and JA related gene expression in the shoots only was measured (Data shown in Chapters 3 and 4).

Table 2-7 Summary of experimental design for time course experiments

Factor		**Level**
Treatment	Control and -K	2
Time point	3, 6, 9, 12, and 15 days after transfer to nutrient solution	5
Replicate	1, 2 and 3 (4)	3
		n=30
Parameters	K concentration, water content, chlorophyll A, chlorophyll B, sucrose, glucose, fructose, malate, glutamate, nitrate and protein content and *LOX2.2*, *LOX2.3*, *LOX2.A*, *AOS*, *AOC*, *JIP23*, *JIP37* and *JIP60* transcript levels	19
Tissue	Shoots and roots (5 plants per treatment per time point)	2

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and time on K concentration, water content and metabolites concentrations, in the shoot and root and JA related gene expression in the shoot tissue. The effect of K was then compared at each time point using a T-test in order to determine at which time point any effects were seen (Appendix 2, Tables S2-2, 2-3 and 2-5). The statistical package Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate tub samples were taken from.

A Pearson product-moment correlation coefficient was also used to investigate the relationship between the various parameters measured (Appendix 3, Table 3-1). The analysis was carried out using Sigma Plot statistical package.

2.8.3. Leaf regions

Experiments were carried out to establish how changes in metabolism and transcript levels in response to K deficiency differed across the second leaf of 14 day old plants. Six segments from each leaf region were sampled from plants grown in control and K free nutrient solution. Samples were analysed from three or four replicates of this experiment depending on parameters being measured, Table 2-8 gives a summary of the experimental design this experiment. The changes in metabolite levels and JA related gene expression were measured (Data shown in Chapters 3 and 4).

Table 2-8 Summary of experimental design for leaf region experiments

Factor		**Level**
Treatment	Control and -K	2
Leaf region	Tip, middle and base	3
Replicate	1, 2 and 3 (4)	3
		n=18
Parameters	K concentration, water content, chlorophyll A, chlorophyll B, sucrose, glucose, fructose, malate and glutamate content and *LOX2.2*, *LOX2.3*, *LOX2.A*, *AOS*, *AOC*, *JIP23*, *JIP37* and *JIP60* transcript level	17

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and leaf region on K content, water content and metabolites concentrations and JA related gene expression in the shoot tissue. The effect of K was then compared for each leaf region using T-test in order to determine in which region any effects were seen (Appendix 2, Tables S2-4 and 2-7). The statistical package Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate tub samples were taken from.

2.8.4. Resupply of K experiment

Experiments were carried out to establish how JA related transcript levels changed after resupply of K. JA related transcript levels in plants grown in K free nutrient solution for 10 days before K resupply were compared to that plants grown in continuously in control or K free nutrient solution (Section 2.1.8). Samples were analysed from three replicate experiments (Section 4.5, Figure 4-10), Table 2-9 gives a summary of the experimental design.

Table 2-9 Summary of experimental design for resupply experiment

Factor		**Level**
Treatment	Control, -K and K-resupply	3
Time point	0, 1, 2 3 and 5 hours after resupply	5
Replicate	1, 2 and 3	3
		n=30
Parameters	K-concentration, *LOX2.3*, *LOX2.A*, *AOS*, *JIP23* and *JIP60* transcript level	6

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and time point on K content and JA related gene expression in the shoot tissue. The effect of K was then compared for each time point using T-test in order to determine at which time effects were seen (Appendix 2, Table S2-8). The statistical package Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate tub samples were taken from.

2.8.5. Pathogen susceptibility

Experiments were carried out to establish how K deficiency affects plants susceptibility to fungal pathogens. Forty segments from each region of the second leaf from plants grown for 14 days in control and K free nutrient solution were inoculated with either *R. secalis* or *Bgh* (Section 2.6) and the number of segments with visible lesions and extent of infection was assessed 3, 6, 9, 12 and 15 days after inoculation. Three replicates were carried out for each experiment (Chapter 5, Figures 5-2 and 5-5), table 2-10 gives a summary of the experimental design for this experiment.

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and leaf region on susceptibility to pathogens. The effect of K was then compared for each leaf region using T-test in order to determine in which region any effects were seen (Appendix 2, Tables 2-9 and 2-10). The statistical package Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate tub samples were taken from.

Table 2-10 Summary of experimental design for effect of K on pathogen susceptibility experiments

Factor		**Level**
Treatment	Control and -K	2
Leaf region	Tip, middle and base	3
Time point	3, 6, 9, 12 ,15 days post inoculation	5
Replicate	1, 2 and 3	3
		n=90
Parameters	Segments with *Bgh* colonies, number of *Bgh* colonies per cm^2, segments with *R. secalis* symptoms, length of *R. secalis* lesions	4

A Pearson product-moment correlation coefficient was also used to investigate the relationship between the susceptibility of the plants to the pathogens and the K content, water content, metabolite concentrations and JA related gene transcript levels (Appendix 3, Table 3-2). The analysis was carried out using Sigma Plot statistical package.

2.8.6. Me-JA and Pathogens

Experiments were carried out to establish how Me-JA affects plants susceptibility to fungal pathogens. Twenty-four segments from the middle of the second leaf from plants grown for 14 days in control nutrient solution and treated with Me-JA for 24 hours were inoculated with either *R. secalis* or *Bgh* (Section 2.6), and extent of infection was assessed 3, 6, 9 and 12 days after inoculation. Three replicates were carried out for each experiment (Chapter 5, Figures 5-3 and 5-6), table 2-11 gives a summary of the experimental design for this experiment.

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and leaf region on susceptibility to pathogens. The effect of K was then compared for each leaf region using T-test in order to determine in which region any effects were seen (Appendix 2, Table S2-10). The statistical package Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate.

Table 2-11 Summary of experimental design for effect of Me-JA on pathogen susceptibility experiments

Factor		Level
Treatment	Control (water) and Me-JA treated	2
Time point	3, 6, 9 and 12 days post inoculation	4
Replicate	1, 2 and 3	3
		n=24
Parameters	Number of *Bgh* colonies per cm^2 and length of *R. secalis* lesions	2

2.8.7. Expression levels in response to pathogens

Experiments were carried out to establish the effect K deficiency and pathogen infection have on JA related gene expression. Detached leaf segments from, the middle of the second leaf from plants grown for 14 days in the control or K free nutrient solution were inoculated with Bgh or *R. secalis*, and samples taken 0, 1, 2, 3 and 4 days post inoculation and the transcript levels of JA related genes determined (Sections 2.4 and 2.6). Three replicates were carried out for each experiment (Chapter 5, Figures 5-4 and 5-7), table 2-12 gives a summary of the experimental design for this experiment.

Table 2-12 Summary of experimental design for K and pathogen effect on JA related gene expression

Factor		Level
Treatment	Control and -K	2
Infection	Mock and inoculated with pathogen	2
Time point	0, 1, 2, 3 and 4 days post inoculation	5
Replicate	1, 2 and 3	3
		n=60
Parameters	*LOX2.A*, *AOS*, *JIP60* and *PAL*	4

The data was analysed using ANOVA to compare the effect of K availability in nutrient solution and leaf region on susceptibility to pathogens. The effect of K was then compared for each leaf region using T-test in order to determine in which region any effects were seen (Appendix 2, Tables S2-11 and S2-13). The statistical package

Genstat was used to carry out the analysis, a block structure was used to account for differences between replicate.

2.8.8. Pearson product moment correlation analysis

In addition to the ANOVAs, Pearson product-moment correlations were carried out on data obtained in order to investigate relationships between variables measured, using Minitab 15 statistical software. The relationships between changes in K-levels, metabolites and JA-related gene expression in the time course experiment (Section 2.8.2, See Appendix Table S3-1 for results). The association between changes in K-levels, metabolites, JA-related gene expression and pathogen infection in the time course experiment (Section 2.8.3 and 2.8.5, See Appendix Table S3-2 for results).

Chapter 3. Physiological and biochemical effects of K-deficiency in Barley

3.1. Introduction

Extensive research has been carried out into the effect of K-deficiency on the physiology of plants, as discussed in the introduction. This research spans a wide range of crop plants as well as the model plant *A. thaliana* (Bailey and Laidlaw, 1998; Evans, 1963; Gregory and Sen, 1937; Leigh and Wyn Jones, 1984; Richards and Coleman, 1952; Stamp and Geisler, 1980), however only a small proportion of this research has been carried out on barley (Epstein *et al.*, 1963; Gregory and Sen, 1937; Richards and Coleman, 1952). Much of the research into the physiological effects of K-deficiency was carried out between the 1930s and 1980s. In the 90s research shifted towards the molecular identification of K transport systems (Anderson *et al.*, 1992; Sentenac *et al.*, 1992), including work on barley (Amtmann *et al.*, 2006; Amtmann *et al.*, 1999; Boscari *et al.*, 2009; Karley and White, 2009). Over the last 10 years large scale analyses of molecular and metabolic responses to nutrient deficiencies using transcriptomics, proteomics and metabolomics have dramatically enhanced our knowledge of how plants adjust their metabolism to the nutrient supply (Amtmann and Armengaud, 2009). Most of this work was carried out with *A. thaliana* (Armengaud *et al.*, 2009), but the models generated from this work can now serve as a starting point for studies with crop species focussing on responses of individual transcripts, proteins and metabolites to a specific combination of stimuli. To maximize further knowledge gained from these studies the obtained data need to be integrated with precise information on plant development, physiology and growth.

3.1.1. Potassium and growth

Several studies have shown a reduction in shoot and root weight of barley during K-starvation (Drew, 1975; Gregory and Sen, 1937; Helal and Mengel, 1979; Humphries, 1951). Leigh and Wyn Jones (1984) combined assessment of growth with the determination of K concentration in the plant and concluded that the critical concentration of K in barley plants is 2 % dry weight, below this value plants were considered to be K-deficient (Leigh and Wyn Jones, 1984). A number of studies have been carried out demonstrating impaired shoot growth in K-starved plants (El Dessougi *et al.*, 2002; Stamp and Geisler, 1980), high salt levels lead to amplification of this effect (Degl'Innocenti *et al.*, 2009; Hafsi *et al.*, 2010). More recent studies have also measured root length. Walker *et al*. (1998) showed a reduction in root cell elongation rate in barley grown in low-K (2µM) solution compared with control (5mM) solution. However, Høgh-Jensen (2003) did not observe an effect of K-supply on root length of barley plants, but did observe an increase in root hair length leading to an overall increase in root surface area. A K-deficiency induced reduction in the number of tillers and ears produced, and hence yield, has also been reported (Gregory and Sen, 1937). Furthermore K-status has been shown to affect water relations in barley, Gregory and Sen (1937) showed that water content as percent dry weight was increased and respiration was decreased in barley plants grown in low K concentrations.

3.1.2. Potassium and plant biochemistry

As described for other species, K-starvation leads to a decrease in chlorophyll concentrations in barley (Helal *et al*., 1975). Leigh *et al*. (1986) showed a decrease in sucrose and reducing sugars and Gregory and Sen (1937) showed a decrease in reducing sugars (extrapolated from hexose concentrations) and sucrose in barley contradictory to results seen for other plant species. K nutrition has also been shown to have a big impact on nitrogen metabolism, such that total nitrogen is lower in both roots and shoots of K-deficient plants (Helal and Mengel, 1979; Høgh-Jensen and Pedersen, 2003). Incorporation of radioactively labelled N indicated that while protein N is decreased, amino N is increased (Gregory and Sen, 1937; Helal and Mengel, 1979; Helal *et al*., 1975). K status also has an effect on the concentrations of specific amino acids (Richards and Berner, 1954). Although all these studies looked at barley under high versus low-K conditions there is quite a lot of variation between results, which is likely to be due to differences in K-supply, other growth conditions (e.g. light and N supply), sampling times and barley variety.

Most of the above mentioned research into the effect of K-deficiency on barley plants was done on relatively mature barley plants, despite the fact that K uptake is largest in young plants. Another weakness of the available body of information is that background conditions are often poorly specified and therefore determination of correlations and causalities is difficult. In this chapter, I will describe the effects of K withdrawal on hydroponically grown barley (*Hordeum vulgare* L. cv Optic) plants on K concentration, plant growth and a variety of metabolites over a time course of 3 weeks after germination. The results establish a well standardised and characterised system, which forms the basis for subsequent comparison of the effects of pathogens on K-sufficient and K-starved plants (Chapter 5).

3.2. K-concentration in K-deprived barley seedlings

Barley (*H. vulgare*, var. Optic) seeds were germinated on paper towel, seedlings transferred 4 days after germination to hydroponic solution containing a minimal medium with 2mM K (control) or without K (-K) and grown for another 3 weeks (for exact composition of growth media see Table 2-1 in Chapter 2). Five plants were harvested at each time point and K content of shoot and root tissue determined by photospectrometry after extraction with HCl (see materials and methods section 2.7). Figure 3-1 shows K concentrations in shoot and root tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Tables S2-2 and S2-3), see chapter 2 section 2.8.2 for more detailed experimental design.

No significant interaction was found between K-availability and time ($p=0.134$), but a significant reduction in K-concentration was measured in the shoots of plants grown in the K-free media compared with those grown in control ($p=0.008$). The shoot tissue (Figure 3-1A) grown in control solution had a low K concentration at the first time point (day 3), being 2.5 % K in dry weight (DW). This may be due to the seed being germinated on paper towel with no nutrients supplied. The K concentrations in the control shoot tissue increased rapidly over time and by 15 days after transfer to solution the K concentration had increased from 2.5 % DW to 4.5 % DW. The shoot tissue grown in –K solution on day 3 was at 1.4 % DW, already lower than in control plants, and continued to fall over time reaching 0.3 % DW by day 15. Although 3 days after transfer to solution the K concentration in –K grown shoots was 56.3 % of that of control shoots the difference was not significant ($p=0.160$). However, by day 6 the K concentration in –K shoots were significantly lower than in control shoots ($p=0.025$), and by day 15 the K concentration in –K shoots was 7.2 % of that in the control plants.

The root tissue (Figure 3-1B) showed a similar overall pattern to the shoot tissue, with the K-concentration in roots from plants grown in the K-free nutrient solution lower than the control, however unlike in the shoots a significant interaction was observed with time ($p<0.001$). Three days after transfer of the seedlings to nutrient solution the root K concentration in control plants was 1.7 % DW and by day 9 it had more than doubled to 4.2 % DW. The K concentration in the K starved root tissue was 0.9 % DW on day 3, already lower than in control plants and thereafter remained stable around 1 % K in DW throughout the experiment. On day 3 there was already a significant difference between the samples (p=0.048), the K concentration in the -K root tissue being 54.7 % of that in the control sample. As the K concentration in the control tissue rose the difference between the samples increased, by day 12 the –K roots only contained 22.5 % of the K in control roots.

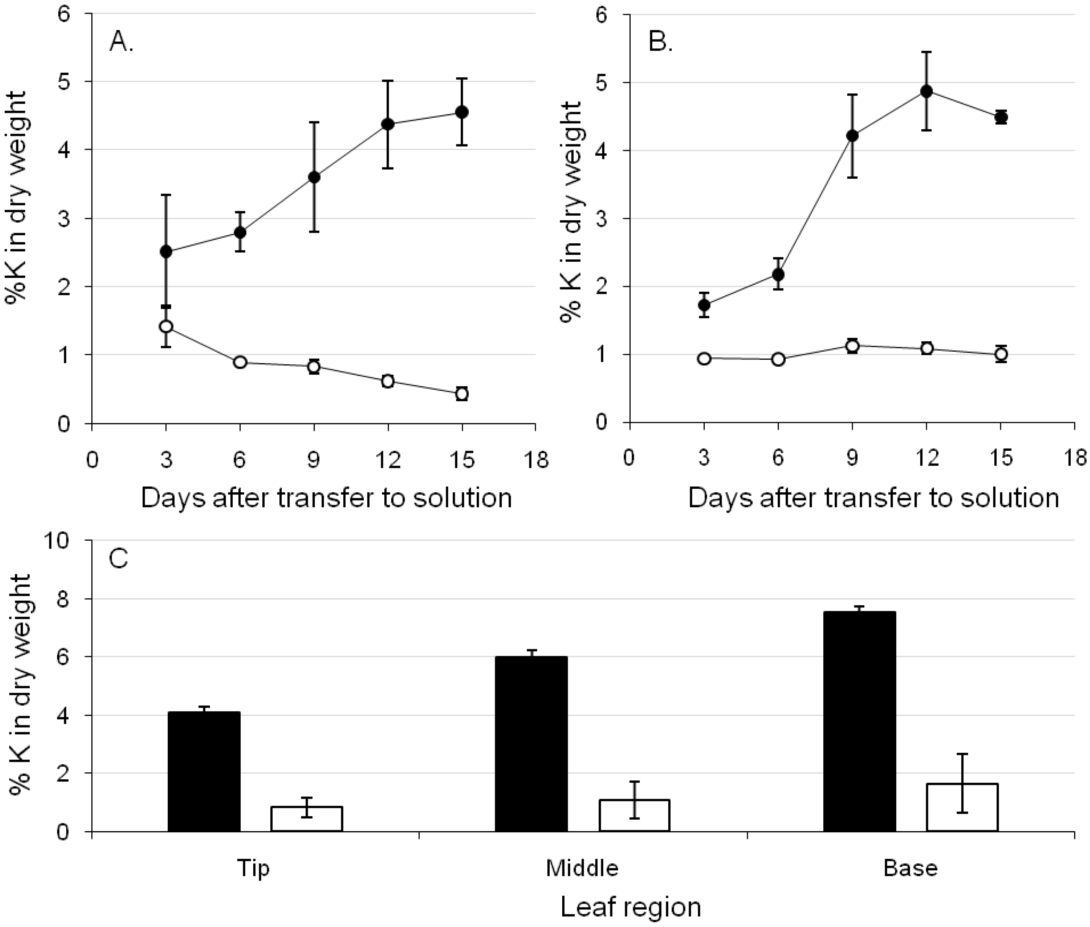


Figure 3-1 K-content in barley tissue. K concentration in A: shoots and B: roots from plants grown in control (●) and -K (○) media solution. 5 plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution and the K concentration determined using a flame photometer, the mean (± SE) of 4 replicate experiments is shown (Chapter 2, Sections 2.1and 2.8.2). C: K concentration in 4 cm leaf regions. Tip, middle and base segments were prepared from 2nd leaves of plants grown for 14 days in nutrient solution (Chapter 2, 2.8.3). 6 leaf segments were pooled for each leaf region and the K concentration determined using a flame photometer, mean (± SE) of 3 replicates shown. Note: in some cases error bars are smaller than the symbols.

In order to investigate how the K is distributed within the leaf, the K concentration was determined for tip, middle and base segments from 2nd leaves of plants grown in control or –K solution for 14 days (Figure 3-1C). The data was analysed using ANOVA (Appendix 2, Table S2-4), see chapter 2 section 2.8.3 for more detailed experimental design. K concentration in the control samples increased from tip to base of the leaf, with the K concentration in the tips segments containing only 54.2 % of that in the base segments, the interaction between responses to K deficiency and leaf region was not significant (p=0.562). There was a significant reduction in K levels in the segments grown in K-free solution compared with control segments (p<0.001); the -K segments contained approximately 20 % of the K in the corresponding control segments (tip 20.3 %, middle 17.9 % and base 21.8 % -K/control).

3.3. Water content of K-starved barley tissue

The effect of K status of barley on water content was determined from the change in weight when tissue was dried. The plants were grown in control or –K solution and the shoot and root tissue was sampled every 3 days as described in Chapter 2 Section 2.1. The tissue was weighed before and after freeze drying and the % water content calculated. The double log of the data was analysed using ANOVA (Appendix 2, Tables S2-2 and S2-3), see chapter 2 section 2.8.2 for more detailed experimental design. There was no change in the water content of the control or –K plants over time (p=0.412) or and no difference between treatments (p=0.121, Table 3-1). The water content of the first and second leaves was also determined separately (data not shown). This showed that the first leaves had lower water content than the second leaves irrespective of K-supply. No difference was observed between first or second leaves grown in control or –K nutrient solution.

Table 3-1 Water content (%) in barley tissue over time.

The water content of plants grown in control and –K nutrient solution. for 3, 6, 9, 12 and 15 days after transfer to nutrient solution, 5 plants were pooled for each condition and tissue type at each time point, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.1and 2.8.2).

Days after transfer to nutrient solution	Shoot		Root	
	Control	-K	Control	-K
3	90.6	92.6	94.87	97.41
6	91.5	89.5	94.31	95.42
9	91.9	90.5	95.54	96.54
12	92.7	92.0	74.47	96.63
15	91.7	90.3	96.98	96.16

The effect of K-deficiency on the water content of different leaf regions was determined, using the tip, middle and base of the second leaf. Segments were cut from the second leaf of seedlings grown for 14 days in control or –K solution as described in material and methods section 2.1. The data was analysed using ANOVA (Appendix 2,

Table S2-4), see chapter 2 section 2.8.3 for more detailed experimental design. The water content in the segments prepared from –K plants was significantly lower and in control segments (p=0.004), a significant reduction in water content was observed from the base to the tip of the leaf (p<0.001). The average water content of the control leaf regions increased from the tip to the base but the difference was not significant (Table 3-2). The reduction in water content from base to tip is also seen in the –K leaf regions, where the difference between the samples was significant (Table 3-2; p=0.004). The middle and base segments were not significantly different from each other but both were significantly different from the tip region, with the comparison of the tip and middle segments resulting in a p-value of 0.028 and between tip and base segment water content giving a p-value of 0.012. There was no significant difference between water content of middle and base segments from plants grown in control and –K nutrient solution. There was a significant difference between growth conditions in water content of the tip region of the leaf (p =0.043).

Table 3-2 Water content (%) in regions of 14 day old 2nd leaves

Leaf region	% water content	
	Control	**-K**
Tip	90.11	86.40
Middle	91.42	90.72
Base	92.12	91.54

3.4. Effect of K withdrawal on growth in barley seedlings.

The effect of K-starvation on the growth of barley (Optic variety) grown in hydroponic solution was investigated by growing plants in control or –K nutrient solution and sampling every 2 days, as described in the Chapter 2 Section 2.1. In addition to allowing the assessment of the effect of K-deficiency on the early stages of growth, establishing reduced growth in plants grown in K-free solution was used to confirm K-starvation. The data was analysed using ANOVA (Appendix 2, Table S2-1), the data for root length and shoot, root and total weight were not normally distributed and were transformed by taking the log10 before analysis, see chapter 2 section 2.8.2 for more detailed experimental design. By day 12 the -K plants were showing slight visual K-deficiency symptoms the leaves and stems appeared thinner and a slightly paler green than the control plants. By day 18, as well as being visually much smaller than the control plants, the –K plants had started to form chlorotic lesions on the first and second leaves and displayed a loss of turgidity throughout the shoot. The effect of the K withdrawal on the growth of the barley plants was quantified by measuring the weight and length of barley shoots (Figure 3-2), leaves (Figure 3-3) and roots (Figure 3-4) grown in control or –K nutrient solution every two days.

3.4.1. Effect of K withdrawal on shoot growth in barley

The shoots of plants grown in K-free nutrient solution were shorter and weighted less than those grown in control solution. The shoot length (Figure 3-2A) of the control plants increased steadily over time, oscillating slightly with the appearance of new leaves, a significant interaction was observed between the two factors ($p<0.001$). The length of the shoots of the K-starved plants also increased albeit at a slower rate than the control plants. There was a significant difference between the shoot lengths of plants grown in the different nutrient solutions from as early as day 6 ($p=0.015$). The difference became considerably larger after day 12. The fresh weight (FW) of the control plant shoots increased rapidly over time, however the interaction between the factors was not significant ($p=0.075$), the rate of increase was smaller in shoots of –K plants (Figure 3-2B). The shoots from –K plants had significantly reduced weight compared to the control plants ($p<0.001$). From day 12 onwards there was a marked further divergence between the shoot weights of the control and –K plants. By day 20 the shoot weight of the -K plants was only half (50.7 %) that of the control plants. Figure 3-2 C shows photographs of representative 20 day old control and –K plants.

The effect of K-deficiency on the growth of individual leaves from the intercalary meristem to the tip of the leaf was investigated as per material and methods section 2.4. Figure 3-3 shows the length of the first (A), second (B) and third (C) leaf from plants grown in control and –K nutrient solution over time. The first leaf was already initiated before the plants were transferred to nutrient solution, and continued to increase in length until day 6, at which point it stopped expanding. No significant interaction between K-availability and time was observed ($p=0.491$), however significant differences were seen with both factors ($p<0.001$) There was little difference in the growth rate of the first leaves of plants grown in control and –K solution indicating that there was sufficient K available from the seed to allow the first leaf to expand fully.

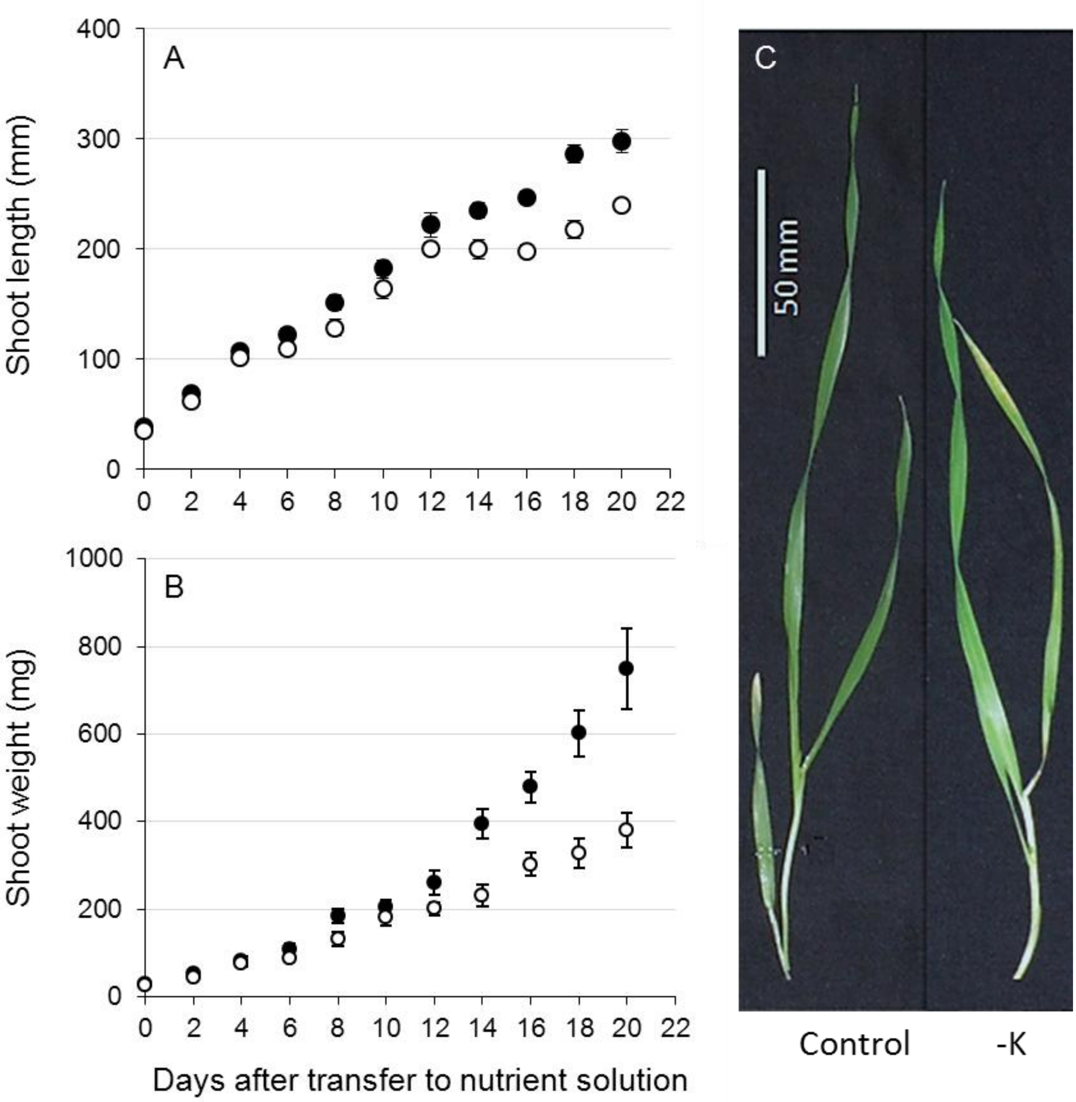


Figure 3-2. Growth of barley shoots under K stress. The A: length and B: fresh weight of shoots from 5 plants grown in control (●) and -K (○) media solution was determined 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days after transfer to nutrient solution, the mean (± SE) of 4 replicate experiments is shown (Chapter 2, Sections 2.1and 2.8.1). C: shows representative samples of control and –K shoots 20 days after transfer to solution.

The second leaves of both control and –K plants were not visible until 4 days into the experiment, at which point there was no significant difference in leaf lengths between plants grown in different K treatments. Once the second leaf was apparent growth was rapid, however the K-starved plants had a slight reduction in growth rate compared with the control plants, and by day 8 there was a significant reduction in length of the second leaf (p= 0.006). By day 14 the second leaves of plants grown in both control and –K conditions were fully expanded. At this point a highly significant reduction in growth (P=0.002) was observed in –K plants. The second leaves of the –K plants had expanded to 85 % that of the control plants. No significant interaction between K-availability and time was observed in the second leaf (p=0.146), however significant differences were seen with both factors individually (p<0.001)

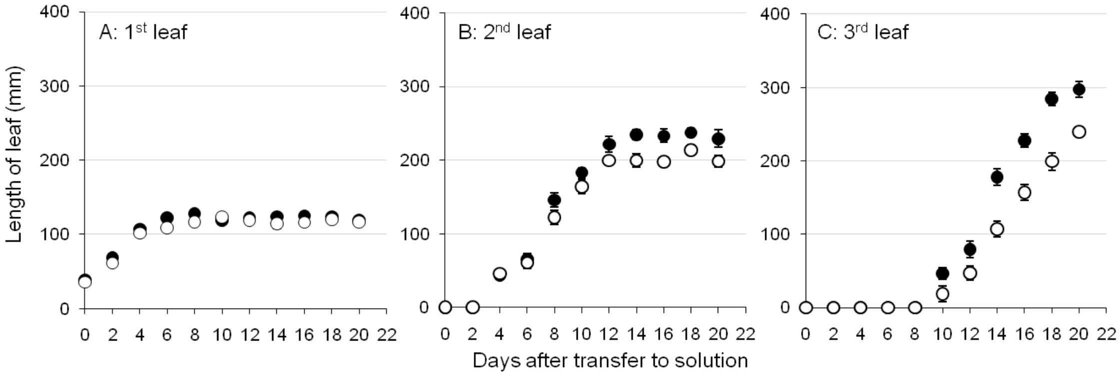


Figure 3-3 Growth of barley leaves under K stress. The length of A: 1st leaves, B: 2nd leaves and C: 3rd leaves from 5 plants grown in control (●) and -K (○) media solution was determined 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days after transfer to nutrient solution, the mean (± SE) of 4 replicate experiments is shown (Chapter 2, Sections 2.1and 2.8.1).

The third leaf was not visible until the tenth day of growth in nutrient solution, at this point there was no significant difference between the leaves under different growth conditions, however by day 12, the third leaves of the –K plants were significantly shorter (p=0.006) than those grown in control solution, with the –K third leaf length at around 60 % that of the control. The growth of the control plants' third leaf slowed before that of the K-starved plants and by day 20 the –K third leaves were 80 % of the length of that of the control plants. A significant interaction between K-availability and time was observed in the tried leaf (p=0.004). The first of the fourth leaves of the control plants became visible on day 18 (data not shown) however no fourth leaf developed on the –K plants until day 20.

3.4.2. Effect of K withdrawal on root growth in barley

The root systems of the plants grown in the –K and control solutions were also sampled every 2 days (Figure 3-4). The roots of plants grown in K-free nutrient solution were longer and weighted less than those grown in control solution. A significant interaction with time was observed for both variables (p<0.001). The roots systems of the plants grown in –K solution were initially of a similar length to those grown in control solution, the –K plants however these plants had fewer, thinner and weaker roots. Both the plants grown in control and the plants grown in –K solution increased in length gradually over time. The longest root of the control and –K plants initially increased at a similar rate, but by day 16 the –K roots had started to grow more rapidly and were 58 % longer than the control plants. The weight of the root system of both the control and –K plants increased over time. The root weight of the –K plants increased at a slower rate than those of the control plants. By day 20 the root weight of the –K plants was only 64 % of that of the control plants.

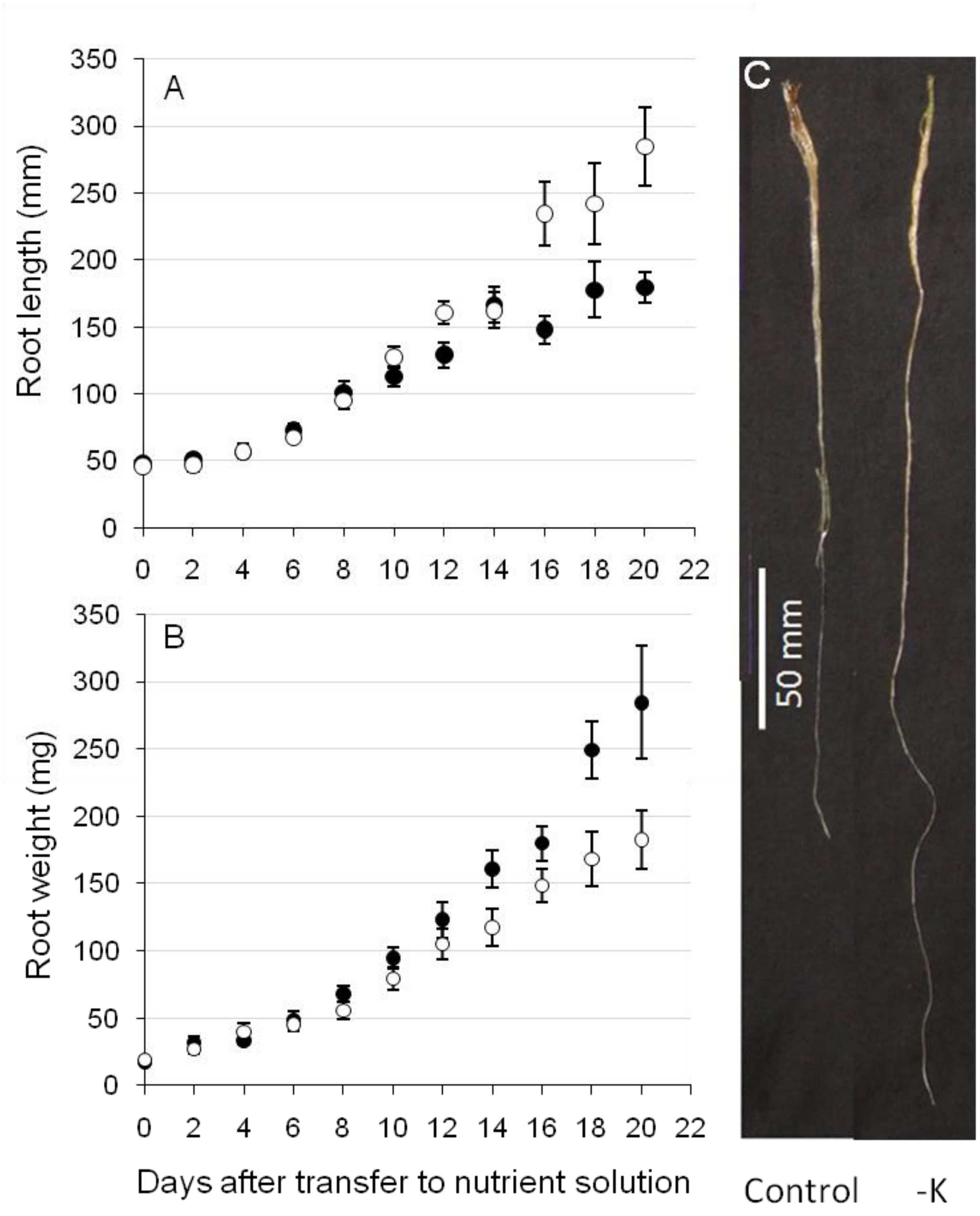


Figure 3-4 Growth of barley roots under K stress. The A: length and B: fresh weight of roots from 5 plants grown in control (●) and -K (○) media solution was determined 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days after transfer to nutrient solution, the mean (± SE) of 4 replicate experiments is shown (Chapter 2, Sections 2.1 and 2.8.1). C: shows representative samples of control and –K roots 20 days after transfer to solution.

3.5. Effect of K withdrawal on metabolites

3.5.1. Chlorophyll concentration in barley tissue

Chlorophyll a and b are vital components of photosynthesis, as they allow the utilisation of light energy. The effect of K-deficiency on chlorophyll concentration in barley seedlings was investigated. Barley plants were grown in control or K-free nutrient solution and the shoots from five plants were sampled every 3 days, the chlorophyll concentration (per fresh weight) was determined as per Chapter 2, Section 2.3.2. Figure 3-5A and B shows Chlorophyll a and b concentration in shoot tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-2), see chapter 2 section 2.8.2 for more detailed experimental design.

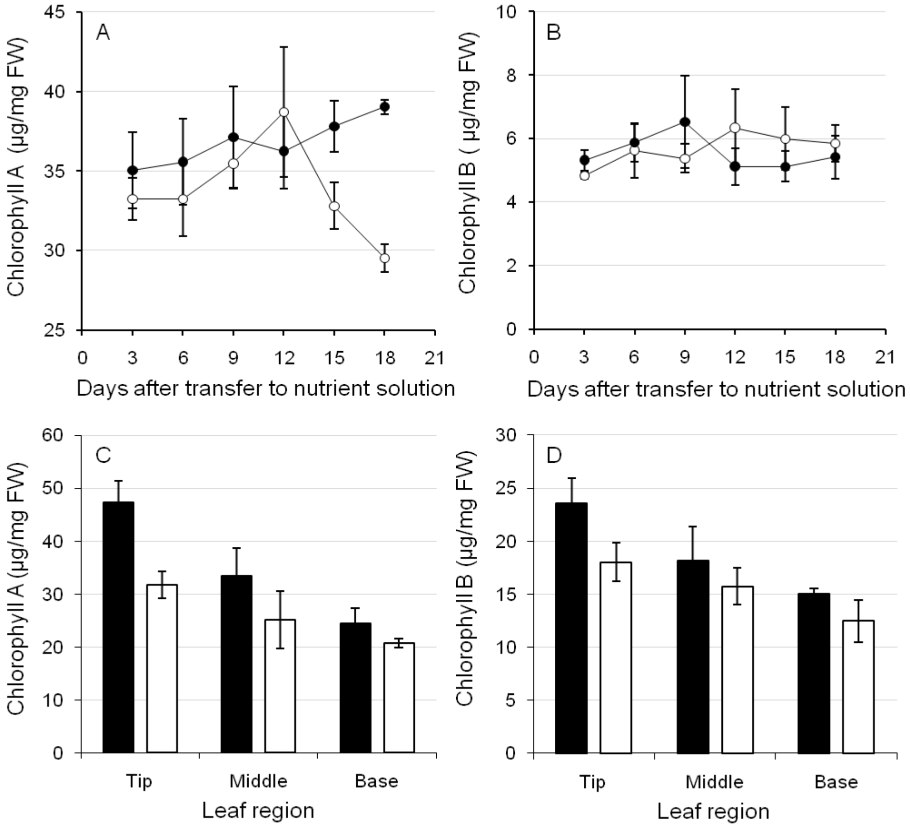


Figure 3-5 K-content in barley tissue. A: chlorophyll a and B: chlorophyll b concentration in shoot tissue from plants grown in control (●) and -K (○) media solution. 5 plants were pooled 3, 6, 9, 12, 15 and 18 days after transfer to nutrient solution and the K concentration determined using uv/vis spec, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.3.1 and 2.8.2). C: Chlorophyll a and D: Chlorophyll b in 4 cm leaf regions. Tip, middle and base segments were prepared from 2nd leaves of plants grown for 14 days in nutrient solution (Chapter 2, 2.8.3). 6 leaf segments were pooled for each leaf region and the K concentration determined using uv/vis spec, mean (± SE) of 3 replicates shown.

No significant interaction between the effect of K-availability and time on Chlorophyll a (p=0.663) or b (p=0.458) was observed. There was no significant change in either Chlorophyll a (p=0.346) or Chlorophyll b (p=0.950) in response to K-deficiency, despite an apparent reduction in Chlorophyll a in the –K shoots after day 15.

The effect of K withdrawal on chlorophyll a and b concentration in first and second leaves of 14 day plants was also investigated separately (data not shown); no difference in chlorophyll concentration was observed between the leaves.

The effect of K-deficiency on chlorophyll concentration in different leaf regions was also investigated. The chlorophyll a and b concentration in tip, middle and base segments from 14 day old second leaves of barley seedling grown in control and –K solution was measured as described in chapter 2 section 2.3.2. Figure 3-5C and D shows Chlorophyll a and b concentration across the leaf, no significant interaction was observed between K-availability and time for either chlorophyll a (p=0.328) or Chlorophyll b (p=674). The data was analysed using ANOVA (Appendix 2, Table S2-4), see chapter 2 section 2.8.3 for more detailed experimental design.

K-starved leaves had reduced chlorophyll a concentration when compared with the control segments (p=0.013), but no significant change in Chlorophyll b was observed (p=0.054). A significant reduction in both chlorophyll a (p=0.004) and b (p=0.016) was observed from the tip to the base of the leaf. The control plants showed a reduction in chlorophyll a and b concentration from the tip to the base of the leaf, the base segments contained 52 % and 64 % of the chlorophyll a and b concentration in the tips, respectively, this was not a significant reduction (p=0.075). Variation in chlorophyll concentration within the leaf of -K plants was small but significant; chlorophyll a (p=0.027) and b (p=0.035) were both higher in the tips than the base segments, with the –K base segments containing 65 % of the chlorophyll A and 69 % of the chlorophyll b in the tip segments. The effect of K-deficiency on chlorophyll concentration was most prominent in the tip with the chlorophyll a concentration in the -K segments 67 % of the control and the chlorophyll b concentration 76 % of the control segments. The base region was affected least, with –K base segments containing 85 % of the chlorophyll a and 83 % of the chlorophyll b of the control plants.

3.5.2. Sugar concentration in barley tissue

The effect of K status on the concentration (per fresh weight) of hexose sugars in barley was investigated. Sugars not only provide a source of energy for the plant, but may also provide energy for pathogenic organisms attacking the plants and therefore increased sugar concentration may increase the attractiveness and create a more favourable environment for plant to pathogens. A number of known effects of K-deficiency (e.g. on photosynthesis, long distance transport and glycolytic enzyme) could alter sugar concentration in plants (Marschner, 1995). Plants grown in control or –K solution were sampled every 3 days and the sucrose, glucose and fructose concentrations in freeze dried shoot and root tissue were determined as described in chapter 2 section 2.3.3. Figure 3-6 shows glucose, fructose and sucrose levels concentration in shoot and root tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-2 and S2-3), see chapter 2, section 2.8.2 for more detailed experimental design. No significant interaction was seen between effect of K-supply and time for sugar concentration in either the shoots or roots.

The glucose concentrations in the shoot tissue were initially high (Figure. 3-6 A), but decreased rapidly during growth ($p<0.001$), by day 9 the concentration had dropped in the control to 15 % and –K to 40 % the initial value (day 3). Over the entire time course glucose concentration in the –K shoots were higher compared to the control plants, however this increase was only significant on days 3 ($p=0.048$) and 12 ($p=0.004$).

The glucose concentration measured in the root tissue was lower overall than that in the shoot tissue (Figure 3-6 B), and as in the shoot tissue, they dropped over time ($p<0.001$). As in shoots the rate of decrease was lower in –K plants than in control plants, resulting in significantly reduced glucose levels in K-starved plants ($p=0.030$). By day 9 the concentration of glucose had dropped to 12.4 % of the day 3 value in the control roots and 36.0 % in the –K roots.

The glucose concentration measured in the root tissue was lower overall than that in the shoot tissue (Figure 3-6 B), and as in the shoot tissue, they dropped over time ($p<0.001$). As in shoots the rate of decrease was lower in –K plants than in control plants, resulting in significantly reduced glucose levels in K-starved plants ($p=0.030$). By day 9 the concentration of glucose had dropped to 12.4 % of the day 3 value in the control roots and 36.0 % in the –K roots.

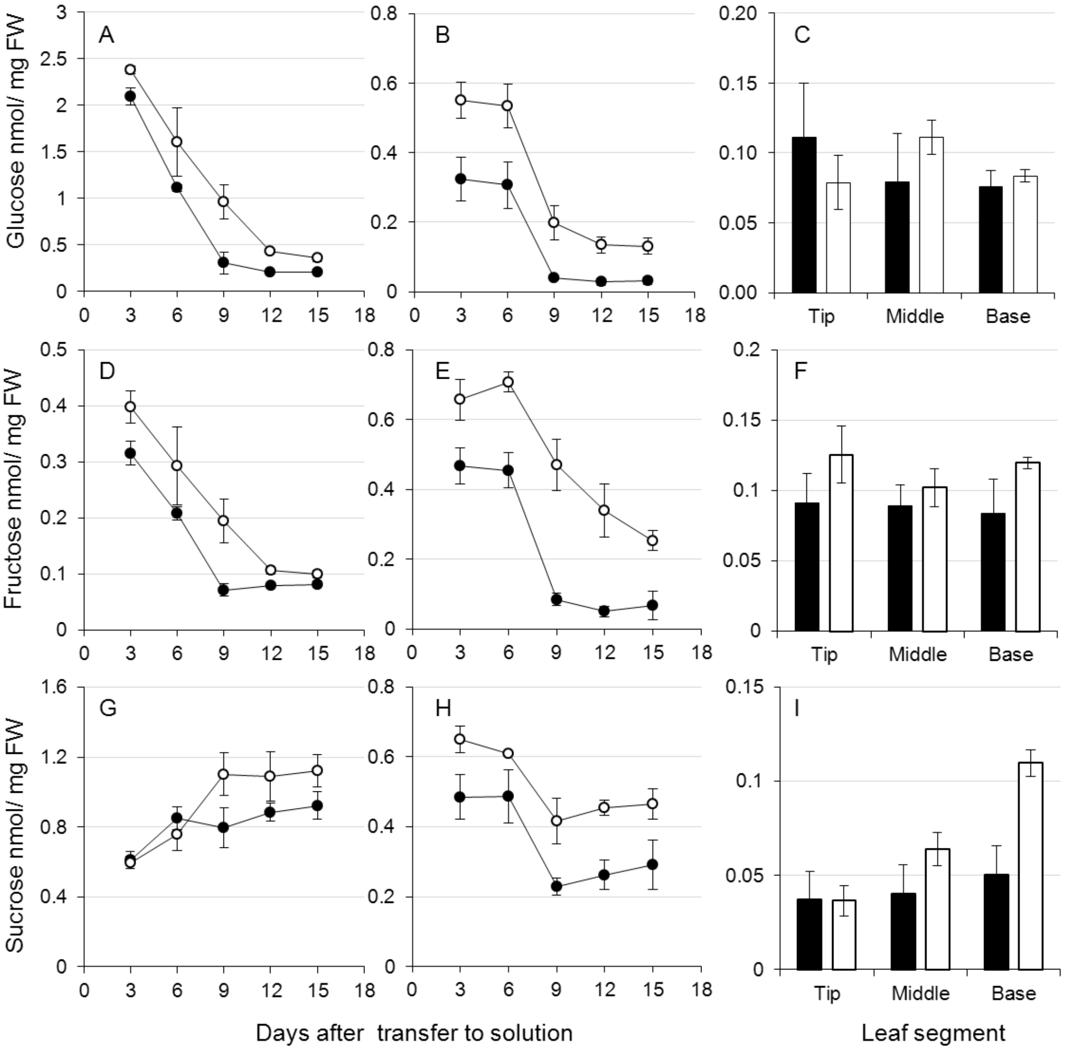


Figure 3-6 Sugar concentration in barley tissue. A, B, C: glucose, D, E, F: fructose and G, H, I: sucrose concentrations in A, D, G: shoots and B, E, H: roots from plants grown in control (●) and -K (○) media. 5 plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution and the sugar concentration determined, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.3.3 and 2.8.2). C, F, I: 4 cm leaf regions from plants grown in control (●) and -K (○) media. Tip, middle and base segments were prepared from 2nd leaves of plants grown for 14 days in nutrient solution (Chapter 2, 2.8.3). 6 leaf segments were pooled for each leaf region and the K concentration determined, mean (± SE) of 3 replicates shown.

The fructose concentration in both the shoots and roots showed a similar pattern to the glucose concentration (Figure 3-6 C and D) although at much lower concentrations. The fructose concentration in the shoots were highest at the 3 day time point and then rapidly dropped ($p<0.001$). By day 9 the fructose concentration in the control shoots was 22.6 % of those of the day 3 shoot samples, after this they remained constant. The concentration in the –K shoot tissue decreased more slowly over time, reaching 26.7 %

of the day 3 concentration by day 12. The fructose concentration in the –K shoots was higher than in control shoots as early as day 3, with the –K shoot tissue containing 26.4 % more fructose than the control shoots, however this was not statistically significant until day 15 (p=0.012).

The fructose concentration in the roots was generally higher than that in the shoots. Both the control and –K root samples contained high concentration of fructose until day 6. Subsequently fructose concentration decreased over time and reached the minimum concentration seen during this time course on day 12 in control plants (11 % of the day 3). By contrast the Fructose concentration in the –K plants was still decreasing at the end of the experiment on day 15. The fructose concentration in the K-starved roots was significantly higher than in the control roots (p=0.007). –K roots contained a significantly higher concentration of fructose than in the control already at day 6 and remained consistently higher throughout the experiment.

Sucrose concentration in the shoot tissue of both control and –K plants showed a very different pattern to that of the glucose and fructose. The sucrose concentration was initially low and increased over the course of the experiment (p=0.003), this increase was small with a 51 % increase in the control plants and a 88 % in the –K plants between days 3 and 15, however this change was only significant in –K plants (p=0.029). There was no significant difference between the sucrose concentration in the control and –K shoots (p=0.114). By day 9 the concentration in the –K plant were significantly higher than in the control plants at the same time point (p=0.012).

The sucrose concentration in both the control and –K root tissue showed a similar pattern over time as that of glucose and fructose, although the drop was less severe. The sucrose level for both treatments were at their highest on day 3. A sharp drop in sucrose concentration was seen between days 6 and 9 after this the concentration remained constant. There was no significant difference between the sucrose concentration in the control and –K roots (p=0.078).

The effect of K-deficiency on sugar content in different areas of the second leaf was also investigated. Figure 3-6 shows glucose, fructose and sucrose levels concentration across the second leaf of 14 day old plants. The data was analysed using ANOVA (Appendix 2, Table S2-4), see chapter 2, section 2.8.3 for more detailed experimental design. No significant change in glucose or fructose concentration was observed between the difference leaf regions. However, an increase in sucrose was measured from the tip to the base of the 2nd leaf from plant grown in –K nutrient solution ($p<0.001$), this was not the case in the leaves from control plants ($p=0.317$). The fructose and sucrose concentration in the K-starved segments were significantly higher than in the control segments across the leaf ($p=0.034$ and $p=0.015$ respectively), but there was no change in glucose concentration between treatments ($p=0.906$).

3.5.3. Malate concentration in shoot and root tissue

Malate is an important intermediate of the tricarboxylic acid cycle (TCA cycle), also known as the citric acid cycle or Krebs cycle. The TCA cycle is an important process in cellular respiration, and consists of a series of reactions allowing the breakdown of carbohydrates to carbon dioxide and water to generate ATP and reducing equivalents. Malate concentration was measured using enzyme assays (as described in the Chapter 2, Section 2.3.4). Figure 3-7 shows malate levels concentration in shoot and root tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-2 and S2-3), see chapter 2, section 2.8.2 for more detailed experimental design.

No significant interaction was seen between the effect of K-availability and time on malate in shoot tissue ($p=0.392$), and no significant change was observed in response to K-starvation ($p=0.078$). The malate concentration in the control samples changed significantly over the time course ($p=0.040$). A transient rise in malate concentration was seen in the control samples peaking at day 9, at which point the concentration had increased by 39 % over the day 3 samples. After day 9 the malate concentration dropped again; by day 15 the malate concentration in the control shoots was 54 % of the day 3 concentration. No significant change in malate concentration was seen in the –K samples, however a peak was still observed on day 9. The shoots of K-deprived plants had a significantly lower concentration of malate than control shoots as early as day 3 ($p = 0.039$). After the peak at day 9 the malate concentration in the –K shoots dropped more slowly than in the control, and by day 12 they was no significant difference between the two treatments ($p=0.320$).

A significant interaction was seen between the effect of K-availability and time on malate in shoot tissue ($p=0.036$). The malate concentration in control roots increased rapidly between day 3 and day 6 with a 151 % increase in concentration. Concentrations decreased again after day 6; by day 12 the malate concentration was 26 % of that on day 6. These changes in concentration were significant over the time course sampled (p=0.005). The transient accumulation of malate did not occur in the –K roots, instead a slow but significant decrease was seen over time (p=0.039). By day 15 the malate concentration was 35 % of that on day 3. The only time there was a significant difference between the root malate concentration in control and –K plants was on day 6 (p=0.003) when the control samples reached the maximum concentration; at this point the malate concentration in the –K roots were 54 % of the control samples.

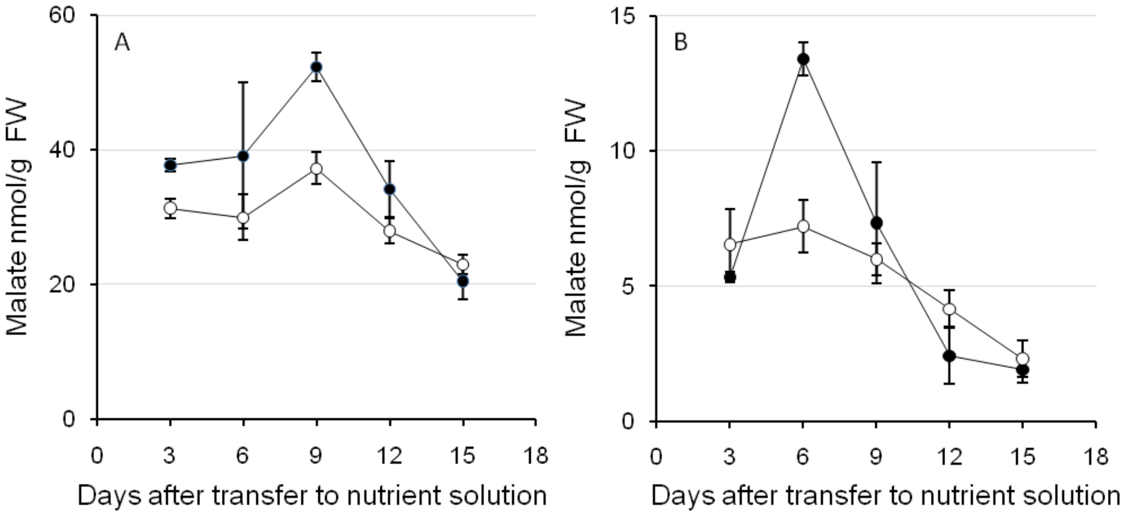


Figure 3-7 Malate concentration in barley tissue. Malate concentration in A: shoot and B: root tissue from plants grown in control (●) and -K (○) media. 5 plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution and the K concentration determined, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.3.1 and 2.8.2).

3.5.4. Glutamate concentration in shoot and root tissue

Glutamate is an amino acid which plays an important role in N assimilation and photorespiration where it incorporates N from ammonium to form glutamine. It is a major nitrogen source for pathogens and pests. Glutamate concentration was measured using enzyme assays (as described in chapter 2, section 2.3.5). Figure 3-8 shows glutamate concentration in shoot and root tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-2 and S2-3), see chapter 2, section 2.8.2 for more detailed experimental design.

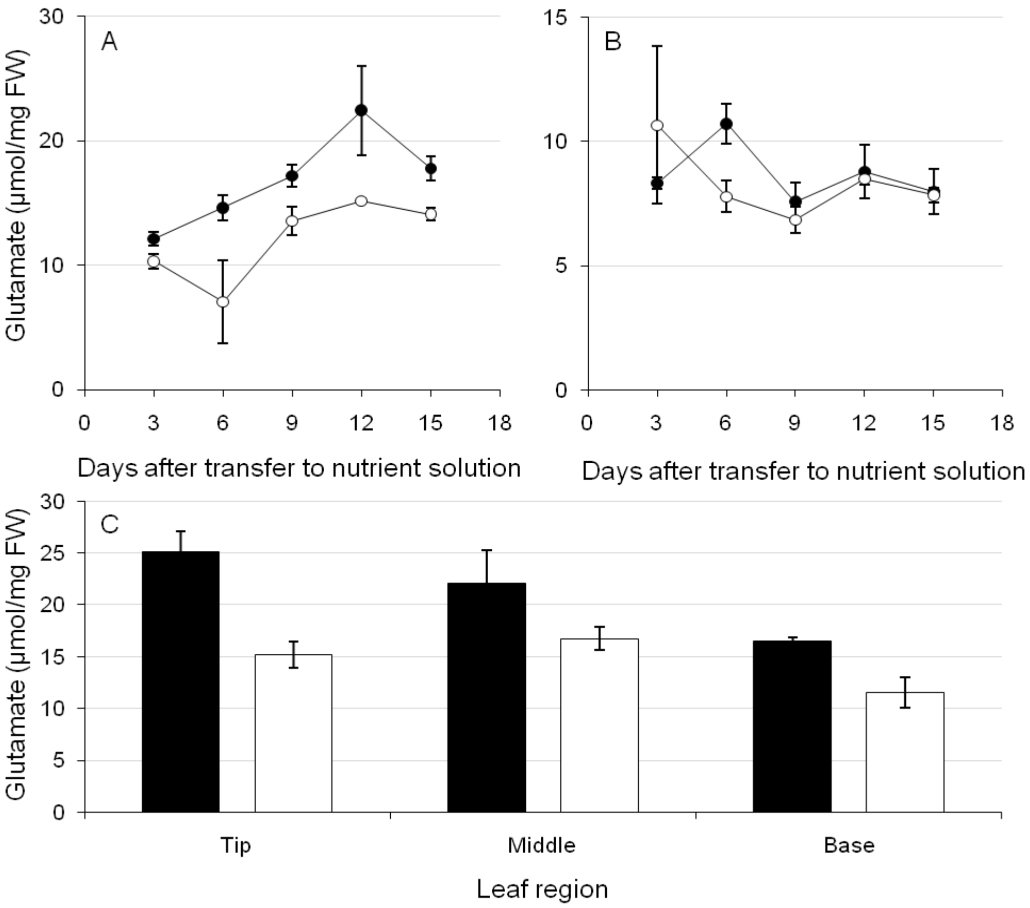


Figure 3-8 Glutamate concentration in barley tissue. Glutamate concentration in A: shoot and B: root tissue from plants grown in control (●) and -K (○) media. 5 plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution and the K concentration determined, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.1and 2.8.2). C: glutamate concentration in 4 cm leaf regions. Tip, middle and base segments were prepared from 2nd leaves of plants grown for 14 days in nutrient solution (Chapter 2, 2.8.3). 6 leaf segments were pooled for each leaf region and the K concentration determined, mean (± SE) of 3 replicates shown. Note: in some cases error bars are smaller than the symbols.

No significant interaction was seen between the effect of K-availability and time on glutamate in either shoot (p=0.418) or root tissue (p=0.348). No significant change was observed in response to K-starvation (p=0.060), but the glutamate levels did increase significantly over time (p<0.001). There was little difference in glutamate concentration between the control and root samples from –K plants (p=0.848).

The effect of K-deficiency on different leaves and different areas of the second leaf was also investigated. No difference in glutamate concentration between first and second leaves was detected in control or –K plants (data not shown). Figure 3-8 shows glutamate concentration across the second leaf of 14 day old plants. The data was analysed using ANOVA (Appendix 2, Table S2-4), see chapter 2, section 2.8.3 for more detailed experimental design. No significant interaction was seen between the effect of K-availability and leaf region on glutamate concentration (p=0.369). There was however a significant reduction in glutamate levels in the K-free segments (p=0.001) and glutamate concentration decreased from the tip to the base of the leaf. The glutamate concentration decreased slightly from the tip to the base of the 2nd leaf from control plants (Figure 3-8 C). There was no significant difference in glutamate concentration between the tip, middle and base segments of the 2nd leaf from –K plants (p=0.128).

3.5.5. Protein concentration in barley seedling tissue

Total protein concentration in the plant tissue was determined using Bio-Rad protein assay dye reagent (materials and methods section 2.3.7). Figure 3-9 shows glutamate levels concentration in shoot and root tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-2 and S2-3), see chapter 2, section 2.8.2 for more detailed experimental design.

Protein concentration in the control shoot tissue was stable throughout the experiment (p=0.516). There was a little more variation in the protein content of the leaves from the –K plants, with a large drop between day 3 and day 6 and almost complete recovery by day 9, however this was still not significant (p=0.275). There was no significant difference in protein content between shoot tissue grown in control and –K solution (p=0.680).

There was no significant change in protein concentration over the time course for root of either control (p=0.316) or –K (p=0.391) plants. Although the protein concentration in the –K plants were constantly higher than in the control plants this was not significant at any point during the experiment.

The protein concentration in 14 day old first and second leaves of barley seedlings were also measured (data not shown). There was a slight increase in protein concentration in the K-starved first leaves compared with control first leaves, but no difference between treatments in the second leaves. Figure 3-9 shows protein concentration across the second leaf of 14 day old plants. The data was analysed using ANOVA (Appendix 2, Table S2-4), see chapter 2, section 2.8.3 for more detailed experimental design. No significant interaction was seen between the effect of K-availability and leaf region on protein concentration (p=0.260) and no significant effect of K-deficiency was observed (p=0.711). There was however a significant reduction in protein level from the tip to base of the leaf (p=0.015). The protein concentration in the control plants decreased from the tip to the base of the second leaf (p=0.030), with the middle segments containing 77 % and the base segments containing 66 % of protein in the tip segments. The drop in protein concentration from tip to base was more pronounced in the –K plants, with the middle segments containing 59 % and base segments containing 29 % of the protein in the tip.

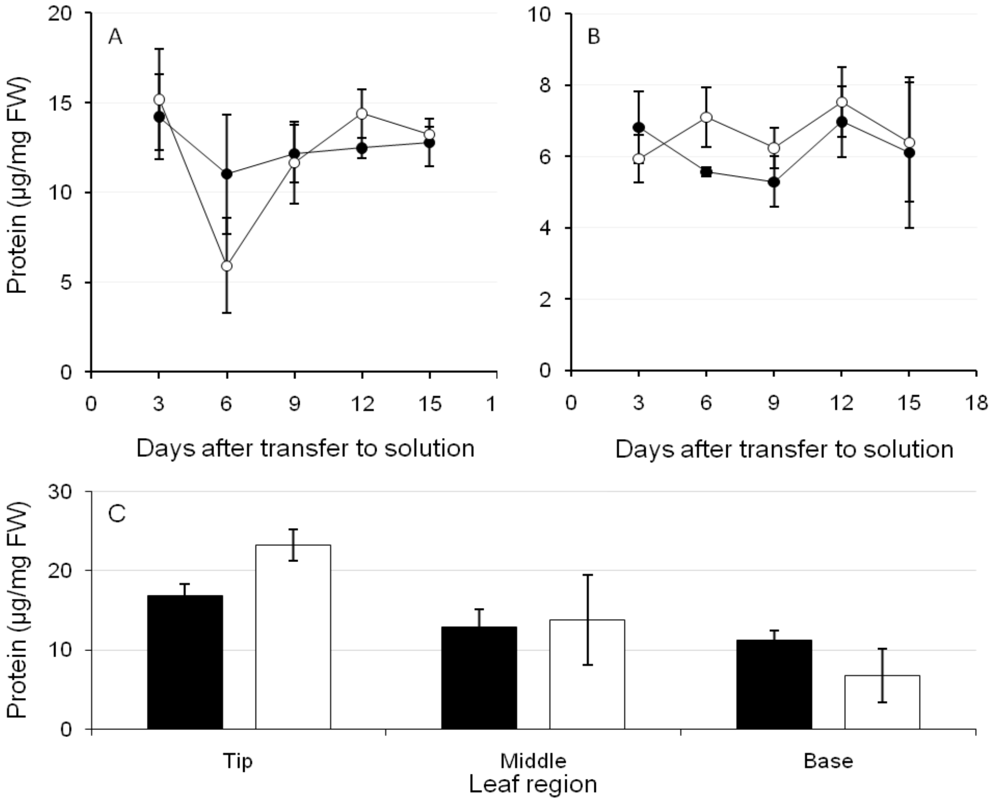


Figure 3-9 Protein concentration in barley tissue. Protein concentration in A: shoot and B: root tissue from plants grown in control (●) and -K (○) media. 5 plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution and the protein determined, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.1and 2.8.2). C: protein concentration in 4 cm leaf regions. Tip, middle and base segments were prepared from 2nd leaves of plants grown for 14 days in nutrient solution (Chapter 2, 2.8.3). 6 leaf segments were pooled for each leaf region and the protein determined, mean (± SE) of 3 replicates shown. Note: in some cases error bars are smaller than the symbols.

3.5.6. Nitrate concentration in barley seedling tissue

Nitrogen is needed for the production of amino acids to make proteins, nucleic acids and many secondary metabolites. Plants are not able to fix atmospheric nitrogen themselves. The major form in which nitrogen is taken up by plants is nitrate. Nitrate like malate is mostly stored in the vacuoles and it is transported in the xylem from roots to shoots. The nitrate concentration was measured using enzyme assays (as described in chapter 2, section 2.3.5). Figure 3-10 shows nitrate concentration in shoot and root tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-2 and S2-3), the data for nitrate concentration in the root was not normally distributed and was transformed by taking the log10 before analysis, see chapter 2, section 2.8.2 for more detailed experimental design.

No significant interaction was seen between the effect of K-availability and time on nitrate concentration in either shoot (p=0.784) or root tissue (p=0.183). No significant change in nitrate concentration was observed in response to K-availability in either shoot (p=0.405) or root (p=0.958) tissue.

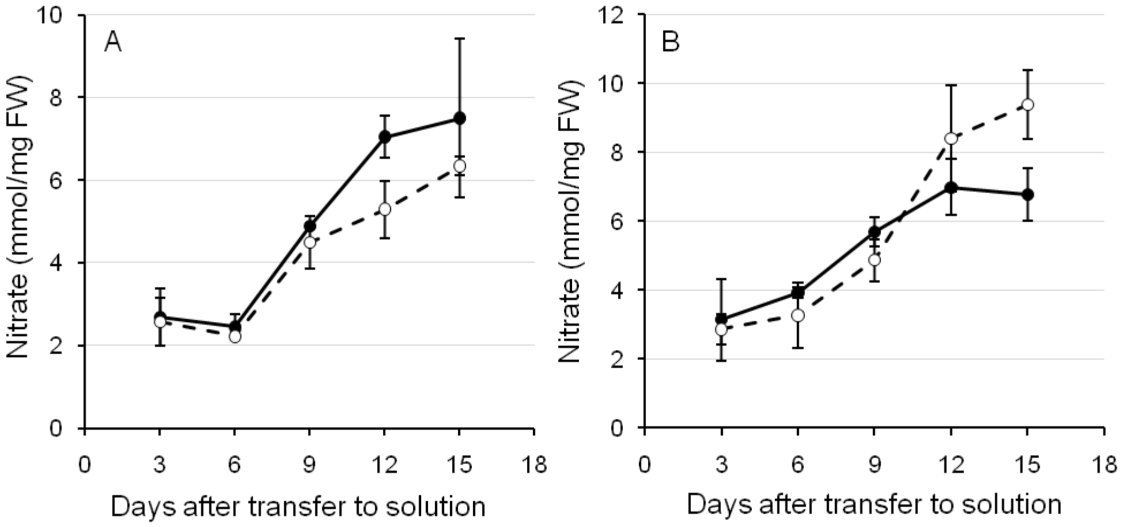


Figure 3-10 Nitrate concentration in barley tissue. Protein concentration in A: shoot and B: root tissue from plants grown in control (●) and -K (○) media. 5 plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution and the nitrate concentration determined, the mean (± SE) of 3 replicate experiments is shown (Chapter 2, Sections 2.3.1 and 2.8.2).

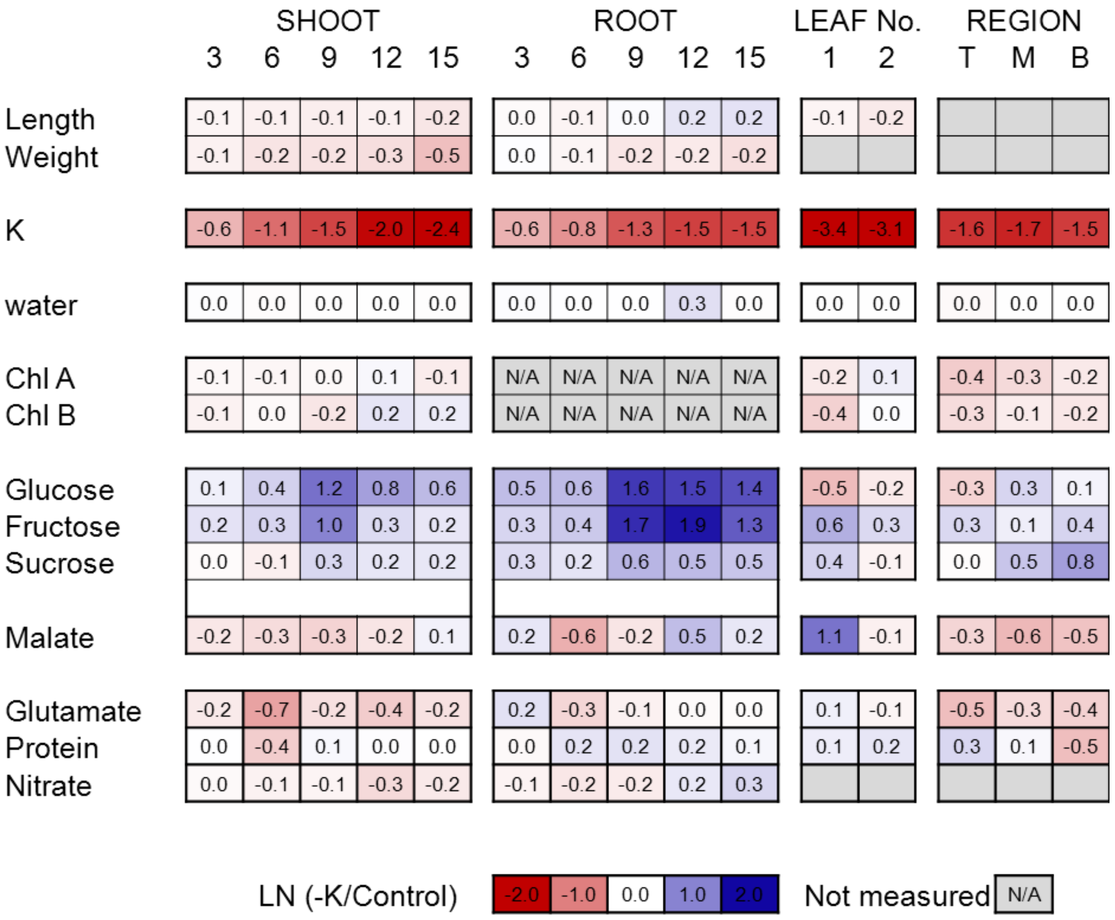
3.6. Discussion and conclusions

In this chapter the effect of K-deficiency on growth, water content and metabolite concentration in a variety of different barley tissues was determined (see Figure 3-11 for over view of results in heat map format). Although the various parameters have been measured before in barley with respect to K-concentration, no one study has looked at all the parameters studied here and considered both shoot or root tissue, nor the more localised effect of K-deficiency on the leaves. Much of the work previously carried out in barley and other plants has shown similar patterns to those obtained here, for example an increase in hexose sugars in K-starved plants. The only recent study to comprehensively look at the effect of K-deficiency in plants was carried out on *A. thaliana* by Armengaud *et al.* (2009), during which the effects of K withdrawal and resupply on a wide variety of metabolite concentration in shoot and root tissue were investigated.

The results obtained showed a clear change in physiological properties of plants deprived of K. As described previously (Drew, 1975; Helal and Mengel, 1979; Leigh and Wyn Jones, 1984), K withdrawal led to reduced shoot growth and an increase in root length but reduced density and diameter. The lack of K had very little effect on the growth of the plants until day 12, as no K was supplied to the –K plants at any point this implies that K available from the seed was sufficient for normal growth up until this point. As early as day 3 the –K plants were already below the 2 % of DW critical K concentration suggested by Leigh and Wyn Jones (1984) indicating that the plants in this system were able to grow normally at least for a short time at K concentration below this. No effect was seen on the growth rate of either shoot or root tissue until the shoot tissue reached below 0.8 % K in DW indicating that this could be the critical concentration for this system, however the K concentration in the root tissue was maintained at around 1% K in DW throughout the time course carried out.   It is unlikely that growth was a factor in the changes of metabolite concentration in response to K withdrawal as with the exception of the chlorophyll concentration all changes were seen before any effect on growth.

A comparison of the results the 2009 study published by Armengaud *at al.* and the data in this chapter shows both similarities and differences in response to K-deficiency in the model plant *Arabidopsis* and barley. Both species showed a rapid decrease in K concentration, little change in water content or chlorophyll concentration and a rapid increase in hexose sugars after K withdrawal.

The Armengaud *et al.* (2009) study found that the malate concentration in the shoot tissue initially decreased slightly but then increased after day 14 in the –K plants. My results for shoot tissue also showed an initial decrease in malate concentration, and a subsequent rise was observed during the final time point, 15 days after transfer to solution. However in *Arabidopsis* root tissue, the malate concentration decreased in the –K tissue with the decrease becoming more pronounced over time. In barley a slight increase in the malate concentration in the root tissue under K stress was observed, after a transient peak at day 6 in the control plants.



	SHOOT					ROOT					LEAF No.		REGION		
	3	6	9	12	15	3	6	9	12	15	1	2	T	M	B
Length	-0.1	-0.1	-0.1	-0.1	-0.2	0.0	-0.1	0.0	0.2	0.2	-0.1	-0.2			
Weight	-0.1	-0.2	-0.2	-0.3	-0.5	0.0	-0.1	-0.2	-0.2	-0.2					
K	-0.6	-1.1	-1.5	-2.0	-2.4	-0.6	-0.8	-1.3	-1.5	-1.5	-3.4	-3.1	-1.6	-1.7	-1.5
water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Chl A	-0.1	-0.1	0.0	0.1	-0.1	N/A	N/A	N/A	N/A	N/A	-0.2	0.1	-0.4	-0.3	-0.2
Chl B	-0.1	0.0	-0.2	0.2	0.2	N/A	N/A	N/A	N/A	N/A	-0.4	0.0	-0.3	-0.1	-0.2
Glucose	0.1	0.4	1.2	0.8	0.6	0.5	0.6	1.6	1.5	1.4	-0.5	-0.2	-0.3	0.3	0.1
Fructose	0.2	0.3	1.0	0.3	0.2	0.3	0.4	1.7	1.9	1.3	0.6	0.3	0.3	0.1	0.4
Sucrose	0.0	-0.1	0.3	0.2	0.2	0.3	0.2	0.6	0.5	0.5	0.4	-0.1	0.0	0.5	0.8
Malate	-0.2	-0.3	-0.3	-0.2	0.1	0.2	-0.6	-0.2	0.5	0.2	1.1	-0.1	-0.3	-0.6	-0.5
Glutamate	-0.2	-0.7	-0.2	-0.4	-0.2	0.2	-0.3	-0.1	0.0	0.0	0.1	-0.1	-0.5	-0.3	-0.4
Protein	0.0	-0.4	0.1	0.0	0.0	0.0	0.2	0.2	0.2	0.1	0.1	0.2	0.3	0.1	-0.5
Nitrate	0.0	-0.1	-0.1	-0.3	-0.2	-0.1	-0.2	-0.2	0.2	0.3					

LN (-K/Control) -2.0 -1.0 0.0 1.0 2.0 Not measured N/A

Figure 3-11 Effects of K-deficiency on barley. Changes in metabolite concentration in barley tissue grown in -K solution. 5 plants were pooled for each sample and the mean of 3 replicate experiments are shown. Blue indicates an increase and red indicates a decrease in –K plants compared with control plants as indicated in colour bar; white indicates no change. Numbers are **the** natural logarithm of fold-changes.

Armengaud et al (2004) showed a decrease in both glutamate and nitrate concentration in the *Arabidopsis* shoot and root tissue in the K-starved plants. A decrease in nitrogen metabolism has also been observed in barley plants (Helal *et al.*, 1975), in this chapter I have confirmed this effect also occurs in the shoots grown in this system. However, little change was seen in glutamate and a slight increase in nitrate concentration was seen in the root tissue. There is no significant change in protein concentration in either the shoot or root tissue of barley, however the –K roots have constantly higher protein concentration throughout the experiment than seen in the control plants, in *Arabidopsis* plants the protein concentration in the shoot and root tissue decreased in response to K-deficiency. These changes imply different strategies in nitrogen metabolism in response to K-deficiency in the two species.

The biggest change in metabolite concentration was seen in hexose sugar concentration which in contrast to previous results published for barley (Gregory and Sen, 1937; Leigh *et al.*, 1986) was increased in both shoots and roots. It has been suggested that the increases are due to impaired long distance transport mechanisms under low K conditions (Cakmak, 1994). However, if this was the case a decrease in sugar concentration in the roots would be expected. Armengaud et al (2009) suggested that the increase may be due to impaired sugar usage in the roots leading to a build-up of sugar concentration. If the increase in shoot sugar concentration inhibits photosynthesis through negative feedback this could explain the slight decrease in chlorophyll concentration observed towards the end of the time course in the –K shoots and in the tips of the leaves. There are a variety of other processes that are effected by K-deficiency that could also influence the chlorophyll concentration in the K-starved plants, these include enzyme inhibition such as RuBisCo (Peoples and Koch, 1979). The reduction in chlorophyll concentration seen here were small but studies on older barley plants have shown much larger decreases (Helal *et al.*, 1975; Stamp and Geisler, 1980), implying that the reductions found here were the early stages of chlorophyll concentration reduction.

The transport of nitrate into and in the xylem utilises K^+ as a counter ion (Blevins *et al.*, 1978; Rufty *et al.*, 1981). The inhibition of transport of nitrate to the above ground tissue could explain the increase in nitrate concentration in the root tissue and the decrease in the shoot tissue in the later time points.

Chapter 4. Effect of K deficiency on Jasmonate levels in Barley plants

4.1. Introduction

Potassium (K) starvation has been shown to have a wide range of effects on plants including an increase in accumulation of jasmonate and its conjugates (Amtmann *et al.*, 2006; Amtmann *et al.*, 2008; Armengaud *et al.*, 2004; Armengaud *et al.*, 2010; Armengaud *et al.*, 2009; Troufflard *et al.*, 2010). Armengaud *et al.* (2004) carried out full genome microarray analysis to investigate the effect of long-term K-starvation and short-term K re-supply after starvation in *Arabidopsis* seedlings. They identified a wide range of genes that showed changes in transcript levels in response to changes in K-availability, and using iterative group analysis (Breitling *et al.*, 2004a) revealed a number of re-occurring and highly significant groups of functionally related genes that responded to K. The most prominent of the groups contained genes related to signalling and synthesis of jasmonic acid. In addition, genes involved in stress adaptation, calcium-signalling, transport and cell wall biosynthesis also responded to K status. In this chapter the regulation of JA-related gene expression in barley plants grown with different K-supply is investigated.

In *A. thaliana* a number of JA-biosynthesis genes were up-regulated during K-starvation and/or down-regulated after K re-supply (Armengaud *et al.*, 2004). These genes encoded type-2 lipoxygenase (LOX2, At3g45140), allene-oxide synthase (AOS, At5g42650), allene-oxide cyclase (AOC, At3g25760) and 12-oxo-Phytodienoic acid reductase (OPR3, At2g06050). A number of JA-responsive genes also altered transcript abundance with change in K availability. These included vegetative storage proteins (VSP1, At5g24780 and VSP2, At5g24770), JA-induced thionin (THI2.1, At1g72260) and Chlorophyllase (CORI1/CHL1, At1g19670). Changes in expression of genes involved in other JA-related processes were also seen including polyamine metabolism (AtADC2, At4g34710), glucosinolate synthesis and degradation (methythioalkylmalate synthase-like gene, At5g23020 and cytochrome-P450-dependent mono-oxygenases, CYP79B2, At4g39950 and CYP79B3, At2g22330) and defence mechanisms (polygalacturonase inhibiting proteins, aspartic proteinases, protease-inhibitors and FAD-related oxidoreductases).

Further evidence of an increase in JA in response to K-starvation was obtained when the transcript abundance of JA-biosynthesis genes for *Arabidopsis* plants grown in control and K-free media were measured using qPCR. Transcript levels of JA-biosynthesis genes *LOX2*, *AOS*, *AOC1* and *OPR3* and JA reporter genes *VSP*, *Thi2.1* and *PDF1.2* were increased in the K-deprived plants, while other lipoxygenases not involved in JA-biosynthesis, such as *LOX1* (At1g55020) and *LOX3* (At1g17420), showed no difference in transcript abundances between growth conditions (Cao *et al.*, 2006; Troufflard *et al.*, 2010). Troufflard *et al.* (2010) used liquid chromatography-mass spectrometry to measure the oxylipin abundance in the 14 day old tissue, demonstrating an increase in JA, OPDA, 9-HOD and 13-HOD in the K-deprived plants. The effects of a mutation in the COI1, an important component of JA signal recognition (Devoto and Turner, 2005; Di Cera, 2006; Xie *et al.*, 1998), on the JA increase in response to K deprivation were investigated using microarrays (Armengaud *et al.*, 2010). Comparison of transcriptional responses to K-starvation and K re-supply between wild-type and Coi1-16 *Arabidopsis* plants allowed the authors to separate COI-independent from COI1-dependent responses to K-supply. Both known and novel targets for COI1-dependent JA signalling were identified (Armengaud *et al.*, 2010). In the same study wild-type plants were found to be less damaged by thrips when grown in –K conditions than when grown in K-sufficient conditions, while *coi1* mutants had far more bites than the wild-type plant in any K condition. The authors proposed that increased JA enhanced the defence potential of the –K plants, and that this effect was at least partly dependent on JA signalling.

The microarray experiment carried out by Armengaud *et al.* (2004) provided no evidence for the involvement in K responses of any other hormones except auxin, which may be a result of (JA-dependent) altered indole-glucosinolate biosynthesis competing with auxin biosynthesis (Armengaud *et al.*, 2004). However, an increase in ethylene (ET) has been measured in *Arabidopsis* plants 6 h after K withdrawal (Shin and Schachtman, 2004) and was shown to be required for inducing the expression of the K-dependent genes such as the high-affinity K transporter HAK5 (Jung *et al.*, 2009). The effect of withdrawal of other nutrients on typical K-dependent genes such as *LOX2* and *VSP2* has also been investigated; no change was seen in *Arabidopsis* plants grown in media low in nitrogen-, phosphorus- or calcium for 14 days, indicating that the JA-response was specific for low K (Troufflard *et al.*, 2010).

The finding that low K-supply activates the JA pathway with potential downstream effects on plant defence against herbivores and pathogens could have major implications for nutrient-disease interactions in the field. It is therefore essential to find out whether the interaction between K and JA observed in Arabidopsis also occurs in crop species, in particular in non-Brassica species. This chapter presents data on JA-related gene expression in response to K-starvation of barley plants.

4.2. Selection of reference gene for qPCR analysis of K-regulated genes

In order to establish which gene to use as a reference gene for subsequent qPCRs, concentrations of three transcripts in RNA samples from plants grown in control and –K conditions were compared. Ubiquitin (*UBQ*, M60175), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*, M36650) and α-tubulin (*α-TUB*, HvU40042) were selected as possible reference genes, and their transcript levels were measured in cDNA prepared from 1µg of RNA of tissue from barley plants grown in either control or –K nutrient solution and sampled at 3, 6, 9, 12 and 15 days after transfer to the nutrient solution. All data were normalised to the day 3 control sample, to allow for differences in expression levels for between genes. In Figure 4-1A a box plot shows the spread of the measured transcript levels of the three genes. There were high levels of variability in the UBQ transcript levels compared to the *GAPDH* and *α-TUB* and therefore *UBQ* was discounted as a reference gene.

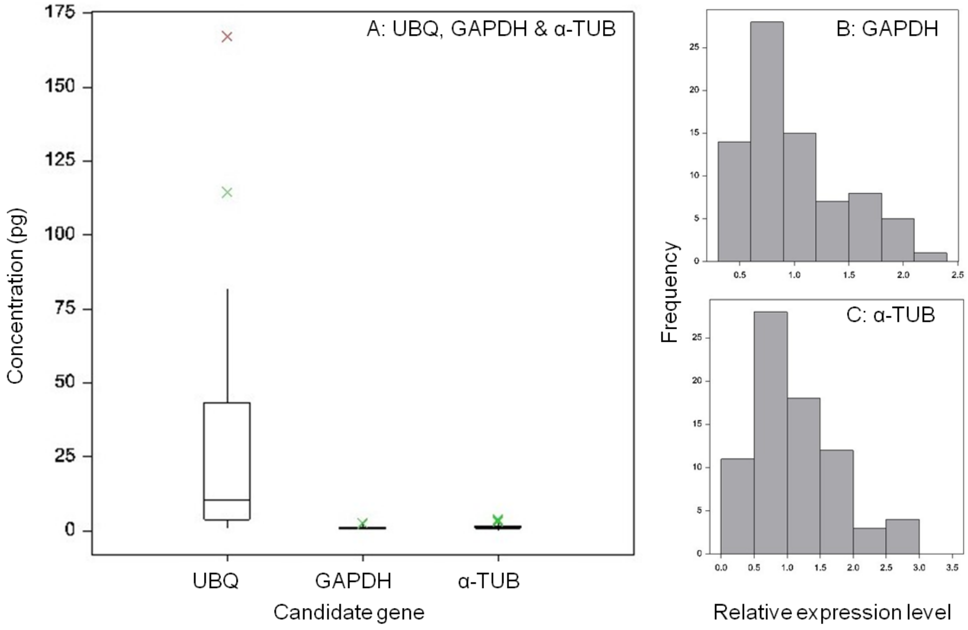


Figure 4-1 Comparison of reference transcripts for qPCR. To determine the most suitable candidate gene for use as a reference gene for subsequent qPCRs transcript levels of ubiquitin (*UBQ*), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and α-tubulin (*α-TUB*) relative to day-3 control samples, for barley plants grown in full nutrient control and –K nutrient solution for 3, 6, 9, 12 and 15 days were compared using a box plot (A). Histograms are shown for B: *GAPDH* and C*: α-TUB*. For *GAPDH* and *α-TUB* data from 3 replicates of the time course experiment (for experimental design see Chapter 2, Section 2.8.2). is shown, for *UBQ* the data for just one replicate is shown.

The transcript levels of *GAPDH* and *TUB* were more consistent across the different samples compared with the *UBQ*. To establish which of these genes should be used the frequency distribution for the same data was investigated. The *GAPDH* frequency distribution (Figure 4-1B) showed less spread in the measured transcript levels than the TUB frequency distribution (Figure 4-1C), but the data were more skewed towards the lower values. Although the data for *α-TUB* were also slightly skewed to values below one the bias was smaller than for the *GAPDH* and therefore it was decided to use α-TUB as a reference gene for further qPCRs.

4.3. Transcript levels of JA-related genes in K starved barley

4.3.1. Transcript levels of JA-biosynthesis genes in K starved barley

In order to establish if the increase in JA-biosynthesis gene expression observed in *Arabidopsis* plants under K stress also occurs in barley plants the transcript levels of JA-biosynthesis genes were measured by qPCR (Chapter 2, Section 2.4). Barley seedlings were grown in control or K-free nutrient solution and the shoots and roots from 5 plants were pooled every 3 days. Transcript levels of *LOX2* (three different genes, *LOX2:Hv2* AJ507212, *LOX2:Hv:3* AJ507213 and *LOX2.A*, AK362687.1), *AOS* (AJ250864) and *AOC* (AJ308488) in the shoot tissue were determined using qPCR. Figure 4-3 shows JA related gene transcript levels in shoot tissue over a time course of 2 weeks, normalised to *α-TUB*. The data was analysed using ANOVA (Appendix 2, Table S2-5), the data *LOX2.2*, *LOX2.3*, and *AOC* were not normally distributed and were transformed by taking the log10 before analysis, see chapter 2, section 2.8.2 for more detailed experimental design. No JA-related gene expression was seen in the root tissue (data not shown).

The transcript levels for three *LOX2* genes were analysed. All three sequences have higher similarity to the Arabidopsis *LOX2* gene than to any other Arabidopsis genes encoding lipoxygenases. *LOX2.2* and *LOX2.3* were identified by Bachmann *et al.* (2002) as *LOX2* genes and shown to be responsive to JA treatment. A third *LOX2* gene was identified during the course of this project (see Chapter 6), and BLAST searches showed its closest homolog to be the rice *LOX2* gene and its closest homolog in *Arabidopsis* to be *LOX2*. A phylogenetic tree was produced using CLC main work bench software (CLC bio) to compare the similarity of the three barley *LOX2* genes with *LOX2* genes from other plants including Arabidopsis and rice (Figure 4-2).

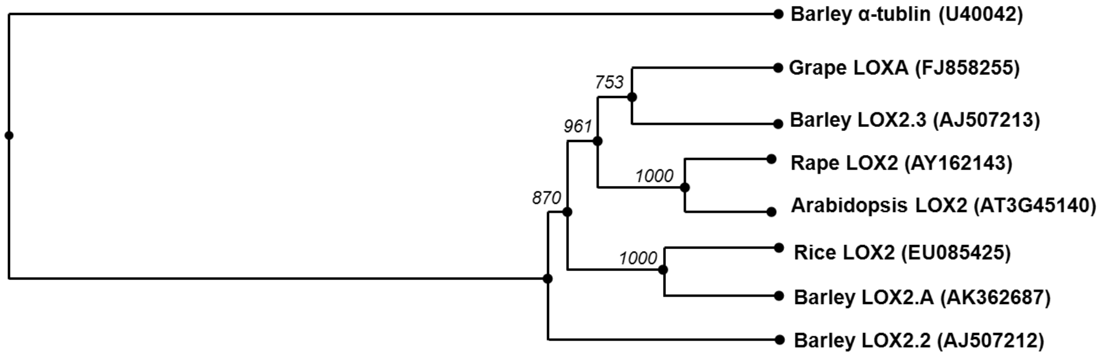


Figure 4-2 Tree showing differences between LOX2 genes. The relationship between LOX2.A, previously identifed barley LOX2 genes and LOX genes from other species is shown. Locus identifiers are shown in barackets and bootstrap values in italics.

The ANOVA showed a significant interaction between the effect of K-availability and time on *LOX2.2* transcript level (p<0.001). The *LOX2.2* transcript level in the control plants decreased over time, such that by day 12 the *LOX2.2* level was 13 % of that on day 3. The *LOX2.2* transcript level in the K-starved plants remained stable throughout the experiment. Little difference was seen between the *LOX2.2* transcript levels in control and –K plants before day 12. On days 12 and 15 *LOX2.2* expression in –K plants was considerably (9 and 10-fold respectively) higher than that in control samples (Figure 4-3).

No significant interaction between the effect of K-availability and time on *LOX2.3* transcript level (p=0.350) and no significant change was seen in response to K supply (p=0.467). *LOX2.3* expression increased slightly over time in both control and –K plants. Although there appeared to be a slightly higher level of *LOX2.3* expression in the –K samples throughout the experiment, this was not statistically significant.

No significant interaction between the effect of K-availability and time on *LOX2.A* transcript level (p=0.052) and no significant change was seen in response to K supply (p=0.070). Control plants showed a slight drop in *LOX2.A* expression over time. In the –K samples *LOX2.A* transcript levels increased significantly over time, doubling between day 3 and day 15. No difference in *LOX2.A* expression was seen between the control and –K samples until day 9, when the *LOX2.A* transcript levels were two fold higher in the –K plants, a significant difference (p=0.027). By day 15 the *LOX2.A* transcript levels were 4 fold higher in the –K plants compared to the control at the same time point, a highly significant difference (p=0.003).

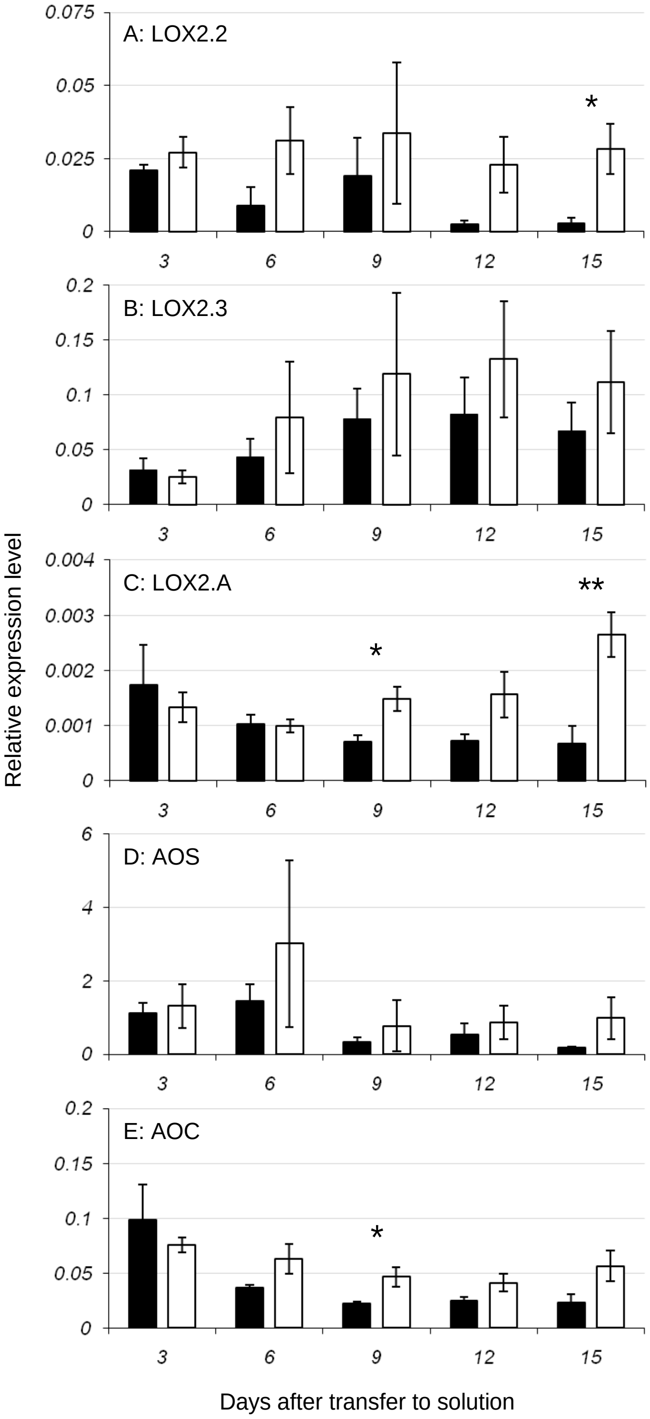


Figure 4-3. Transcript levels of JA-biosynthesis genes in barley shoot tissue. Transcript levels of genes encoding lipoxygenase 2 genes (A: *LOX2.2*, B; *LOX2.3*, and C: *LOX2.A*), allene oxidase synthase (D: *AOS*) and allene oxidase cyclase (E: *AOC*) relative to α-tubulin in shoot tissue from plants grown in control (■) and -K (□) media. Five plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution, the mean (± SE) of data from four independently grown and treated batches of plants are shown (for experimental design and statistical analysis see Chapter 2, Sections 2.1.2 and 2.8.2). Significant differences between control and –K samples at a 5 % (*) and 0.5 % (**) confidence interval are marked with asterisks.

No significant interaction between the effect of K-availability and time on *AOS* transcript level was seen (p=0.749). The control plants showed a highly significant decrease in *AOS* expression over time (p=0.001); by day 15 the *AOS* expression in the control plants was 15 % of that on day 3. No significant difference was seen in the *AOS* expression between time points in the –K samples (p=0.293), however, a peak in *AOS* expression was observed in two of the four replicates on day 6. Although the *AOS* transcript level was higher in the –K samples compared with the control samples after day 3, there was no significant difference between the samples either throughout the experiment (p=0.430). .

No significant interaction between the effect of K-availability and time on *AOC* transcript level (p=0.157). The *AOC* transcript levels were significantly higher in the K starved plants than in the control plants (p=0.007). Higher levels of *AOC* transcript in –K plants compared to the control plants were apparent as early as day 6, when the –K plants *AOC* level was double that of the control plants. The *AOC* transcript levels in the –K plants remained higher than in the control thoughtout experiment. A significant change in AOC transcript levels over time was also demonstrated (p<0.001). A significant decrease in *AOC* transcript level in control plants was observed, the majority of this increase was seen during the early time points, by day 6 the *AOC* transcript levels had dropped to 37 % of that observed on day 3. No significant change was seen in *AOC* transcript levels in the –K plants.

4.3.2. Transcript levels of 'JA-induced' genes in K starved barley

In order to confirm the increase in JA-related gene expression in the K-deprived plants the transcript levels of three so-call 'JA-induced proteins' (JIPs) were measured, *JIP23* (BM816519), *JIP37* (X82937) and *JIP60* (BM815987). Both protein and transcript levels of JIPs have been shown to be increased in response to JA (Andresen *et al.*, 1992; Chaudhry *et al.*, 1994; Weidhase *et al.*, 1987). Many are of unknown function, and they are therefore named according to their size (e.g. JIP23 is a M_r 23,000 peptide). Barley seedlings were grown in control or K-free nutrient solution and the shoots from 5 plants were sampled every 3 days. The transcript levels of *JIP23*, *JIP37* and *JIP60* were measured using qPCR. Figure 4-4 shows JA induced gene transcript levels in shoot tissue over a time course of 2 weeks, normalised to *α-TUB*. The data was analysed using ANOVA (Appendix 2, Table S2-5), as the data was not normally distributed it was transformed by taking the log10 before analysis, see chapter 2, section 2.8.2 for more detailed experimental design.

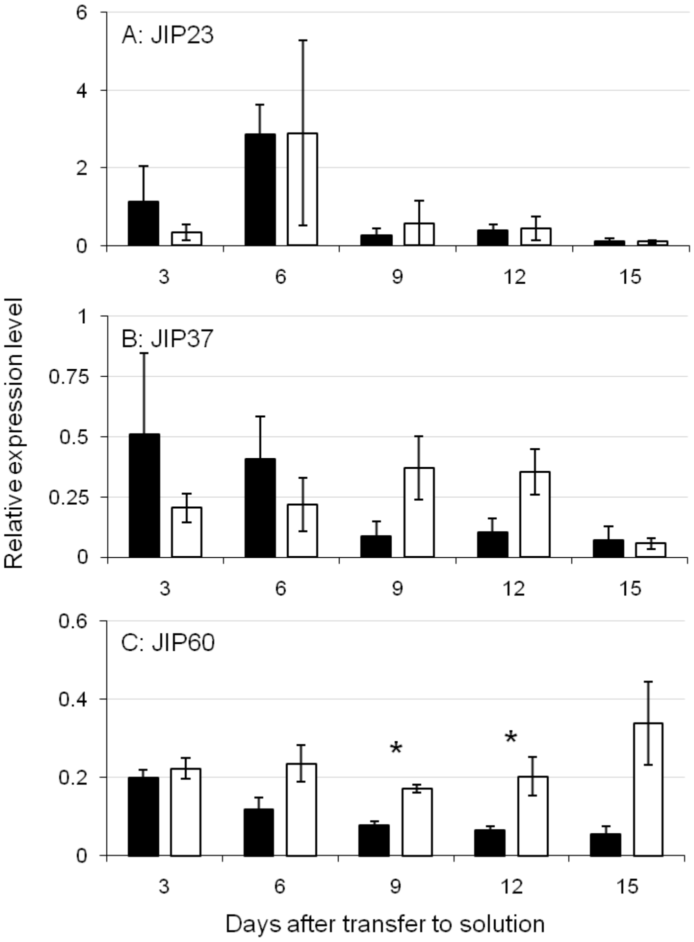


Figure 4-4. JA-induced gene transcript levels in barley shoot tissue. Transcript levels of genes encoding Jasmonate Induced Proteins A: *JIP23*, B: *JIP37* and C: *JIP60*, relative to α-tubulin in shoot tissue from plants grown in control (■) and -K (□) media. Five plants were pooled 3, 6, 9, 12 and 15 days after transfer to nutrient solution, the mean (± SE) of data from four independently grown and treated batches of plants are shown (for detail of experimental design and statistical analysis see Chapter 2, Sections 2.1.2 and 2.8.2). Significant differences between control and –K samples at a 5 % (*) and 0.5 % (**) confidence interval are marked with asterisks.

No significant interaction between the effect of K-availability and time on *JIP23* transcript level was seen (p=0.770) and there was no significant difference between the control and –K samples (p=0.784). There was little change in the *JIP23* transcript levels in either the control or –K samples over time, except a peak that was seen in some but not all replicates at day 6 for both treatments.

No significant interaction between the effect of K-availability and time on *JIP37* transcript level was seen (p=0.0.087) By day 9 the *JIP37* transcript level was higher in the –K plants compared with the control plants, and remained higher at day 12 however no significant difference in *JIP37* transcript level were observed (p=0.509). By day 15 the *JIP37* transcript levels had dropped to the level seen in the control plants. A significant change in *JIP37* transcript level was observed over time (p<0.001).

A significant interaction between the effect of K-availability and time on *JIP60* transcript level was seen (p=0.0.004). As early as day 6 the *JIP60* transcript levels in –K plants were higher than in the control plants. By day 9 the *JIP60* transcript levels in the –K plants were double those in the control plants at the same time point, There was a drop in *JIP60* expression in control plants over time; by day 15 the *JIP60* transcript level was 27 % of that at day 3. By contrast, there was little change in the *JIP60* level over time in –K plants.

4.3.3. Hormone levels in K starved barley

To assess the effects of K deficiency on the concentration of JA and other plant stress hormones the levels of JA, ABA, SA and SA-glycoside were determined spectrophotometrically by the Metabolomics and Proteomics Mass Spectrometry Facility at Exeter University. Three replicates of freeze dried samples collected after 3, 9 and 15 days growth in control or –K nutrient solution were analysed. Figure 4-5 shows Hormone concentration in shoot tissue over a time course of 2 weeks. The data was analysed using ANOVA (Appendix 2, Table S2-5), see chapter 2, section 2.8.2 for more detailed experimental design. However, no results were obtained for JA in some replicates, making statistical analysis of the data impossible, the results that were obtained are shown in Figure 4-5A.

The JA levels in plants grown in both control and –K solutions decreased between days 3 and 9, but then increased by day 15 (Figure 4-5A). The samples collected 3 and 9 days after transfer to nutrient solution showed similar JA levels in the control and –K plants. However, by day 15 the JA levels in the –K plants were higher than that seen in the control plants, with the JA levels in the –K plants increasing by 44 % of the control plant concentration.

No significant interaction between the effect of K-availability and time on *ABA* concentration was seen (p=0.0.198) and there was no significant difference between the control and –K samples (p=0.091). Initially there was no difference in the ABA levels between the plants grown in the two treatments, but by day 9 the ABA levels in the –K plants were double those in the control plants, a significant difference (p=0.033). The higher levels in the –K plants seem to be due to the lack of the reduction in ABA levels over time seen in the control plants rather than a real increase. The ABA concentration in the control plants declined significantly over time (p=0.007), with the levels in the day 15 shoot samples only 47 % of those in the day 3 samples (Figure 4-5B). The –K samples showed no significant change in ABA levels over time (p= 0.802).

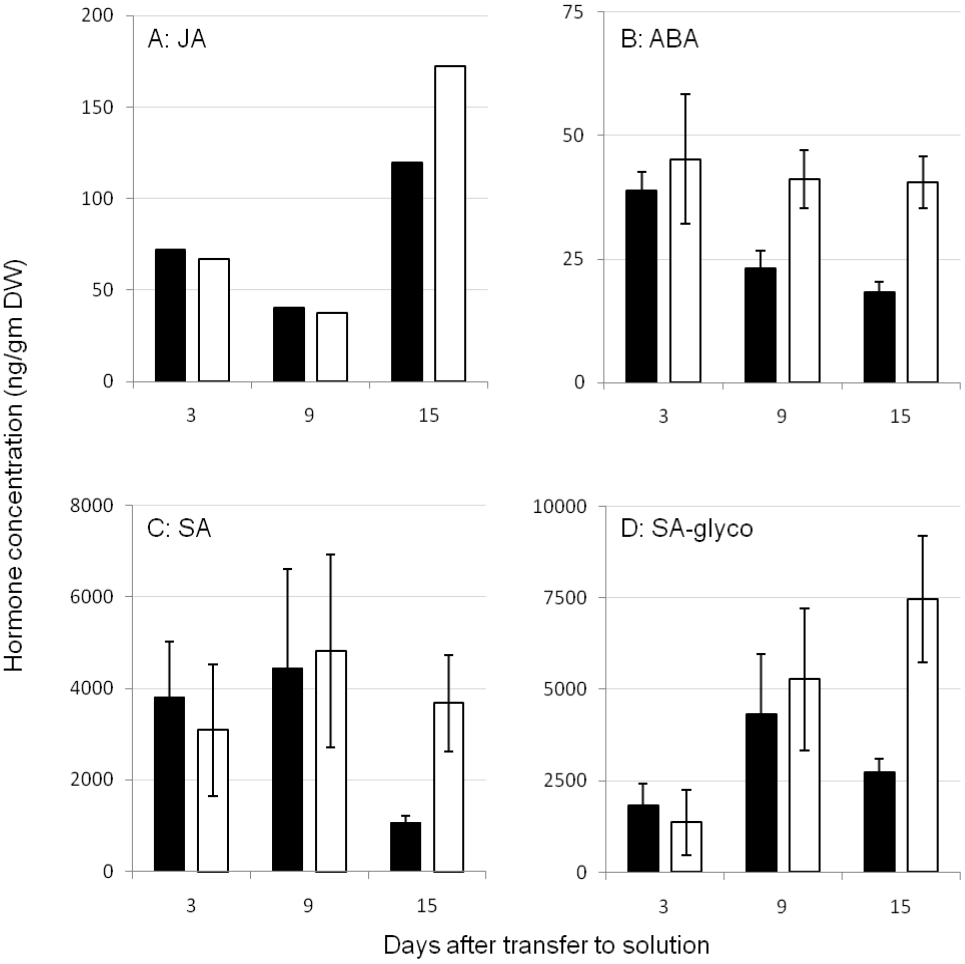


Figure 4-5. Hormone levels in barley shoot tissue. Levels of A: JA, B: ABA, C:SA and D SA-glycoside in shoot tissue from plants grown in control (■) and -K (□) media. Five plants were pooled 3, 9 and 15 days after transfer to nutrient solution and the K concentration determined, the mean (± SE) of data from three independently grown and treated plant batches is shown (for detail of experimental design and statistical analysis see Chapter 2, Sections 2.5 and 2.8.2), except for JA for which only one sample or the mean of two replicate samples are shown.

No significant interaction between the effect of K-availability and time on *SA* concentration was seen (p=0.389) and there was no significant difference between the control and –K samples (p=0.412). On days 3 and 9 the SA levels in the plants showed no difference between treatments (Figure 4-5C). On day 15, however, the –K plants had higher SA levels than the control plants at the same time point, however, this difference was not significant (p=0.153). There was no significant change in SA levels over the course of the experiment in the plants grown in either control or –K solution (p=0.247 and p= 0.550 respectively).

No significant interaction between the effect of K-availability and time on *SA* concentration was seen (p=0.116) and there was no significant difference between the control and –K samples (p=0.159). Over early time points an increase in SA-glyco was observed for both the control and –K plants, however, between day 9 and 15 the SA-glyco levels in the control plans decreased, while the levels in the –K samples continued to increase (Figure 4-5D). On days 3 and 9 there was little difference between control and –K plants (p=0.693 and p=0.402 respectively), but on day 15 the –K plants contained 3 times more SA-glyco than the control plants, however, this difference was not significant (p=0.142).

4.3.4. JA-related gene expression in different leaves of K starved barley plants

To establish whether the increase in JA-related gene expression observed in K-starved plants occurs in both the first and second leaves, barley plants were grown in control or K-free nutrient solution and the first and second leaves were sampled after 14 days (Chapter 2, Section 2.1.4). The expression levels of three *LOX2* genes, *AOS* and *AOC* and JA-induced genes *JIP23, JIP37* and *JIP60* were measured using qPCR and compared to that of α-tubulin (Chapter 2, Section 2.4). By day 14 the first leaf was fully expanded, and K withdrawal had little effect on the length of the leaf, while the second leaf was just reaching full length and there was significant reduction in growth in the K-deficient plants (Chapter 3). It is also worth noting that at this time point both leaves had a large reduction in K concentration in the plants grown in K-free media, and that the K levels in the second leaves were slightly higher than in the first leaves for both growth conditions. Only 2 replicates were carried out for this experiment, both replicates are shown for each condition (Figure 4-6). Due to low number of replicates not statistical analysis was carried out.

There was no difference in transcript level of *LOX2.2* between leaves for the control plants in the first replicate, but the second replicate showed lower levels in the second leaf. There seemed to be slightly less *LOX2.2* transcript level in the second leaf compared with the first leaf in the –K plants in both replicates. There was slightly more in *LOX2.2* transcript levels in the first and second leaves from –K plants compared to the control leaves in both replicates. This was prominent in the first leaves, where the percentage of K in dry weight was lower.

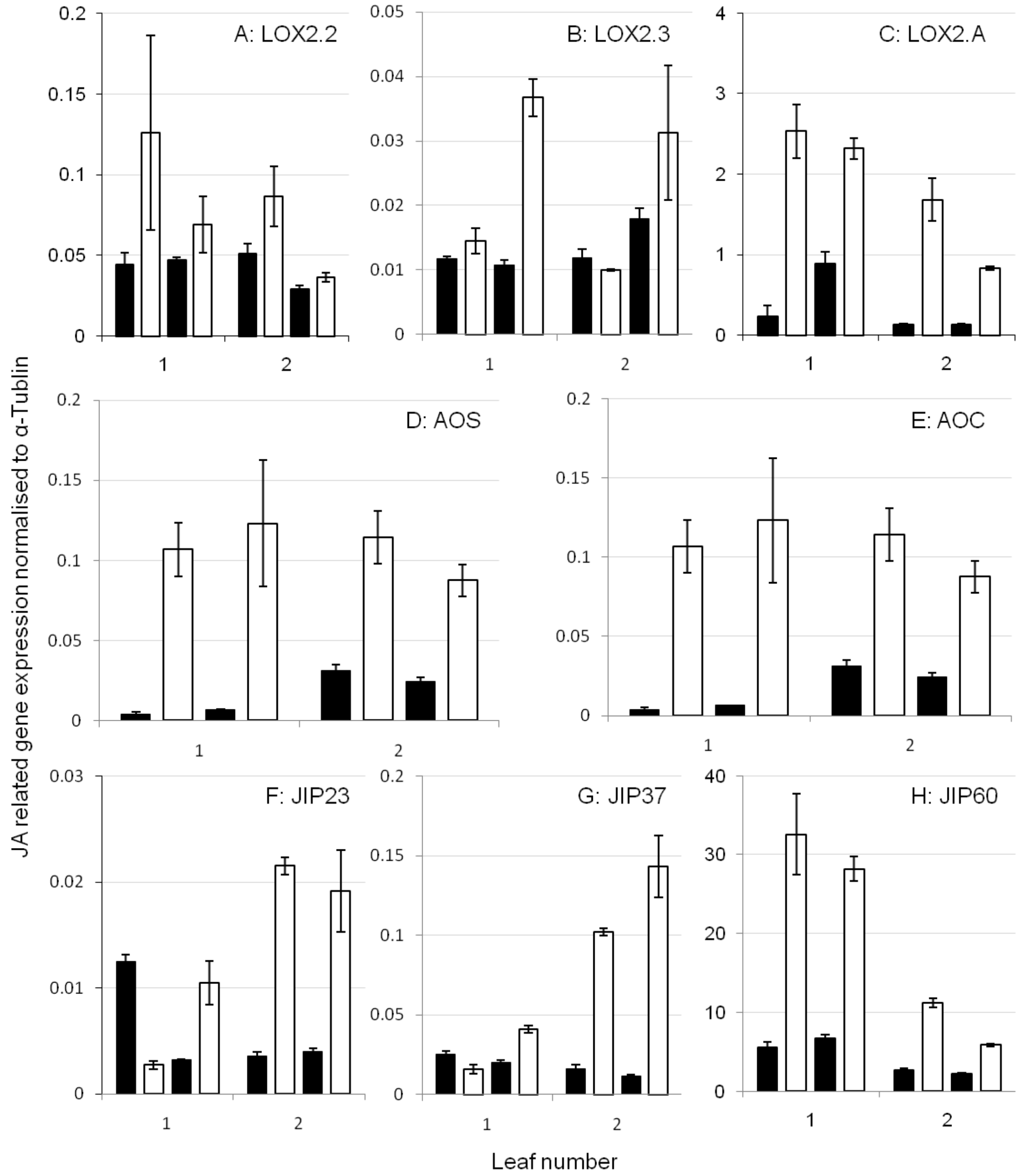


Figure 4-6. Transcript levels of JA-related genes in first and second leaves of barley. Transcript levels of JA-biosynthesis genes (A: *LOX2.2*, B: *LOX2.3*, C: *LOX2.A*, D: *AOS* and E: *AOC*) and JA-induced genes (F: *JIP23*, G: *JIP37* and H: *JIP60*) relative to α-tubulin in the first (1) and second (2) leaf from plants grown in control (■) and -K (□) media. 1st and 2nd leaf tissue from five plants grown in control or –K nutrient solution were pooled for each sample (Chapter 2, Section 2.1.4). Data from two independently grown and treated plant batches of plants are shown. Error bars represent the mean (± SE) of technical replicates (Chapter 2, Section 2.4.7).

The *LOX2.3* transcript levels were equal in the first and second leaves in the first replicate for both control and –K plants. But the second replicate showed higher *LOX2.3* expression in the second leaf of control plants compared to the first leaf. Little difference in *LOX2.3* expression was seen between leaves in the –K samples. In the second replicate, the plants suffering from K-starvation had more *LOX2.3* transcript in the first leaves than the control, however, this was not seen in the first replicate. There was no difference in *LOX2.3* expression between treatments in the second leaf samples.

The *LOX2.A* transcript levels were lower in the second leaves than in the first leaves for both growth conditions. The expression of *LOX2.A* was greater in both the first and second leaves of the –K plants compared to the control plants. The *LOX2.A* expression was highest in the second leaves where a 6-fold increase was seen in the mean value for –K plants over the control compared with a 3-fold increase in the first leaf samples.

The mean *AOS* transcript level was five times higher in the second leaf than the first for the plants grown in control solution (Figure 4-6D). The plants grown in K-free solution showed little difference in *AOS* expression between leaves. *AOS* transcript level was 21 fold greater in –K leaves compared to the control leaves. There was also an increase in mean *AOS* transcript level in the –K second leaves compared to the control second leaves but with a 6-fold increase this was much smaller than in the first leaves

In the control plants the *AOC* transcript level in the second leaf was slightly higher than that in the first leaf in the first replicate, but this was not repeated in the second replicate. Interestingly, in the –K plants the first replicate showed a higher level of *AOC* expression in the second leaf than in the first, but this was reversed in the second replicate. An increase in *AOC* transcript level was seen in both leaves under K stress compared to the control, with a mean increase of 4-fold in the first leaf and 3-fold in the second leaf in –K plants compared with the control plants.

There was a lot of variation in *JIP23* transcript levels for all the samples (Figure 4-6F), therefore no conclusions can be drawn from these data.

The *JIP37* transcript levels in the first leaves were slightly higher than in the second leaves for the control plants, however, in the –K plants the mean *JIP37* transcript levels were 4-fold higher in the second leaves than in the first leaves (Figure 4-6G). There was no change in the *JIP37* transcript level between growth conditions in the first leaves, but in the second leaves the mean *JIP37* levels were 9 times higher in the leaves of –K plants than in the leaves of control plants.

The transcript level of *JIP60* in the second leaves was considerably lower than that observed in the first leaf in both control and K-deficient plants (Figure 4-6H). The *JIP60* transcript levels were higher in leaves of plants grown in K-free media than in leaves of the control plants. This difference was more pronounced in the first leaf where a 5- fold difference was observed in the mean abundance of *JIP60* transcripts, while in the second leaves a 4-fold difference was observed.

4.3.5. Expression of JA-related genes in different regions of leaves of K starved barley plants

In order to establish if the K-dependent changes in JA-related gene expression were evenly distributed throughout the leaf, the abundance of transcripts of JA-related genes were quantified in samples from different areas of the second leaf (Figure 4-7). Barley seedlings were grown for 14 days in control and K-free nutrient solutions and 4 cm sections were taken from the tip, middle and base of the second leaf. The transcript levels of JA-biosynthesis genes *LOX2.3*, *LOX2.A*, *AOS* and *AOC* and JA-induced genes *JIP23*, *JIP37* and *JIP60* were quantified using qPCR. Figure 4-7 shows JA related gene transcript levels tip, middle and base segments, normalised to *α-TUB*. The data was analysed using ANOVA (Appendix 2, Table S2-5), the data for *AOS*, *AOC*, *JIP23* and *JIP37* were not normally distributed and were transformed by taking the log10 before analysis, see chapter 2, section 2.8.3 for more detailed experimental design. No significant interaction between the effect of K-availability and leaf region on the transcript levels of any JA related gene measured was observed (p=0.142).

There was no significant difference between the *LOX2.3* transcript abundance in leaves from the control and –K plants (p=0.466). There was however a significant reduction in *LOX2.3* expression from tip to base (p=0.005). The expression of *LOX2.3* in leaves of the control plants decreased from tip to base (Figure 4-7A, p=0.055); the middle segment had 51 % of the transcript abundance measured in the tip segment, and the base segment had 55 % of the transcript abundance seen in the middle segment. There was little change in *LOX2.3* transcript levels between the tip and middle segments in the –K plants, but the base segment had much lower *LOX2.3* expression with a transcript level 35 % of the tip segment and 31 % of the middle segment (Figure 4-7B).

In all segments there were higher levels of *LOX2.A* transcript in the –K plants compared to the control plants (Figure 4-7A), however, this was not significant (p=0.087). The difference increased from tip to base, in the tip 2 fold greater *LOX2.A* expression was observed, while the middle and base segments 4-fold and 7-fold greater *LOX2.A* expression was observed, respectively, than in control plants. There was a significant reduction in LOX2.3 expression from tip to base to the leaf (p=0.002). No significant change in *LOX2.A* expression was observed in the control plants from base to tip (p=0.073). However, a significant decrease was seen in the transcript abundance of *LOX2.A* from the tip to the base of the -K leaves (p=0.025), the *LOX2.A* transcript level in the middle segment was 53 % and base segment 38 % of the level in the tip segments.

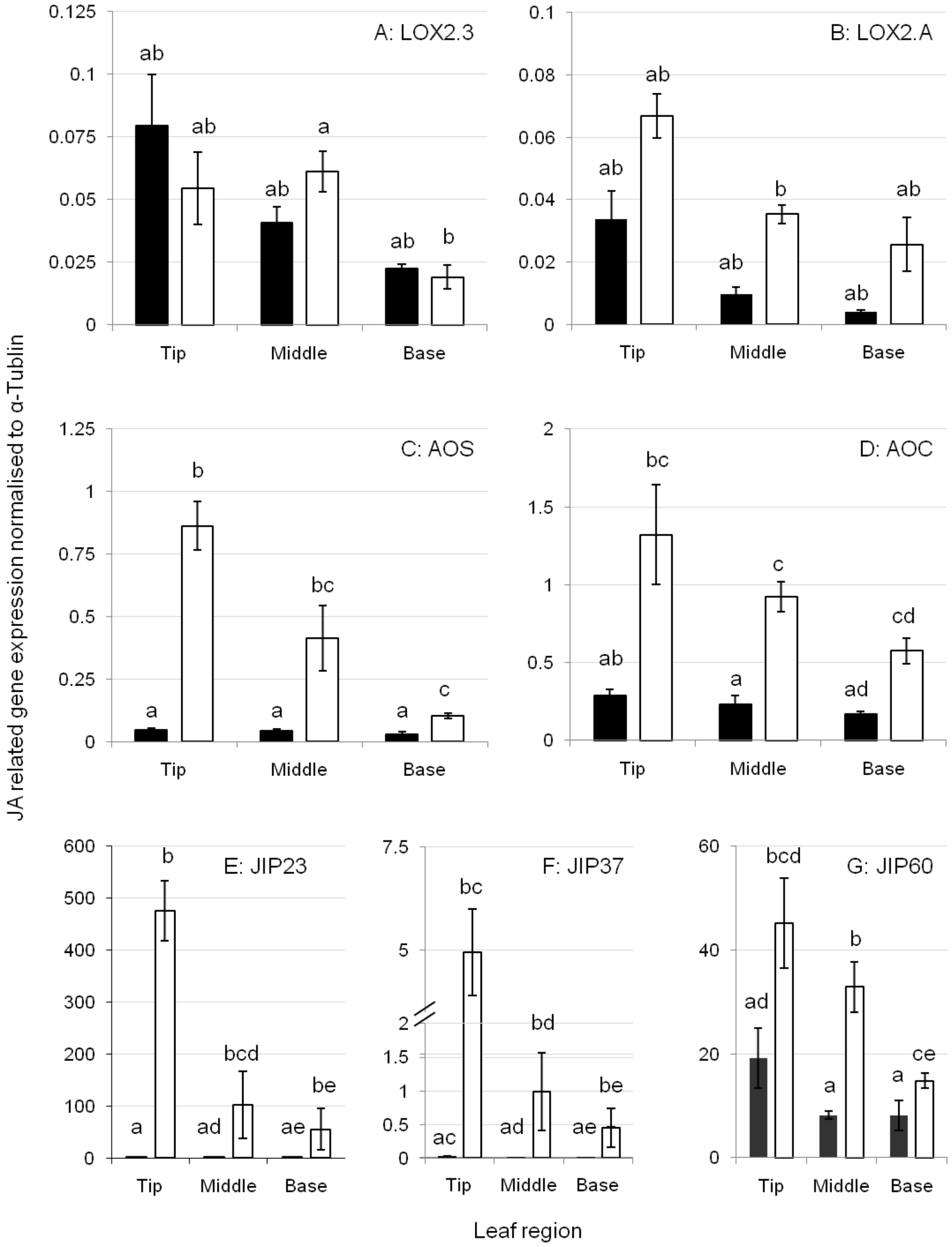


Figure 4-7 Transcript levels of JA-related genes in tip middle and base regions of barley second leaves. Transcript levels of JA-biosynthesis genes (A: *LOX2.3*, B: *LOX2.A*,C: *AOS* and D: *AOC*) and JA-induced genes (E*: JIP23*, F: *JIP37* and G: *JIP60*) relative to α-tubulin in tip, middle and base regions of second leaf tissue from plants grown in control (■) and -K (□) media. Six leaf segments were pooled and the transcript levels of JA related genes determined using qPCR, the mean (± SE) of data from three independently grown and treated plants batches is shown (for detail of experimental design and statistical analysis see Chapter 2, Sections 2.1.5 and 2.8.3).

In all segments greater *AOS* transcript abundances were seen in the –K segments compared to the control plants (Figure 4-7C, p<0.001). An increase in *AOS* expression in response to K-starvation was measured in the tip, middle and base segments where an 18-fold, 10-fold and 3-fold increases were observed respectively. A significant change in *AOS* transcript levels was also seen across the leaf (p=0.004). The *AOS* transcript level in the control samples did not change across the leaf. The *AOS* expression in the –K leaves were reduced from the tip to the base of the leaf. Expression of *AOS* in the middle segments were 48 % and in the base segments 12 % of that in the tip segments.

Greater *AOC* transcript abundance was seen in the leaves from plants grown in K-free solution (p<0.001). The increase in *AOC* transcript levels in the –K leaves over control leaves was 5-fold in the tip segments, 4-fold in the middle segments was and 3-fold in the base segments. A significant reduction in AOC transcript level from the tip to the base of the leaf was observed (p=0.044).

A large increase in *JIP23* transcript abundance was seen in the leaves from the –K plants compared to the control plants (p<0.001), with the tips demonstrating an increase of 867-fold in the –K plants compared to the control plants. A smaller but still substantial increase was seen the middle and base segments with 215-fold and 184-fold increases in *JIP23* levels compared to the corresponding control samples. A decrease in *JIP23* transcript levels was seen from tip to base of the leaves (p=0.035), the *JIP23* expression in the base segments prepared from control plants was only 55 % of that in the tip. The –K segments showed larger decrease in *JIP23* expression level from the tip to base of the leaves, with the middle segments containing 22 % and the base 12 % the *JIP23* transcript levels in the tip segments.

JIP37 showed a statistically significant increase in transcript levels in the –K plants compared with the control plants for all segments (p<0.001). The *JIP37* transcript levels were increased 211-fold in the tip, 61-fold in the middle and 98-fold in the base segments compared to the corresponding control segments. A reduction in *JIP37* level was observed from the tip to the base of the leaves (p=0.005). This was most evident in the –K plants were the *JIP37* transcript in the base segments was only 9 % of that in the tip segments (p=0.057).

All the segments showed an increase in *JIP60* transcript levels in the –K leaves compared with the control leaves (p=0.092). The increase was smaller than observed for the other JIPs, with the tips and base showing a 2-fold, the middle segments a 4-fold increase in expression compared to the control samples. A significant change in *JIP60* transcript levels was observed across the leaf (p=0.005). The *JIP60* transcript levels did not change significantly over the length of the control leaf (p=0.193). However, the leaf segments from the plants grown in K-free solution showed a significant reduction in *JIP60* transcript levels from the tip to the base to the leaf segments (P=0.027), with the middle segment demonstrating 73 % and the base segment 33 % of the transcript levels in the tip segment.

4.4. JA-related gene expression in barley under other nutrient stress

To establish if the increase in some JA-related gene expression observed for K-deficiency is also seen for deficiencies of other nutrients, barley plants were grown in nutrient solutions lacking other nutrients. After germination plants were grown for 10 days in the control, K-free, low-Ca, N-free, P-free and S-free nutrient solution before the shoot tissue was harvested (Chapter 2, Section 2.1.1). qPCR was used to determine the transcript levels of JA-related genes in each sample (Figure 4-8). Only two replicates were completed for this experiment and the results for both replicates are shown. Due to low number of replicates no statistical analysis was carried out.

The mean *LOX2.3* transcript level in the –K plants was 52 % higher than that seen in the control plants. A slight increase in the mean *LOX2.3* transcript levels was also seen in the –Ca (14 %), -P (12 %) and –S (4 %) plants after 10 days compared to the control plants. *LOX2.3* expression in the plants grown in the –N nutrient solution was reduced to less than half that seen in the control plants in the first replicate, however, no change in expression was seen in the second replicate compared with the control plants.

In the first replicate there was very little change in *LOX2.A* expression with the different growth conditions. In the second replicate, the *LOX2.A* expression level in the –K plants was 2-fold higher than that seen in the control plants, after 10 days growth in the nutrient solutions. An increase in *LOX2.A* expression was also seen in plants grown in solutions lacking other nutrients. The biggest increase was seen in the –N plants which had *LOX2.A* levels elevated by 4-fold compared to the control plants. As with *LOX2.3*, the lowest increase was seen in the –S plants, where the *LOX2.A* transcript level increased by 12 % over that of the control samples.

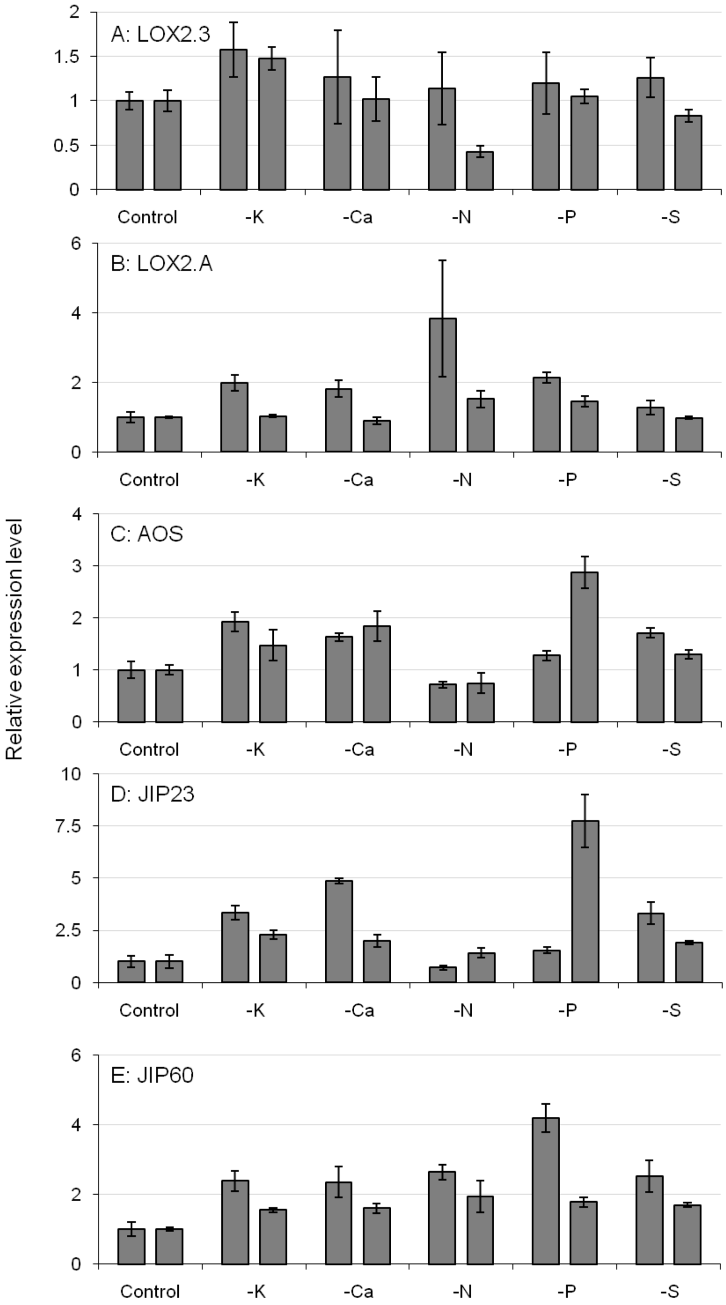


Figure 4-8 Transcript levels of JA-related genes under deficiency for different mineral nutrients. Transcript levels of JA-biosynthesis genes (A: *LOX2.3*, B: *LOX2.A* and C: *AOS*) and JA-induced genes (D: *JIP23* and E: *JIP60*) relative to α-tubulin in shoot tissue from plants grown in control, K-free (-K), low Ca (–Ca), N-Free (-N), P-free (-P) and S-free (-S) nutrient solution for 10 days (Chapter 2, Section 2.1.1). 8 plants were pooled for each time point, the data from two independently grown and treated plants batches are shown. The data was normalised to the control values, error bars represent the mean (± SE) of technical replicates (Chapter 2. Section 2.4).

The mean *AOS* transcript level in the plants grown in K-free solution was increased 2-fold compared to the plants grown in control solution. The mean levels of *AOS* transcript were also increased 2-fold in the –Ca and –S when compared to the control. The withdrawal of P lead to a 3 fold increase in *AOS* in the first replicate, but only a slight increase (27 %) in the second replicate. The plants grown in the N-free solution showed a reduced mean *AOS* transcript level, reaching only 73 % of that observed in the control plants.

The transcript levels of *JIP23* also showed an increase in the plants grown in the K-free solution when compared to the control, resulting in a mean *JIP23* transcript level in the –K plants double that of the control plants. An increase in *JIP23* levels was also seen in the –Ca, -P and –S plants, however the level of this increase varied a lot between replicates. As with the *AOS* expression levels the *JIP23* level in P starved tissue was much higher in the first replicate than the second with 8-fold and 2-fold increases respectively. Very little changes in *JIP23* transcript levels were seen in the plants grown in the N-free solution compared to the control plants.

The *JIP60* transcript levels in the –K plants were double that observed in the control plants. A similar increase in mean *JIP60* expression was seen in the low Ca, -N and –S plants (approximately 2-fold) compared to the control plants. The plants grown in solution lacking in P also showed an increase in *JIP60* levels, but once again the magnitude of this increase varied between replicates, with a 2-fold and 4-fold increases in the first and second replicates respectively.

4.5. JA-related gene expression in barley after re-supply of K

To establish if the increase in JA-related gene expression in plants deprived of K was reversible, plants were grown for 10 days in K-free nutrient solution before being transferred to full nutrient control solution, alongside full nutrient and –K controls. The shoot tissue was harvested at different time points (0, 1, 2 and 5 days) after re-supply of K (See chapter 2, Sections 2.1.8 and 2.8.4). The K concentration (Figure 4-9) and the JA-related gene expression (Figure 4-10) were determined. All samples were taken at the same time of the day. Data from K-resupplied plants were compared to those from plants grown in full nutrient control throughout the entire experiment (control) and from plants remaining in K-free nutrient solution (-K). The data obtained was analysed using ANOVA (Appendix 2, Table S2-5), as the results for the JA related gene transcript levels were not normally distributed and therefore was transformed by taking the log10 before analysis, see chapter 2, section 2.8.4 for more detailed experimental design.

The ANOVA showed a significant interaction between the effect of K availability and time on the K concentration in shoots ($p<0.001$). The control shoot samples contained around 6.5 % K in dry weight on the day of re-supply and no significant change was seen in K levels at later time points (p=0.612). Before re-supply the –K shoot samples contained around 1 % K in dry weight which was 14 % of that seen in the control samples, a highly significant difference (p<0.001). There was no significant change in K levels in the -K plants over the period of this experiment (p=0.208). The K levels in the control plants were significantly higher during the course of the experiment (p<0.001). The shoot tissue from K-resupplied plants still contained low levels of K 24 hours after re-supply and the concentration in dry weight was not significantly different to that seen in the –K plants (p=0.835) and still significantly lower than in the control plants (p=0.025). By day 2 after K re-supply, shoot K levels in the resupplied plants had increased to 3 times that of the –K plants. However, it was still only 63 % of that seen in the control plants (Figure 4-9A). Although there was still no significant difference between the resupplied and –K plants (p=0.088), this was probably due to variation in K levels in the –K plants. Between day 2 and day 5 a small additional increase in shoot K concentration was observed for the K-resupplied plants. At the end of the experiment, 5 days after K-resupply, shoot K-concentrations in K-resupplied plants were significantly higher than in the –K plants (p=0.002) but still significantly lower than in shoots of the control plants (p=0.004).

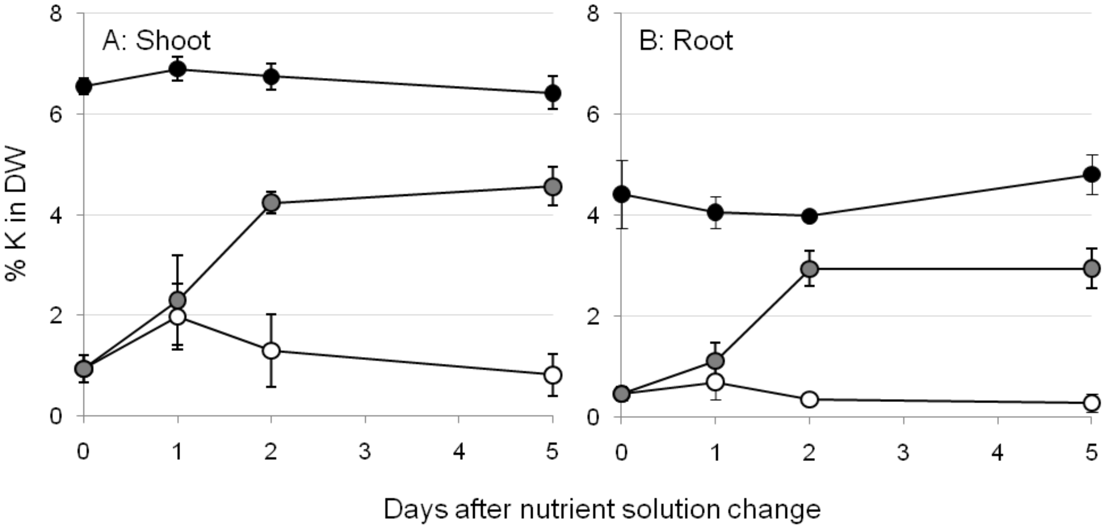


Figure 4-9 K-levels in barley after K re-supply. The K concentration was determined in A: shoot and B: root tissue from plants grown in control medium (●), -K medium (○) or –K medium for 10 days before transfer to control medium at time point 0 (●). Five plants were pooled 0, 1, 2 and 5 days after K was resupplied and the K concentration determined (Chapter 2, Section 2.2), the mean (± SE) of data from three independently grown and treated batches of plants is shown (for experimental design and statistical analysis see Chapter 2, Sections 2.1.8 and 2.8.4).

A significant interaction between the effect of K availability and time on the K concentration in roots ($p<0.001$) was also seen. Although the overall K concentration was lower in the root tissue than in the shoot tissue, similar kinetics of K accumulation after K re-supply were observed in both tissues. There was no significant variation in root K levels during the experiment in control ($p=0.367$) or –K ($p=0.340$) plants. The root K concentration of the K-resupplied plants increased significantly over the course of the experiment ($p=0.006$). 24 hours after K-re-supply the root K level in the resupplied plants was only slightly higher than in the –K plants, but by day 2 it had reached 8.6 times that of the –K plants. The K concentration in the tissue resupplied with K was 27 % of the control K concentration on day 1, this had risen to 74 % by day 2 but had not increased further by day 5; at all time points measured the K level in the control plants were significantly higher than in the resupplied plants.

In both the shoot and root the K concentration of the K-resupplied plants did not increase further after day 2 implying that at this stage net K uptake and growth had reached a new balance.

The *LOX2.3* transcript showed a high variation making it difficult to draw conclusions from the data (Figure 4-10A). The response of transcript abundance to K-resupply for *LOX2.A*, *AOS* and *JIP60* were very similar (Figure 4-10B, C, E). At all time points the –K plants had higher transcript levels than the control plants, although large variation between batches of –K grown plants reduced the significance of this difference, particularly on days 2, 3 and 5. Transcript levels of K-resupplied plants were still similar to those of –K plants 24 hours after re-supply but considerably lower thereafter. Again the significance of this difference remains to be proven for some time points due to large variation between the values obtained for –K plants. No significant changes in *LOX2.3* or *AOS* expression were observed in response to either K-supply or over time.

No significant interaction between the effect of K-availability and time on LOX2.A expression were observed ($p=0.104$), however, despite large variation in the LOX2.A transcript levels in the –K tissue, a significant change in response to K was seen ($p=0.002$).

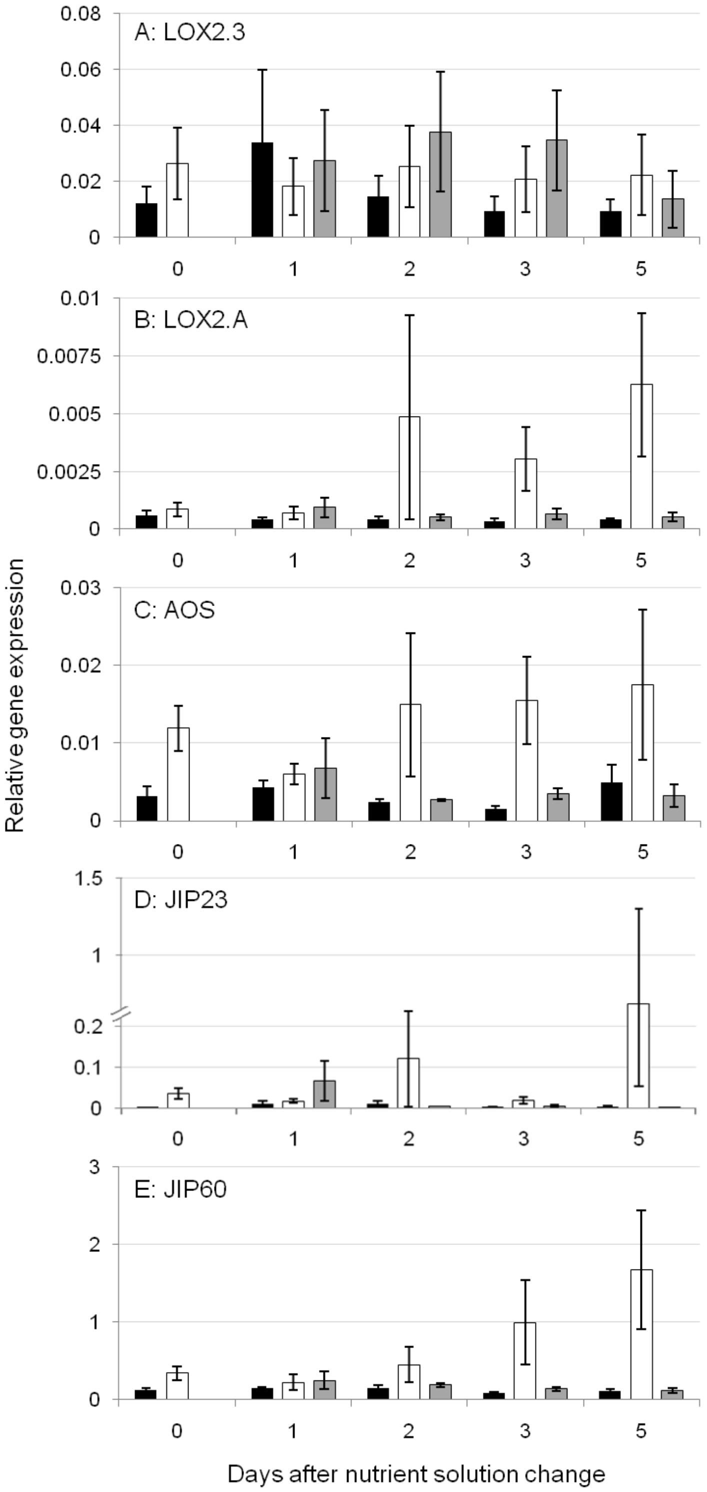


Figure 4-10. Transcript levels of JA-related genes in barley after K re-supply. Transcript levels of JA-biosynthesis genes (A: *LOX2.3*, B: *LOX2.A* and C: *AOS*) and JA-induced genes (D: *JIP23* and E: *JIP60*) relative to α-tubulin in shoot tissue from plants grown in control (■),-K (□) nutrient solution and plants grown in –K solution for 10 days before transfer to full nutrient solution (▨). Five plants were pooled 0, 1, 2 and 5 days after K was resupplied and the level of JA related gene expression determined using qPCR (Chapter 2, Section 2.4), the mean (± SE) of data from three independently grown and treated batches of plants is shown (for experimental design and statistical analysis see Chapter 2, Sections 2.1.8 and 2.8.4).

JIP23 expression also varied considerably but transcript levels, however a significant interaction between the effect of K-availability and time was observed (0.042). The *JIP23* expression in the plants resupplied with K almost doubled between day 0 and day 1, but after this a rapid reduction in transcript abundance was seen by day 2 and *JIP23* expression was only 5 % of that seen 24 hours after re-supply. The *JIP23* levels in the plants resupplied with K after 10 days were elevated 1 day after re-supply with a 7.2 fold increase compared to the control plants. However, 2 days after re-supplying K the abundance of *JIP23* transcripts had dropped to 36 % of that seen in the control plants, remaining low for the rest of the experiment.

A significant interaction between the effect of K-availability and time was also observed for JIP60 (p= 0.015). The *JIP60* transcript abundance in the –K plants was always higher than in the control plants but large variation between batches of –K plants meant that this difference in average levels was hard to evaluate. As with the other genes looked at the *JIP60* transcript levels had dropped to around those of the control plants by 2 days after re-supply and remained low for the remainder of the experiment, the JIP60 expression levels in the resupplied plants were around 43 % of those of the –K plants by day 2 and as low as 7 % by 5 days after re-supply.

4.6.Conclusion and discussion

In this chapter the response of several JA-biosynthesis and JA-induced genes to K-deficiency was investigated. The main aim of this work was to translate knowledge gained from Arabidopsis to barley. However, the work expanded on previous experiments with Arabidopsis by analysing individual leaves and leaf zones thereby allowing us to link the results on gene expression with the data obtained from K measurements in the previous tissues (Chapter 3).

4.6.1. Election and performance of JA-related genes

A number of JA-related genes were selected to be used use as marker genes for JA including genes encoding for important enzymes in the biosynthesis pathway leading to JA (3 *LOX2* genes, *AOS*, and *AOC*) and genes previously reported to change transcript levels in response to JA treatment (*JIP23*, *JIP37* and *JIP60*).

Initially two barley *LOX2* genes (*LOX2.2*; AJ507212 and *LOX2.3*; AJ507212) were selected based on a high homology with the Arabidopsis *LOX2* gene and published data classifying them as barley *LOX2* genes (Bachmann *et al.*, 2002). The Barley LOX2 genes with close homology to the *AtLOX2* gene (At3g45140) shown to be up-regulated in response to K-deficiency (Armengaud *et al.*, 2004; Armengaud *et al.*, 2010; Troufflard *et al.*, 2010) were identified using a BLAST search (Altschul *et al.*, 1990). However, when quantified using qPCR *LOX2.2* and *LOX2.3* displayed high level of variation between replicates making it difficult to draw conclusions (Figure 4-3A and B). A further *LOX2* gene was selected with more constancy between replicate experiments. *LOX2.A* was identified as a candidate for an additional barley *LOX2* gene during the microarray studies described in chapter 6. The closest homolog of *LOX2.A* was the rice *LOX2* gene (EU08542) and its closest match in Arabidopsis was *LOX2* (At3g45140). The *LOX2.A* transcript was more constant and showed an increase in response to K-starvation (Figure 4.3C) and it therefore was selected for use as a marker for K-deficiency in the barley, and is also likely to represent the functional homologue of *LOX2* in Arabidopsis.

Barley homologs of the Arabidopsis *AOS* and *AOC* genes were identified using a BLAST search and were confirmed in the literature (Maucher *et al.*, 2000; Maucher *et al.*, 2004). *AOC* also showed a consistent response to K-starvation while *AOS* was more variable (although still generally responsive to K; Figure 4.3D and E).

Although a number of marker genes downstream of JA have been identified in Arabidopsis these genes have not yet been identified and sequenced in barley, however, a number of genes have been identified as JA-responsive. The *JIP* genes were selected on the basis of having been reported to respond to JA treatment, although little is known about their function (Andresen *et al.*, 1992; Chaudhry *et al.*, 1994; Leopold *et al.*, 1996; Müller-Uri *et al.*, 2002; Reinbothe *et al.*, 1994). JIP23 has been shown to be involved in JA-related transcription and JIP37 is closely related to it, while JIP60 has been shown to be a ribosome inactivating protein (Chaudhry *et al.*, 1994; Leopold *et al.*, 1996; Müller-Uri *et al.*, 2002). *JIP23* and *JIP37* transcript levels were very variable both between replicates and when compared to the expression level of other genes in the same samples. They did not display a consistent response to K-starvation (Figure 4-4). As little is known about their function it is possible that these genes form part of JA signal cascades that are independent of the response to low K. The abundance of *JIP60* transcripts, however, were consistently higher in K-starved plants and showed a lot less variation between replicates than the *JIP23* and *JIP37* genes. The expression levels for this gene were consistent with those seen for both *LOX2.A* and *AOC*, and hence these three genes were used as marker genes in further experiments (Chapter 5).

4.6.2. Response of JA-related transcripts to K-starvation and re-supply

No difference between control and –K plants was observed in transcript level of any of the JA-related genes over the first six days of plant growth in low K, despite the K concentration in –K plants being lower than in control plants on day 6 (Chapter 3, Figure 3-1). This finding indicates that there is a threshold of internal K-content below which transcriptional responses in the JA-pathway are induced. By day 9 the transcript levels of all the genes measured were higher in the –K plants than in the control plants, although the extent of this increase varied between genes (Figure 4-3 and 4-4). The difference between –K and control plants with respect to JA-related transcript levels persisted and in many cases further increased up to at least day 15 (the last day assessed here). The important conclusion from this chapter is therefore that barley did appear to up-regulate JA-related genes in response to low K as previously reported for Arabidopsis. The barley genes *LOX2.A*, *AOC* and *JIP60* can be used in the future as JA-related marker genes for K-starvation in barley.

When barley seedlings were resupplied with K after 10 days of growth in –K solution the increase in JA-related gene expression was reversed further supporting a direct link between K-supply and gene expression (Figure 4-10). However, the difference between K-resupplied and non-resupplied plants was not seen until 2 days after medium change, whereas in Arabidopsis a reduction in JA-related gene expression was seen within 2-6 hours after K-re-supply (Armengaud *et al.*, 2004). However, concentration in plants resupplied with K increased more slowly in barley than in Arabidopsis; no change in K-concentration was seen in either the roots or shoots of barley after 24 hours of re-supply (compare Figure 4-9), while contents in Arabidopsis roots and shoots had reached around 50 % control levels at this time point (compare Figure 2 in (Armengaud *et al.*, 2004). The slow return of transcript levels of JA-related genes to control values in barley is therefore likely to be due to slow increase in K concentration after resupply of K compared to Arabidopsis. It should also be mentioned that this experiment was carried out before the full dataset for the starvation time course was available. The choice of day 10 as the time point for re-supply was based on a clear difference in K-concentrations between –K and control plants (see Figure 3-1 in chapter 3). However, as evident in Figures 4-2 and 4-3 of this chapter at this stage the difference in JA-related transcript levels was not yet significant and in hindsight re-supply should have been administered at a later time point.

4.6.3. Effect of K-supply on hormone levels

The increase of *LOX2*, *AOS* and *AOC* transcripts during K-starvation suggests (Figure 4-3) an increase of JA-biosynthesis, and the increase of *JIP60* transcript indicates elevated JA levels in –K plants (Figure 4-4). However, JA measurements in the same tissue detected no increase in JA at day 9 and only a slight increase (1.4 fold) on day 15 (Figure 4-5). In some of the samples (including various time points for both –K and control plants) no JA was detected and as a result the level of replication was insufficient for statistical analysis. A possible explanation is that the tissue samples were stored for a long time at -80 °C before freeze drying, possibly allowing some breakdown of the JA. Unfortunately, due to machine failure at the Mass Spectrometry facility a full experimental set of 3 replicates per time point could not be achieved within the time frame of this PhD.

It should be noted that the increase in JA levels in K-starved *Arabidopsis* plants was also relatively small (1.8-fold) yet clearly significant (Troufflard *et al.*, 2010). Furthermore, Troufflard *et al.* (2010) observed a much stronger K-response in the JA precursors 9-hydroxy-12-oxo-octade cadienoic acid (9-HOD), 13-HOD and OPDA. These oxylipins have been reported to act as signals in their own right (Böttcher and Pollmann, 2009; Stintzi *et al.*, 2001; Taki *et al.*, 2005; Vellosillo *et al.*, 2007) and their concentrations should be determined in barley in the future.

Several other plant hormones were also measured (Figure 4-5). K-starved plants displayed higher levels of ABA compared to the control plants as early as day 9. ABA is an important signal in salt and drought-stress. However, no significant difference in water content was observed between control and –K plants in chapter 3 (Table 3-1). It is interesting that shortage in K, which is the main solute required for osmotic adjustment during salt and water stress, also induces an ABA response in the absence of water stress. An increase in SA and SA-glycoside levels in the –K plants than was also unexpected as no indication for an increase in SA-related genes was found in Arabidopsis (Armengaud et al., 2004). Furthermore, JA and SA have been reported to act antagonistically, therefore, if anything a reduction in SA levels was expected in the –K plants (Berrocal-Lobo *et al.*, 2002; Harms *et al.*, 1998; Niki *et al.*, 1998; Norton *et al.*, 2007). However, synergistic effects of JA and SA have also been found (Mur *et* *al.,* 2006; Ton *et al.,* 2002; Van Wees *et al.*, 2008) and it is becoming increasingly clear that the two hormones interact with each other in a much more complex way than originally thought. Clearly, the observed increase in SA compounds needs to be taken into account when assessing the effect of K nutrition on defence responses in barley.

4.6.4. Local responses to K within the shoot and within the leaf

In addition to looking at the effects of K-starvation on the JA-related gene expression in whole shoot tissue, the levels in first and second leaves of 14 day old plants were also assessed (Figure 4-6). The differences in JA-related gene expression between –K and control plants were in general larger in these samples than in the whole shoot samples, possibly due to the majority of the change occurring in the leaves. The JA-related transcript levels were higher in the –K plants in almost all samples, except for *LOX2.3* and *JIP23* for which a large level of variability made it difficult to interpret the data obtained. The increase in JA-related gene expression was higher in the first leaf indicating that JA is accumulated to a higher level in the first leaf than in the second leaf. This may be related to the lower concentration (47 %) of K in the first leaf than in the second leaf in plants grown in –K solution (Figure 3-1, chapter 3). It is important to note that the second leaf showed more physical deficiency symptoms, such as paler colour and necrotic patches (data not shown). The results obtained here therefore suggest that transcript levels of JA-related genes are directly linked to K-content rather than downstream events. It was nevertheless decided to use the second leaf for further experiments as it grows longer thereby allowing better resolution of changes within the leaf.

The JA-related gene expression in response to K-deficiency was measured in the tip, middle and base segments of 14 day old barley plants (Figure 4-7). For all genes analysed except for *LOX2.3*, which again showed variation between replicates and little change with K status, transcript levels were generally higher in the –K samples compared to the control samples. Within the second leaf, transcript levels were highest in the tip samples, depleting towards the base of the leaf. This gradient was seen in both control and –K plants but was much steeper in the latter. The gene expression pattern correlated with the local concentration of K within the leaves, which decreased from base to tip in control and particularly –K plants (see Figure 3-1 in chapter 3 and Appendix 3, Table S3-2). However, at this stage other differences between and within the leaves of the plants (e.g. turgor, growth rates, apoplastic pH) cannot be excluded as the cause of the differences in JA-related transcript levels. In conclusion, transcript levels of most of the selected JA-related genes in whole shoots, individual leaves and leaf segments generally followed the amount of K both in time and leaf region. The specific factors linking tissue K-concentration with gene expression are unknown at this stage and are further discussed in Chapter 7.

4.6.5. Response of JA-related transcripts to other nutrients

A high level of variation in the JA-related gene expression was seen in response to the withdrawal of other mineral nutrients, however most samples showed some increase (Figure 4-8). This is in contrast to the results obtained with *Arabidopsis* plants grown in nutrient media lacking P, N S or Ca, which showed no change in transcript abundance of *LOX2* and *VSP2* (Troufflard *et al.*, 2010). In barley, the withdrawal of phosphorus (P) resulted in some of the largest increases in JA-related gene expression, however, only in one of the two replicate experiments. Only a small increase in JA-related gene expression was seen in plants deprived of sulphur. One of the shortcomings of this experiment is that at the chosen time point (10 days) JA-related gene expression in the –K plants was only marginally increased over the control and hence all differences measured may reflect normal variation around the control value. Clearly, the issue of nutrient specificity requires much more detailed analysis in the future involving longer exposures to deficiency conditions, more replicates and a thorough analysis of tissue ion contents, growth rates and other physiological indicators of deficiency.

Chapter 5. Potassium deficiency and Pathogen infection in Barley

5.1. Introduction

Potassium availability is known to have an effect on the susceptibility of barley to a number of different pathogens including fungi, insects, and bacteria (Perrenoud, 1990). In the majority of cases higher K leads to reduced infection, but this is not always the case, and examples of higher or unchanged infection in response to higher K availability have also been reported (Imas and Magen, 2000). In 2008, Amtmann *et al.*, suggested that the increased concentrations of JA seen in K starved plants might play a role in defence against pathogen attack and this group have since demonstrated reduced susceptibility to thrips in K starved *A. thaliana* plants (Armengaud *et al.*, 2010). In this chapter I investigate the effect of K-deficiency, and the resultant increase in JA concentration, on the infection of barley by two contrasting fungal pathogens.

5.1.1. The effect of K nutrition on wounding and herbivore attack

Increased K supply in the field or greenhouse almost always leads to enhanced defence against insect pathogens, apparent as reduced insect feeding, damage or reproduction (Perrenoud, 1990). In 1990, a study by the International Potash Institute compared results from 175 trials looking at the effect of increased K-fertilisation on aphids in a number of plant species (Perrenoud, 1990). Of these trials 115 showed reduced development of the insects or reduced damage, while 46 showed increased insect attack. Higher K availability was found to lead to higher susceptibility to some insects, including thrips *Frankliniella* sp. and aphids *Lipaphis erysimi, Schizaphis graminum* and *Toxoptera graminum* (Perrenoud, 1990).

5.1.2. JA, wounding and herbivore attack

Although JA is involved in plant responses to a number of abiotic and biotic stresses, it is particularly well known as a signal for wounding and herbivore attack (Chen, 2008; Gatehouse, 2002; León *et al.*, 2001; Wasternack *et al.*, 2006). Induction of the JA signalling pathway in response to wounding by herbivores promotes the production of a number of defence proteins. This has been well studied in tomato plants leading to identification of a number of JA-responsive proteins, including protease inhibitors, leucine aminopeptidases and threonine deaminases (Chen *et al.*, 2004; Chen *et al.*, 2005; Walling, 2000). JA-induced proteins that have been shown to be produced in response to mechanical wounding include thionin in barley (Andresen *et al.*, 1992) and trypsin inhibitors in tomato (Farmer *et al.*, 1992). While some of the responses of plants to herbivores are shared with the wounding response, other responses are related to herbivore-specific stimuli, such as the composition of insect saliva (Miles, 1999; Walling, 2000). The JA-signalling response to herbivore attack includes both indirect (volatile emissions) and direct (formation of defence proteins and small compounds) defence mechanisms (Halitschke and Baldwin, 2004). The release of volatiles has been demonstrated to lead to the induction of defence mechanisms in neighbouring plants (Farmer and Ryan, 1990).

5.1.3. *R. secalis* and Plant K Status

Although the resistance mechanisms to the hemi-biotrophic fungal pathogen *Rhynchosporium secalis* are well studied in barley (Zhan *et al.*, 2008), little is known about the effect of nutrients on *R. secalis* infection. A little work has been carried out looking at the effect of nitrogen on *R. secalis* infection. Jenkyn and Griffiths (1976) found that increased nitrogen availability reduced the levels of *R. secalis* infection in both Cambrinus and Proctor barley cultivars. The same group showed that susceptibility was negatively correlated with the total nitrogen content of barley leaves and positively correlated with water soluble carbohydrates (Jenkyn and Griffiths, 1978). In 1975, Ayres and Jones used ^{86}Rb as a chemical analog of K in barley plants infected with *R. secalis*, and demonstrated increased ^{86}Rb accumulation in infected regions, leading to stomatal opening and increased transpiration (Ayres and Jones, 1975).

5.1.4. *Rhynchosporium secalis* and JA

A large proportion of the current knowledge of resistance strategies in barley to *R. secalis* is focused on the gene-for-gene interactions (Zhan *et al.*, 2008) and some work has been carried out looking into the effect of plant hormones. Allen and Lyon (1978) looked at the effect of *R. secalis* infection on accumulation of growth regulators such as auxin, gibberellin and cytokinin. They showed that after 10 days no change was seen in the auxin and cytokinin concentration, but substantially higher gibberellin concentrations were observed. Weiskorn *et al.* (2002) investigated the effect of the SA analogs, bion (benzo-(1,2,3)-thiadiazole-7-carbothoic acid **S**-methyl ester, BTH) and dichloroisonicotinic acid (DCINA), and of JA on *R. secalis* infection. High variation between replicates was seen but the overall results indicated a reduction of infection in the plants treated with each of the three chemicals. However, *R. secalis* infection does not appear to induce JA-biosynthesis in barley leaves; no change in expression of the JA biosynthesis gene *LOX2Hv1* was observed in either the epidermal or the mesophyll cells of infected leaves (Steiner-Lange *et al.*, 2003).

5.1.5. Powdery mildew and K

In 1990 the International Potash Institute released a review of the effects of K on plant health, drawing together the results from many field and glasshouse studies looking at the effect of K on plant diseases (Perrenoud, 1990). For barley, one of the most studied fungal diseases is powdery mildew (*Blumeria graminis* f. Sp. *hordei*). The manner in which K-supply affected *Bgh* infection varied between the different studies. In most studies (17/31) higher K was found to lead to lower *Bgh* infection. However, in nine studies, an increase in *Bgh* with higher K was found and five studies found no effect of K supply on infection (Perrenoud, 1990). More recently Brennan and Jayansena (2007) reported less *Bgh* on barley crops with K fertilizer applied compared with those grown in K-depleted soils, and an increased grain yield was obtained from the plants supplied K. By contrast, Wiese *et al.* (2003) observed that the application of K fertilizer to barley plants had little effect on powdery mildew colony numbers.

5.1.6. Powdery mildew and JA

Treatment of barley leaves with JA (Schweizer *et al.*, 1993) and Me-JA (Walters *et al.*, 2002) reduces susceptibility to *Bgh*. Schweizer *et al.* (1993) suggested that the higher resistance to *Bgh* of JA treated plants was due to antifungal properties of JA rather than induced resistance. However, Walters *et al.* (2002) demonstrated that treatment of the first leaf with Me-JA led to higher phenylalanine ammonia lyase (PAL) and peroxidase activity and to lower *Bgh* infection on second leaves. Treatment of barley leaves with other oxylipins such as colneleic acid, 9,12,13-trihydroxy-11(E)-octadecenoic acid and 9,12,13-trihydroxy-10(E)-octadecenoic acid led to lower *Bgh* infection on the treated leaf (Cowley and Walters, 2005). The treatment with oxylipins also acted systemically; when the first leaves were treated with etheroleic acid, colneleic acid or 9,12,13-Trihydroxy-11(E)-octadecenoic acid a reduction in *Bgh* infection was seen on the second leaves (Cowley and Walters, 2005).

5.1.7. Aim

In this chapter, I report the effects of low-K supply on the infection of barley by two fungal pathogens, *Bgh* and *R. secalis*, and compare the effects observed with those obtained by JA treatment to investigate whether they could be due to the higher JA in K-starved plants, as suggested by the work presented in Chapter 3. In addition, the effect of both pathogens on JA related gene expression in control and –K plants is reported. Before focussing on fungal pathogens I will describe a set of experiments in which wounding was applied to mimic the damage caused by herbivores during feeding and thus investigate whether the elevated JA concentration in –K plants modulates the response of JA-related transcripts to wounding.

5.2. K-deficiency and JA response to wounding

The second leaves of 14 day old barley plants were wounded approximately 1 cm from the leaf tip (see Chapter 2, Section 2.1.7). The second leaves from 5 plants were pooled 0, 1, 2, 3, 4, 6 and 8 hours after wounding and qPCR was used to measure the transcript abundance of JA-related genes in the samples (Chapter 2, Section 2.4). The experiments were carried out with two independently grown batches of plants and the results from both replicates are shown separately (Figure 5-1). Due to low number of replicates no statistical analysis was carried out.

A transient increase of *LOX2.A* transcript abundance was seen in both control and –K plants in response to wounding with the increase in –K plants being twice that in the control plants (Figure 5-1A). The abundance of *LOX2.A* transcripts in the control plants increased directly after wounding, by 25 % and 40 % during the first hour. The transcript abundance then quickly decreased to below that seen before wounding. By 2 hours after wounding the *LOX2.A* abundance was 36 % and 41 % of control plants before wounding, and remained constant after this. A similar fold increase was seen in *LOX2.A* expression in the –K plants one hour after wounding, with an increase of 49 % and 21 % over the initial levels. The decline of *LOX2.A* expression was slower in –K plants and did not level out until 4 hours after wounding, when the transcript was 51% and 31% of -K plants before wounding. The initial *LOX2.A* expression before wounding were 6-fold and 5-fold higher in the –K than in the control plants in replicates 1 and 2 respectively. By 2 hours after wounding the *LOX2.A* expression were 13-fold and 10-fold higher in the –K plants than in the control plants.

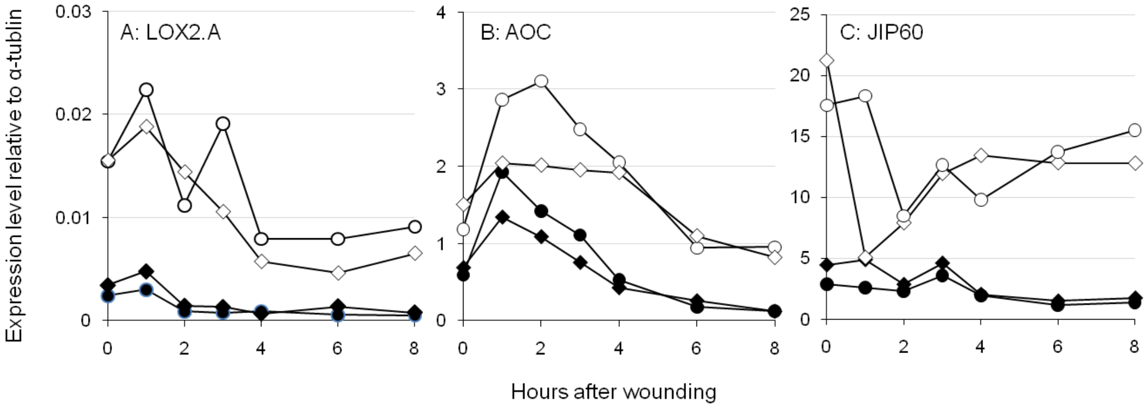


Figure 5-1 JA related gene expression and wounding in –K plants. Transcript abundance of JA related genes A: LOX2.A, B: AOC and C: JIP60 relative to α-tubulin in 2nd leaf tissue from plants grown in control (●,◆) and -K (○,◇) media for 10 days before being wounded with a pair of forceps (Chapter 2, Section 2.1.7). The transcript levels before wounding, 1, 2, 3, 4, 6 and 8 hours after wounding are shown. Leaves from five plants were pooled for each time point, 2 replicates shown.

A transient increase in the abundance of *AOC* transcripts was also seen in response to wounding in control and –K plants (Figure 5-1B). The abundance of *AOC* transcripts peaked after 1 hour in the control plants, increasing by 3-fold and 2-fold in the two replicates. The transcript abundance dropped after 1 hour but the decline was slower than for the *LOX2.A* transcript, the decline continued until 8 hours after wounding when the expression for the two replicates reached 21 % and 17 % of the initial values. The *AOC* expression in the –K samples also increased initially. In the first replicate the *AOC* expression increased 2-fold after 2 hours, while a smaller increase was seen in the second replicate with an increase of just 1.4 % after 1 hour and no further increase after this. The *AOC* transcript abundance in both samples gradually decreased reaching 79 % and 73 % of the initial values 6 hours after wounding. The abundance of *AOC* transcripts in the –K plants before wounding was approximately 2-fold that of the control plants for both replicates. The *AOC* expression in control plants increased more rapidly than in the –K plants, resulting in a reduction in the difference between the two treatments, the –K samples were 1.5-fold that of the control plants an hour after wounding. The slower decline in *AOC* expression in the –K plants resulted in a larger difference in *AOC* transcript abundance in later time points, with the –K samples reaching 8-fold and 7-fold higher transcript concentration than the control plants by after 8 hours.

Little change in the abundance of *JIP60* transcripts was seen during the course of the experiment in the control samples (Figure 5-1C). In the –K samples, which displayed 5-6 fold higher *JIP60* transcript abundance at the beginning of the experiment, there was a decrease in *JIP60* expression 1 to 2 hours after wounding, after which the expression returned to around the 60 % of the initial values, remaining at this level for the remainder of the experiment.

5.3. *R. secalis* infection in K deficient barley plants

5.3.1. K-deficiency and *R. secalis* infection

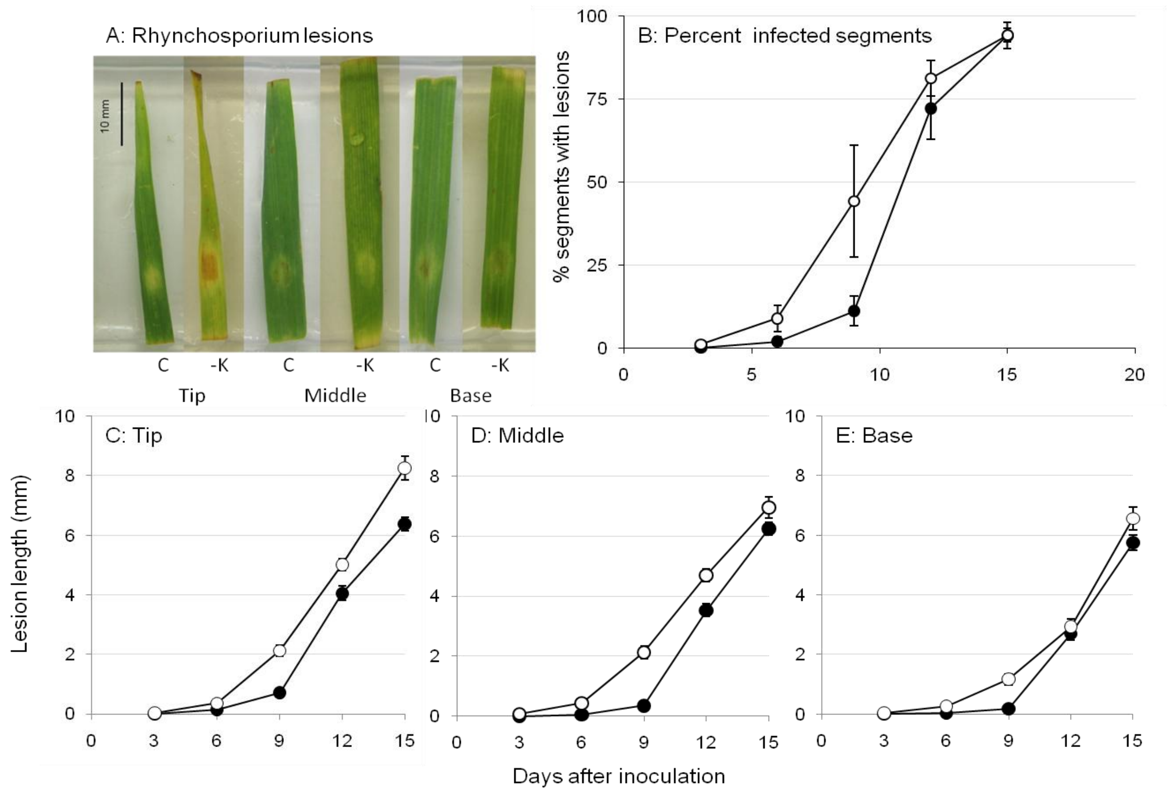


Figure 5-2. *R. secalis* infection in barley leaves under K stress. Detached leaf segments were prepared from the tip middle and base regions of the second lead of plants grown for 14 days in control (●) or –K (○) nutrient solution. A: *R. secalis* lesions on tip, middle and base segments from leaves grown in control and –K nutrient solution, 12 days after inoculation. B: The percentage of control (●) and –K (○) leaf segments inoculated with *R. secalis* with visible lesions 3, 6, 9, 12 and 15 days after inoculation (Chapter 2, Section 2.6.1). The length of *R. secalis* lesions formed on C: tip, D: middle and E: base segments cut from second leaves of barley plants grown for 14 days in control (●) or –K (○) nutrient solution 3, 6, 9, 12 and 15 days after inoculation. The mean (± SE) of 3 replicate experiments is show. Note: in some cases error bars are smaller than the symbols, for experimental design and statistical analysis see Chapter 2, Section 2.8.5).

To establish the effect of K-deficiency on *R. secalis* infection, detached tip, middle and base leaf segments were prepared from the second leaf of barley plants grown in control or –K nutrient solution for 14 days (for details see Chapter 2, section 2.1). The segments were each inoculated with 10 µl of 10^6 spores/ml spore solution and the length of any visible lesion was measured every 3 days (Section 2.6.1, Figure 5-2). Figure 5-2 shows *R. secalis* infection levels 3, 6, 9, 12 and 15 days post inoculation. The data was analysed using ANOVA (Appendix 2, Table S2-9), the data was not normally distributed and was transformed by taking the log10 before analysis, see chapter 2, section 2.8.5 for more detailed experimental design.

K-starvation led to earlier formation of visible *R. secalis* lesions on the leaf segments. No lesions were visible on the control segments on day 3, and by day 9 only 12 % of control segments had visible lesions (Figure 5-1 B). However, by day 15, 94 % of leaf segments had visible lesions. Visible symptoms of *R. secalis* were seen on the –K segments as early as day 3, when 1 % of segments had visible lesions. Nine days after inoculation 45 % of the -K segments had visible lesions, 4 times as many as the control segments. By day 15 the number of segments with visible lesions on the control and –K segments equalised, with around 94 % of segments displaying visible lesions.

5.3.2. JA and *R. secalis* infection

In order to investigate if JAs have any effect on *R. secalis* infection, segments from the middle of the second leaf of barley plants grown in full nutrient control solution were floated for 24 hours in a 45 µM solution of methyl-jasmonate (Me-JA) or water (control) as described in the Chapter 2, section 2.6.3. The detached leaf segments were then inoculated with 10 µl of spore suspension (10^6 spores/ml) and the lengths of the visible lesions were measured every 3 days. Figure 5-3 shows the effect of Me-JA treatment on *R. secalis* infection levels 3, 6, 9, 12 and 15 days post inoculation. The data was analysed using ANOVA (Appendix 2, Table S2-10), the data was not normally distributed and was transformed by taking the log10 before analysis, see chapter 2, section 2.8.6 for more detailed experimental design.

There was a significant interaction between the effect of Me-JA treatment and days since inoculation ($p=0.006$) on Rhynchosporium lesion size. Lesions were first visible on the segments treated with Me-JA 6 days after inoculation with *R. secalis*, when 4 segments had visible lesions. By day 12 the length of lesions on the Me-JA treated leaf segments was 15 % larger than those measured on the control plants. The lesion size in the segments treated with Me-JA remained marginally larger than in the control plants.

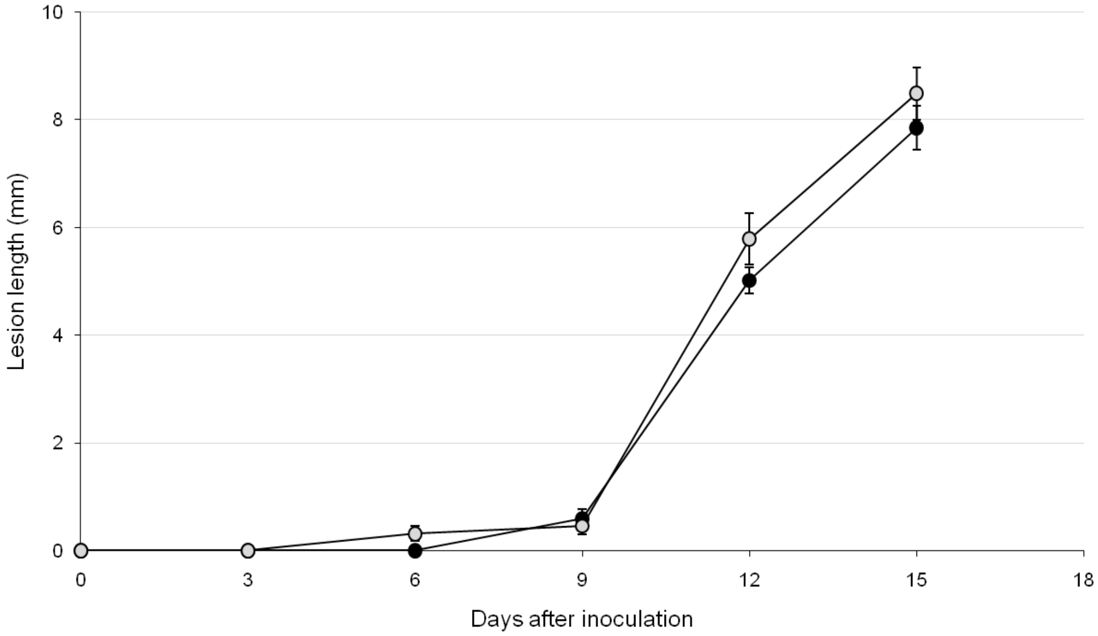


Figure 5-3. Methyl Jasmonate and *R. secalis* infection. Middle leaf segments from 14 day old second leaves, grown in control nutrient solution were floated on 45 µM Me-JA (●) or water (●) for 24 hour before inoculation with 10^6 spores/ml solution of *R. secalis* spores (Chapter 2 Sections 2.6.1 and 2.6.3). The length of the lesions formed on 24 detached leave segments was measured 3, 6, 9, 12 and 15 days post inoculation. The mean (± SE) of 3 replicate experiments is shown. for experimental design and statistical analysis see Chapter 2, Section 2.8.6).

5.3.3. JA related gene expression and *R. secalis* infection

To investigate the effect of *R. secalis* infection on the transcription of JA-related genes, detached leaves from control and –K plants were infected with *R. secalis* and the expression of JA-related genes measured using qPCR (see Chapter 2, sections 2.4 and 2.6.1). The middle segments were selected for these experiments because (a) the growth conditions had a greater effect on the amount of infection here than in the base segments, (b) they had a larger surface area than the tip segments, and (c) they remained flatter than the other segments when placed on agar, which prevented displacement of the spore solution. Figure 5-4 shows the effect of K starvation and R. secalis infection on LOX2, AOS, JIP60 and PAL transcription 0, 1, 2, 3 and 4 days post inoculation. The data was analysed using ANOVA (Appendix 2, Table S2-11), the data was not normally distributed and was transformed by taking the log10 before analysis, see chapter 2, section 2.8.7 for more detailed experimental design.

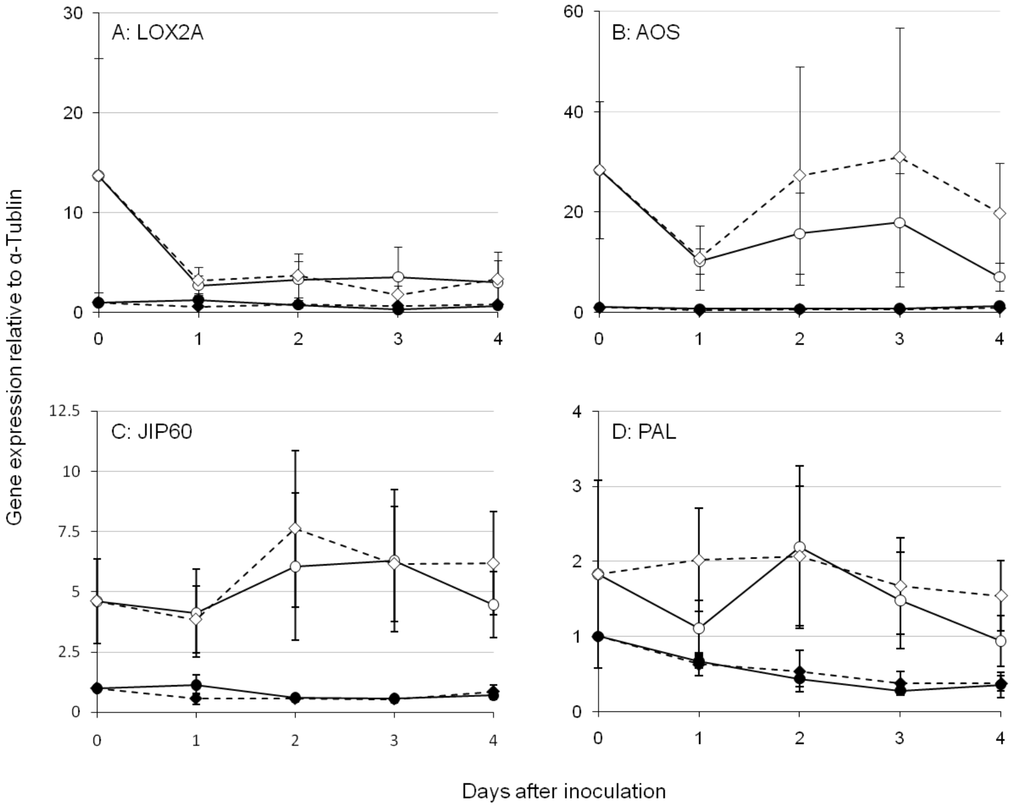


Figure 5-4 JA related gene expression after *R. secalis* infection. Transcript abundance of JA related genes A: *LOX2A*, B: *AOS* and C: *JIP60* and pathogen related gene D: *PAL* relative to α-tubulin in middle segments of the second leaf from plants grown in control (●,◆) and -K (○,◇) media for 14 days, and either mock-inoculated (control; ●,○) or *R. secalis* inoculated (◆,◇). Five detached leaf segments were pooled for each time point, 0, 1, 2, 3 and 4 days post inoculation and the transcript levels determined using qPCR. Data represent the mean (± SE) of 4 replicates (for experimental design and statistical analysis see Chapter 2, Section 2.8.7).

No significant interaction of the effect of K-availability, pathogen infection and time on JA related gene expression was observed. As shown previously (Chapter 4) the transcript abundance of *LOX2.A* ($p=0.002$), *AOS* ($p<0.001$) and *JIP60* ($p<0.001$) were significantly higher in the –K plants than in the control plants. Interestingly the pathogen related gene PAL was also up regulated in response to K-starvation. After treatment with *R. secalis* solution or water, transcript of *LOX2.A* and *AOS* dropped considerably in – K plants but still remained significantly higher than in control plants. Apparently the treatment itself had an effect on JA signalling, independent of the fungus. *R. secalis* infection had no significant effect on the abundance of *LOX2.A* ($p=0.677$), *AOS* ($p=0.680$), *JIP60* ($p=0.966$) or *PAL* ($p=0.107$) transcripts in either control or –K segments.

5.4. Powdery mildew infection in K starved barley plants

5.4.1. K-deficiency and Powdery mildew

To investigate the effect of K-deficiency on the severity of *Bgh* infection in tip, middle and base segments of barley leaves, detached leaf segments were prepared from plants grown in control or –K nutrient solution and inoculated with powdery mildew spores (for details see as per Materials and Methods section 2.6.2). The severity of infection was assessed by counting the number of mildew colonies on each segment every 3 days. The number of colonies per cm^2 was calculated for each segment to account for differences in size between segments. Figure 5-5 shows *Bgh* infection levels 3, 6, 9, 12 and 15 days post inoculation. The data was analysed using ANOVA (Appendix 2, Table S2-9), the data was not normally distributed and was transformed by taking the log10 before analysis, see chapter 2, section 2.8.5 for more detailed experimental design.

Visible *Bgh* colonies formed later on the K-deficient leaves than on the control leaves (Figure 5-5B). Colonies were first visible on control segments on day 6, when 8 % of tip, 34 % of middle and 22 % of base segments had one or more visible colonies. The number of segments with colonies increased over time, and by 15 days after inoculation 80 % of the control segments had visible colonies (Figure 5-5 B). The colonies on the –K segments were slower to appear; on day 6 there were no colonies on the tip segments and just 3 % of the middle and 5 % on the base segments had visible symptoms. By 15 days after inoculation 50 % of –K segments had visible colonies. The number of –K segments with visible colonies was lower for the tip (20 %) than the base (81 %) of the leaf, although there were less visible colonies on the control tips (74 %) than the base (92%) the effect was much smaller.

A significant interaction was observed between the effects of K-availability, leaf region and length of time since inoculation on colonies per cm^2 ($p<0.001$). K-deficient barley plants were clearly less susceptible to *Bgh* infection than the K-sufficient plants (Figure 5-5). The K-deficient segments had significantly fewer colonies than the control segments throughout the experiment. As early as day 6, there were fewer visible colonies on the -K tip, middle and base segments than the control segments. The difference in colony number between –K and control plants was considerably decreased from the tip to the base of the leaf. Expressed as a percentage of the control, the average colony number per cm^2 on the –K segments on day 15 was 14 % for the tip, 36% for the middle and 77% for the base.

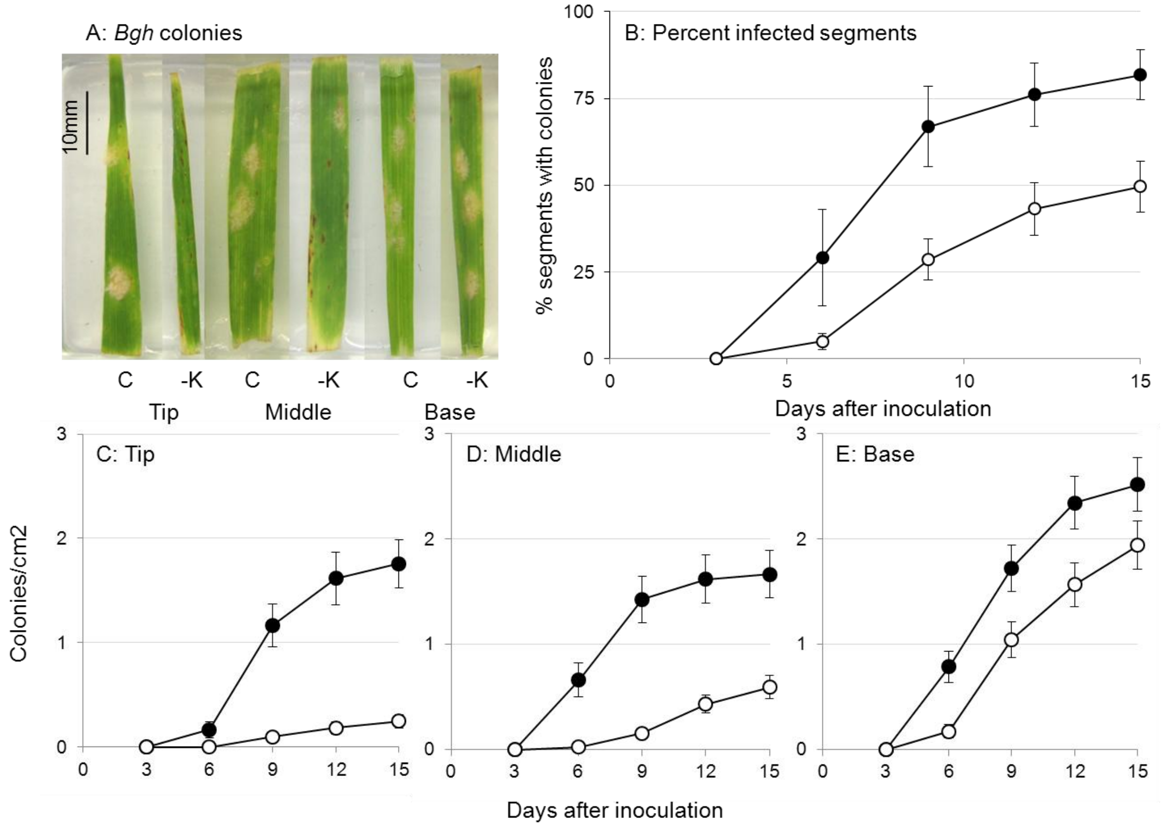


Figure 5-5. Powdery mildew infection in barley leaves under K stress. Detached leaf segments were prepared from the tip middle and base regions of the second lead of plants grown for 14 days in control (●) or –K (○) nutrient solution. The leaf segments were inoculated with Bgh spores using an inoculation column. A: *Bgh* colonies on tip, middle and base segments from leaves grown in control and –K nutrient solution, 12 days after inoculation. B: Number of leaf segments that showed visible colonies with time after inoculation with *Bgh* (in % of total number of inoculated segments). The number of *Bgh* colonies formed on C: tip, D: middle and E: base segments 0, 3, 6, 9, 12 and 15 days post inoculation. The mean (± SE) of 3 replicate experiments is show. Note: in some cases error bars are smaller than the symbols, for experimental design and statistical analysis see Chapter 2, Sections 2.6.2 and 2.8.5).

The severity of powdery mildew infection (measured as average number of colonies per cm^2) increased from the tip to the base of the leaf in both control and –K plants (Figure 5-5 C-E). In control leaves colony numbers differed little between tip and middle segments but the base segments had 44 % and 51 % more colonies per cm^2 than the tip and middle segments respectively. The average number of colonies per cm^2 on –K segments also increased from the tip to the base of the leaf. By day 15 there were 0.25 colonies/cm^2 on the tip, 0.59 colonies/cm^2 on the middle and 1.9 colonies/cm^2 on the base segments from –K plants.

5.4.2. JA and powdery mildew infection

It has previously been reported that treatment of barley with Me-JA leads to lower *Bgh* infection (Section 5.1.6). To establish a possible role for JA in linking K-deficiency with decreased susceptibility to *Bgh* it was important to repeat the experiment here with the same plants and growth conditions as used previously (Chapter 2, Sections 2.1). Segments from the middle of the second leaf of barley plants grown in control solution were floated for 24 hours on water or on a 45 µM solution of methyl-jasmonate (Me-JA). The detached leaf segments were then inoculated with powdery mildew spores (for details see Chapter 2, section 2.6.3). To assess the severity of infection, the number of successful colonies on each leaf segment was counted every 3 days. Figure 5-6 shows the effect of Me-JA treatment on *Bgh* infection levels 3, 6, 9 and 12 days post inoculation. The data was analysed using ANOVA (Appendix 2, Table S2-10), see chapter 2, section 2.8.6 for more detailed experimental design.

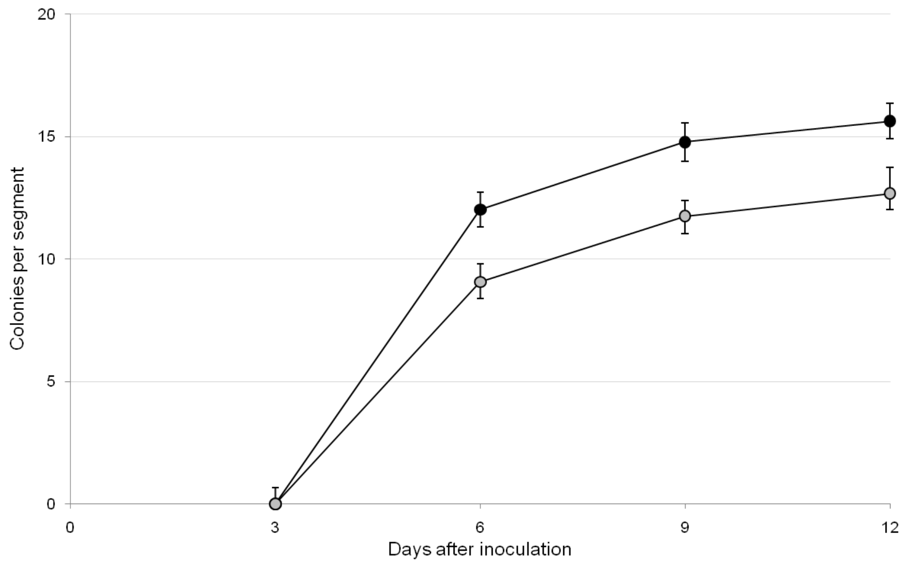


Figure 5-6. Methyl Jasmonate and powdery mildew infection. Middle segments from second leaves of 14 day old barley plants grown in control solutions were floated on water (closed circles) or on 45 µM Me-JA (open circles) for 24 hours before inoculation with powdery mildew spores (Chapter 2, Section 2.6.26 and 2.6.3). The number of colonies on each of 24 segments were counted every 3 days. The means (± SE) of 3 replicate experiments are shown, for experimental design and statistical analysis see Chapter 2, Section 2.8.6).

There was a significant interaction between the effect of Me-JA treatment and days since inoculation on Rhynchosporium lesion size ($p<0.001$). Treatment with Me-JA led to a reduction in *Bgh* infection compared with water treated controls (Figure 5-6). Colonies were first seen on day 6 on both the Me-JA treated and control segments, but there were fewer (76 %) colonies on the Me-JA treated segments compared to the control segments. By day 12, colony numbers on the Me-JA treated segments were slightly lower than on the control segments, with the Me-JA treated segments having 82 % the number of visible colonies seen on the control segments.

5.4.3. JA related gene expression and powdery mildew infection

To investigate the effect of *Bgh* infection on the transcription of JA-related genes, detached leaves prepared from the second leaf from control and –K plants were infected with *Bgh* and the expression of JA-related genes measured using qPCR (see Chapter 2, sections 2.4 and 2.6.2). Figure 5-4 shows the effect of K starvation and *R. secalis* infection on LOX2, AOS, JIP60 and PAL transcription 0, 1, 2, 3 and 4 days post inoculation. The data was analysed using ANOVA (Appendix 2, Table S2-13), the data obtained for LOX2.A and AOS was not normally distributed and was transformed by taking the log10 before analysis, see chapter 2, section 2.8.7 for more detailed experimental design.

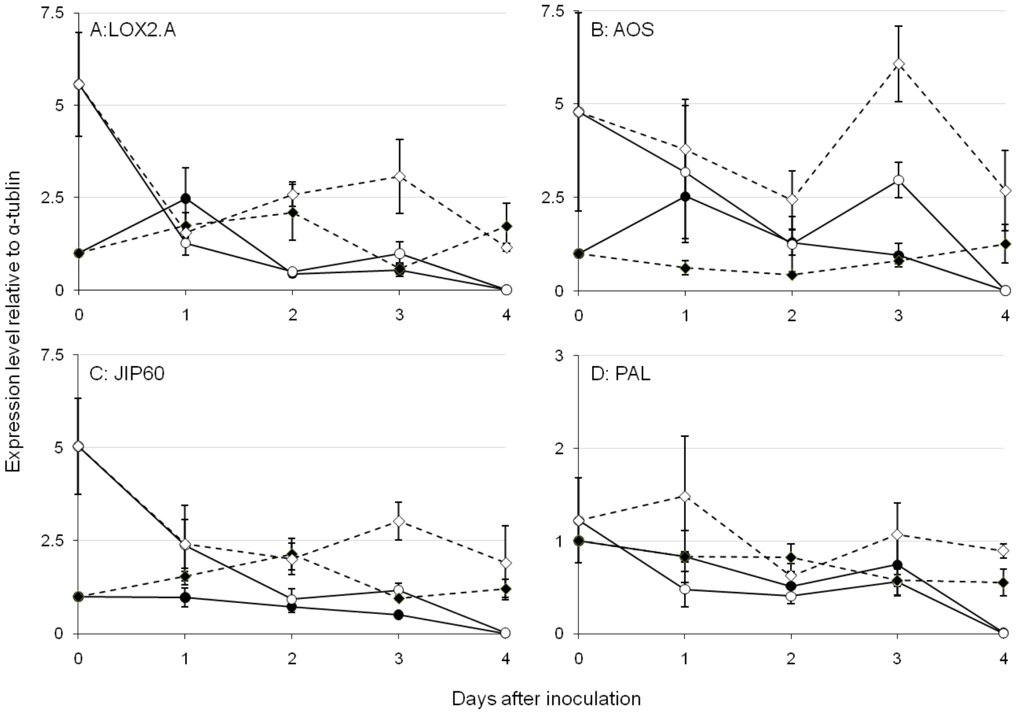


Figure 5-7 JA related gene expression after *Bgh* infection.

Transcript abundance of JA related genes A: *LOX2A*, B: *AOS* and C: *JIP60* and pathogen related gene D: *PAL* relative to α-tubulin in middle segments of the second leaf from plants grown in control (●,◆) and -K (○,◇) media for 14 days, either mock-inoculated (control; ●,○) or *Bgh* inoculated (◆,◇). Five detached leaf segments were pooled for each time point, 0, 1, 2, 3 and 4 days post inoculation and the transcript levels determined using qPCR. Data represent the mean (± SE) of 4 replicates (for experimental design and statistical analysis see Chapter 2, Section 2.8.7).

No significant interaction of the effect of K-availability, pathogen infection and time on JA related gene expression was observed. The inoculation, independent of the presence of fungus in the applied solution, led again to a drop of expression of *LOX2.A*, *AOS* and *JIP60* in the – K plants and largely abolished the difference in transcript abundance between –K and control plants that was evident at time point 0 (Figure 5-7). This finding, together with a large variation of transcript concentration between replicates and time points, made it difficult to extract any clear conclusions from the experiments. Keeping these problems in mind, the data indicated higher expression in *Bgh*-inoculated plants than in non-infected plants, with clear interaction between the effect of *Bgh* infection and time on *LOX2.A* ($p<0.001$) and AOS ($p<0.001$) transcript abundance. However only AOS transcript levels were significantly affected by interaction between K-availability and Bgh infection ($p=0.011$).

5.5. Discussion and conclusion

In this chapter the effect of K-starvation and Me-JA treatment on severity of infection of both *Bgh* and *R secalis* was described. In addition, the response of JA-related genes to powdery mildew and *R. secalis* infection and wounding (to mimic herbivore attack) was investigated and compared between plants grown in control conditions and plants grown in low K conditions.

5.5.1. Increase in JA after wounding extended in –K plants

Armengaud *et al* (2010) demonstrated a reduction in thrips bites in K-starved *A. thaliana* plants when compared to K-replete controls. Since JA treatment has been shown to reduce plant susceptibility to thrips (Abe *et al.*, 2009) it is possible that the effect of low K was due to increased JA-concentration. Unfortunately, Arabidopsis JA-signalling mutants (*coi1.16*) were damaged so quickly and so badly by the thrips that they could not be used to test the necessity of JA signalling for the low-K effect. The Amtmann group also investigated whether the effect of K nutrition on the susceptibility in *A. thaliana* to thrips was due to a difference in the production of glucosinolates. Indeed, low-K plants contained less glucosinolates (particularly of the indole type) and this difference was dependent on functional JA-signalling (i.e. it was less pronounced in *coi1.16* mutants; (Troufflard *et al.*, 2010). However, mutants in the production of indole glucosinolates (cyp79b2/b3) grown in low-K conditions showed the same decrease in susceptibility to thrips as wild type plants, suggesting that higher indole glucosinolate concentration did not underlie the effect of low K on susceptibility to thrips.

This thesis focussed on fungal pathogens rather than insect herbivores but the JA-marker genes identified in Chapter 3 allowed the effect of wounding on these genes to be tested, and whether this was modulated by the K status of the plants. While wounding is only one component of herbivore attack (saliva components being important additional stimuli) these experiments provided a first hint of whether JA-signalling is involved in K-herbivore interactions in barley.

The results showed enhanced JA related gene expression response to wounding in the K-starved plants (Figure 5-1). In both the control and –K plants an increase in JA-related gene expression was seen 1 h after wounding. Despite higher transcript concentrations in –K plants at the beginning of the experiment, the relative change in transcript concentrations and the overall shape of the response over time were similar in the –K and control plants indicating that the effect of the wounding on gene expression was additive to the effect of K-starvation. Furthermore, transcript levels in the –K plants returned to initial levels more slowly after wounding than the control plants. This suggests a stronger and prolonged JA-signal in response to wounding in the K-starved plants compared to the K-sufficient plants. Whether or not a higher constitutive JA concentration and a prolonged JA signal after wounding would enhance the plant's defence potential against herbivorous insects' remains to be proven.

Further work should now be carried out looking at the response of barley JA marker genes to insect pathogens such as aphids. The molecular experiments should be complemented by investigating preferences of insects to control and –K plants. To understand the role of JA as a mobile defence signal it would also be interesting to look at the effect of K-starvation on systemic wound signalling by assessing effects of K-nutrition on JA-marker gene expression in unwounded leaves from wounded plants.

5.5.2. K-deficient plants are more susceptible to *R. secalis*

The role of JA in plant defence responses differs depending on the pathogen. Early research on this topic suggested a fundamental difference between plant defence mechanisms against biotrophic and necrotrophic fungi characterising the former as SA -dependent and the latter as JA-dependent (McDowell and Dangl, 2000). More recent research shows that the distinction between the JA and the SA pathways, and between the defence mechanisms against biotrophs and necrotrophic fungi, is more complicated than originally thought due to multiple roles of JA and cross-talk of the pathways (Lorenzo and Solano, 2005). A fundamental question guiding this thesis is whether an increase of JA caused by abiotic stress (here K-deficiency) has different effects on pathogen susceptibility depending on the specific role of JA for plant defence against the pathogen in question. This question was addressed by testing the effects of low-K nutrition and Me-JA application on two different fungal pathogens of barley representing different lifestyles, one being a hemi-biotroph (*R. secalis*) and one being a full biotroph (*Bgh*). Indeed previous reports indicated that the two fungi have different responses to JA treatment, however, perhaps surprisingly, the biotroph *Bgh* infection was reduced by the JA and Me-JA treatment (Schweizer *et al.*, 1993; Walters *et al.*, 2002).

K-starvation led to increased susceptibility of barley to *R. secalis*; *R. secalis* lesions were seen earlier and were significantly larger on the leaf segments from plants grown in –K nutrient solution (Figure 5-2). Treatment of leaf segments with Me-JA had little effect on lesion size (if anything they were slightly increased, Figure 5-3). This result differs from a previous study (Weiskorn *et al.*, 2002) which observed a decrease of *R. secalis* infection in JA-treated plants, albeit with a high level of variation. The difference between the results presented here and results of Weiskorn *et al*, (2002) stresses the importance of measuring the effect of JA on fungal infection in the same growth conditions, plant developmental stage and infection protocol as used for the other experiments in this project. The results obtained here indicate that the observed effect of K-deficiency on *R. secalis* infection is independent of JA.

The lack of transcriptional response of JA-related genes to *R. secalis* infection (Figure 5-4) further supports the notion that JA does not play a major role in the defence of barley against *R. secalis,* at least not at the infection stage assessed here. *R. secalis* acts as a biotroph during the initial growth of hyphae, but later changes to a necrotrophic phase during which it leads to the collapse of host cells (For a more in depth discussion on the *R. secalis* life cycle see Chapter 1, Section 1.5.3.1). The sampling period spanned the first 4 days after inoculation. Based on previous assessments of the *R. secalis* infection (Jones and Ayres, 1974; Linsell *et al.*, 2010; Lyngs Jørgensen *et al.*, 1993) and observations that no pale green lesions had formed on the leaf segments at the time of sampling it is most likely that the *R. secalis* is still in the biotrophic phase of its life cycle. The lack of response of JA marker genes indicates the increased susceptibility to *R. secalis* in –K plants is likely to be a result of other changes caused by low K. Bearing in mind previous findings (Jenkyn and Griffiths, 1976), good candidates are K-dependent changes in N and C metabolites and K-dependent changes of stomatal opening (for further discussion see Chapter 7).

The observation that *R. secalis* symptoms were less pronounced in the base than in the tip of the leaf (in both control and –K plants) is interesting as it is in accordance with the observed K gradient within the leaf (see Figure 3-1 in Chapter 3 and Appendix 3, Figure S3-2). Thus, a negative relationship between K content and *R. secalis* infection is apparent both at organ and at tissue level and spans a wide range of K-concentrations in the leaf.

5.5.3. K-deficient plants are less susceptible to *Bgh* infection

The effect of K-deficiency and Me-JA treatment on infection of barley by the biotrophic fungus *Bgh* was also investigated in this chapter. Low levels of K-fertilization in the field often lead to a decrease in *Bgh* infection in barley plants (Perrenoud, 1990). However, the effect of K varies a lot between published trials, indicating that timing and conditions of infection have a big impact on the outcome. Reduced *Bgh* infection in response to JA treatment has also been reported (Schweizer *et al.*, 1993; Walters *et al.*, 2002). No study to date has measured the effects of K and JA on *Bgh* infection using identical growth conditions, plant material, inoculation protocols etc., and it is therefore impossible to assess the relationship between JA and K effects on plant susceptibility to *Bgh* based on published data.

In the experiments reported in this chapter, both K-deficiency and Me-JA treatment led to reduced *Bgh* infection in barley plants (Figures 5-5 and 5-6). The number of segments with visible *Bgh* colonies and the number of colonies per cm^2 were both reduced in –K plants compared to the control plants (Figure 5-5). The reduction in the number of colonies indicated increased resistance to *Bgh* response in low-K plants. The effect of K on *Bgh* infection was largest in the tips and smallest in the base segments. Considering that the tissue K concentration is lower in the leaf tip than at the base of the leaf (Figure 3-1) a positive correlation between K concentration and *Bgh* infection can be concluded at tissue level (Appendix 3, Table S3-2). Treatment of leaf segments with Me-JA led to reduced *Bgh* infection compared to mock treated controls, indicating that increased JA concentration enhances the resistance of barley to *Bgh*. A reduction in *bgh* infection in response to JA and Me-JA treatment has previously been reported (Schweizer *et al.*, 1993; Walters *et al.*, 2002), and in *A. thaliana* the JA constitutuive expressor of JA responses (*cer1*) has been shown to be less suceptable to Arabidopsis powdery mildew (*Erysiphe cichoracearum*) infection compared with wild type plants (Ellis *et al.*, 2002). Hence, lower susceptibility to *Bgh* in K-starved barley plants may indeed be due to increased JA concentration in these plants. Increase in JA concentration stimulates the expression of a wide variety of defence related genes in A. thaliana including vegitiative storage proteins (VSP) and plant defensin (PDF1.2) which maybe responsible for the reduced suceptability to bgh in –K plant (Berger *et al.*, 1995; Penninckx *et al.*, 1996). However, Schweizer *et al*. (1993) suggested that JA maybe directly inhibiting appressoria differentiation of the fungus. It is important to note that transcript abundance of JA-related genes differed in the leaf regions (particularly of K-deficient plants) being highest in the tip and lowest at the base of the leaf (Figure 4-7 in Chapter 4), a positive correlation was observed between the JA related gene expression in the detached leaf segment before inoculation and the rate and severity of Bgh infection (Appendix 3 Table S3-2).

Transcripts of JA-related genes showed a lot of variation in their abundance, and a fast response to the inoculation procedure (independent of fungus being present; Figures 5-4 and 5-7). It is therefore not possible to make firm statements about the effects of *Bgh* on the expression of the selected JA-related genes or to assess whether K modulated these responses. Nevertheless the data suggest an increase in the expression of JA-related genes during *Bgh* infection.

In conclusion, the experiments carried out in this study suggested a negative relationship between barley leaf tissue K concentration and infection by *R. secalis* (a hemi-biotrophic fungus), and no effect of JA on *R. secalis* infection. Thus, in the case of *R. secalis,* K-deficiency has an adverse effect on plant health which is likely to be based on physiological or metabolic changes in the plant. By contrast, a positive relationship was indicated between barley leaf tissue K-concentration and infection by *Bgh* (a biotrophic fungus), and a significant reduction in *Bgh* infection was achieved by JA treatment. Thus, in the case of powdery mildew, K-deficiency has a beneficial effect on plant health which may be caused by an increase in JA in the leaf tissue. Additional experiments, indicated an additive and prolonged effect of low K on the expression of wound-induced (JA-related) genes after mechanical wounding. Whether this effect translates into lower susceptibility of K-deficient barley plants to herbivorous insects requires further experimentation.

Chapter 6: Transcriptional changes of Barley in response to K-starvation and exposure to *R. secalis*

6.1. Introduction low-K

6.1.1. Background and aim

Microarray technology allows the simultaneous measurements of a large number of transcripts, enabling scientists to investigate in a fast and efficient manner how different conditions and treatments influence the transcriptome of an organism, tissue or cell type. In this chapter I will describe data obtained from microarray experiments designed to investigate the effects of K-starvation and *Rhynchosporium secalis* infection on detached leaf segments prepared from barley plants grown in hydroponics for 14 days.

The effects of K-deficiency on crop yield and health have been studied extensively, but much less research has been carried out to understand how changes at the level of physiology and biochemistry are underscored by gene expression in crops. A small number of microarray studies have been carried out in the model plant *A. thaliana*. For example Gierth *et al.* (2005) used microarrays to look at the effect of short term K deprivation on transcript levels in *A. thaliana* roots. They found surprisingly few changes in samples taken up to 96 hours after K withdrawal, despite a 60% drop in K in the root tissue. Amongst the genes that had altered expression in response to K-starvation was the KUP/HAK/KT transporter *AtHAK5*, which was consistently upregulated after 48 hours of K deprivation. Hampton *et al*. (2004) used microarrays to compare transcriptional changes in response to K-deficiency and cesium toxicity in roots and shoots. Armengaud *et al.* (2004) carried out microarray experiments looking at the changes in the *A. thaliana* root and shoot transcriptomes in response to longer term (14 days) K deprivation and subsequent resupply (6-24 hours). A large number of K-responsive genes were identified in this study, particularly those with functions in cell wall biochemistry, calcium signalling, plant defence, and JA signalling. To-date, no microarray studies looking at the effects of *R. secalis* infection on transcriptional profiles has been published.

6.1.2. Aims of chapter

In this chapter I report the effects of K-deficiency and/or *R. secalis* infection on gene regulation in barley plants. I also report genes that show a differential response to *R. secalis* infection in control and K-starved plants to identify possible mechanisms for the change in susceptibility to *R. secalis* in K-starved plants.

6.1.3. Experimental setup

The microarray experiment was set up in order to investigate changes in gene expression in response to K-deficiency and *R. secalis* and any interaction between the two responses. Four replicate experiments were carried out by growing and treating separate batches of plants. In each replicate experiment, mock-infected and *R. secalis*-infected leaf segments from control and K-starved plants were sampled 2 and 4 days after inoculation (Figure 6-1). Barley plants were grown in full nutrient control or K-free nutrient solution for 14 days. Detached leaf segments were prepared from the middle of the second leaf of each plant and inoculated with *R. secalis* spore solution as described previously (Materials and Methods section 2.6.1). Five segments were harvested and pooled 2 and 4 days after inoculation. RNA was prepared and labelled as described previously (Materials and Methods section 2.4.1 and 2.7).

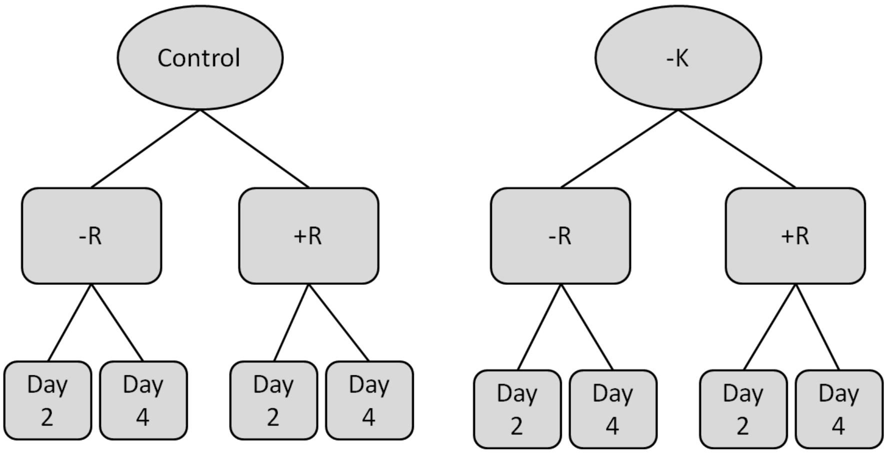


Figure 6-1 Experimental set up. Plants were grown in control or –K nutrient solution for 14 days, before the preparation of detached leaf segments from the middle of the second leaf. The leaf segments were inoculated with three 10 µL drops of 10^6 spores/ ml solution of *R. secalis* (+R) or mock inoculated with water. Five leaf segments were pooled from each set of conditions 2 and 4 days after inoculcation. The experiment was carried out in four replicates.

The SCRI_Hv35_44K_v1 (Agilent design 020599) microarray used here was designed based on the HarvEST assembly 35 and represents 42,302 barley sequences (for more information see Materials and Methods section 2.7.6). Two differently labelled RNA samples were hybridized to each array according to a balanced design. 16 arrays were used each with 2 samples making 32 samples in total.

6.2. Statistical properties of the microarray dataset

The Agilent Genespring software was used to carry out principal component analysis (PCA) on the raw data (dye signal intensity) obtained from the microarrays (Figure 6-2). A clear separation was observed between the control and K-starved samples, indicating that a large proportion of the variation in the data can be accounted for by K availability (Figure 6-2 A). Very little separation was observed based on *R. secalis* infection (Figure 6-2B) or days after inoculation with *R. secalis* (Figure 6-2C), an indication that these factors had less effect on the transcriptome of the barley plants than K availability. At the level of individual transcripts very few significant differences between the day 2 and day 4 samples were found; these samples were therefore treated as replicates during further analysis of the data (Sections 6.3. to 6.5.).

Due to the high number of samples, the microarray hybridization was carried out on two consecutive days with slides 29 and 30 hybridised on the first day and 31 and 32 on the second day. This could explain the observed separation between the data obtained from the two pairs of slides (Figure 2-6D). It is also worth noting that hybridization of the two pairs of slides was carried out by different people, which may also lead to variation due to different skill and experience. Furthermore, slides 29 and 30 were the last two slides from one box and 31 and 32 were taken from a different box, although all slides were from the same production batch and had been stored together. The technical variation between the two pairs of slides may obscure smaller changes induced by the treatments and future experiments should seek to avoid the possible sources of artefacts listed above. Prior to further analysis the data were normalised to the medium signal across all arrays.

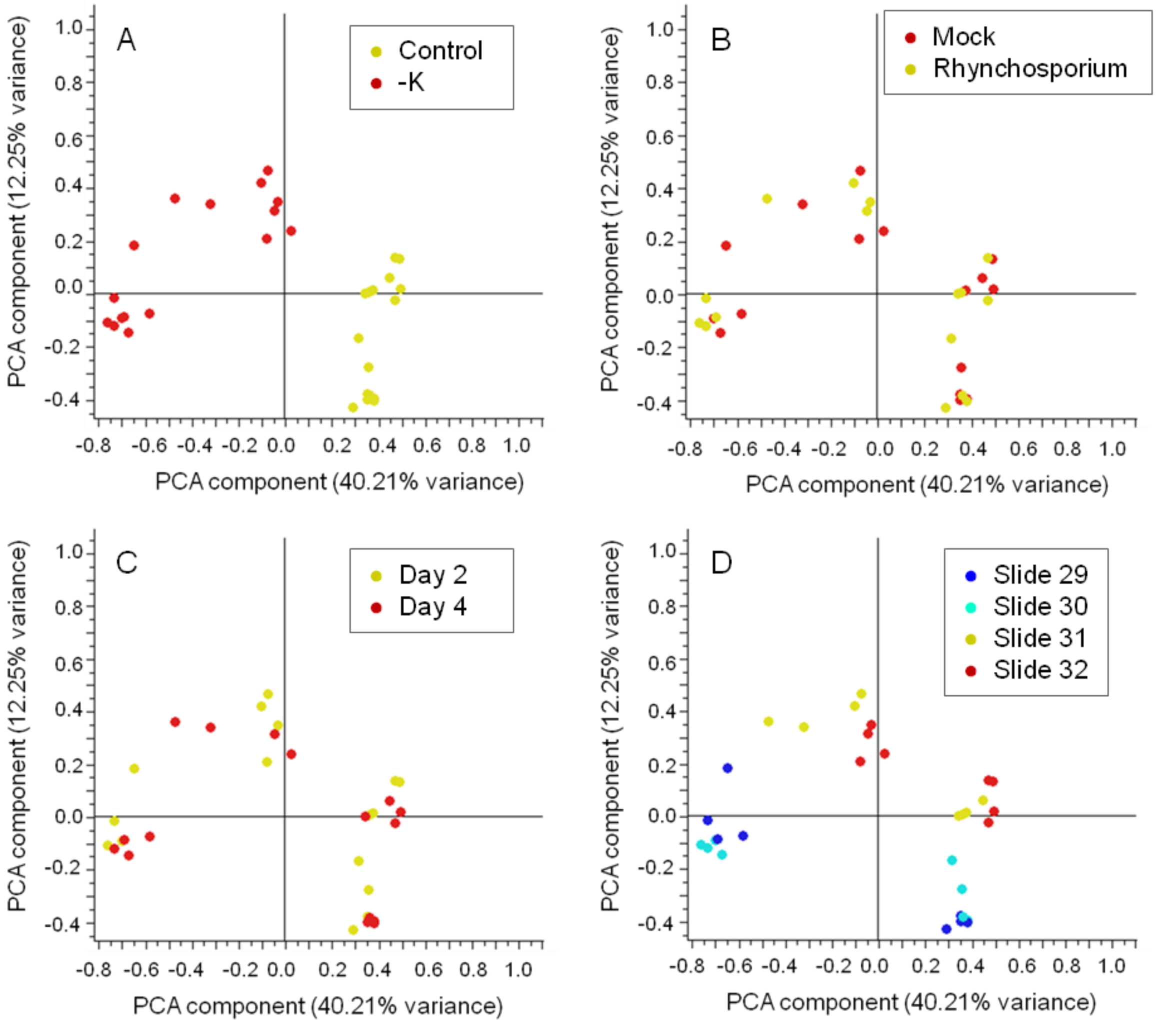


Figure 6-2 PCA analysis of microarray results. Principal component analysis (PCA) was carried out using the Agilent Genespring software. The grouping of the microarray results based on the effect of K treatment (A), *R. secalis* infection (B), time since inoculation (C) and microarray slide (D) are shown.

6.3. Changes in gene regulation in response to K-deficiency

To investigate changes in gene regulation in response to K-deficiency in both the *R. secalis* infected and mock infected tissue the data were subjected to a two-way ANOVA and a correction for multiple testing (Benjamini and Hochberg, 1995; Bonferroni, 1936) to obtain lists of differentially expressed genes with a given significance with respect to K treatment. The lists of differentially expressed genes contained 17002 genes with a p-value of less than 0.05, and 2736 genes with a p-value of less than 0.005. The latter was used for functional analysis.

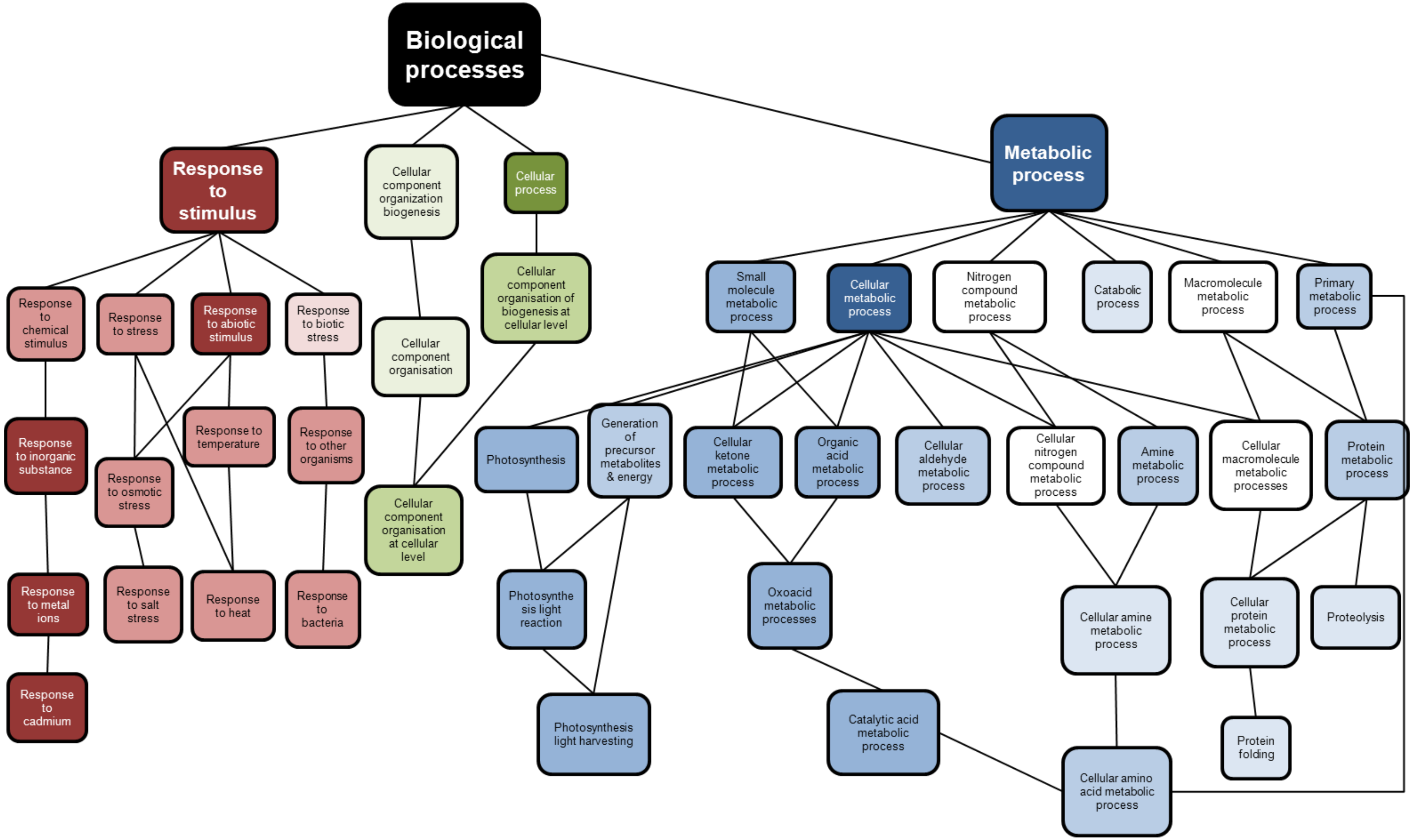


Figure 6-3 Biological processes affected by K deficiency in Barley.
The closest match to *A. thaliana* was identified for genes with altered expression levels in response to K-starvation when $p<0.005$, corrected for false discoveries using Bonferroni multiple testing correction. The data was entered into AmiGO GO term enrichment program. Three main categories were identified as changed in response to K-starvation response to stimuli (red), cellular processes (green) and metabolic processes (blue). The darker the shade the more significant the enrichment.

The closest protein match in the *A. thaliana* TAIR9 database was identified for genes with altered expression levels and was used to categorise the data into biological processes based on GO terms using Amigo17 GO terms enrichment tool (Ashburner *et al.*, 2000) with a p-value greater than 0.01 (Boyle *et al.*, 2004). A representation of the functional categories harbouring K-responsive genes is shown in Figure 6-3. The GO term analysis identified three broad categories of K-responsive genes: response to stimuli ($p=4.84e^{-25}$), cellular processes ($p=1.47e^{-17}$) and metabolic processes ($p=3.09e^{-25}$, Figure 6-3). The first category could be further split into a number to subcategories: response to abiotic stress, chemical stimulus and biotic stress. By far the most significant of these categories was response to abiotic stress which accounted for 9 % of the K-responsive transcripts ($p=4.35e^{-11}$). 48 % of the identified genes expressed differentially were involved in cellular processes, particularly in cellular component organisation or biogenesis. 47% of K-responsive genes were involved in metabolic processes such as photosynthesis and energy generation as well as the metabolic processing of nitrogen, organic acids, aldehydes, ketones and proteins.

In order to investigate the effect of K-deficiency on gene regulation in barley further, the gene list was split into genes that were upregulated and those that were downregulated in response to K-starvation using the GO terms with the Agilent Genespring software (For full gene lists seen Appendix 4, Tables S4-1 and S4-2). The lists obtained both contained a similar number of differentially expressed genes in response to K-starvation, with the 1282 genes upregulated and 1454 genes downregulated. However some of these genes were replicated and others did not have close matches in the Tair9 data base. The new lists were then entered into the Amigo GO terms enrichment program as described above.

6.3.1. Genes upregulated in response to K-deficiency

The genes that were upregulated in response to K-deficiency could be split into the same three categories identified earlier for all genes changed in response to K (Table 6-1), however there was a high level of overlap between the genes involved in cellular and metabolic processes, so these groups were combined to reduce repetition. The majority of the genes upregulated in response to low K were classified in this category of cellular and metabolic processes, which was largely made up of genes involved in cellular metabolic (35%) and primary metabolic processes (35%). The genes "upregulated in response to stimuli" formed a much smaller but highly significant group (p=1.94 x e^{-9}) which made up just 22% of all genes upregulated in response to K-deficiency. This category could be further split into three smaller but still highly significant groups of genes involved in response to abiotic stress (p=6.95 x e^{-9}), stress (p=3.50 x e^{-8}) and chemical stimuli (p=6.36 x e^{-8}).

It is worth noting that a number of JA related genes were upregulated in response to K-starvation in these experiments (marked in red in table 6-1). These include the JA biosynthesis genes *LOX2*, *AOS* and *AOC3* and *JAZ*, which suppresses JA related gene expression by *MYC2* (Chini *et al.*, 2007; Thines *et al.*, 2007). No barley *JIP* genes were identified as having altered regulation in response to K-starvation during this analysis, possibly due to differences in JA induced genes in *A. thaliana* and barley, although *JIP60* was part of the list of genes upregulated in response to K-starvation produced identified by the ANOVA.

K-starvation also lead to greater expression of a number of pathogen-related genes, including general pathogen-related genes encoding, for example, PR proteins and PAL. Two *Mlo* genes, *Mlo1* and *Mlo15*, were also upregulated in response to K-starvation.

Table 6-1 Gene categories upregulated upon K-deficiency. Genes upregulated in response to K-starvation with p values < 0.005 corrected for false discoveries using Bonferroni multiple testing correction. The best match in *A. thaliana* was identified and entered into the AmiGO GO term enrichment programme. JA-related genes are highlighted in red and selected pathogen related genes in blue.

Primary group	**Secondary Group**	**Genes upregulated after –K**
Response to stimuli	**Response to abiotic stimuli**	BPM2 GSTF10 AT1G70420 NPY2 AT5G66560 EB1C PAL1 GSTU19 ADH1 CP1 ABCB4 FAR1 PBA1 PHB3 LIP1 ZIFL1 AT1G03230 AT2G43090 COX6B PR4 GDH2 NIA1 AS1 P5CS1 SLT1 GLR2.8 SCR DRIP2 GAMMA CA2 RANGAP2 PP5.2 GLR3.3 AT5G23540 UGT74E2 SAT32 AT1G03220 AVP1 RSR4 PAL2 AT1G73230 ZTL AT2G39980 ERD9 NQR LOX2 GT72B1 PIP2A SOT12 UGT73C1 NFXL1 UGT78D2 TIL OSM34 Response to Temperature stimulus LOS1 CSDP1 HSP18.2 ZF3 ANNAT7 HSP17.4 RBOHD AOX1A MTHSC70-2 RHL41 BOB1 HSP70 CCR1 LOS2 HSP60 CPN10 HPT1 FES1 SUMO1 FRO1 HSP23.6-MITO DREB2A VTC2 MBF1C HVA22E HSP81-2 RPT2a AT5G51440 ANNAT2 TIL
	Response to stress	AOS ASA1 AT4G11290 LOS1 GSTF10 WRKY11 LOX1 CSDP1 HSP18.2 ZF3 PAL1 APX3 HSP17.4 PEPR1 AT3G09560 GSTU19 RBOHD AOX1A MLO15 JAZ1 POP2 WIN1 MTHSC70 BOB1 NPR3 PBF1 HSP70 PUB24 WRKY70 AT1G01170 TYRDC CCR1 AT5G55070 CRK19 HSP60 LCR68 RBP-DR1 AT3G54470 ALDH6B2 ELI3-2 AT1G72300 OPCL1 DRIP2 CPN10 HPT1 UGE1 PR1 ELI3-1 AT5G23540 RNS1 AlaAT1 MLO1 AT1G03220 AT2G15130 FES1 SUMO1 RAP2.2 HSP23.6-MITO PAL2 DREB2A PHB4 VTC2 LOX2 MBF1C MDAR1 CERK1 AT3G03270 RPT2a PIP2A AT5G51440 AT5G44380 SAR3 KIN10 AT5G05340. Response to osmotic/ salt stress BPM2 ZF3 ANNAT7 ADH1 CP1 FAR1 PBA1 PHB3 MTHSC70-2 RHL41 AT1G03230 AT2G43090 COX6B PR4 GDH2 AS1 P5CS1 SLT1 LOS2 GAMMA CA2 RANGAP2 AT5G23540 UGT74E2 SAT32 AT1G03220 AVP1

		FRO1 RSR4 AT1G73230 NQR GT72B1 HVA22E HSP81-2 SOT12 NFXL1 ANNAT2 TIL OSM34
	Response to chemical stimuli	AT4G33540 BPM2 PAP1 AOS ARF19 ASA1 AT4G11290 WRKY11 LOX1 HSP18.2 ZF3 ANNAT7 PAL1 APX3 PEPR1 PMZ GSH2 BRU6 ABCB4 JAZ1 WRKY46 PHB3 RHL41 PR4 GDH2 PDR11 PUB24 RALF23 WRKY70 P5CS1 AT5G55070 CRK19 AT3G53600 RBP-DR1 GRX480 RLK ALDH6B2 DRIP2 PDR12 PR1 UGT74E2 TPS1 AVP1 RAP2.2 RHM1 RSR4 PAL2 DREB2A UGT75B1 ERD9 VTC2 LOX2 GT72B1 MBF1C HVA22E HSP81-2 CERK1 PIP2A SOT12 AT5G44380 SAR3 NFXL1 UGT74F2 KIN10 ANNAT2 AMY1 AT5G05340 Response to metal ion GSTF10 ADK1 ROC2 AT1G56190 GSTU19 ADH1 AT2G41380 POP2 PBA1 MTHSC70-2 AT4G13780 AT5G65750 ATARCA GSTL3 GDH2 PAC1 HSP70 OASB AS1 LOS2 SYNC1 HSP60 AT3G54470 GSTU8 AT2G33590 CCoAOMT1 AlaAT1 GSTU13 HSP23.6-MITO ATS9 emb2742 MDAR1 TUA5 RPT2a AT1G07590 AT3G48990 GAD
Cellular and metabolic processes	**Small molecule metabolic processes**	PRMT3 FIB1 AT1G56190 HMG1 AT3G22910 AT5G09550 AT4G29680 GDI2 PRMT6 PFK3 AT3G54470 ALA2 PFK2 UGE1 AT5G08690 PLDALPHA2 TPK2 RHM1 PYRD TRM11 LOX2 TSBtype2 URH1 AT1G19340 Organic acid / cellular ketone metabolic process AOS THA2 ACX2 ASA1 AT1G75980 ATBCAT-5 LOX1 CER1 ACL CER4 PAL1 mtACP2 GSH2 FAR1 POP2 OMR1 ATKRS-1 AT4G13780 TSB2 PEX12 AT4G39280 OASB MTO1 TYRDC P5CS1 FAH1 OPCL1 AT2G20340 HPT1 AT1G51720 AT4G16800 NADP-ME4 AlaAT1 AOC4 OVA9 AT3G13930 GAD4 CHAT RSR4 UGT75B1 LACS4 AT4G31810 VTC2 LOX2 TSBtype2 AAE7 AAO3 GPAT5 NFXL1 UGT74F2
	Cellular metabolic processes	RPT1A AT1G67000 AT3G60210 AT1G54380 AT5G09380 RPL18 AOS THA2 AT4G31460 AT5G03030 ACX2 bZIP16 ASA1 PRMT3 FD3 AT5G09770 GSTF10 BIM2 AT1G75980 WRKY11 AT1G09640 ATBCAT-5 FBW2 FIB1 LOX1 CER1 CSDP1 AT4G25890 AT1G16040 ATS2 ROC2 AT1G56190 AT5G60390 ACL CER4 PAL1 PEPKR2 RLK4 AT2G34480 AT1G12010 PEPR1 ZYP1a UBP6 mtACP2 AT5G45775 GSH2 GSTU19 RBOHD ADH1 AT4G31860 ARP HMG1 RFC2 AT4G16710 AT5G35400 AOX1A AT5G55140 DFR AT2G37650 UBQ1 AT1G07070 ALDH2C4 NRPB2 WRKY46 FAR1 AT3G29010 AT1G70600 POP2 AT3G22910 SUS5 KIWI AT4G11160 AT5G60670 PBA1 AT2G34140 OMR1 ATKRS-1 AT1G16740 MTHSC70-2 ACLA-3 CKS1 UBQ6 CIPK12 ERF1-1 AT4G13780 AT5G09550 BOB1 WRKY57 AT1G74050 AT3G13580 TSB2 RSZ33 AT3G53740 AT2G32060 AT3G57490 AT5G39850 AT4G34670 PEX12 AT2G40010 AT5G20170 DGK5 emb2171 UGT73C6 PBF1 PAC1 AT4G29680 PAD2 RHL2 HSP70 AT4G39280 PUB24 GDI2 NIA1 PRMT6 CSTF64 OASB WRKY55 WRKY70 MTO1 HAI2 AT2G24360 TYRDC AS1 CCR1 ERO1 P5CS1 LAP3 CRK19 CHR1 AT1G74270 PFK3 AT3G53600 AT3G06680 AT5G52650 AT3G54470 EIF3G1 AT5G61170 UBP2 THY-2 FAH1 ERO2 UPL6 EMB1080 AT2G09990 AT1G72300 ALA2 OPCL1 EIF2 AT3G60340 RPN1A SAMDC RTL1 GAMMA CA2 CPN10 AT2G20340 HPT1 XTH29 GSTU8 PFK2 UGE1 AT1G51720 AT5G23540 RNS1 UGT74E2 AT5G06410 TPS1 AT4G16800 AT2G33590 AT1G01660 AT3G01800 NADP-ME4 CCoAOMT1 AlaAT1 AOC4 AT5G08690 CIP8 UFD1 PLDALPHA2 AT1G20370 AT2G04520 OVA9 AT3G13930 emb2386 GAD4 LPP3 CHAT PKDM7D UBA 2 AT1G54290 GSTU13 DMR6 CDT1A SUMO1 AT4G33865 TPK2 CKI1 RAP2.2 RHM1 ATS9 ACS2 RSR4 PBB2 PAL2 DREB2A PYRD NAC103 AT1G52300 AT4G15000 ATGSTF13 UGT75B1 ZTL TTN1 ERD9 AT3G56370 ARC6 LACS4 AT5G56940 AT4G31810 VTC2 AT5G24510 BRL1 TRM11 LOX2 rps15ae GT72B1 MBF1C ASK2 TPPF AT5G23535 NFU4 CRK3 MDAR1 TSBtype2 AT2G01250 HSP81-2 AAE7 RGLG1 AAO3 GPAT5 OTP80 emb1624 BB AT4G03230 RLK1 RPT2a URH1 PGY2 AT2G45730 AT5G62300 NFXL1 AT3G05560 AT4G27250 GSTF3 SLD5 AT3G04920 UGT74F2 AT3G16780 GFA2 SC35 PGY1 CRK34 DTX35 GSTU18 AT1G19340
	Catabolic processes	RPT1A THA2 ACX2 GSTF10 AT1G75980 FBW2 CER1 AT1G56190 PAL1 GSTU19 POP2 PBA1 UBQ6 AT5G09550 PEX12 PBF1 PAC1 PAD2 GDI2 LYM2 PFK3 UBP2 RPN1A GSTU8 PFK2 AT5G23540 AlaAT1 UFD1 AT3G14075 UBA 2 GSTU13 ATS9 PBB2 ATGSTF13 ZTL ERD9 ASD1 AT4G31810 GT72B1 ASK2 MDAR1 ftsh4 RPT2a URH1 GSTF3 ftsh3 GSTU18
	Primary metabolic processes	AT1G54380 AT5G09380 AOS THA2 AT5G56590 ACX2 bZIP16 ASA1 BGAL8 BIM2 AT1G75980 WRKY11 ATBCAT-5 FIB1 LOX1 CER1 CSDP1 AT1G28580 ATS2 AT1G56190 CER4 AT1G23460 ZYP1a mtACP2 AT3G09560 GSH2 ARP HMG1 RFC2 AT4G16710 AT5G35400 AT3G11210 AT2G37650 NRPB2 WRKY46 FAR1 POP2 AT3G22910 BGLU11 SUS5 KIWI AT2G34140 OMR1 CKS1 AT5G09550 WRKY57 TSB2 RSZ33 AT2G32060 AT4G34670 PEX12 AT5G20170 PAC1 AT4G29680 RHL2 GDI2 CSTF64 AT4G20430 OASB WRKY55 WRKY70 MTO1 TYRDC AS1 P5CS1 CHR1 AT5G45910 PFK3 AT3G53600 AT3G54470 UBP2 FAH1 3BETAHSD/D2 AT5G24318

	AT3G48950 AT1G09390 ALA2 OPCL1 RXF12 AT1G28600 RTL1 AT2G20340 HPT1 XTH29 PFK2 UGE1 AT1G51720 AT3G23510 TPS1 AT4G16800 NADP-ME4 AlaAT1 AOC4 AT5G08690 PLDALPHA2 AT1G20370 emb2386 GAD4 AT3G14075 LPP3 CHAT PKDM7D CDT1A RAP2.2 RHM1 RSR4 DREB2A PYRD NAC103 UGT75B1 LACS4 AT4G31810 ORP4B LOX2 AT3G26430 MBF1C TPPF TSBtype2 AAE7 AAO3 GPAT5 OTP80 AT3G55430 RPT2a URH1 SOT12 NFXL1 SLD5 UGT74F2 SC35 CWINV2 AT1G19340
	Protein metabolic process
	RPT1A AT1G67000 AT3G60210 RPL18 AT4G31460 AT5G03030 PRMT3 AT5G09770 AT3G61540 AT1G09640 FBW2 AT4G25890 AT1G16040 ROC2 AT5G60390 PEPKR2 RLK4 AT2G34480 PEPR1 UBP6 AT5G45775 AT4G31860 NCLPP7 AT5G55140 UBQ1 AT1G07070 AT3G29010 AT1G70600 AT4G11160 AT5G60670 PBA1 ATKRS-1 AT1G16740 MTHSC70-2 UBQ6 CIPK12 ERF1-1 AT4G13780 MC9 BOB1 DegP9 AT1G74050 AT3G13580 AT3G53740 AT2G32060 AT3G57490 AT5G39850 AT4G34670 AT2G40010 emb2171 PBF1 PAC1 PAD2 HSP70 AT4G39280 PUB24 PRMT6 AT4G20430 HAI2 AT2G24360 ERO1 CRK19 AT1G74270 AT3G06680 AT5G52650 XCP1 EIF3G1 AT5G61170 scpl2 UBP2 ERO2 UPL6 EMB1080 SCPL34 AT2G09990 AT1G72300 EIF2 AT3G60340 RPN1A CPN10 AT5G23540 AT1G67420 AT5G06410 AT3G59080 AT1G01660 AT3G01800 CIP8 UFD1 AT2G04520 OVA9 emb2386 UBA 2 AT1G54290 SUMO1 AT3G14067 AT4G33865 ATS9 PBB2 AT1G52300 AT4G15000 ZTL TTN1 AT3G56370 ARC6 SCPL51 AT5G56940 AT5G24510 BRL1 rps15ae ASK2 AT5G23535 CRK3 AT2G01250 HSP81-2 emb1624 BB AT5G19740 AT4G03230 RLK1 ftsh4 RPT2a scpl29 PGY2 AT2G45730 AT5G62300 AT3G05560 AT3G04920 AT3G16780 GFA2 PGY1 CRK34 ftsh3

6.3.2. Genes downregulated in response to K-deficiency

Of the genes downregulated in response to K-deficiency 48 % were assigned a function in metabolic processes (p=6.42 x e^{-16}) and 50 % were assigned a function in cellular processes (p=2.60 x e^{-13}) (Table 6-2). There was a considerable overlap between these two categories. The most significant sub-group within the genes with both functions was a small group of genes related to photosynthesis (p=3.71 e^{-20}) which makes up just 5 % of genes downregulated in response to K-starvation. Other functional assignments within the cellular/metabolic process group of downregulated genes were tetrapyrrole biosynthesis and glyceraldehyde-3-phosphate metabolism.

There were two JA related genes in the list of genes downregulated in response to K-deficiency (highlighted in red in table 6-2); *COI1* and JAR1. In *A. thaliana*, *COI1* is involved in the removal of *JAZ* from *MYC* allowing transcription of JA related genes (Chini *et al.*, 2007; Thines *et al.*, 2007). *JAR1* is involved in the formation of JA conjugates, catalysing the addition of Ile and ACC to JA (Staswick *et al.*, 1992; Staswick and Tiryaki, 2004).

Table 6-2 Gene categories downregulated upon K-deficiency. Genes downregulated in response to K-starvation with p values < 0.005 Corrected for false discoveries using Bonferroni multiple testing correction. The best match in *A. thaliana* was identified and entered into AmiGO GO term enrichment programme. JA-related genes are highlighted in red and selected pathogen related genes in blue.

Primary group	Secondary Group	Genes downregulated after –K
Metabolic process	**Primary metabolic process**	CAF1 WAK5 AT5G46390 SSI2 CRK24 SS4 RPL16 ISI1 SERAT1;1 BPEp ARF2 GSL12 AT5G14460 ENA AT5G39990 PLA2A ACC1 emb1067 AT3G22450 SEX4 AT2G35840 AT3G62110 FMO GS-OX5 SWN HDS AT2G03200 CRS1 LUT1 AT4G27270 CKL2 EMB2247 GLYR2 DWF4 AT1G01760 AT5G16810 PEPR1 AT1G29880 AT2G03500 LIP2 TLP18.3 AT4G24810 CEV1 CSP41A AK-HSDH II cpHsc70-1 AT1G55140 HMG1 AT5G50260 SIGA AT4G29100 AT5G16650 AT2G35390 PGL1 U1-70K RIF10 FTSH1 SD2-5 AT3G13040 AT3G27110 AT3G54210 ARA12 AT3G57120 EDD1 MYB48 ISPF AT5G10290 PPCK1 SEC AT5G04710 AT5G65240 AT1G76050 AT3G63490 AGL19 AT5G53340 LYC ftsh7 ELF5A-3 AT2G27420 AT4G17740 AT5G01720 UEV1D-4 AT2G24590 AT3G08980 AT1G20810 RAP2.9 TIM AT5G22080 AT1G36730 FTSH11 AT5G13240 HSL1 AT3G59350 AT1G66430 FBA2 KCS2 AT2G41710 KAS I AT1G80030 VAR1 ERF1-3 AT5G64380 GSTL2 HRS1 CSTF64 SD1-13 AT3G24530 AT2G34080 OTP51 SPL2 AT2G26800 AT3G12700 AT2G40120 GCP1 MYB59 SPPL2 EMB2761 LUT2 AT1G35340 AT5G18230 CHR1 AT4G29000 AT5G57610 AT3G48820 U2A' RXW8 CRR22 PHR2 3BETAHSD/D2 COI1 AT5G20220 OTP84 AT3G20230 AT5G21326 BAS1 AT2G39670 AT3G58140 GR CKB1 AT3G09830 AT3G15520 EMB2730 AT3G06950 AT1G67280 emb2768 OASA1 FBP7 AT2G42750 FPA ZFP7 NRPB5 WRKY20 BGAL13 AT5G59010 RNR1 GAPA-2 AT3G23750 AT2G31010 CPN60A TLP7 AT3G61790 JAR1 ZML1 WRKY3 LSF1 TPS1 UBC11 AT3G57190 MRL1 AT1G70820 AT5G18610 AT5G17670 AT1G30680 AT5G37930 AT2G43560 AT5G35100 AT2G01060 PDH-E1 ALPHA AT1G63770 PK1B SCPL19 UBC5 AT1G71070 HCF109 AT5G59700 FC2 AT5G62620 MHK SPO11-2 OTP81 AT2G13440 AT2G02570 CRK10 CPK1 AT2G19940 AT4G31390 AT4G26555 HPL1 CDT1A AT4G39780 OVA4 AT3G17410 SMO1-1 AT5G66530 DEG8 UBC32 AT2G44830 AT5G22850 TLP3 RAP2.12 AVA-P2 AT4G25290 NADK3 AT1G71810 ARAD1 GS2 AT1G20650 ISA3 AT2G03390 AT3G06180 GME ASD1 AT1G73170 NRPE5 AT4G36195 RPS7.1 AT4G38960 CIPK4 NOA1 AT3G13120 SIG2 EMB1030 AT5G52010 GATB AT1G60230 CYP97A3 AT5G58300 AT1G77020 AT4G37510 HB-1 AUR3 TPPD AT5G35170 ORP1D RPL12-C CRSH EGY2 XPL1 AT3G27180 AT3G59890 EBS AT4G09350 AT4G19830 CPHSC70-2EAT SHOCK PROTEIN 70-2 AT4G36390 KDSB RPL15 AK-HSDH I AT2G28970 CRK26 AT3G10060 PHOT2 AT2G33550 AT3G05350 OASC DET1 AT5G52660 emb2726 RAP2.4 AT1G12230 DEGP1 OTP86 AT5G13510 AT2G33800 CRT3
Metabolic process	**Primary metabolic process**	CAF1 WAK5 AT5G46390 SSI2 CRK24 SS4 RPL16 ISI1 SERAT1;1 BPEp ARF2 GSL12 AT5G14460 ENA AT5G39990 PLA2A ACC1 emb1067 AT3G22450 SEX4 AT2G35840 AT3G62110 FMO GS-OX5 SWN HDS AT2G03200 CRS1 LUT1 AT4G27270 CKL2 EMB2247 GLYR2 DWF4 AT1G01760 AT5G16810 PEPR1 AT1G29880 AT2G03500 LIP2 TLP18.3 AT4G24810 CEV1 CSP41A AK-HSDH II cpHsc70-1 AT1G55140 HMG1 AT5G50260 SIGA AT4G29100 AT5G16650 AT2G35390 PGL1 U1-70K RIF10 FTSH1 SD2-5 AT3G13040 AT3G27110 AT3G54210 ARA12 AT3G57120 EDD1 MYB48 ISPF AT5G10290 PPCK1 SEC AT5G04710 AT5G65240 AT1G76050 AT3G63490 AGL19 AT5G53340 LYC ftsh7 ELF5A-3 AT2G27420 AT4G17740 AT5G01720 UEV1D-4 AT2G24590 AT3G08980 AT1G20810 RAP2.9 TIM AT5G22080 AT1G36730 FTSH11 AT5G13240 HSL1 AT3G59350 AT1G66430 FBA2 KCS2 AT2G41710 KAS I AT1G80030 VAR1 ERF1-3 AT5G64380 GSTL2 HRS1 CSTF64 SD1-13 AT3G24530 AT2G34080 OTP51 SPL2 AT2G26800 AT3G12700 AT2G40120 GCP1 MYB59 SPPL2 EMB2761 LUT2 AT1G35340 AT5G18230 CHR1 AT4G29000 AT5G57610 AT3G48820 U2A' RXW8 CRR22 PHR2 3BETAHSD/D2 COI1 AT5G20220 OTP84 AT3G20230 AT5G21326 BAS1 AT2G39670 AT3G58140 GR CKB1 AT3G09830 AT3G15520 EMB2730 AT3G06950 AT1G67280 emb2768 OASA1 FBP7 AT2G42750 FPA ZFP7 NRPB5 WRKY20 BGAL13 AT5G59010 RNR1 GAPA-2 AT3G23750 AT2G31010 CPN60A TLP7 AT3G61790 JAR1 ZML1 WRKY3 LSF1 TPS1 UBC11 AT3G57190 MRL1 AT1G70820 AT5G18610 AT5G17670 AT1G30680 AT5G37930 AT2G43560 AT5G35100 AT2G01060 PDH-E1 ALPHA AT1G63770 PK1B SCPL19 UBC5 AT1G71070 HCF109 AT5G59700 FC2 AT5G62620 MHK SPO11-2 OTP81 AT2G13440 AT2G02570 CRK10 CPK1 AT2G19940 AT4G31390 AT4G26555 HPL1 CDT1A AT4G39780 OVA4 AT3G17410 SMO1-1 AT5G66530 DEG8 UBC32 AT2G44830 AT5G22850 TLP3 RAP2.12 AVA-P2 AT4G25290 NADK3 AT1G71810 ARAD1 GS2 AT1G20650 ISA3

		AT2G03390 AT3G06180 GME ASD1 AT1G73170 NRPE5 AT4G36195 RPS7.1 AT4G38960 CIPK4 NOA1 AT3G13120 SIG2 EMB1030 AT5G52010 GATB AT1G60230 CYP97A3 AT5G58300 AT1G77020 AT4G37510 HB-1 AUR3 TPPD AT5G35170 ORP1D RPL12-C CRSH EGY2 XPL1 AT3G27180 AT3G59890 EBS AT4G09350 AT4G19830 CPHSC70-2EAT SHOCK PROTEIN 70-2 AT4G36390 KDSB RPL15 AK-HSDH I AT2G28970 CRK26 AT3G10060 PHOT2 AT2G33550 AT3G05350 OASC DET1 AT5G52660 emb2726 RAP2.4 AT1G12230 DEGP1 OTP86 AT5G13510 AT2G33800 CRT3
	Pigment biosynthesis process	LUT1 AT1G74470 CH1 GUN5 RIF10 ISPF LYC POR C LUT2 OMT1 PCB2 HEMA1 CYP97A3
	Cellular metabolic processes	CAF1 WAK5 GCH THIC FdC1 SSI2 CRK24 SS4 RPL16 SERAT1;1 BPEp ARF2 GSL12 AT5G14460 ENA PLA2A ACC1 emb1067 AT3G22450 SEX4 AT2G35840 FMO GS-OX5 SWN HDS CRS1 LUT1 EFE AT4G27270 CKL2 AT1G74470 EMB2247 GLYR2 AT1G01760 AT5G16810 PEPR1 AT1G29880 AT2G03500 LIP2 CH1 AT4G24810 CEV1 CSP41A AK-HSDH II cpHsc70-1 DGK7 AT1G55140 HMG1 SIGA AT4G29100 GUN5 AT5G16650 PDX2 AT2G35390 PGL1 U1-70K RIF10 SD2-5 CAT2 AT3G13040 IMPL1 AT3G54210 ARA12 AT3G57120 EDD1 MYB48 ISPF AT5G10290 PPCK1 SEC AT5G65240 AT4G28830 AT1G76050 AT3G63490 AGL19 AT1G29810 AT5G53340 TPK1 LYC ELF5A-3 SBP1 AT5G01720 UEV1D-4 AT2G24590 FMN/FHY AT1G20810 HEMD RAP2.9 AAE14 POR C CKX1 AT5G22080 AT1G36730 AT5G22020 FTSH11 AT5G13240 HSL1 AT3G59350 AT1G66430 FBA2 KCS2 AT2G41710 KAS I AT1G80030 ERF1-3 AT4G25310 GSTL2 HRS1 CSTF64 SD1-13 NTRC SPL2 AT2G26800 CKX6 AT2G40120 MYB59 EMB2761 AT5G18230 NIA2 CHR1 OMT1 AT4G29000 GLY2 AT5G57610 AT3G48820 U2A' CRR22 PHR2 MPK6 COI1 AT5G20220 OTP84 AT3G20230 AT5G21326 AT2G39670 AT3G58140 GR CKB1 AT3G09830 AT3G15520 EMB2730 AT3G06950 emb2768 OASA1 FBP7 AT2G42750 FPA ZFP7 NRPB5 WRKY20 AT5G59010 RNR1 PCB2 HEMA1 GAPA-2 AT3G23750 AT2G31010 CPN60A TLP7 AT3G61790 JAR1 ZML1 WRKY3 LSF1 YUC8 TPS1 UBC11 AT3G57190 MRL1 AT5G18610 AT5G17670 AT1G30680 AT5G37930 AT2G43560 ALDH3F1 AT5G35100 AT2G01060 PDH-E1 ALPHA PK1B RBOH F UBC5 HCF109 AT5G59700 ECHID FC2 AT5G62620 MHK SPO11-2 OTP81 AT2G13440 AT2G02570 CRK10 CPK1 AT2G19940 AT4G31390 AT4G26555 HPL1 CDT1A AT4G39780 OVA4 AT3G17410 AT5G66530 UBC32 AT2G44830 TLP3 RAP2.12 AVA-P2 AT4G25290 NADK3 AT1G71810 ARAD1 GS2 AT1G20650 ISA3 AT2G03390 AT3G06180 GME NRPE5 RPS7.1 AT4G38960 CIPK4 NOA1 AT3G13120 CAX3 SIG2 EMB1030 AT5G52010 GATB LIN2 AT1G60230 CYP97A3 AT5G58300 AT1G77020 AT4G37510 HB-1 AUR3 TPPD AT5G35170 RPL12-C CRSH XPL1 AT3G27180 AT3G59890 EBS AT4G09350 AT4G19830 CPHSC70-2EAT SHOCK PROTEIN 70-2 AT4G36390 KDSB AGO1 RPL15 AK-HSDH I REF4 AT2G28970 CRK26 AT3G10060 PHOT2 AT2G33550 OASC DET1 AT5G52660 emb2726 RAP2.4 OTP86 AT5G13510 AT2G33800 CRT3 Photosynthesis NDF2 LHCB2.2 TLP18.3 PSAF AT1G77090 NDHF FTSH1 LHCA3 AGY1 TIM LHB1B2 PGR5-LIKE A VAR1 TROL LHCB2.3 PSBP-1 OTP51 FNR1 LUT2 LHCA2 LPA1 PSBC AT4G30720 AT5G11450 PSBD AT1G76450 ABC4 DEG8 LHB1B1 LHCB4.1 STN8 YCF9 PPL2 LHCA1 PQL3 DEGP1 AT3G56650 CPFTSY
Cellular Process	**Cellular component organisation**	FAB1A PEX14 AT1G48900 SCC3 SWI3C PTAC4 CcdA CRR6 CCB4 APG2 ELF5A-3 AGY1 TIM TIC110 TUA6 NDH-O CPSRP54 ERF1-3 OTP51 JAC1 CHR1 SCY1 LPA1 CRL ABIL1 EMB2730 AT5G58260 CPN60A AT3G57190 ARO1 HCF109 PFI HCF136 SPO11-2 CDT1A ATK5 VAM3 AT2G30620 CRR7 NOA1 SCO1 AUR3 PHOT2 CPFTSY
	Protein complex subunit organisation	AT1G48900 CcdA CRR6 CCB4 ELF5A-3 NDH-O CPSRP54 ERF1-3 OTP51 LPA1 ABIL1 AT5G58260 AT3G57190 HCF109 PFI HCF136 CRR7

6.4. Changes in defence mechanisms during K-starvation

In order to investigate the way in which K-deficiency affects defence signalling, changes in JA-related and selected other defence related genes in response to both K-starvation and *R. secalis* were looked at in more detail.

JA biosynthesis genes (*LOX2*, *AOC3*, *AOS* and *OPR3*) and a Jasmonate-induced protein, JIP60, featured in the list of genes upregulated in response to K-starvation with a p-value of less than 0.05 (Figure 6-4). The microarray data confirmed the higher JA-related gene expression in K-starved plants observed by qPCR (chapter 4). The microarrays also indicated a lower expression of *JIP23* in response to –K, which showed high levels of variation in response to K-starvation in the qPCR measurements.

Four further JA-related genes were identified as differentially expressed in response to –K: coronatine-insensitive protein (*COI1*), Jasmonate ZIM domain (*JAZ*), Jasmonate resistant 1 (*JAR1*) and jasmonate O-methyltransferase (*JMT*). The COI1 and JAZ proteins are both involved in the JA sensing by the SCF^{COI1} complex (Chini *et al.*, 2007; Thines *et al.*, 2007) JAZ is involved in the suppression of the transcription factor MYC2 in *A. thaliana*. In barley the *JAZ* transcript was elevated in the K-starved samples compared to the control regardless of *R. secalis* infection. COI1 in *A. thaliana* forms part of the protein complex which removes the suppression by JAZ in response JA-Ile allowing the transcription of JA responsive genes. In barley, *COI1* transcript was lower in the K-starved plants compared with the control plants in both infected and uninfected samples.

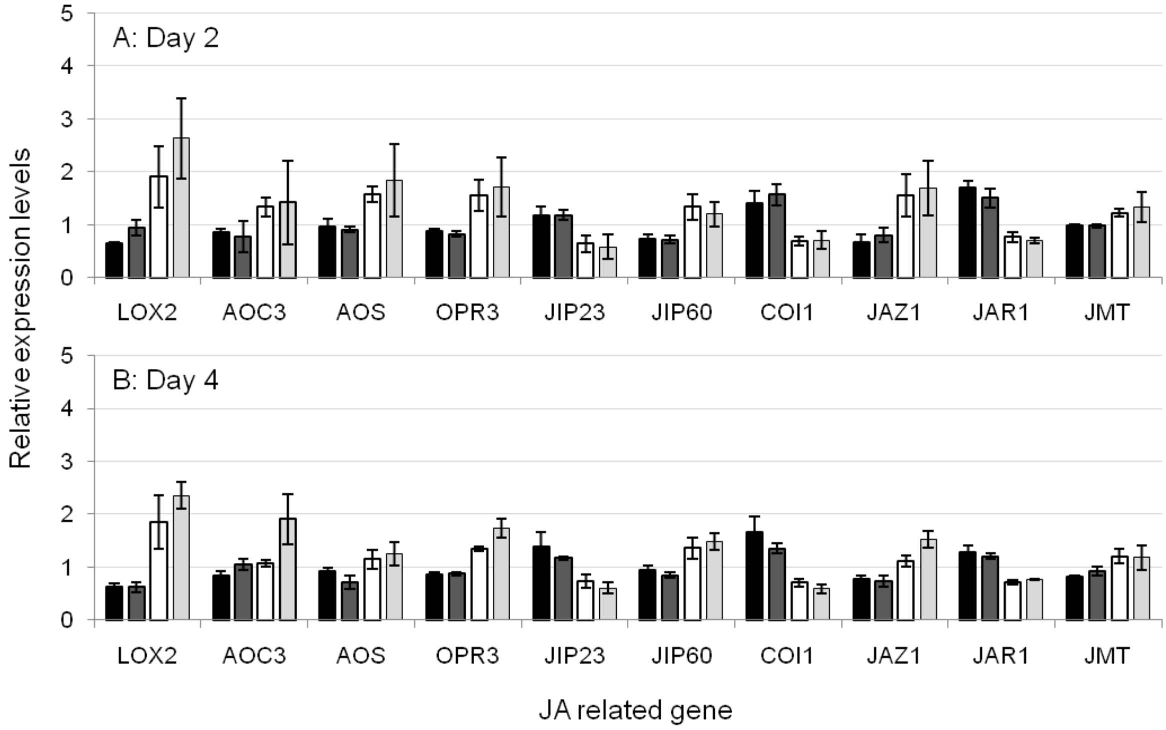


Figure 6-4 Changes in JA related gene expression in response to –K and *R. secalis*. Expression profiles of JA related genes from data obtained by microarray analysis of control mock infected (■), control *R. secalis* infected (■), -K mock infected (□) and –K *R. secalis* infected (■) samples at 2 (A) and 4 (B) days after infection. The mean (± SE) of 4 replicate experiments are shown.

The remaining two JA-related genes are both involved with formation of JA conjugates. JAR1 catalyses the addition of an isoleucine group to JA resulting in the formation of Jasmonate-isoleucine (JA-Ill) and is suppressed during K-starvation in barley. JMT adds a methyl group to JA forming Methyl Jasmonate (Me-JA); slightly higher *JMT* transcript was observed in the K-starved samples.

No change was seen in the expression of any JA-related gene in response to *R. secalis* in either the control or K-starved tissue on day 2. By day 4 slightly higher *AOC, OPR3* and *JAZ* was observed in response to *R. secalis* infection in the K-starved plants only.

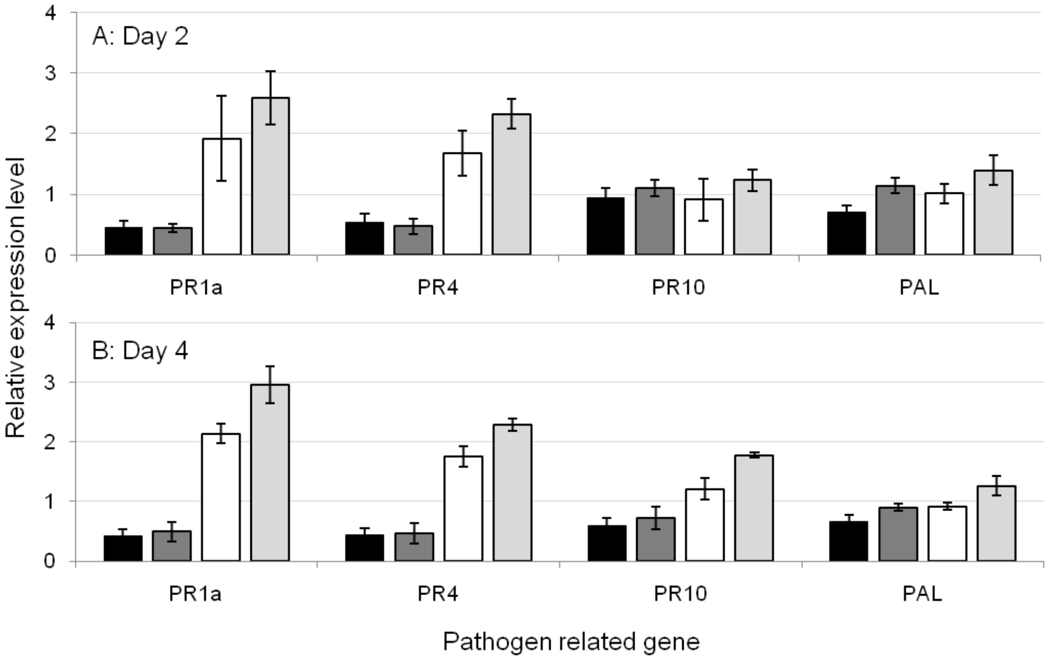


Figure 6-5 Changes in defence related gene expression in response to –K and *R. secalis*. Expression profiles of defence related genes from data obtained from the microarray analysis of control mock infected (■), control *R. secalis* infected (▩), -K mock infected (□) and –K *R. secalis* infected (░) samples at 2 and 4 days after infection. The mean (± SE) of 4 replicate experiments are shown.

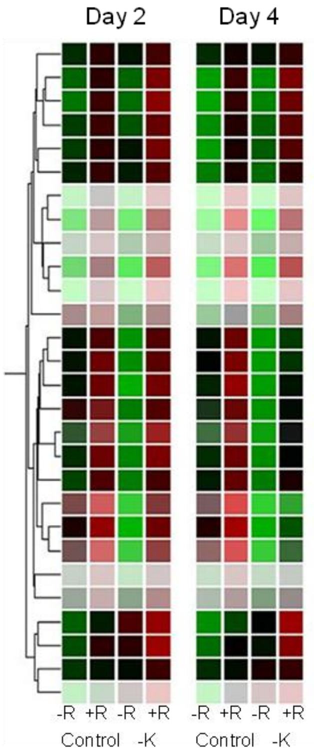
The changes in the expression of four pathogen responsive genes in the four treatments were also examined (Figure 6-5), these included Pathogen related (*PR*) genes as well as Phenylalanine ammonia lyase (*PAL*). The expression profiles of *PR1a* and *PR4* were similar; both were upregulated in response to K-starvation in both infected and uninfected tissue. There was little change in *PR1* or *PR4* expression in response to *R. secalis* in the control samples, however, by day 4 the expression in the *R. secalis* infected K-starved samples was higher than that of the mock inoculated K-starved samples. Little change was seen in the abundance of *PR10* transcript 2 days after inoculation, however by 4 days after inoculation the expression was higher in the K-starved samples compared with the control, the largest change in the *R. secalis* infected tissue. The *PAL* response to both K-starvation and *R. secalis* infection was small, however, higher expression was measured in the K-starved samples regardless of infection at both time points. Higher expression was also seen in response to *R. secalis* infection in both control and K-starved samples. The changes were accumulative resulting in the infected K-starved samples having the highest *PAL* expression of all the samples.

6.5. Changes in gene regulation in response to *R. secalis* infection

The ANOVA also identified genes that demonstrated a change in expression in response to *R. secalis* infection with a p-value of less than 0.05. 28 transcripts were identified as being significantly changed in response to *R. secalis* infection (Figure 6-6 and Appendix 4, Table S4-3).

Three main functional categories of genes in this group were blue copper proteins (*BCP*), germin like proteins and a group of fungal proteins. BPCs are copper-binding proteins with electron carrier activity (Jansen *et al.*, 2005). Germin-like proteins have been identified in defence mechanisms against a number of plant pathogens including Bgh, *R. secalis* and rice blast (Hurkman and Tanaka, 1996; Zhang *et al.*, 1995) and they could be involved in the reactive oxygen species (ROS) response. The final group of proteins with altered expression in *R. secalis* infected tissue compared to the mock infected tissue was a group of fungal proteins, which might have been included on the microarray because of extraction from contaminated barley tissue. The hybridization signal is likely to derive from *R. secalis* present in the infected leaf segments.

Other genes with altered expression in response to *R. secalis* include genes homologous to the rice vacuolar sorting receptor protein, PUP3 (a purine transporter), an electron transporter and a peroxidase 1 precursor.



Best hit NCBI nr	Best hit Rice PP5	Description	Top TAIR9 protein hit	P-value
gb|EAZ31950.1| 7e-61 hypothetical protein OsJ_015433 [Oryza sativa]	LOC_Os04g52190.1 2e-62 vacuolar sorting receptor 7 precursor putative expressed	U35_44k_v1_23158	AT1G30900	0.0417
ref|NP_001060957.1| 4e-39 Os08g0137800 [Oryza sativa]	LOC_Os08g04340.1 8e-41 blue copper protein precursor putative expressed	U35_44k_v1_16426	AT2G32300	0.00491
ref|NP_001060957.1| 1e-31 Os08g0137800 [Oryza sativa]	LOC_Os08g04340.1 2e-33 blue copper protein precursor putative expressed	U35_44k_v1_16427	AT3G27200	4.19E-05
ref|NP_001064000.1| 6e-37 Os09g0572700 [Oryza sativa]	LOC_Os09g39940.1 1e-38 blue copper protein precursor putative expressed	U35_44k_v1_7374	AT2G31050	0.0036
gb|EAZ25868.1| 1e-42 hypothetical protein OsJ_009351 [Oryza sativa]	LOC_Os03g08880.1 3e-44 ATPUP3 putative expressed	U35_44k_v1_22645	AT1G28220	0.00491
gb|ABU56004.1| 9e-39 blue copper-binding protein [Dasypyrum villosum]	LOC_Os09g39940.1 2e-35 blue copper protein precursor putative expressed	U35_44k_v1_15289	AT1G22480	0.00491
ref|XP_001913063.1| 5e-18 unnamed protein product [Podospora anserina]	LOC_Os02g14059.1 1e-18 60S ribosomal protein L11-1 putative expressed	U35_44k_v1_45719	AT5G45775	0.00731
ref|XP_391117.1| 6e-46 hypothetical protein FG10941.1 [Gibberella zeae PH-1]	LOC_Os05g06770.1 2e-41 40S ribosomal protein S27a putative expressed	U35_44k_v1_13667	AT2G47110	6.45E-05
ref|XP_387656.1| 1e-94 hypothetical protein FG07480.1 [Gibberella zeae PH-1]	LOC_Os12g21798.1 1e-66 40S ribosomal protein S3a putative expressed	U35_44k_v1_35988	AT4G34670	0.000172
ref|XP_388987.1| 2e-48 EF1A_TRIRE ELONGATION FACTOR [Gibberella zeae PH-1]	LOC_Os03g08060.2 5e-37 elongation factor 1-alpha putative expressed	U35_44k_v1_12026	AT5G60390	2.05E-05
ref|XP_382718.1| e-100 hypothetical protein FG02542.1 [Gibberella zeae PH-1]	LOC_Os08g13690.1 1e-57 60S ribosomal protein L7-1 putative expressed	U35_44k_v1_9648	AT2G44120	4.47E-06
No hits found	No hits found	U35_44k_v1_9182		0.0417
gb|ABG46235.1| e-119 germin-like protein 4c [Hordeum vulgare subsp. vulgare]	LOC_Os08g08990.1 1e-101 germin-like protein subfamily 1 member 11 precursor putative	U35_44k_v1_14950	AT5G39110	0.0417
gb|ABG46235.1| e-117 germin-like protein 4c [Hordeum vulgare subsp. vulgare]	LOC_Os08g08990.1 2e-99 germin-like protein subfamily 1 member 11 precursor putative	U35_44k_v1_14954	AT5G39110	0.00619
gb|ABG46236.1| 2e-13 germin-like protein 4d [Hordeum vulgare subsp. vulgare]	LOC_Os12g05870.1 4e-15 germin-like protein subfamily 1 member 8 precursor putative	U35_44k_v1_33564		0.00999
gb|EAZ05530.1| 9e-36 hypothetical protein OsI_026762 [Oryza sativa]	LOC_Os08g04350.1 2e-37 uclacyanin-2 precursor putative expressed	U35_44k_v1_4430	AT2G32300	0.0161
ref|NP_001061164.1| 2e-78 Os08g0190100 [Oryza sativa]	LOC_Os08g09080.2 6e-80 germin-like protein subfamily 1 member 11 precursor putative	U35_44k_v1_13229	AT5G39110	0.00491
gb|AAG00425.1|AF250933_1 1e-89 germin A [Hordeum vulgare]	LOC_Os08g08990.1 3e-75 germin-like protein subfamily 1 member 11 precursor putative	U35_44k_v1_14956	AT5G39110	0.000615
gb|EAY85319.1| 0.0 hypothetical protein OsI_006552 [Oryza sativa]	LOC_Os02g17090.1 0.0 cucumisin precursor putative expressed	U35_44k_v1_16231	AT4G10540	0.0036
ref|NP_001063614.1| 5e-88 Os09g0507300 [Oryza sativa]	LOC_Os09g32952.1 9e-90 L-ascorbate oxidase precursor putative expressed	U35_44k_v1_6811	AT4G39830	0.0231
gb|AAG00425.1|AF250933_1 e-122 germin A [Hordeum vulgare]	LOC_Os08g08990.1 1e-105 germin-like protein subfamily 1 member 11 precursor putative	U35_44k_v1_14951	AT5G39110	0.0183
gb|EAY96461.1| 1e-31 hypothetical protein OsI_017694 [Oryza sativa]	LOC_Os05g04490.1 4e-33 peroxidase 1 precursor putative expressed	U35_44k_v1_12830	AT2G18140	0.0124
No hits found	LOC_Os01g42960.1 7e-06 electron transporter putative expressed	U35_44k_v1_30734		0.0177
gb|EAZ34182.1| 4e-13 hypothetical protein OsJ_017665 [Oryza sativa]	LOC_Os05g32440.1 5e-15 expressed protein	U35_44k_v1_31426		0.00491
gb|EAY94895.1| 2e-85 hypothetical protein OsI_016128 [Oryza sativa]	LOC_Os04g44250.1 4e-87 cytokinin-O-glucosyltransferase 3 putative expressed	U35_44k_v1_8568	AT2G36790	0.0344
ref|NP_001047373.1| e-125 Os02g0605900 [Oryza sativa]	LOC_Os02g39330.1 1e-127 endochitinase PR4 precursor putative expressed	U35_44k_v1_20965	AT3G54420	0.0183
sp|A2Z7B3|GME1_ORYSI 0.0 GDP-mannose 3,5-epimerase 1	LOC_Os10g28200.1 0.0 GDP-mannose 35-epimerase 1 putative expressed	U35_44k_v1_462	AT5G28840	0.0344
gb|AAF34811.1|AF005089_1 9e-26 oxalate oxidase [Triticum aestivum]	LOC_Os08g08980.1 1e-23 germin-like protein subfamily 1 member 7 precursor putative	U35_44k_v1_31611		0.0256

Figure 6-6 Altered gene expression in response to *R. secalis*. Genes with changes in expression levels in response to Rhynchosporium infection at P<0.05 were sorted using 3 main categories of genes could be identified blue copper proteins (🟦), genes from fungi (🟨) and germin like proteins (🟩).

Genes encoding four blue copper proteins were shown to have significantly altered transcript levels in response to *R. secalis* infection. All four responded to both K status and *R. secalis* infection in a similar way (Figure 6-7), it is possible that they are replicates of the same gene, however the closest homolog in rice and *A. thaliana* are not the same for each (Figure 6-6). The blue copper protein genes showed little change in expression in response to K-deficiency. The expression of these genes was significantly higher in the *R. secalis* infected tissue compared to the mock infected samples for both K-free and control tissue (for p-values see Figure 6-6). Despite there being no significant change in the expression of blue copper protein genes in response to K-deficiency, the K-free plants showed a stronger response to *R. secalis* at both time points for all the blue copper protein.

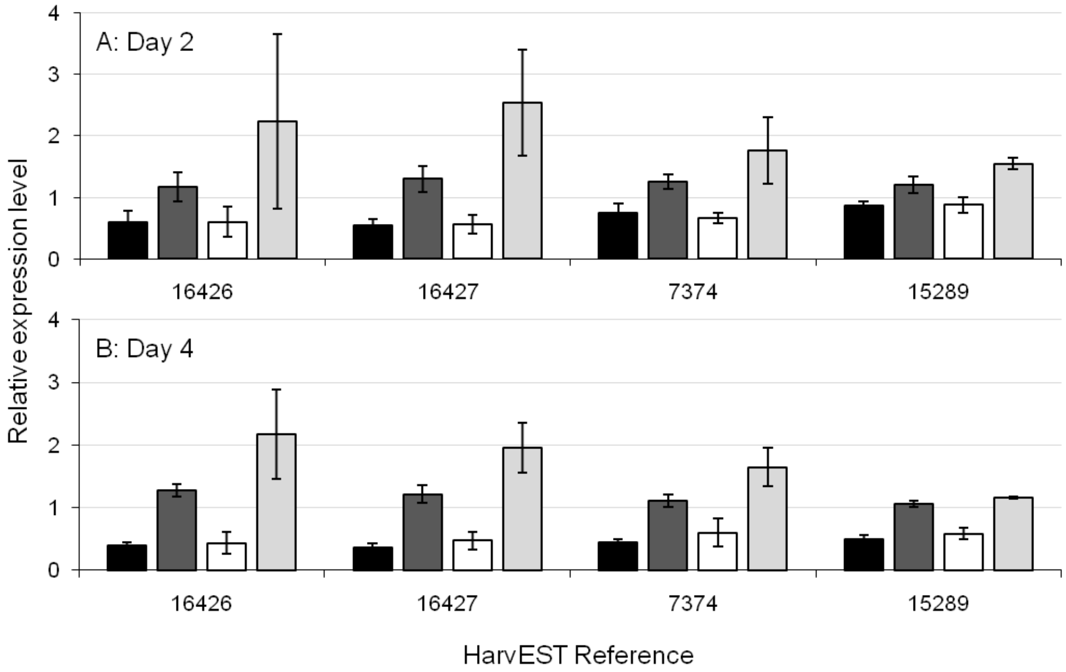


Figure 6-7. Response patterns of blue copper protein genes with altered expression in response *R. secalis* infection. Expression profiles of genes with altered expression in response to both –K and *R. secalis* from data obtained from the microarray analysis of control mock infected (■), control *R. secalis* infected (■), -K mock infected (□) and –K *R. secalis* infected (■) samples at 2 and 4 days after infection. The mean (± SE) of 4 replicate experiments are shown.

Seven transcripts encoding germin like proteins (GLPs) were identified by the microarray experiment as being upregulated in response to *R. secalis* infection (Figure 6-8). Six of these proteins can be classed as type 4 GLPs, which can be further split in to GLP 4d (DQ647623) and GLP 4c (DQ647622). Sequence alignment using Clustal W (Chenna *et al.*, 2003) showed that the *4c GLP* sequences were very similar and are likely to be replicates of the same transcript. All the *GLP type 4* genes showed similar expression patterns. Lower expression was seen in K-free samples compared to the control samples for both time points. *R. secalis* infection resulted in higher transcript in both K-free and control tissue, but this was much smaller in the K-deficient plants than in the control 4 days after inoculcation indicating that K-deficiency leads to a faster decline of a transient response of *GLP*s to *R. secalis* infection.

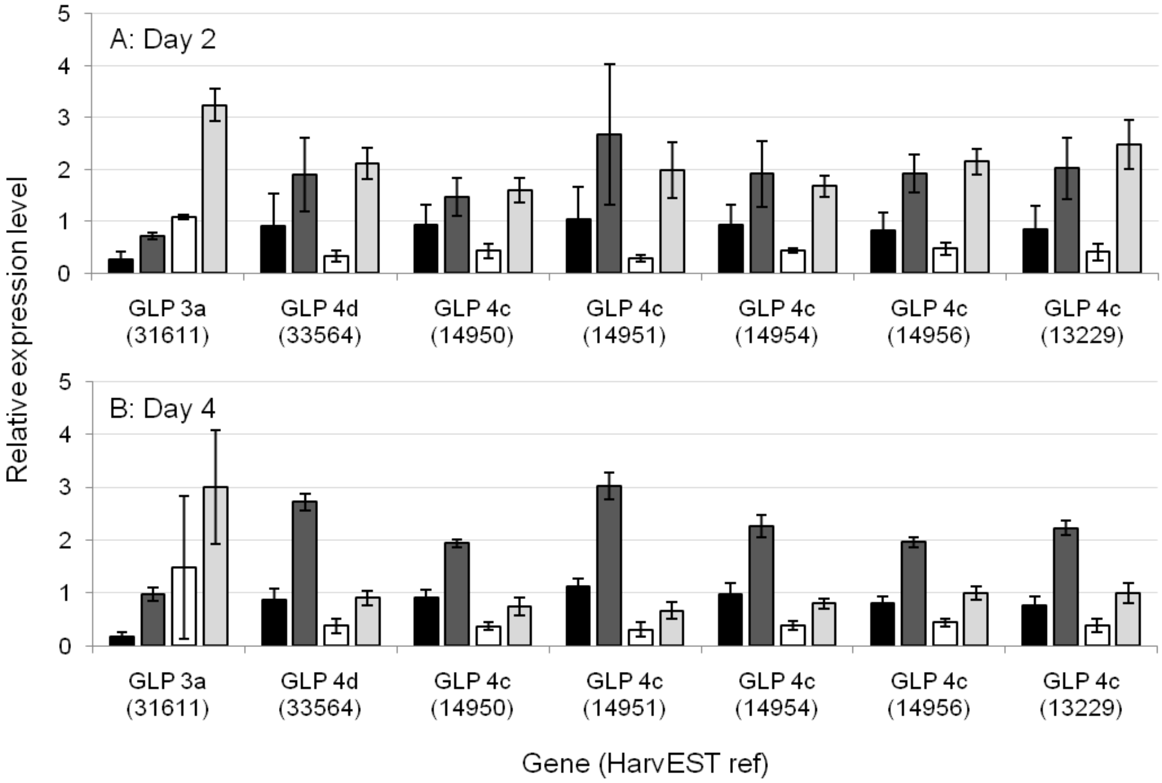


Figure 6-8. Response patterns of germin like protein genes with altered expression in response *R. secalis* infection. Expression profiles of genes with altered expression in response to both –K and *R. secalis* from data obtained from the microarray analysis of control mock infected (■), control *R. secalis* infected (■), -K mock infected (□) and –K *R. secalis* infected (■) samples at 2 and 4 days after infection. The mean (± SE) of 4 replicate experiments are shown.

The *GLP3a* gene showed a very different response pattern to the *type 4 GLPs*. Higher *GLP3a* transcript was observed in response to K-deficiency on both day 2 (4-fold) and 4 (8-fold) in the uninfected tissue. *R. secalis* infection also led to higher *GLP3a* expression, with control plants showing 3-fold and 5-fold higher expression in response to *R. secalis* on days 2 and 4 respectively. The two stresses combined resulted in higher *GLP3a* expression compared to the mock infected control samples on both day 2 (13 fold) and day 4 (17 fold), indicating that the response is additive.

There was little expression of the putative fungal genes in the mock infected tissue regardless of K status (Figure 6-6). The expression of all putative fungal genes was much higher in the *R. secalis* infected tissue. Although the best matches for these genes from the NCBI database were not *R. secalis* genes, they were fungal genes and it is likely that *R. secalis* will contain close homologues. The presence of putative fungal genes thus confirmed the fungal infection in the *R. secalis* inoculated tissue but not the mock inoculated samples. It is worth noting that the expression of the fungal genes was higher in the infected K-deficient samples compared to the control samples on day 2, but not on day 4.

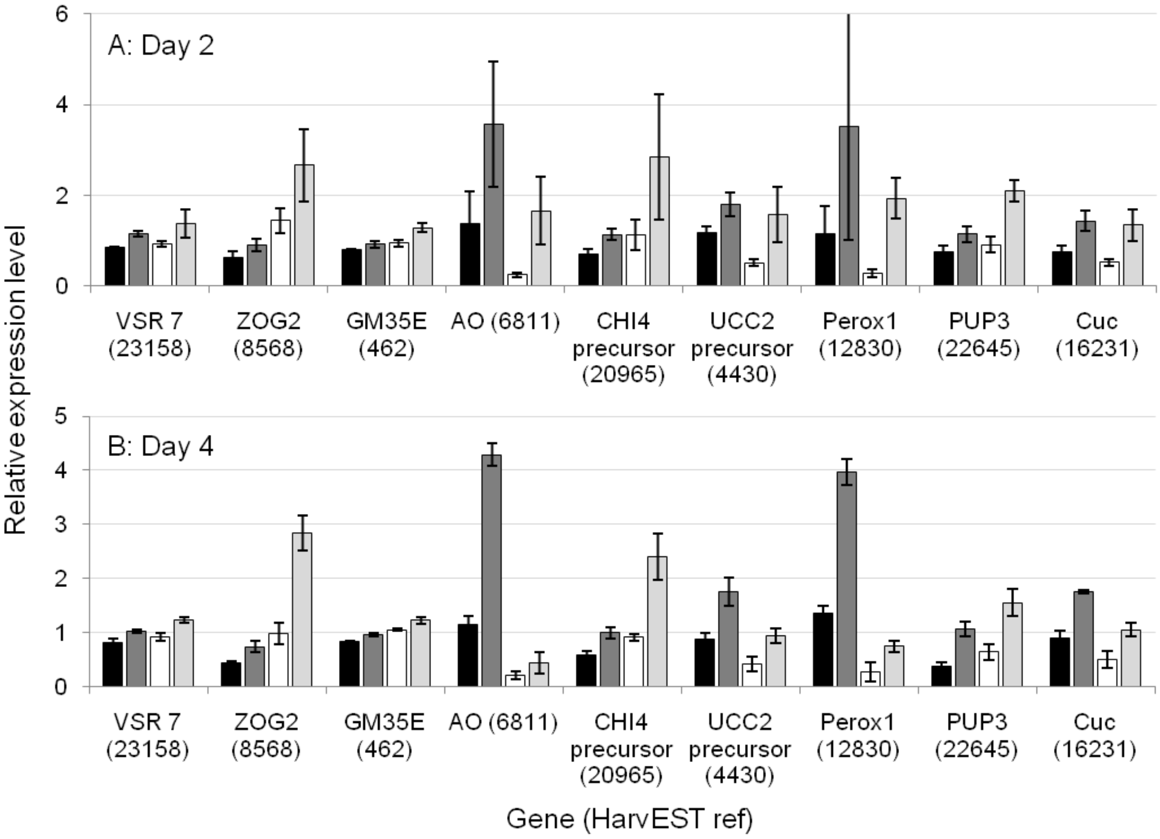


Figure 6-9. Response patterns of other genes with altered expression in response *R. secalis* infection. Expression profiles of genes with altered expression in response to both –K and *R. secalis* from data obtained from the microarray analysis of control mock infected (■), control *R. secalis* infected (■), -K mock infected (□) and –K *R. secalis* infected (■) samples at 2 and 4 days after infection. The mean (± SE) of 4 replicate experiments are shown.

There was little difference in the expression patterns of the rest of the genes changed in response to *R. secalis* between the two time points (Figure 6-9). The majority of the genes were upregulated in response to low-K although often only very slightly. By contrast genes encoding precursors of L-ascorbate oxidase (*AO*), uclacyanin-2 (*UCC2* precursor), peroxidase 1 (*Perox1*) and cucumisin (*Cuc*) were all downregulated in response to K-starvation in both the infected and uninfected tissue. Transcripts of all these genes were more abundant in leaves challenged with *R. secalis* in both the control and K-free tissue.

6.6. Discussion and conclusions

In this chapter the results of microarray experiments measuring the effect of K-deficiency and *R. secalis* infection on the transcriptome of barley leaves are presented. The individual effects of each treatment were analysed as well as the modulation of *R. secalis*-induced changes by K-deficiency.

6.6.1. Response to K-deficiency

K-deficiency has a substantial effect on the barley transcriptome as demonstrated by the large number of genes that show changes in expression when K is withdrawn (Table 6-1 and 6-2). The majority of K-responsive genes identified have likely functions in cellular and metabolic processes indicating a profound re-programming of plant metabolism at the level of transcriptional regulation. Genes related to organic acid, ketone, and protein metabolic processes were all upregulated in response to K-starvation (Table 6-1). The group of genes most significantly downregulated in response to K-starvation were those related to photosynthesis but a number of genes involved in cellular component organisation and tetrapyrrole biosynthesis were also downregulated (Table 6-2). The results obtained here for barley are in agreement with studies on transcriptional changes caused by a deficiency of essential mineral nutrients in *A. thaliana* all of which found that the adjustment of primary metabolism and photosynthesis to nutrient deficiency involves the concerted transcriptional regulation of many enzymes (Amtmann and Armengaud, 2009; Hoefgen and Nikiforova, 2008; Morcuende *et al.*, 2007; Tschoep *et al.*, 2009). In particular, it was shown in a previous study on *A. thaliana* that long-term K-deficiency caused transcript changes that could be integrated with changes in enzyme activities and metabolite concentrations to establish a model of the direct effects of K-deficiency and the metabolic adjustments of the plants (Armengaud *et al.*, 2009). While a strong effect of low-K on genes involved in primary metabolic processes is common to *A. thaliana* and barley the lists of regulated shoot genes are not identical. While the differences could reflect different metabolic and regulatory pathways in the two species they could also be due to the uncertainty of assigning the barley sequences to homologous sequences in *A. thaliana* that might not be the correct functional prologues. Another difficulty in interpreting transcript changes relies in the fact that it is not clear which of the changes are instrumental in regulating specific metabolic pathways and which are the consequence of changes in metabolite concentration achieved by other regulatory means (e.g. posttranslational). The presented data on transcriptional responses of barley to low K will have to be complemented by a more detailed kinetic analysis of transcripts, metabolites and enzyme activities and a better understanding of gene functions to allow us to build a model of genes involved in metabolic “re-programming” in response to K-deficiency.

The other main group of genes with changed expression in response to K-starvation contained genes known to respond to environmental stimuli, including biotic and biotic stress as well as chemical stimuli (Figure 6-3 and Table 6-1). This group is only present in the list of genes upregulated in response to K-starvation (Table 6-1). It includes genes that are responsive to temperature stress, osmotic pressure, salt stress and metal ions and are therefore likely to have fundamental functions in adjusting plant physiology and metabolism to any stress that limits growth. Many JA biosynthesis genes are featured in this group, confirming the results obtained by qPCR (Chapter 4) and supporting the notion that JA is an important component of plant responses to low-K.

6.6.2. Response to *R. secalis* infection and its modification by K

A relatively small number of genes showed changes in regulation in response to *R. secalis* (Figure 6-6). It is possible that the more subtle changes in response to *R. secalis* have been lost due to the technical variation between arrays (Section 6.2, Figure 6-2). Twenty-eight genes were identified having altered transcript levels, the majority of which fitted into three main categories: blue copper proteins, germin-like proteins and fungal genes (Figure 6-6).

The blue copper proteins (BCPs) are a group of copper binding protein with electron carrier activity, and have been reported to be upregulated in response to pathogens such as powdery mildew in barley (Jansen *et al.*, 2005) . They make up 4 of the 28 genes upregulated in barley leaves in response to *R. secalis* infection, with uclacyanin-2 precursor possibly being a fifth (Figures 6-6- and 6-7). Although there was no change in expression of *BCP*s in response to K in the uninfected tissue, the increase in *BCP* transcript in response to *R. secalis* is larger in plants grown in K-free solution compared to the control plants (figure 6-7). BCPs could therefore only be responsible for the higher susceptibility of plants to *R. secalis* if they had a negative effect on resistance, which is unlikely as they are upregulated in response to *R. secalis*. Instead it is possible that these genes are particularly sensitive to *R. secalis* infection and therefore respond more strongly in the more susceptible plants early on in the infection process.

Three genes encoding germin-like proteins (GLPs) were responsive to *R. secalis* infection (Figure 6-6 and Figure 6-8). GLPs are a functionally and taxonomically diverse group of proteins that have previously been shown to be involved in defence against pathogens in plants (Himmelbach *et al.*, 2010; Zimmermann *et al.*, 2006). GLPs have oxalate oxidase activity and may be involved in reactive oxygen species production in response to pathogens (Dumas *et al.*, 1995). Type 4 GLPs have previously been shown to be expressed in barley in response to *R. secalis* (Steiner-Lange *et al.*, 2003). Although expression of genes encoding type 4 GLPs was lower in uninfected K-starved plants compared to the control plants, *GLP* expression was not altered by K status in infected tissue on day 2 (Figure 6-8). However, by day 4 the expression had dropped in the K-starved plants while remaining high in the controls. It is possible that this decline indicates a shorter response of the *GLP* genes in the K-starved plants, leading to higher infection, but it may also be due to the *R. secalis* in the plants moving into the necrotrophic phase more quickly, making programmed cell death ineffective as a defence mechanism.

6.6.3. Problems and further work

The microarray experiments identified a large number of genes that were upregulated in response to K-deficiency some of which were highly expressed and it is possible that some small changes in gene expression were overlooked by the choosing a very low p-value as a cut-off. Indeed the number of genes identified as K responsive at $p<0.05$ was considerably larger than the list extracted for $p<0.005$. Future analysis of the larger list containing nearly 15000 genes in combination with metabolomics and proteomics studies should provide a sound framework for understanding the effects of low-K in greater detail. It is also worth noting that the data shown in this chapter was obtained from the analysis of the microarray results, and should be confirmed using qPCR, however this was not possible during the time allowed for this PhD.

Due to the limited knowledge of gene functions in barley it is not yet possible to carry out GO term analysis on lists of barley sequences. To obtain an idea of the processes affected by transcript changes in barley the functional annotation of the closest homolog in *A. thaliana* had to be used leading to potential misinterpretations as the *A. thaliana* homolog may not have the same function as the barley gene. Furthermore, some of the barley genes did not have a close homolog in *A. thaliana* and therefore were lost from the analysis. However, current efforts by the barley sequencing consortium in sequencing and annotating the barley genome will lead to increased availability of resourses in the future (Schulte *et al.*, 2009).

Very little correlation was seen between changes in gene expression in response to K-supply and *R. secalis* infection and no significant interaction between the 2 factors was detected (Data not shown). It should be noted the very little is known about the molecular processes underlying the different phases of *R. secalis* invasion and of the plant's defence against the fungus. The differentially expressed genes identified in this first microarray study will be useful markers to carry out an extended analysis of transcriptional regulation in *R. secalis* infected barley plants over a longer period of time and in different parts of the plant.

Considering that K-deficiency had a stronger effect on mildew than *R. secalis* it would in hindsight have been better to carry out the microarray analysis for this pathogen. While not possible within the time frame of this project a microarray experiment using a similar design as the one used here to assess K-pathogen interactions should be carried out for mildew.

Chapter 7: Discussion

In the field, crops are subject to a wide variety of biotic and abiotic stresses, often simultaneously. In order to manage these stresses effectively it is important to understand not only the plants' responses to individual stresses but also the way in which these responses interact. More intensive, modern farming methods can lead to depletion of minerals in the soil and increased pathogen density. It has previously been demonstrated that the mineral nutrient status of a plant can have a significant effect on susceptibility to pathogen attack (Perrenoud, 1990). Potassium is an important plant nutrient that is often deficient in highly cultivated soils, leading to large quantities of fertiliser being applied to soils to maintain plant production. Much of the current knowledge of effects of K-supply on plant metabolism, gene expression and pest resistance has been obtained from the model plant *Arabidopsis thaliana* (Armengaud *et al.*, 2004; Armengaud *et al.*, 2009; Hammond *et al.*, 2003; Hampton *et al.*, 2004). In order for this knowledge to be applicable to agriculture it is important to assess whether crop plants respond to K in the same manner as the model plant. During this project I therefore investigated the effect of K-deficiency on growth, metabolite concentrations, gene expression and pathogen susceptibility in barley. More specifically, I was interested whether K-deficiency in barley induces a JA response, as observed for *A. thaliana*, and whether this response affects pathogen susceptibility. This question is particularly relevant because production of secondary compounds downstream of JA-signalling is likely to differ between the two species. For example, glucosinolate concentrations have been reported to increase in a JA-dependent manner in K-deficient *A. thaliana* plants (Troufflard *et al.*, 2010), but this response is unlikely to play a major role for non-Brassica species. The results obtained in this thesis show that despite possible differences in downstream events, an induction of JA biosynthesis genes does occur in K-deficient barley plants (Chapter 4, Figures 4-3, 4-6 and 4-7). Furthermore, the study provides strong evidence that the effect of K-deficiency on pathogen susceptibility is determined by the JA-sensitivity of the pathogen (Chapter 5).

7.1. Challenges of knowledge transfer from *A. thaliana* to barley

Transfer of knowledge from the model plant *A. thaliana* to the cereal crop barley presents a number of problems. Many resources that are available in *A. thaliana* do not exist for barley, most importantly the barley genome has not yet been full sequenced. Nevertheless, in May 2011, 501,620 public barley ESTs were available, an increase of almost 23,000 since 2009 when 478,734 ESTs provided partial sequence information for around 50,000 tentative unigenes (Schulte *et al.*, 2009; Sreenivasulu *et al.*, 2008). Due to the large size of the barley genome, at around 5,500 MB (Sreenivasulu *et al.*, 2008), sequencing the full genome is a challenge, although advances in sequencing technology have made it a realistic goal and a consortium of scientists is set to achieve this (Mayer *et al.*, 2011; Schulte *et al.*, 2009; Wicker *et al.*, 2006; Wicker *et al.*, 2009). The limited knowledge of genes involved in JA signalling in barley, and the limited sequence data available made the selection of marker genes for JA responses difficult. Many of the well known JA marker genes in *A. thaliana* either don't exist in barley or have not yet been sequenced.

Although sequence data were available for many of the genes involved in JA biosynthesis, they were often not well characterised at functional level. In *A. thaliana*, several genes encode 13-lipoxygenases, which catalyse the first committed step in JA synthesis in *A. thaliana*, but the *AtLOX2* gene is the only one that responds to K-deficiency (Armengaud *et al.*, 2004; Troufflard *et al.*, 2010). The nomenclature of the LOX genes is generally confusing and the usage of both letters (van Mechelen *et al.*, 1999) and numbers (Bachmann *et al.*, 2002) for the barley genes makes it difficult to pinpoint the 'correct' barley lipoxygenase gene (See Chapter 4, Section 4.3.1 and Figure 4-2). Three genes were investigated during this study; *LOX2Hv2* (*LOX2.2*) and *LOX2Hv3* (*LOX2.3*) had previously been identified as being JA responsive (Bachmann *et al.*, 2002), however they gave inconsistent results in response to K-starvation, and powdery mildew (Chapter 4, Figure 4-3 and data not shown). The microarray studies described in Chapter 6 identified a close homolog to the rice *OsLOX2* gene and the *A. thaliana AtLOX2* gene (See Chapter 4, Figure 4-2), which was consistently up-regulated in response to K-deficiency, wounding and powdery mildew infection (Chapter 4, Figures 4-3 and 4-7 and Chapter 5, Figure 5-1 and 5-7).

Allene-oxide synthase (AOS) and allene oxide cyclase (AOC) catalyse subsequent steps of JA biosynthesis. In *A. thaliana*, the single genes encoding AOS, *AtAOS*, and *AtAOC1* are strongly up-regulated during K-deficiency (Armengaud *et al.*, 2004; Troufflard *et al.*, 2010). One barley gene was identified for each enzyme and both genes showed consistently higher during K-deficiency (Chapter 4, Figures 4-3, 4-6 and 4-7).

None of the commonly used JA downstream or responsive genes in *A. thaliana* have been described in barley. However, a diverse group of genes that are responsive to JA treatment have been identified in barley, and are referred to as 'JA induced proteins' (JIPs) (Andresen *et al.*, 1992; Chaudhry *et al.*, 1994; Weidhase *et al.*, 1987). Many of these proteins are not functionally characterised, and they are named based on the size of the gene product. Due to the lack of information on the role of these proteins and the complexity of JA responses it is difficult to know in which JA responses these genes might be involved. Three JIP proteins were selected to be tested during this study (*JIP23, JIP37 and JIP60*) with varying results (Chapter 4). *JIP23* expression was inconsistent in its response to K-starvation and showed little response to wounding or powdery mildew infection (Chapters 4 and 5). *JIP60* transcript abundance was higher in response to K-starvation and powdery mildew infection but not wounding (Chapters 4 and 5). *JIP37* also showed higher transcript abundance in response to K-starvation but not as strongly as *JIP60* (Chapter 4).

In summary, *LOX2.A*, *AOS* and *JIP60* genes from barley were identified here as suitable marker genes for JA-biosynthesis in response to low K, wounding and powdery mildew. These genes provide tools for further studies into the interaction between various stress response pathways in barley and the role of JA therein.

7.2. K-deficiency and susceptibility to pathogens

K-deficiency leads to a number of physiological, transcriptional and metabolic changes to plants, many of which could influence susceptibility and attractiveness to pathogens. Results of studies looking at the effect of K-deficiency on pathogen infection are variable not only between plant and pathogen species but also between different studies looking at the same host-pathogen interaction (Perrenoud, 1990), indicating a delicate balance between factors that determine the outcome of an interaction. This is, to my knowledge, the first study in which the effects of K-deficiency on tissue K-content, metabolite concentrations, hormone concentration, transcript abundance and pathogen susceptibility, as well as the effect of JA treatment on pathogen susceptibility, were determined in plants grown under identical controlled environmental conditions, using the same tissues, treatments and sampling time points. The data obtained were therefore directly comparable and can be used to discuss possible factors linking external K supply with pathogen susceptibility.

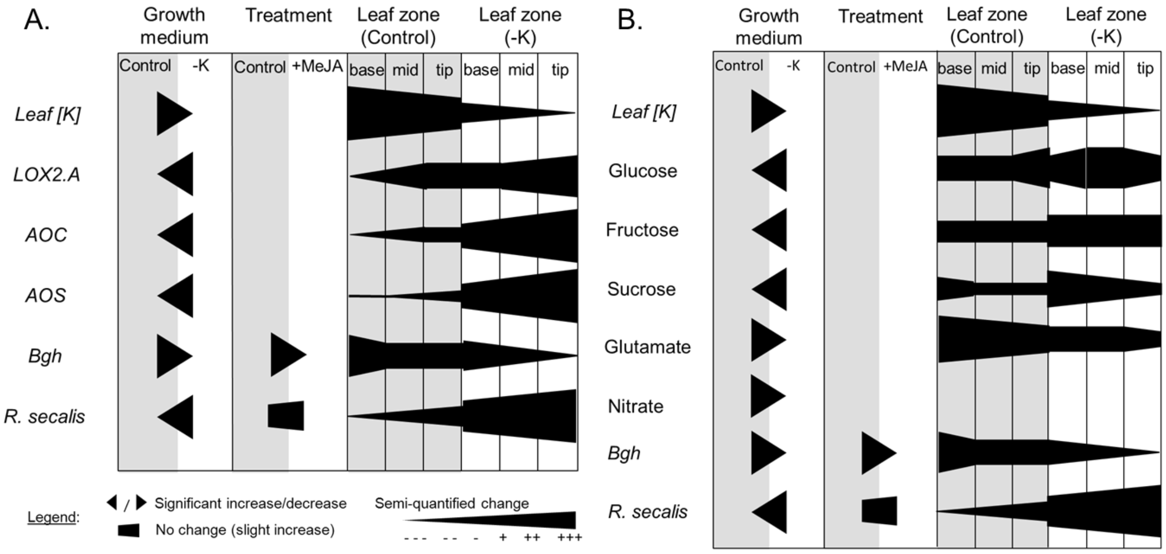


Figure 7-1. Semi-quantitative summary of the main results obtained in this thesis. A. Levels of tissue K, transcripts of JA-biosynthesis genes and pathogens in whole shoots and leaf segments of plants grown in control and K-free (-K) conditions. B. Levels of tissue K, metabolites and pathogens in whole shoot (left panels) and leaf segments (right panels) of plants grown in control and K-free (-K) conditions. Black triangles in the left panels indicate changes in levels over the period of observation. In the right panels, values, measured in different leaf segments were classified into six categories ranging from high (+++) to low (---) and changes between these are shown as a sequence of trapeze bars.

In Chapter 5 it was demonstrated that K-deficient plants appeared more susceptible to *R. secalis* infection but less susceptible to *Bgh*, indicating that K-deficiency affects the plants response to these pathogens differently. To assist the discussion of which K-dependent parameters could explain the effects of K-deficiency on *R. secalis* and *Bgh*, Figure 7-1 summarises in a semi-quantitative manner the most important results obtained in this thesis including whole-leaf data averaged over the assessed time period of growth in control and low-K conditions (left panels) and data from different leaf zones (right panels) as well as the effect of externally applied JA (centre panels). Figure 7-1A shows that out results indicated that *Bgh* infection was positively correlated with tissue K-content and negatively correlated with both JA-related gene expression (Also see Appendix 3) and Me-JA treatment. By contrast *R. secalis* infection is negatively correlated to tissue K-content and positively correlated to with both JA-related gene expression and Me-JA treatment. No link was found between *R. secalis* infection levels externally applied JA. Figure 7-1B indicates a positive relationship between *Bgh* infection and nitrogen metabolism, and a negative association between the concentration of hexose sugars in the shoot tissue, however this is not echoed on the different leaf regions and Pearson correlations showed no significant correlation (Appendix 3), where little change in sugar concentration was observed across the leaf, except for sucrose which had a positive relationship with *Bgh* infection. The opposite relationship was seen for *R. secalis* infection with a positive relationship to sugar concentration and a negative association with nitrate metabolism.

7.2.1. How does K-deficiency lead to increased susceptibility to *R. secalis*?

Hemi-biotrophic fungi like *R. secalis* acquire nutrients from the host plants, initially from living cells and during later stages from dead tissue. During this study the severity of infection was assessed by measuring the length of the lesions formed when *R. secalis* causes the collapse of the host cells during the necrotrophic phase. However, the size and rate of development of these lesions are likely to be influenced by developmental rate during the biotrophic phase.

Using Rb as a homologue for K, it has been shown that K accumulates around stomata during *R. secalis* infection (Ayres and Jones, 1975), the negative correlation between K concentration and *R. secalis* infection indicates that *R. secalis* is not directly dependant on high K concentration. However, it is possible that the increased K accumulation plays a part in plant defences against the pathogen, K-deficiency may limit this response resulting in increased *R. secalis* infection.

The concentrations of hexose sugars were higher in K-starved plants (Chapter 3, Figure 3-5), therefore the availability of these sugars for uptake by the pathogen is higher. Previous studies have demonstrated higher *R. secalis* mycelium growth and spore production at higher glucose concentrations (Jenkyn and Griffiths, 1976; Olutiola and Ayres, 1973). It is possible that the higher concentration of hexose sugars in the –K plants led to higher uptake of these sugars and resulted in increased growth of *R. secalis*. However, the smallest change in susceptibility between the –K and control samples was seen in the segments taken from the base of the leaf where the increase in sugar concentration in response to K-starvation was largest (Figure 7-1B). Therefore, it is unlikely that the increase in sugar concentration in response to K-starvation is solely responsible for the increased susceptibility to *R. secalis.*

Lower glutamate and nitrate concentration in the K-starved plants compared to the control samples indicate lower nitrogen concentration in the plant (Figure 7-1B). Pathogens such as *R. secalis* acquire all the nitrogen they require from the host plant and it has previously been demonstrated that host plants with higher nitrogen concentrations are more susceptible to infection by *R. secalis* (Jenkyn and Griffiths, 1978). Therefore, it is unlikely that lower nitrogen concentration in the K-starved plants would be responsible for the higher susceptibility to infection of *R. secalis*.

K-starvation also led to increased expression of JA-related genes in both *A. thaliana* (Armengaud *et al.*, 2004; Armengaud *et al.*, 2010; Troufflard *et al.*, 2010) and barley plants (Chapter 4 and Figure 7-1A). JA signalling plays an important role in the response of plants to both biotic and abiotic stress and is known to inhibit other responses to pathogens, such as SA signalling (Harms *et al.*, 1998; Loake and Grant, 2007; Norton *et al.*, 2007). As discussed in Chapter 5 very little work has been carried out looking at the effect of JA on *R. secalis* in barley but Weiskorn *et al.* (2002) showed a reduction in *R. secalis* infection in response to JA treatment. However, the experiments reported in this thesis showed treatment of leaf segments grown in full nutrient solution with Me-JA had no significant effect on *R. secalis* infection (Chapter 5, Figure 5-3). If anything, a slightly higher *R. secalis* infection was observed in JA-treated leaves. No change in JA-related gene expression was seen in response to *R. secalis* infection (Steiner-Lange *et al.*, 2003). This indicates that JA-signalling is not involved in plant response to *R. secalis* infection and it is therefore unlikely that increased JA concentration in response to K-starvation is either directly or indirectly responsible for the increased susceptibility to *R secalis*.

It is also possible that physical factors altered by K-deficiency influence the susceptibility of barley plants to *R. secalis*. Fourteen days after transfer to nutrient solution (when the leaf segments were prepared for infection) the second leaf of plants grown in K-free solution was significantly shorter than that of plants grown in full nutrient solution (see Chapter 3, Section 3.4.1) and comparison of areas of leaf segments from the two treatments showed that the tip, middle and base segments of leaves from –K plants were smaller than the corresponding segments of plants grown in full nutrient solution (data not shown). In the field, it is possible the smaller surface area of the –K plants could lead to a change in levels of infection, but this is unlikely to have affected infection during the experiments reported in Chapter 5 as each leaf segment was inoculated with equal concentration and volume of spore solution and the lesions did not reach the leaf margins. It is possible that other physical attributes of the leaves could have been affected by K-starvation such as cell wall structure or cuticle thickness, however, these factors were not investigated as part of this thesis. Plants with thicker cuticles have been shown to be more resistant to *R. secalis* than those with thinner cuticles (Ayres and Owen, 1971).

Other possible reasons for increased susceptibility of K-deficient plants to *R. secalis* were identified in the microarray study described in Chapter 6. Two main groups of genes encoding germin like proteins (GLPs) and blue copper proteins (BCPs), were found to have significantly changed transcript abundance in response to *R. secalis* infection indicating they may be involved in the plant response to this pathogen. Both these groups have previously been shown to be involved in responses to pathogens (Zimmermann et al., 2006; Himmelbach et al., 2010; Jansen et al., 2005).

In barley, GLPs can be split into six sub-families (Davidson *et al.*, 2009), two of which were identified here as being responsive to *R. secalis* and also had altered gene expression in response to K-starvation (Chapter 6, Figures 6-6 and 6-8). It has previously been shown that *GLP3* and *GLP4* transcript abundance are increased in response to *R. secalis* infection (Dumas *et al.*, 1995; Steiner-Lange *et al.*, 2003), and the microarray analysis confirmed this to be the case in our system, indicating a role for both genes in the plants' defence against *R. secalis*. Zimmermann *et al.* (2006) showed that silencing of *GLP3* and over expression of *GLP4* lead to increased resistance to powdery mildew infection, indicating that low *GLP3* expression and high *GLP4* expression enhance resistance. In uninfected leaves, K-starvation led to higher *GLP3* expression and low *GLP 4* expression, which may have contributed to the higher susceptibility to *R. secalis* infection (Chapter 6, Figure 6-8). It is also worth noting that *GLP*s have been shown to be down regulated by JA, while being up-regulated in response to SA and the fungal pathogen *Alternaria brassicicola* in *A. thaliana* (Schenk *et al.*, 2000).

The microarray study also identified a number of genes encoding BCPs that where up-regulated in response to *R. secalis* infection (Chapter 6, Figure 6-6 and 6-7). *BCP* transcripts have previously been shown to accumulate after powdery mildew (Jansen *et al.*, 2005) and stripe rust infection in cereals (Coram *et al.*, 2010), but had not previously been linked to *R. secalis*. *BCP*s have also been shown to be induced in response to JA, but not SA or ET (Schenk *et al.*, 2000), but there was no change in *BCP* expression between the control and –K uninfected samples (Chapter 6, Figure 6-7). The observed increase in *BCP* expression in response to *R. secalis* was larger in K-starved plants Chapter 6, Figure 6-7). As K-starved plants are more susceptible to *R. secalis* it is unlikely that BCPs play a role in defence. It is more likely that they are symptomatic and hence increased response reflects a higher rate of infection.

7.2.2. How does K-deficiency lead to decreased susceptibility to *Bgh*?

Bgh is an obligate biotroph and therefore obtains all its nutrients from the living host cells. Plants infected with *Bgh* accumulate increased concentration of glucose, sucrose and fructose, with a larger increase being observed in susceptible plants (Hwang and Heitefuss, 1986; Swarbrick *et al.*, 2006). The major carbon source for barley *Bgh* is glucose, meaning sucrose must be metabolised in the leaf before uptake by the fungus (Mendgen and Nass, 1988; Sutton *et al.*, 1999). Results obtained in chapter 3 indicated that glucose concentration in K-starved plants was higher than in the control plants (Figure 7-1B), and it is unlikely that the changes in sugar concentration are responsible for the reduced susceptibility to *Bgh.*

It has previously been shown that low concentration of nitrogen in plants can lead to reduced susceptibility to *Bgh* infection (Jensen and Munk, 1997; Last, 1962; Wiese *et al.*, 2003). In Chapter 3 it was demonstrated that K-starvation led to lower concentrations of nitrate and glutamate, indicating lower N metabolism in the tissue. Therefore, it is possible that low N availability was a contributing factor to the low susceptibility to *Bgh* seen in K-starved plants. However, the correlation between K-deficiency induced glutamate increase in different leaf regions and level of infection was poor (Figure 7-1B, Appendix 3, Table S3-2).

K-starved plants had higher JA-related gene expression indicating an accumulation of JA in the K-starved plants (Figure 7-1A). Treatment with JA or Me-JA leads to reduced susceptibility to *Bgh* in plants (Schweizer *et al.*, 1993; Walters *et al.*, 2002), implying that the accumulation of JA in the K-deficient plants may lead to enhanced resistance to *Bgh*. The increase in JA-related gene expression in response to K-deficiency increased from the base to the tip of the leaf and was indirectly proportional to *Bgh* infection observed (Figure 7-1A, Appendix 3, Table S3-2), further indicating that JA accumulation in response to K may have an effect on the susceptibility of barley plants to powdery mildew.

As with *R. secalis*, it is possible that physical factors could affect *Bgh* infection. However, the reduced number of powdery mildew colonies on the –K plants observed in Chapter 5 was not due to the smaller leaf area resulting in fewer spores landing on the leaf surface as the number of colonies per square centimetre was used to account for any variation in segment size. However, in the field where the whole leaf is exposed to *Bgh* it may lead to reduced colony numbers. Water content is also unlikely to be responsible for the reduction in *Bgh* infection in the –K tissue as there was little change between the control and –K samples (Chapter 3, Table 3-1). It is possible that other unmeasured physical attributes could be affected by K-starvation and impact upon susceptibility to *Bgh*.

It is also worth noting that a number of genes that were up-regulated in response to K-deficiency in the microarray experiment discussed in Chapter 6 are also involved in plant response to pathogens and may influence susceptibility to *Bgh*. These genes include general pathogen related genes such as *PAL* and a number of *PR* proteins (Chapter 6 and Appendix 4, Table S4-1). Inhibition of *PAL* has been shown to increase susceptibility of barley to powdery mildew (Carver *et al.*, 1994). In addition to this a number of specific powdery mildew resistant genes were significantly changed in response to K-starvation when $p<0.05$. *MLO3* was up regulated, while *MLA1*, *MLA10*, and homologs of the wheat powdery mildew resistance genes *PM3b* and *PM3g* were all down regulated in response to K-deficiency.

Quantitative correlation analysis was carried out using a Pearson product-moment correlation for the data obtained for the shoot and leaf region data obtained during the study, the results are shown in the Appendix 3 Tables S3-1 and S3-2.

7.3. Further work

In this thesis it has been demonstrated that K-deficiency leads to a number of physiological, biochemical and transcriptional changes in barley plants. It has also been established that K-deficiency has different effects on pathogens with different infection strategies. Results suggested that an increase in JA in response to K-starvation might play an important role in reduced susceptibly to *Bgh* infection. In order to investigate the role of JA in defence against *Bgh* during K-starvation barley further, JA mutants could be used. At present there are no known JA mutants in barley, however TILLING populations could be used to identify mutants or JA genes could be knocked down. In addition to studying defence responses, plants deficient in JA signalling could be used to investigate the effect of the K-dependent JA response on other biochemical and physiological changes during K-starvation.

During the work presented in this thesis, JA concentration in the K-starved samples was measured along with a number of other plants hormones (Chapter 4, Figure 4-5). In 2010

Troufflard *et al*. showed that although JA concentration was increased in K-deficient *A. thaliana* plants, other oxylipins such as 12-oxo-octadecadienoic acid (OPDA), 9-hydroxy-12-oxo-octadecadienoic acid (9-HOD) and 13-hydroxy-12-oxo-octadecadienoic acid (13-HOD) were increased to a much higher level. These oxylipins are able to act as signal molecules in their own right and may be important in the plants response to K-deficiency independent of JA. These other oxylipins should be measured in barley plants to establish if they are also up-regulated in barley and identify any differences to the response seen in *A. thaliana.*

During this study the effects of K-deficiency and JA on two fungal pathogens of barley with different infection strategies were investigated and it was demonstrated that the effect was different on different pathogens. In order to investigate this further it would be interesting to look at the effect on other pathogens, with similar life strategies or modes of attack as those used during this study. In addition to this, the consequences of the increase in JA in response to K-starvation on the susceptibility of plants to other pathogen types, such as herbivorous insects, could be investigated. As insects are often more mobile and more able to move from plant to plant, this would also allow preference tests to be carried out to establish if K-deficient plants are more or less attractive to herbivores.

Appendices

Appendix 1. Sequences and primer locations

The mRNA sequence obtained from NCBI the database for all genes studied are shown below. The Forward (Red) and reverse (Green) primers used for qPCRs are highlighted.

Gene: Alpha-tubulin
GenBank: U40042.1
Locus: HVU40042
Length: 1629 bp
Definition: Hordeum vulgare alpha tubulin (tubA) mRNA, complete cds.

```
   1 gaattcggca cgagcgtact cgcctctctc cgcgcacacg agctctcgcc cccttcctcc
  61 aacccatctc gccagcggcg cagcccaacc acccgccaca atgagggagt gcatctcgat
 121 ccacatcggc caggccggca tccaggtcgg aaacgcgtgc tgggagctct actgcctcga
 181 gcatggcatt cagcctgatg gtcagatgcc cggtgacaag accgttgggg gaggtgatga
 241 tgctttcaac accttcttca gcgagactgg tgctgggaag cacgtccccc gtgcagtctt
 301 tgttgatctt gagcctactg tgattgatga ggtgaggact ggtgcttacc gccagctctt
 361 ccaccctgag cagcttatca gtggcaagga ggatgcagcc aacaacttcg cccgtggtca
 421 ttacaccatt ggcaaggaga ttgttgatct gtgccttgac cgtatcagga agctgtccga
 481 caactgcact ggtctccagg gcttccttgt cttcaatgct gttggaggtg gaactggctc
 541 tggccttggt tctcttctcc tagagcgtct ctctgttgac tatggaaaga agtccaagct
 601 tggggttcac agtgtaccat ctccccaggt gtccacctct gttgttgagc catacaacag
 661 tgtcctgtcc acccactccc tccttgagca caccgatgtc tctatcctgc ttgacaatga
 721 ggccatctat gacatctgcc gccgctccct tgacattgag cgcccaacat acaccaacct
 781 caacaggctt gtttctcagg tcatatcatc actgactgct tccctgaggt ttgacggtgc
 841 tctgaatgtt gatgtgaatg agttccaaac caacctggtg ccctacccaa ggatccactt
 901 catgctttcc tcctatgccc cagtgatatc agcagagaag gcttaccatg agcagctgtc
 961 tgttgccgag atcaccaaca gtgcattcga gccttcctcc atgatggcca agtgtgaccc
1021 ccgccatggc aagtacatgg cctgctgtct catgtaccgt ggggatgtcg tgcccaagga
1081 cgtcaacgct gctgtggcca ccatcaagac caagcgcact atccagtttg ttgactggtg
1141 ccccactggc ttcaagtgcg gtatcaacta ccagccacct ggtgtcgtcc caggggggcga
1201 ccttgccaag gtccagaggg ctgtgtgcat gatctccaac tccaccagtg ttgttgaggt
1261 cttctcccgc atcgaccaca agtttgacct gatgtacgcc aagcgtgcct ttgtccactg
1321 gtatgtgggt gagggtatgg aggagggaga gttctctgag gcccgtgagg accttgctgc
1381 cctggagaag gactatgaag aagttggtgc tgagttcgac gatggtgagg atggtgacga
1441 gggtgatgag tactagagcc tgcctcctgg tgctttccca aggcgtacta ctgctatcct
1501 atgatctgcc caagcggctt tatctgttat ctgtctgttt gaatgtttgc tttgtggtgt
1561 ttgttttaca acctgttgtg ttgtaagaac atattggtct gtctgaacct aatgtctcgt
1621 gccgaattc
```

Gene: Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)
GenBank: M36650.1
Locus: BLYGAPDH
Length: 1141 bp
Definition: Barley glyceraldehyde-3-phosphate dehydrogenase mRNA, 3' end.

```
   1 gtcaacgacc cgttcatcac caccgactac atgacctaca tgttcaagta tgacactgtc
  61 cacggacagt ggaagcacca tgaagttaag gtgaaggact ccaagaccct tctcttcggt
 121 gagaaggagg ttgctgtgtt tggttgcaga aaccccgagg agattccatg ggccgctgct
 181 ggtgctgagt acgttgtgga gtccaccggt gttttcactg acaaggacaa ggctgcagct
 241 cacattaagg gtggtgccaa gaaggtcatc atttctgctc ccagcaagga cgctcccatg
 301 tttgtctgtg gtgtcaacga gaaggaatac aagtcagaca tcgacattgt ctccaatgct
 361 agctgcacca ccaactgtcc tgctcctctt gctaaggtta tcaatgacag gtttggcatt
 421 gttgagggtt tgatgaccac tgtccatgcc atgactgcta cccagaagac tgttgatggt
```

```
 481 ccttcaagca aggactggag aggtggaagg gctgctagct tcaacatcat tccaagcagc
 541 actggtgctg caaaggccgt tggcaaggtg ctcccagaac ttaacggaaa gttgactgga
 601 atggccttcc gtgttcccac tgttgatgtt tctgttgttg atctgactgt tagacttgcc
 661 aagccagcca cctatgagca gattaaggct gctatcaagg aggagtctga gggaaacctc
 721 aagggcattt tgggttatgt cgatgaggac cttgtttcca ctgacttcca gggtgacagc
 781 aggtccagca tctttgatgc caaggccggg attgctctga acgacaactt tgtcaagctt
 841 gtctcatggt acgacaacga gtggggatac agcacccgtg tggtcgacct catccgccac
 901 atgcacagca ccaagtaaat gagccaaagc atgaagatac agggagtgtg gtttgcccca
 961 gagaagagaa gagtgtacaa cctcttccga gaataaattt ttgtatggaa ttatggcaac
1021 taaaaaaaac cttttattgg atgatcctga tggttggttg agcttagcgg ctcacatttt
1081 ggtggtatta tgtacttgct tgaactaaat catgagttat tttcactcat cgtcatgggt
1141 t
```

Gene: 18S ribosomal RNA 18S
GenBank: AY552749.1
Locus: AY552749
Length: 1014 bp
Definition: Hordeum vulgare subsp. vulgare 18S ribosomal RNA gene, partial

```
   1 cctgagaaac ggctaccaca tccaaggaag gcagcaggcg cgcaaattac ccaatcctga
  61 cacggggagg tagtgacaat aaataacaat accgggcgca ttagtgtctg gtaattggaa
 121 tgagtacaat ctaaatccct taacgaggat ccattggagg gcaagtctgg tgccagcagc
 181 cgcggtaatt ccagctccaa tagcgtatat ttaagttgtt gcagttaaaa agctcgtagt
 241 tggaccttgg gccgggtcgg ccggtccgcc tcacggcgag caccgaccta ctcgaccctt
 301 cggccggcat cgcgctccta gccttaattg gccgggtcgt gttttcggca tcgttacttt
 361 gaagaaatta gagtgctcaa agcaagccat cgctctggat acattagcat gggataacat
 421 cataggattc cggtcctatt gtgttggcct tcgggatcgg agtaatgatt aatagggaca
 481 gtcgggggca ttcgtatttc atagtcagag gtgaaattct tggatttatg aaagacgaac
 541 aactgcgaaa gcatttgcca aggatgtttt cattaatcaa gaacgaaagt tgggggctcg
 601 aagacgatca gataccgtcc tagtctcaac cataaacgat gccgaccagg gatcggcgga
 661 tgttgcttat aggactccgc cggcacctta tgagaaatca aagtctttgg gttccggggg
 721 gagtatggtc gcaaggctga aacttaaagg aattgacgga agggcaccac caggcgtgga
 781 gcctgcggct taatttgact caacacgggg aaacttacca ggtccagaca tagcaaggat
 841 tgacagactg agagctcttt cttgattcta tgggtggtgg tgcatggccg ttcttagttg
 901 gtggagcgat ttgtctggtt aattccgtta acgaacgaga cctcagcctg ctaactagct
 961 atgcggagcc atccctccgc agctagcttc ttagagggac tatggccgtt tagg
```

Gene: Lipoxygenase 2 (*LOX2:Hv:2*)
GenBank: AJ507212.1
Locus: AJ507212
Length: 3023 bp
Definition: Hordeum vulgare mRNA for lipoxygenase 2.

```
   1 ggcacgagat caccctttcc tccccttgct tactaccaca acgtaccata gctacattcc
  61 tcttagtaat agctagttag ctagccacgg ccacaatgca gacggcaacc aagcctctgg
 121 taggcgcacg tgccgtcccg ttgagccgga gggcatcgtt cctcgtggca gaggctcgcc
 181 ggaaaccgag cacgaacgcc cgccgcacca gggttggcag caccagcacc accacgacca
 241 ccaccaccat cttaaccgac gtcaatgggc cagccctcac cacggtcgcc aagccgggcc
 301 accagtacga cctcaagcag accgtcgaga tgaaggccac ggtgtcggtg cacatgaaga
 361 gtttctggtg gtcggatgag aagaaggaaa gggcccgtga ttgggcgtac gatctcattc
 421 tcggctcatg gctgaccctt gagctcgtca gctccgagtt ggatccaaaa acggggcagg
 481 aacatgatgt aatctccggc aaactcaagc actcgcgtga aaccgaaaag gattacgact
 541 tatatgaagc catctttacg tgccggcatc gtttggcccc atcgggcgcc gtgcgcctgg
 601 tgaactacca ccacaccgag atgttgctag gagaagtcaa gatcttccct gccggcgaag
 661 atccaacaaa gtcatccgcc gtcacgttat tccactgcca atcctggatc gacccctcac
 721 attgcagccc cgacaagcgt accttcttcc ctgtcgagaa atcctacatc ccgtcgcaga
```

```
 781 caccaaaggg tgtggagaag ttgcgcaaga gtgaactgga ggccctccgc ggcaatggct
 841 gcggcgagcg caagaaacat gaccgcatct acgattacga cgtatacaac gacctcggca
 901 aacctgagtc caagcggccc gtgctcggcg gcaaggagca cccctaccca cggcgctgcc
 961 gcacgggccg cccacgcagc aagacagatc cgtcctcgga ggaggagtcc cacaagaagg
1021 gcgaaatgta cgtgccccgg gacgagacct tcaccgagcg caaggagcaa gcgttcctca
1081 caaagcaatt gttgtcacag ctgcacggat tatgcactgg cttgaaggtg aacaaggaca
1141 tcctccccag ctttcccacc ttggcctcca tcgacgcctt gtacgacgac gacttcagga
1201 accagcccgt gcagcctgaa gggggcaagg tcaggctcat cctggatttg cttgccaagg
1261 agctggttca tctggttaag ctcgaagggg cggagttcgt ggaggggata cgtcgagtct
1321 tcaagttcga aacccccgag attcatgaca tggacaagct tgcttggttc agagacgagg
1381 agtttgcgcg gcaaaccctt gcagggatga acccactcag catccaacta gtcacggagt
1441 tgcctatcgt cagcaagctg gacgagctta agtacggccc ggcggactcc cttatcacta
1501 aggagctgat tgaaaagcag atcaaccgta tcatgacggc cgaagaggcc gtggctcaga
1561 aaaagctgtt catgctggac taccacgact tgctgctacc atacgtacac agggtgcgca
1621 agctggacaa caagaccatg tacgggtcac gcactctctt cttcctcgcc gacgacggca
1681 cgctccggcc gatcgccatc gagctgacgc ggcccaagtc tccacacaag cagcagtggc
1741 gcaaggtctt cacgccggga tcgggataca gcggcagcgt cacggggtcc tgggagtggc
1801 agctcgccaa gatccatgtc ctatcccacg acaccggcta ccaccagctt gtgagccact
1861 ggctgaggac gcactgctgc gtcgagccgt acgtcattgc ggcgaaccgg cagctgagcc
1921 agatgcatcc catctaccgc ctgctacacc cgcacttccg cttcaccatg gagatcaacg
1981 cccaggcacg aggcatgctc atctgcgccg acggcatcat cgagaagacc ttctcgccgg
2041 gggagttcag catggagatc agctccgcgg cgtacgacaa gcagtggcgg ttcgacatgg
2101 aggcactgcc ggaggacctc atacggaggg gcatggcggt cagaggggag gatggcaagc
2161 tggagctggc aatagaggac tacccgtacg ccaacgatgg cctgctggtc tgggatgcca
2221 tcaagcagtg ggcgtcggac tatgtggcgc actactaccc gtgcgcggtg gacatcgtcg
2281 acgatgagga gctccaggat tggtggacgg aggtgcgcac caaaggccac cctgacaagc
2341 aggacgagcc atggtggccg gagctggact gccacgagag cctggtccag gtcctggcca
2401 ccatcatgtg ggtcacctcg gcacatcatg ccgccgtcaa cttcgggcag taccccatgg
2461 ctgggtacgt cccgaaccac ccgagcattg cccggaggaa catgccgtgt gagatgggac
2521 cggaggagat gctcgcgttc aaggcggcgc cagagaaggt gtggttggac acgttgccgt
2581 ctcaactcca gacagtcatg gtcatggcga cattggacct cctctcgtcg cacgcgtcgg
2641 acgaggagta catgggcacg caccaggagc cggcttggca aagggatggg gaggtcgaca
2701 aggcgtttca ggttttccag aagaagatga gagacatcgc ggagcaggtc gaagagtgga
2761 acaaggacga cagccggaga aaccgccacg gtgccggcgt agtgccgtac gtgctgctca
2821 ggccattgaa cggtaatccg atggacgcta aaacggtgat ggagatgggc attccgaaca
2881 gcatttccat ttaatcagaa tgagatgcat acatgtttca tatagtgtca aatgtaacac
2941 aattaataga acttattgaa ttcacatttg tcgcaataag aaaagacgac atttttttgt
3001 tacatttata acatagtgaa act
```

Gene: Lipoxygenase 2 (*LOX2:Hv:3*)
GenBank: AJ507213.1
Locus: AJ507213
Length: 2967 bp
Definition: Hordeum vulgare mRNA for lipoxygenase 2.

```
   1 gagcagcgga agaacataac gtagatattg gaccagctcg atccatcggc agcagcggct
  61 agctagccct tcccgggcca gccatgatcc atctgaagca gcctcttgtg ctctccgcgc
 121 agagcagcaa tgttgcctcg ccgctcttcg tcgcgggcgg ccagcagagg cgggcgtccg
 181 gcgcagggag gacctgctct gggcgccggc tcagcgcgcg aaggataagc tgcgcgtcga
 241 ccgaggaggc cgtcggcgtc tcgacgtccg tgacgaccaa ggagagggcg ctgacggtga
 301 cggccatcgt gaccgcgcag gtgcccacct ccgtgtacgt cgcccgcggc ctcgacgaca
 361 tccaggacct cttcggcaag acgcttctgc tcgagctcgt cagctccgag cttgacccca
 421 agacgggaag ggagagggag agagtgaagg ggtttgcgca catgacgctc aaggagggga
 481 cgtacgaggc caagatgtcg gtgccggcgt cgttcgggcc ggtgggcgcg gtgctggtgg
 541 agaacgagca ccacagggag atgttcatca aggacatcaa gctcatcacc ggcggcgacg
 601 agagcaccgc catcaccttc gacgtcgcct cctgggtgca ctccaagttc gacgaccccg
 661 agccgcgcgc cttcttcacc gtcaagtcat acctgccgtc gcagacgccg ccgggaatcg
 721 aggcgctgag gaagaaggag ctggagacgc tgcgtggcga cgggcacagc gagcgcaagt
 781 tccacgagcg cgtctacgac tacgacacct acaacgacct cggcgaccct gacaagaaca
```

```
 841 tcgaccacaa acgccccgtg ctgggcacca aggagcaccc ctaccctcgc cggtgccgca
 901 ccggccggcc aaagaccctc tacgacccgg agacggagac gaggagctcg ccggtgtacg
 961 tgccgcgcga cgagcagttc tcggacgtca aggggcggac gttcagcgcg acgacgctgc
1021 ggtcggggct gcacgccatc ctgccggccg tggcgccgct gctcaacaac tcgcatggct
1081 tctcgcactt cccggccatc gacgccctct acagcgacgg catcccgctc cccgtcgacg
1141 ggcacggcgg caactccttc aacgtcatca acgacgtcat tcctcgtgtc gtccagatga
1201 tcgaggacac caccgagcac gtcctccgct tcgaggtccc cgagatgctt gagagggacc
1261 gattttcgtg gttcagagac gaggagttcg cgaggcagac gctcgcaggg ctcaacccta
1321 tctgcatccg ccgcctcacg gaattcccca tcgtgagcaa gctcgacccg gcggtgtacg
1381 ggccggcgga gtcggcgctg agcaaggaga tcctggagaa gatgatgaac gggcgcatga
1441 cggtggagga ggcgatggag aagaagcggc tgttcctgct ggactaccac gacgtgttcc
1501 tgccgtacgt gcacagggtg cgggagctgc cggacacgac gctgtacggg tcccgcaccg
1561 tcttcttcct gagcgacgag ggcacgctga tgccgctggc catcgagctg acgcggccgc
1621 agtcgccgac caagccgcag tggaagcgcg ccttcacgca cggctccgac gccaccgagt
1681 cgtggctgtg gaagctggcc aaggctcacg tgctgaccca cgacaccggc taccaccagc
1741 tcgtcagcca ctggctgcgc acgcacgcct gcgtcgagcc ctacatcatc gccaccaacc
1801 ggcagctcag ccggatgcac ccggtgtacc gcctgctgca cccgcacttc cgctacacca
1861 tggagatcaa cgcgctggcc cgggaggccc tcatcaacgc cgacggcatc atcgaggagg
1921 ccttcctggc tggcaagtac tccatcgagc tcagctccgt agcctacggc gccgcctggc
1981 agttcaacac ggaggcgctg ccggaggacc tcatcaaccg gggctcgcc gtgcgcaggg
2041 acgacggcga gctcgagctc gccatcaagg actacccgta cgccgacgac gggctcctca
2101 tctggggctc catcaagcag tgggcgtccg actacgtgga cttctactac aagtcggacg
2161 gggacgtcgc cggcgacgag gagctgcggg cgtggtggga ggaggtgcgc accaaggggc
2221 acgcggacaa gaaggacgag ccgtggtggc ccgtgtgcga caccaaggag aacctcgtcc
2281 agatcctgac catcatcatg tgggtcacgt ccggccacca cgccgccgtc aacttcggtc
2341 agtaccatta cgccgggtac ttccccaacc gtccgacggt ggtgcggagg aacatcccgg
2401 tggaggagaa ccgggacgac gagatgaaga agttcatggc caggccggag gaggtgctgc
2461 tgcagagcct cccctcgcag atgcaggcca tcaaggtgat ggcgacgctg gacatcctct
2521 cctcgcactc cccgacgag gagtacatgg gagagtacgc tgagccggcg tggctggccg
2581 agcccatggt gaaggcggca ttcgagaagt tcagcggcag gctcaaggag gcggagggca
2641 ccatcgacat gcgaaacaac aacccggaga acaagaacag gtgtggcgcc ggcatcgtgc
2701 cgtacgagct gctcaagccg ttctcagaac caggggtcac tgggagaggc atccccaaca
2761 gcatctccat ttgattgttg ccccaggata agatatcatt gcacgtagga ttattaggaa
2821 taagatgtac agtatgtacg cacccagcag cgattgctac cggacatgac acggcccggg
2881 agaggcatct actagtgtgt ccctgctatt gtatgccgtg aattagtact aataaggtta
2941 tttgaattgt gacctcaaaa aaaaaaa
```

Gene: Predicted protein (*LOX2.A*)
GenBank: AK362687.1
Locus: AK362687
Length: 3017 bp
Definition: Hordeum vulgare subsp. vulgare mRNA for predicted protein, complete cds, clone: NIASHv2009G18.

```
  1 gaaaccatcc agcctccaga gccaccacca caccatcggg agtcgtcgca tcgtcccctc
 61 tttctcctcc tccacttgca gcaccggcag cggcctccgc tcgtcttcgc tttccgctgg
121 gctccgtggc ggtgcgcagc tgatcgtgcg gtgctcctcc gttggcccgt ccgaggtggt
181 gccgaccagt agtagtagcg gcggcggcgg cgacggtgtg cttcttccgc catggcagga
241 aagggcggtg cttcggtgcg cgtcagggcg gtggcgacgg ttaaggtcac cgttggtggg
301 ttcctcgatg ggctgaggcc gtcgaggacg ctggacgatg tgaaggacct catcggcagg
361 tccatggaga tcgagctcgt cagcgccgag cttgacgcca agacggggga ggagaagcag
421 acgatcaaga gctacgccca caaggtggcc gacaacgacg tccaagtcgt aacctacgaa
481 gccgacttca acgtgccggc ggggttcggc cccgtcggcg ccgtgctggt ctccaacgag
541 cacggcacgg agatgttcct ggaggacgtg aaggtggtca ccgccggcgg caactcgccg
601 cccgacgtca tccgctgcga ctcctggctg ccgcccaagt ccggcgacgc caaccgcgtc
661 ttcttcgcca acaagcctta cctgccgagc cagactcctc cgggtctcca ggcctaccgg
721 aagaaggacc tcgccaagaa gcgcggtgac ggcaccgggc agaggaaggc caccgaccgg
781 gtctacgact acgacgtgta caacgacctc gggtccggcg aggagctcgg tgcctccggc
841 tcccgccccg tcctcggcgg caacaagcag ttcccctacc cgcggcgctg ccgcaccggc
901 cgcccccgat ccaccaaagg tacgtacgta tacacgacaa agtcaagaga tactacgtac
```

```
 961 gtactcctac agtgcatgaa cgtgtgtggt acatgggtaa ctcgtgcaaa ttgcgtacgt
1021 gcagacccgc agtcggagac gaggagcggc gacgtctacg tgccgaggga cgaggccttc
1081 tcggaggtga agaacgtgca gttctcggtg aagacgctgc agtcggtgct ccacgcggcg
1141 gtgccggcgg tgcagtcgac gctcatcgac cccaaccagg gcttcccctc cttcttcgtc
1201 atcgacaagc tcttcgagga cggggttgag ctgccgcgcg ccgaggatct cggcttcctc
1261 cgcgccgccg tgccgcgcct cctcgagttc ctccgcgacg gccccggcga caaactcctc
1321 ctcttcgacg cccccgcaaa tgtccagaag gacaagttcg cgtggttgcg ggacgaggag
1381 ttcgccaggg agacgctggc cgggatcaat ccgtacgcca tcgagcttgt caaggaattt
1441 cctctcaaga gcaagctgga cccggcggtg tacgggccgg ccgagtcggc gatcaccgcc
1501 gagctgctgg aggctcagat ggggcacgcc atgacggtgc ccgaggcggt gaagaacaag
1561 aggctcttca tgctcgactt ccacgacctg ttcctgccct acgtgcacaa gatccgcgcc
1621 ctgcagcaca ccaccatgta cggctcccgc accatcatgt tcctcaccga cgacggcacg
1681 ctgcgcctcc tcgccatcga gctcacccgg ccggcctcac ccatgatgcc gcagtggagg
1741 caggtcttca cttcctccac ggacaccacc aagtcgtggc tgtggcggat ggccaagtcc
1801 cacgtccgcg cccacgacgc cggccaccac gagctcgtca cccactggct gcgcacgcac
1861 tgcgccgtcg agccctacat cctcgcggcc aacaggcagc tcagcgagat gcaccccatc
1921 ttccagctgc tgcgcccgca cttccgctac accatgcgga tcaacgcgct cgcacgctcc
1981 gccctcatca acggcggcgg catcatcgag ctcaccttct ccccgcagag gtacgccatg
2041 gagctcagct ccgtcgccta cgacaagctc tggcgcttcg acatggaggc cctccccgcc
2101 gacctcgtcc gcaggggcat ggcggaggag gaccccacgg cggagcacgg actcaagctc
2161 gccatcaaag actacccgtt cgccaacgac gggctcctca tctgggacgc catcaagggg
2221 tgggttcagg cgtacgtttc cagctactac ccgaccgccg ccagcgtcac gggcgacgcg
2281 gagctgcagg ctttctggac ggaggtgcgc acagagggac acgccgacaa gaaggacgcg
2341 ccgtggtggc ccaagctcga cacgccggag agcctcgcgc acacgctcac caccatcatc
2401 tgggtcgcgg cggcgcacca cgcggccgtc aacttcgggc agtacgactt cggcggctac
2461 ttcccgaacc gcccctccat cgcacgcacc aacatgccgg tggaggagcc cgtggacgcc
2521 gccgccttcg acaagttcct ggacaacccg gaccaggcgc tccgggaatg cttcccgtcg
2581 caggtgcagg caacgctggt gatggcggtg ctggacgtgc tgtccagcca ctcccccgac
2641 gaggagtacc tgggagggat ggagactgcc ccgtggggcg gcgacaccgc ggtgcgggcg
2701 gcgtacgtga ggttcaacga gcagcttaag gcggtggaag ggatcatcga cggaaggaac
2761 aagaacagga agctcaagaa ccggtgcggc gccggcatcg tgccgtacca gctcatgaaa
2821 ccattctcgc agcccggcgt cacgggcaag ggcattccca acagcacctc catctaatca
2881 aacatagctt ccgtgaatct agtagacttc gttgtccgtt gttaaatgta cgtatttatt
2941 taagctccac acttcatatc tgtatgtgta tatccattct attaataagt tcagatttac
3001 tacttatttc gcatccg
```

Gene: Allene oxide synthase (*AOS*)
GenBank: AJ250864.1
Locus: AJ250864
Length: 1819 bp
Definition: Hordeum vulgare mRNA for allene oxide synthase.

```
  1 cctcctcgct ccaatcttgt ctgcatcgag agcgcgagat ccaaagttcc aaacccaacc
 61 agcctagcta gcagcagagt tagatgaacc agagcggcat ggcgcgcagc gacgagggct
121 ccctggtgcc gcgggaggtg ccgggcagct acggcctgcc gtttgtctcg gccatccgcg
181 accgcctcga cttctactac ttccagggcc aggacaagta cttcgagtcc cgcgtcgaga
241 agtacggctc caccgtcgtc cgcatcaacg tgccgccggg ccccttcatg gcgcgcgatc
301 cccgcgtggt cgccgtgctc gacgccaaga gcttccccgt gctctttgac gtcaccaagg
361 tcgagaagaa gaacctcttc accggcacct acatgccctc cacctccctc accggcggct
421 tcccggtctg ctcctacctc gacccctccg agcccaccca caccaaggtc aagcagctgc
481 tcttctccct cctcgcctcg cgcaaggacg ccttcatccc ggccttccgc tcccacttct
541 cctcgctgct cgccaccgtc gagtcgcagc tccttctcag cggcaagtcc aacttcaaca
601 cgctcaacga cgccacctcc ttcgagttca tcggcgacgg ctacttcggc gtgctcccct
661 ccgcctcaga cctcggcacc accggcccgg ccaaggccgc caagtggctc atattccagc
721 tccacccgct cgtcacgctc ggcctcccca tgatcctcga ggagccgctc ctccacaccg
781 tgcacctccc gcccttcctc gtcagcggcg actacaaggc gctgtacaag tacttcttcg
841 ccgccgcgac caaggcgctc gacaccgccg agggcctcgg gctgaagcgg gacgaggcgt
901 gccacaacct gctgttcgcc accgtcttca acagctacgg cggcctcaag gtgctgctcc
961 cggggatcct cgcgcgcatc gccgactccg gagagaagtt ccacaagaag ctcgtcacgg
```

```
1021 agatacgcgc cgccgtggcg gaggccggcg gcaaggtgac gatagaggcg ctggagaaga
1081 tggagctgac caagtcggcg gtgtgggagg cgctgcgtct ggacccggcc gtcaagttcc
1141 agtacggccg cgccaaggcg gacatgaaca tcgagagcca cgacgcggtg ttcgccgtga
1201 agaaggggga gatgctgttc gggtaccagc cgtgtgccac aaaggacccc cgggtgttcg
1261 gccccacggc gagggagttc gtcggcgacc ggttcgtcgg gaaggagggg agcaagctgc
1321 tgaagtacgt gtactggtcg aacgggcggg agaccgagag ccccagcgtg cacaacaagc
1381 agtgcccagg caagaacctg gtcgtgctcg tcggccggct cctggtggtg gagctgttcc
1441 tccggtacga caccttcacg gccaaagtcg gcctcgacct tctcggcacc aaggttgagt
1501 tcaccggcgt caccaaggca acgtccggtg tggcagacgc tgtttaaatc cttcaccgga
1561 cggccatcgg tgacaaaggg atcaacgcag ggcagcaacg atccttacac cgtcatcacg
1621 tacgtacgta cgtgcctcgc tatatctacc ttagtagtgt acgtacgagt cagtcatgca
1681 tgtgcatgca tcttgttcgg ttcttcgcag cattgtgccc tcgtgtatgt gtgcgtgtgt
1741 tgtgtttact tttggcaatc ttgcccagtg taataagccg gacttttcta caataagatt
1801 cccccttttg gcgtacggt
```

Gene: Allene oxide cyclase (*AOC*)
GenBank: AJ308488.1
Locus: AJ308488
Length: 920 bp
Definition: Hordeum vulgare mRNA for allene oxide cyclase.

```
  1 cggatccaag agtatcatcg tcggttagcc gtcgccacca ccaccaagag gcaacaccgc
 61 catggcagtg cgcccttcct ccgtctccgt ccgggccggt gcgtccgtct cggcgaagct
121 gacccccttgg cgggccgcga gggctgggct cggtggcagg gtcagcgtca gctcgggcag
181 gaggtgcggc ggccccgtgc gggcgtcgct cttctcgccc aagcctgcgg tggccatgga
241 cgcgcggccg agcaaggtgc aggagctgca cgtgtacgag ctcaacgagc gcgaccgcga
301 gagccccgcc tacctccggc tgagcgccaa ccagagccag aacgcgctcg gcgacctcgt
361 ccccttcacc aacaaggtgt acaacgggag cctggacaag cggatcggga tcacggcggg
421 gatctgcatc ctgatccagc acgtgccgga gcgcaacggc gaccgctacg aggccatcta
481 cagcatgtac ttcggcgact acggccacat cagcctgcag gggccctacc tcacctacga
541 ggagtcctac ctcgccgtca caggaggctc cggcgtcttc gagggcgtct acgggcaggt
601 caagctcaac cagatcgtct tccccttcaa gatcttctac actttctacc tcaagggcat
661 cccggacctg ccaaaggagc tgctctgcac gcccgtcccg ccttccccca ccgtcgagcc
721 aacgcccgcc gccaaggcca ccgagccaca cgcatgcctc aacaacttca ctgactagct
781 gtacctgtga tctactctac ctagctatgt tatgtacacc aactgctcgt gcagtcgtgc
841 aagttgaatg aatctccgtg tgttgcaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
901 aaaaaaaaaa aaaaaaaaaa
```

Gene: 23 KD Jasmonate induced protein (*JIP23*)
GenBank: BM816519.1
Locus: BM816519
Length: 921 bp
Definition: HB105D06_SK.ab1 HB Hordeum vulgare subsp. vulgare cDNA clone HB105D06_SK.ab1 similar to 23 KD JASMONATE-INDUCED PROTEIN gi|282989|pir|S22514 jasmonate-induced protein 1 - barleyJASMONATE-INDUCED PROTEIN HOMOLOG gi|576625|gb|AAA86977.1| (U15657), mRNA sequence.

```
  1 cccgggctgc aggaattcgg cacgaggaag cgaactagct agtacaccta tatatcacag
 61 tgtgtgtgca aagcaatggc ctctggagtg tttggtaccc ccatttcagc gcagacggtg
121 atagccactg gagagtataa ggaacccatt acccaaaaag atgttgcaga ctatgccatg
181 aagatgatca acgccggtgg taaggatgtt aacgcgcaaa agttcgtcga caacctcaag
241 gagaggtacg gtaacggaat agctgtaaaa tgcctcctct acaatgccac tggtgccact
301 ttgaactttg ctaagtacaa cgattggcac ggccatatct atgatacacc ctacccatca
361 gatattcaga atgggcaatg gggtgcattc ctccacgtcc acccaagtgg agctgccgct
421 ggttcagctg gtgccgttgt gtatcgtagc aagatcccct ccagcagcag ctcctgcgat
```

```
481 tggttgttct cttggaccgt cccctacatt ggtggcaacg gggtgtacac tgaaatccgc
541 gaggaagggc actacccaag tgtgggaagc tgggattata tctacaatgt gaagctgaaa
601 aattcaagtg tcacctctat tgatagcaac tatggatacg tttccaaggc tgacatcggt
661 gaaggcacta ccatgaacgc acgtggagtt ttcgagtttc cctactanat ctcgttgcaa
721 gcagcgagtt gttattggtg gtcttcaagt cttgtgtggc gtncatgtac gcctgngttg
781 gtttttttctt tactgtcttt atggggcatt tttatagatc tgatgatcca cgaataagcg
841 aaggaagatg gggaccanag agatgtaccg nngtaatgga ttttattnta tatggcgntg
901 ggaatcagat ggccttcttt
```

Gene: 37 KD Jasmonate-induced protein (*JIP37*)
GenBank: X82937.1
Locus: X82937
Length: 1334 bp
Definition: H.vulgare mRNA for jasmonate induced protein.

```
   1 cccttacctt gtgctcgtgg tgaagctgat ccttccattg cttggtgccc ccaagctacc
  61 ccaaggctta catcatgtca ggtgacctag ttgatttaag tccagggcag ttgcataagc
 121 tagccgatct gatccatcga caagaagtcc agaagcttca agagctcgag ttcaactcgt
 181 atgcagagca acaaaagtat ctccgtgacg ccaatgatgc ccgcgataag gtgtatcaca
 241 tccttgacag tgcacgggac atgatagcac aaaccgaggc tgagaaggat gccaccaagc
 301 aagatattgg caaggatgtc tatgactact gcaccaaggc catcggaata tctctccaat
 361 tcatccggag ttacaacacc cgtctcacct accttgacaa gctcaagacc cacagtgatg
 421 atctcatcaa gcagctaaag ttcctcaatc tagccaccca acagaaggag gcccagcgcc
 481 ttgcccttga ggctggcatg tataagaaag ccacgctgga gaatgccaaa aagtttcaac
 541 actttgcacc aaatcaattc tcaaaatggc tcaaggaaaa caaaatcatg tttgaggatc
 601 ttgtgcaaga aaatatgtca aagcttggtt ttagggggc tttcaaaaac ttggatgata
 661 tccaaaagct acaggtatat gacaatatta ttgcggaagc gggacaagga aaatctgtgg
 721 tgacttactc atttgaagct ttgggaaaag tcggggtggc agttttagtt tttacagcag
 781 ctgcaatggt gtgggacata tacacagcag aagataagct ggaggcagcg gttagggatt
 841 cagtaaatgc gttaactgca gtagttaacc ttgaggtggg agaaatagtt actactgctg
 901 tagaagctgg attcgtagca ctagacatcg aaattgcttc tgcggctgtc acggtaattg
 961 gaggagtcgt cggttttgga attggtgcgc tcattgggat agctgcaggt gcgctgcttg
1021 acttgatttt cagcagtgga actagcaagg tgaagataac agacgggctt acagtttgcc
1081 gtgtggcgcc tatgcctcat ggtctcgaac tcgctcgtct agttaagcac aattaccctg
1141 aactttaaac tgatcatgct actactatga tctttgctat gctatcttct gctcttagtg
1201 ktagctacta ctgtcgtgag tatgtaagtg cctagtgtgc actcctaaaa taatacccccc
1261 cgcccctgtc gggaaactat tgtattcatg atgccatgat gaggaataaa agtcatgttg
1321 attcaaaaaa aaaa
```

Gene: 60 KD Jasmonate induced protein (*JIP60*)
GenBank: BM815987.1
Locus: BM815987
Length: 858 bp
Definition: HB106E12_SK.ab1 HB Hordeum vulgare subsp. vulgare cDNA clone HB106E12_SK.ab1 similar to hypothetical protein[Oryza sativa],60 KD JASMONATE-INDUCED PROTEIN [Hordeum vulgare],hypothetical protein[Oryza sativa],kda jasmonate-induced protein [Hordeum vulgare=barley, cv. Bonus, Peptide, 560 aa], mRNA sequence.

```
  1 cccgggctgc aggaattcgg cacgaggggc cgaaagaaaa ttccccaagt tctattttgt
 61 ctgggcaaga gcgacatggc ggccgccacc ggctacggtg acgccccgac cgacgagcag
121 ctgctcgcgt acgctgagct acccagacaa ggccgctaca tggcggaggt gttcgccgtg
181 cgcatccccg ccaccgccgg agcagacccg cccagcggca ccatctcttt ccacggcggc
241 cactgcagca gcgacttcat ttacagcccg gaagaagaac acgcctcgca gcaaaccagc
301 tgcgacagcc agggtaacat cgtgctcact gggccatcgg tggccacctc ggcatacagt
361 ccaattgtct tcagcctcga cctccatgag ggacaagccg acgaggagga ggaagagaac
421 accgcgagga tagtctgcga caccattggc ggcgacttct ctaactacaa cagggccata
```

```
 481 tcggagactg tacgcacagg ctatggcccc gcggaggtga tctacgccgt cctaagtaat
 541 ggcatccaag gccgggtcga cgtgaagctt tctcgcctgc agtcccgaga cgaagaggtt
 601 ctcgtcggca ggatcgttgc tcgcagcaag ctgttcgacg tcggttgcgt gctcttctac
 661 aaggaggccg ctaacaagca gggagtgcac gtgcgaccgg nggagccggt tccgttggcg
 721 aggcatgcac tcgccttgcc gctgcacatg ccgctgacga ttgagcttga ccttcgtcgc
 781 ggtggatccc gagatgaaat tgttagaggc aagcttgagt tcaaactgcc atcgacggcc
 841 tgcatacggg aacgtctg
```

Gene: Phenylalanine ammonia-lyase (*PAL*)
GenBank: Z49147.1
Locus: Z49147
Length: 1777 bp
Definition: H.vulgare partial PAL mRNA for phenylalanine ammonia-lyase (1777 bp).

```
   1 ctcctcttca acacaagtct ttcttcaaga cacagatcaa tccagataca catacaccgg
  61 cctactttca gaatccagac acatacacca gctagttcac ctgtttagaa ctacttctct
 121 gcatatctcg atggagtgcg agaacgcaca cgttgccgcc aacggcgatg gcttgtgcgt
 181 ggcgcagcca gcgcgggccg acccactcaa ctggggcaag gcggcggagg agctgtccgg
 241 gagccatttg gatgccgtga agcggatggt ggaggagtac cgtaagccgg tggtgaccat
 301 ggagggcgcc agcctgacca tcgccatggt cgccgcggtg gctgccggca acgacaccag
 361 ggtggagctc gatgagtccg cccgcggccg cgtcaaggag agcagcgact gggtcatgaa
 421 cagcatgatg aacggcaccg acagctatgg tgtcaccacc ggcttcggcg ccacctctca
 481 ccggaggacc aaggagggcg gcgctctgca gagagagctc atccgattcc ttaacgccgg
 541 agccttcggc accggcaccg atggccacgt tctgcctgcc gcgacgacga gggcggcgat
 601 gctcgtccga gtaaatacct tgctgcaggg atattcaggc attcgctttg agatcctgga
 661 gacgattgcc acacttctca atgccaacgt gacaccatgc ctgccgctcc ggggcacgat
 721 caccgcgtct ggtgacctcg taccgctttc gtacatcgcg ggcctggtca ccggccgccc
 781 aaactccgtg gcaaccgctc cagatggcac gaaggttaat gccgcggagg catttaagat
 841 cgccggcatc cagcatggct tcttcgagct gcagccgaag gaaggccttg ccatggtgaa
 901 tggcacggcg gtaggctctg ggcttgcatc catggtgctt ttcgaggcta acgtccttag
 961 cctccttgcc gaggtcctgt cggccgtctt ctgcgaggtg atgaatggca agccggagta
1021 caccgatcac ttgacccaca agctgaagca ccaccctgga cagatcgagg ctgccgccat
1081 catggagcac attcttgaag gcagctccta catgatgcta gcaaagaagc tcggtgagct
1141 tgacccattg atgaagccaa agcaagatag gtatgcactc cgcacatcgc ctcagtggct
1201 tggccctcag attgaggtca tccgtgctgc caccaagtcg atcgagcggg agatcaactc
1261 tgtcaatgac aacccactca tcgacgtttc tcgtggcaag gctatccacg gtggcaactt
1321 ccagggcaca cctattggtg tgtccatgga caacaccagg cttgccattg ctgcgatcgg
1381 taaactcatg tttgcccagt tctcagagct agtgaatgac ttctacaaca acggtctgcc
1441 ttccaacctc tccggcgggc gcaacccaag tttggactat ggcttcaagg gtgctgagat
1501 tgccatggcg tcatactgtt ccgagcttca gttcctgggc aaccctgtga ccaaccatgt
1561 ccagagcgca gagcagcaca accaagatgt caactctctt ggccttatct cctcaaggaa
1621 gactgccgag gccattgaca tactcaagct catgtcctcg acattttgg tcgcattgtg
1681 ccaggctatc gacctccgcc acctcgagga gaatgtcaag aatgctgtaa agagctgtgt
1741 gaagacggtg gccaggaaga cactaagcac tgatagc
```

Appendix 2. ANOVA results

All values show are p-values. The data obtained for some variables were not normally distributed and were there for transformed by taking the Log10 (marked with * or double log **).

Table S2-1 Growth of barley plants under K-stress

		Time point (days)											**Whole exp**		
		0	2	4	6	8	10	12	14	16	18	20	Control/-K	Time	Inter
length	**total**	0.462	0.224	0.519	0.042	0.042	0.797	0.493	0.059	0.142	0.950	0.094	0.815	<0.001	0.095
	shoot	0.200	0.268	0.309	0.015	0.001	0.050	0.013	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	leaf 1	0.200	0.238	0.309	0.014	0.077	0.423	0.444	0.155	0.057	0.509	0.658	<0.001	<0.001	0.491
	leaf 2			0.840	0.519	0.006	0.050	0.013	0.002	0.001	0.015	0.023	<0.001	<0.001	0.146
	leaf 3						0.113	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004
	leaf 4											<0.001			
	root*												<0.001	<0.001	<0.001
weight	**total***												<0.001	<0.001	<0.001
	shoot*												<0.001	<0.001	0.075
	root*												<0.001	<0.001	<0.001

Table S2-2 Biochemical and physiological effects of K-stress on shoots

	Time points					**Whole experiment**			**Over time**	
	3	6	9	12	15	+/- K	Time	Inter	Cont	-K
% K in DW **						0.121	0.412	0.118		
% water	0.196	0.169	0.342	0.499	0.444	0.265	0.464	0.371	0.715	0.432
Chl A	0.251	0.037	0.607	0.720	0.045	0.346	0.638	0.663	0.912	0.477
Chl B	0.181	0.808	0.372	0.471	0.234	0.950	0.782	0.458	0.670	0.541
Glucose	0.048	0.286	0.156	0.004	0.087	0.084	<0.001	0.377	<0.001	<0.001
Fructose	0.145	0.357	1.330	0.126	0.012	0.111	<0.001	0.312	<0.001	0.002
Sucrose	0.594	0.140	0.012	0.226	0.364	0.114	0.003	0.239	0.134	0.029
Malate	0.039	0.402	0.006	0.320	0.220	0.078	<0.001	0.392	0.040	0.300
Glutamate	0.132	0.154	0.006	0.166	0.112	0.060	<0.001	0.418	0.043	0.035
Protein	0.856	0.636	0.621	0.886	0.983	0.680	0.103	0.822	0.516	0.275
Nitrate	0.920	0.447	0.633	0.206	0.582	0.405	<0.001	0.784	0.015	0.001

Table S2-3 Biochemical and physiological effects of K-stress on roots

	Time points					**Whole experiment**			**Over time**	
	3	6	9	12	15	+/- K	Time	interact	Control	-K
% K in DW	0.048	0.048	0.014	0.010	0.002	<0.001	<0.001	<0.001	<0.001	0.057
% water **						0.329	0.097	0.004		
Glucose	0.164	0.043	0.079	0.020	0.094	0.030	<0.001	0.375	<0.001	<0.001
Fructose	0.100	0.017	0.048	0.043	0.053	0.007	<0.001	0.187	<0.001	0.002
Sucrose	0.045	0.257	0.169	0.047	0.034	0.078	<0.001	0.891	<0.001	0.021
Malate	0.420	0.003	0.700	0.313	0.276	0.813	<0.001	0.036	0.005	0.039
Glutamate	0.524	0.160	0.549	0.481	0.894	0.848	0.429	0.384	0.387	0.495
Protein	0.779	0.344	0.057	0.105	0.844	0.152	0.119	0.769	0.316	0.391
Nitrate	0.823	0.588	0.451	0.496	0.265	0.350	0.296	0.577	0.005	0.470

Table S2-4 Biochemical and physiological effects of K-stress across the leaf

	Segments			**Whole exp**			**Across leaf**		**Control**			**-K**		
	Tip	Mid	Base	+/- K	Seg	Inter	Control	-K	T/M	T/B	M/B	T/M	T/B	M/B
% K in DW	0.023	0.031	0.046	<0.001	0.060	0.562	0.044	0.313	0.084	0.012	0.137	0.458	0.340	0.254
% water	0.043	0.334	0.225	0.004	<0.001	0.027	0.217	0.004	0.392	0.163	0.385	0.028	0.012	0.412
Chl A	0.140	0.006	0.360	0.013	0.004	0.328	0.075	0.098	0.283	0.068	0.179	0.206	0.027	0.479
Chl B	0.306	0.276	0.334	0.054	0.016	0.674	0.189	0.028	0.415	0.076	0.396	0.089	0.035	0.202
Glucose	0.255	0.408	0.436	0.906	0.892	0.475	0.775	0.319	0.703	0.545	0.911	0.341	0.842	0.073
Fructose	0.232	0.621	0.323	0.034	0.656	0.666	0.939	0.530	0.936	0.303	0.862	0.395	0.795	0.421
Sucrose	0.974	0.367	0.099	0.015	0.011	0.071	0.317	<0.001	0.744	0.344	0.090	0.047	0.006	0.002
Glutamate	0.063	0.131	0.105	0.001	0.017	0.369	0.175	0.128	0.612	0.059	0.213	0.340	0.308	0.099
Protein	0.149	0.831	0.431	0.711	0.015	0.260	0.228	0.088	0.398	0.119	0.515	0.179	0.030	0.449

Table S2-5 JA-related gene expression in shoots under K-stress

	Time points					**Whole exp**			**Over time**	
	3	6	9	12	15	+/- K	Time	Interaction	Control	-K
LOX2.2 *						0.003	<0.001	<0.001		
LOX2.3*						0.467	<0.001	0.350		
LOX2.A	0.980	0.778	0.027	0.074	0.003	0.070	0.382	0.052	0.698	0.036
AOS	0.746	0.486	0.558	0.246	0.212	0.430	0.033	0.749	0.001	0.293
AOC *						0.007	<0.001	0.157		
JIP23*						0.784	<0.001	0.770		
JIP37 *						0.509	<0.001	0.087		
JIP60 *						0.002	0.014	0.004		

Table S2-6 Hormone levels in shoots during K-stress

	Time points			**Whole experiment**			**Overtime**	
	3	9	15	Control/-K	Time	Inter	Control	-K
ABA	0.564	0.033	0.018	0.091	0.035	0.198	0.007	0.802
SA	0.649	0.338	0.153	0.412	0.213	0.389	0.247	0.550
SA-glyco	0.693	0.402	0.142	0.159	0.027	0.116	0.306	0.061

Table S2-7 JA-related gene expression across the leaf during K-stress

	Segments			**Whole exp**			**Across leaf**	
	Tip	Middle	Base	+/- K	segment	interaction	Control	-K
LOX2.3	0.121	0.115	0.608	0.466	0.005	0.142	0.055	0.052
LOX2.A	0.168	0.001	0.150	0.087	0.002	0.688	0.073	0.025
AOS *				<0.001	0.004	0.090		
AOC *				<0.001	0.044	0.792		
JIP23*				<0.001	0.035	0.287		
JIP37 *				<0.001	0.003	0.230		
JIP60	0.232	0.049	0.064	0.092	0.005	0.107	0.193	0.027

Table S2-8 JA-related gene expression in shoots after K-resupply

	All			Control/ -K			Control/Resupply			-K/Resupply			Overtime		
	Treatment	Time	Inter	Treatment	Time	Inter	Treatment	Time	Inter	Treatment	Time	Inter	Control	-K	RS
%K Shoots	<0.001	0.004	<0.001	<0.001	0.194	0.781	<0.001	<0.001	<0.001	<0.001	0.004	0.002	0.612	0.208	0.005
%K Roots	<0.001	0.005	<0.001	<0.001	0.778	0.121	<0.001	0.004	0.007	<0.001	0.003	0.001	0.367	0.340	0.006
LOX2.3*	0.074	0.409	0.358												
LOX2.A*	0.020	0.632	0.104												
AOS*	0.103	0.118	0.057												
JIP23*	0.051	0.063	0.042												
JIP60*	0.047	0.682	0.015												

Table S2-9 *R. secalis* lesion length and *B. graminis* colony numbers on K-stressed leaves

	Lesion length *	**Colonies/cm^2 ***
Control/-K	<0.001	<0.001
Segment	0.084	<0.001
Time	<0.001	<0.001
Control/-K v Segment	<0.001	<0.001
Control/-K v Time	<0.001	<0.001
Segment v Time	<0.001	<0.001
Control/-K v Segment v Time	<0.001	<0.001

Table S2-10. Infection levels of *R. secalis* and *B. graminis* after Me-JA treament

	Time point					**Whole experiment**		
	3	6	9	12	15	Treatment	time	Interaction
R. secalis*						<0.001	<0.001	0.006
Bgh	*	0.003	0.003	0.001	n/a	<0.001	<0.001	<0.001

Table S2-11 JA-related gene expression in response to *R. secalis* during K-stress

	*LOX2.A**	*AOS**	*JIP60**	*PAL**
Control/-K	0.002	<0.001	<0.001	<0.001
Infection	0.677	0.680	0.966	0.107
Time	0.023	0.559	0.666	<0.001
Control/-K v Infection	0.500	0.919	0.286	0.335
Control/-K v Time	0.191	0.116	0.408	<0.001
Infection v Time	0.779	0.942	0.788	0.679
Control/-K v Infection v Time	0.533	0.793	0.967	0.722

Table S2-13 JA-related gene expression in response to *B. graminis* during K-stress

	*LOX2.A**	*AOS**	*JIP60*	*PAL*
Control/-K	<0.001	<0.001	<0.001	0.351
Infection	<0.001	<0.001	<0.001	0.006
Time	<0.001	<0.001	<0.001	0.002
Control/-K v Infection	0.311	0.011	0.394	0.097
Control/-K v Time	<0.001	0.150	<0.001	0.851
Infection v Time	<0.001	<0.001	0.079	0.190
Control/-K v Infection v Time	0.278	0.581	0.481	0.524

Appendix 3. Pearson product moment correlation analysis

Table S3-1 Analysis of correlation of changes in biochemistry and gene expression in shoot tissue during K-stress.
Significant ($p<0.05$) positive correlations are shown in green and negative correlations in red, darker colour indicate a more significant correlation

	% K	% water	Chl A	Chl B	Total Chl	Glucose	Fructose	Sucrose	Malate	Glutamate	Protein
% K	*										
% water	0.193	*									
Chl A	0.181	0.260	*								
Chl B	-0.049	0.153	0.251	*							
Total Chl	0.147	0.275	0.961	0.508	*						
Glucose	-0.246	-0.060	-0.255	-0.109	-0.258	*					
Fructose	-0.276	-0.120	-0.293	-0.088	-0.286	0.977	*				
Sucrose	-0.279	-0.002	0.423	0.145	0.417	-0.588	-0.567	*			
Malate	0.130	0.004	0.259	0.215	0.291	0.063	-0.012	-0.132	*		
Glutamate	0.574	0.230	0.353	0.029	0.323	-0.600	-0.633	0.387	0.111	*	
Protein	-0.021	0.283	0.158	0.093	0.167	0.075	0.090	-0.003	0.079	0.200	*
Nitrate	0.267	0.047	0.226	-0.029	0.192	-0.708	-0.685	0.456	-0.234	0.621	0.160
LOX2.2	-0.503	0.055	-0.037	-0.063	-0.051	0.541	0.481	-0.099	-0.071	-0.438	-0.168
LOX2.3	-0.249	0.057	0.200	-0.184	0.125	-0.365	-0.401	0.542	-0.007	0.213	-0.004
LOX2.A	-0.603	-0.170	-0.398	-0.039	-0.365	0.273	0.226	0.129	-0.106	-0.363	-0.003
AOS	-0.319	0.198	-0.113	-0.050	-0.114	0.389	0.382	-0.034	0.104	-0.169	0.440
AOC	-0.260	-0.021	-0.262	-0.070	-0.253	0.698	0.620	-0.280	0.055	-0.408	0.094
JIP23	-0.169	0.025	0.023	-0.065	0.002	0.213	0.136	-0.002	0.049	-0.202	-0.646
JIP37	-0.024	0.099	-0.015	0.005	-0.012	0.098	0.055	-0.034	-0.152	-0.126	-0.523
JIP60	-0.501	-0.058	-0.175	0.144	-0.115	0.382	0.374	0.077	-0.146	-0.387	0.037
PAL	-0.245	0.155	-0.191	0.124	-0.135	0.087	0.088	0.096	-0.112	-0.038	0.178

Table S3-1 Continued.

	LOX2.2	*LOX2.3*	*LOX2.A*	*AOS*	*AOC*	*JIP23*	*JIP37*	*JIP60*	*PAL*
% K									
% water									
Chl A									
Chl B									
Total Chl									
Glucose									
Fructose									
Sucrose									
Malate									
Glutamate									
Protein									
Nitrate									
LOX2.2	*								
LOX2.3	0.101	*							
LOX2.A	0.347	0.198	*						
AOS	0.370	0.451	0.313	*					
AOC	0.477	-0.177	0.670	0.338	*				
JIP23	0.558	0.167	0.020	0.353	0.174	*			
JIP37	0.140	-0.012	0.037	0.096	-0.049	0.548	*		
JIP60	0.428	0.044	0.637	0.220	0.579	0.065	-0.121	*	
PAL	-0.072	0.297	0.286	0.636	0.238	0.082	0.281	0.107	*

Table S3-2 Analysis of correlation of changes in biochemistry and gene expression in detached leaf segments during K-stress.
Significant ($p<0.05$) positive correlations are shown in green and negative correlations in red, darker colour indicate a more significant correlation.

		Metabolites									JA related gene expression						
		% K	**% Water**	**Chl A**	**Chl B**	**Glucose**	**Fructose**	**sucrose**	**glutamate**	**protein**	***LOX2.3***	***LOX2.A***	***AOS***	***AOC***	***JIP23***	***JIP37***	***JIP60***
Metabolites	**% K**	*															
	% water	0.507	*														
	Chl A	0.102	-0.256	*													
	Chl B	0.029	-0.292	0.914	*												
	Glucose	-0.062	-0.120	-0.130	-0.185	*											
	Fructose	-0.416	-0.211	-0.179	-0.373	0.197	*										
	Sucrose	-0.258	0.217	-0.481	-0.477	-0.054	0.299	*									
	Glutamate	0.367	0.073	0.829	0.854	-0.188	-0.496	-0.484									
	Protein	-0.257	-0.605	0.610	0.512	-0.106	0.215	-0.545	0.306								
JA related gene expression	***LOX2.3***	-0.057	-0.184	0.494	0.442	0.056	-0.112	-0.482	0.443	0.334	*						
	LOX2.A	-0.696	-0.737	0.163	0.210	0.006	0.354	-0.157	-0.134	0.435	0.364	*					
	AOS	-0.606	-0.778	-0.157	-0.073	0.021	0.252	-0.180	-0.306	0.447	0.209	0.752	*				
	AOC	-0.731	-0.594	-0.136	-0.073	0.001	0.514	-0.032	-0.405	0.414	0.184	0.717	0.707	*			
	JIP23	-0.636	-0.821	-0.019	0.045	-0.009	0.348	-0.215	-0.278	0.601	0.034	0.739	0.914	0.678	*		
	JIP37	-0.577	-0.794	-0.026	0.045	-0.017	0.252	-0.197	-0.222	0.551	0.038	0.696	0.912	0.545	0.977	*	
	JIP60	-0.548	-0.590	-0.092	-0.043	-0.013	0.218	-0.121	-0.153	0.353	0.428	0.750	0.878	0.607	0.716	0.751	*
Lesion Length	**Day 3**	-0.485	-0.109	-0.059	0.066	-0.004	0.057	0.297	-0.098	-0.059	-0.017	0.323	0.062	0.177	0.076	0.103	0.169
	Day 6	-0.757	-0.304	0.048	0.069	0.232	0.524	0.111	-0.207	0.345	-0.151	0.536	0.315	0.525	0.459	0.372	0.247
	Day 9	-0.737	-0.455	0.009	0.031	0.295	0.538	0.008	-0.262	0.489	-0.065	0.452	0.491	0.713	0.591	0.468	0.317
	Day 12	-0.562	-0.690	0.233	0.370	0.190	0.231	0.033	0.129	0.427	0.147	0.518	0.567	0.538	0.545	0.525	0.455
	Day 15	-0.727	-0.689	-0.002	0.089	-0.008	0.349	0.255	-0.185	0.296	0.049	0.607	0.663	0.622	0.618	0.594	0.518
Segments with lesions	**Day 3**	-0.513	-0.173	-0.069	0.068	0.003	0.048	0.300	-0.122	-0.065	-0.054	0.379	0.122	0.176	0.145	0.179	0.201
	Day 6	-0.734	-0.478	0.103	0.136	0.239	0.539	0.009	-0.215	0.461	-0.164	0.598	0.411	0.622	0.593	0.486	0.260
	Day 9	-0.716	-0.471	0.022	0.046	0.285	0.535	0.001	-0.257	0.498	-0.053	0.433	0.481	0.735	0.581	0.449	0.295
	Day 12	-0.444	-0.545	0.333	0.428	0.172	0.331	-0.059	0.243	0.457	0.162	0.422	0.443	0.472	0.465	0.421	0.330
	Day 15	-0.288	-0.393	0.438	0.462	-0.022	0.178	-0.049	0.345	0.279	0.233	0.355	0.246	0.162	0.245	0.243	0.149
Number of colonies	**Day 3**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Day 6	0.513	0.406	-0.042	-0.127	0.023	-0.183	0.351	0.059	-0.324	-0.619	-0.738	-0.646	-0.614	-0.549	-0.522	-0.752
	Day 9	0.390	0.185	0.113	0.174	-0.161	-0.373	0.235	0.198	-0.293	-0.493	-0.429	-0.488	-0.457	-0.421	-0.411	-0.658
	Day 12	0.338	0.130	0.125	0.159	-0.111	-0.334	0.268	0.140	-0.252	-0.475	-0.407	-0.491	-0.423	-0.413	-0.404	-0.646
	Day 15	0.315	0.147	0.100	0.123	-0.121	-0.301	0.309	0.106	-0.289	-0.473	-0.387	-0.504	-0.413	-0.432	-0.427	-0.648
Segments with colonies	**Day 3**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Day 6	0.593	0.387	-0.002	0.051	-0.418	-0.299	0.138	0.232	-0.228	-0.518	-0.633	-0.495	-0.528	-0.426	-0.401	-0.632
	Day 9	0.667	0.400	0.048	0.023	-0.066	-0.278	0.242	0.206	-0.399	-0.503	-0.710	-0.692	-0.690	-0.624	-0.592	-0.791
	Day 12	0.625	0.512	0.007	-0.016	-0.022	-0.253	0.331	0.163	-0.493	-0.526	-0.699	-0.814	-0.683	-0.738	-0.715	-0.830
	Day 15	0.628	0.543	0.040	-0.010	-0.016	-0.259	0.316	0.170	-0.470	-0.530	-0.699	-0.841	-0.729	-0.746	-0.721	-0.849

Table S3-2 Continued.

		Lesion Length					Segments with lesions					Number of colonies					Segments with colonies				
		Day3	**Day6**	**Day9**	**Day12**	**Day15**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Metabolites	**% K**											*									
	% water											*									
	Chl A											*									
	Chl B											*									
	Glucose											*									
	Fructose											*									
	Sucrose											*									
	Malate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Glutamate											*									
	Protein											*									
	Nitrate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Jasmonate related gene expression	***LOX2.3***											*									
	LOX2.A											*									
	AOS											*									
	AOC											*									
	JIP23											*									
	JIP37											*									
	JIP60											*									
Lesion length	**Day 3**	*										*									
	Day 6	0.479	*									*									
	Day 9	0.085	0.823	*								*									
	Day 12	0.304	0.455	0.586	*							*									
	Day 15	0.299	0.455	0.562	0.858	*						*									
Segments with lesions	**Day 3**	0.991	0.494	0.089	0.324	0.337	*					*									
	Day 6	0.397	0.954	0.855	0.526	0.476	0.423	*				*									
	Day 9	0.053	0.782	0.995	0.599	0.566	0.054	0.831	*			*									
	Day 12	0.131	0.482	0.624	0.928	0.725	0.142	0.557	0.637	*		*									
	Day 15	0.094	0.282	0.280	0.707	0.685	0.121	0.282	0.279	0.788	*	*									
Number of colonies	**Day 3**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Day 6	-0.33	-0.35	-0.31	-0.28	-0.24	-0.34	-0.38	-0.29	-0.26	-0.07	*	*								
	Day 9	-0.16	-0.28	-0.32	-0.04	0.005	-0.14	-0.28	-0.30	-0.05	0.24	*	0.813	*							
	Day 12	-0.04	-0.24	-0.30	-0.02	0.034	-0.03	-0.24	-0.28	-0.07	0.23	*	0.801	0.969	*						
	Day 15	-0.01	-0.23	-0.32	-0.05	0.043	0.002	-0.24	-0.23	-0.10	0.222	*	0.797	0.965	0.995	*					
segments with colonies	**Day 3**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Day 6	-0.22	-0.36	-0.39	-0.19	-0.19	-0.23	-0.36	-0.37	-0.09	0.119	*	0.727	0.789	0.725	0.705					
	Day 9	-0.26	-0.43	-0.48	-0.26	-0.28	-0.26	-0.45	-0.46	-0.19	0.080	*	0.896	0.871	0.849	0.842		0.863			
	Day 12	-0.06	-0.35	-0.50	-0.34	-0.37	-0.08	-0.39	-0.49	-0.30	-0.06	*	0.859	0.814	0.821	0.826		0.774	0.951		
	Day 15	-0.10	-0.32	-0.49	-0.42	-0.44	-0.11	-0.37	-0.49	-0.37	-0.13		0.867	0.795	0.791	0.799		0.736	0.926	0.984	

Appendix 4. Microarray results

Table S4-1 Genes upregulated in response to K-starvation.

Expression values relative to the medium for all data, of genes upregulated in K-starved (-K) plants with p-values < 0.005 corrected for false discoveries using Bonferroni multiple testing correction. Results for control (C) and K-starved (-K) detached leaf segments mock inoculation with water (mock) or inoculated with *R. secalis* (+ *RS*), two and four days post inoculation are shown.

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_376_PI390587928	0.612	2.215	0.825	2.074	0.358	1.684	0.288	1.346	expressed protein	
CUST_392_PI390587928	0.651	2.528	0.766	2.525	0.515	2.366	0.578	2.453	acid phosphatase 1 precursor putative expressed	AT4G25150.1
CUST_6729_PI390587928	0.313	1.800	0.284	2.591	0.286	1.683	0.976	2.922	-	
CUST_18613_PI390587928	0.500	3.233	0.653	4.155	0.458	3.484	0.549	5.412	cytochrome P450 71D7 putative expressed	AT5G32440.1
CUST_35524_PI390587928	0.792	1.177	0.788	1.078	0.820	1.277	0.798	1.397	pyridoxin biosynthesis protein ER1 putative expressed	AT5G01410.1
CUST_25350_PI390587928	0.863	1.815	0.783	1.656	0.519	1.345	0.736	1.547	SET domain containing protein	AT2G18850.1
CUST_19651_PI390587928	0.773	1.491	0.774	1.386	0.769	1.288	0.740	1.346	plant-specific domain TIGR01568 family protein expressed	AT1G06920.1
CUST_21363_PI390587928	0.483	3.866	0.647	4.097	0.494	3.973	0.395	3.679	nodulin putative expressed	AT5G25250.1
CUST_27425_PI390587928	0.903	1.669	0.782	1.487	0.708	1.199	0.697	1.372	expressed protein	AT2G31725.1
CUST_11811_PI390587928	0.633	2.561	0.776	3.243	0.720	1.910	0.623	3.436	ZIM motif family protein expressed	
CUST_15021_PI390587928	0.516	3.203	0.563	2.133	0.602	2.667	0.573	3.363	charged multivesicular body protein 2a putative expressed	AT5G44560.1
CUST_36407_PI390587928	0.368	2.820	0.531	3.175	0.451	3.105	0.427	3.682	glutamate dehydrogenase 2 putative expressed	AT5G07440.2
CUST_21240_PI390587928	0.743	1.728	0.451	1.984	0.626	1.596	0.618	1.951	F-box domain containing protein expressed	
CUST_20607_PI390587928	0.755	3.001	0.938	2.577	0.594	2.487	0.488	1.879	BAP2 putative expressed	
CUST_12663_PI390587928	0.525	2.290	0.556	2.689	0.599	2.106	0.747	3.089	-	
CUST_9061_PI390587928	0.871	1.419	0.839	1.448	0.746	1.240	0.788	1.492	rho guanine nucleotide exchange factor putative expressed	AT4G16510.1
CUST_36690_PI390587928	0.730	1.522	0.753	1.625	0.677	1.593	0.695	1.645	peptidase family M28 containing protein expressed	AT1G67420.1
CUST_28761_PI390587928	0.767	1.561	0.912	1.609	0.873	1.525	0.842	1.789	-	
CUST_6143_PI390587928	0.899	1.395	0.939	1.444	0.813	1.264	0.842	1.327	tetratricopeptide-like helical putative expressed	
CUST_23566_PI390587928	0.689	1.203	0.787	1.578	0.760	1.317	0.891	1.590	-	
CUST_17867_PI390587928	0.815	1.464	0.812	1.333	0.697	1.152	0.659	1.209	RHL2 putative expressed	AT5G02820.1
CUST_4520_PI390587928	0.770	1.710	0.690	1.678	0.574	1.281	0.634	1.393	60S ribosomal protein L12 putative expressed	AT5G60670.1
CUST_14398_PI390587928	0.499	2.448	0.363	1.643	0.294	1.565	0.323	1.412	protein HVA22 putative expressed	AT5G50720.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_19640_PI390587928	0.626	1.401	0.630	1.249	0.623	1.328	0.587	1.675	ubiquitin-protein ligase putative expressed	
CUST_17883_PI390587928	0.675	1.381	0.742	1.292	0.548	1.441	0.550	1.717	alpha-L-fucosidase 2 precursor putative expressed	AT1G09390.1
CUST_28606_PI390587928	0.651	1.949	0.674	1.742	0.688	1.481	0.665	1.840	SKP1-like protein 1B putative expressed	AT5G42190.1
CUST_16706_PI390587928	0.796	1.551	0.824	1.702	0.735	1.724	0.784	2.531	-	
CUST_15467_PI390587928	0.681	1.525	0.759	1.888	0.743	1.400	0.729	1.590	U5 small nuclear ribonucleoprotein 200 kDa helicase putative expressed	AT2G42270.1
CUST_3832_PI390587928	0.682	1.656	0.733	1.859	0.561	1.433	0.670	2.122	-	
CUST_21182_PI390587928	0.778	1.713	0.812	1.351	0.883	1.752	0.828	1.988	17.4 kDa class I heat shock protein 3 putative expressed	AT3G46230.1
CUST_6611_PI390587928	0.664	2.364	0.481	2.035	0.655	2.156	0.811	4.194	win2 precursor putative expressed	AT3G04720.1
CUST_41562_PI390587928	0.785	1.194	0.808	1.760	0.668	1.144	0.769	1.743	-	
CUST_26679_PI390587928	0.641	2.194	0.760	2.833	0.695	5.886	0.751	4.711	lipid binding protein putative expressed	
CUST_7633_PI390587928	0.897	1.920	0.792	1.753	0.660	1.294	0.684	1.340	60S ribosomal protein L6 putative expressed	AT1G74050.1
CUST_15514_PI390587928	0.803	2.427	0.758	2.182	0.582	1.574	0.604	2.041	60S ribosomal protein L33-B putative expressed	AT1G74270.1
CUST_37418_PI390587928	0.703	1.721	0.789	1.595	0.843	2.316	0.734	2.126	expressed protein	AT4G13400.1
CUST_576_PI390587928	0.759	2.965	0.646	1.960	0.481	3.246	0.581	3.085	caffeoyl-CoA O-methyltransferase 2 putative expressed	AT4G34050.1
CUST_20478_PI390587928	0.804	1.632	0.809	1.255	0.692	1.181	0.608	1.520	seed maturation protein putative expressed	AT4G23630.1
CUST_4668_PI390587928	0.010	1.320	0.029	1.784	0.051	1.104	0.051	3.471	ZIM motif family protein expressed	
CUST_25518_PI390587928	0.162	7.538	0.380	5.848	0.185	6.670	0.114	6.107	-	
CUST_25534_PI390587928	0.698	1.695	0.593	1.527	0.626	1.343	0.597	1.443	-	
CUST_38286_PI390587928	0.387	2.725	0.391	3.965	0.342	2.128	0.794	6.013	alpha-amylase/trypsin inhibitor putative expressed	AT4G11650.1
CUST_2284_PI390587928	0.821	1.541	0.657	1.513	0.969	1.672	0.870	1.665	sulfate transporter 3.3 putative expressed	AT1G23090.1
CUST_34299_PI390587928	0.809	1.626	0.839	1.482	0.731	1.527	0.837	1.757	nucleotide pyrophosphatase/phosphodiesterase putative expressed	AT5G50400.1
CUST_9554_PI390587928	0.477	1.922	0.696	1.796	0.425	1.582	0.512	1.683	RER1A protein putative expressed	AT4G39220.1
CUST_16227_PI390587928	0.661	1.565	0.857	1.581	0.506	1.167	0.570	1.341	chaperonin CPN60-1 mitochondrial precursor putative expressed	AT3G23990.1
CUST_42291_PI390587928	0.936	1.442	0.786	1.400	0.747	1.234	0.845	1.277	expressed protein	AT5G39600.1
CUST_24203_PI390587928	0.048	3.959	0.111	6.248	0.160	4.092	0.154	5.608	mitochondrial chaperone BCS1 putative expressed	AT3G50930.1
CUST_11825_PI390587928	0.797	1.161	0.865	1.396	0.824	1.243	0.912	1.602	phospholipid-transporting ATPase 2 putative expressed	AT5G44240.1
CUST_38163_PI390587928	0.661	3.003	0.491	2.423	0.558	2.875	0.862	4.399	catalytic/ hydrolase putative expressed	AT5G02230.2
CUST_11048_PI390587928	0.797	1.412	0.716	1.159	0.732	1.301	0.697	1.305	glyoxylate reductase putative expressed	AT2G45630.2
CUST_24810_PI390587928	0.767	1.472	0.846	1.374	0.802	1.388	0.846	1.727	calmodulin binding protein putative expressed	
CUST_26838_PI390587928	0.531	3.282	0.462	2.492	0.531	2.547	0.462	3.385	expressed protein	
CUST_13916_PI390587928	0.368	4.516	0.300	3.665	0.298	3.755	0.331	4.424	expressed protein	
CUST_17142_PI390587928	0.183	2.318	0.215	1.497	0.769	5.583	0.294	3.902	cytochrome P450 86A1 putative expressed	AT4G39480.1
CUST_11702_PI390587928	0.779	1.485	0.799	1.137	0.821	1.386	0.763	1.384	RALF precursor putative expressed	AT3G16570.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_26668_PI390587928	0.520	1.486	0.780	2.375	0.664	1.357	0.815	2.043	CRK10 putative	AT4G23270.1
CUST_18739_PI390587928	0.788	1.233	0.850	1.162	0.865	1.211	0.800	1.310	UBP15 putative expressed	
CUST_3635_PI390587928	0.623	2.369	0.560	3.021	0.533	1.539	0.482	1.950	OsWRKY76 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	
CUST_36097_PI390587928	0.913	1.411	0.920	1.431	0.916	1.349	0.877	1.478	ATP-binding cassette sub-family E member 1 putative expressed	AT4G19210.1
CUST_1421_PI390587928	0.705	1.131	0.695	1.401	0.804	1.309	0.729	1.517	-	
CUST_21489_PI390587928	0.436	2.448	0.653	3.264	0.418	2.312	0.500	3.689	peroxisomal-coenzyme A synthetase putative expressed	AT3G48990.1
CUST_22744_PI390587928	0.548	1.541	0.733	1.571	0.545	1.468	0.558	1.984	annexin-like protein RJ4 putative expressed	AT5G65020.1
CUST_41412_PI390587928	0.421	3.531	0.559	4.370	0.464	3.525	0.435	5.635	cytokinin-O-glucosyltransferase 1 putative expressed	AT2G15480.1
CUST_41885_PI390587928	0.628	1.675	0.848	1.919	0.632	1.359	0.658	2.288	lipid binding protein putative expressed	AT1G62790.2
CUST_37757_PI390587928	0.832	1.499	0.732	1.413	0.756	1.243	0.769	1.426	-	
CUST_35527_PI390587928	0.368	2.211	0.459	2.362	0.572	2.231	0.510	2.881	expressed protein	AT3G57450.1
CUST_2304_PI390587928	0.300	4.101	0.326	4.462	0.596	3.683	0.314	5.905	potassium transporter 13 putative expressed	AT2G30070.1
CUST_34319_PI390587928	0.769	3.769	0.910	4.184	0.628	2.360	0.622	3.286	multidrug resistance-associated protein 3 putative expressed	AT3G13080.1
CUST_12431_PI390587928	0.808	1.270	0.873	1.254	0.795	1.108	0.758	1.077	plasminogen activator inhibitor 1 RNA-binding protein putative expressed	AT4G16830.3
CUST_39864_PI390587928	0.722	1.383	0.814	1.621	0.790	1.534	0.771	1.761	kinesin motor domain containing protein expressed	AT5G23910.1
CUST_13500_PI390587928	0.805	1.671	0.881	1.787	0.825	1.349	0.677	1.453	far upstream element-binding protein 1 putative expressed	AT4G10070.1
CUST_20019_PI390587928	0.850	1.277	0.951	1.236	0.805	1.217	0.857	1.392	expressed protein	AT1G35220.1
CUST_15949_PI390587928	0.769	1.495	0.767	1.607	0.718	1.428	0.608	1.652	OsWRKY45 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	AT3G56400.1
CUST_36303_PI390587928	0.741	1.871	0.718	1.876	0.679	1.515	0.672	2.035	ubiquitin-protein ligase putative expressed	AT4G08980.5
CUST_33898_PI390587928	0.684	2.525	0.657	2.798	0.444	1.584	0.472	3.640	anthocyanidin 53-O-glucosyltransferase putative expressed	AT3G16520.2
CUST_41858_PI390587928	0.858	1.187	0.875	1.131	0.844	1.232	0.845	1.395	proteasome subunit beta type 7-A precursor putative expressed	AT5G40580.2
CUST_7929_PI390587928	0.850	1.476	0.776	1.415	0.800	1.286	0.805	1.528	cardiolipin synthetase putative expressed	
CUST_297_PI390587928	0.686	1.595	0.656	1.456	0.638	1.691	0.606	1.801	metal ion binding protein putative expressed	AT5G23760.1
CUST_2936_PI390587928	0.615	1.836	0.699	2.075	0.725	2.232	0.671	2.691	-	
CUST_16257_PI390587928	0.553	2.078	0.685	2.723	0.623	2.136	0.642	3.062	ATPase coupled to transmembrane movement of substances putative expressed	AT1G66950.1
CUST_32737_PI390587928	0.849	1.930	0.746	1.655	0.623	1.267	0.632	1.346	40S ribosomal protein S29 putative expressed	AT4G33865.1
CUST_15842_PI390587928	0.932	1.679	0.698	1.355	0.682	1.227	0.815	1.408	NOL1/NOP2/sun family protein expressed	AT2G22400.1
CUST_3974_PI390587928	0.690	1.942	0.683	2.090	0.749	1.721	0.692	1.899	-	
CUST_41735_PI390587928	0.071	5.912	0.064	3.453	0.039	6.792	0.010	3.519	expressed protein	AT2G31945.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_8237_PI390587928	0.769	1.136	0.772	1.092	0.865	1.204	0.826	1.262	pseudouridylate synthase/ tRNA-pseudouridine synthase putative expressed	AT5G35400.1
CUST_36010_PI390587928	0.530	2.518	0.630	2.400	0.530	2.272	0.462	2.571	acetylornithine aminotransferase mitochondrial precursor putative expressed	AT1G80600.1
CUST_23632_PI390587928	0.787	1.788	0.739	1.703	0.690	1.472	0.722	1.863	DNA binding protein putative expressed	AT5G09380.1
CUST_92_PI390587928	0.435	4.125	0.181	3.859	0.240	3.502	0.115	3.025	glucan endo-13-beta-glucosidase precursor putative expressed	AT5G24318.1
CUST_26698_PI390587928	0.731	1.155	0.853	1.206	0.791	1.095	0.735	1.236	lipin N-terminal conserved region family protein expressed	AT3G09560.3
CUST_40373_PI390587928	0.827	1.645	0.811	1.525	0.700	1.246	0.746	1.541	pentatricopeptide repeat putative expressed	AT1G80150.1
CUST_4904_PI390587928	0.391	3.824	0.592	3.486	0.474	3.309	0.318	2.864	OsWRKY62 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	
CUST_4687_PI390587928	0.860	1.163	0.837	1.038	0.809	1.187	0.824	1.241	proteasome subunit beta type 1 putative expressed	AT3G60820.1
CUST_16571_PI390587928	0.425	2.736	0.509	2.620	0.459	4.708	0.396	4.609	boron transporter-like protein 2 putative expressed	AT1G74810.1
CUST_40420_PI390587928	0.497	2.096	0.803	2.807	0.712	2.691	0.592	2.893	expressed protein	
CUST_3712_PI390587928	0.795	2.519	0.845	2.611	0.650	1.561	0.666	1.987	glycosylphosphatidylinositol anchor biosynthesis protein 11 putative expressed	AT1G16040.1
CUST_7482_PI390587928	0.543	1.516	0.526	1.352	0.603	1.134	0.518	1.552	16.9 kDa class I heat shock protein 2 putative expressed	AT5G59720.1
CUST_27814_PI390587928	0.279	3.794	0.328	4.229	0.306	3.346	0.320	4.793	transparent testa 12 protein putative expressed	AT5G52450.1
CUST_41256_PI390587928	0.820	2.328	0.662	1.626	0.878	1.462	0.820	1.315	-	
CUST_3542_PI390587928	0.583	2.569	0.688	2.608	0.587	2.758	0.618	2.323	-	
CUST_30548_PI390587928	0.636	1.331	0.528	1.457	0.623	2.192	0.805	1.989	receptor-like protein kinase putative expressed	AT4G00340.1
CUST_2334_PI390587928	0.410	2.783	0.140	2.096	0.043	2.142	0.085	1.755	-	
CUST_34349_PI390587928	0.822	1.098	0.932	1.293	0.864	1.134	0.815	1.181	adhesion regulating molecule conserved region family protein expressed	AT2G26590.3
CUST_27644_PI390587928	0.839	1.773	0.876	1.670	0.627	1.150	0.648	1.410	expressed protein	AT1G61870.1
CUST_31586_PI390587928	0.758	2.477	0.755	2.577	0.569	1.873	0.550	2.075	60S acidic ribosomal protein P1 putative expressed	AT5G24510.1
CUST_6151_PI390587928	0.762	1.468	0.793	1.834	0.785	1.409	0.814	1.309	cell division cycle protein 20 putative expressed	AT4G33270.1
CUST_15038_PI390587928	0.843	1.668	0.825	1.601	0.752	1.195	0.748	1.474	pentatricopeptide repeat protein PPR1106-17 putative expressed	AT2G25580.1
CUST_33173_PI390587928	0.862	1.381	0.838	1.407	0.688	1.181	0.744	1.296	dihydroorotate dehydrogenase mitochondrial precursor putative expressed	AT5G23300.1
CUST_20235_PI390587928	0.878	1.182	0.875	1.090	0.854	1.221	0.844	1.318	OsGrx_S15.1 - glutaredoxin subgroup II expressed	AT3G15660.2
CUST_12866_PI390587928	0.870	1.972	0.654	1.809	0.684	1.528	0.777	1.831	tetratricopeptide-like helical putative expressed	AT5G16860.1
CUST_11067_PI390587928	0.830	1.883	0.740	2.003	0.743	1.661	0.739	1.659	cell division protein ftsZ putative expressed	AT2G36250.2
CUST_27490_PI390587928	0.568	2.065	0.623	2.037	0.667	1.881	0.592	2.541	-	
CUST_49_PI390587928	0.722	1.335	0.907	1.498	0.798	1.264	0.766	1.295	CDPK-related protein kinase putative expressed	AT2G46700.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_25260_PI390587928	0.255	1.081	0.709	3.235	0.180	1.481	0.980	2.995	germin-like protein subfamily 1 member 7 precursor putative expressed	
CUST_42171_PI390587928	0.447	1.880	0.509	1.607	0.815	2.771	0.647	2.636	-	
CUST_8242_PI390587928	0.345	1.661	0.428	2.273	0.735	2.620	0.837	5.597	alanine aminotransferase 2 putative expressed	AT1G17290.1
CUST_11705_PI390587928	0.779	3.115	0.637	3.762	0.521	2.874	0.759	6.308	cytochrome P450 72A1 putative expressed	AT3G14630.1
CUST_18960_PI390587928	0.862	1.737	0.814	1.762	0.586	1.315	0.636	1.387	conserved hypothetical protein	
CUST_19966_PI390587928	0.021	1.262	0.023	1.948	0.034	2.564	0.121	2.540	ZFP16-2 putative expressed	AT3G53600.1
CUST_5293_PI390587928	0.688	1.281	0.915	2.226	0.710	1.219	0.817	2.553	transferrin receptor-like dimerisation domain containing protein expressed	AT5G19740.1
CUST_12743_PI390587928	0.420	3.528	0.686	4.238	0.495	2.337	0.421	4.287	-	
CUST_20703_PI390587928	0.774	1.959	0.742	1.605	0.628	1.524	0.667	1.841	expressed protein	AT4G17540.1
CUST_20533_PI390587928	0.789	1.379	0.855	1.349	0.589	1.082	0.650	1.354	-	
CUST_4723_PI390587928	0.634	1.863	0.670	1.847	0.648	2.190	0.685	3.544	anthocyanidin 3-O-glucosyltransferase putative expressed	AT5G49690.1
CUST_10847_PI390587928	0.803	1.698	0.778	1.642	0.576	1.100	0.590	1.340	60S ribosomal protein L7-2 putative expressed	AT3G13580.1
CUST_22810_PI390587928	0.473	2.380	0.385	2.971	0.563	2.037	0.537	2.702	alcohol dehydrogenase 2 putative expressed	AT1G77120.1
CUST_1758_PI390587928	0.814	1.365	0.805	1.314	0.805	1.206	0.824	1.440	mitochondrial import inner membrane translocase subunit TIM14 putative expressed	AT5G03030.1
CUST_24838_PI390587928	0.242	4.211	0.260	4.648	0.105	4.427	0.163	5.524	cysteine synthase putative expressed	AT2G43750.1
CUST_36008_PI390587928	0.686	1.353	0.648	1.295	0.988	1.350	0.766	1.434	senescence-associated protein DH putative expressed	AT2G23810.1
CUST_5015_PI390587928	0.738	1.574	0.711	1.270	0.675	2.074	0.627	1.761	pyrophosphate-energized vacuolar membrane proton pump putative expressed	AT1G15690.1
CUST_17963_PI390587928	0.809	1.660	0.872	1.532	0.767	1.387	0.727	1.684	26S proteasome non-ATPase regulatory subunit 11 putative expressed	AT1G29150.1
CUST_32628_PI390587928	0.811	1.271	0.879	1.451	0.826	1.243	0.791	1.381	DNA-directed RNA polymerase II 135 kDa polypeptide putative expressed	AT4G21710.1
CUST_3865_PI390587928	0.574	1.514	0.795	1.625	0.581	1.280	0.619	1.830	ferredoxin-3 chloroplast precursor putative expressed	AT2G27510.1
CUST_19550_PI390587928	0.784	2.329	0.683	2.071	0.535	1.492	0.571	1.793	40S ribosomal protein S3a putative expressed	AT4G34670.1
CUST_24498_PI390587928	0.595	1.894	0.679	2.262	0.732	2.444	0.725	2.851	lysine decarboxylase-like protein putative expressed	AT5G11950.2
CUST_8921_PI390587928	0.542	3.611	0.849	5.189	0.481	2.975	0.396	4.629	CYP710A1 putative expressed	AT2G34500.1
CUST_23523_PI390587928	0.814	1.484	0.852	1.553	0.900	1.459	0.824	1.694	jmjC domain containing protein expressed	AT1G08620.1
CUST_41472_PI390587928	0.694	1.761	0.543	1.928	0.539	1.763	0.654	1.901	pathogen-related protein putative expressed	AT1G78780.2
CUST_14185_PI390587928	0.277	2.362	0.359	2.039	0.555	2.338	0.427	2.593	rab GDP dissociation inhibitor alpha putative expressed	AT3G59920.1
CUST_10198_PI390587928	0.657	1.552	0.824	1.828	0.687	1.212	0.774	1.924	protein binding protein putative expressed	AT1G74180.1
CUST_39605_PI390587928	0.567	3.254	0.777	5.458	0.316	2.479	0.437	4.267	2-oxoglutarate dehydrogenase E1 component mitochondrial precursor putative expressed	AT5G65750.1
CUST_14232_PI390587928	0.854	1.792	0.761	1.746	0.682	1.462	0.729	1.733	expressed protein	AT3G15420.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_19241_PI390587928	0.803	1.996	0.745	2.040	0.576	1.334	0.581	1.480	guanine nucleotide-binding protein beta subunit-like protein putative expressed	
CUST_26466_PI390587928	0.415	3.525	0.345	2.990	0.491	2.406	0.474	3.275	expressed protein	
CUST_42169_PI390587928	0.832	2.143	0.788	2.187	0.575	1.386	0.590	1.594	-	
CUST_33250_PI390587928	0.659	1.548	0.885	1.520	0.835	1.571	0.790	1.967	cp protein putative expressed	AT5G37070.1
CUST_21894_PI390587928	0.505	2.047	0.760	1.911	0.515	1.692	0.413	1.673	calmodulin-like protein 41 putative expressed	AT1G76640.1
CUST_4876_PI390587928	0.683	1.668	0.711	1.493	0.711	1.442	0.736	1.652	-	
CUST_13981_PI390587928	0.713	1.484	0.757	1.687	0.728	1.798	0.852	1.322	glucan endo-13-beta-glucosidase precursor putative	AT1G79480.2
CUST_2646_PI390587928	0.762	1.604	0.946	1.790	0.867	1.381	0.819	1.570	calmodulin binding protein putative expressed	
CUST_12757_PI390587928	0.613	1.592	0.830	1.909	0.580	1.246	0.690	1.813	MLO-like protein 1 putative expressed	
CUST_3901_PI390587928	0.874	1.433	0.830	1.420	0.847	1.343	0.816	1.521	NADP-dependent malic enzyme chloroplast precursor putative expressed	AT1G79750.1
CUST_10543_PI390587928	0.120	4.312	0.239	5.525	0.140	5.699	0.118	6.512	indole-3-acetate beta-glucosyltransferase putative expressed	AT2G43820.1
CUST_35746_PI390587928	0.499	1.677	0.435	1.901	0.439	1.523	0.525	1.923	secretory protein putative expressed	AT2G15130.1
CUST_16419_PI390587928	0.640	2.304	0.664	2.607	0.604	2.393	0.769	2.229	activating signal cointegrator 1 complex subunit 3 putative expressed	AT5G61140.1
CUST_25603_PI390587928	0.904	1.602	0.808	1.363	0.738	1.194	0.830	1.570	50S ribosomal protein L24 putative expressed	AT5G23535.1
CUST_20424_PI390587928	0.550	1.439	0.594	1.474	0.823	1.778	0.866	2.069	-	
CUST_19548_PI390587928	0.822	2.957	0.827	2.786	0.641	1.887	0.602	2.343	40S ribosomal protein S3a putative expressed	AT4G34670.1
CUST_9453_PI390587928	0.584	2.013	0.729	2.536	0.300	1.504	0.414	1.912	expressed protein	
CUST_7727_PI390587928	0.871	1.316	0.857	1.153	0.758	1.109	0.734	1.205	transcription factor BTF3 putative expressed	AT1G73230.1
CUST_25310_PI390587928	0.455	3.895	0.667	4.892	0.378	2.438	0.452	6.024	expressed protein	AT4G05020.1
CUST_27338_PI390587928	0.729	2.205	0.399	1.854	0.378	1.494	0.403	1.746	hydrolase putative expressed	AT5G18860.1
CUST_6519_PI390587928	0.226	7.144	0.371	7.715	0.422	32.771	0.168	19.514	polygalacturonase precursor putative expressed	AT1G23460.1
CUST_22191_PI390587928	0.365	1.332	0.305	1.838	0.362	3.137	0.753	3.274	regulatory protein putative expressed	AT4G19970.1
CUST_23197_PI390587928	0.516	1.253	0.478	1.384	0.624	1.707	0.639	1.758	-	
CUST_22782_PI390587928	0.768	1.832	0.810	1.636	0.642	1.187	0.605	1.437	-	
CUST_37251_PI390587928	0.640	1.787	0.596	1.491	0.546	1.276	0.530	1.455	glycine-rich protein 2b putative expressed	AT4G36020.1
CUST_29913_PI390587928	0.729	1.390	0.740	1.382	0.693	1.181	0.641	1.285	OsIAA6 - Auxin-responsive Aux/IAA gene family member expressed	AT3G16500.1
CUST_16960_PI390587928	0.797	1.415	0.926	1.781	0.891	1.520	0.804	1.785	ubiquitin interaction motif family protein expressed	AT4G11860.1
CUST_29136_PI390587928	0.715	1.460	0.660	1.616	0.829	1.373	0.827	1.472	heavy metal-associated domain containing protein expressed	
CUST_38087_PI390587928	0.641	1.920	0.793	2.239	0.692	1.789	0.569	1.785	10-deacetylbaccatin III 10-O-acetyltransferase putative expressed	AT3G03480.1
CUST_38709_PI390587928	0.654	1.258	0.692	1.437	0.707	1.268	0.779	1.606	metabolite transport protein csbC putative expressed	AT1G79820.3
CUST_7716_PI390587928	0.364	2.017	0.380	1.818	0.513	3.012	0.406	2.554	subtilisin-chymotrypsin inhibitor 2 putative expressed	
CUST_15613_PI390587928	0.812	1.282	0.860	1.283	0.693	1.147	0.739	1.280	SCO1 protein homolog mitochondrial precursor putative expressed	AT4G39740.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_20624_PI390587928	0.513	2.264	0.542	1.930	0.463	3.217	0.431	4.855	mannitol dehydrogenase putative expressed	AT4G37980.1
CUST_30140_PI390587928	0.542	1.405	0.600	1.833	0.788	2.089	0.858	2.399	receptor-like protein kinase precursor putative expressed	AT1G73080.1
CUST_8631_PI390587928	0.506	2.614	0.416	1.691	0.472	2.424	0.527	2.818	-	
CUST_41352_PI390587928	0.797	1.464	0.831	1.493	0.876	1.428	0.827	1.571	-	
CUST_11286_PI390587928	0.757	1.186	0.860	1.421	0.815	1.286	0.760	1.237	expressed protein	AT4G00800.1
CUST_10311_PI390587928	0.874	1.516	0.781	1.351	0.785	1.341	0.772	1.578	conserved hypothetical protein	AT2G43780.2
CUST_37224_PI390587928	0.892	1.468	0.826	1.313	0.713	1.404	0.782	1.423	ribosome recycling factor putative expressed	AT3G01800.1
CUST_19121_PI390587928	0.747	2.957	0.757	2.950	0.781	1.904	0.761	2.875	pentatricopeptide repeat protein PPR986-12 putative expressed	AT5G59200.1
CUST_35514_PI390587928	0.562	3.121	0.814	5.032	0.515	2.179	0.488	3.815	transferase putative expressed	AT5G42830.1
CUST_12962_PI390587928	0.224	4.622	0.236	4.247	0.115	3.391	0.090	4.022	harpin-induced 1 putative expressed	
CUST_3313_PI390587928	0.819	1.921	0.874	1.949	0.778	1.996	0.851	2.661	leucoanthocyanidin reductase putative expressed	AT4G27250.1
CUST_33083_PI390587928	0.802	1.595	0.787	1.464	0.614	1.710	0.797	1.775	amidase putative	AT4G34880.1
CUST_38107_PI390587928	0.604	2.188	0.873	2.770	0.552	1.741	0.660	3.467	peroxisomal membrane protein PMP22 putative expressed	AT4G14305.1
CUST_2105_PI390587928	0.926	1.627	0.887	1.396	0.811	1.281	0.788	1.435	50S ribosomal protein L20 putative expressed	AT1G16740.1
CUST_5948_PI390587928	0.765	1.319	0.739	1.258	0.748	1.248	0.703	1.305	survival motor neuron containing protein expressed	AT1G54380.1
CUST_22796_PI390587928	0.768	1.608	0.689	1.554	0.836	1.578	0.871	1.870	-	
CUST_120_PI390587928	0.681	1.480	0.652	1.498	0.601	1.288	0.568	1.359	WD-repeat protein 74 putative expressed	AT1G29320.1
CUST_23849_PI390587928	0.486	2.243	0.558	2.285	0.638	2.461	0.499	2.413	-	
CUST_14914_PI390587928	0.848	1.806	0.729	1.903	0.651	1.556	0.776	1.955	DNA binding protein putative expressed	AT5G09380.1
CUST_24887_PI390587928	0.797	1.894	0.742	1.731	0.714	1.301	0.684	1.445	phosphate carrier protein mitochondrial precursor putative expressed	AT5G14040.1
CUST_27666_PI390587928	0.871	1.611	0.773	1.404	0.708	1.122	0.706	1.338	60S ribosomal protein L29 putative expressed	AT3G06680.1
CUST_27682_PI390587928	0.730	1.252	0.764	1.293	0.730	1.118	0.702	1.284	ubiquitin-like protein SMT3 putative expressed	AT4G26840.1
CUST_18529_PI390587928	0.457	1.793	0.648	2.083	0.739	1.743	0.859	2.837	receptor-like protein kinase putative	AT5G60900.1
CUST_2892_PI390587928	0.714	1.502	0.793	1.465	0.741	1.917	0.615	1.757	ABA-responsive protein putative expressed	AT5G13200.1
CUST_3898_PI390587928	0.212	3.162	0.288	6.734	0.482	5.927	0.566	13.575	expressed protein	AT2G39050.1
CUST_10114_PI390587928	0.608	1.504	0.372	1.589	0.584	1.964	0.486	1.973	mannitol dehydrogenase putative expressed	AT4G37990.1
CUST_10587_PI390587928	0.729	1.666	0.891	1.673	0.938	1.938	0.856	2.224	palmitoyl-protein thioesterase 1 precursor putative expressed	AT3G60340.2
CUST_41675_PI390587928	0.702	1.537	0.777	1.383	0.843	2.101	0.860	1.917	-	
CUST_3759_PI390587928	0.917	1.318	0.715	1.235	0.728	1.335	0.735	1.304	tetratricopeptide-like helical putative expressed	AT2G37320.1
CUST_1529_PI390587928	0.109	8.447	0.115	8.138	0.079	6.121	0.163	12.717	multidrug resistance protein 4 putative expressed	AT2G47000.1
CUST_5532_PI390587928	0.521	1.444	0.514	1.393	0.494	1.198	0.469	1.282	60S acidic ribosomal protein P1 putative expressed	AT5G24510.1
CUST_3775_PI390587928	0.666	1.498	0.677	1.726	0.709	1.380	0.646	1.568	CID11 putative expressed	AT1G53650.2
CUST_12190_PI390587928	0.729	2.390	0.591	2.213	0.468	1.741	0.483	1.848	SET domain containing protein expressed	AT3G56570.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_41536_PI390587928	0.543	1.826	0.571	2.373	0.607	1.855	0.509	2.332	pathogenesis-related protein PRMS precursor putative expressed	AT4G33720.1
CUST_22411_PI390587928	0.233	3.756	0.271	4.587	0.553	12.542	0.306	5.730	-	
CUST_22209_PI390587928	0.570	1.375	0.544	1.568	0.698	1.579	0.905	1.672	expressed protein	AT1G74170.1
CUST_40344_PI390587928	0.733	1.416	0.798	1.518	0.737	1.265	0.753	1.549	eukaryotic translation initiation factor 4B putative expressed	AT3G26400.1
CUST_32415_PI390587928	0.682	1.571	0.910	2.677	0.701	1.312	0.776	2.146	4-coumarate--CoA ligase 1 putative expressed	AT1G20510.1
CUST_32431_PI390587928	0.272	3.292	0.439	4.542	0.203	1.765	0.348	5.058	esterase PIR7B putative expressed	AT2G23600.1
CUST_27048_PI390587928	0.544	2.312	0.677	2.668	0.633	1.947	0.596	3.285	cyanogenic beta-glucosidase precursor putative expressed	AT1G02850.2
CUST_34002_PI390587928	0.589	3.001	0.654	3.215	0.618	2.127	0.653	2.547	plant-specific domain TIGR01615 family protein expressed	AT2G39650.1
CUST_8491_PI390587928	0.490	4.952	0.526	4.191	0.531	4.707	0.506	3.526	lysyl-tRNA synthetase putative expressed	AT3G11710.1
CUST_27080_PI390587928	0.875	1.515	0.848	1.377	0.751	1.224	0.730	1.241	nucleolar protein involved in pre-mRNA processing putative expressed	AT2G46230.1
CUST_2274_PI390587928	0.442	3.811	0.438	3.720	0.666	5.171	0.598	7.573	Zn-dependent hydrolases including glyoxylases putative expressed	AT4G33540.1
CUST_34289_PI390587928	0.854	1.497	0.784	1.507	0.841	1.248	0.866	1.369	nuclear pore protein 84 / 107 containing protein expressed	AT3G14120.2
CUST_25354_PI390587928	0.732	1.300	0.823	1.422	0.684	1.659	0.774	1.715	pyrophosphate-energized vacuolar membrane proton pump putative expressed	AT1G15690.1
CUST_40019_PI390587928	0.374	2.041	0.363	1.897	0.375	2.290	0.287	2.724	-	
CUST_15180_PI390587928	0.746	1.278	0.782	1.536	0.801	1.318	0.803	1.451	interferon-related developmental regulator family protein expressed	AT1G27760.3
CUST_37084_PI390587928	0.587	1.320	0.772	1.617	0.653	1.182	0.705	1.645	DNA-binding protein MNB1B putative expressed	AT1G20693.3
CUST_41850_PI390587928	0.454	2.177	0.462	2.042	0.560	2.744	0.465	2.659	alpha-amylase isozyme C2 precursor putative expressed	AT4G25000.1
CUST_24162_PI390587928	0.304	2.182	0.905	8.516	0.325	1.106	0.414	6.054	aromatic-L-amino-acid decarboxylase putative expressed	AT2G20340.1
CUST_8943_PI390587928	0.601	2.377	0.596	2.454	0.693	3.618	0.646	3.651	expressed protein	AT1G22260.1
CUST_9981_PI390587928	0.759	1.920	0.704	1.734	0.613	1.303	0.611	1.341	protein gar2 putative expressed	AT3G18610.1
CUST_32527_PI390587928	0.414	4.260	0.474	5.594	0.556	5.546	0.503	5.737	flavonol 4-sulfotransferase putative expressed	AT2G03760.1
CUST_25652_PI390587928	0.482	1.707	0.555	1.925	0.389	1.553	0.335	2.194	-	
CUST_4833_PI390587928	0.748	1.546	0.676	1.279	0.739	1.558	0.729	1.631	TPR Domain containing protein expressed	
CUST_24444_PI390587928	0.806	1.830	0.875	1.878	0.736	1.389	0.699	1.553	F-box domain containing protein expressed	
CUST_13736_PI390587928	0.434	3.194	0.480	3.346	0.344	2.524	0.328	4.376	-	
CUST_40837_PI390587928	0.815	1.416	0.886	1.574	0.796	1.325	0.740	1.719	HIRA-interacting protein 5 putative expressed	AT3G20970.1
CUST_18559_PI390587928	0.812	2.298	0.707	1.797	0.684	1.338	0.620	1.433	BPM putative expressed	AT2G29200.1
CUST_41402_PI390587928	0.680	2.369	0.730	3.351	0.701	2.415	0.665	2.939	transcriptional repressor NF-X1 putative expressed	AT1G10170.1
CUST_31685_PI390587928	0.569	3.368	0.451	2.620	0.492	1.949	0.411	2.145	glutathione S-transferase 6 putative expressed	AT2G30870.1
CUST_17367_PI390587928	0.374	4.798	0.553	5.273	0.530	6.462	0.312	7.567	serine carboxypeptidase 1 precursor putative expressed	AT1G73300.1
CUST_28626_PI390587928	0.789	1.602	0.783	1.286	0.819	1.626	0.669	1.442	inositolphosphorylceramide-B C-26 hydroxylase putative expressed	AT2G34770.1
CUST_25157_PI390587928	0.881	1.728	0.743	1.534	0.769	1.381	0.785	1.468	-	

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_22394_PI390587928	0.845	1.615	0.920	1.712	0.844	1.267	0.780	1.900	60S ribosomal protein L24 mitochondrial precursor putative expressed	AT4G31460.1
CUST_19505_PI390587928	0.805	1.605	0.785	1.459	0.681	1.346	0.699	1.512	cleavage and polyadenylation specificity factor 73 kDa subunit putative expressed	
CUST_28456_PI390587928	0.473	9.038	0.593	8.166	0.539	8.627	0.583	5.997	lysyl-tRNA synthetase putative expressed	AT3G11710.1
CUST_12842_PI390587928	0.130	7.522	0.164	5.609	0.117	6.411	0.136	6.934	endothelial differentiation-related factor 1 putative expressed	AT3G24500.1
CUST_18313_PI390587928	0.569	1.794	0.664	2.334	0.498	1.170	0.480	1.680	xylanase inhibitor putative expressed	AT1G03230.1
CUST_20041_PI390587928	0.709	2.348	0.616	2.243	0.520	1.492	0.549	1.708	GTP binding protein putative expressed	AT5G58370.2
CUST_6461_PI390587928	0.233	2.806	0.317	4.486	0.248	3.467	0.445	6.705	ripening-related protein 2 precursor putative expressed	
CUST_38476_PI390587928	0.794	1.988	0.832	1.955	0.796	1.733	0.724	1.988	expressed protein	
CUST_21063_PI390587928	0.736	1.614	0.757	1.761	0.698	1.465	0.672	1.780	-	
CUST_13134_PI390587928	0.634	1.793	0.678	1.674	0.642	1.498	0.575	1.820	eukaryotic translation initiation factor 1A putative expressed	AT2G04520.1
CUST_27094_PI390587928	0.631	2.045	0.780	2.763	0.609	1.535	0.745	2.756	sialin putative expressed	AT2G38060.1
CUST_37284_PI390587928	0.578	1.359	0.707	1.231	0.734	1.417	0.659	1.668	anthocyanidin 53-O-glucosyltransferase putative expressed	AT3G16520.2
CUST_2895_PI390587928	0.789	1.705	0.879	1.647	0.797	2.123	0.684	1.950	ABA-responsive protein putative expressed	AT5G13200.1
CUST_13555_PI390587928	0.627	1.496	0.819	1.609	0.710	1.641	0.632	1.597	-	
CUST_7749_PI390587928	0.778	1.225	0.764	1.106	0.778	1.223	0.739	1.224	legumin-like protein putative expressed	AT1G07750.1
CUST_4986_PI390587928	0.784	1.358	0.867	1.593	0.748	1.385	0.694	1.464	RNA-binding protein precursor putative expressed	AT5G61030.1
CUST_15662_PI390587928	0.843	1.695	0.670	1.403	0.777	1.965	0.752	1.833	glycosyltransferase 6 putative expressed	AT2G22900.1
CUST_30327_PI390587928	0.834	1.284	0.821	1.322	0.803	1.398	0.823	1.423	-	
CUST_6573_PI390587928	0.649	2.209	0.698	1.805	0.721	2.379	0.568	2.073	expressed protein	
CUST_26401_PI390587928	0.734	1.824	0.760	1.876	0.497	1.136	0.510	1.625	60S ribosomal protein L19-3 putative expressed	AT3G16780.1
CUST_14454_PI390587928	0.801	1.669	0.684	1.540	0.629	1.703	0.633	1.590	conserved hypothetical protein	
CUST_29653_PI390587928	0.868	1.482	0.806	1.330	0.821	1.238	0.754	1.337	protein arginine N-methyltransferase 6 putative expressed	AT3G20020.2
CUST_32604_PI390587928	0.688	1.481	0.781	1.554	0.663	1.148	0.760	1.265	NOL1/NOP2/sun family protein expressed	AT2G22400.1
CUST_15476_PI390587928	0.490	2.022	0.518	2.179	0.457	1.702	0.429	2.079	ABC-2 type transporter family protein	
CUST_20200_PI390587928	0.720	1.461	0.945	1.461	0.757	1.841	0.766	2.282	pyrimidine-specific ribonucleoside hydrolase rihA putative expressed	AT1G05620.1
CUST_7626_PI390587928	0.760	1.568	0.800	1.380	0.742	1.249	0.682	1.422	nuclear migration protein nudC putative expressed	AT5G53400.1
CUST_36878_PI390587928	0.750	1.795	0.833	1.967	0.664	1.757	0.600	1.796	26S proteasome regulatory subunit rpn1 putative expressed	AT2G20580.1
CUST_37273_PI390587928	0.915	2.322	0.816	2.140	0.648	1.839	0.672	1.851	60S ribosomal protein L18a putative expressed	AT2G34480.1
CUST_17630_PI390587928	0.838	1.467	0.651	1.279	0.676	1.307	0.717	1.449	expressed protein	
CUST_33286_PI390587928	0.821	1.601	0.808	1.742	0.819	1.545	0.803	1.676	ribosomal protein S11 containing protein expressed	
CUST_29174_PI390587928	0.783	1.579	0.899	1.597	0.740	1.198	0.739	1.401	nucleic acid binding protein putative expressed	AT3G62240.1
CUST_20178_PI390587928	0.634	2.567	0.702	2.798	0.568	1.659	0.565	2.922	TMV response-related gene product putative expressed	

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_11243_PI390587928	0.853	1.596	0.925	1.601	0.789	1.217	0.733	1.321	ATP binding protein putative expressed	AT4G11160.1
CUST_41123_PI390587928	0.331	5.708	0.383	6.349	0.304	4.732	0.342	8.073	mitochondrial chaperone BCS1 putative expressed	AT3G50930.1
CUST_17290_PI390587928	0.875	2.010	0.781	1.761	0.770	1.492	0.789	1.528	ran GTPase activating protein putative expressed	AT5G19320.1
CUST_25064_PI390587928	0.936	2.153	0.836	1.932	0.637	1.413	0.647	1.594	membrane-associated salt-inducible protein like putative expressed	
CUST_14947_PI390587928	0.892	1.695	0.882	1.597	0.630	1.194	0.666	1.387	60S ribosomal protein L10a-1 putative expressed	AT2G27530.2
CUST_30852_PI390587928	0.900	1.460	0.868	1.417	0.776	1.255	0.797	1.640	cytochrome c oxidase polypeptide Vc putative expressed	AT2G47380.1
CUST_19179_PI390587928	0.886	1.710	0.778	1.510	0.748	1.216	0.799	1.512	bifunctional dihydrofolate reductase-thymidylate synthase putative expressed	AT4G34570.1
CUST_35084_PI390587928	0.772	1.441	0.783	1.115	0.826	1.402	0.765	1.385	RALF precursor putative expressed	
CUST_4666_PI390587928	0.594	2.215	0.648	2.181	0.696	1.931	0.748	2.584	ZIM motif family protein expressed	
CUST_19786_PI390587928	0.598	3.809	0.367	2.014	0.397	2.638	0.310	2.878	-	
CUST_40872_PI390587928	0.771	2.132	0.773	1.789	0.854	2.034	0.781	2.668	hypothetical protein	
CUST_23759_PI390587928	0.773	1.178	0.704	1.140	0.764	1.227	0.721	1.261	expressed protein	
CUST_41909_PI390587928	0.848	1.338	0.944	1.230	0.780	1.271	0.764	1.449	dihydroflavonol-4-reductase putative expressed	
CUST_23775_PI390587928	0.575	2.028	0.719	1.968	0.557	1.971	0.553	2.346	retinoid-inducible serine carboxypeptidase precursor putative expressed	AT2G27920.1
CUST_2956_PI390587928	0.597	1.792	0.696	1.932	0.778	1.760	0.734	2.123	-	
CUST_13616_PI390587928	0.637	2.878	0.670	3.234	0.593	2.325	0.584	2.907	expressed protein	
CUST_7981_PI390587928	0.381	3.639	0.483	4.302	0.372	5.196	0.275	2.927	calcium-transporting ATPase 13 plasma membrane-type putative expressed	AT3G22910.1
CUST_6757_PI390587928	0.331	2.042	0.288	2.919	0.291	1.687	1.090	3.142	-	
CUST_5549_PI390587928	0.847	2.451	0.781	2.376	0.589	1.742	0.568	1.873	retrotransposon protein putative Ty1-copia subclass	AT5G61170.1
CUST_2313_PI390587928	0.857	1.545	0.789	1.703	0.580	1.211	0.724	1.439	ATPase family AAA domain-containing protein 3 putative expressed	AT5G16930.1
CUST_955_PI390587928	0.789	1.280	0.942	1.351	0.827	1.436	0.811	1.325	proline-rich protein putative expressed	AT5G14540.1
CUST_33795_PI390587928	0.615	1.575	0.626	1.472	0.632	1.307	0.516	1.416	translation initiation factor putative expressed	AT4G38710.1
CUST_29683_PI390587928	0.123	41.579	0.180	28.698	0.111	21.218	0.086	22.543	-	
CUST_1641_PI390587928	0.810	1.453	0.786	1.418	0.730	1.176	0.746	1.240	eukaryotic translation initiation factor 2 gamma subunit putative expressed	AT1G04170.1
CUST_19354_PI390587928	0.649	1.433	0.773	1.416	0.671	1.420	0.682	1.451	DNA repair protein rhp16 putative expressed	AT1G05120.1
CUST_17124_PI390587928	0.820	1.221	0.736	1.084	0.796	1.112	0.701	1.116	cleavage stimulation factor 64 putative expressed	AT1G71800.1
CUST_4064_PI390587928	0.847	1.157	0.866	1.188	0.908	1.231	0.850	1.156	CUE domain containing protein expressed	
CUST_31910_PI390587928	0.549	1.973	0.595	1.588	0.507	1.726	0.462	2.512	heat shock 22 kDa protein mitochondrial precursor putative expressed	AT5G51440.1
CUST_31117_PI390587928	0.752	1.798	0.814	1.687	0.776	1.887	0.716	2.351	glucan endo-13-beta-glucosidase precursor putative expressed	AT1G78520.1
CUST_2354_PI390587928	0.379	5.306	0.379	7.122	0.138	5.683	0.093	6.339	remorin putative expressed	AT5G23750.2

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_39378_PI390587928	0.894	1.660	0.723	1.419	0.794	1.506	0.819	1.468	replication factor C subunit 4 putative expressed	AT1G63160.1
CUST_25994_PI390587928	0.678	1.810	0.656	1.445	0.585	1.430	0.550	1.458	-	
CUST_16266_PI390587928	0.824	1.413	0.819	1.441	0.694	1.120	0.744	1.368	dynamin-related protein 3A putative expressed	AT2G14120.3
CUST_16282_PI390587928	0.387	4.095	0.659	8.331	0.294	2.515	0.291	5.611	tyrosine/DOPA decarboxylase 2 putative expressed	AT2G20340.1
CUST_23811_PI390587928	0.916	1.811	0.853	1.675	0.706	1.206	0.740	1.604	-	
CUST_7208_PI390587928	0.752	1.777	0.827	2.001	0.634	1.278	0.631	1.603	neutral/alkaline invertase putative expressed	AT1G22650.1
CUST_33768_PI390587928	0.442	4.107	0.517	4.469	0.619	4.545	0.399	4.753	protein phosphatase 2C putative expressed	AT1G07430.1
CUST_960_PI390587928	0.590	5.013	0.571	4.550	0.363	3.075	0.305	2.795	ocs element-binding factor 1 putative expressed	
CUST_13769_PI390587928	0.738	1.510	0.633	1.383	0.666	2.120	0.785	2.594	ATP binding protein putative expressed	AT1G63640.1
CUST_11539_PI390587928	0.527	1.697	0.609	1.854	0.721	1.749	0.713	1.884	POT family protein expressed	AT1G22540.1
CUST_18825_PI390587928	0.731	2.065	0.615	1.735	0.534	1.285	0.571	1.539	40S ribosomal protein S24 putative expressed	AT3G04920.1
CUST_16580_PI390587928	0.545	1.625	0.845	1.985	0.680	1.814	0.567	1.766	hydrolase acting on glycosyl bonds putative expressed	AT5G05460.1
CUST_15356_PI390587928	0.780	1.614	0.660	1.637	0.595	1.214	0.593	1.333	60S ribosomal protein L18 putative expressed	AT3G05590.1
CUST_11369_PI390587928	0.778	2.056	0.895	2.028	0.747	1.714	0.685	1.833	steroid nuclear receptor ligand-binding putative expressed	AT4G12680.1
CUST_15372_PI390587928	0.329	3.071	0.444	5.626	0.293	4.958	0.297	7.126	-	
CUST_38531_PI390587928	0.666	1.878	0.554	2.121	0.767	2.397	0.869	3.102	monooxygenase putative expressed	AT4G38540.1
CUST_41249_PI390587928	0.804	1.422	0.841	1.291	0.769	1.160	0.765	1.439	nucleic acid binding protein putative expressed	AT1G76940.1
CUST_2296_PI390587928	0.837	1.211	0.745	1.209	0.770	1.390	0.752	1.301	RAB member of RAS oncogene family-like 3 putative expressed	AT5G64813.1
CUST_21591_PI390587928	0.391	2.635	0.447	2.978	0.384	3.020	0.448	4.016	protein P21 putative expressed	AT4G11650.1
CUST_9566_PI390587928	0.750	1.166	0.695	1.177	0.725	1.111	0.810	1.283	-	
CUST_6330_PI390587928	0.867	1.312	0.742	1.586	0.774	1.355	0.817	1.479	rhomboid family protein expressed	AT3G58460.1
CUST_17447_PI390587928	0.782	1.244	0.769	1.126	0.892	1.373	0.793	1.513	gene X-like protein putative expressed	AT3G12550.1
CUST_29410_PI390587928	0.428	3.244	0.601	3.800	0.541	3.740	0.482	4.294	glutamate dehydrogenase 2 putative expressed	AT5G07440.2
CUST_20197_PI390587928	0.880	1.538	0.871	1.573	0.874	1.614	0.860	1.791	ubiquitin fusion degradation protein 1 putative expressed	AT2G21270.3
CUST_5642_PI390587928	0.671	1.103	0.911	1.343	0.638	1.175	0.698	1.270	sugar transport protein 1 putative expressed	AT1G11260.1
CUST_38222_PI390587928	0.634	1.768	0.653	1.712	0.701	1.870	0.760	2.182	NAC domain-containing protein 78 putative expressed	
CUST_19570_PI390587928	0.776	1.559	0.887	1.330	0.889	1.426	0.780	1.578	casein kinase I isoform delta-like putative expressed	AT4G14340.1
CUST_2220_PI390587928	0.843	1.242	0.912	1.131	0.814	1.099	0.793	1.244	26S protease regulatory subunit 7 putative expressed	AT1G53750.1
CUST_172_PI390587928	0.809	1.637	0.777	1.422	0.706	1.819	0.720	1.647	expressed protein	
CUST_25300_PI390587928	0.715	1.536	0.794	1.437	0.779	1.527	0.789	1.950	catalytic/ hydrolase putative expressed	AT2G38740.1
CUST_30839_PI390587928	0.703	1.812	0.737	1.703	0.556	1.354	0.601	1.698	heat shock 70 kDa protein mitochondrial precursor putative expressed	AT5G09590.1
CUST_2050_PI390587928	0.896	1.710	0.887	1.638	0.688	1.083	0.742	1.290	tankyrase 1 putative expressed	AT3G09890.1
CUST_19198_PI390587928	0.631	4.974	0.664	6.164	0.373	2.940	0.385	6.788	protein induced upon tuberization putative expressed	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_39105_PI390587928	0.750	1.617	0.591	1.478	0.535	2.301	0.313	1.903	oxidoreductase putative expressed	AT4G09670.1
CUST_21143_PI390587928	0.707	2.346	0.671	2.472	0.601	1.967	0.564	2.473	-	
CUST_10995_PI390587928	0.728	3.882	0.790	4.467	0.337	2.624	0.495	3.278	trehalose-phosphate phosphatase putative expressed	
CUST_31908_PI390587928	0.499	2.047	0.597	1.788	0.429	1.593	0.362	2.468	heat shock 22 kDa protein mitochondrial precursor putative expressed	AT4G25200.1
CUST_12006_PI390587928	0.812	1.161	0.871	1.637	0.774	1.216	0.787	1.371	electron transporter putative expressed	
CUST_24986_PI390587928	0.680	1.448	0.741	1.834	0.769	1.813	0.747	1.969	oxysterol-binding protein OBPa putative expressed	AT4G25850.1
CUST_5163_PI390587928	0.520	2.369	0.601	3.533	0.503	2.239	0.695	4.047	chitin-inducible gibberellin-responsive protein 2 putative expressed	AT2G37650.1
CUST_36172_PI390587928	0.551	1.257	0.612	1.447	0.707	1.234	0.735	1.815	dihydroflavonol-4-reductase putative expressed	AT5G42800.1
CUST_4732_PI390587928	0.551	2.350	0.488	2.448	0.802	2.355	0.685	2.129	zinc finger homeodomain protein 1 putative expressed	AT3G28917.1
CUST_5754_PI390587928	0.590	1.985	0.795	2.324	0.547	1.738	0.513	2.256	glutathione S-transferase GSTU6 putative expressed	AT1G10370.1
CUST_19106_PI390587928	0.441	3.848	0.688	6.462	0.188	3.065	0.512	7.539	potassium transporter 5 putative expressed	AT4G13420.1
CUST_1814_PI390587928	0.688	2.715	0.689	2.605	0.471	1.521	0.700	3.231	TMV response-related gene product putative expressed	
CUST_19713_PI390587928	0.594	2.616	0.562	2.382	0.455	2.064	0.447	2.097	elongation factor 2 putative expressed	AT1G56070.1
CUST_35889_PI390587928	0.556	2.206	0.693	2.575	0.669	2.087	0.597	4.186	-	
CUST_14550_PI390587928	0.513	1.443	0.536	1.265	0.710	1.973	0.778	1.550	male sterility protein 2 putative expressed	AT4G33790.1
CUST_25762_PI390587928	0.554	2.615	0.673	2.394	0.628	2.603	0.592	2.977	F-box domain containing protein expressed	AT1G61340.1
CUST_27317_PI390587928	0.311	2.329	0.283	2.532	0.297	2.028	0.404	2.153	-	
CUST_40443_PI390587928	0.896	1.303	0.971	1.334	0.891	1.192	0.860	1.279	potassium transporter 14 putative expressed	AT5G09400.1
CUST_2496_PI390587928	0.758	1.538	0.711	1.423	0.602	1.093	0.625	1.338	40S ribosomal protein S10 putative expressed	AT5G52650.1
CUST_34744_PI390587928	0.817	1.220	0.954	1.229	0.848	1.199	0.804	1.167	ATPase family AAA domain-containing protein 1 putative expressed	AT4G27680.1
CUST_28339_PI390587928	0.707	3.894	0.691	3.122	0.619	2.763	0.584	2.941	3-isopropylmalate dehydratase small subunit 2 putative expressed	AT2G43090.1
CUST_12150_PI390587928	0.644	1.501	0.838	2.019	0.724	1.485	0.806	2.293	expressed protein	AT2G33570.1
CUST_5306_PI390587928	0.870	1.418	0.915	1.426	0.763	1.201	0.741	1.261	hydrolase NUDIX family protein expressed	AT4G11980.1
CUST_5779_PI390587928	0.133	5.637	0.156	4.724	0.147	5.743	0.115	5.917	-	
CUST_25607_PI390587928	0.787	1.640	0.831	1.491	0.867	1.739	0.731	2.114	transparent testa 12 protein putative expressed	AT4G25640.1
CUST_39583_PI390587928	0.892	1.742	0.854	1.472	0.884	1.473	0.895	1.930	late embryogenesis abundant protein putative expressed	
CUST_2373_PI390587928	0.331	2.280	0.528	2.992	0.106	2.220	0.445	2.662	cytochrome P450 94A2 putative expressed	AT3G56630.1
CUST_32174_PI390587928	0.562	1.620	0.669	1.352	0.798	2.035	0.725	2.031	EH-domain-containing protein 1 putative expressed	AT3G20290.2
CUST_10845_PI390587928	0.729	2.599	0.660	2.061	0.487	1.415	0.495	1.788	peptidyl-prolyl cis-trans isomerase CYP19-3 putative expressed	AT3G56070.2
CUST_6190_PI390587928	0.524	3.875	0.493	3.704	0.457	2.865	0.419	3.540	-	
CUST_3442_PI390587928	0.867	1.380	0.958	1.367	0.860	1.167	0.871	1.458	ectonucleotide pyrophosphatase/phosphodiesterase 1 putative expressed	AT4G29680.1
CUST_42178_PI390587928	0.538	1.963	0.670	2.564	0.552	2.305	0.609	2.977	amino acid permease putative expressed	AT2G01170.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_23053_PI390587928	0.885	2.988	0.624	2.588	0.558	2.016	0.578	1.659	Ser/Thr-rich protein T10 in DGCR region putative expressed	AT4G38260.1
CUST_37044_PI390587928	0.407	1.548	0.607	1.478	0.345	1.855	0.318	1.643	AN1-type zinc finger protein 2B putative expressed	AT3G28210.1
CUST_19398_PI390587928	0.193	2.621	0.155	1.734	0.414	4.082	0.462	6.036	hypothetical protein	
CUST_27358_PI390587928	0.191	3.976	0.311	6.336	0.201	3.777	0.317	6.444	gibberellin receptor GID1L2 putative expressed	AT5G06570.2
CUST_41017_PI390587928	0.656	2.622	0.711	2.046	0.780	2.100	0.865	3.083	-	
CUST_34872_PI390587928	0.641	1.104	0.782	1.327	0.827	1.201	0.828	1.265	pollen signalling protein with adenylyl cyclase activity putative expressed	
CUST_11558_PI390587928	0.572	1.918	0.638	2.003	0.609	1.871	0.743	2.978	anthranilate N-benzoyltransferase protein 1 putative expressed	
CUST_5954_PI390587928	0.566	1.841	0.778	1.955	0.781	1.978	0.707	2.367	ribosomal RNA apurinic site specific lyase putative	
CUST_11590_PI390587928	0.349	1.514	0.552	1.839	0.466	1.462	0.448	2.574	B12D protein expressed	AT3G29970.1
CUST_6976_PI390587928	0.685	1.662	0.787	1.709	0.544	1.220	0.779	1.290	NOL1/NOP2/sun family protein expressed	AT4G40000.1
CUST_19866_PI390587928	0.325	6.881	0.331	5.911	0.276	5.482	0.241	6.781	expressed protein	
CUST_34547_PI390587928	0.708	1.415	0.848	1.344	0.744	1.702	0.580	1.433	ABA-responsive protein putative expressed	AT5G13200.1
CUST_17466_PI390587928	0.881	1.589	0.825	1.552	0.765	1.335	0.752	1.360	cell division control protein 48 homolog B putative expressed	AT2G03670.1
CUST_32837_PI390587928	0.542	2.818	0.624	2.914	0.354	2.241	0.297	1.762	protein binding protein putative	AT1G03670.1
CUST_18721_PI390587928	0.460	2.474	0.558	2.893	0.339	2.341	0.316	2.319	-	
CUST_22663_PI390587928	0.888	1.480	0.781	1.266	0.801	1.404	0.837	1.525	expressed protein	AT5G49410.2
CUST_14734_PI390587928	0.714	1.595	0.807	1.589	0.637	1.345	0.616	1.692	hsc70-interacting protein putative expressed	AT3G17880.1
CUST_19604_PI390587928	0.717	1.570	0.816	1.635	0.739	1.422	0.640	1.690	phosphatidylinositol 3- and 4-kinase family protein expressed	AT2G46500.2
CUST_6497_PI390587928	0.923	1.379	0.874	1.250	0.779	1.093	0.840	1.263	uncharacterized ACR COG1565 family protein expressed	AT1G04900.1
CUST_26325_PI390587928	0.819	2.418	0.803	2.229	0.721	1.816	0.684	2.110	ubiquitin-activating enzyme E1 3 putative expressed	AT5G06460.1
CUST_206_PI390587928	0.647	1.943	0.671	2.015	0.668	1.714	0.597	2.424	mal d 1-associated protein putative expressed	AT2G35900.1
CUST_17173_PI390587928	0.746	1.579	0.666	1.274	0.609	1.347	0.570	1.406	-	
CUST_27363_PI390587928	0.754	1.636	0.879	1.825	0.766	1.403	0.732	2.156	harpin-induced protein putative expressed	AT2G46150.1
CUST_2557_PI390587928	0.672	1.990	0.734	2.006	0.685	1.937	0.632	2.101	chaperonin putative expressed	
CUST_6405_PI390587928	0.220	5.295	0.172	4.473	0.222	7.694	0.233	9.458	peroxidase 52 precursor putative expressed	AT5G05340.1
CUST_4160_PI390587928	0.049	4.877	0.070	10.011	0.014	4.101	0.156	11.801	-	
CUST_22822_PI390587928	0.819	1.951	0.763	1.721	0.739	1.418	0.763	1.603	-	
CUST_1786_PI390587928	0.605	1.329	0.736	1.596	0.709	1.911	0.566	2.348	expressed protein	AT5G25590.1
CUST_10922_PI390587928	0.743	1.697	0.757	1.641	0.509	1.048	0.519	1.345	60S ribosomal protein L11-1 putative expressed	AT5G45775.2
CUST_36052_PI390587928	0.768	1.274	0.779	1.343	0.724	1.437	0.696	1.570	nucleic acid binding protein putative expressed	AT4G17720.1
CUST_7118_PI390587928	0.726	1.111	0.889	1.328	0.774	1.176	0.904	1.452	phytosulfokine receptor precursor putative expressed	AT1G17240.1
CUST_1477_PI390587928	0.853	1.169	0.894	1.119	0.797	1.236	0.827	1.459	-	
CUST_16597_PI390587928	0.659	1.483	0.530	1.372	0.643	1.174	0.563	1.257	expressed protein	

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_17852_PI390587928	0.707	1.747	0.592	1.881	0.537	2.334	0.534	2.752	anthocyanidin 3-O-glucosyltransferase putative expressed	AT5G17050.1
CUST_684_PI390587928	0.727	1.799	0.665	1.308	0.630	1.482	0.625	1.368	anther-specific proline-rich protein APG putative expressed	
CUST_24620_PI390587928	0.460	6.421	0.554	5.296	0.392	5.365	0.321	5.642	ZFP16-2 putative expressed	AT5G59820.1
CUST_13679_PI390587928	0.635	2.203	0.670	1.519	0.720	3.571	0.632	2.963	OsTIL-1 - Oryza sativa Temperature-induced lipocalin-1 expressed	AT5G58070.1
CUST_8624_PI390587928	0.736	1.222	0.727	1.330	0.856	1.300	0.757	1.510	-	
CUST_1401_PI390587928	0.718	1.295	0.800	1.283	0.737	1.281	0.635	1.330	-	AT5G64920.1
CUST_15282_PI390587928	0.750	1.580	0.849	1.609	0.822	1.573	0.823	1.691	methionyl-tRNA synthetase putative expressed	AT4G13780.1
CUST_24248_PI390587928	0.642	1.511	0.649	1.640	0.603	1.295	0.599	1.520	mitochondrial import inner membrane translocase subunit Tim17 putative expressed	AT2G37410.2
CUST_11295_PI390587928	0.778	1.756	0.842	1.725	0.690	1.217	0.657	1.408	-	
CUST_31016_PI390587928	0.662	1.423	0.750	1.553	0.620	1.303	0.621	1.613	dihydrolipoyllysine-residue acetyltransferase component of pyruvatedehydrogenase complex mitochondrial precursor putative expressed	AT3G13930.1
CUST_26541_PI390587928	0.337	1.096	0.530	2.795	0.298	1.665	0.880	2.626	germin-like protein subfamily 1 member 7 precursor putative expressed	AT5G39110.1
CUST_19634_PI390587928	0.681	1.395	0.855	2.159	0.522	1.306	0.627	1.563	glucan endo-13-beta-glucosidase 4 precursor putative expressed	AT5G56590.1
CUST_26371_PI390587928	0.761	1.122	0.896	1.069	0.823	1.212	0.883	1.459	phosphoglycerate kinase cytosolic putative expressed	AT1G56190.2
CUST_30888_PI390587928	0.730	1.260	0.967	1.372	0.664	1.036	0.810	1.509	receptor-like GPI-anchored protein 2 putative expressed	AT2G17120.1
CUST_33900_PI390587928	0.609	1.619	0.688	2.296	0.677	1.993	0.860	2.969	cytochrome P450 76C4 putative expressed	AT2G45570.1
CUST_22384_PI390587928	0.592	1.528	0.574	1.691	0.783	1.396	0.596	1.660	expressed protein	
CUST_16212_PI390587928	0.889	1.251	0.873	1.235	0.789	1.278	0.879	1.273	glutathione S-transferase GSTF1 putative expressed	AT2G02930.1
CUST_3152_PI390587928	0.450	3.638	0.663	4.569	0.322	3.309	0.275	2.582	lipid phosphate phosphatase 3 chloroplast precursor putative expressed	AT3G02600.2
CUST_4158_PI390587928	0.697	1.231	0.831	1.141	0.778	1.344	0.784	1.420	serine carboxypeptidase K10B2.2 precursor putative expressed	AT4G30810.1
CUST_29011_PI390587928	0.539	1.659	0.542	1.613	0.737	1.793	0.664	1.954	histone H2B.2 putative expressed	AT5G22880.1
CUST_30749_PI390587928	0.838	1.398	0.869	1.275	0.772	1.117	0.760	1.197	heat shock protein 81-3 putative expressed	AT5G56030.1
CUST_33000_PI390587928	0.808	1.238	0.764	1.261	0.842	1.149	0.796	1.333	40S ribosomal protein S26 putative expressed	
CUST_5818_PI390587928	0.553	1.382	0.753	1.447	0.753	1.194	0.694	1.625	calmodulin-like protein 1 putative expressed	AT3G10190.1
CUST_37274_PI390587928	0.973	2.187	0.931	2.200	0.757	1.585	0.778	1.843	60S ribosomal protein L18a putative expressed	AT2G34480.1
CUST_5057_PI390587928	0.890	1.999	0.655	1.759	0.525	1.426	0.545	1.578	expressed protein	
CUST_2869_PI390587928	0.844	1.536	0.777	1.896	0.632	1.364	0.699	1.374	PRE putative expressed	AT1G80680.1
CUST_22496_PI390587928	0.786	1.146	0.783	1.502	0.712	1.182	0.829	1.377	cylicin-1 putative expressed	AT2G03150.1
CUST_22543_PI390587928	0.376	2.083	0.449	1.829	0.266	1.207	0.355	2.449	monooxygenase putative expressed	AT4G38540.1
CUST_915_PI390587928	0.239	2.285	0.273	1.746	0.637	6.608	0.428	4.164	desiccation-related protein PCC13-62 precursor putative expressed	AT1G47980.1
CUST_4976_PI390587928	0.862	1.234	0.892	1.223	0.860	1.092	0.812	1.150	myrosinase precursor putative expressed	AT1G16020.2

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_10627_PI390587928	0.248	4.062	0.279	4.507	0.502	12.207	0.298	5.887	germin-like protein subfamily 1 member 17 precursor putative expressed	AT5G39150.1
CUST_12400_PI390587928	0.549	1.964	0.564	2.151	0.709	1.685	0.616	2.711	-	
CUST_18415_PI390587928	0.582	6.043	0.393	4.567	0.283	3.181	0.248	3.124	expressed protein	AT4G35730.1
CUST_13422_PI390587928	0.463	1.758	0.455	2.698	0.533	2.522	0.554	1.791	sex determination protein tasselseed-2 putative expressed	AT3G26760.1
CUST_6610_PI390587928	0.537	1.681	0.471	2.326	0.436	1.756	0.464	2.288	win2 precursor putative expressed	AT3G04720.1
CUST_32377_PI390587928	0.751	1.518	0.706	1.578	0.664	1.249	0.783	1.883	-	
CUST_41391_PI390587928	0.407	2.326	0.416	1.375	0.576	2.258	0.358	3.060	-	
CUST_1229_PI390587928	0.453	2.602	0.395	3.311	0.599	2.897	0.442	2.236	CBL-interacting serine/threonine-protein kinase 15 putative expressed	AT4G18700.1
CUST_32238_PI390587928	0.389	1.575	0.518	2.488	0.664	1.557	0.612	1.728	seed specific protein Bn15D17A putative expressed	
CUST_6238_PI390587928	0.628	2.348	0.471	1.720	0.615	2.051	0.564	1.863	OsAPx3 - Peroxisomal Ascorbate Peroxidase encoding gene expressed	AT4G35000.1
CUST_1245_PI390587928	0.438	1.886	0.385	1.778	0.584	2.841	0.451	3.438	reticuline oxidase precursor putative expressed	AT1G26390.1
CUST_38285_PI390587928	0.299	2.166	0.312	2.584	0.365	2.067	0.519	3.093	alpha-amylase/trypsin inhibitor putative expressed	AT4G11650.1
CUST_4040_PI390587928	0.653	2.356	0.478	1.715	0.632	1.955	0.529	1.747	OsAPx3 - Peroxisomal Ascorbate Peroxidase encoding gene expressed	AT4G35000.1
CUST_15950_PI390587928	0.734	1.508	0.678	1.582	0.676	1.540	0.554	1.639	-	
CUST_24341_PI390587928	0.647	1.579	0.604	1.573	0.575	1.147	0.544	1.350	-	
CUST_10164_PI390587928	0.846	2.153	0.813	2.013	0.747	1.393	0.808	2.094	glucan endo-13-beta-glucosidase 4 precursor putative expressed	AT5G56590.1
CUST_7307_PI390587928	0.862	2.000	0.858	1.994	0.576	1.253	0.590	1.547	40S ribosomal protein S20 putative expressed	AT5G62300.2
CUST_15204_PI390587928	0.738	1.587	0.843	1.605	0.839	1.646	0.782	1.874	ankyrin-3 putative expressed	AT5G61230.1
CUST_16226_PI390587928	0.717	1.612	0.836	1.614	0.487	1.168	0.577	1.269	chaperonin CPN60-1 mitochondrial precursor putative expressed	AT3G23990.1
CUST_32131_PI390587928	0.632	2.941	0.760	3.121	0.597	2.824	0.670	5.704	-	
CUST_34952_PI390587928	0.535	2.863	0.613	3.518	0.553	3.209	0.546	5.268	-	
CUST_2751_PI390587928	0.837	1.097	0.790	1.034	0.957	1.213	0.910	1.248	-	AT4G30580.1
CUST_3773_PI390587928	0.776	1.360	0.857	1.252	0.815	1.199	0.704	1.383	zinc finger A20 and AN1 domains-containing protein putative expressed	AT1G51200.2
CUST_40512_PI390587928	0.766	1.865	0.710	1.678	0.572	1.241	0.672	1.297	glycosyltransferase putative expressed	AT3G18170.1
CUST_3043_PI390587928	0.633	1.427	0.586	1.533	0.766	1.810	0.938	1.899	expressed protein	AT2G34140.1
CUST_15959_PI390587928	0.707	1.477	0.770	1.273	0.758	1.833	0.853	1.794	transposon protein putative Mutator sub-class expressed	AT5G66560.1
CUST_41380_PI390587928	0.864	1.489	0.873	1.599	0.776	1.205	0.737	1.506	expressed protein	AT1G14060.1
CUST_30672_PI390587928	0.816	1.797	0.770	1.600	0.716	1.252	0.710	1.239	single-stranded DNA-binding protein putative expressed	AT3G18580.1
CUST_7939_PI390587928	0.614	1.814	0.632	1.946	0.573	1.405	0.580	1.881	expressed protein	
CUST_21551_PI390587928	0.628	1.591	0.737	2.110	0.660	1.537	0.705	2.312	endoplasmic oxidoreductin-1 precursor putative expressed	AT2G38960.2
CUST_33769_PI390587928	0.904	1.415	0.881	1.253	0.780	1.147	0.750	1.279	-	
CUST_37772_PI390587928	0.539	2.847	0.481	1.934	0.634	2.295	0.589	2.800	hypothetical protein	
CUST_12430_PI390587928	0.647	1.378	0.707	1.693	0.759	1.204	0.685	1.413	3-hydroxy-3-methylglutaryl-coenzyme A reductase 3 putative expressed	AT1G76490.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_34334_PI390587928	0.740	1.824	0.834	2.327	0.762	1.462	0.739	2.414	cytochrome P450 74A1 chloroplast precursor putative expressed	AT5G42650.1
CUST_26405_PI390587928	0.598	2.717	0.705	2.709	0.530	2.311	0.633	3.161	ubiquitin carboxyl-terminal hydrolase 6 putative expressed	AT1G51710.2
CUST_34350_PI390587928	0.845	1.458	0.816	1.465	0.810	1.402	0.763	1.509	adhesion regulating molecule conserved region family protein expressed	AT2G26590.3
CUST_9356_PI390587928	0.718	2.425	0.620	2.201	0.746	2.042	0.601	2.381	peptidase/ subtilase putative expressed	AT4G20430.1
CUST_25182_PI390587928	0.660	2.450	0.631	2.277	0.611	2.770	0.580	2.956	protein kinase putative expressed	AT1G01660.1
CUST_19514_PI390587928	0.560	2.342	0.574	2.352	0.582	2.700	0.719	2.896	ubiquitin carboxyl-terminal hydrolase family protein expressed	AT1G04860.1
CUST_24021_PI390587928	0.691	2.096	0.799	2.231	0.691	2.089	0.556	2.648	mtN19-like protein putative expressed	AT5G61820.1
CUST_5199_PI390587928	0.868	3.508	0.820	2.839	0.605	2.325	0.636	2.483	elongation factor 1-alpha putative expressed	AT5G60390.3
CUST_36255_PI390587928	0.551	2.442	0.601	3.259	0.421	1.909	0.380	2.678	AER putative expressed	AT2G39980.1
CUST_36069_PI390587928	0.684	1.395	0.745	1.373	0.759	1.336	0.708	1.768	microsomal glutathione S-transferase 3 putative expressed	AT1G65820.1
CUST_22125_PI390587928	0.865	1.520	0.854	1.388	0.721	1.368	0.777	1.665	ATP synthase beta chain mitochondrial precursor putative expressed	AT5G08690.1
CUST_5076_PI390587928	0.654	1.363	0.590	1.389	0.576	1.341	0.626	1.533	glutathione S-transferase GSTF1 putative expressed	AT3G62760.1
CUST_6906_PI390587928	0.755	1.567	0.797	1.615	0.747	1.488	0.713	1.554	expressed protein	AT1G73350.3
CUST_39368_PI390587928	0.803	1.568	0.608	1.389	0.767	1.353	0.777	1.775	calcium-dependent protein kinase putative expressed	AT1G12680.1
CUST_5714_PI390587928	0.344	3.140	0.274	3.359	0.829	2.749	0.349	4.284	expressed protein	AT1G22540.1
CUST_23770_PI390587928	0.827	1.435	0.777	1.420	0.736	1.115	0.737	1.515	expressed protein	AT2G35790.1
CUST_21540_PI390587928	0.744	1.497	0.910	1.621	0.643	1.165	0.721	1.635	ethylene response element binding protein putative expressed	AT3G14230.2
CUST_15841_PI390587928	0.789	1.294	0.903	1.370	0.729	1.263	0.722	1.353	expressed protein	AT1G15780.1
CUST_13425_PI390587928	0.390	1.827	0.380	2.705	0.354	2.463	0.479	1.876	sex determination protein tasselseed-2 putative expressed	AT3G26760.1
CUST_33805_PI390587928	0.368	3.360	0.671	6.785	0.356	1.742	0.693	6.733	-	
CUST_39852_PI390587928	0.770	2.087	0.762	1.997	0.943	2.408	0.823	2.413	expressed protein	
CUST_38053_PI390587928	0.094	7.124	0.114	6.764	0.074	4.470	0.080	5.825	-	
CUST_6613_PI390587928	0.528	1.883	0.532	2.276	0.554	1.812	0.678	3.507	win2 precursor putative expressed	AT3G04720.1
CUST_10492_PI390587928	0.120	4.148	0.155	6.756	0.094	3.474	0.510	11.883	oxalate oxidase 2 precursor putative expressed	AT1G18980.1
CUST_26697_PI390587928	0.769	1.645	0.691	1.483	0.631	1.131	0.602	1.315	expressed protein	
CUST_25722_PI390587928	0.745	1.440	0.715	1.537	0.745	1.345	0.666	1.651	expressed protein	AT5G58920.1
CUST_35695_PI390587928	0.726	1.377	0.740	1.424	0.815	1.444	0.873	1.473	F-box domain containing protein expressed	
CUST_7481_PI390587928	0.686	1.421	0.890	1.627	0.684	1.517	0.833	1.849	-	
CUST_11982_PI390587928	0.782	1.298	0.879	1.303	0.760	1.581	0.738	1.519	glutamate receptor 2.9 precursor putative expressed	AT2G29110.1
CUST_26388_PI390587928	0.732	2.206	0.758	2.763	0.644	1.564	0.629	2.297	importin beta-3 putative expressed	AT5G19820.1
CUST_8348_PI390587928	0.885	2.000	0.869	1.750	0.650	1.342	0.687	1.565	ribonucleoprotein like protein putative expressed	AT5G55550.2
CUST_27643_PI390587928	0.855	1.477	0.858	1.327	0.824	1.350	0.864	1.517	ubiquinone biosynthesis methyltransferase COQ5 mitochondrial precursor putative expressed	AT5G57300.2

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_7172_PI390587928	0.335	3.490	0.425	2.948	0.259	2.368	0.265	3.088	expressed protein	
CUST_25057_PI390587928	0.689	2.040	0.559	2.021	0.604	1.536	0.559	1.623	expressed protein	
CUST_22869_PI390587928	0.330	4.022	0.310	3.903	0.412	4.236	0.274	5.964	-	
CUST_42201_PI390587928	0.783	2.984	0.469	2.655	0.573	2.008	0.647	2.416	60S ribosomal protein L18a putative expressed	AT2G34480.1
CUST_26312_PI390587928	0.705	1.862	0.722	2.167	0.733	1.848	0.784	2.728	expressed protein	
CUST_21086_PI390587928	0.588	1.319	0.730	1.202	0.834	1.366	0.768	1.596	ribonuclease 1 precursor putative expressed	AT2G02990.1
CUST_4270_PI390587928	0.660	1.372	0.932	1.621	0.725	1.150	0.964	1.560	calmodulin binding protein putative expressed	AT5G57580.1
CUST_13950_PI390587928	0.834	1.350	0.600	1.684	0.503	1.191	0.682	1.538	CDT1a protein putative expressed	AT2G31270.1
CUST_39064_PI390587928	0.726	3.735	0.784	4.873	0.425	3.001	0.455	3.261	trehalose-phosphate phosphatase putative expressed	AT4G12430.1
CUST_4861_PI390587928	0.774	1.463	0.831	1.497	0.715	1.343	0.662	1.602	delta 1-pyrroline-5-carboxylate synthetase putative expressed	AT2G39800.1
CUST_10985_PI390587928	0.324	4.477	0.357	4.922	0.157	4.678	0.245	5.463	cysteine synthase putative expressed	
CUST_9093_PI390587928	0.736	2.641	0.691	2.318	0.607	1.983	0.609	2.258	cyclin-dependent kinases regulatory subunit putative expressed	AT2G27960.1
CUST_13764_PI390587928	0.680	1.802	0.766	2.214	0.589	1.599	0.597	2.228	beta-fructofuranosidase insoluble isoenzyme 1 precursor putative expressed	AT3G52600.2
CUST_19779_PI390587928	0.756	1.453	0.805	1.558	0.634	1.191	0.741	1.927	gibberellin receptor GID1L2 putative expressed	AT3G48690.1
CUST_5930_PI390587928	0.511	2.202	0.339	1.560	0.278	1.569	0.248	1.334	protein HVA22 putative expressed	AT5G50720.1
CUST_18820_PI390587928	0.803	1.819	0.759	1.733	0.575	1.128	0.580	1.480	60S ribosomal protein L33-B putative expressed	AT1G74270.1
CUST_27802_PI390587928	0.534	2.035	0.594	2.273	0.556	2.282	0.464	2.506	-	
CUST_38828_PI390587928	0.702	1.353	0.879	1.491	0.757	1.265	0.750	1.805	expressed protein	AT1G75170.2
CUST_21431_PI390587928	0.492	3.183	0.570	3.507	0.640	3.016	0.681	6.146	embryonic abundant protein-like putative expressed	AT2G41380.1
CUST_8857_PI390587928	0.814	1.113	0.879	1.141	0.888	1.121	0.871	1.196	HECT-domain family protein expressed	AT3G17205.2
CUST_39866_PI390587928	0.836	1.529	0.748	1.295	0.791	1.656	0.774	1.437	harpin-induced protein putative expressed	
CUST_31621_PI390587928	0.738	1.207	0.912	1.484	0.796	1.233	0.795	1.440	fas-associated factor 1-like protein putative expressed	AT4G10790.1
CUST_33681_PI390587928	0.305	2.254	0.363	2.849	0.603	3.557	0.307	2.023	agmatine coumaroyltransferase putative expressed	
CUST_8687_PI390587928	0.841	1.364	0.926	1.389	0.768	1.116	0.734	1.218	tubulin beta-3 chain putative expressed	AT5G62700.1
CUST_5249_PI390587928	0.714	1.602	0.714	1.616	0.512	1.209	0.587	2.025	boron transporter-like protein 2 putative expressed	AT1G15460.1
CUST_36258_PI390587928	0.621	1.736	0.563	1.458	0.520	1.476	0.620	2.284	glutathione S-transferase IV putative expressed	AT3G62760.1
CUST_25566_PI390587928	0.325	3.484	0.456	3.455	0.822	4.757	0.543	5.009	RNA-binding protein cabeza putative expressed	AT3G15680.1
CUST_42004_PI390587928	0.836	1.736	0.767	1.452	0.648	1.736	0.726	2.496	-	
CUST_39573_PI390587928	0.484	2.385	0.587	2.237	0.345	2.230	0.279	2.400	-	
CUST_31126_PI390587928	0.806	1.825	0.784	1.586	0.768	1.525	0.743	1.949	expressed protein	AT5G16060.1
CUST_10835_PI390587928	0.716	1.317	0.598	1.186	0.623	1.278	0.553	1.326	-	
CUST_15844_PI390587928	0.518	1.467	0.703	1.614	0.613	1.105	0.644	1.641	RGH1A putative	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_3992_PI390587928	0.861	1.471	0.872	1.332	0.847	1.360	0.818	1.290	-	
CUST_38226_PI390587928	0.486	2.736	0.296	1.944	0.504	4.005	0.506	3.813	expressed protein	
CUST_953_PI390587928	0.935	1.363	0.842	1.124	0.783	1.225	0.786	1.215	SLD5 putative expressed	AT5G49010.1
CUST_32802_PI390587928	0.123	8.105	0.206	11.029	0.386	12.766	0.294	24.427	IN2-1 protein putative expressed	AT5G02790.1
CUST_32009_PI390587928	0.858	1.535	0.569	1.146	0.724	1.353	0.714	1.639	expressed protein	
CUST_15721_PI390587928	0.080	9.800	0.105	8.221	0.080	13.117	0.081	13.995	UBiQuitin family member putative expressed	AT3G52590.1
CUST_17174_PI390587928	0.841	1.575	0.843	1.451	0.736	1.407	0.716	1.740	catalytic/ oxidoreductase acting on NADH or NADPH putative expressed	AT5G61220.1
CUST_41583_PI390587928	0.470	2.816	0.379	2.256	0.392	2.164	0.343	1.998	-	
CUST_34692_PI390587928	0.723	1.619	0.747	1.652	0.511	1.219	0.453	1.247	60S ribosomal protein L18 putative expressed	AT3G05590.1
CUST_35698_PI390587928	0.606	1.949	0.488	1.778	0.359	1.686	0.369	1.603	cell envelope integrity inner membrane protein TolA putative	
CUST_36953_PI390587928	0.870	1.299	0.983	1.294	0.810	1.102	0.858	1.330	electron transporter putative expressed	AT4G08550.1
CUST_4736_PI390587928	0.676	1.916	0.614	1.709	0.783	1.754	0.811	2.304	adagio protein 1 putative expressed	AT5G57360.1
CUST_1500_PI390587928	0.697	2.428	0.571	1.840	0.629	1.747	0.563	1.658	-	
CUST_22792_PI390587928	0.645	2.345	0.651	2.079	0.556	1.702	0.616	1.899	tetratricopeptide-like helical putative	AT5G16860.1
CUST_33515_PI390587928	0.885	1.753	0.858	1.517	0.673	1.211	0.705	1.242	60S ribosomal protein L23 putative expressed	AT3G04400.1
CUST_27599_PI390587928	0.501	2.315	0.556	2.265	0.630	2.803	0.598	2.942	DELLA protein GAI1 putative expressed	AT3G54220.1
CUST_15885_PI390587928	0.415	7.237	0.456	6.206	0.392	5.846	0.405	5.692	valyl-tRNA synthetase putative	
CUST_27646_PI390587928	0.760	1.336	0.715	1.275	0.705	1.203	0.706	1.388	expressed protein	AT1G73940.1
CUST_11239_PI390587928	0.348	2.026	0.313	3.046	0.513	1.485	0.219	1.623	amine oxidase precursor putative expressed	AT4G14940.1
CUST_28684_PI390587928	0.712	1.659	0.835	2.042	0.566	1.295	0.734	1.783	MLO-like protein 1 putative expressed	AT2G44110.2
CUST_26268_PI390587928	0.484	2.691	0.538	2.422	0.481	3.031	0.659	2.777	-	
CUST_39253_PI390587928	0.559	1.492	0.784	1.805	0.608	1.302	0.734	1.931	-	AT2G35530.1
CUST_24070_PI390587928	0.359	2.991	0.493	3.133	0.303	2.524	0.436	3.646	glutamate decarboxylase putative expressed	AT2G02010.1
CUST_13937_PI390587928	0.754	1.364	0.832	1.979	0.668	1.240	0.894	2.325	nucleotide binding protein putative expressed	AT2G20330.1
CUST_4273_PI390587928	0.511	3.639	0.547	3.357	0.456	3.575	0.588	6.444	L-allo-threonine aldolase putative expressed	AT3G04520.1
CUST_15997_PI390587928	0.741	1.679	0.761	1.856	0.635	1.378	0.591	1.604	flavonol synthase/flavanone 3-hydroxylase putative expressed	AT5G24530.1
CUST_5933_PI390587928	0.782	1.337	0.858	1.381	0.662	1.104	0.578	1.165	S-adenosylmethionine-dependent methyltransferase/ methyltransferase putative expressed	AT1G19340.1
CUST_22190_PI390587928	0.693	3.447	0.709	2.955	0.527	1.853	0.463	2.511	WD-repeat protein 50 putative expressed	AT5G14050.1
CUST_17024_PI390587928	0.771	1.747	0.773	1.792	0.757	1.346	0.805	1.708	keratin-associated protein 5-4 putative expressed	
CUST_5156_PI390587928	0.515	2.767	0.908	3.481	0.220	2.637	0.300	2.159	peroxidase 52 precursor putative expressed	AT5G05340.1
CUST_40915_PI390587928	0.726	1.325	0.821	1.361	0.780	1.133	0.740	1.450	cupin RmlC-type putative expressed	AT3G58670.2
CUST_1962_PI390587928	0.777	1.179	0.787	1.398	0.752	1.158	0.709	1.152	expressed protein	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_33977_PI390587928	0.861	2.710	0.739	2.277	0.498	1.451	0.536	1.509	fibrillarin-2 putative expressed	AT5G52470.2
CUST_14059_PI390587928	0.448	1.948	0.703	2.068	0.516	1.816	0.464	2.146	calmodulin-like protein 41 putative expressed	AT1G76640.1
CUST_29290_PI390587928	0.544	2.241	0.640	2.174	0.554	1.860	0.449	1.901	-	
CUST_31017_PI390587928	0.808	1.250	0.808	1.336	0.800	1.172	0.780	1.107	uridine 5-monophosphate synthase putative expressed	AT3G54470.1
CUST_23073_PI390587928	0.327	2.747	0.413	1.712	0.703	2.526	0.691	3.362	microtubule-associated protein RP/EB family member 3 putative expressed	AT5G67270.1
CUST_10695_PI390587928	0.824	1.511	0.863	1.509	0.798	1.336	0.897	1.501	CAB2 putative expressed	AT1G16560.4
CUST_20885_PI390587928	0.676	3.455	0.600	3.008	0.618	2.795	0.599	2.968	snRNP protein putative expressed	AT3G06190.1
CUST_15719_PI390587928	0.048	14.680	0.081	11.544	0.047	15.959	0.050	14.224	UBiQuitin family member putative expressed	AT3G52590.1
CUST_3867_PI390587928	0.591	1.495	0.750	1.539	0.603	1.323	0.594	1.673	ferredoxin-3 chloroplast precursor putative expressed	AT2G27510.1
CUST_13978_PI390587928	0.709	1.175	0.750	1.744	0.782	1.411	0.856	1.828	kinesin-4 putative expressed	AT5G27550.1
CUST_38086_PI390587928	0.577	1.625	0.643	1.520	0.729	1.775	0.829	2.207	rab GDP dissociation inhibitor alpha putative expressed	AT5G09550.1
CUST_29726_PI390587928	0.712	6.256	0.798	6.380	0.599	4.431	0.590	2.711	FIP1 putative	
CUST_38910_PI390587928	0.769	1.409	0.864	2.267	0.628	1.255	0.936	2.672	expressed protein	AT1G73380.1
CUST_33684_PI390587928	0.459	1.542	0.557	1.351	0.571	1.816	0.680	1.894	-	AT3G14630.1
CUST_3728_PI390587928	0.525	1.844	0.615	2.302	0.591	1.919	0.685	2.853	expressed protein	AT5G06270.1
CUST_30262_PI390587928	0.673	1.524	0.706	1.575	0.699	1.736	0.752	2.251	DNA-binding protein putative expressed	AT4G00200.1
CUST_25569_PI390587928	0.325	3.560	0.421	3.327	0.797	4.638	0.512	4.800	RNA-binding protein cabeza putative expressed	AT3G15680.1
CUST_31315_PI390587928	0.051	6.291	0.034	11.775	0.604	5.430	0.068	10.449	sulfate transporter 3.5 putative expressed	AT5G19600.1
CUST_1344_PI390587928	0.233	3.751	0.357	8.282	0.614	6.995	0.591	14.865	expressed protein	
CUST_34614_PI390587928	0.699	2.558	0.716	2.509	0.640	2.046	0.617	2.292	elongation factor 1-alpha putative expressed	AT5G60390.3
CUST_25446_PI390587928	0.931	1.692	0.773	1.288	0.874	1.320	0.795	1.183	zinc finger protein putative expressed	AT3G15680.1
CUST_40096_PI390587928	0.837	1.315	0.925	1.155	0.711	1.130	0.769	1.331	tubulin alpha-2 chain putative expressed	AT5G19780.1
CUST_3388_PI390587928	0.901	2.153	0.803	2.132	0.618	1.480	0.716	1.574	expressed protein	
CUST_8630_PI390587928	0.886	2.045	0.835	1.987	0.685	1.649	0.613	1.811	F-box domain containing protein expressed	
CUST_36658_PI390587928	0.449	2.383	0.683	3.415	0.438	1.880	0.565	4.181	transposon protein putative unclassified expressed	AT3G22370.1
CUST_10854_PI390587928	0.663	1.366	0.751	1.771	0.592	1.501	0.618	1.789	-	
CUST_23046_PI390587928	0.614	1.401	0.796	1.617	0.600	1.239	0.552	1.426	adenosine 3-phospho 5-phosphosulfate transporter 1 putative expressed	AT3G46180.1
CUST_31022_PI390587928	0.368	2.388	0.584	2.553	0.424	1.907	0.304	2.418	naringenin2-oxoglutarate 3-dioxygenase putative expressed	AT5G24530.1
CUST_25914_PI390587928	0.683	1.560	0.670	1.606	0.789	1.434	0.731	1.902	zinc finger C-x8-C-x5-C-x3-H type family protein expressed	AT2G33835.1
CUST_13967_PI390587928	0.482	2.662	0.491	2.689	0.407	2.439	0.292	1.597	hsp20/alpha crystallin family protein expressed	
CUST_18199_PI390587928	0.824	1.506	0.832	1.945	0.759	1.418	0.764	1.670	global transcription factor group E putative expressed	AT3G27260.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_35157_PI390587928	0.651	1.446	0.686	1.322	0.781	1.386	0.844	1.515	histone H2B.1 putative expressed	AT5G22880.1
CUST_21587_PI390587928	0.813	1.936	0.775	1.731	0.740	1.534	0.639	1.407	ubiquitin-protein ligase/ zinc ion binding protein putative expressed	AT4G37110.1
CUST_10895_PI390587928	0.325	3.209	0.374	5.000	0.263	4.657	0.261	6.594	Bowman-Birk type wound-induced proteinase inhibitor WIP1 precursor putative	
CUST_13674_PI390587928	0.604	3.112	0.669	3.468	0.546	2.760	0.459	4.050	esterase precursor putative expressed	AT3G26430.1
CUST_2859_PI390587928	0.572	2.424	0.570	2.035	0.635	2.130	0.679	2.565	vegetatible incompatibility protein HET-E-1 putative expressed	
CUST_20442_PI390587928	0.722	1.484	0.825	1.677	0.687	1.458	0.721	1.791	anthocyanidin 3-O-glucosyltransferase putative expressed	AT3G21760.1
CUST_11274_PI390587928	0.834	1.141	0.775	1.088	0.867	1.131	0.805	1.275	-	AT5G65000.1
CUST_41844_PI390587928	0.735	1.408	0.773	1.335	0.701	1.338	0.664	1.671	OsGrx_C2.2 - glutaredoxin subgroup I expressed	AT5G40370.1
CUST_7915_PI390587928	0.808	2.463	0.496	1.358	0.959	1.977	0.785	1.816	-	
CUST_23741_PI390587928	0.505	3.827	0.610	4.991	0.448	2.612	0.349	6.257	flavonol-3-O-glycoside-7-O-glucosyltransferase 1 putative expressed	AT2G36750.1
CUST_19582_PI390587928	0.485	2.120	0.753	2.535	0.674	2.576	0.738	3.035	-	
CUST_26318_PI390587928	0.756	1.334	0.775	1.218	0.850	1.298	0.738	1.418	expressed protein	AT4G22740.2
CUST_36524_PI390587928	0.766	1.307	0.726	1.405	0.718	1.117	0.659	1.171	asparaginyl-tRNA synthetase cytoplasmic 3 putative expressed	AT5G56680.1
CUST_32537_PI390587928	0.683	1.367	0.685	1.492	0.632	1.390	0.595	1.556	-	
CUST_41535_PI390587928	0.482	2.251	0.462	2.917	0.430	2.447	0.467	3.126	pathogenesis-related protein PRMS precursor putative expressed	AT4G25790.1
CUST_7606_PI390587928	0.595	1.968	0.679	2.001	0.646	1.817	0.753	3.044	NADP-specific glutamate dehydrogenase putative expressed	AT1G51720.1
CUST_7622_PI390587928	0.652	2.925	0.729	3.491	0.650	2.353	0.579	2.897	elongation factor 2 putative expressed	AT1G56070.1
CUST_25476_PI390587928	0.826	1.621	0.839	1.588	0.717	1.111	0.722	1.302	nop14-like family protein expressed	
CUST_31191_PI390587928	0.705	2.499	0.660	2.077	0.476	1.377	0.481	1.707	peptidyl-prolyl cis-trans isomerase CYP19-3 putative expressed	AT3G56070.2
CUST_15924_PI390587928	0.961	1.818	0.730	1.218	0.725	1.305	0.801	1.520	expressed protein	AT4G35850.1
CUST_22101_PI390587928	0.788	1.778	0.740	1.789	0.567	1.196	0.570	1.391	60S ribosomal protein L36-2 putative expressed	AT3G53740.4
CUST_29915_PI390587928	0.714	1.409	0.774	1.762	0.643	1.380	0.750	1.395	OsIAA6 - Auxin-responsive Aux/IAA gene family member expressed	
CUST_32851_PI390587928	0.502	2.368	0.574	2.053	0.860	2.314	0.708	3.016	-	
CUST_15163_PI390587928	0.755	1.901	0.823	2.321	0.798	1.972	0.801	1.913	activating signal cointegrator 1 complex subunit 3 putative expressed	AT5G61140.1
CUST_2087_PI390587928	0.625	1.645	0.844	1.922	0.565	1.237	0.697	1.850	MLO-like protein 1 putative expressed	AT4G02600.2
CUST_9357_PI390587928	0.772	1.752	0.699	1.471	0.764	1.514	0.777	2.029	cytokinin-O-glucosyltransferase 2 putative expressed	AT3G02100.1
CUST_10559_PI390587928	0.621	1.981	0.639	2.311	0.784	2.311	0.765	2.787	ABC transporter family protein expressed	AT3G60160.1
CUST_41647_PI390587928	0.598	1.258	0.660	1.210	0.805	1.322	0.977	1.541	-	
CUST_33734_PI390587928	0.622	1.784	0.514	1.868	0.526	1.876	0.539	3.236	cyclin N-terminal domain containing protein	AT4G31860.1
CUST_26796_PI390587928	0.670	1.453	0.694	1.153	0.805	1.552	0.635	1.342	inositolphosphorylceramide-B C-26 hydroxylase putative expressed	AT4G20870.1
CUST_33750_PI390587928	0.864	1.344	0.903	1.322	0.897	1.261	0.920	1.533	sterol-4-alpha-carboxylate 3-dehydrogenase decarboxylating putative expressed	AT2G26260.2

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_30769_PI390587928	0.505	2.510	0.546	1.847	0.543	2.128	0.396	1.466	aquaporin TIP2.2 putative expressed	AT3G16240.1
CUST_22856_PI390587928	0.397	3.191	0.710	4.359	0.469	2.473	0.510	3.711	mitogen-activated protein kinase kinase kinase 2 putative expressed	
CUST_740_PI390587928	0.816	1.701	0.791	1.726	0.793	1.690	0.775	1.875	threonine dehydratase biosynthetic chloroplast precursor putative expressed	AT3G10050.1
CUST_19782_PI390587928	0.684	1.235	0.869	1.108	0.780	1.160	0.852	1.532	pyrimidine-specific ribonucleoside hydrolase rihA putative expressed	AT2G36310.1
CUST_18574_PI390587928	0.662	1.948	0.686	2.177	0.588	1.606	0.619	2.564	glutathione S-transferase GSTU6 putative expressed	AT1G10360.1
CUST_22547_PI390587928	0.691	1.532	0.665	1.146	0.921	1.676	0.772	1.610	apurinic endonuclease-redox protein putative expressed	AT2G41460.1
CUST_25326_PI390587928	0.783	1.385	0.794	1.979	0.707	1.312	0.809	2.193	ACS-like protein putative expressed	AT4G23850.1
CUST_36740_PI390587928	0.646	1.690	0.911	1.666	0.684	2.033	0.526	1.942	-	
CUST_3268_PI390587928	0.791	1.245	0.897	1.341	0.767	1.205	0.781	1.515	expressed protein	AT3G57890.1
CUST_8510_PI390587928	0.617	1.621	0.772	1.726	0.492	1.711	0.472	2.187	cytochrome c oxidase copper chaperone putative expressed	AT1G53030.1
CUST_18621_PI390587928	0.627	1.229	0.525	1.463	0.748	1.934	0.753	1.780	expressed protein	
CUST_16158_PI390587928	0.653	1.880	0.726	2.038	0.698	2.460	0.758	2.458	glutathione S-transferase GSTF1 putative expressed	AT3G62760.1
CUST_41231_PI390587928	0.387	6.531	0.611	6.965	0.453	5.352	0.334	7.541	expressed protein	
CUST_28609_PI390587928	0.528	1.227	0.500	1.635	0.543	1.231	0.567	1.454	expressed protein	
CUST_5545_PI390587928	0.874	2.229	0.775	2.023	0.572	1.423	0.611	1.789	40S ribosomal protein S12 putative expressed	AT2G32060.2
CUST_18451_PI390587928	0.489	1.826	0.545	1.984	0.547	2.036	0.523	2.417	-	
CUST_6583_PI390587928	0.632	1.349	0.745	1.364	0.842	1.459	0.702	1.757	amino acid transporter putative expressed	AT5G09220.1
CUST_35176_PI390587928	0.805	1.267	0.810	1.349	0.803	1.116	0.887	1.473	histone H2A putative expressed	AT5G02560.1
CUST_18312_PI390587928	0.595	1.906	0.664	2.385	0.493	1.170	0.507	1.767	xylanase inhibitor putative expressed	AT1G03220.1
CUST_39210_PI390587928	0.836	1.225	0.840	1.160	0.811	1.067	0.854	1.247	expressed protein	AT4G38150.2
CUST_10898_PI390587928	0.369	3.629	0.406	5.491	0.282	5.216	0.269	6.918	Bowman-Birk type wound-induced proteinase inhibitor WIP1 precursor putative	
CUST_39497_PI390587928	0.427	2.526	0.560	2.835	0.456	2.159	0.524	3.038	calcium ion binding protein putative	AT5G39670.1
CUST_13693_PI390587928	0.770	2.918	0.609	2.457	0.567	2.061	0.580	4.220	OsGrx_I1 - glutaredoxin subgroup III expressed	AT1G28480.1
CUST_6274_PI390587928	0.840	1.560	0.769	1.463	0.732	1.232	0.745	1.505	CDT1a protein putative expressed	
CUST_31066_PI390587928	0.513	2.242	0.604	1.934	0.788	3.024	0.677	3.151	expressed protein	AT1G07040.1
CUST_40825_PI390587928	0.727	1.389	0.702	1.301	0.912	1.758	0.805	1.659	expressed protein	AT5G58000.1
CUST_1872_PI390587928	0.356	5.720	0.421	6.307	0.505	6.505	0.397	9.472	carbohydrate transporter/ sugar porter/ transporter putative expressed	AT5G13750.1
CUST_11983_PI390587928	0.295	1.893	0.337	1.986	0.307	3.610	0.151	3.246	boron transporter-like protein 2 putative expressed	AT1G15460.1
CUST_28567_PI390587928	0.874	1.399	0.883	1.208	0.826	1.261	0.763	1.139	expressed protein	AT1G07970.1
CUST_39981_PI390587928	0.868	1.602	0.848	1.760	0.609	1.251	0.672	1.210	transferase transferring glycosyl groups putative expressed	AT5G06550.1
CUST_40702_PI390587928	0.492	2.176	0.544	2.312	0.667	2.199	0.699	2.550	seven in absentia protein family protein	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_3777_PI390587928	0.740	1.628	0.731	1.862	0.760	1.486	0.707	1.703	CID11 putative expressed	AT1G32790.1
CUST_38804_PI390587928	0.218	3.738	0.208	3.484	0.144	2.858	0.090	2.565	flower-specific gamma-thionin precursor putative expressed	AT2G02130.1
CUST_22382_PI390587928	0.708	1.567	0.663	1.724	0.518	2.205	0.556	2.568	-	
CUST_27406_PI390587928	0.674	3.479	0.778	4.272	0.600	2.541	0.530	2.370	-	
CUST_41538_PI390587928	0.455	1.922	0.446	2.596	0.420	2.134	0.493	2.948	pathogenesis-related protein PRMS precursor putative expressed	AT2G14610.1
CUST_29652_PI390587928	0.288	2.513	0.397	2.552	0.338	2.441	0.311	2.560	leucoanthocyanidin reductase putative expressed	
CUST_10482_PI390587928	0.594	1.528	0.734	1.518	0.912	1.821	0.858	2.080	phytosulfokine receptor precursor putative expressed	
CUST_32417_PI390587928	0.482	2.188	0.671	2.652	0.549	1.376	0.487	2.952	pnFL-2 putative expressed	AT1G19180.1
CUST_3654_PI390587928	0.680	1.456	0.587	1.336	0.621	1.170	0.614	1.333	-	
CUST_6433_PI390587928	0.847	1.243	0.838	1.294	0.705	1.068	0.757	1.335	protein yrdA putative expressed	AT1G47260.1
CUST_398_PI390587928	0.783	1.273	0.878	1.130	0.869	1.243	0.815	1.332	protein-O-fucosyltransferase 1 putative expressed	AT3G05320.1
CUST_34477_PI390587928	0.828	1.423	0.845	1.152	0.819	1.350	0.708	1.432	transcriptional factor TINY putative expressed	
CUST_26548_PI390587928	0.856	1.190	0.852	1.191	0.794	1.151	0.902	1.299	amidase putative expressed	AT4G34880.1
CUST_8493_PI390587928	0.595	1.728	0.843	3.202	0.554	2.777	0.362	2.048	cytochrome P450 78A3 putative	AT3G61880.1
CUST_38511_PI390587928	0.741	1.309	0.766	1.376	0.763	1.135	0.839	1.608	histone H2B.2 putative expressed	AT5G22880.1
CUST_41229_PI390587928	0.752	1.249	0.860	1.180	0.890	1.183	0.848	1.424	nucleic acid binding protein putative expressed	AT4G17720.1
CUST_20347_PI390587928	0.769	1.297	0.770	1.049	0.745	1.071	0.739	1.178	50S ribosomal protein L12-2 chloroplast precursor putative expressed	
CUST_6124_PI390587928	0.718	2.100	0.739	2.491	0.644	1.775	0.651	2.260	expressed protein	AT1G70420.1
CUST_20177_PI390587928	0.820	1.206	0.871	1.212	0.865	1.268	0.841	1.208	transmembrane BAX inhibitor motif-containing protein 4 putative expressed	AT1G03070.1
CUST_20768_PI390587928	0.744	1.330	0.828	1.603	0.826	1.185	0.809	1.400	expressed protein	AT3G21310.1
CUST_11833_PI390587928	0.897	1.391	0.830	1.334	0.722	1.110	0.751	1.297	50S ribosomal protein L17 putative expressed	AT5G09770.1
CUST_41122_PI390587928	0.707	1.865	0.699	1.848	0.608	1.832	0.717	1.801	glutathione S-transferase GSTF1 putative expressed	AT3G62760.1
CUST_10050_PI390587928	0.557	1.870	0.605	1.745	0.634	2.086	0.662	1.997	expressed protein	
CUST_39193_PI390587928	0.880	1.129	0.837	1.203	0.848	1.176	0.910	1.331	calcium ion binding protein putative expressed	AT5G08580.1
CUST_25824_PI390587928	0.902	1.215	0.963	1.302	0.827	1.155	0.872	1.306	vesicle-fusing ATPase putative expressed	AT4G04910.1
CUST_38032_PI390587928	0.855	2.669	0.861	2.622	0.738	1.946	0.751	2.289	elongation factor 1-alpha putative expressed	AT5G60390.3
CUST_22891_PI390587928	0.564	1.446	0.661	1.559	0.533	1.094	0.604	1.595	expressed protein	
CUST_8061_PI390587928	0.466	4.370	0.460	3.576	0.535	5.448	0.507	5.251	hypothetical protein	
CUST_15984_PI390587928	0.525	12.255	0.516	8.252	0.302	8.946	0.299	7.844	MYND finger family protein expressed	AT3G21820.1
CUST_36089_PI390587928	0.870	1.163	0.910	1.098	0.889	1.240	0.891	1.270	proteasome subunit beta type 6 precursor putative expressed	AT4G31300.1
CUST_28953_PI390587928	0.889	1.715	0.949	2.093	0.841	1.546	0.896	2.060	expressed protein	
CUST_30665_PI390587928	0.870	2.882	0.700	2.113	0.525	1.659	0.558	1.633	fibrillarin-2 putative expressed	AT5G52470.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_4665_PI390587928	0.309	2.376	0.514	2.844	0.588	2.169	0.503	3.323	ZIM motif family protein expressed	
CUST_20491_PI390587928	0.615	3.356	0.572	3.748	0.306	2.050	0.486	4.235	sex determination protein tasselseed-2 putative expressed	AT3G51680.1
CUST_30681_PI390587928	0.632	1.271	0.607	0.963	0.791	1.442	0.719	1.526	multiple stress-responsive zinc-finger protein ISAP1 putative expressed	AT4G12040.2
CUST_23774_PI390587928	0.612	1.849	0.693	1.783	0.589	1.798	0.641	2.255	retinoid-inducible serine carboxypeptidase precursor putative expressed	AT2G27920.3
CUST_29789_PI390587928	0.625	2.041	0.732	2.591	0.641	2.332	0.740	2.694	AMP-binding protein putative expressed	AT3G16910.1
CUST_21544_PI390587928	0.783	1.471	0.924	1.593	0.765	1.240	0.742	1.628	endoplasmic oxidoreductin-1 precursor putative expressed	AT1G72280.1
CUST_31548_PI390587928	0.539	2.062	0.552	1.959	0.598	2.767	0.493	3.301	-	
CUST_21374_PI390587928	0.805	1.649	0.705	1.405	0.709	2.036	0.697	1.626	xylem cysteine proteinase 2 precursor putative expressed	
CUST_15691_PI390587928	0.883	2.321	0.582	1.953	0.521	1.699	0.596	1.878	-	
CUST_38850_PI390587928	0.726	2.578	0.516	1.339	0.579	2.803	0.738	3.150	L-ascorbate oxidase precursor putative expressed	AT5G21105.1
CUST_6633_PI390587928	0.581	1.537	0.585	1.526	0.541	1.197	0.514	1.388	60S ribosomal protein L27a-3 putative expressed	AT1G70600.1
CUST_42133_PI390587928	0.778	1.669	0.827	1.739	0.792	1.573	0.770	1.837	mitochondrial folate transporter/carrier putative expressed	AT5G66380.1
CUST_26244_PI390587928	0.947	1.926	0.840	1.931	0.691	1.307	0.747	1.546	-	
CUST_15536_PI390587928	0.704	9.861	0.491	6.370	0.784	8.850	0.713	11.644	ATP8 putative expressed	AT1G23550.1
CUST_35195_PI390587928	0.810	1.657	0.812	1.677	0.614	1.278	0.610	1.368	expressed protein	AT4G00231.1
CUST_2003_PI390587928	0.806	1.466	0.806	1.616	0.777	1.296	0.753	1.508	expressed protein	
CUST_17123_PI390587928	0.435	3.139	0.615	3.106	0.579	4.445	0.424	2.847	strictosidine synthase 1 precursor putative expressed	AT3G59530.2
CUST_38509_PI390587928	0.369	3.840	0.335	3.149	0.341	2.845	0.423	4.034	expressed protein	
CUST_38525_PI390587928	0.529	2.112	0.355	2.949	0.307	1.399	0.920	2.516	-	
CUST_29357_PI390587928	0.940	2.748	0.651	1.924	0.507	1.442	0.592	1.744	chaperonin CPN60-1 mitochondrial precursor putative expressed	AT3G23990.1
CUST_42010_PI390587928	0.812	1.451	0.802	1.717	0.737	1.629	0.841	1.497	serine/arginine-rich protein putative expressed	
CUST_14205_PI390587928	0.721	1.419	0.833	1.444	0.821	1.360	0.737	1.573	xyloglucan endotransglucosylase/hydrolase protein 30 precursor putative expressed	AT4G18990.1
CUST_39377_PI390587928	0.638	4.005	0.800	4.588	0.663	6.440	0.506	9.275	-	
CUST_41136_PI390587928	0.788	1.247	0.753	1.244	0.815	1.093	0.858	1.287	UBP26 putative expressed	AT3G49600.1
CUST_33767_PI390587928	0.893	1.377	0.828	1.350	0.813	1.126	0.833	1.185	tRNA (guanine-N(1)-)-methyltransferase putative expressed	AT2G45730.1
CUST_943_PI390587928	0.962	1.434	0.873	1.375	0.791	1.073	0.813	1.313	expressed protein	AT1G75980.1
CUST_25838_PI390587928	0.868	1.379	0.774	1.217	0.770	1.107	0.749	1.221	expressed protein	
CUST_16686_PI390587928	0.323	4.864	0.408	5.501	0.302	3.395	0.425	7.118	-	
CUST_7037_PI390587928	0.914	2.109	0.682	1.486	0.668	1.306	0.729	1.464	40S ribosomal protein S29 putative expressed	AT4G33865.1
CUST_39068_PI390587928	0.876	1.601	0.683	1.318	0.653	1.441	0.657	1.073	expressed protein	AT4G37445.1
CUST_4912_PI390587928	0.566	2.359	0.646	2.391	0.522	3.581	0.513	2.689	reticuline oxidase precursor putative expressed	AT5G44400.1
CUST_34682_PI390587928	0.442	2.051	0.709	2.451	0.520	2.318	0.545	2.266	-	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_19799_PI390587928	0.884	1.242	0.812	1.158	0.682	1.246	0.909	1.332	expressed protein	AT1G07590.1
CUST_29989_PI390587928	0.612	1.810	0.900	1.676	0.720	2.149	0.807	2.374	branched-chain-amino-acid aminotransferase putative expressed	AT5G65780.1
CUST_18824_PI390587928	0.768	2.801	0.732	2.410	0.616	1.437	0.613	1.801	60S ribosomal protein L33-B putative expressed	AT1G07070.1
CUST_33474_PI390587928	0.796	1.525	0.904	1.504	0.741	1.158	0.720	1.473	-	
CUST_33490_PI390587928	0.584	1.511	0.789	1.817	0.826	1.642	0.714	1.832	expressed protein	
CUST_33505_PI390587928	0.737	1.186	0.842	1.167	0.862	1.063	0.848	1.215	expressed protein	
CUST_12623_PI390587928	0.724	1.621	0.822	1.643	0.716	1.387	0.768	1.601	triacylglycerol lipase putative expressed	AT3G14075.1
CUST_21101_PI390587928	0.685	1.824	0.653	1.840	0.576	1.381	0.536	1.579	60S acidic ribosomal protein P3 putative expressed	AT4G25890.1
CUST_28828_PI390587928	0.765	1.672	0.787	1.777	0.739	1.464	0.724	1.828	expressed protein	AT1G80000.2
CUST_18670_PI390587928	0.798	1.520	0.826	1.391	0.708	1.224	0.855	1.584	histone H4 putative expressed	AT5G59970.1
CUST_11431_PI390587928	0.688	1.529	0.704	1.479	0.678	1.372	0.682	1.648	-	AT5G14530.1
CUST_31578_PI390587928	0.834	1.610	0.719	1.247	0.665	1.156	0.688	1.258	expressed protein	AT2G31410.1
CUST_33351_PI390587928	0.795	1.365	0.792	1.334	0.899	1.213	0.757	1.383	-	
CUST_5594_PI390587928	0.785	1.269	0.790	1.395	0.849	1.345	0.851	1.676	myb-like DNA-binding domain containing protein expressed	
CUST_14008_PI390587928	0.415	7.032	0.619	10.034	0.387	5.334	0.238	13.056	cytochrome P450 72A1 putative expressed	AT2G46950.1
CUST_9395_PI390587928	0.807	1.748	0.730	1.734	0.655	1.477	0.660	1.672	structural constituent of ribosome putative expressed	AT5G55140.1
CUST_22000_PI390587928	0.834	1.821	0.797	1.767	0.627	1.312	0.787	1.578	elongation factor 1-gamma 2 putative expressed	AT1G09640.1
CUST_7212_PI390587928	0.569	1.720	0.615	1.903	0.753	2.848	0.653	3.648	dihydroflavonol-4-reductase putative expressed	AT1G15950.1
CUST_73_PI390587928	0.589	1.635	0.679	1.700	0.673	1.904	0.634	2.577	-	
CUST_6004_PI390587928	0.741	1.481	0.713	1.560	0.683	1.191	0.686	1.377	tRNA pseudouridine synthase family protein expressed	AT1G20370.1
CUST_3241_PI390587928	0.796	1.482	0.831	1.362	0.785	1.411	0.937	1.739	CRK6 putative expressed	
CUST_5316_PI390587928	0.476	2.015	0.574	2.619	0.412	1.349	0.494	2.540	glutathione S-transferase GSTU6 putative expressed	AT1G10370.1
CUST_37347_PI390587928	0.674	2.363	0.828	3.100	0.509	1.929	0.557	3.621	transposon protein putative unclassified expressed	AT3G22370.1
CUST_31907_PI390587928	0.753	1.817	0.844	1.945	0.721	1.666	0.694	1.660	glutaminyl-tRNA synthetase putative expressed	AT1G25350.1
CUST_30668_PI390587928	0.843	2.904	0.647	2.122	0.463	1.453	0.506	1.583	fibrillarin-2 putative expressed	AT5G52470.1
CUST_13804_PI390587928	0.926	1.770	0.783	1.566	0.684	1.136	0.702	1.361	60S ribosomal protein L37 putative expressed	AT1G52300.1
CUST_5146_PI390587928	0.215	4.789	0.354	9.130	0.206	4.941	0.350	10.820	enolase 1 putative expressed	AT2G36530.1
CUST_33967_PI390587928	0.574	1.547	0.535	1.461	0.525	1.275	0.522	1.377	60S acidic ribosomal protein P1 putative expressed	AT5G24510.1
CUST_28817_PI390587928	0.503	1.358	0.468	1.301	0.790	1.557	0.657	1.595	-	
CUST_28833_PI390587928	0.668	1.635	0.759	1.665	0.740	1.368	0.694	1.616	dehydration-responsive element-binding protein 2C putative expressed	AT5G05410.2
CUST_30545_PI390587928	0.649	1.716	0.687	1.507	0.682	1.615	0.699	2.163	transposon protein putative Pong sub-class	
CUST_22399_PI390587928	0.808	1.751	0.825	1.742	0.688	1.367	0.771	1.720	SURF1 putative expressed	
CUST_7844_PI390587928	0.661	2.837	0.873	3.121	0.686	2.191	0.639	3.597	diacylglycerol kinase putative expressed	AT2G20900.2

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_31614_PI390587928	0.563	1.634	0.572	2.515	0.506	1.731	0.693	2.274	Ser/Thr protein kinase putative expressed	AT1G67000.1
CUST_14486_PI390587928	0.619	1.673	0.678	1.958	0.774	1.767	0.619	1.349	anthocyanidin 3-O-glucosyltransferase putative expressed	AT2G22590.1
CUST_41587_PI390587928	0.180	5.975	0.245	6.394	0.202	6.390	0.290	7.645	-	
CUST_18520_PI390587928	0.700	1.415	0.715	1.228	0.823	1.440	0.798	1.387	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 3-like 1 putative expressed	AT5G05130.1
CUST_39906_PI390587928	0.527	2.634	0.762	2.990	0.534	1.692	0.581	3.202	conserved hypothetical protein	
CUST_14347_PI390587928	0.779	1.485	0.851	1.658	0.888	1.409	0.812	1.737	ATP-binding cassette sub-family F member 2 putative expressed	AT5G60790.1
CUST_27316_PI390587928	0.338	2.475	0.304	2.740	0.334	2.230	0.452	2.351	phytosulfokine receptor precursor putative expressed	AT3G56370.1
CUST_21115_PI390587928	0.857	1.568	0.861	1.475	0.720	1.241	0.701	1.354	eukaryotic translation initiation factor 6 putative expressed	AT3G55620.1
CUST_33551_PI390587928	0.681	1.439	0.727	1.454	0.646	1.652	0.750	1.518	carbon catabolite derepressing protein kinase putative expressed	AT3G01090.2
CUST_1318_PI390587928	0.716	1.481	0.700	1.348	0.786	1.298	0.605	1.473	-	
CUST_5321_PI390587928	0.868	1.574	0.951	1.528	0.868	1.504	0.830	1.969	4-nitrophenylphosphatase putative expressed	AT5G47760.1
CUST_31088_PI390587928	0.656	1.652	0.610	1.443	0.856	1.710	0.768	1.988	1-aminocyclopropane-1-carboxylate oxidase putative expressed	AT1G06645.1
CUST_14193_PI390587928	0.863	1.235	0.815	1.140	0.869	1.161	0.787	1.165	pentatricopeptide repeat protein PPR986-12 putative expressed	AT2G37320.1
CUST_34100_PI390587928	0.254	9.669	0.401	8.382	0.146	9.001	0.348	8.893	dehydrin Rab16C putative expressed	
CUST_20396_PI390587928	0.556	1.959	0.610	2.193	0.410	1.539	0.432	2.140	mitochondrial import inner membrane translocase subunit TIM14 putative expressed	AT5G03030.1
CUST_41309_PI390587928	0.581	1.453	0.770	1.551	0.741	1.565	0.578	1.472	flavonoid 3-monooxygenase putative expressed	AT5G57260.1
CUST_29423_PI390587928	0.520	3.353	0.754	5.804	0.589	3.064	0.650	7.148	potassium transporter 5 putative expressed	AT4G13420.1
CUST_33381_PI390587928	0.805	1.851	0.742	1.502	0.584	1.147	0.485	1.278	-	
CUST_34978_PI390587928	0.609	2.279	0.776	3.207	0.684	2.163	0.755	3.644	phytosulfokine receptor precursor putative expressed	AT1G72300.1
CUST_3985_PI390587928	0.605	2.355	0.646	2.644	0.616	3.208	0.554	2.411	strictosidine synthase 1 precursor putative expressed	AT3G59530.2
CUST_2233_PI390587928	0.747	1.277	0.756	1.446	0.797	1.678	0.828	1.773	OsWRKY68 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	AT4G31550.1
CUST_7242_PI390587928	0.688	1.815	0.764	2.004	0.804	1.860	0.750	2.256	AMP-binding protein putative expressed	AT3G16910.1
CUST_12919_PI390587928	0.624	1.569	0.679	1.458	0.874	1.831	0.840	1.559	cytochrome P450 86A2 putative expressed	AT2G23180.1
CUST_31012_PI390587928	0.686	2.549	0.699	2.586	0.587	1.824	0.591	2.123	mitochondrial glycoprotein expressed	AT5G02050.1
CUST_17944_PI390587928	0.879	3.092	0.721	3.454	0.518	1.920	0.575	2.261	BPM putative expressed	AT2G29200.1
CUST_3271_PI390587928	0.600	1.137	0.687	1.379	0.624	1.130	0.773	1.426	receptor-like protein kinase putative	AT5G60900.1
CUST_24121_PI390587928	0.552	1.361	0.635	1.615	0.782	1.397	0.807	1.945	dihydroflavonol-4-reductase putative expressed	AT5G42800.1
CUST_30868_PI390587928	0.878	1.504	0.908	1.368	0.748	1.209	0.716	1.520	lysosomal protective protein precursor putative expressed	AT5G23210.1
CUST_3069_PI390587928	0.542	4.123	0.534	2.850	0.753	5.752	0.604	4.553	calcium-binding protein CAST putative expressed	AT4G20780.1
CUST_28986_PI390587928	0.796	2.381	0.707	2.149	0.649	1.467	0.670	1.816	40S ribosomal protein S16 putative expressed	AT2G09990.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_4931_PI390587928	0.420	2.548	0.465	2.921	0.468	2.968	0.268	3.079	carboxylic ester hydrolase putative expressed	AT5G23870.1
CUST_3707_PI390587928	0.335	3.022	0.495	3.722	0.363	1.967	0.292	3.067	hypothetical protein	
CUST_39725_PI390587928	0.753	1.242	0.840	1.328	0.710	1.045	0.772	1.280	serine-threonine kinase receptor-associated protein putative expressed	AT3G15610.1
CUST_41453_PI390587928	0.562	2.362	0.592	2.303	0.769	2.618	0.731	3.590	alanine--glyoxylate aminotransferase 2 homolog 2 mitochondrial precursor putative expressed	AT3G08860.1
CUST_19865_PI390587928	0.338	5.299	0.357	5.172	0.273	3.952	0.270	5.532	expressed protein	
CUST_2531_PI390587928	0.885	1.371	0.814	1.477	0.736	1.195	0.761	1.367	mitochondrial prohibitin complex protein 1 putative expressed	AT5G40770.1
CUST_18657_PI390587928	0.420	3.064	0.762	4.711	0.325	1.798	0.313	3.658	zinc-finger protein 1 putative expressed	AT5G43170.1
CUST_17465_PI390587928	0.704	1.238	0.714	1.176	0.790	1.272	0.765	1.270	signal recognition particle receptor beta subunit putative expressed	AT5G05670.1
CUST_28677_PI390587928	0.665	1.336	0.881	1.364	0.670	1.465	0.755	1.643	ribonucleoprotein putative expressed	AT4G03110.1
CUST_31628_PI390587928	0.446	1.345	0.553	1.606	0.741	1.769	0.652	2.162	esterase precursor putative expressed	AT5G45910.1
CUST_21454_PI390587928	0.838	1.122	0.874	1.162	0.848	1.153	0.967	1.499	electron transporter putative expressed	AT5G11640.1
CUST_39432_PI390587928	0.826	1.894	0.660	1.653	0.640	1.472	0.600	1.487	50S ribosomal protein L20 putative expressed	AT1G16740.1
CUST_1657_PI390587928	0.813	1.081	0.883	1.108	0.840	1.138	0.915	1.233	glutathione synthetase chloroplast precursor putative expressed	AT5G27380.1
CUST_13541_PI390587928	0.815	2.029	0.854	1.935	0.617	1.342	0.565	1.486	ATP binding protein putative expressed	
CUST_12317_PI390587928	0.617	1.805	0.893	2.400	0.763	2.079	0.796	2.451	-	
CUST_2679_PI390587928	0.607	1.468	0.914	2.205	0.603	1.146	0.751	2.119	phenylalanine ammonia-lyase putative expressed	AT3G53260.1
CUST_35461_PI390587928	0.550	1.434	0.730	1.659	0.764	1.440	0.767	1.942	lipoxygenase 4 putative expressed	AT1G55020.1
CUST_23359_PI390587928	0.527	1.937	0.677	1.997	0.673	1.416	0.781	2.226	RING-H2 finger protein ATL2L putative expressed	
CUST_23375_PI390587928	0.838	1.179	0.817	1.472	0.794	1.354	0.872	1.298	XI-I putative expressed	AT4G33200.1
CUST_29623_PI390587928	0.680	2.068	0.708	1.694	0.767	1.725	0.703	1.439	-	
CUST_22214_PI390587928	0.799	1.288	0.774	1.429	0.751	1.252	0.722	1.571	OsFtsH3 - Oryza sativa FtsH protease homologue of AtFtsH3/10 expressed	AT2G29080.1
CUST_40924_PI390587928	0.613	1.238	0.578	1.549	0.765	1.424	0.673	1.923	6-phosphofructokinase 2 putative expressed	AT5G47810.1
CUST_40940_PI390587928	0.764	1.392	0.779	1.440	0.605	1.144	0.678	1.190	prohibitin putative expressed	AT3G27280.1
CUST_23827_PI390587928	0.730	3.171	0.770	3.512	0.518	2.103	0.541	2.615	pumilio-family RNA binding repeat containing protein expressed	AT3G10360.1
CUST_27037_PI390587928	0.595	1.576	0.720	1.911	0.457	1.320	0.560	2.189	monodehydroascorbate reductase putative expressed	AT3G52880.1
CUST_40754_PI390587928	0.863	1.508	0.688	1.706	0.649	1.206	0.706	1.440	homogentisate geranylgeranyl transferase putative expressed	AT2G18950.1
CUST_15137_PI390587928	0.797	1.286	0.862	1.448	0.773	1.243	0.726	1.400	prefoldin putative expressed	AT5G55860.1
CUST_28713_PI390587928	0.716	1.562	0.718	1.390	0.663	1.297	0.640	1.358	mitochondrial import receptor subunit TOM7-1 putative expressed	
CUST_13530_PI390587928	0.838	1.452	0.774	1.475	0.751	1.274	0.705	1.205	protease Do-like 9 putative expressed	AT5G40200.1
CUST_23487_PI390587928	0.673	1.402	0.676	2.066	0.498	1.220	0.579	1.721	CSLA4 - cellulose synthase-like family A; mannan synthase expressed	AT5G03760.1
CUST_27978_PI390587928	0.416	2.488	0.559	3.226	0.376	2.383	0.306	3.001	transposon protein putative Mutator sub-class expressed	AT2G14820.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_38142_PI390587928	0.836	2.035	0.791	1.646	0.646	1.186	0.647	1.568	60S ribosomal protein L22-2 putative expressed	AT3G05560.3
CUST_32469_PI390587928	0.652	2.412	0.694	2.841	0.718	2.725	0.774	4.450	reticuline oxidase precursor putative expressed	AT4G20860.1
CUST_37711_PI390587928	0.616	2.894	0.480	2.990	0.451	2.152	0.464	2.623	expressed protein	
CUST_8155_PI390587928	0.213	5.332	0.284	4.714	0.549	7.366	0.351	9.094	nuclear protein putative expressed	
CUST_2561_PI390587928	0.683	2.001	0.721	1.994	0.676	1.924	0.633	2.093	-	
CUST_13678_PI390587928	0.662	2.163	0.672	1.481	0.795	3.686	0.689	2.989	OsTIL-1 - Oryza sativa Temperature-induced lipocalin-1 expressed	AT5G58070.1
CUST_27871_PI390587928	0.570	2.062	0.643	2.490	0.570	1.974	0.634	2.555	gibberellin receptor GID1L2 putative expressed	AT5G06570.2
CUST_5828_PI390587928	0.775	2.136	0.693	1.910	0.888	1.653	0.703	1.586	-	
CUST_528_PI390587928	0.304	2.613	0.393	2.895	0.332	3.067	0.400	3.308	dihydroflavonol-4-reductase putative expressed	AT4G27250.1
CUST_15265_PI390587928	0.318	3.616	0.370	3.622	0.368	3.258	0.354	3.419	division protein putative expressed	AT5G42480.1
CUST_17542_PI390587928	0.734	1.534	0.710	1.275	0.785	1.425	0.781	1.471	copine-6 putative expressed	AT3G01650.1
CUST_39446_PI390587928	0.602	2.830	0.387	1.831	0.685	3.350	0.769	4.808	reticuline oxidase precursor putative expressed	AT5G44380.1
CUST_26493_PI390587928	0.838	1.250	0.968	1.337	0.780	1.080	0.741	1.227	OsFtsH5 - Oryza sativa FtsH protease homologue of AtFtsH4 expressed	AT2G26140.1
CUST_37689_PI390587928	0.856	1.289	0.787	1.323	0.817	1.205	0.792	1.377	expressed protein	AT2G44820.1
CUST_1230_PI390587928	0.849	1.742	0.659	1.314	0.693	1.987	0.695	1.636	xylem cysteine proteinase 2 precursor putative expressed	AT4G35350.1
CUST_26540_PI390587928	0.749	1.286	0.836	1.264	0.735	1.207	0.790	1.405	transposon protein putative unclassified expressed	AT1G65730.1
CUST_20867_PI390587928	0.501	2.652	0.778	3.529	0.524	1.782	0.621	3.585	-	
CUST_12938_PI390587928	0.892	1.659	0.784	1.472	0.746	1.243	0.756	1.379	CRS1 / YhbY domain containing protein expressed	
CUST_5535_PI390587928	0.607	1.828	0.568	1.767	0.638	1.826	0.610	2.001	glutathione S-transferase parA putative expressed	AT1G78380.1
CUST_2720_PI390587928	0.586	2.441	0.803	2.694	0.770	2.668	0.785	3.636	alcohol dehydrogenase 1 putative expressed	AT1G77120.1
CUST_17840_PI390587928	0.810	1.632	0.831	1.797	0.802	1.424	0.790	1.643	loricrin putative expressed	AT5G58470.2
CUST_31179_PI390587928	0.705	1.341	0.721	1.197	0.755	1.307	0.661	1.395	protein translation factor SUI1 putative expressed	AT1G54290.1
CUST_29010_PI390587928	0.817	1.313	0.849	1.202	0.762	1.208	0.724	1.211	sec12-like protein 2 putative expressed	AT2G01470.1
CUST_19868_PI390587928	0.307	5.381	0.339	5.455	0.267	3.776	0.239	5.665	expressed protein	
CUST_4764_PI390587928	0.654	2.127	0.569	1.637	0.541	1.437	0.430	1.535	cystatin putative expressed	AT5G47550.1
CUST_36235_PI390587928	0.911	1.392	0.917	1.293	0.824	1.358	0.847	1.599	expressed protein	AT2G25310.1
CUST_14906_PI390587928	0.860	1.450	0.899	1.314	0.849	1.363	0.843	1.409	mitochondrial uncoupling protein 4 putative expressed	AT1G14140.1
CUST_39014_PI390587928	0.653	1.573	0.971	4.079	0.704	1.449	0.695	3.548	indole-3-acetic acid-amido synthetase GH3.8 putative expressed	AT4G37390.1
CUST_25630_PI390587928	0.494	1.467	0.635	1.365	0.586	1.744	0.591	1.940	CER1 putative expressed	AT1G02205.2
CUST_30811_PI390587928	0.805	1.912	0.723	2.250	0.812	1.742	0.829	2.078	-	
CUST_23904_PI390587928	0.663	1.460	0.771	1.470	0.758	1.519	0.887	2.239	expressed protein	AT3G22550.1
CUST_30641_PI390587928	0.625	1.498	0.760	1.686	0.662	1.475	0.764	1.973	-	
CUST_35650_PI390587928	0.718	1.572	0.703	1.667	0.608	1.203	0.655	1.411	60S ribosomal protein L9 putative expressed	AT1G33140.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_4455_PI390587928	0.388	2.337	0.469	2.419	0.607	2.374	0.537	3.015	-	
CUST_24756_PI390587928	0.805	1.185	0.919	1.164	0.792	1.122	0.788	1.252	membrane protein putative expressed	AT5G07250.2
CUST_6499_PI390587928	0.334	2.851	0.319	2.864	0.347	3.103	0.324	4.401	esterase PIR7A putative expressed	AT2G23610.1
CUST_9984_PI390587928	0.837	2.035	0.719	1.715	0.691	1.641	0.744	2.559	cytochrome c oxidase subunit putative expressed	AT1G22450.1
CUST_33754_PI390587928	0.768	1.384	0.790	1.203	0.817	1.609	0.852	1.404	eukaryotic translation initiation factor 2C 2 putative expressed	AT1G31280.1
CUST_9798_PI390587928	0.498	1.519	0.692	1.576	0.738	1.367	0.688	1.363	isoamyl acetate-hydrolyzing esterase putative expressed	AT3G11210.1
CUST_28620_PI390587928	0.704	1.301	0.764	1.300	0.659	1.016	0.608	1.196	3-hydroxyisobutyryl-CoA hydrolase/ catalytic putative expressed	AT4G31810.1
CUST_28387_PI390587928	0.847	1.460	0.958	1.311	0.741	1.160	0.750	1.590	cytochrome c oxidase polypeptide Vb mitochondrial precursor putative expressed	AT3G15640.2
CUST_5354_PI390587928	0.915	1.498	0.874	1.462	0.754	1.152	0.757	1.386	nucleolin putative expressed	AT5G54580.1
CUST_37369_PI390587928	0.829	1.276	0.911	1.344	0.787	1.069	0.765	1.336	co-chaperone protein HscB mitochondrial precursor putative expressed	AT5G06410.1
CUST_2606_PI390587928	0.744	1.299	0.801	1.256	0.705	1.171	0.665	1.233	-	
CUST_33615_PI390587928	0.601	1.555	0.969	2.500	0.582	1.438	0.747	2.321	-	
CUST_23255_PI390587928	0.302	6.620	0.302	6.180	0.320	6.527	0.297	6.365	-	
CUST_7445_PI390587928	0.759	1.635	0.891	1.929	0.705	1.245	0.674	1.895	abhydrolase domain-containing protein 5 putative expressed	AT4G24160.1
CUST_37262_PI390587928	0.879	1.525	0.680	1.129	0.756	1.328	0.825	1.566	-	AT2G01620.1
CUST_28141_PI390587928	0.557	1.383	0.842	2.298	0.502	1.511	0.804	2.137	phenylalanine ammonia-lyase putative expressed	AT2G37040.1
CUST_8328_PI390587928	0.639	1.529	0.893	1.771	0.722	1.650	0.931	1.874	nucleus protein putative expressed	AT4G38495.1
CUST_24729_PI390587928	0.778	1.749	0.811	1.700	0.679	1.222	0.680	1.452	membrane-associated salt-inducible protein like putative expressed	AT3G13160.1
CUST_38922_PI390587928	0.644	1.276	0.822	1.237	0.758	1.517	0.792	2.159	expressed protein	
CUST_24761_PI390587928	0.290	4.627	0.346	5.458	0.134	4.950	0.209	5.621	cysteine synthase putative expressed	AT2G43750.1
CUST_4964_PI390587928	0.790	2.355	0.684	2.011	0.400	1.373	0.454	1.554	40S ribosomal protein S11 putative expressed	AT3G48930.1
CUST_3756_PI390587928	0.785	1.666	0.740	1.652	0.734	1.585	0.801	1.956	set1 complex component swd2 putative expressed	AT5G14530.1
CUST_17093_PI390587928	0.766	1.747	0.846	1.944	0.866	2.087	0.832	2.205	oxysterol-binding protein OBPa putative expressed	AT4G25850.1
CUST_18099_PI390587928	0.653	1.714	0.867	2.103	0.727	1.507	0.776	1.993	preproMP73 putative expressed	
CUST_32551_PI390587928	0.637	2.502	0.784	3.239	0.703	1.863	0.612	3.389	-	
CUST_37000_PI390587928	0.346	2.200	0.375	2.520	0.289	2.907	0.235	3.798	protein ASYMMETRIC LEAVES1 putative expressed	AT2G37630.1
CUST_33604_PI390587928	0.840	1.811	0.840	1.725	0.782	1.331	0.786	1.836	-	
CUST_5847_PI390587928	0.805	1.638	0.824	1.922	0.973	1.784	0.764	2.085	nucleic acid binding protein putative expressed	AT1G67210.1
CUST_27921_PI390587928	0.087	6.391	0.146	6.294	0.101	4.321	0.102	8.470	multidrug resistance protein 4 putative expressed	AT4G18050.1
CUST_2425_PI390587928	0.562	2.377	0.691	2.548	0.696	3.128	0.498	2.634	acyl-coenzyme A oxidase 1 putative expressed	AT5G65110.1
CUST_6916_PI390587928	0.833	1.453	0.736	1.317	0.793	1.227	0.777	1.380	-	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_37925_PI390587928	0.788	1.288	0.771	1.274	0.788	1.402	0.817	1.527	dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex mitochondrial precursor putative expressed	AT5G55070.1
CUST_22742_PI390587928	0.628	1.751	0.800	1.734	0.630	1.694	0.623	2.174	annexin-like protein RJ4 putative expressed	AT5G10230.1
CUST_25521_PI390587928	0.515	3.654	0.500	3.180	0.456	3.311	0.493	3.802	-	
CUST_26543_PI390587928	0.615	1.725	0.648	1.537	0.549	1.199	0.542	1.577	-	
CUST_5724_PI390587928	0.805	1.213	0.898	1.172	0.749	1.214	0.746	1.469	dihydroflavonol-4-reductase putative expressed	AT2G33590.1
CUST_14596_PI390587928	0.640	6.257	0.535	4.858	0.382	3.366	0.449	3.889	-	
CUST_35494_PI390587928	0.226	2.680	0.151	2.831	0.321	4.579	0.234	4.315	-	
CUST_22556_PI390587928	0.527	3.983	0.539	3.828	0.480	2.918	0.473	3.714	RNA-binding protein-like putative expressed	
CUST_18630_PI390587928	0.536	2.050	0.635	2.849	0.643	1.963	0.746	2.656	expressed protein	
CUST_30299_PI390587928	0.771	1.572	0.857	1.585	0.823	1.348	0.543	1.320	F-box domain containing protein expressed	
CUST_33125_PI390587928	0.892	1.393	0.876	1.349	0.813	1.168	0.825	1.205	-	
CUST_9402_PI390587928	0.821	1.321	0.815	1.352	0.783	1.369	0.741	1.461	expressed protein	AT5G20170.1
CUST_11035_PI390587928	0.727	1.562	0.868	1.442	0.698	1.387	0.703	1.732	-	
CUST_18290_PI390587928	0.431	3.223	0.457	3.089	0.316	2.506	0.248	2.015	glutathione S-transferase C-terminal domain containing protein expressed	AT2G30870.1
CUST_21241_PI390587928	0.537	8.285	0.495	7.156	0.291	6.891	0.382	7.039	phenylalanyl-tRNA synthetase alpha chain putative expressed	AT4G39280.1
CUST_36254_PI390587928	0.782	1.196	0.821	1.144	0.761	1.193	0.769	1.351	NADH-ubiquinone oxidoreductase 18 kDa subunit mitochondrial precursor putative expressed	AT5G67590.1
CUST_30814_PI390587928	0.750	1.208	0.789	1.144	0.765	1.084	0.792	1.305	esterase precursor putative expressed	AT5G45910.1
CUST_5261_PI390587928	0.823	1.443	0.923	1.488	0.765	1.193	0.741	1.375	chaperone protein dnaJ putative expressed	AT5G48030.1
CUST_37276_PI390587928	0.889	2.077	0.873	2.048	0.705	1.499	0.734	1.794	60S ribosomal protein L18a putative expressed	AT2G34480.1
CUST_4037_PI390587928	0.788	1.349	0.854	1.328	0.751	1.245	0.692	1.192	cyclopropane fatty acid synthase putative expressed	AT3G23510.1
CUST_19157_PI390587928	0.831	1.701	0.851	1.534	0.786	1.440	0.769	1.706	RNA polymerase II transcriptional coactivator KIWI putative expressed	AT5G09250.1
CUST_6283_PI390587928	0.669	1.433	0.819	1.817	0.644	1.296	0.741	1.799	transposon protein putative unclassified expressed	AT1G65730.1
CUST_29363_PI390587928	0.438	2.373	0.317	1.778	0.336	2.100	0.233	1.734	-	
CUST_5075_PI390587928	0.714	1.484	0.549	1.169	0.567	1.171	0.598	1.499	glutathione S-transferase GSTF1 putative expressed	AT3G62760.1
CUST_33896_PI390587928	0.558	1.571	0.537	1.273	0.733	2.053	0.834	1.641	male sterility protein 2 putative expressed	AT5G22500.1
CUST_23737_PI390587928	0.725	2.311	0.846	2.836	0.579	1.896	0.547	2.075	expressed protein	AT3G16810.1
CUST_8965_PI390587928	0.445	4.443	0.246	3.827	0.542	5.918	0.614	8.379	Zn-dependent hydrolases including glyoxylases putative expressed	AT4G33540.1
CUST_31496_PI390587928	0.731	2.015	0.672	1.814	0.553	1.288	0.584	1.591	-	
CUST_4521_PI390587928	0.626	3.885	0.325	2.559	0.335	2.019	0.432	2.693	60S ribosomal protein L27 putative expressed	AT4G15000.1
CUST_10645_PI390587928	0.836	1.273	0.883	1.131	0.772	1.137	0.750	1.203	calcium-binding mitochondrial protein Anon-60Da putative expressed	AT1G65540.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_16676_PI390587928	0.366	1.870	0.451	1.633	0.684	1.859	0.668	2.308	-	
CUST_37014_PI390587928	0.407	2.673	0.345	2.052	0.369	2.113	0.282	1.802	expressed protein	
CUST_25860_PI390587928	0.359	2.333	0.335	1.856	0.444	3.043	0.340	2.535	-	
CUST_22391_PI390587928	0.671	1.439	0.730	1.603	0.796	1.622	0.826	2.218	gibberellin receptor GID1L2 putative expressed	AT1G47480.1
CUST_12232_PI390587928	0.566	1.759	0.518	1.752	0.661	2.626	0.846	2.445	cytochrome P450 72A1 putative expressed	AT3G14630.1
CUST_14292_PI390587928	0.930	1.274	0.871	1.324	0.858	1.158	0.883	1.153	signal recognition particle receptor alpha subunit putative expressed	AT4G30600.1
CUST_593_PI390587928	0.437	2.704	0.632	3.394	0.470	2.602	0.508	3.722	-	
CUST_608_PI390587928	0.823	1.774	0.802	1.587	0.673	1.202	0.663	1.133	guanine nucleotide-binding protein-like 3 putative expressed	AT3G07050.1
CUST_32442_PI390587928	0.528	2.315	0.637	2.417	0.377	1.677	0.612	2.377	harpin-induced 1 putative expressed	
CUST_27765_PI390587928	0.818	1.346	0.743	1.503	0.696	1.143	0.692	1.301	homogentisate geranylgeranyl transferase putative expressed	AT2G18950.1
CUST_36747_PI390587928	0.372	4.443	0.746	7.984	0.295	2.288	0.415	6.591	-	
CUST_2269_PI390587928	0.765	1.311	0.765	1.358	0.765	1.132	0.728	1.346	hypothetical protein	
CUST_7511_PI390587928	0.622	1.664	0.661	1.541	0.499	1.224	0.473	1.441	-	
CUST_7278_PI390587928	0.825	1.469	0.791	1.283	0.706	1.155	0.697	1.350	40S ribosomal protein S20 putative expressed	AT5G62300.2
CUST_38287_PI390587928	0.322	2.680	0.472	3.073	0.289	2.368	0.389	3.205	glutamate decarboxylase putative expressed	AT5G17330.1
CUST_31064_PI390587928	0.591	2.118	0.623	2.396	0.517	1.961	0.499	2.340	Ser/Thr protein phosphatase family putative expressed	
CUST_16197_PI390587928	0.690	2.445	0.691	2.739	0.491	1.859	0.588	2.687	ATG1 putative expressed	
CUST_15206_PI390587928	0.743	1.626	0.801	1.615	0.828	1.772	0.806	2.060	ankyrin-3 putative expressed	AT5G61230.1
CUST_15222_PI390587928	0.779	1.376	0.806	1.230	0.754	1.189	0.787	1.286	NOL1/NOP2/sun family protein expressed	
CUST_25412_PI390587928	0.775	1.260	0.678	1.322	0.810	1.382	0.821	1.336	14-3-3-like protein S94 putative expressed	AT5G38480.1
CUST_40062_PI390587928	0.835	1.141	0.912	1.225	0.817	1.079	0.906	1.335	-	
CUST_6133_PI390587928	0.686	2.213	0.725	2.541	0.494	1.822	0.578	2.373	expressed protein	AT1G47500.1
CUST_29213_PI390587928	0.858	1.102	0.946	1.233	0.850	1.048	0.794	1.121	ATP binding protein putative expressed	AT4G12790.5
CUST_3945_PI390587928	0.670	1.733	0.664	1.799	0.640	1.529	0.719	2.091	-	AT3G19780.1
CUST_12257_PI390587928	0.834	1.656	0.838	1.547	0.762	1.303	0.755	1.370	expressed protein	AT5G62440.1
CUST_34954_PI390587928	0.799	1.281	0.942	1.254	0.814	1.201	0.767	1.211	-	
CUST_39403_PI390587928	0.798	2.379	0.808	2.103	0.503	1.358	0.490	1.584	protein arginine N-methyltransferase 3-like protein putative expressed	AT3G12270.1
CUST_30967_PI390587928	0.781	1.212	0.874	1.193	0.912	1.182	0.796	1.201	interferon-related developmental regulator family protein expressed	
CUST_25242_PI390587928	0.392	1.638	0.477	2.331	0.589	1.877	0.636	2.623	multidrug resistance-associated protein 3 putative expressed	AT3G60160.1
CUST_16105_PI390587928	0.716	2.219	0.751	2.498	0.492	1.494	0.640	3.216	basic blue protein precursor putative expressed	AT2G02850.1
CUST_27286_PI390587928	0.416	1.989	0.422	1.734	0.529	2.250	0.463	2.815	indole-3-acetate beta-glucosyltransferase putative expressed	AT4G15550.1
CUST_26855_PI390587928	0.593	2.956	0.810	4.268	0.501	2.217	0.558	4.858	expressed protein	AT1G57610.2
CUST_13917_PI390587928	0.053	7.539	0.216	22.247	0.130	9.785	0.226	23.710	polyphenol oxidase II chloroplast precursor putative expressed	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_6010_PI390587928	0.741	1.462	0.876	1.246	0.944	1.754	0.869	1.546	methylglutaconyl-CoA hydratase mitochondrial precursor putative expressed	AT4G16800.1
CUST_18926_PI390587928	0.857	1.229	0.856	1.249	0.858	1.240	0.873	1.303	splicing factor arginine/serine-rich 2 putative expressed	AT5G64200.2
CUST_1879_PI390587928	0.616	1.694	0.738	1.705	0.658	1.563	0.669	2.928	retinal dehydrogenase 1 putative expressed	AT3G24503.1
CUST_16999_PI390587928	0.732	1.345	0.794	1.325	0.886	1.393	0.964	1.760	expressed protein	AT4G35170.1
CUST_41886_PI390587928	0.733	1.948	0.940	2.232	0.631	1.478	0.718	2.691	lipid binding protein putative expressed	
CUST_17580_PI390587928	0.669	2.792	0.612	2.856	0.486	1.854	0.392	2.151	expressed protein	
CUST_30488_PI390587928	0.803	1.331	0.739	1.054	0.737	1.203	0.689	1.288	30S ribosomal protein S16 putative expressed	AT5G56940.1
CUST_13593_PI390587928	0.658	1.893	0.876	2.937	0.610	1.736	0.454	2.125	ubiquitin-protein ligase putative expressed	
CUST_4519_PI390587928	0.713	1.981	0.619	1.797	0.604	1.383	0.589	1.642	60S ribosomal protein L12 putative expressed	AT5G60670.1
CUST_2305_PI390587928	0.627	2.235	0.516	1.587	0.678	1.815	0.651	2.104	-	
CUST_23830_PI390587928	0.239	4.763	0.278	4.187	0.201	2.535	0.160	4.153	-	
CUST_19702_PI390587928	0.617	1.647	0.651	1.446	0.551	1.555	0.546	1.527	ankyrin-like protein putative expressed	AT3G04710.2
CUST_40539_PI390587928	0.601	1.523	0.716	1.673	0.500	1.291	0.445	1.608	expressed protein	
CUST_30412_PI390587928	0.619	1.727	0.756	1.814	0.740	1.453	0.824	1.714	phosphatase DCR2 putative expressed	AT5G63140.1
CUST_39679_PI390587928	0.704	2.116	0.851	2.115	0.688	1.845	0.688	2.600	expressed protein	AT5G23520.1
CUST_17589_PI390587928	0.710	1.568	0.665	1.588	0.856	1.635	0.706	1.753	-	
CUST_36257_PI390587928	0.473	2.508	0.451	2.592	0.443	2.057	0.428	3.172	glutathione S-transferase IV putative expressed	AT3G62760.1
CUST_24543_PI390587928	0.633	1.943	0.724	1.785	0.560	1.319	0.590	2.102	transposon protein putative CACTA En/Spm sub-class	
CUST_36304_PI390587928	0.313	1.494	0.343	1.338	0.353	1.337	0.649	2.433	TMV response-related gene product putative expressed	AT5G66580.1
CUST_9569_PI390587928	0.068	3.123	0.248	3.837	0.111	5.028	0.272	10.216	-	
CUST_14214_PI390587928	0.724	1.589	0.768	1.962	0.769	1.465	0.736	1.734	CID11 putative expressed	AT1G53650.2
CUST_1915_PI390587928	0.535	2.138	0.685	2.120	0.729	2.517	0.796	2.962	disulfide oxidoreductase/ monooxygenase/ oxidoreductase putative	AT1G21430.1
CUST_14230_PI390587928	0.813	1.259	0.884	1.239	0.846	1.194	0.823	1.273	expressed protein	AT3G58800.1
CUST_1931_PI390587928	0.638	1.908	0.942	2.637	0.637	1.858	0.621	2.352	lipoxygenase 7 chloroplast precursor putative expressed	AT3G45140.1
CUST_15843_PI390587928	0.565	1.250	0.716	1.535	0.618	1.115	0.690	1.598	RGH1A putative	
CUST_41922_PI390587928	0.818	1.582	0.889	1.718	0.808	1.272	0.770	1.350	ATP-dependent RNA helicase DDX23 putative expressed	
CUST_34798_PI390587928	0.670	1.375	0.961	1.917	0.676	1.119	0.864	1.647	lysM receptor-like kinase putative expressed	AT3G21630.1
CUST_38055_PI390587928	0.702	1.442	0.638	1.305	0.609	1.152	0.592	1.288	nucleoside diphosphate kinase 1 putative expressed	
CUST_26916_PI390587928	0.613	1.198	0.760	1.556	0.837	1.547	0.820	1.487	OsWRKY3 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	AT1G69310.2
CUST_38071_PI390587928	0.614	3.157	0.711	3.884	0.532	2.625	0.665	3.268	multidrug resistance-associated protein 3 putative expressed	AT3G13080.1
CUST_30874_PI390587928	0.854	1.344	0.875	1.477	0.816	1.335	0.824	1.620	ATOZI1 putative expressed	AT1G01170.2

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_8675_PI390587928	0.519	3.193	0.401	2.610	0.151	2.134	0.176	2.533	endo-14-beta-xylanase putative expressed	AT1G58370.1
CUST_21737_PI390587928	0.764	1.694	0.801	1.719	0.809	1.528	0.763	1.765	serine/threonine-protein phosphatase 2A 72/130 kDa regulatory subunitB putative expressed	AT5G44090.1
CUST_13793_PI390587928	0.400	2.637	0.504	3.411	0.806	2.804	0.561	3.085	cytochrome P450 74A2 putative expressed	AT5G42650.1
CUST_13808_PI390587928	0.651	1.665	0.612	1.641	0.570	1.281	0.584	1.408	40S ribosomal protein S2 putative expressed	AT3G57490.1
CUST_17578_PI390587928	0.732	1.300	0.817	1.416	0.723	1.359	0.704	1.605	-	
CUST_27768_PI390587928	0.104	3.444	0.198	2.527	0.293	3.603	0.329	3.203	glycerol-3-phosphate acyltransferase 8 putative	AT3G11430.1
CUST_18833_PI390587928	0.784	1.639	0.882	2.155	0.516	1.151	0.708	1.820	ubiquitin-protein ligase putative expressed	AT3G11840.1
CUST_22775_PI390587928	0.724	1.792	0.662	1.819	0.665	1.329	0.518	1.760	-	
CUST_5757_PI390587928	0.656	1.806	0.522	1.580	0.488	1.593	0.531	1.557	-	AT3G04710.2
CUST_6779_PI390587928	0.414	2.131	0.545	2.448	0.537	1.676	0.787	3.508	ATP binding protein putative expressed	AT4G03230.1
CUST_25601_PI390587928	0.726	1.595	0.772	1.856	0.723	2.037	0.626	1.842	sulfated surface glycoprotein 185 precursor putative expressed	
CUST_27645_PI390587928	0.814	1.771	0.755	1.632	0.664	1.389	0.718	1.852	expressed protein	AT1G73940.1
CUST_7360_PI390587928	0.779	1.794	0.749	1.652	0.631	1.182	0.707	1.385	60S ribosomal protein L23 putative expressed	AT3G04400.1
CUST_5618_PI390587928	0.480	3.312	0.549	2.851	0.670	2.845	0.740	3.839	-	
CUST_25229_PI390587928	0.837	1.643	0.703	1.522	0.663	1.218	0.626	1.371	ubiquitin fusion protein putative expressed	AT3G52590.1
CUST_28497_PI390587928	0.616	1.652	0.611	1.607	0.499	1.467	0.434	1.586	mannitol dehydrogenase putative expressed	AT4G37980.1
CUST_26267_PI390587928	0.356	2.706	0.435	2.378	0.329	2.551	0.417	2.465	-	
CUST_82_PI390587928	0.270	2.005	0.296	3.054	0.356	1.574	0.287	1.864	amine oxidase precursor putative expressed	AT1G31710.1
CUST_4272_PI390587928	0.481	4.031	0.565	3.936	0.440	4.086	0.551	7.633	L-allo-threonine aldolase putative expressed	AT3G04520.1
CUST_30847_PI390587928	0.629	1.340	0.875	1.943	0.617	1.089	0.845	1.907	catalytic/ hydrolase putative expressed	AT2G36290.1
CUST_12153_PI390587928	0.600	1.141	0.703	1.483	0.757	1.123	0.971	1.724	receptor-like serine-threonine protein kinase putative expressed	AT4G11530.1
CUST_30863_PI390587928	0.552	2.661	0.551	1.765	0.674	2.320	0.668	3.157	solute carrier family 35 member B3 putative expressed	AT4G23010.1
CUST_28374_PI390587928	0.583	2.834	0.538	3.256	0.512	2.089	0.473	3.952	D-3-phosphoglycerate dehydrogenase chloroplast precursor putative expressed	AT4G34200.1
CUST_27943_PI390587928	0.835	1.906	0.854	2.130	0.713	1.205	0.726	1.751	-	
CUST_28965_PI390587928	0.659	1.852	0.661	1.625	0.722	1.709	0.676	1.777	cysteine-type peptidase putative expressed	AT2G27350.2
CUST_17800_PI390587928	0.920	1.206	0.943	1.326	0.759	1.172	0.735	1.454	CBS domain containing protein expressed	AT2G14520.1
CUST_23179_PI390587928	0.392	3.525	0.398	3.211	0.354	2.939	0.388	3.378	expressed protein	AT5G61140.1
CUST_5932_PI390587928	0.629	1.570	0.817	2.302	0.592	1.748	0.763	2.670	alphaalpha-trehalose-phosphate synthase putative expressed	AT1G78580.1
CUST_9142_PI390587928	0.788	1.377	0.779	1.214	0.663	1.003	0.679	1.225	RNA-binding post-transcriptional regulator csx1 putative expressed	AT5G19350.2
CUST_40914_PI390587928	0.311	2.139	0.413	2.339	0.434	1.616	0.662	2.632	nitrate reductase putative expressed	AT1G77760.1
CUST_10864_PI390587928	0.811	1.325	0.779	1.225	0.703	1.110	0.718	1.276	TPR Domain containing protein expressed	AT1G33400.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_38239_PI390587928	0.596	3.448	0.675	4.022	0.634	3.616	0.552	3.914	ubiquitin-protein ligase/ zinc ion binding protein putative	AT4G37420.1
CUST_5016_PI390587928	0.819	1.350	0.819	1.247	0.815	1.637	0.853	1.658	pyrophosphate-energized vacuolar membrane proton pump putative expressed	AT1G15690.1
CUST_3882_PI390587928	0.726	2.574	0.825	3.427	0.740	2.016	0.794	3.593	1-aminocyclopropane-1-carboxylate synthase putative expressed	AT1G01480.2
CUST_23524_PI390587928	0.845	1.450	0.780	1.257	0.743	1.230	0.798	1.327	50S ribosomal protein L17 putative expressed	AT5G09770.1
CUST_26303_PI390587928	0.806	1.680	0.876	1.390	0.698	1.269	0.760	1.737	expressed protein	AT3G63270.1
CUST_32506_PI390587928	0.229	8.037	0.213	5.712	0.222	6.884	0.231	7.200	expressed protein	
CUST_25568_PI390587928	0.294	3.266	0.411	3.237	0.759	4.505	0.493	4.668	RNA-binding protein cabeza putative expressed	AT3G15680.1
CUST_4749_PI390587928	0.647	1.535	0.830	2.017	0.582	1.524	0.668	2.316	phosphatidylinositol transfer protein CSR1 putative expressed	AT1G01630.1
CUST_41473_PI390587928	0.637	2.117	0.556	2.401	0.492	1.945	0.555	2.798	pathogen-related protein putative expressed	AT1G78780.2
CUST_9790_PI390587928	0.520	3.266	0.730	3.752	0.394	2.319	0.406	2.836	expressed protein	
CUST_33358_PI390587928	0.686	1.902	0.651	1.745	0.750	2.014	0.792	2.308	NAC domain-containing protein 78 putative expressed	
CUST_37361_PI390587928	0.646	1.385	0.690	1.408	0.729	1.328	0.781	1.568	expressed protein	AT2G32760.2
CUST_39389_PI390587928	0.946	1.238	0.902	1.132	0.870	1.082	0.795	1.185	expressed protein	AT3G52860.1
CUST_11268_PI390587928	0.510	2.217	0.777	2.807	0.492	1.829	0.510	2.274	L-ascorbate oxidase precursor putative expressed	AT4G39830.1
CUST_16510_PI390587928	0.708	1.496	0.727	1.201	0.738	1.378	0.779	1.514	-	
CUST_11859_PI390587928	0.752	1.469	0.724	1.213	0.901	1.727	0.970	2.016	-	AT5G66160.1
CUST_31021_PI390587928	0.639	1.392	0.863	1.387	0.630	1.412	0.870	1.490	glycoside hydrolase family 28 putative expressed	AT3G48950.1
CUST_33826_PI390587928	0.830	1.482	0.771	1.547	0.885	1.424	0.857	1.853	serine/threonine protein kinase putative expressed	AT2G24360.1
CUST_1034_PI390587928	0.712	1.278	0.823	1.502	0.653	1.379	0.724	1.609	-	
CUST_42233_PI390587928	0.801	1.630	0.713	1.231	0.786	1.548	0.691	1.411	inositolphosphorylceramide-B C-26 hydroxylase putative expressed	AT2G34770.1
CUST_34880_PI390587928	0.511	2.689	0.415	2.117	0.280	1.902	0.264	1.720	-	
CUST_5169_PI390587928	0.241	3.774	0.268	3.254	0.162	2.994	0.117	2.300	harpin-induced protein putative expressed	
CUST_30738_PI390587928	0.767	1.287	0.858	1.339	0.782	1.267	0.807	1.364	phospholipase D alpha 2 putative expressed	AT1G52570.1
CUST_19688_PI390587928	0.687	1.759	0.691	1.778	0.618	1.484	0.603	1.597	protein SEY1 putative expressed	
CUST_13704_PI390587928	0.281	3.855	0.395	5.312	0.430	6.653	0.331	5.316	cis-zeatin O-glucosyltransferase putative expressed	AT4G01070.1
CUST_19735_PI390587928	0.635	1.455	0.577	1.399	0.649	1.406	0.600	1.312	glutamate receptor 3.3 precursor putative expressed	AT1G42540.1
CUST_35640_PI390587928	0.701	1.546	0.704	1.650	0.627	1.241	0.625	1.373	60S ribosomal protein L9 putative expressed	AT1G33140.1
CUST_33410_PI390587928	0.356	2.826	0.708	2.851	0.401	3.546	0.266	2.219	expressed protein	
CUST_24699_PI390587928	0.556	1.674	0.665	1.938	0.753	2.239	0.556	1.573	DNA binding protein putative expressed	
CUST_6706_PI390587928	0.395	2.931	0.482	2.676	0.365	2.864	0.448	2.659	-	
CUST_2231_PI390587928	0.798	1.319	0.846	1.342	0.769	1.058	0.783	1.399	retrotransposon protein putative LINE subclass expressed	AT4G11060.1
CUST_24576_PI390587928	0.024	2.731	0.019	6.391	0.134	4.838	0.018	5.728	expressed protein	

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_29585_PI390587928	0.513	1.592	0.598	1.517	0.773	2.088	0.642	1.958	serine/threonine-protein kinase BRI1-like 1 precursor putative expressed	AT1G55610.2
CUST_6536_PI390587928	0.653	1.544	0.782	1.441	0.932	2.105	0.721	2.151	UDP-glucose 4-epimerase putative expressed	AT1G12780.1
CUST_5297_PI390587928	0.835	1.504	0.854	1.374	0.747	1.145	0.738	1.138	periodic tryptophan protein 1 putative expressed	AT4G18905.1
CUST_13411_PI390587928	0.821	1.493	0.762	1.016	0.954	1.582	0.552	1.765	beta-galactosidase precursor putative expressed	AT2G28470.1
CUST_33070_PI390587928	0.499	2.759	0.581	2.819	0.788	3.664	0.561	3.524	RNA-binding region-containing protein 1 putative expressed	AT1G78260.2
CUST_19426_PI390587928	0.718	1.903	0.758	1.636	0.654	1.383	0.726	1.831	40S ribosomal protein S27a putative expressed	AT2G47110.1
CUST_33574_PI390587928	0.776	1.644	0.812	1.890	0.703	1.464	0.628	2.014	-	AT3G45970.1
CUST_26163_PI390587928	0.512	2.783	0.666	3.955	0.825	4.516	0.697	5.959	glutathione S-transferase putative expressed	AT3G09270.1
CUST_3130_PI390587928	0.763	2.316	0.732	1.814	0.524	1.277	0.479	1.322	-	
CUST_12810_PI390587928	0.526	1.743	0.894	2.023	0.318	1.266	0.358	2.157	expressed protein	AT3G57450.1
CUST_2379_PI390587928	0.584	2.470	0.814	3.880	0.656	1.620	0.612	3.624	anthranilate synthase component I-1 chloroplast precursor putative expressed	AT5G05730.1
CUST_15269_PI390587928	0.777	1.152	0.878	1.209	0.713	1.021	0.756	1.177	expressed protein	AT5G58100.1
CUST_38444_PI390587928	0.811	1.324	0.866	1.303	0.776	1.362	0.763	1.406	proteasome subunit alpha type 4 putative	AT3G22110.1
CUST_37252_PI390587928	0.662	1.859	0.655	1.656	0.601	1.331	0.595	1.508	glycine-rich protein 2b putative expressed	AT4G36020.1
CUST_23075_PI390587928	0.845	1.261	0.808	1.148	0.882	1.282	0.843	1.348	expressed protein	AT1G68680.1
CUST_7872_PI390587928	0.653	1.365	0.771	1.672	0.700	1.436	0.678	1.703	aldehyde oxidase 1 putative expressed	AT2G27150.2
CUST_27700_PI390587928	0.811	1.371	0.883	1.304	0.717	1.464	0.768	1.560	26S protease regulatory subunit 4 putative expressed	AT4G29040.1
CUST_26949_PI390587928	0.809	1.651	0.834	1.609	0.581	1.292	0.722	2.195	adenylate kinase A putative expressed	AT5G63400.1
CUST_21972_PI390587928	0.881	1.597	0.875	1.401	0.663	1.330	0.879	1.505	chromatin complex subunit A101 putative expressed	AT5G66750.1
CUST_15007_PI390587928	0.731	1.534	0.778	1.385	0.829	1.477	0.778	1.936	OsWRKY46 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	AT2G40740.1
CUST_35947_PI390587928	0.536	1.461	0.507	1.446	0.635	1.636	0.698	1.883	esterase precursor putative expressed	AT1G28580.1
CUST_38151_PI390587928	0.317	11.803	0.325	4.842	0.521	19.541	0.324	15.183	late embryogenesis abundant protein group 3 putative expressed	AT1G52690.2
CUST_7717_PI390587928	0.304	1.710	0.332	1.580	0.362	2.090	0.314	1.993	subtilisin-chymotrypsin inhibitor 2 putative expressed	
CUST_16589_PI390587928	0.667	1.779	0.865	2.533	0.604	1.424	0.656	2.377	glycerolphosphate mutase putative expressed	AT3G05170.1
CUST_11596_PI390587928	0.718	2.359	0.961	2.958	0.693	2.176	0.708	2.800	glucan endo-13-beta-glucosidase precursor putative expressed	AT3G55430.1
CUST_32525_PI390587928	0.714	1.810	0.777	1.858	0.767	1.319	0.704	2.110	glutathione S-transferase GSTU6 putative expressed	AT1G27130.1
CUST_7547_PI390587928	0.638	3.561	0.313	3.760	0.508	3.380	0.494	5.888	-	
CUST_15428_PI390587928	0.324	3.115	0.276	1.829	0.954	7.165	0.497	5.100	cytochrome P450 86A1 putative expressed	AT4G39480.1
CUST_4784_PI390587928	0.650	1.727	0.702	1.733	0.655	1.663	0.625	1.569	-	
CUST_2554_PI390587928	0.489	1.154	0.632	1.312	0.745	1.660	0.835	1.457	male sterility protein 2 putative expressed	AT4G33790.1
CUST_12681_PI390587928	0.589	2.742	0.786	2.630	0.306	3.320	0.194	2.909	alpha-N-arabinofuranosidase 1 precursor putative expressed	AT3G10740.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_28886_PI390587928	0.744	1.562	0.755	1.559	0.643	1.312	0.632	1.428	SLT1 protein putative expressed	AT2G37570.1
CUST_5837_PI390587928	0.564	1.675	0.755	1.887	0.712	2.071	0.709	2.161	monoglyceride lipase putative expressed	AT2G39420.1
CUST_7610_PI390587928	0.573	1.718	0.738	1.763	0.705	2.118	0.650	2.005	methylmalonate-semialdehyde dehydrogenase putative expressed	AT2G14170.1
CUST_41337_PI390587928	0.863	1.295	0.811	1.084	0.788	1.069	0.797	1.240	6-phosphofructokinase putative expressed	AT4G26270.1
CUST_34632_PI390587928	0.347	1.636	0.345	1.461	0.648	3.339	0.571	3.219	caffeoyl-CoA O-methyltransferase 1 putative expressed	AT4G26220.1
CUST_7408_PI390587928	0.772	1.187	0.784	1.153	0.784	1.089	0.779	1.201	expressed protein	AT4G16170.1
CUST_28747_PI390587928	0.633	4.997	0.563	4.052	0.415	3.091	0.350	3.897	60S ribosomal protein L33-B putative expressed	AT1G07070.1
CUST_8276_PI390587928	0.788	4.097	0.565	3.056	0.288	1.918	0.282	2.296	peroxidase 39 precursor putative expressed	AT4G11290.1
CUST_16173_PI390587928	0.834	1.100	0.962	1.189	0.846	1.236	0.834	1.273	sucrose cleavage protein-like putative expressed	AT4G26620.1
CUST_20923_PI390587928	0.423	3.992	0.379	3.684	0.477	4.390	0.436	4.355	-	
CUST_35952_PI390587928	0.788	1.510	0.838	1.357	0.753	1.389	0.706	1.684	ER lumen protein retaining receptor C28H8.4 putative expressed	AT1G75760.1
CUST_34153_PI390587928	0.773	1.515	0.716	1.457	0.611	1.329	0.784	1.432	RNA-binding post-transcriptional regulator csx1 putative expressed	AT5G19350.2
CUST_12824_PI390587928	0.706	1.731	0.716	1.756	0.766	1.590	0.737	1.903	sodium/hydrogen exchanger 2 putative expressed	AT3G05030.2
CUST_26240_PI390587928	0.521	3.663	0.479	4.915	0.343	2.592	0.523	7.239	glutathione S-transferase GSTU6 putative expressed	AT1G10370.1
CUST_13676_PI390587928	0.659	3.026	0.797	4.339	0.638	2.775	0.658	5.161	esterase precursor putative expressed	AT3G26430.1
CUST_36819_PI390587928	0.777	1.490	0.705	1.381	0.667	1.373	0.594	1.467	expressed protein	AT2G20825.1
CUST_26070_PI390587928	0.025	3.080	0.064	6.445	0.073	5.647	0.050	7.395	eukaryotic peptide chain release factor subunit 1-1 putative expressed	AT5G47880.2
CUST_6_PI390587928	0.590	2.211	0.675	2.394	0.458	1.788	0.496	2.737	-	
CUST_24888_PI390587928	0.588	1.256	0.527	1.348	0.718	1.685	0.774	1.774	hypothetical protein	
CUST_35052_PI390587928	0.827	1.335	0.737	1.249	0.741	1.187	0.666	1.157	queuine tRNA-ribosyltransferase domain containing 1 putative expressed	
CUST_11966_PI390587928	0.525	2.903	0.652	3.785	0.385	1.630	0.500	3.289	-	
CUST_22705_PI390587928	0.488	4.428	0.763	6.062	0.551	5.160	0.403	12.008	non-cyanogenic beta-glucosidase precursor putative expressed	AT1G02850.5
CUST_36090_PI390587928	0.807	1.351	0.857	1.204	0.864	1.295	0.843	1.512	proteasome subunit beta type 6 precursor putative expressed	AT4G31300.1
CUST_41846_PI390587928	0.843	1.443	0.733	1.302	0.831	1.347	0.789	1.425	HMG1/2-like protein putative expressed	
CUST_10301_PI390587928	0.801	1.263	0.833	1.333	0.850	1.218	0.827	1.362	interferon-related developmental regulator family protein expressed	
CUST_19584_PI390587928	0.441	2.188	0.607	2.242	0.552	2.318	0.584	2.916	-	
CUST_4968_PI390587928	0.639	1.506	0.719	1.443	0.714	1.634	0.682	1.571	-	
CUST_28566_PI390587928	0.533	3.861	0.629	4.221	0.456	3.756	0.415	3.501	ATPase 3 putative expressed	AT3G28510.1
CUST_2770_PI390587928	0.794	1.230	0.776	1.350	0.620	1.216	0.719	1.279	mitochondrial prohibitin complex protein 2 putative expressed	
CUST_18896_PI390587928	0.612	1.457	0.668	1.890	0.391	0.939	0.663	1.527	reticuline oxidase precursor putative expressed	AT4G20860.1
CUST_5347_PI390587928	0.346	2.944	0.341	2.146	0.403	3.746	0.305	3.137	caspase putative expressed	AT5G04200.1
CUST_17719_PI390587928	0.562	9.796	0.500	4.867	0.979	10.031	0.610	8.072	subtilisin-like protease precursor putative expressed	AT3G14067.1
CUST_1376_PI390587928	0.563	2.443	0.604	2.823	0.653	2.287	0.790	3.033	-	

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_13260_PI390587928	0.783	1.274	0.701	1.299	0.713	1.372	0.734	1.367	TPR Domain containing protein expressed	AT1G76630.1
CUST_13058_PI390587928	0.640	1.501	0.803	1.778	0.625	1.664	0.892	1.910	Leucine Rich Repeat family protein	
CUST_21018_PI390587928	0.763	1.229	0.939	1.299	0.805	1.142	0.741	1.184	MYB4 putative expressed	
CUST_9684_PI390587928	0.612	1.371	0.742	1.401	0.676	1.211	0.565	1.210	Zn-finger RanBP-type containing protein expressed	
CUST_37255_PI390587928	0.666	1.845	0.662	1.670	0.588	1.277	0.595	1.492	glycine-rich protein 2b putative expressed	AT4G36020.1
CUST_30016_PI390587928	0.763	1.145	0.743	1.117	0.753	1.237	0.718	1.182	VTC2 putative expressed	AT4G26850.1
CUST_7315_PI390587928	0.739	3.648	0.637	3.220	0.496	1.874	0.511	2.474	40S ribosomal protein S9 putative expressed	AT5G39850.1
CUST_5101_PI390587928	0.777	1.478	0.863	1.550	0.815	1.376	0.859	1.544	-	
CUST_6107_PI390587928	0.869	1.707	0.803	1.581	0.614	1.178	0.672	1.374	ATPase family AAA domain-containing protein 3 putative expressed	AT3G03060.1
CUST_37132_PI390587928	0.617	1.884	0.653	1.910	0.750	1.913	0.581	1.820	nodulin-like protein putative expressed	AT2G34357.1
CUST_15803_PI390587928	0.678	2.661	0.716	3.447	0.462	1.981	0.460	1.780	expressed protein	AT4G35240.1
CUST_3935_PI390587928	0.799	1.185	0.804	1.337	0.809	1.131	0.852	1.346	lipoate protein ligase-like protein putative expressed	AT3G29010.1
CUST_10608_PI390587928	0.852	1.793	0.828	1.813	0.691	1.368	0.679	1.579	serine/threonine-protein phosphatase 5 putative expressed	AT2G42810.1
CUST_36988_PI390587928	0.860	1.501	0.793	1.332	0.728	1.138	0.725	1.264	RNA methylase putative expressed	AT3G26410.1
CUST_5995_PI390587928	0.891	1.465	0.850	1.236	0.795	1.297	0.780	1.141	expressed protein	AT5G27330.1
CUST_32560_PI390587928	0.681	1.813	0.720	1.957	0.480	1.479	0.590	1.870	expressed protein	AT2G37860.3
CUST_16702_PI390587928	0.598	2.316	0.364	1.733	0.252	1.683	0.255	1.343	sucrose synthase 2 putative expressed	AT5G37180.1
CUST_19954_PI390587928	0.481	2.193	0.668	2.274	0.498	1.851	0.419	2.515	Q-rich domain protein putative expressed	
CUST_11476_PI390587928	0.619	1.394	0.846	2.044	0.674	1.422	0.688	1.978	aspartic proteinase nepenthesin-2 precursor putative expressed	AT3G59080.2
CUST_4850_PI390587928	0.674	1.325	0.842	1.346	0.815	1.297	0.901	1.620	mitochondrial 2-oxoglutarate/malate carrier protein putative expressed	AT2G22500.1
CUST_9066_PI390587928	0.636	2.134	0.645	2.077	0.747	1.884	0.720	2.183	-	
CUST_1869_PI390587928	0.737	1.152	0.891	1.174	0.823	1.212	0.857	1.535	expressed protein	AT1G20970.1
CUST_39660_PI390587928	0.396	1.460	0.626	1.670	0.722	1.887	0.517	1.687	calcium-transporting ATPase 9 plasma membrane-type putative expressed	AT5G57110.2
CUST_16532_PI390587928	0.631	1.622	0.869	2.098	0.678	1.490	0.684	2.105	-	
CUST_41403_PI390587928	0.540	2.707	0.650	3.073	0.579	2.582	0.531	3.156	transcriptional repressor NF-X1 putative expressed	AT1G10170.1
CUST_40870_PI390587928	0.672	1.326	0.718	1.391	0.650	1.136	0.574	1.210	protein binding protein putative expressed	AT2G30580.1
CUST_33273_PI390587928	0.810	1.459	0.810	1.576	0.821	1.502	0.800	1.467	mitochondrion protein putative expressed	AT2G16460.1
CUST_28596_PI390587928	0.867	1.303	0.890	1.202	0.922	1.164	0.893	1.302	protein phosphatase type 2A regulator/ signal transducer putative expressed	AT5G53500.1
CUST_27621_PI390587928	0.259	6.584	0.283	5.793	0.074	3.394	0.044	3.948	S-adenosylmethionine decarboxylase proenzyme putative expressed	AT3G02470.4
CUST_28627_PI390587928	0.737	1.497	0.647	1.647	0.714	1.329	0.883	1.821	expressed protein	
CUST_18453_PI390587928	0.436	1.482	0.501	2.088	0.442	1.997	0.584	2.286	GEX1 putative expressed	AT5G55490.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_37625_PI390587928	0.683	1.552	0.738	1.760	0.695	1.200	0.677	1.345	F-box domain containing protein expressed	
CUST_5424_PI390587928	0.559	1.906	0.574	1.871	0.524	2.763	0.607	3.153	esterase precursor putative expressed	AT1G28600.2
CUST_8203_PI390587928	0.809	1.413	0.868	1.704	0.864	1.349	0.737	1.561	auxin response factor 19 putative expressed	AT1G19220.1
CUST_22256_PI390587928	0.763	1.128	0.815	1.138	0.757	1.215	0.787	1.341	alanine aminotransferase 2 putative expressed	AT1G72330.1
CUST_6462_PI390587928	0.634	1.624	0.773	1.714	0.666	1.332	0.605	1.796	RHM1 putative expressed	AT1G78570.1
CUST_34017_PI390587928	0.536	1.998	0.601	1.717	0.512	1.807	0.632	1.977	metal ion binding protein putative expressed	AT1G01490.2
CUST_39499_PI390587928	0.733	1.776	0.883	2.523	0.634	1.588	0.825	2.349	transposon protein putative unclassified expressed	AT1G65730.1
CUST_19182_PI390587928	0.718	1.949	0.699	1.556	0.900	1.542	0.732	1.328	expressed protein	
CUST_41849_PI390587928	0.863	1.236	0.938	1.180	0.853	1.125	0.835	1.210	26S proteasome non-ATPase regulatory subunit 14 putative expressed	AT5G23540.1
CUST_2896_PI390587928	0.620	1.738	0.646	1.706	0.690	1.536	0.581	1.335	CTP synthase putative expressed	AT3G12670.1
CUST_36140_PI390587928	0.831	1.964	0.762	1.839	0.568	1.073	0.810	2.444	expressed protein	
CUST_7206_PI390587928	0.614	1.665	0.813	2.118	0.656	1.316	0.613	1.805	respiratory burst oxidase protein D putative expressed	AT5G47910.1
CUST_7782_PI390587928	0.813	2.306	0.842	2.467	0.775	2.287	0.867	3.002	jasmonate-induced protein putative	
CUST_16902_PI390587928	0.406	2.814	0.591	3.105	0.601	3.333	0.406	3.506	expressed protein	AT5G39530.1
CUST_36001_PI390587928	0.744	1.565	0.745	1.417	0.596	1.065	0.668	1.344	60S ribosomal protein L7-2 putative expressed	AT2G01250.2
CUST_3795_PI390587928	0.683	1.445	0.775	2.270	0.635	1.560	0.689	1.684	CID11 putative expressed	
CUST_11676_PI390587928	0.459	3.891	0.322	2.644	0.324	3.527	0.324	3.955	harpin-induced protein putative expressed	
CUST_8835_PI390587928	0.701	1.487	0.690	1.214	0.587	1.146	0.562	1.333	40S ribosomal protein S15a putative expressed	AT4G29430.1
CUST_17940_PI390587928	0.335	1.416	0.431	1.443	0.518	2.527	0.424	2.160	lectin-like receptor kinase 7 putative expressed	AT2G37710.1
CUST_7036_PI390587928	0.729	1.192	0.759	1.192	0.824	1.473	0.780	1.583	expressed protein	
CUST_16716_PI390587928	0.369	2.980	0.414	2.513	0.389	2.825	0.291	2.487	OsWRKY47 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	AT2G46400.1
CUST_35400_PI390587928	0.613	2.837	0.674	3.787	0.423	2.674	0.401	2.904	peptidyl-prolyl cis-trans isomerase FKBP-type family protein expressed	
CUST_803_PI390587928	0.696	1.680	0.785	1.908	0.795	1.667	0.805	1.531	cytokinin-N-glucosyltransferase 1 putative expressed	AT3G11340.1
CUST_36879_PI390587928	0.730	1.543	0.719	1.562	0.673	1.210	0.644	1.301	eukaryotic translation initiation factor 3 subunit 4 putative expressed	AT3G11400.1
CUST_34649_PI390587928	0.642	3.013	0.689	2.407	0.589	1.971	0.566	2.348	elongation factor 1-alpha putative expressed	AT5G60390.3
CUST_17754_PI390587928	0.724	1.402	0.776	1.147	0.734	1.298	0.621	1.312	hypersensitive-induced response protein putative expressed	AT5G62740.1
CUST_6435_PI390587928	0.785	1.484	0.824	1.356	0.715	1.117	0.666	1.279	vegetatible incompatibility protein HET-E-1 putative expressed	AT1G71840.1
CUST_4678_PI390587928	0.649	1.633	0.635	1.876	0.744	1.285	0.795	2.282	allene oxide cyclase 4 chloroplast precursor putative expressed	AT1G13280.1
CUST_9920_PI390587928	0.776	1.722	0.834	1.668	0.776	1.725	0.716	1.920	aminotransferase y4uB putative expressed	AT3G22200.1
CUST_10329_PI390587928	0.443	1.452	0.500	1.361	0.658	1.362	0.527	1.668	expressed protein	
CUST_18823_PI390587928	0.712	1.902	0.698	1.649	0.601	1.215	0.596	1.533	60S ribosomal protein L33-B putative expressed	AT1G74270.1
CUST_7473_PI390587928	0.807	1.477	0.825	1.420	0.768	1.178	0.816	1.414	UDP-3-O- glucosamine N-acyltransferase putative expressed	AT4G21220.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_27301_PI390587928	0.736	1.637	0.632	1.451	0.577	1.401	0.577	1.519	acyl carrier protein mitochondrial precursor putative expressed	AT1G65290.1
CUST_8495_PI390587928	0.744	2.797	0.682	2.854	0.700	2.810	0.604	4.491	indole-3-acetate beta-glucosyltransferase putative expressed	AT1G05560.1
CUST_38513_PI390587928	0.810	2.771	0.658	2.504	0.567	1.656	0.724	2.719	mitochondrial 2-oxoglutarate/malate carrier protein putative expressed	AT2G22500.1
CUST_30539_PI390587928	0.864	1.952	0.724	1.800	0.714	1.555	0.728	1.602	-	
CUST_35548_PI390587928	0.800	1.513	0.754	1.435	0.839	1.535	0.932	1.996	transposon protein putative unclassified expressed	AT1G65730.1
CUST_19202_PI390587928	0.575	4.328	0.881	5.955	0.505	3.562	0.461	4.192	acyl-activating enzyme 11 putative expressed	AT1G21540.1
CUST_510_PI390587928	0.756	1.791	0.880	1.825	0.821	1.689	0.790	2.193	hypothetical protein	
CUST_37135_PI390587928	0.425	1.656	0.247	1.252	0.388	2.210	0.517	1.962	1-aminocyclopropane-1-carboxylate oxidase 1 putative expressed	AT1G12010.1
CUST_15029_PI390587928	0.341	4.822	0.469	5.786	0.539	5.476	0.554	8.623	thaumatin-like protein 1 precursor putative expressed	AT1G75800.1
CUST_41124_PI390587928	0.037	5.483	0.062	6.250	0.036	5.644	0.040	7.680	indole-3-acetate beta-glucosyltransferase putative expressed	AT1G05680.1
CUST_39242_PI390587928	0.713	1.839	0.754	1.959	0.701	1.712	0.671	2.171	expressed protein	AT5G42570.1
CUST_33802_PI390587928	0.241	3.555	0.507	5.039	0.310	2.109	0.303	5.110	aromatic-L-amino-acid decarboxylase putative expressed	AT4G28680.2
CUST_34824_PI390587928	0.317	2.552	0.362	2.076	0.133	2.113	0.112	1.574	cell envelope integrity inner membrane protein TolA putative	
CUST_25081_PI390587928	0.708	3.656	0.826	4.771	0.352	2.429	0.642	2.916	pumilio-family RNA binding repeat containing protein expressed	AT2G29190.1
CUST_7041_PI390587928	0.881	1.309	0.865	1.250	0.811	1.156	0.783	1.152	splicing factor arginine/serine-rich 4 putative expressed	AT2G37340.1
CUST_38050_PI390587928	0.541	2.026	0.850	2.621	0.751	2.234	0.839	3.228	expressed protein	AT3G50190.1
CUST_27917_PI390587928	0.820	1.601	0.627	1.105	0.810	1.470	0.759	1.431	expressed protein	AT4G09060.1
CUST_36915_PI390587928	0.793	1.264	0.782	1.335	0.676	1.125	0.671	1.210	cystathionine beta-lyase putative expressed	AT3G01120.1
CUST_37937_PI390587928	0.882	1.695	0.806	1.438	0.695	1.293	0.691	1.391	-	AT2G18850.1
CUST_22770_PI390587928	0.858	1.526	0.768	1.190	0.774	1.285	0.844	1.494	proteasome subunit alpha type 7 putative expressed	AT5G66140.1
CUST_2973_PI390587928	0.215	8.672	0.244	7.670	0.172	6.016	0.255	11.781	multidrug resistance protein 4 putative expressed	AT4G18050.1
CUST_4528_PI390587928	0.590	1.023	0.606	1.230	0.688	1.536	0.749	1.177	eukaryotic translation initiation factor 2C 2 putative expressed	
CUST_12409_PI390587928	0.796	1.790	0.853	1.586	0.715	1.735	0.723	2.181	subtilisin-like protease precursor putative expressed	AT3G14067.1
CUST_32774_PI390587928	0.449	2.180	0.442	2.596	0.710	3.049	0.716	2.715	plant-specific domain TIGR01568 family protein expressed	
CUST_956_PI390587928	0.942	1.799	0.820	1.625	0.631	1.228	0.702	1.233	transferase transferring glycosyl groups putative expressed	AT5G06550.1
CUST_10498_PI390587928	0.799	1.176	0.820	1.290	0.844	1.361	0.865	1.485	peroxisome assembly protein 12 putative expressed	AT3G04460.1
CUST_13510_PI390587928	0.492	3.129	0.518	4.188	0.407	2.894	0.450	4.923	glutathione S-transferase GSTU6 putative expressed	AT1G10370.1
CUST_38666_PI390587928	0.905	2.676	0.890	2.666	0.566	1.633	0.562	2.003	60S ribosomal protein L6 putative expressed	AT1G74050.1
CUST_15538_PI390587928	0.508	1.373	0.710	1.605	0.695	1.605	0.578	1.564	protein binding protein putative expressed	
CUST_9430_PI390587928	0.538	2.643	0.617	2.734	0.517	1.827	0.580	2.973	caltractin putative expressed	AT5G49480.1
CUST_22306_PI390587928	0.896	1.396	0.853	1.598	0.848	1.327	0.926	1.539	expressed protein	AT3G17030.1
CUST_32481_PI390587928	0.594	3.153	0.653	3.063	0.695	3.087	0.645	3.647	endoribonuclease Dicer putative expressed	AT4G15417.1
CUST_17598_PI390587928	0.354	7.451	0.166	4.261	0.551	7.405	0.107	5.040	globulin precursor putative expressed	

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_23328_PI390587928	0.584	2.230	0.621	2.772	0.562	1.951	0.679	2.656	mitotic spindle checkpoint protein MAD2 putative expressed	AT3G25980.1
CUST_10966_PI390587928	0.807	1.680	0.790	1.848	0.631	1.230	0.450	1.856	cytochrome c putative expressed	AT1G22840.1
CUST_32326_PI390587928	0.620	1.716	0.735	1.673	0.659	1.548	0.681	2.954	-	AT3G24503.1
CUST_4081_PI390587928	0.782	1.443	0.887	1.345	0.837	1.231	0.885	1.915	phenazine biosynthesis protein putative expressed	AT4G02860.1
CUST_20379_PI390587928	0.750	1.342	0.923	1.554	0.835	1.581	0.776	1.421	transferase transferring glycosyl groups putative expressed	AT4G15240.1
CUST_19216_PI390587928	0.808	1.601	0.850	1.510	0.669	1.196	0.643	1.287	ribonucleoprotein like protein putative expressed	AT1G58470.1
CUST_32342_PI390587928	0.491	1.547	0.679	1.661	0.517	1.560	0.491	1.752	NAD(P)H-dependent oxidoreductase putative expressed	AT1G59950.1
CUST_3393_PI390587928	0.348	2.137	0.281	1.848	0.207	2.088	0.146	1.629	small heat shock-like protein putative expressed	
CUST_22013_PI390587928	0.848	1.416	0.791	1.124	0.896	1.553	0.840	1.654	protein yippee-like OJ1003C07.11 putative expressed	AT3G60210.1
CUST_34838_PI390587928	0.741	1.270	0.892	1.332	0.750	1.240	0.795	1.191	zinc finger C3HC4 type family protein expressed	AT5G12310.1
CUST_17943_PI390587928	0.750	2.614	0.698	2.353	0.578	1.533	0.527	1.720	nucleolar protein NOP5 putative expressed	AT5G27120.1
CUST_12748_PI390587928	0.636	3.386	0.463	2.769	0.522	1.580	0.457	1.980	-	
CUST_16751_PI390587928	0.607	1.817	0.740	2.300	0.604	1.553	0.799	2.339	glutathione S-transferase GSTF2 putative expressed	AT3G62760.1
CUST_42037_PI390587928	0.357	2.027	0.403	2.131	0.529	1.845	0.698	2.227	expressed protein	AT1G09575.1
CUST_34668_PI390587928	0.693	1.887	0.692	1.460	0.706	2.006	0.681	2.271	aquaporin PIP2.3 putative expressed	AT3G53420.2
CUST_28985_PI390587928	0.839	1.278	0.782	1.166	0.871	1.214	0.879	1.330	histone H2B.2 putative expressed	AT5G22880.1
CUST_20570_PI390587928	0.766	1.121	0.794	1.073	0.802	1.284	0.693	1.286	carbohydrate transporter/ sugar porter putative expressed	AT2G16120.1
CUST_10194_PI390587928	0.796	1.207	0.895	1.384	0.809	1.260	0.773	1.233	-	
CUST_8391_PI390587928	0.785	1.231	0.820	1.190	0.714	1.249	0.716	1.480	NADPH quinone oxidoreductase 1 putative expressed	AT3G27890.1
CUST_4419_PI390587928	0.876	2.572	0.674	2.390	0.482	1.526	0.737	1.587	60S ribosomal protein L19-3 putative expressed	AT1G02780.1
CUST_18533_PI390587928	0.426	2.368	0.510	2.265	0.645	1.610	0.544	2.333	RING-H2 finger protein ATL2L putative expressed	
CUST_15111_PI390587928	0.785	1.415	0.805	1.208	0.688	1.141	0.665	1.430	UDP-N-acetylglucosamine transferase subunit alg13 putative expressed	AT4G16710.2
CUST_7718_PI390587928	0.652	1.482	0.803	1.476	0.559	1.226	0.551	1.973	ATP-citrate synthase putative expressed	AT1G09430.1
CUST_5520_PI390587928	0.713	1.807	0.643	1.907	0.743	2.167	0.793	2.361	NAC domain-containing protein 78 putative expressed	AT5G64060.1
CUST_25115_PI390587928	0.784	1.600	0.712	1.738	0.688	1.293	0.685	1.373	60S acidic ribosomal protein P0 putative expressed	AT2G40010.1
CUST_30872_PI390587928	0.836	1.306	0.818	1.233	0.902	1.131	0.787	1.479	ATOZI1 putative expressed	AT1G01170.2
CUST_36343_PI390587928	0.805	1.411	0.787	1.351	0.783	1.233	0.719	1.306	ATP-dependent Clp protease proteolytic subunit mitochondrial precursor putative expressed	AT5G23140.1
CUST_31909_PI390587928	0.496	2.250	0.631	1.881	0.423	1.685	0.416	2.724	heat shock 22 kDa protein mitochondrial precursor putative expressed	AT5G51440.1
CUST_37179_PI390587928	0.804	2.018	0.882	1.907	0.598	1.262	0.693	1.732	eukaryotic translation initiation factor 3 subunit 5 putative expressed	AT2G39990.1
CUST_30733_PI390587928	0.360	1.924	0.299	2.274	0.448	2.109	0.483	2.291	-	
CUST_2976_PI390587928	0.572	3.180	0.628	3.219	0.498	2.552	0.555	3.167	-	
CUST_31771_PI390587928	0.382	2.213	0.392	2.448	0.345	1.866	0.395	2.915	transposon protein putative unclassified expressed	AT1G64940.1

Table S4-1 Genes upregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_36811_PI390587928	0.296	2.422	0.453	2.883	0.562	2.438	0.656	4.001	F-box domain containing protein expressed	
CUST_29096_PI390587928	0.592	1.556	0.698	1.693	0.550	1.311	0.812	1.880	thaumatin-like protein precursor putative expressed	AT4G11650.1
CUST_10718_PI390587928	0.761	1.621	0.813	1.762	0.626	1.216	0.641	2.063	thiamin pyrophosphokinase 1 putative expressed	AT2G44750.2
CUST_7862_PI390587928	0.494	1.846	0.610	1.586	0.815	2.106	0.632	2.139	heat shock cognate 70 kDa protein putative expressed	AT3G12580.1
CUST_24709_PI390587928	0.630	1.445	0.903	2.657	0.438	0.991	0.744	2.840	cytokinin-O-glucosyltransferase 3 putative expressed	AT2G36790.1
CUST_6670_PI390587928	0.825	2.491	0.866	2.558	0.600	2.133	0.577	2.163	elongation factor 1-alpha putative expressed	AT5G60390.3
CUST_2683_PI390587928	0.633	1.484	0.894	2.004	0.517	1.507	0.782	2.089	phenylalanine ammonia-lyase putative expressed	AT3G53260.1
CUST_8931_PI390587928	0.187	1.616	0.578	1.795	0.587	2.010	0.361	1.848	-	
CUST_26832_PI390587928	0.529	1.981	0.485	1.369	0.789	2.083	0.763	2.675	USP family protein putative expressed	AT3G03270.2
CUST_2560_PI390587928	0.802	2.567	0.780	2.134	0.643	1.688	0.627	2.171	chaperonin putative expressed	AT1G14980.1
CUST_23177_PI390587928	0.735	2.289	0.569	1.327	0.551	2.238	0.495	2.048	OTU-like cysteine protease family protein expressed	AT3G57810.3
CUST_35597_PI390587928	0.705	1.487	0.752	1.971	0.739	1.550	0.811	1.704	regulatory protein NPR1 putative expressed	AT5G45110.1
CUST_39615_PI390587928	0.936	1.912	0.849	1.701	0.827	1.637	0.788	1.661	arsenical pump-driving ATPase putative expressed	AT3G10350.1
CUST_24432_PI390587928	0.720	1.617	0.682	1.583	0.594	1.218	0.615	1.433	protein binding protein putative expressed	AT5G22480.1
CUST_7414_PI390587928	0.797	1.299	0.919	1.893	0.720	1.232	0.910	2.183	nucleoside transporter putative expressed	AT4G05120.1
CUST_5014_PI390587928	0.776	1.289	0.739	1.134	0.740	1.519	0.759	1.504	pyrophosphate-energized vacuolar membrane proton pump putative expressed	AT1G15690.1
CUST_11138_PI390587928	0.825	1.202	0.832	1.317	0.766	1.222	0.912	1.372	leucine carboxyl methyltransferase 1 putative expressed	AT1G02100.1
CUST_16940_PI390587928	0.515	1.742	0.810	2.612	0.505	1.271	0.759	2.189	PDR5-like ABC transporter putative expressed	AT1G15520.1
CUST_17185_PI390587928	0.692	2.041	0.786	3.633	0.704	2.001	0.780	3.527	tryptophan synthase beta chain 2 putative expressed	AT5G38530.1
CUST_27966_PI390587928	0.682	1.471	0.596	1.220	0.698	1.230	0.690	1.394	-	
CUST_10553_PI390587928	0.930	1.443	0.929	1.181	0.820	1.189	0.816	1.368	cytochrome b5 putative expressed	AT1G26340.1
CUST_31955_PI390587928	0.736	1.870	0.618	1.681	0.512	1.377	0.587	1.913	expressed protein	AT4G17540.1
CUST_11591_PI390587928	0.403	1.511	0.623	1.782	0.531	1.364	0.480	2.343	B12D protein expressed	AT3G29970.1
CUST_5194_PI390587928	0.666	2.092	0.737	1.892	0.745	2.202	0.667	2.189	-	
CUST_14905_PI390587928	0.793	1.075	0.791	1.279	0.877	1.257	0.828	1.199	-	
CUST_30593_PI390587928	0.850	1.505	0.663	1.416	0.711	1.385	0.778	1.422	cleavage and polyadenylation specificity factor 5 putative expressed	AT4G25550.1
CUST_40783_PI390587928	0.523	2.286	0.719	2.435	0.828	3.384	0.740	3.510	-	
CUST_21456_PI390587928	0.778	1.413	0.874	1.371	0.806	1.447	0.789	1.622	proline iminopeptidase putative expressed	AT3G61540.1
CUST_40830_PI390587928	0.610	1.422	0.714	1.502	0.656	1.574	0.536	1.656	calcium ion binding protein putative expressed	AT4G38810.2
CUST_7892_PI390587928	0.761	2.410	0.712	2.124	0.735	2.092	0.747	3.077	protein binding protein putative expressed	AT3G63530.2
CUST_866_PI390587928	0.540	1.999	0.760	3.329	0.521	2.121	0.707	3.088	ATPase coupled to transmembrane movement of substances putative expressed	AT1G66950.1

Table S4-1 Genes upregulated in response to K-starvation **(Continued).**

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_14581_PI390587928	0.905	1.577	0.880	1.605	0.846	1.303	0.776	1.337	RNA binding protein putative expressed	
CUST_37724_PI390587928	0.769	2.871	0.729	2.317	0.838	1.783	0.778	1.552	-	
CUST_39970_PI390587928	0.128	3.093	0.040	3.658	0.083	2.499	0.057	3.264	-	
CUST_20311_PI390587928	0.825	1.227	0.806	1.321	0.742	1.210	0.884	1.415	tubulin folding cofactor D putative expressed	AT3G60740.1
CUST_19388_PI390587928	0.677	2.004	0.706	1.397	0.803	3.332	0.684	2.728	OsTIL-1 - Oryza sativa Temperature-induced lipocalin-1 expressed	AT5G58070.1
CUST_22339_PI390587928	0.825	1.426	0.791	1.397	0.700	1.150	0.725	1.223	expressed protein	AT5G11010.3
CUST_32529_PI390587928	0.152	3.095	0.151	2.827	0.190	3.162	0.148	3.089	flavonol 4-sulfotransferase putative expressed	AT2G03760.1
CUST_41496_PI390587928	0.876	1.485	0.788	1.287	0.750	1.108	0.723	1.266	ATP-binding cassette sub-family F member 2 putative expressed	AT5G60790.1
CUST_17205_PI390587928	0.706	1.441	0.770	2.130	0.730	1.477	0.672	1.746	CSLA3 - cellulose synthase-like family A; mannan synthase expressed	AT5G03760.1
CUST_29471_PI390587928	0.246	2.126	0.527	2.968	0.390	1.902	0.423	3.074	cytochrome P450 94A1 putative expressed	AT2G27690.1
CUST_32982_PI390587928	0.798	2.896	0.694	2.844	0.545	1.908	0.620	2.577	-	
CUST_14086_PI390587928	0.743	1.596	0.879	1.880	0.591	1.124	0.710	1.959	transmembrane 9 superfamily protein member 2 precursor putative expressed	AT4G12650.1
CUST_8466_PI390587928	0.622	4.831	0.864	7.633	0.384	3.148	0.429	7.558	expressed protein	
CUST_23861_PI390587928	0.751	1.494	0.858	1.316	0.708	1.265	0.732	1.496	-	
CUST_8482_PI390587928	0.354	2.876	0.539	2.918	0.365	2.478	0.419	3.998	metal ion binding protein putative expressed	AT1G01490.2
CUST_1243_PI390587928	0.396	2.656	0.453	2.893	0.395	2.998	0.476	4.110	-	
CUST_33258_PI390587928	0.724	2.272	0.753	2.284	0.650	1.675	0.608	2.030	surfeit 6 putative expressed	AT5G05210.2
CUST_40772_PI390587928	0.571	1.630	0.836	2.388	0.624	1.217	0.656	1.909	anthocyanidin 53-O-glucosyltransferase putative expressed	AT1G01390.1
CUST_31060_PI390587928	0.853	1.648	0.928	1.484	0.898	1.685	0.804	1.775	coiled-coil domain-containing protein 25 putative expressed	AT5G11500.1
CUST_37075_PI390587928	0.789	2.096	0.728	2.061	0.548	1.338	0.576	1.572	guanine nucleotide-binding protein beta subunit-like protein putative expressed	AT1G18080.1
CUST_24153_PI390587928	0.797	1.275	0.864	1.484	0.869	1.198	0.795	1.340	transcription factor BIM2 putative expressed	AT1G69010.1
CUST_23722_PI390587928	0.609	2.309	0.519	2.592	0.458	1.885	0.352	1.814	INDETERMINATE-related protein 9 putative expressed	
CUST_34934_PI390587928	0.533	1.817	0.560	1.322	0.644	1.867	0.795	2.517	-	
CUST_7742_PI390587928	0.761	1.329	0.730	1.130	0.839	1.529	0.769	1.448	chloroplastic quinone-oxidoreductase putative expressed	AT4G13010.1
CUST_12860_PI390587928	0.827	1.319	0.877	1.254	0.781	1.156	0.775	1.167	expressed protein	AT3G07660.1
CUST_4777_PI390587928	0.578	2.099	0.554	2.712	0.633	1.705	0.481	2.629	tryptophan synthase beta chain 1 putative expressed	AT4G27070.1
CUST_29630_PI390587928	0.180	4.746	0.203	4.639	0.148	4.345	0.199	5.163	-	
CUST_12674_PI390587928	0.622	2.846	0.563	2.273	0.393	2.307	0.333	1.987	cell envelope integrity inner membrane protein TolA putative	
CUST_41532_PI390587928	0.546	1.872	0.567	2.396	0.588	1.846	0.545	2.503	pathogenesis-related protein PRMS precursor putative expressed	AT4G33720.1

Table S4-2 Genes down regulated in response to K-starvation.

Expression values relative to the medium for all data, of genes downregulated in K-starved (-K) plants with p-values < 0.005 corrected for false discoveries using Bonferroni multiple testing correction. Results for control (C) and K-starved (-K) detached leaf segments mock inoculation with water (mock) or inoculated with *R. secalis* (+ *RS*), two and four days post inoculation are shown.

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_25457_PI390587928	1.377	0.867	1.266	0.738	1.151	0.709	1.127	0.649	aminopeptidase/ metalloexopeptidase putative expressed	AT3G05350.1
CUST_34641_PI390587928	1.195	0.659	1.235	0.630	1.672	0.720	1.250	0.592	expressed protein	
CUST_12519_PI390587928	3.576	0.660	2.278	0.283	3.252	0.383	1.707	0.173	early nodulin 93 putative expressed	AT5G25940.1
CUST_19790_PI390587928	1.749	0.707	1.613	0.718	1.536	0.684	1.424	0.630	TPR Domain containing protein expressed	AT1G02910.1
CUST_40170_PI390587928	1.233	0.703	1.088	0.679	1.342	0.785	1.258	0.815	-	AT5G10460.1
CUST_26526_PI390587928	1.454	0.364	1.529	0.325	1.437	0.440	1.660	0.231	chlorophyll a-b binding protein chloroplast precursor putative expressed	AT3G61470.1
CUST_14579_PI390587928	1.253	0.656	1.217	0.486	1.459	0.654	1.197	0.549	expressed protein	AT5G20100.1
CUST_35477_PI390587928	1.749	0.793	1.158	0.686	1.427	0.829	1.620	0.722	expressed protein	
CUST_30484_PI390587928	1.749	0.719	1.453	0.661	1.159	0.595	1.251	0.503	acid phosphatase putative expressed	AT3G01310.1
CUST_41207_PI390587928	1.173	0.703	1.136	0.734	1.097	0.661	1.166	0.666	coatomer subunit alpha putative expressed	AT1G62020.1
CUST_28586_PI390587928	1.220	0.821	1.275	0.829	1.327	0.756	1.163	0.743	clathrin binding protein putative expressed	AT4G18060.1
CUST_24126_PI390587928	1.554	0.478	1.549	0.375	2.106	0.494	1.714	0.467	-	
CUST_12412_PI390587928	1.639	0.620	1.317	0.469	1.410	0.571	1.499	0.481	-	AT1G03475.1
CUST_7310_PI390587928	1.161	0.909	1.291	0.833	1.230	0.843	1.168	0.820	expressed protein	AT1G27510.1
CUST_39325_PI390587928	1.268	0.615	1.136	0.601	1.358	0.622	1.324	0.542	OsGrx_S14 - glutaredoxin subgroup II expressed	
CUST_19449_PI390587928	1.435	0.783	1.283	0.684	1.257	0.797	1.194	0.737	bile acid sodium symporter putative expressed	AT3G56160.1
CUST_38133_PI390587928	1.418	0.698	1.266	0.605	1.520	0.746	1.379	0.733	eukaryotic translation initiation factor 5A-2 putative expressed	AT1G69410.1
CUST_25211_PI390587928	1.241	0.851	1.206	0.822	1.205	0.771	1.276	0.854	coatomer subunit alpha putative expressed	AT2G21390.1
CUST_16_PI390587928	1.476	0.532	1.293	0.451	1.647	0.837	1.492	0.539	-	
CUST_18304_PI390587928	1.225	0.888	1.137	0.692	1.131	0.710	1.134	0.759	OsMPK6 - putative MAPK based on amino acid sequence homology expressed	AT2G43790.1
CUST_27255_PI390587928	1.251	0.588	1.310	0.475	1.230	0.633	1.105	0.438	peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein expressed	AT5G35100.1
CUST_40956_PI390587928	2.059	0.623	1.667	0.667	1.946	0.549	2.141	0.494	-	ATCG01010.1
CUST_33001_PI390587928	1.841	0.836	1.544	0.812	1.500	0.814	1.541	0.667	SET domain containing protein expressed	AT5G14260.2
CUST_29346_PI390587928	1.548	0.576	1.605	0.541	1.424	0.505	1.435	0.425	CBS domain containing protein expressed	
CUST_10953_PI390587928	1.283	0.786	1.177	0.719	1.310	0.696	1.301	0.786	-	
CUST_6857_PI390587928	1.581	0.438	1.848	0.414	1.839	0.554	1.474	0.463	phosphate transporter 1 putative expressed	AT1G68740.1
CUST_30627_PI390587928	2.304	0.458	2.504	0.289	3.969	0.595	2.906	0.427	MYB59 putative expressed	AT3G46130.1
CUST_10310_PI390587928	1.169	0.796	1.068	0.661	1.183	0.786	1.119	0.725	-	
CUST_2933_PI390587928	1.307	0.717	1.237	0.569	1.352	0.650	1.130	0.581	expressed protein	AT1G11540.1
CUST_14584_PI390587928	1.038	0.768	1.102	0.835	1.140	0.723	1.127	0.800	PX domain containing protein expressed	AT3G48195.1
CUST_41902_PI390587928	3.039	0.427	3.432	0.529	2.998	0.542	2.233	0.267	indole-3-acetate beta-glucosyltransferase putative expressed	AT3G02100.1
CUST_40663_PI390587928	1.221	0.635	1.199	0.512	1.215	0.748	1.142	0.648	GIF2 putative expressed	AT4G00850.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_28015_PI390587928	1.588	0.432	1.249	0.336	1.815	0.496	1.503	0.253	-	
CUST_932_PI390587928	1.536	0.632	1.404	0.648	1.355	0.688	1.341	0.617	threonyl-tRNA synthetase putative expressed	AT2G04842.1
CUST_8794_PI390587928	1.748	0.491	1.758	0.643	1.937	0.751	1.698	0.512	-	
CUST_6580_PI390587928	1.411	0.825	1.317	0.805	1.198	0.836	1.207	0.636	expressed protein	AT5G02250.1
CUST_15700_PI390587928	1.156	0.640	1.222	0.740	1.274	0.771	1.214	0.689	hypothetical protein	
CUST_19470_PI390587928	1.412	0.741	1.361	0.652	1.475	0.695	1.355	0.624	protein kinase putative expressed	AT4G24810.2
CUST_35204_PI390587928	1.708	0.804	1.650	0.547	2.737	0.779	1.933	0.470	multidrug resistance protein 22 putative expressed	AT3G28345.1
CUST_17590_PI390587928	1.209	0.768	1.177	0.883	1.053	0.803	1.203	0.881	ATP binding protein putative expressed	AT2G38840.1
CUST_19145_PI390587928	1.183	0.916	1.166	0.701	1.321	0.783	1.182	0.724	expressed protein	AT1G55340.2
CUST_15158_PI390587928	1.285	0.642	1.239	0.480	1.248	0.517	1.162	0.429	ATNAP8 putative expressed	AT4G25450.3
CUST_40231_PI390587928	1.780	0.813	1.511	0.608	1.420	0.585	1.131	0.509	cysteine-type peptidase putative expressed	AT3G57810.3
CUST_24342_PI390587928	1.186	0.653	1.204	0.489	1.438	0.908	1.391	0.738	-	
CUST_5063_PI390587928	1.570	0.550	1.387	0.383	1.504	0.546	1.349	0.418	expressed protein	AT3G59040.1
CUST_33324_PI390587928	1.423	0.888	1.206	0.713	1.289	0.801	1.172	0.625	OCP3 putative expressed	AT5G11270.1
CUST_32085_PI390587928	1.351	0.794	1.325	0.724	1.346	0.711	1.417	0.744	preprotein translocase secA subunit chloroplast precursor putative expressed	AT4G01800.1
CUST_6085_PI390587928	1.357	0.791	1.403	0.756	1.282	0.701	1.489	0.863	quinone oxidoreductase putative expressed	AT4G21580.1
CUST_38333_PI390587928	1.517	0.835	1.377	0.663	1.401	0.703	1.267	0.641	anaphase-promoting complex subunit 11 putative expressed	AT3G05870.1
CUST_20962_PI390587928	1.224	0.750	1.270	0.720	1.294	0.820	1.243	0.731	3-5 exonuclease eri-1 putative expressed	
CUST_283_PI390587928	1.698	0.492	1.727	0.429	2.569	0.666	2.567	0.586	expressed protein	AT2G25737.1
CUST_39371_PI390587928	1.835	0.671	1.608	0.461	1.292	0.544	1.346	0.624	expressed protein	AT4G16060.1
CUST_11809_PI390587928	1.476	0.727	1.080	0.454	1.243	0.609	1.203	0.511	alanyl-tRNA synthetase putative expressed	AT5G22800.1
CUST_29026_PI390587928	1.329	0.662	1.254	0.534	1.358	0.647	1.218	0.578	MYB transcription factor putative expressed	AT2G01060.1
CUST_33761_PI390587928	1.765	0.715	1.248	0.393	1.449	0.573	1.216	0.445	ferredoxin-6 chloroplast precursor putative expressed	AT1G32550.1
CUST_27269_PI390587928	1.435	0.795	1.324	0.790	1.451	0.742	1.230	0.687	hypothetical protein	AT5G38060.1
CUST_35229_PI390587928	1.272	0.768	1.151	0.743	1.335	0.740	1.556	0.852	expressed protein	AT1G02816.1
CUST_9229_PI390587928	1.113	0.677	1.164	0.644	1.282	0.766	1.139	0.657	expressed protein	
CUST_21845_PI390587928	1.321	0.614	1.217	0.613	1.400	0.590	1.321	0.548	DNA gyrase subunit B putative expressed	
CUST_35245_PI390587928	1.765	0.578	1.604	0.437	1.580	0.324	1.369	0.302	-	
CUST_3805_PI390587928	1.229	0.671	1.621	0.765	1.309	0.435	1.450	0.506	peroxidase 2 precursor putative expressed	AT5G05340.1
CUST_26901_PI390587928	1.377	0.699	1.243	0.616	1.586	0.847	1.295	0.629	-	
CUST_12724_PI390587928	1.396	0.677	1.218	0.575	1.376	0.682	1.283	0.533	-	
CUST_33622_PI390587928	1.267	0.727	1.196	0.722	1.491	0.740	1.363	0.692	mTERF family protein expressed	
CUST_11532_PI390587928	1.380	0.610	1.298	0.601	1.389	0.588	1.238	0.528	expressed protein	
CUST_4704_PI390587928	1.281	0.728	1.326	0.746	1.475	0.678	1.352	0.771	homeodomain leucine zipper protein CPHB-5 putative expressed	AT3G01470.1
CUST_12570_PI390587928	1.548	0.549	1.321	0.437	1.436	0.566	1.396	0.375	expressed protein	AT4G01050.1
CUST_22760_PI390587928	1.763	0.702	1.673	0.535	1.625	0.833	1.634	0.516	photosystem I reaction center subunit III chloroplast precursor putative expressed	AT1G31330.1
CUST_13592_PI390587928	1.484	0.687	1.498	0.597	1.560	0.654	1.416	0.605	tubby-related protein 1 putative expressed	AT1G53320.1
CUST_26561_PI390587928	1.518	0.606	1.713	0.537	1.758	0.490	1.848	0.325	ribulose bisphosphate carboxylase large chain precursor putative expressed	ATCG00490.1
CUST_442_PI390587928	1.201	0.683	1.175	0.786	1.355	0.859	1.270	0.699	ras-related protein Rab-18 putative expressed	AT1G43890.3
CUST_10170_PI390587928	1.182	0.807	1.252	0.798	1.231	0.694	1.089	0.722	expressed protein	AT3G11850.2

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_37820_PI390587928	1.353	0.529	1.168	0.405	1.461	0.557	1.318	0.471	mTERF family protein expressed	
CUST_1112_PI390587928	1.611	0.596	1.645	0.405	1.311	0.462	1.194	0.336	cytochrome P450 97B3 putative expressed	AT1G31800.1
CUST_11223_PI390587928	1.251	0.787	1.276	0.812	1.432	0.712	1.379	0.562	expressed protein	
CUST_8855_PI390587928	1.500	0.629	1.384	0.471	1.644	0.658	1.417	0.415	beta-propeller domains of methanol dehydrogenase type putative expressed	AT1G54780.1
CUST_17270_PI390587928	1.200	0.704	1.287	0.636	1.310	0.714	1.187	0.643	casein kinase II subunit beta-4 putative expressed	AT5G47080.1
CUST_15040_PI390587928	1.747	0.839	1.333	0.540	1.811	0.757	1.747	0.727	transcription regulator putative expressed	AT2G13690.1
CUST_1399_PI390587928	2.164	0.753	1.414	0.622	2.112	0.715	2.122	0.828	-	
CUST_30411_PI390587928	2.334	0.127	3.183	0.387	1.425	0.081	4.195	0.520	-	
CUST_4178_PI390587928	1.367	0.591	1.419	0.551	1.308	0.513	1.144	0.425	amino acid binding protein putative expressed	AT2G39570.1
CUST_17301_PI390587928	1.310	0.554	1.342	0.339	1.377	0.553	1.262	0.467	2-hydroxy-3-oxopropionate reductase putative expressed	AT1G17650.1
CUST_4194_PI390587928	1.708	0.433	1.615	0.460	1.653	0.477	1.556	0.432	expressed protein	AT1G75100.1
CUST_27290_PI390587928	1.523	0.714	1.370	0.742	1.308	0.631	1.198	0.544	CDK5RAP1-like protein putative expressed	AT4G36390.1
CUST_5247_PI390587928	1.570	0.614	1.537	0.380	1.291	0.470	1.204	0.439	peptidyl-prolyl cis-trans isomerase FKBP-type family protein expressed	AT3G10060.1
CUST_22079_PI390587928	1.167	0.649	1.423	0.626	1.202	0.854	1.122	0.731	catalase-1 putative expressed	AT4G35090.1
CUST_30039_PI390587928	1.414	0.744	1.491	0.693	1.451	0.782	1.301	0.732	expressed protein	AT4G20740.1
CUST_41267_PI390587928	1.231	0.908	1.287	0.762	1.396	0.709	1.300	0.807	fructokinase-2 putative expressed	AT1G66430.1
CUST_31108_PI390587928	1.346	0.539	1.372	0.550	1.479	0.623	1.539	0.551	expressed protein	AT3G22450.1
CUST_5124_PI390587928	2.203	0.402	2.197	0.337	1.742	0.489	1.529	0.340	peptide chain release factor 2 putative expressed	AT3G57190.1
CUST_37932_PI390587928	1.461	0.671	1.592	0.458	1.528	0.766	1.537	0.710	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase putative expressed	AT2G24270.1
CUST_41688_PI390587928	1.208	0.591	1.327	0.542	1.887	0.595	1.843	0.661	-	
CUST_29242_PI390587928	1.689	0.480	1.844	0.289	3.536	0.568	2.873	0.463	osmotin-like protein precursor putative expressed	AT2G28790.1
CUST_40713_PI390587928	1.539	0.732	1.442	0.579	1.261	0.619	1.237	0.619	NHL repeat protein putative expressed	AT1G56500.1
CUST_24824_PI390587928	1.421	0.619	1.356	0.475	1.388	0.601	1.131	0.433	expressed protein	AT3G27180.1
CUST_16087_PI390587928	1.126	0.843	1.101	0.872	1.231	0.739	1.079	0.775	-	
CUST_8828_PI390587928	1.219	0.515	1.360	0.771	1.278	0.530	1.280	0.672	expressed protein	AT3G02910.1
CUST_11732_PI390587928	1.376	0.441	1.166	0.352	1.151	0.469	1.140	0.311	expressed protein	AT5G44600.1
CUST_12738_PI390587928	1.257	0.687	1.254	0.661	1.739	0.665	1.388	0.640	-	AT3G51860.1
CUST_19520_PI390587928	1.303	0.677	1.279	0.527	1.248	0.677	1.186	0.552	carboxyl-terminal-processing protease precursor putative expressed	AT5G46390.2
CUST_3649_PI390587928	2.429	0.345	1.998	0.194	2.416	0.295	2.114	0.227	ribulose bisphosphate carboxylase small chain C chloroplast precursor putative expressed	AT1G67090.1
CUST_34658_PI390587928	2.775	0.199	2.495	0.155	2.771	0.118	3.456	0.152	cytokinin dehydrogenase 1 precursor putative expressed	AT2G41510.1
CUST_27953_PI390587928	1.505	0.831	1.228	0.622	1.250	0.687	1.217	0.590	-	
CUST_5926_PI390587928	2.204	0.451	2.187	0.553	1.579	0.386	3.220	0.392	terpene synthase 7 putative expressed	AT1G70080.1
CUST_11562_PI390587928	1.398	0.887	1.185	0.778	1.186	0.657	1.215	0.654	syntaxin 23 putative expressed	AT5G46860.1
CUST_27767_PI390587928	1.826	0.777	1.437	0.552	1.335	0.726	1.291	0.518	-	AT3G59890.1
CUST_26559_PI390587928	1.229	0.752	1.093	0.736	1.194	0.833	1.265	0.697	-	
CUST_10215_PI390587928	1.450	0.697	1.419	0.619	1.436	0.643	1.153	0.449	cytochrome P450 97B2 putative expressed	AT4G15110.1
CUST_24175_PI390587928	1.242	0.866	1.274	0.899	1.413	0.849	1.257	0.776	signal transducer putative expressed	AT3G15470.1
CUST_13467_PI390587928	1.174	0.809	1.155	0.696	1.255	0.818	1.196	0.838	-	
CUST_8365_PI390587928	1.203	0.876	1.132	0.640	1.122	0.739	1.145	0.827	complex 1 protein containing protein expressed	AT1G76065.1
CUST_30985_PI390587928	1.443	0.606	1.453	0.491	1.467	0.558	1.339	0.523	expressed protein	AT1G22850.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_65_PI390587928	1.524	0.802	1.269	0.605	1.284	0.733	1.240	0.585	expressed protein	
CUST_4239_PI390587928	1.637	0.482	1.384	0.354	1.468	0.493	1.228	0.336	expressed protein	AT5G48790.1
CUST_39251_PI390587928	1.412	0.791	1.324	0.648	1.558	0.821	1.378	0.669	-	
CUST_33050_PI390587928	1.757	0.585	1.608	0.389	1.904	0.679	1.405	0.406	-	
CUST_27926_PI390587928	1.218	0.862	1.163	0.786	1.212	0.838	1.144	0.846	expressed protein	AT5G65120.1
CUST_30862_PI390587928	1.245	0.900	1.272	0.794	1.336	0.838	1.190	0.819	NEDD8-conjugating enzyme Ubc12-like putative expressed	AT4G36800.2
CUST_37339_PI390587928	1.185	0.685	1.211	0.554	1.352	0.778	1.293	0.713	ubiquinone biosynthesis protein ubiB putative expressed	AT4G31390.2
CUST_8103_PI390587928	1.354	0.690	1.226	0.539	1.324	0.674	1.202	0.506	-	AT3G45050.3
CUST_6346_PI390587928	1.383	0.487	1.303	0.346	1.533	0.573	1.343	0.327	senescence-associated protein DIN1 putative expressed	AT2G17850.1
CUST_9125_PI390587928	1.263	0.671	1.302	0.670	1.326	0.772	1.238	0.605	-	
CUST_28187_PI390587928	1.430	0.554	1.428	0.411	1.279	0.517	1.340	0.491	ATA15 protein putative expressed	AT1G66330.2
CUST_1928_PI390587928	1.742	0.446	1.678	0.574	1.338	0.313	1.564	0.193	bifunctional aspartokinase/homoserine dehydrogenase 2 chloroplast precursor putative expressed	AT4G19710.2
CUST_13812_PI390587928	1.332	0.783	1.293	0.572	1.367	0.728	1.324	0.705	2-cys peroxiredoxin BAS1 chloroplast precursor putative expressed	AT5G06290.1
CUST_32953_PI390587928	1.359	0.922	1.163	0.763	1.485	0.819	1.257	0.697	-	
CUST_22640_PI390587928	1.727	0.592	1.302	0.439	1.800	0.704	1.916	0.548	-	
CUST_30600_PI390587928	1.510	0.697	1.326	0.495	1.515	0.790	1.512	0.588	nicotinate phosphoribosyltransferase-like protein putative expressed	
CUST_4600_PI390587928	1.204	0.418	1.300	0.461	1.418	0.397	1.697	0.442	carbonic anhydrase chloroplast precursor putative expressed	AT5G14740.2
CUST_31606_PI390587928	6.570	0.318	3.782	0.115	5.150	0.068	2.987	0.050	chymopapain precursor putative	AT5G50260.1
CUST_24901_PI390587928	1.555	0.706	1.380	0.404	1.410	0.491	1.162	0.453	expressed protein	AT2G21960.1
CUST_26441_PI390587928	1.244	0.705	1.179	0.795	1.177	0.686	1.170	0.628	aldehyde dehydrogenase 3B1 putative expressed	AT4G36250.1
CUST_31622_PI390587928	1.699	0.620	1.808	0.403	1.556	0.475	1.396	0.315	phospholipid hydroperoxide glutathione peroxidase 1 chloroplast precursor putative expressed	AT2G25080.1
CUST_22454_PI390587928	1.497	0.628	1.540	0.723	1.467	0.758	1.378	0.618	-	
CUST_24715_PI390587928	1.569	0.687	1.687	0.669	1.581	0.775	1.330	0.560	cytochrome P450 74A4 putative expressed	AT4G15440.1
CUST_27510_PI390587928	1.657	0.724	1.234	0.684	1.364	0.711	1.578	0.570	SFR2 putative expressed	AT3G06510.2
CUST_28516_PI390587928	1.263	0.854	1.259	0.824	1.363	0.868	1.342	0.841	DNA-directed RNA polymerases II 24 kDa polypeptide putative expressed	AT3G22320.1
CUST_19348_PI390587928	1.315	0.591	1.402	0.546	1.344	0.652	1.229	0.470	CAO putative expressed	AT2G47450.1
CUST_1465_PI390587928	2.108	0.697	1.648	0.481	1.866	0.487	1.601	0.447	-	
CUST_34719_PI390587928	1.288	0.698	1.213	0.648	1.374	0.701	1.240	0.606	signal recognition particle 54 kDa protein 2 putative expressed	
CUST_35253_PI390587928	1.683	0.715	1.304	0.769	1.417	0.755	1.473	0.549	permease 1 putative expressed	
CUST_19364_PI390587928	1.191	0.875	1.076	0.904	1.278	0.922	1.187	0.947	expressed protein	
CUST_22315_PI390587928	1.207	0.584	1.158	0.519	1.382	0.685	1.132	0.607	DCL protein chloroplast precursor putative expressed	AT3G46630.1
CUST_30074_PI390587928	1.765	0.549	1.357	0.353	1.538	0.518	1.559	0.429	oxygen-evolving enhancer protein 3 chloroplast precursor putative	AT2G01918.1
CUST_18203_PI390587928	1.660	0.711	1.460	0.501	1.499	0.498	1.227	0.432	xylogen protein 1 putative expressed	AT2G13820.2
CUST_16276_PI390587928	1.084	0.764	1.173	0.839	1.107	0.701	1.143	0.736	expressed protein	AT5G66840.1
CUST_14062_PI390587928	1.300	0.867	1.235	0.863	1.164	0.719	1.272	0.864	impaired sucrose induction 1-like protein putative expressed	AT4G27750.1
CUST_3993_PI390587928	1.165	0.687	1.291	0.715	1.324	0.731	1.318	0.755	PHD zinc finger protein putative expressed	AT2G27980.1
CUST_177_PI390587928	1.351	0.752	1.463	0.613	1.437	0.801	1.330	0.659	PCB2 putative expressed	AT5G18660.1
CUST_29075_PI390587928	1.242	0.580	1.295	0.568	1.545	0.706	1.364	0.593	serine/threonine-protein kinase MHK putative expressed	AT4G13020.4

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_41770_PI390587928	1.450	0.690	1.563	0.604	1.523	0.606	1.273	0.508	amino acid binding protein putative expressed	AT2G39570.1
CUST_6042_PI390587928	1.139	0.783	1.308	0.866	1.162	0.763	1.085	0.714	transferase transferring glycosyl groups putative expressed	AT1G80260.1
CUST_16153_PI390587928	1.389	0.411	1.791	0.701	1.190	0.354	1.558	0.598	elicitor-responsive protein 1 putative expressed	AT3G55470.2
CUST_15722_PI390587928	1.315	0.757	1.219	0.797	1.257	0.873	1.285	0.771	resistance protein putative expressed	
CUST_3854_PI390587928	1.641	0.589	1.366	0.405	1.451	0.522	1.287	0.354	endonuclease putative expressed	AT2G15820.1
CUST_24704_PI390587928	1.583	0.695	1.367	0.399	1.392	0.554	1.200	0.379	rf1 protein mitochondrial precursor putative expressed	AT3G53700.1
CUST_26950_PI390587928	1.297	0.703	1.248	0.575	1.342	0.678	1.135	0.516	RING finger and CHY zinc finger domain-containing protein 1 putative expressed	AT5G22920.1
CUST_4099_PI390587928	1.650	0.649	1.548	0.497	1.487	0.630	1.287	0.562	glutamine amidotransferase subunit pdxT putative expressed	AT5G60540.1
CUST_24534_PI390587928	1.186	0.882	1.225	0.899	1.039	0.766	1.116	0.863	-	
CUST_34724_PI390587928	1.553	0.725	1.372	0.638	1.309	0.655	1.146	0.492	ATP-dependent RNA helicase rhlE putative expressed	AT4G09730.1
CUST_17036_PI390587928	1.169	0.664	1.121	0.748	1.155	0.618	1.069	0.664	-	
CUST_8740_PI390587928	1.867	0.535	1.686	0.363	1.437	0.403	1.882	0.366	expressed protein	
CUST_20563_PI390587928	1.239	0.761	1.169	0.690	1.298	0.599	1.194	0.560	-	
CUST_4753_PI390587928	1.118	0.853	1.172	0.795	1.310	0.805	1.161	0.797	F-box domain containing protein expressed	
CUST_12634_PI390587928	1.105	0.826	1.109	0.661	1.234	0.820	1.138	0.745	protein tyrosine phosphatase-like protein PTPLB putative expressed	AT5G59770.1
CUST_33532_PI390587928	1.305	0.668	1.340	0.532	1.469	0.548	1.286	0.520	-	
CUST_17457_PI390587928	1.456	0.676	1.262	0.561	1.353	0.592	1.159	0.588	receptor-like kinase putative expressed	
CUST_27647_PI390587928	1.179	0.805	1.233	0.734	1.388	0.735	1.261	0.694	S-adenosylmethionine-dependent methyltransferase putative expressed	AT4G28830.1
CUST_40773_PI390587928	1.376	0.516	1.192	0.442	1.526	0.667	1.535	0.509	serine/threonine protein kinase putative expressed	AT4G23320.1
CUST_18712_PI390587928	1.237	0.843	1.133	0.826	1.239	0.865	1.215	0.783	retrotransposon protein putative unclassified	AT2G28970.1
CUST_30630_PI390587928	1.296	0.646	1.344	0.518	1.397	0.622	1.189	0.543	signal peptide peptidase-like 2B putative expressed	AT1G63690.1
CUST_5636_PI390587928	1.354	0.736	1.313	0.618	1.453	0.823	1.201	0.540	expressed protein	AT1G28760.1
CUST_11272_PI390587928	1.243	0.804	1.355	0.656	1.332	0.828	1.293	0.805	OsFtsH9 - Oryza sativa FtsH protease homologue of AtFtsH11 expressed	AT5G53170.1
CUST_34415_PI390587928	1.168	0.685	1.344	0.689	1.412	0.812	1.383	0.719	disulfide oxidoreductase/ monooxygenase putative expressed	AT1G12140.1
CUST_10064_PI390587928	1.218	0.555	1.076	0.339	2.116	0.650	1.995	0.563	expressed protein	AT5G47920.1
CUST_37683_PI390587928	1.407	0.782	1.515	0.661	1.478	0.817	1.427	0.587	expressed protein	AT1G44000.1
CUST_19580_PI390587928	1.331	0.606	1.446	0.607	1.254	0.656	1.234	0.656	OsFtsH1 - Oryza sativa FtsH protease homologue of AtFtsH1/5 expressed	AT1G50250.1
CUST_11117_PI390587928	1.501	0.506	1.414	0.421	1.752	0.662	1.386	0.608	-	AT5G65660.1
CUST_32488_PI390587928	1.371	0.806	1.390	0.641	1.397	0.741	1.243	0.655	NADH dehydrogenase putative expressed	AT5G08740.1
CUST_8261_PI390587928	1.131	0.639	1.368	0.708	1.609	0.719	1.245	0.584	expressed protein	
CUST_18372_PI390587928	1.081	0.788	1.237	0.874	1.270	0.809	1.354	0.858	xylosyltransferase 1 putative expressed	AT1G71070.1
CUST_29568_PI390587928	1.349	0.892	1.152	0.745	1.297	0.896	1.271	0.832	expressed protein	AT5G39530.1
CUST_2044_PI390587928	1.601	0.563	1.508	0.455	1.552	0.613	1.630	0.516	transposon protein putative unclassified	AT4G38180.1
CUST_20115_PI390587928	1.385	0.833	1.173	0.790	1.407	0.879	1.247	0.713	expressed protein	
CUST_37560_PI390587928	1.567	0.380	1.194	0.299	2.298	0.420	1.721	0.287	expressed protein	AT1G19910.1
CUST_5343_PI390587928	1.722	0.399	1.923	0.384	2.009	0.276	1.970	0.276	calcium-dependent protein kinase 2 putative expressed	AT1G08650.1
CUST_31918_PI390587928	1.530	0.762	1.592	0.887	1.445	0.601	1.401	0.588	EMB1381 putative expressed	AT2G31340.1
CUST_9128_PI390587928	1.427	0.728	1.531	0.716	1.140	0.648	1.077	0.671	2-C-methyl-D-erythritol 24-cyclodiphosphate synthase chloroplast precursor putative expressed	AT1G63970.1
CUST_16003_PI390587928	1.511	0.664	1.387	0.614	1.467	0.629	1.366	0.526	NBS-LRR type disease resistance protein putative expressed	AT3G07040.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		**+ *RS***		**mock**		**+ *RS***			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_13815_PI390587928	1.244	0.462	1.428	0.314	1.314	0.564	1.322	0.413	glutamine synthetase chloroplast precursor putative expressed	AT5G35630.3
CUST_27215_PI390587928	1.222	0.648	1.239	0.650	1.332	0.722	1.265	0.564	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor putative expressed	AT5G16000.1
CUST_19085_PI390587928	1.183	0.835	1.344	0.827	1.203	0.783	1.255	0.836	palmitoyltransferase pfa3 putative expressed	
CUST_377_PI390587928	1.317	0.774	1.254	0.780	1.480	0.766	1.423	0.795	expressed protein	AT3G48210.1
CUST_13645_PI390587928	1.252	0.505	1.495	0.500	1.426	0.574	1.217	0.408	signal recognition particle 54 kDa protein 2 putative expressed	AT1G48900.1
CUST_40155_PI390587928	1.300	0.528	1.271	0.352	1.584	0.585	1.519	0.536	transposon protein putative unclassified expressed	AT4G35250.1
CUST_6817_PI390587928	1.230	0.745	1.446	0.842	1.275	0.793	1.153	0.709	protein kinase domain containing protein expressed	AT5G59700.1
CUST_25879_PI390587928	1.036	0.679	1.269	0.800	1.268	0.727	1.155	0.764	-	
CUST_39279_PI390587928	1.236	0.790	1.126	0.864	1.230	0.757	1.203	0.758	-	
CUST_6833_PI390587928	1.311	0.831	1.107	0.716	1.191	0.758	1.156	0.683	pentatricopeptide repeat protein PPR986-12 putative expressed	AT4G37380.1
CUST_24096_PI390587928	1.192	0.642	1.118	0.616	1.054	0.618	1.105	0.614	-	
CUST_7855_PI390587928	3.122	0.401	3.768	0.421	3.861	0.370	3.748	0.126	-	
CUST_2862_PI390587928	1.138	0.779	1.137	0.759	1.130	0.827	1.072	0.700	protein phosphatase 2C isoform beta putative expressed	AT1G18030.1
CUST_14528_PI390587928	1.409	0.915	1.376	0.735	1.276	0.751	1.125	0.672	expressed protein	AT5G24314.1
CUST_29152_PI390587928	1.401	0.757	1.451	0.791	1.617	0.724	1.498	0.718	DRE binding factor 1 putative expressed	AT4G39780.1
CUST_3884_PI390587928	2.549	0.597	1.908	0.434	2.500	0.534	2.168	0.434	S-adenosylmethionine-dependent methyltransferase/ methyltransferase/ thiopurine S-methyltransferase putative expressed	AT2G43940.1
CUST_37112_PI390587928	3.396	0.391	2.848	0.240	2.293	0.126	1.878	0.068	1-aminocyclopropane-1-carboxylate oxidase 1 putative expressed	AT1G05010.1
CUST_21955_PI390587928	1.718	0.831	1.485	0.652	1.332	0.710	1.397	0.703	NHL repeat protein putative expressed	AT1G56500.1
CUST_29743_PI390587928	1.672	0.300	1.889	0.174	1.794	0.355	1.710	0.204	carbonic anhydrase chloroplast precursor putative expressed	AT5G14740.2
CUST_16588_PI390587928	1.257	0.721	1.330	0.578	1.351	0.655	1.408	0.766	DNA-binding protein SMUBP-2 putative expressed	AT5G35970.1
CUST_34738_PI390587928	1.301	0.722	1.246	0.583	1.374	0.708	1.102	0.609	MYB transcription factor putative expressed	AT2G01060.1
CUST_24564_PI390587928	1.372	0.850	1.374	0.753	1.729	0.618	1.514	0.571	-	
CUST_37533_PI390587928	1.423	0.665	1.100	0.504	1.358	0.811	1.332	0.627	cytokinin-N-glucosyltransferase 1 putative expressed	AT3G11340.1
CUST_722_PI390587928	1.199	0.681	1.175	0.649	1.322	0.724	1.205	0.708	methyl-CpG binding domain containing protein expressed	
CUST_24611_PI390587928	1.353	0.692	1.223	0.541	1.525	0.660	1.282	0.401	-	
CUST_39810_PI390587928	1.247	0.692	1.145	0.778	1.428	0.849	1.324	0.716	-	
CUST_16449_PI390587928	1.124	0.558	1.552	0.536	1.725	0.628	2.154	0.656	receptor-like protein kinase precursor putative expressed	AT1G04970.2
CUST_41289_PI390587928	1.368	0.501	1.358	0.428	1.415	0.530	1.365	0.427	shikimate kinase family protein expressed	
CUST_12680_PI390587928	1.500	0.731	1.328	0.642	1.419	0.704	1.487	0.789	CDT1a protein putative expressed	AT2G31270.1
CUST_39608_PI390587928	1.201	0.899	1.123	0.808	1.093	0.794	1.044	0.767	phosphoribosylformylglycinamidine synthase chloroplast precursor putative expressed	AT1G74260.1
CUST_23186_PI390587928	1.663	0.703	1.623	0.652	1.539	0.786	1.528	0.694	calcineurin B-like protein 10 putative expressed	AT4G33000.2
CUST_2383_PI390587928	1.680	0.633	1.326	0.754	1.485	0.527	1.213	0.434	lycopene epsilon cyclase chloroplast precursor putative expressed	AT5G57030.1
CUST_35637_PI390587928	1.853	0.770	1.836	0.558	1.655	0.775	1.263	0.738	-	
CUST_27693_PI390587928	1.338	0.933	1.246	0.663	1.294	0.831	1.294	0.882	ferredoxin-thioredoxin reductase catalytic chain chloroplast precursor putative expressed	AT2G04700.1
CUST_39454_PI390587928	1.239	0.642	1.364	0.588	1.396	0.621	1.261	0.626	-	AT3G08690.1
CUST_25510_PI390587928	1.475	0.825	1.355	0.663	1.363	0.831	1.103	0.561	small multi-drug export protein putative expressed	AT2G02590.1
CUST_34259_PI390587928	1.373	0.493	1.263	0.403	1.689	0.537	1.612	0.415	-	AT1G73080.1
CUST_3266_PI390587928	1.244	0.582	1.201	0.576	1.357	0.796	1.304	0.564	40S ribosomal protein S3a putative expressed	

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_42235_PI390587928	1.067	0.763	1.103	0.596	1.298	0.817	1.310	0.776	sugar transporter type 2a putative expressed	AT4G35300.3
CUST_20906_PI390587928	1.184	0.741	1.109	0.638	1.258	0.859	1.153	0.627	DNA binding protein putative expressed	
CUST_4351_PI390587928	1.107	0.629	1.244	0.694	1.304	0.723	1.214	0.741	secondary cell wall-related glycosyltransferase family 47 putative expressed	AT5G16890.1
CUST_20752_PI390587928	1.250	0.902	1.143	0.818	1.209	0.806	1.233	0.697	expressed protein	
CUST_36973_PI390587928	1.517	0.555	2.138	0.650	2.218	0.591	2.290	0.649	peptide transporter PTR2 putative expressed	AT4G21680.1
CUST_9174_PI390587928	2.475	0.587	2.184	0.411	1.841	0.486	1.443	0.289	14-dihydroxy-2-naphthoate octaprenyltransferase putative expressed	AT1G60600.2
CUST_36196_PI390587928	1.250	0.576	1.154	0.520	1.318	0.570	1.213	0.460	SFR2 putative expressed	AT3G06510.2
CUST_14728_PI390587928	1.557	0.729	1.648	0.746	1.730	0.698	1.479	0.580	chaperone protein dnaJ putative expressed	AT5G16650.1
CUST_35642_PI390587928	1.152	0.767	1.345	0.843	1.366	0.854	1.276	0.742	expressed protein	AT2G35800.1
CUST_37095_PI390587928	0.996	0.776	1.198	0.825	1.227	0.714	1.216	0.743	nucleic acid binding protein putative expressed	AT4G26480.1
CUST_31655_PI390587928	1.425	0.633	1.430	0.596	1.297	0.674	1.311	0.538	-	
CUST_32677_PI390587928	1.226	0.814	1.211	0.811	1.318	0.789	1.233	0.744	CCR4-NOT transcription complex subunit 3 putative expressed	AT5G18230.2
CUST_21512_PI390587928	1.349	0.931	1.299	0.801	1.378	0.795	1.202	0.685	pyruvate dehydrogenase E1 component alpha subunit putative expressed	AT1G01090.1
CUST_41659_PI390587928	2.592	0.663	2.692	0.748	1.238	0.427	1.783	0.548	ATFP4 putative expressed	AT4G05030.1
CUST_34768_PI390587928	1.287	0.769	1.422	0.707	1.279	0.667	1.156	0.528	-	
CUST_14419_PI390587928	1.452	0.608	1.569	0.588	1.393	0.576	1.230	0.515	GTP-binding protein LepA containing protein expressed	AT5G08650.1
CUST_12174_PI390587928	1.185	0.570	1.188	0.746	1.352	0.818	1.293	0.644	-	
CUST_4340_PI390587928	1.176	0.729	1.125	0.713	1.319	0.799	1.174	0.704	receptor protein kinase TMK1 precursor putative expressed	AT3G23750.1
CUST_5346_PI390587928	1.179	0.890	1.230	0.783	1.235	0.780	1.103	0.785	expressed protein	AT4G30200.3
CUST_24641_PI390587928	1.129	0.745	1.159	0.706	1.156	0.722	1.085	0.735	casein kinase II subunit beta-4 putative expressed	AT5G47080.1
CUST_7607_PI390587928	1.220	0.450	1.164	0.448	1.250	0.520	1.216	0.395	GDP-mannose 35-epimerase 2 putative expressed	AT5G28840.1
CUST_15473_PI390587928	1.357	0.773	1.182	0.600	1.638	0.891	1.589	0.755	-	
CUST_25477_PI390587928	1.282	0.609	1.214	0.482	1.205	0.539	1.220	0.519	expressed protein	
CUST_19305_PI390587928	1.447	0.524	1.348	0.693	1.432	0.714	1.167	0.448	-	
CUST_23247_PI390587928	1.107	0.742	1.213	0.760	1.192	0.733	1.195	0.813	exostosin-like putative expressed	AT2G35100.1
CUST_28272_PI390587928	1.574	0.592	1.420	0.655	1.273	0.630	1.270	0.382	glycyl-tRNA synthetase 2 chloroplast/mitochondrial precursor putative expressed	AT3G48110.1
CUST_30031_PI390587928	1.219	0.861	1.149	0.745	1.163	0.844	1.191	0.726	stachyose synthase precursor putative expressed	AT3G57520.1
CUST_25338_PI390587928	1.162	0.618	1.293	0.642	1.397	0.764	1.210	0.604	ATP binding protein putative	AT4G23180.1
CUST_39531_PI390587928	1.460	0.588	1.821	0.424	1.593	0.641	1.522	0.497	-	
CUST_16963_PI390587928	1.451	0.681	1.392	0.596	1.577	0.519	1.413	0.628	hydroxymethylglutaryl-CoA lyase mitochondrial precursor putative expressed	AT2G26800.2
CUST_2088_PI390587928	1.190	0.691	1.321	0.739	1.950	0.635	1.702	0.462	bZIP protein putative expressed	
CUST_32106_PI390587928	1.248	0.683	1.168	0.672	1.299	0.711	1.251	0.668	cytochrome c oxidase assembly protein ctaG putative expressed	AT1G02410.1
CUST_37131_PI390587928	1.293	0.735	1.251	0.759	1.306	0.819	1.323	0.774	peroxin Pex14 putative expressed	AT5G62810.1
CUST_16808_PI390587928	1.135	0.845	1.257	0.827	1.131	0.746	1.132	0.852	expressed protein	
CUST_15009_PI390587928	1.196	0.776	1.165	0.702	1.198	0.791	1.142	0.754	expressed protein	AT2G01110.1
CUST_9374_PI390587928	1.291	0.812	1.219	0.706	1.418	0.900	1.356	0.890	serine/threonine-protein kinase 12 putative expressed	AT2G45490.1
CUST_2151_PI390587928	1.246	0.688	1.215	0.514	1.530	0.812	1.359	0.606	glutathione transferase putative expressed	AT5G44000.1
CUST_15041_PI390587928	1.369	0.692	1.491	0.653	1.602	0.703	1.400	0.646	transcription regulator putative expressed	AT2G13690.1
CUST_41680_PI390587928	1.897	0.433	1.454	0.426	1.353	0.312	1.455	0.270	geranylgeranyl hydrogenase putative expressed	AT1G74470.1
CUST_11629_PI390587928	1.206	0.818	1.256	0.739	1.372	0.866	1.224	0.867	ZAC putative expressed	AT4G21160.4

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_34804_PI390587928	1.327	0.431	1.221	0.351	1.421	0.431	1.222	0.344	-	
CUST_14928_PI390587928	1.397	0.533	1.780	0.409	1.548	0.618	1.479	0.460	ribulose bisphosphate carboxylase/oxygenase activase chloroplast precursor putative expressed	AT2G39730.3
CUST_27089_PI390587928	1.616	0.633	1.589	0.504	1.357	0.597	1.190	0.461	MAR binding filament-like protein 1 putative expressed	AT3G16000.1
CUST_12714_PI390587928	1.265	0.625	1.257	0.616	1.374	0.783	1.383	0.696	-	
CUST_7612_PI390587928	1.632	0.624	1.582	0.369	1.457	0.630	1.543	0.521	4-nitrophenylphosphatase putative expressed	AT5G36790.2
CUST_12730_PI390587928	1.621	0.642	1.365	0.594	1.697	0.886	1.621	0.619	-	
CUST_12528_PI390587928	4.124	0.858	2.267	0.317	3.140	0.496	1.562	0.160	early nodulin 93 putative expressed	
CUST_33426_PI390587928	1.696	0.478	1.447	0.348	1.623	0.449	1.350	0.345	digalactosyldiacylglycerol synthase 2 putative expressed	
CUST_29957_PI390587928	1.263	0.677	1.211	0.662	1.481	0.877	1.360	0.755	ulp1 protease family C-terminal catalytic domain containing protein	
CUST_18575_PI390587928	1.463	0.634	1.285	0.422	1.352	0.832	1.388	0.557	serine/threonine-protein kinase SNT7 chloroplast precursor putative expressed	AT5G01920.1
CUST_8968_PI390587928	1.248	0.775	1.388	0.786	1.402	0.731	1.274	0.772	-	
CUST_38769_PI390587928	2.020	0.704	2.164	0.551	1.586	0.341	1.161	0.270	purple acid phosphatase precursor putative expressed	AT2G27190.1
CUST_11166_PI390587928	1.200	0.705	1.279	0.594	1.269	0.658	1.144	0.616	peptide chain release factor 2 putative expressed	AT5G36170.2
CUST_33303_PI390587928	1.328	0.835	1.193	0.682	1.644	0.712	1.355	0.628	-	
CUST_28595_PI390587928	1.257	0.797	1.124	0.755	1.248	0.774	1.170	0.713	F-box domain containing protein expressed	
CUST_34325_PI390587928	1.241	0.646	1.156	0.567	1.353	0.649	1.233	0.538	F-box domain containing protein expressed	
CUST_6568_PI390587928	1.900	0.668	1.654	0.747	2.009	0.747	1.952	0.574	methylase putative expressed	
CUST_14481_PI390587928	1.799	0.670	1.593	0.541	1.886	0.559	1.455	0.454	alphaalpha-trehalose-phosphate synthase putative expressed	AT1G78580.1
CUST_5407_PI390587928	1.657	0.997	1.233	0.664	1.387	0.876	1.227	0.685	metalloendopeptidase putative expressed	AT5G05740.2
CUST_13273_PI390587928	1.574	0.819	1.535	0.698	1.617	0.741	1.315	0.504	major facilitator superfamily antiporter putative expressed	AT5G13740.1
CUST_24012_PI390587928	1.325	0.772	1.147	0.712	1.136	0.623	1.166	0.579	pentatricopeptide repeat protein PPR868-14 putative expressed	AT5G44230.1
CUST_29509_PI390587928	1.756	0.620	1.394	0.662	1.623	0.797	1.623	0.522	expressed protein	
CUST_4215_PI390587928	1.368	0.732	1.245	0.557	1.370	0.726	1.399	0.617	peptidyl-prolyl cis-trans isomerase CYP37 chloroplast precursor putative expressed	
CUST_17121_PI390587928	1.244	0.788	1.356	0.866	1.248	0.809	1.107	0.726	cadmium-induced protein putative expressed	AT4G19070.1
CUST_27311_PI390587928	1.337	0.751	1.456	0.624	1.423	0.654	1.209	0.626	ethylene-responsive element binding protein 2 putative expressed	AT1G53910.3
CUST_18143_PI390587928	1.876	0.590	1.505	0.391	1.741	0.522	1.926	0.510	microtubule-associated protein MAP65-1a putative expressed	AT2G01910.1
CUST_1857_PI390587928	1.441	0.513	1.475	0.472	1.626	0.517	1.492	0.563	dehydrodolichyl diphosphate synthase 6 putative expressed	AT2G17570.1
CUST_5300_PI390587928	1.328	0.732	1.337	0.649	1.466	0.725	1.205	0.614	PPR2 putative expressed	AT3G06430.1
CUST_2926_PI390587928	1.258	0.783	1.283	0.782	1.332	0.812	1.227	0.790	casein kinase I isoform delta-like putative expressed	AT1G72710.1
CUST_1687_PI390587928	1.234	0.690	1.163	0.652	1.277	0.764	1.333	0.753	zinc finger protein putative expressed	AT5G20220.2
CUST_24767_PI390587928	1.358	0.670	1.276	0.506	1.416	0.753	1.337	0.535	hypothetical protein	
CUST_36730_PI390587928	1.178	0.781	1.167	0.579	1.099	0.655	1.073	0.672	formamidopyrimidine-DNA glycosylase putative expressed	
CUST_29792_PI390587928	1.585	0.657	1.528	0.615	1.079	0.643	1.234	0.543	cytochrome P450 97B3 putative expressed	AT1G31800.1
CUST_34973_PI390587928	1.372	0.930	1.338	0.848	1.308	0.891	1.255	0.855	acyl-desaturase chloroplast precursor putative expressed	AT2G43710.1
CUST_25852_PI390587928	1.235	0.702	1.297	0.662	1.360	0.615	1.320	0.752	ATP/GTP-binding protein putative expressed	AT4G21210.1
CUST_40517_PI390587928	1.334	0.829	1.550	0.828	1.348	0.770	1.136	0.672	expressed protein	AT5G49710.3
CUST_37022_PI390587928	1.223	0.812	1.193	0.787	1.151	0.741	1.137	0.804	DNA cytosine methyltransferase MET2a putative expressed	
CUST_3841_PI390587928	1.140	0.657	1.304	0.598	1.474	0.712	1.439	0.626	expressed protein	AT2G33550.1
CUST_1611_PI390587928	1.235	0.692	1.222	0.550	1.242	0.677	1.125	0.573	expressed protein	AT1G71460.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_41571_PI390587928	1.120	0.762	1.114	0.695	1.237	0.756	1.218	0.692	-	
CUST_26688_PI390587928	1.380	0.495	1.507	0.416	1.443	0.527	1.400	0.417	-	
CUST_36894_PI390587928	1.325	0.726	1.137	0.448	1.223	0.693	1.239	0.682	heat shock protein binding protein putative expressed	AT4G09350.1
CUST_19308_PI390587928	1.496	0.566	1.234	0.491	1.425	0.456	1.294	0.419	zinc ribbon 1 putative	
CUST_3655_PI390587928	1.478	0.557	1.564	0.396	1.695	0.629	1.481	0.595	dihydrolipoyl dehydrogenase mitochondrial precursor putative expressed	AT1G48030.2
CUST_16545_PI390587928	1.326	0.863	1.312	0.732	1.455	0.858	1.367	0.789	lycopene beta cyclase chloroplast precursor putative expressed	AT3G10230.2
CUST_10328_PI390587928	1.410	0.840	1.577	0.647	1.576	0.721	1.517	0.642	-	
CUST_4220_PI390587928	1.609	0.869	1.178	0.675	1.311	0.799	1.424	0.685	uroporphyrinogen-III synthase putative expressed	AT2G26540.1
CUST_3702_PI390587928	1.140	0.581	1.219	0.511	1.391	0.638	1.184	0.501	cyclin-like F-box putative expressed	AT2G26850.1
CUST_4538_PI390587928	1.400	0.437	1.478	0.320	1.472	0.494	1.593	0.325	oxygen-evolving enhancer protein 3-1 chloroplast precursor putative expressed	AT4G21280.1
CUST_3299_PI390587928	1.123	0.727	1.121	0.726	1.098	0.669	1.133	0.679	expressed protein	
CUST_11196_PI390587928	1.116	0.757	1.178	0.827	1.170	0.702	1.109	0.705	F-box domain containing protein expressed	
CUST_37118_PI390587928	2.445	0.423	2.291	0.260	2.436	0.215	2.255	0.133	1-aminocyclopropane-1-carboxylate oxidase 1 putative expressed	AT1G05010.1
CUST_19015_PI390587928	1.469	0.539	1.227	0.610	1.458	0.813	1.356	0.507	-	
CUST_323_PI390587928	1.438	0.699	1.233	0.654	1.358	0.595	1.093	0.501	-	
CUST_39178_PI390587928	1.416	0.576	1.262	0.358	1.502	0.570	1.304	0.351	-	AT3G49170.1
CUST_2201_PI390587928	1.222	0.755	1.181	0.760	1.290	0.792	1.234	0.735	auxin response factor 2 putative expressed	AT5G62000.3
CUST_39225_PI390587928	1.431	0.653	1.378	0.365	1.495	0.497	1.347	0.472	protein phosphatase 2C homolog 7 putative expressed	AT2G30170.1
CUST_8232_PI390587928	1.355	0.631	1.577	0.757	1.359	0.499	1.409	0.603	expressed protein	AT5G51920.1
CUST_20662_PI390587928	1.583	0.663	1.515	0.673	1.747	0.765	1.613	0.721	expressed protein	AT5G04460.1
CUST_12733_PI390587928	1.908	0.691	1.899	0.513	1.594	0.539	1.263	0.394	-	
CUST_21731_PI390587928	1.194	0.704	1.352	0.735	1.286	0.712	1.231	0.648	O-sialoglycoprotein endopeptidase putative expressed	AT2G45270.1
CUST_31921_PI390587928	1.633	0.665	1.553	0.554	1.620	0.581	1.247	0.499	palmitoyl-protein thioesterase 1 precursor putative expressed	
CUST_37936_PI390587928	1.167	0.827	1.174	0.685	1.200	0.793	1.218	0.760	cyclin delta-2 putative expressed	AT2G22490.1
CUST_25989_PI390587928	1.492	0.513	1.332	0.367	1.433	0.498	1.200	0.326	-	
CUST_10333_PI390587928	1.331	0.752	1.271	0.772	1.360	0.780	1.344	0.732	-	
CUST_29199_PI390587928	1.236	0.942	1.150	0.837	1.192	0.809	1.324	0.896	expressed protein	AT5G12470.1
CUST_6943_PI390587928	1.505	0.855	1.312	0.595	1.333	0.722	1.404	0.649	-	AT1G73100.1
CUST_13585_PI390587928	1.149	0.295	1.432	0.182	2.324	0.230	2.642	0.208	abscisic stress ripening protein 2 putative expressed	
CUST_30496_PI390587928	1.251	0.870	1.202	0.807	1.226	0.798	1.168	0.720	expressed protein	
CUST_16884_PI390587928	1.853	0.259	2.353	0.302	1.775	0.299	1.627	0.177	-	
CUST_31581_PI390587928	5.821	0.363	3.167	0.076	4.138	0.159	2.852	0.017	cysteine proteinase EP-B 1 precursor putative expressed	AT2G27420.1
CUST_40547_PI390587928	1.399	0.470	1.439	0.457	1.477	0.485	1.314	0.393	expressed protein	
CUST_9413_PI390587928	1.830	0.435	1.531	0.317	1.672	0.366	1.590	0.200	expressed protein	AT4G01150.1
CUST_36419_PI390587928	1.532	0.658	1.465	0.500	1.331	0.561	1.169	0.488	expressed protein	AT1G70200.1
CUST_6650_PI390587928	1.091	0.588	1.161	0.653	1.271	0.744	1.068	0.546	-	
CUST_34190_PI390587928	1.922	0.674	1.175	0.603	1.593	0.499	1.395	0.456	-	
CUST_19338_PI390587928	1.547	0.591	1.747	0.549	1.529	0.438	1.484	0.446	TPR-containing protein kinase putative expressed	AT5G59010.1
CUST_40408_PI390587928	2.033	0.881	1.570	0.598	1.402	0.565	1.503	0.647	F-box domain containing protein expressed	
CUST_24519_PI390587928	2.069	0.823	1.610	0.447	1.625	0.663	1.470	0.457	50S ribosomal protein L17 putative expressed	AT3G54210.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_17108_PI390587928	1.684	0.579	1.756	0.450	1.584	0.696	1.508	0.517	RNA binding S1 putative expressed	AT3G23700.1
CUST_13930_PI390587928	1.360	0.563	1.364	0.429	1.692	0.687	1.198	0.401	xyloglucan endotransglucosylase/hydrolase protein 30 precursor putative expressed	
CUST_37287_PI390587928	1.438	0.490	1.315	0.405	1.674	0.716	1.551	0.489	small nuclear ribonucleoprotein Sm D3 putative expressed	AT1G20580.1
CUST_28352_PI390587928	1.355	0.691	1.205	0.598	1.520	0.590	1.546	0.448	-	
CUST_27128_PI390587928	1.393	0.669	1.455	0.582	1.364	0.530	1.210	0.522	katanin p80 WD40-containing subunit B1 homolog 1 putative expressed	AT5G08390.1
CUST_9104_PI390587928	2.694	0.366	1.849	0.202	2.490	0.367	1.927	0.213	tubby protein putative expressed	AT2G47900.2
CUST_28166_PI390587928	2.477	0.467	1.713	0.266	1.761	0.500	1.690	0.367	-	
CUST_28182_PI390587928	1.673	0.226	1.757	0.343	2.634	0.118	2.285	0.127	phosphate transporter 1 putative expressed	AT1G68740.1
CUST_14813_PI390587928	1.226	0.796	1.269	0.749	1.223	0.849	1.225	0.780	protease Do-like 1 chloroplast precursor putative expressed	AT3G27925.1
CUST_15260_PI390587928	1.315	0.444	1.338	0.358	1.536	0.426	1.264	0.411	-	
CUST_1162_PI390587928	1.271	0.741	1.316	0.693	1.360	0.680	1.230	0.629	XAP-5 protein putative expressed	AT2G21150.1
CUST_928_PI390587928	1.324	0.496	1.349	0.495	1.522	0.606	1.442	0.485	toc64 putative expressed	
CUST_30989_PI390587928	1.459	0.704	1.664	0.648	1.682	0.758	1.795	0.695	MYB59 putative expressed	AT5G59780.3
CUST_16873_PI390587928	1.126	0.780	1.208	0.632	1.246	0.760	1.178	0.723	meiotic recombination protein SPO11 putative expressed	AT1G63990.1
CUST_17879_PI390587928	1.354	0.726	1.250	0.577	1.334	0.725	1.140	0.651	-	
CUST_37211_PI390587928	1.702	0.613	1.416	0.408	1.442	0.472	1.465	0.379	peroxiredoxin-5 mitochondrial precursor putative expressed	AT3G52960.1
CUST_38808_PI390587928	1.447	0.724	1.411	0.726	1.463	0.737	1.317	0.622	U1 snRNP 70K protein putative expressed	AT3G50670.1
CUST_25871_PI390587928	1.388	0.819	1.402	0.763	1.348	0.780	1.273	0.680	expressed protein	
CUST_13939_PI390587928	1.136	0.795	1.300	0.835	1.298	0.826	1.143	0.892	amino acid transporter-like protein putative expressed	AT3G30390.2
CUST_774_PI390587928	2.405	0.346	1.986	0.291	2.078	0.358	1.696	0.286	-	
CUST_22937_PI390587928	1.120	0.716	1.388	0.873	1.391	0.851	1.275	0.719	expressed protein	
CUST_41605_PI390587928	1.183	0.616	1.126	0.500	1.242	0.593	1.273	0.527	anthranilate synthase component II putative expressed	
CUST_9098_PI390587928	1.377	0.916	1.359	0.708	1.523	0.781	1.504	0.798	lipase putative expressed	AT1G10740.1
CUST_7661_PI390587928	1.348	0.739	1.321	0.688	1.842	0.689	1.487	0.666	vacuolar cation/proton exchanger 1a putative expressed	AT3G51860.1
CUST_11571_PI390587928	1.582	0.848	1.481	0.717	1.399	0.881	1.205	0.632	esterase/lipase/thioesterase family active site protein putative expressed	AT5G17670.1
CUST_10208_PI390587928	1.247	0.860	1.217	0.686	1.283	0.828	1.219	0.780	YDA putative expressed	AT1G63700.1
CUST_30573_PI390587928	1.826	0.614	1.573	0.404	1.353	0.473	1.247	0.361	GTP binding protein putative expressed	AT1G56050.1
CUST_24168_PI390587928	1.699	0.651	1.619	0.616	1.538	0.572	1.240	0.488	expressed protein	
CUST_18469_PI390587928	1.615	0.614	1.319	0.403	1.215	0.572	1.173	0.353	-	
CUST_1119_PI390587928	1.917	0.505	1.712	0.271	1.892	0.615	2.032	0.428	hydroxyacid oxidase 1 putative expressed	AT3G14130.1
CUST_10224_PI390587928	1.226	0.527	1.283	0.616	1.147	0.526	1.198	0.511	expressed protein	
CUST_35613_PI390587928	1.354	0.773	1.347	0.653	1.353	0.648	1.507	0.683	expressed protein	AT4G23440.1
CUST_37657_PI390587928	1.696	0.500	1.769	0.463	1.661	0.444	1.735	0.339	photosystem II D2 protein putative	ATCG00280.1
CUST_21266_PI390587928	1.465	0.722	1.453	0.636	1.547	0.633	1.367	0.562	ATP binding protein putative expressed	AT5G58300.2
CUST_18346_PI390587928	1.380	0.715	1.199	0.669	1.457	0.806	1.503	0.655	tRNA modification GTPase trmE putative expressed	
CUST_11900_PI390587928	1.248	0.756	1.250	0.826	1.324	0.708	1.123	0.569	inositol 145-trisphosphate 5-phosphatase putative expressed	AT2G43900.1
CUST_2018_PI390587928	1.216	0.740	1.391	0.708	1.349	0.745	1.215	0.662	DNA polymerase family B exonuclease domain containing protein expressed	
CUST_39260_PI390587928	1.470	0.469	1.524	0.383	1.390	0.385	1.376	0.300	serine/threonine kinase-like protein putative expressed	AT5G48540.1
CUST_6021_PI390587928	1.295	0.479	1.361	0.426	1.885	0.614	1.463	0.391	peroxidase 1 precursor putative expressed	AT1G05260.1
CUST_6494_PI390587928	1.319	0.714	1.363	0.687	1.384	0.739	1.209	0.689	light-mediated development protein DET1 putative expressed	AT4G10180.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_27344_PI390587928	1.282	0.922	1.131	0.836	1.268	0.899	1.258	0.798	-	
CUST_30855_PI390587928	1.385	0.646	1.506	0.566	1.453	0.667	1.338	0.562	elongation factor Tu chloroplast precursor putative expressed	AT4G20360.1
CUST_8065_PI390587928	1.293	0.794	1.252	0.496	1.385	0.586	1.287	0.450	expressed protein	AT1G79770.1
CUST_13758_PI390587928	1.152	0.772	1.274	0.640	1.455	0.602	1.487	0.784	rapid alkalinization factor 1 precursor putative expressed	AT3G16570.1
CUST_5940_PI390587928	1.226	0.837	1.186	0.781	1.378	0.785	1.296	0.938	expressed protein	AT3G27460.2
CUST_33968_PI390587928	1.200	0.629	1.255	0.620	1.297	0.718	1.251	0.633	3-5 exonuclease eri-1 putative expressed	AT1G04980.1
CUST_2975_PI390587928	1.627	0.751	1.248	0.652	1.268	0.669	1.322	0.548	ATNAP8 putative expressed	AT4G25450.1
CUST_37209_PI390587928	1.387	0.645	1.355	0.795	1.503	0.673	1.385	0.647	expressed protein	AT2G36835.1
CUST_15912_PI390587928	1.243	0.524	1.327	0.436	1.594	0.674	1.438	0.496	expressed protein	
CUST_25869_PI390587928	1.651	0.694	1.381	0.747	1.296	0.734	1.467	0.635	cytochrome P450 97B2 putative expressed	AT4G15110.1
CUST_16764_PI390587928	1.506	0.734	1.468	0.775	1.294	0.864	1.501	0.593	phospholipase A1 putative expressed	
CUST_24724_PI390587928	1.283	0.885	1.283	0.837	1.320	0.867	1.300	0.850	protein SPATULA putative expressed	AT1G59640.1
CUST_4927_PI390587928	1.375	0.577	1.548	0.485	1.604	0.451	1.678	0.317	protein binding protein putative expressed	ATCG00490.1
CUST_33691_PI390587928	1.238	0.767	1.362	0.764	1.231	0.799	1.260	0.646	expressed protein	AT1G33290.2
CUST_10563_PI390587928	1.658	0.774	1.368	0.627	1.688	0.848	1.504	0.759	adenylate kinase A putative expressed	AT5G63400.1
CUST_41465_PI390587928	1.207	0.540	1.253	0.368	1.595	0.549	1.444	0.495	transaldolase putative expressed	AT1G12230.1
CUST_19404_PI390587928	1.248	0.765	1.393	0.886	1.034	0.617	1.220	0.901	riboflavin biosynthesis protein ribAB chloroplast precursor putative expressed	AT5G64300.1
CUST_2527_PI390587928	1.311	0.675	1.180	0.734	1.368	0.820	1.304	0.653	-	
CUST_22138_PI390587928	1.471	0.836	1.153	0.669	1.227	0.690	1.115	0.523	mTERF family protein expressed	AT5G55580.1
CUST_40273_PI390587928	1.270	0.651	1.305	0.773	1.314	0.687	1.170	0.566	retrotransposon protein putative unclassified	
CUST_32360_PI390587928	1.446	0.424	1.706	0.291	1.632	0.336	1.476	0.308	-	ATCG00300.1
CUST_7382_PI390587928	1.303	0.586	1.532	0.598	1.596	0.824	1.344	0.663	expressed protein	AT4G17240.1
CUST_27210_PI390587928	1.412	0.522	1.318	0.392	1.440	0.481	1.312	0.344	expressed protein	
CUST_10829_PI390587928	1.440	0.319	1.346	0.252	1.499	0.277	1.574	0.266	-	
CUST_12298_PI390587928	1.133	0.762	1.085	0.629	1.144	0.711	1.183	0.686	stress regulated protein putative expressed	AT5G27290.1
CUST_4448_PI390587928	1.533	0.554	1.401	0.454	1.494	0.463	1.507	0.433	expressed protein	AT1G59840.2
CUST_35473_PI390587928	1.513	0.463	1.618	0.352	1.920	0.541	1.801	0.307	-	AT2G40410.2
CUST_29301_PI390587928	1.366	0.478	1.550	0.567	1.643	0.695	1.324	0.603	phosphoglycerate kinase cytosolic putative expressed	
CUST_10115_PI390587928	1.650	0.283	1.429	0.299	1.990	0.254	1.538	0.197	-	
CUST_11930_PI390587928	2.314	0.847	1.979	0.615	1.512	0.578	1.356	0.520	-	
CUST_10131_PI390587928	1.171	0.756	1.239	0.667	1.164	0.682	1.098	0.698	expressed protein	AT3G58010.1
CUST_33834_PI390587928	1.244	0.698	1.097	0.783	1.329	0.726	1.237	0.811	N-rich protein putative expressed	AT3G27090.1
CUST_42225_PI390587928	1.472	0.647	1.451	0.542	1.928	0.582	1.488	0.429	senescence-associated protein-like putative expressed	AT3G12090.1
CUST_29131_PI390587928	1.138	0.815	1.195	0.757	1.249	0.820	1.107	0.745	WD-repeat protein-like putative expressed	AT3G13340.1
CUST_13974_PI390587928	1.430	0.686	1.366	0.749	1.130	0.581	1.255	0.614	AIR9 putative expressed	AT2G34680.1
CUST_3117_PI390587928	1.284	0.759	1.305	0.642	1.358	0.726	1.353	0.795	lipoate-protein ligase putative expressed	AT1G04640.2
CUST_7120_PI390587928	1.172	0.647	1.083	0.565	1.359	0.701	1.332	0.598	-	
CUST_17029_PI390587928	1.724	0.795	1.669	0.640	1.672	0.814	1.437	0.731	naphthoate synthase putative expressed	AT1G60550.1
CUST_21594_PI390587928	1.279	0.602	1.017	0.512	1.318	0.595	1.269	0.576	alpha-soluble NSF attachment protein putative expressed	
CUST_8003_PI390587928	1.367	0.567	1.293	0.577	1.403	0.678	1.237	0.478	RNA polymerase sigma factor rpoD putative expressed	AT1G64860.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_4576_PI390587928	1.261	0.848	1.130	0.630	1.321	0.803	1.175	0.815	thiamin pyrophosphokinase 1 putative expressed	AT1G02880.3
CUST_5831_PI390587928	1.294	0.650	1.298	0.510	1.301	0.594	1.134	0.545	CRS2-associated factor 1 putative expressed	AT2G20020.1
CUST_29917_PI390587928	1.680	0.579	1.749	0.509	1.451	0.470	1.554	0.489	epoxide hydrolase 2 putative expressed	AT3G51000.1
CUST_25442_PI390587928	1.158	0.856	1.170	0.819	1.312	0.894	1.146	0.834	RING-H2 finger protein ATL5D putative expressed	AT4G10160.1
CUST_33402_PI390587928	1.749	0.534	1.734	0.495	1.414	0.306	1.402	0.294	protein SUR2 putative expressed	
CUST_38884_PI390587928	1.324	0.878	1.090	0.921	1.402	0.818	1.324	0.818	NADPH oxidase putative expressed	AT1G64060.1
CUST_38915_PI390587928	1.373	0.406	1.430	0.442	1.498	0.576	1.341	0.471	phosphate transporter 1 putative expressed	AT3G23430.1
CUST_8440_PI390587928	1.703	0.582	1.902	0.454	2.629	0.413	2.368	0.286	tonoplast dicarboxylate transporter putative expressed	AT5G47560.1
CUST_10073_PI390587928	1.297	0.740	1.390	0.590	1.598	0.732	1.292	0.554	ATP binding protein putative expressed	AT5G28680.1
CUST_37692_PI390587928	1.124	0.634	1.254	0.570	1.955	0.828	1.654	0.568	PHD-finger family protein expressed	AT3G14740.2
CUST_11095_PI390587928	1.241	0.828	1.192	0.609	1.391	0.772	1.330	0.744	tricarboxylate transport protein mitochondrial precursor putative expressed	AT5G01340.1
CUST_3261_PI390587928	1.341	0.616	1.458	0.546	1.343	0.518	1.192	0.587	sucrose transport protein SUC4 putative expressed	AT1G09960.1
CUST_6528_PI390587928	1.116	0.694	1.072	0.728	1.202	0.789	1.232	0.833	protein kinase APK1B chloroplast precursor putative expressed	AT2G28930.3
CUST_4283_PI390587928	1.646	0.721	1.482	0.561	1.942	0.924	1.751	0.711	NAD(P)H-dependent oxidoreductase putative expressed	AT1G59950.1
CUST_18179_PI390587928	1.486	0.590	1.326	0.355	1.353	0.560	1.329	0.438	PAP fibrillin family protein expressed	
CUST_38559_PI390587928	1.411	0.694	1.495	0.673	1.373	0.583	1.263	0.575	preprotein translocase secA subunit chloroplast precursor putative expressed	AT4G01800.1
CUST_13186_PI390587928	1.327	0.788	1.335	0.828	1.363	0.910	1.259	0.731	-	AT2G13440.1
CUST_41495_PI390587928	1.591	0.702	1.477	0.478	1.356	0.684	1.157	0.467	50S ribosomal protein L1 putative expressed	AT3G63490.1
CUST_40504_PI390587928	1.242	0.832	1.425	0.812	1.302	0.781	1.234	0.779	protein kinase domain containing protein expressed	AT5G57610.1
CUST_23376_PI390587928	1.863	0.417	1.772	0.354	3.531	0.543	2.999	0.459	-	
CUST_15463_PI390587928	2.287	0.741	2.074	0.528	1.768	0.469	1.395	0.295	-	
CUST_30921_PI390587928	1.058	0.642	1.318	0.617	1.252	0.701	1.208	0.655	ubiquitin-conjugating enzyme E2 variant 1 putative expressed	AT3G52560.1
CUST_8131_PI390587928	1.664	0.733	1.559	0.481	1.421	0.618	1.275	0.439	ruBisCO large subunit-binding protein subunit alpha chloroplast precursor putative expressed	AT2G28000.1
CUST_40318_PI390587928	1.822	0.615	1.631	0.487	1.467	0.483	1.523	0.451	thylakoid lumen protein chloroplast precursor putative expressed	AT1G76450.1
CUST_39628_PI390587928	2.151	0.627	2.000	0.687	1.691	0.511	1.453	0.497	amino acid carrier putative expressed	AT5G09220.1
CUST_36159_PI390587928	2.112	0.443	1.735	0.371	1.527	0.422	1.407	0.260	FAD dependent oxidoreductase putative expressed	
CUST_6390_PI390587928	1.280	0.645	1.299	0.552	1.192	0.569	1.097	0.526	ATP binding protein putative expressed	AT3G58140.1
CUST_31182_PI390587928	1.342	0.608	1.361	0.552	1.486	0.545	1.438	0.607	serine-rich protein putative expressed	
CUST_5182_PI390587928	1.274	0.558	1.181	0.610	1.387	0.704	1.207	0.517	expressed protein	
CUST_19078_PI390587928	1.936	0.536	1.359	0.277	1.588	0.501	1.538	0.300	catalytic/ hydrolase putative expressed	AT3G10840.1
CUST_386_PI390587928	1.430	0.546	1.411	0.512	1.358	0.416	1.260	0.352	ATP binding protein putative expressed	AT2G40120.1
CUST_5027_PI390587928	1.475	0.683	1.740	0.658	1.273	0.633	1.152	0.469	subtilisin-like protease precursor putative expressed	AT5G67360.1
CUST_27979_PI390587928	1.446	0.785	1.384	0.770	1.257	0.721	1.226	0.664	-	AT1G54520.1
CUST_8933_PI390587928	1.203	0.712	1.232	0.745	1.290	0.759	1.135	0.633	-	
CUST_10582_PI390587928	1.347	0.595	1.554	0.534	1.827	0.702	1.670	0.559	-	
CUST_36520_PI390587928	1.635	0.457	1.726	0.350	2.534	0.573	1.916	0.401	expressed protein	
CUST_20803_PI390587928	1.490	0.624	1.467	0.454	1.414	0.562	1.129	0.392	expressed protein	AT1G55140.1
CUST_39772_PI390587928	1.559	0.545	1.408	0.260	1.375	0.386	1.418	0.391	expressed protein	AT5G63500.1
CUST_41484_PI390587928	1.364	0.938	1.325	0.843	1.184	0.788	1.109	0.799	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase SEC putative	AT3G04240.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
									expressed	
CUST_3568_PI390587928	1.482	0.524	1.302	0.341	1.529	0.550	1.621	0.413	ubiquitin carboxyl-terminal hydrolase 4 putative expressed	AT2G22310.1
CUST_11465_PI390587928	1.241	0.586	1.446	0.539	1.513	0.503	1.406	0.424	ATP binding protein putative expressed	
CUST_7602_PI390587928	1.418	0.472	1.417	0.491	1.826	0.504	1.922	0.406	carbonic anhydrase chloroplast precursor putative expressed	AT5G14740.2
CUST_27686_PI390587928	1.130	0.843	1.130	0.775	1.127	0.740	1.080	0.783	diacylglycerol kinase putative expressed	AT4G30340.1
CUST_12518_PI390587928	4.318	0.682	2.377	0.260	3.370	0.373	1.826	0.082	early nodulin 93 putative expressed	
CUST_16521_PI390587928	1.281	0.660	1.272	0.743	1.411	0.853	1.427	0.661	expressed protein	AT2G26760.1
CUST_41361_PI390587928	1.141	0.826	1.176	0.783	1.333	0.793	1.169	0.774	zinc finger CCCH-type with G patch domain protein putative expressed	AT2G24830.1
CUST_33246_PI390587928	1.176	0.728	1.152	0.678	1.214	0.707	1.138	0.726	expressed protein	AT5G51020.1
CUST_189_PI390587928	1.703	0.766	1.505	0.700	1.286	0.720	1.207	0.768	indole-3-acetic acid-amido synthetase GH3.5 putative expressed	AT2G46370.3
CUST_21937_PI390587928	1.547	0.568	1.350	0.338	1.454	0.503	1.271	0.336	50S ribosomal protein L15 chloroplast precursor putative expressed	AT3G25920.1
CUST_22959_PI390587928	1.257	0.804	1.212	0.634	1.532	0.780	1.455	0.709	expressed protein	AT3G55580.1
CUST_8129_PI390587928	1.465	0.890	1.301	0.778	1.270	0.829	1.244	0.742	stromal 70 kDa heat shock-related protein chloroplast precursor putative expressed	AT4G24280.1
CUST_36375_PI390587928	1.090	0.674	1.229	0.765	1.252	0.769	1.182	0.717	-	
CUST_21767_PI390587928	1.484	0.638	1.686	0.597	1.320	0.600	1.140	0.430	UDP-glucose 6-dehydrogenase putative expressed	AT5G15490.1
CUST_26232_PI390587928	1.303	0.619	1.325	0.617	1.421	0.767	1.221	0.598	-	
CUST_4174_PI390587928	1.269	0.487	1.331	0.462	1.171	0.427	1.079	0.368	amino acid binding protein putative expressed	AT2G39570.1
CUST_5196_PI390587928	1.211	0.844	1.377	0.770	1.116	0.619	1.469	0.780	N-acetyl-gamma-glutamyl-phosphate reductase chloroplast precursor putative expressed	AT2G19940.2
CUST_35790_PI390587928	1.244	0.809	1.317	0.822	1.280	0.837	1.208	0.770	protein kinase domain containing protein expressed	AT5G57610.1
CUST_13901_PI390587928	1.379	0.696	1.356	0.756	1.763	0.710	1.418	0.633	ids4-like protein putative expressed	AT2G45130.1
CUST_36252_PI390587928	1.366	0.770	1.359	0.942	1.167	0.762	1.122	0.641	ATTIC110/TIC110 putative expressed	AT1G06950.1
CUST_34613_PI390587928	1.125	0.664	1.379	0.757	1.483	0.807	1.471	0.745	-	AT1G17840.1
CUST_30626_PI390587928	1.734	0.471	2.251	0.349	3.147	0.625	2.383	0.504	MYB59 putative expressed	AT3G46130.1
CUST_19746_PI390587928	1.395	0.801	1.124	0.760	1.137	0.710	1.144	0.621	terminal acidic SANT 1 putative expressed	AT1G36160.1
CUST_26700_PI390587928	1.723	0.887	1.460	0.610	1.478	0.725	1.419	0.597	chloroplast 30S ribosomal protein S10 putative expressed	AT3G13120.1
CUST_31648_PI390587928	1.776	0.522	1.583	0.225	2.361	0.434	2.179	0.360	fasciclin-like arabinogalactan protein 7 precursor putative expressed	AT2G04780.2
CUST_5664_PI390587928	1.389	0.748	1.535	0.668	1.278	0.653	1.213	0.667	expressed protein	AT4G02920.2
CUST_25965_PI390587928	1.189	0.803	1.382	0.837	1.646	0.791	1.400	0.739	-	
CUST_14800_PI390587928	1.083	0.804	1.184	0.732	1.164	0.880	1.099	0.825	expressed protein	AT3G54360.1
CUST_18010_PI390587928	1.192	0.758	1.079	0.730	1.337	0.838	1.328	0.707	rf1 protein mitochondrial precursor putative expressed	AT1G09900.1
CUST_37742_PI390587928	1.089	0.758	1.134	0.634	1.539	0.860	1.478	0.632	PINHEAD protein putative expressed	AT1G48410.2
CUST_28574_PI390587928	1.295	0.671	1.322	0.669	1.305	0.639	1.124	0.673	vesicle-associated membrane protein 712 putative expressed	AT4G32150.1
CUST_36550_PI390587928	1.578	0.891	1.352	0.757	1.532	0.829	1.514	0.703	-	
CUST_26864_PI390587928	1.896	0.775	1.977	0.899	2.532	0.618	2.754	0.651	-	
CUST_31355_PI390587928	1.470	0.503	1.259	0.363	1.246	0.490	1.237	0.354	expressed protein	AT5G44600.1
CUST_3598_PI390587928	2.083	0.624	1.819	0.537	1.650	0.361	1.580	0.341	protein SUR2 putative expressed	
CUST_18261_PI390587928	1.294	0.584	1.269	0.577	1.453	0.725	1.387	0.552	serine/threonine-protein kinase NAK putative expressed	AT5G18610.1
CUST_33399_PI390587928	1.541	0.752	1.458	0.577	1.347	0.637	1.280	0.501	PHD-finger family protein expressed	AT5G23120.1
CUST_34654_PI390587928	1.425	0.774	1.416	0.857	1.436	0.910	1.317	0.788	GATA transcription factor 25 putative expressed	AT3G21175.2
CUST_14321_PI390587928	3.128	0.425	3.612	0.536	2.997	0.527	2.323	0.269	indole-3-acetate beta-glucosyltransferase putative expressed	AT3G02100.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_41189_PI390587928	1.287	0.463	1.205	0.380	1.645	0.513	1.745	0.642	carbonic anhydrase chloroplast precursor putative expressed	AT4G33580.1
CUST_9723_PI390587928	1.719	0.306	1.993	0.186	1.821	0.363	1.750	0.207	carbonic anhydrase chloroplast precursor putative expressed	AT5G14740.2
CUST_16365_PI390587928	1.953	0.310	2.612	0.101	2.140	0.118	1.859	0.109	OsWAK83 - OsWAK pseudogene expressed	AT1G21230.1
CUST_10941_PI390587928	1.308	0.775	1.197	0.726	1.415	0.745	1.300	0.759	-	
CUST_19160_PI390587928	1.551	0.480	1.351	0.399	1.598	0.559	1.542	0.446	-	
CUST_25347_PI390587928	1.175	0.765	1.208	0.823	1.266	0.814	1.137	0.707	-	
CUST_23910_PI390587928	1.769	0.760	1.376	0.561	1.381	0.701	1.322	0.510	preprotein translocase secY subunit chloroplast precursor putative expressed	AT2G18710.1
CUST_12969_PI390587928	1.486	0.646	1.261	0.663	1.782	0.897	1.640	0.733	-	
CUST_31062_PI390587928	1.363	0.653	1.340	0.580	1.364	0.713	1.241	0.517	sialin putative expressed	AT4G00370.1
CUST_15173_PI390587928	1.245	0.677	1.271	0.668	1.362	0.705	1.159	0.625	serine acetyltransferase 3 mitochondrial precursor putative expressed	AT5G56760.1
CUST_42258_PI390587928	1.460	0.582	1.343	0.394	1.528	0.747	1.407	0.690	-	
CUST_20929_PI390587928	1.367	0.842	1.360	0.712	1.200	0.806	1.161	0.710	C-terminal protease precursor putative expressed	AT4G17740.2
CUST_37093_PI390587928	1.150	0.840	1.201	0.765	1.360	0.744	1.282	0.717	nucleic acid binding protein putative expressed	AT5G56140.1
CUST_9414_PI390587928	1.682	0.403	1.433	0.298	1.560	0.339	1.422	0.172	expressed protein	AT4G01150.1
CUST_17280_PI390587928	1.483	0.795	1.452	0.507	1.319	0.614	1.252	0.573	glyoxalase/bleomycin resistance protein/dioxygenase putative expressed	AT5G57040.1
CUST_11047_PI390587928	1.202	0.688	1.180	0.737	1.352	0.643	1.286	0.674	signal transducer putative expressed	
CUST_21797_PI390587928	1.583	0.576	1.292	0.335	1.849	0.560	1.961	0.351	protein binding protein putative expressed	
CUST_29041_PI390587928	1.209	0.682	1.428	0.674	1.361	0.534	1.295	0.442	nitrate reductase 1 putative expressed	AT1G37130.1
CUST_4779_PI390587928	1.155	0.802	1.178	0.759	1.093	0.759	1.173	0.851	SNARE domain containing protein expressed	AT1G79590.2
CUST_38800_PI390587928	1.702	0.322	1.826	0.359	1.848	0.382	1.675	0.320	expressed protein	
CUST_37001_PI390587928	1.324	0.918	1.350	0.847	1.228	0.798	1.116	0.696	pseudouridylate synthase/ transporter putative expressed	AT5G14460.1
CUST_4795_PI390587928	1.440	0.778	1.561	0.772	1.509	0.715	1.355	0.707	metabolite transport protein csbC putative expressed	AT1G05030.1
CUST_39822_PI390587928	1.357	0.772	1.249	0.538	1.295	0.743	1.215	0.622	expressed protein	AT5G62140.1
CUST_38023_PI390587928	1.724	0.554	1.387	0.382	1.425	0.563	1.362	0.393	expressed protein	AT5G48790.1
CUST_27891_PI390587928	1.367	0.632	1.228	0.470	1.500	0.663	1.465	0.554	-	
CUST_19776_PI390587928	1.518	0.742	1.327	0.726	1.534	0.687	1.475	0.570	-	AT4G17840.1
CUST_11298_PI390587928	1.240	0.836	1.152	0.780	1.331	0.869	1.224	0.833	expressed protein	
CUST_1738_PI390587928	1.287	0.803	1.032	0.818	1.282	0.887	1.185	0.806	-	
CUST_39986_PI390587928	1.515	0.755	1.304	0.618	1.191	0.587	1.325	0.381	3-N-debenzoyl-2-deoxytaxol N-benzoyltransferase putative expressed	AT3G62160.1
CUST_2272_PI390587928	1.363	0.762	1.214	0.648	1.257	0.737	1.256	0.706	multisynthetase complex auxiliary component p43 putative expressed	AT3G59980.1
CUST_5571_PI390587928	1.262	0.574	1.440	0.476	1.451	0.433	1.362	0.450	phosphoethanolamine N-methyltransferase putative expressed	
CUST_24160_PI390587928	1.310	0.696	1.287	0.514	1.275	0.573	1.157	0.503	calcium homeostasis regulator CHoR1 putative expressed	AT3G55250.1
CUST_38825_PI390587928	1.208	0.686	1.390	0.672	1.120	0.559	1.230	0.595	prolyl carboxypeptidase like protein putative expressed	AT4G36195.1
CUST_26421_PI390587928	1.461	0.594	1.361	0.444	1.536	0.575	1.491	0.304	protochlorophyllide reductase B chloroplast precursor putative expressed	AT1G03630.2
CUST_27443_PI390587928	1.561	0.509	1.515	0.510	1.802	0.492	1.729	0.415	-	ATCG00790.1
CUST_36394_PI390587928	1.383	0.887	1.268	0.636	1.266	0.801	1.228	0.731	stromal 70 kDa heat shock-related protein chloroplast precursor putative expressed	AT5G49910.1
CUST_35403_PI390587928	1.387	0.783	1.317	0.643	1.259	0.758	1.199	0.745	inositol-1-monophosphatase putative expressed	AT1G31190.1
CUST_30970_PI390587928	1.510	0.682	1.453	0.541	1.464	0.693	1.278	0.557	expressed protein	AT5G11450.1
CUST_17300_PI390587928	1.786	0.538	1.638	0.429	1.957	0.511	1.521	0.353	chlorophyllide a oxygenase chloroplast precursor putative expressed	AT1G44446.1
CUST_14319_PI390587928	1.507	0.645	1.509	0.479	1.538	0.639	1.400	0.454	polyphosphoinositide binding protein putative expressed	AT5G63060.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_41996_PI390587928	1.334	0.811	1.150	0.646	1.554	0.831	1.436	0.835	-	
CUST_8498_PI390587928	1.100	0.759	1.203	0.678	1.288	0.809	1.117	0.756	heterogeneous nuclear ribonucleoprotein U-like protein 1 putative expressed	
CUST_4054_PI390587928	1.521	0.670	1.453	0.551	1.348	0.533	1.400	0.547	-	
CUST_10971_PI390587928	1.224	0.779	1.301	0.726	1.327	0.721	1.183	0.608	chloride channel-like protein CLC-g putative expressed	AT5G33280.1
CUST_31076_PI390587928	1.624	0.609	1.549	0.457	2.015	0.736	1.885	0.539	alginate regulatory protein AlgP putative expressed	
CUST_2888_PI390587928	2.275	0.332	1.631	0.266	1.892	0.246	1.952	0.155	chlorophyll a-b binding protein 2 chloroplast precursor putative expressed	AT2G34420.1
CUST_31900_PI390587928	1.957	0.623	1.397	0.619	1.883	0.705	1.237	0.733	expressed protein	
CUST_37138_PI390587928	1.385	0.701	1.518	0.768	1.222	0.718	1.154	0.826	hydroxymethylbutenyl 4-diphosphate synthase putative expressed	AT5G60600.1
CUST_14803_PI390587928	1.602	0.611	1.486	0.324	1.694	0.707	1.606	0.458	calcium ion binding protein putative expressed	AT2G39470.2
CUST_39975_PI390587928	1.449	0.421	1.378	0.397	1.884	0.417	1.678	0.397	-	
CUST_29816_PI390587928	1.400	0.691	1.395	0.649	1.624	0.895	1.238	0.566	plant-specific domain TIGR01570 family protein expressed	
CUST_37761_PI390587928	2.635	0.409	2.633	0.274	2.528	0.260	1.891	0.128	-	
CUST_7774_PI390587928	1.160	0.769	1.283	0.770	1.336	0.800	1.263	0.873	ras-related protein Rab-18 putative expressed	AT1G43890.3
CUST_27602_PI390587928	1.222	0.719	1.179	0.689	1.309	0.711	1.157	0.632	expressed protein	AT2G48110.1
CUST_33790_PI390587928	1.137	0.840	1.202	0.773	1.287	0.859	1.135	0.802	VIP2 protein putative expressed	AT3G05545.1
CUST_11684_PI390587928	1.095	0.699	1.033	0.659	1.320	0.744	1.108	0.660	-	AT5G23110.1
CUST_37606_PI390587928	1.129	0.780	1.143	0.844	1.359	0.884	1.184	0.736	-	
CUST_24653_PI390587928	1.913	0.660	1.670	0.460	1.326	0.590	1.362	0.606	expressed protein	AT4G16060.1
CUST_35849_PI390587928	1.428	0.816	1.239	0.672	1.516	0.801	1.395	0.707	-	
CUST_5878_PI390587928	1.218	0.699	1.234	0.595	1.407	0.731	1.137	0.642	-	
CUST_37436_PI390587928	1.644	0.699	1.221	0.407	1.706	0.778	1.597	0.624	expressed protein	
CUST_641_PI390587928	1.156	0.712	1.342	0.878	1.167	0.717	1.205	0.634	fatty acid elongase putative expressed	AT5G43760.1
CUST_33481_PI390587928	1.389	0.609	1.533	0.602	1.504	0.673	1.475	0.695	elongation factor Tu mitochondrial precursor putative expressed	
CUST_18598_PI390587928	1.716	0.619	1.590	0.450	1.710	0.576	1.311	0.370	expressed protein	AT3G29290.1
CUST_17623_PI390587928	1.103	0.725	1.057	0.683	1.413	0.864	1.146	0.813	-	
CUST_6320_PI390587928	1.336	0.767	1.376	0.576	1.395	0.648	1.297	0.584	glutathione S-transferase N-terminal domain containing protein expressed	AT5G03880.1
CUST_38335_PI390587928	1.320	0.665	1.329	0.586	1.530	0.719	1.382	0.610	-	
CUST_9572_PI390587928	1.229	0.642	1.145	0.761	1.203	0.668	1.372	0.679	signal transducer putative expressed	AT3G15470.1
CUST_2147_PI390587928	1.525	0.461	1.661	0.606	1.554	0.548	1.473	0.424	potassium transporter 10 putative expressed	AT1G70300.1
CUST_16261_PI390587928	1.334	0.805	1.330	0.876	1.419	0.754	1.298	0.789	expressed protein	
CUST_30984_PI390587928	1.318	0.571	1.402	0.509	1.319	0.572	1.354	0.533	expressed protein	AT1G22850.1
CUST_64_PI390587928	1.518	0.829	1.235	0.533	1.397	0.725	1.422	0.621	expressed protein	AT1G60230.1
CUST_42170_PI390587928	2.545	0.514	2.792	0.503	2.864	0.357	2.160	0.446	expressed protein	
CUST_5462_PI390587928	1.560	0.627	1.468	0.542	1.439	0.649	1.203	0.461	RNA polymerase sigma factor rpoD putative expressed	AT1G08540.1
CUST_25850_PI390587928	1.396	0.530	1.768	0.409	1.519	0.607	1.478	0.459	ribulose bisphosphate carboxylase/oxygenase activase chloroplast precursor putative expressed	AT2G39730.1
CUST_12119_PI390587928	1.787	0.544	1.639	0.423	1.775	0.529	1.666	0.462	ATATH13 putative expressed	AT5G64940.2
CUST_33017_PI390587928	1.145	0.610	1.342	0.534	1.320	0.760	1.238	0.629	glutathione-regulated potassium-efflux system protein kefB putative expressed	AT4G04850.1
CUST_17144_PI390587928	1.350	0.471	1.504	0.496	1.235	0.494	1.312	0.414	homeodomain leucine zipper protein CPHB-5 putative expressed	AT3G01470.1
CUST_5276_PI390587928	1.318	0.873	1.241	0.739	1.235	0.851	1.135	0.780	ATP-dependent protease Clp ATPase subunit putative expressed	AT3G26580.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_21677_PI390587928	1.209	0.592	1.236	0.626	1.319	0.735	1.216	0.572	zinc finger RING-type putative expressed	AT1G11950.1
CUST_10512_PI390587928	1.516	0.561	1.355	0.436	1.340	0.528	1.179	0.313	chlorophyll a-b binding protein 6A chloroplast precursor putative expressed	AT3G54890.1
CUST_27148_PI390587928	1.484	0.611	1.305	0.437	1.307	0.519	1.252	0.360	transposon protein putative CACTA En/Spm sub-class expressed	AT2G40070.2
CUST_30084_PI390587928	1.211	0.698	1.208	0.605	1.225	0.643	1.141	0.632	protein lap1 putative expressed	AT3G11330.1
CUST_6889_PI390587928	1.226	0.632	1.094	0.551	1.469	0.771	1.355	0.715	cyclin-A1 putative expressed	
CUST_29969_PI390587928	1.473	0.691	1.571	0.742	1.734	0.719	1.466	0.575	protein binding protein putative expressed	AT1G78230.1
CUST_4115_PI390587928	1.180	0.768	1.154	0.806	1.239	0.826	1.253	0.891	brain protein 44 putative expressed	AT4G22310.1
CUST_11550_PI390587928	1.442	0.620	1.263	0.473	1.474	0.664	1.363	0.568	60S ribosomal protein L28 putative expressed	
CUST_37168_PI390587928	1.213	0.835	1.267	0.772	1.287	0.821	1.119	0.718	tat pathway signal sequence family protein expressed	AT1G77090.1
CUST_2965_PI390587928	1.223	0.806	1.213	0.697	1.287	0.842	1.141	0.767	cleavage stimulation factor 64 putative expressed	
CUST_26003_PI390587928	1.329	0.797	1.347	0.620	1.521	0.741	1.325	0.800	-	
CUST_40742_PI390587928	1.298	0.906	1.243	0.826	1.365	0.756	1.228	0.765	nucleus protein putative expressed	
CUST_23629_PI390587928	1.191	0.768	1.192	0.920	1.151	0.795	1.138	0.789	expressed protein	AT3G24740.2
CUST_16738_PI390587928	1.446	0.797	1.332	0.638	2.033	0.753	1.663	0.729	transposon protein putative unclassified	
CUST_13502_PI390587928	1.211	0.786	1.271	0.712	1.360	0.893	1.239	0.758	expressed protein	AT5G64840.1
CUST_12263_PI390587928	1.401	0.567	1.434	0.436	1.520	0.528	1.408	0.395	expressed protein	AT5G37360.1
CUST_2656_PI390587928	1.456	0.368	1.523	0.330	1.445	0.444	1.641	0.228	chlorophyll a-b binding protein chloroplast precursor putative expressed	AT3G61470.1
CUST_16584_PI390587928	1.456	0.868	1.214	0.715	1.484	0.847	1.372	0.825	mitochondrial import inner membrane translocase subunit TIM16 putative expressed	AT3G59280.1
CUST_39743_PI390587928	1.987	0.400	2.018	0.245	3.296	0.504	2.657	0.398	-	
CUST_32504_PI390587928	1.201	0.902	1.191	0.738	1.193	0.768	1.125	0.763	protein kinase Pti1 putative expressed	AT3G59350.3
CUST_8750_PI390587928	1.311	0.809	1.150	0.841	1.190	0.776	1.102	0.701	nucleic acid binding protein putative expressed	AT1G76940.1
CUST_24358_PI390587928	1.269	0.835	1.315	0.732	1.246	0.762	1.115	0.732	charged multivesicular body protein 6 putative expressed	AT5G09260.1
CUST_5312_PI390587928	1.217	0.827	1.320	0.755	1.227	0.702	1.076	0.608	-	
CUST_29430_PI390587928	1.583	0.250	1.391	0.204	1.849	0.356	1.738	0.246	cytochrome P450 724B1 putative expressed	AT3G50660.1
CUST_37157_PI390587928	1.243	0.794	1.194	0.809	1.212	0.818	1.144	0.816	photolyase/blue-light receptor PHR2 putative expressed	AT2G47590.1
CUST_24219_PI390587928	1.224	0.465	1.411	0.492	1.456	0.585	1.398	0.419	patatin class 1 precursor putative expressed	AT2G26560.1
CUST_28222_PI390587928	1.815	0.699	1.583	0.447	1.246	0.562	1.344	0.618	expressed protein	AT4G16060.1
CUST_29228_PI390587928	1.239	0.572	1.249	0.589	1.384	0.658	1.262	0.558	disease resistance protein RPM1 putative expressed	
CUST_3976_PI390587928	1.194	0.677	1.283	0.717	1.459	0.824	1.389	0.690	-	
CUST_22005_PI390587928	1.138	0.782	1.194	0.734	1.375	0.771	1.268	0.772	MYBR5 putative expressed	
CUST_16306_PI390587928	1.348	0.647	1.264	0.690	1.311	0.694	1.358	0.592	galactosyltransferase/ transferase transferring hexosyl groups putative expressed	AT5G62620.2
CUST_11873_PI390587928	1.526	0.749	1.278	0.626	1.496	0.673	1.336	0.603	zinc ribbon 1 putative	AT1G68730.1
CUST_25848_PI390587928	3.154	0.165	2.922	0.105	3.418	0.052	3.996	0.091	cytokinin dehydrogenase 1 precursor putative expressed	AT3G63440.1
CUST_5003_PI390587928	1.114	0.718	1.424	0.741	1.293	0.753	1.313	0.675	acid phosphatase putative expressed	AT4G29260.1
CUST_16913_PI390587928	1.435	0.710	1.254	0.729	1.371	0.671	1.257	0.548	expressed protein	AT5G07950.1
CUST_20855_PI390587928	1.206	0.656	1.247	0.654	1.615	0.620	1.386	0.628	-	
CUST_29090_PI390587928	1.650	0.239	1.927	0.129	1.753	0.183	1.449	0.102	carotenoid cleavage dioxygenase 1 putative expressed	AT4G19170.1
CUST_37050_PI390587928	1.420	0.691	1.540	0.715	1.376	0.560	1.236	0.573	methylase putative expressed	AT3G60910.1
CUST_223_PI390587928	1.748	0.890	1.389	0.600	1.480	0.663	1.313	0.664	zinc finger C3HC4 type family protein expressed	AT2G45530.1
CUST_30859_PI390587928	1.121	0.746	1.258	0.798	1.329	0.866	1.093	0.796	1-phosphatidylinositol-4-phosphate 5-kinase/ zinc ion binding protein putative expressed	AT4G33240.2

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_39871_PI390587928	1.868	0.399	1.734	0.265	2.165	0.296	1.617	0.182	CALS1 putative expressed	AT5G13000.2
CUST_25694_PI390587928	1.365	0.733	1.376	0.678	1.391	0.705	1.177	0.616	expressed protein	AT2G37920.1
CUST_4098_PI390587928	1.554	0.658	1.580	0.523	1.411	0.627	1.311	0.580	glutamine amidotransferase subunit pdxT putative expressed	AT5G60540.1
CUST_3900_PI390587928	1.691	0.556	1.609	0.368	1.539	0.460	1.367	0.372	elongation factor G chloroplast precursor putative expressed	AT1G62750.1
CUST_11548_PI390587928	1.819	0.618	1.795	0.610	1.653	0.748	1.559	0.352	expressed protein	AT5G01530.1
CUST_3730_PI390587928	1.329	0.766	1.296	0.623	1.246	0.696	1.104	0.519	nuclear WD protein putative expressed	AT1G80480.1
CUST_23325_PI390587928	1.523	0.756	1.380	0.619	1.370	0.661	1.226	0.581	uncharacterised protein family protein expressed	AT2G34090.1
CUST_19872_PI390587928	1.444	0.717	1.264	0.620	1.509	0.707	1.446	0.574	transcription factor ZmGLK1 putative expressed	
CUST_36814_PI390587928	1.545	0.430	1.688	0.321	2.133	0.422	1.664	0.392	-	
CUST_2336_PI390587928	1.646	0.833	1.447	0.595	1.541	0.792	1.380	0.745	rf1 protein mitochondrial precursor putative expressed	AT1G09900.1
CUST_29876_PI390587928	1.133	0.628	1.099	0.534	1.092	0.528	1.113	0.551	pRGR1 putative expressed	AT5G23550.1
CUST_17503_PI390587928	1.642	0.642	1.578	0.814	1.726	0.737	1.623	0.621	cytokinin-N-glucosyltransferase 1 putative expressed	AT3G55700.1
CUST_39407_PI390587928	1.482	0.784	1.228	0.676	1.187	0.692	1.142	0.650	-	
CUST_13299_PI390587928	1.468	0.877	1.233	0.702	1.329	0.839	1.264	0.768	expressed protein	AT1G47740.2
CUST_19547_PI390587928	1.983	0.611	1.829	0.454	1.549	0.601	1.377	0.497	esterase putative expressed	AT1G29840.1
CUST_6200_PI390587928	1.328	0.680	1.432	0.645	1.406	0.716	1.288	0.575	-	
CUST_42157_PI390587928	1.293	0.804	1.190	0.765	1.410	0.845	1.350	0.814	expressed protein	AT5G40550.1
CUST_42173_PI390587928	1.237	0.616	1.298	0.576	1.427	0.645	1.303	0.626	sialyltransferase-like protein putative expressed	AT3G48820.1
CUST_4257_PI390587928	1.291	0.628	1.202	0.559	1.449	0.934	1.434	0.675	squamosa promoter-binding-like protein 10 putative expressed	AT5G43270.1
CUST_22328_PI390587928	1.977	0.639	1.379	0.541	1.900	0.871	1.874	0.507	-	
CUST_39300_PI390587928	1.703	0.309	1.824	0.208	1.734	0.367	1.674	0.222	-	
CUST_41299_PI390587928	1.258	0.666	1.276	0.695	1.325	0.744	1.173	0.599	CRS1 putative expressed	AT5G16180.1
CUST_37373_PI390587928	1.407	0.663	1.262	0.446	1.461	0.494	1.620	0.615	-	
CUST_17040_PI390587928	1.706	0.840	1.563	0.815	1.379	0.776	1.193	0.650	mTERF family protein expressed	AT5G64950.1
CUST_28236_PI390587928	1.583	0.784	1.403	0.622	1.715	0.901	1.464	0.635	-	
CUST_11887_PI390587928	1.144	0.760	1.230	0.855	1.250	0.808	1.188	0.836	ubiquinone biosynthesis protein ubiB putative expressed	
CUST_16896_PI390587928	1.258	0.489	1.646	0.899	1.029	0.455	1.751	0.657	-	
CUST_23850_PI390587928	1.046	0.614	1.205	0.643	1.244	0.714	1.124	0.651	golgi-localized protein GRIP putative expressed	AT5G66030.1
CUST_31577_PI390587928	3.475	0.173	2.442	0.139	5.113	0.212	4.637	0.165	-	
CUST_37841_PI390587928	1.196	0.750	1.420	0.677	1.169	0.738	1.119	0.720	kinesin-1 putative expressed	AT4G05190.1
CUST_10726_PI390587928	1.165	0.628	1.319	0.554	1.253	0.598	1.075	0.558	lipid binding protein putative expressed	AT3G20270.1
CUST_39869_PI390587928	1.601	0.465	1.646	0.410	1.313	0.363	1.480	0.410	cytokinin-O-glucosyltransferase 2 putative expressed	AT2G36970.1
CUST_13505_PI390587928	1.349	0.892	1.116	0.805	1.284	0.875	1.334	0.746	serine/threonine-protein kinase AFC2 putative expressed	AT4G24740.1
CUST_22456_PI390587928	1.263	0.473	1.297	0.493	1.385	0.642	1.379	0.490	-	
CUST_31640_PI390587928	1.791	0.416	1.720	0.414	1.683	0.283	1.388	0.315	-	
CUST_37655_PI390587928	1.500	0.538	1.327	0.352	1.249	0.488	1.159	0.349	peptidyl-prolyl cis-trans isomerase FKBP-type family protein expressed	AT1G20810.1
CUST_4889_PI390587928	1.076	0.798	1.193	0.728	1.181	0.686	1.077	0.737	af10-protein putative expressed	AT5G11710.1
CUST_22472_PI390587928	1.459	0.660	1.556	0.524	1.559	0.643	1.421	0.561	nuc-1 negative regulatory protein preg putative expressed	AT5G07450.1
CUST_6662_PI390587928	1.483	0.583	1.679	0.488	1.427	0.504	1.525	0.393	CBS domain containing protein expressed	
CUST_40389_PI390587928	1.169	0.870	1.291	0.852	1.275	0.836	1.132	0.735	F-box domain containing protein expressed	

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_7684_PI390587928	1.363	0.526	1.284	0.299	1.321	0.503	1.297	0.399	calcium sensing receptor putative expressed	AT5G23060.1
CUST_39932_PI390587928	1.707	0.637	1.891	0.683	1.389	0.618	1.281	0.532	expressed protein	AT3G07565.1
CUST_23525_PI390587928	1.267	0.824	1.163	0.799	1.206	0.821	1.167	0.695	expressed protein	
CUST_23541_PI390587928	1.282	0.792	1.138	0.804	1.162	0.821	1.145	0.697	expressed protein	AT3G54750.1
CUST_24547_PI390587928	1.344	0.812	1.286	0.714	1.318	0.752	1.176	0.662	-	
CUST_11594_PI390587928	1.346	0.594	1.627	0.516	1.911	0.645	1.742	0.558	expressed protein	AT5G57460.1
CUST_19886_PI390587928	1.345	0.843	1.224	0.850	1.340	0.889	1.353	0.822	reticulon putative expressed	
CUST_9806_PI390587928	1.251	0.649	1.060	0.418	1.366	0.618	1.235	0.617	expressed protein	AT1G10140.1
CUST_8567_PI390587928	1.441	0.800	1.245	0.652	1.696	0.853	1.285	0.620	-	
CUST_39576_PI390587928	1.258	0.728	1.363	0.778	1.275	0.659	1.317	0.566	fatty acid elongase putative expressed	AT1G04220.1
CUST_8583_PI390587928	1.318	0.749	1.317	0.715	1.360	0.757	1.196	0.680	nodulin-like protein putative expressed	AT5G07050.1
CUST_35636_PI390587928	1.829	0.765	1.854	0.561	1.644	0.788	1.255	0.737	-	
CUST_37207_PI390587928	1.219	0.699	1.150	0.527	1.193	0.667	1.137	0.517	oxygen-evolving enhancer protein 2 chloroplast precursor putative expressed	AT1G06680.1
CUST_22024_PI390587928	1.457	0.563	1.221	0.413	1.411	0.560	1.238	0.424	-	
CUST_19577_PI390587928	1.379	0.503	1.375	0.491	1.443	0.579	1.410	0.504	3-5 exonuclease eri-1 putative expressed	
CUST_7236_PI390587928	1.477	0.834	1.415	0.736	1.334	0.689	1.302	0.689	bifunctional thioredoxin reductase/thioredoxin putative expressed	AT2G41680.1
CUST_26298_PI390587928	1.431	0.614	1.332	0.514	1.620	0.644	1.397	0.504	-	
CUST_16932_PI390587928	1.280	0.586	1.551	0.564	1.508	0.666	1.419	0.670	nodulin-like family protein expressed	AT4G19450.1
CUST_9513_PI390587928	1.626	0.583	1.786	0.516	2.362	0.723	1.739	0.568	-	
CUST_12945_PI390587928	0.982	0.476	1.411	0.588	1.324	0.456	1.631	0.609	-	AT1G72190.1
CUST_26345_PI390587928	1.533	0.595	1.409	0.476	1.243	0.564	1.393	0.494	NHL repeat protein putative expressed	AT1G56500.1
CUST_3312_PI390587928	1.401	0.571	1.345	0.480	1.348	0.581	1.221	0.434	expressed protein	AT4G34830.1
CUST_30878_PI390587928	1.209	0.803	1.254	0.763	1.341	0.708	1.233	0.667	TLD family protein expressed	AT5G06260.1
CUST_7082_PI390587928	1.346	0.691	1.209	0.519	1.320	0.640	1.191	0.526	vegetative storage protein putative expressed	AT2G29760.1
CUST_8088_PI390587928	1.387	0.863	1.256	0.707	1.423	0.837	1.331	0.835	OsMPK14 - putative MAPK based on amino acid sequence homology expressed	AT1G10210.2
CUST_3903_PI390587928	2.673	0.290	1.896	0.086	2.448	0.094	1.517	0.046	O-methyltransferase ZRP4 putative expressed	AT4G35160.1
CUST_4909_PI390587928	1.943	0.517	1.869	0.371	2.946	0.644	2.725	0.595	expressed protein	AT2G25737.1
CUST_42064_PI390587928	1.115	0.562	1.235	0.585	1.273	0.697	1.180	0.519	desiccation-associated protein putative	
CUST_39144_PI390587928	2.006	0.535	1.645	0.585	1.400	0.439	1.782	0.534	flavoprotein wrbA putative expressed	AT4G27270.1
CUST_9157_PI390587928	1.524	0.700	1.246	0.570	1.385	0.706	1.309	0.647	nucleic acid binding protein putative expressed	AT2G02570.4
CUST_18262_PI390587928	1.263	0.703	1.353	0.649	1.615	0.864	1.282	0.629	serine/threonine-protein kinase NAK putative expressed	AT5G18610.1
CUST_16032_PI390587928	2.191	0.799	2.013	0.765	2.050	0.679	2.021	0.484	photosystem II D2 protein putative expressed	ATCG00270.1
CUST_31963_PI390587928	1.387	0.899	1.367	0.771	1.394	0.756	1.339	0.719	eukaryotic translation initiation factor 5 putative expressed	AT1G36730.1
CUST_26813_PI390587928	1.114	0.608	1.125	0.627	1.200	0.734	1.158	0.649	-	
CUST_25030_PI390587928	1.568	0.603	1.256	0.572	1.554	0.779	1.500	0.764	-	
CUST_11428_PI390587928	1.618	0.855	1.522	0.753	1.500	0.759	1.565	0.741	protein kinase putative expressed	AT4G24810.2
CUST_40775_PI390587928	1.182	0.675	1.240	0.707	1.316	0.737	1.301	0.784	secondary cell wall-related glycosyltransferase family 47 putative expressed	AT5G16890.1
CUST_24886_PI390587928	1.592	0.630	1.523	0.688	1.466	0.474	1.228	0.434	leucine-rich repeat-containing protein 40 putative expressed	
CUST_36056_PI390587928	1.282	0.828	1.214	0.515	1.191	0.682	1.162	0.661	2-hydroxy-3-oxopropionate reductase putative expressed	AT3G25530.1
CUST_25451_PI390587928	1.282	0.637	1.308	0.596	1.295	0.601	1.277	0.609	protein cbxX chromosomal putative expressed	AT3G24530.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_26457_PI390587928	1.669	0.435	1.540	0.275	1.758	0.424	1.422	0.268	plant-specific domain TIGR01615 family protein expressed	AT2G38820.2
CUST_2891_PI390587928	1.119	0.503	1.283	0.386	1.751	0.771	1.533	0.774	rhythmically expressed gene 2 protein putative expressed	AT2G41250.1
CUST_26504_PI390587928	1.140	0.869	1.207	0.826	1.191	0.762	1.103	0.803	TENA/THI-4 family protein expressed	AT5G32470.1
CUST_18560_PI390587928	1.196	0.685	1.208	0.622	1.282	0.710	1.128	0.684	expressed protein	AT2G06005.1
CUST_28750_PI390587928	1.167	0.853	1.101	0.751	1.314	0.970	1.396	0.894	sugar transporter type 2a putative expressed	AT4G35300.1
CUST_5468_PI390587928	1.320	0.626	1.253	0.524	1.216	0.577	1.189	0.503	-	AT5G52970.1
CUST_9502_PI390587928	1.300	0.613	1.186	0.692	1.273	0.709	1.233	0.588	MAR binding filament-like protein 1 putative expressed	AT3G16000.1
CUST_7745_PI390587928	1.401	0.598	1.580	0.575	1.558	0.629	1.339	0.529	CP5 putative expressed	AT1G64720.1
CUST_38584_PI390587928	1.552	0.632	1.455	0.513	1.390	0.648	1.254	0.497	50S ribosomal protein L1 putative expressed	AT3G63490.2
CUST_5345_PI390587928	1.229	0.816	1.238	0.698	1.222	0.663	1.150	0.681	polygalacturonase putative expressed	AT3G62110.1
CUST_3115_PI390587928	1.307	0.914	1.214	0.846	1.077	0.778	1.099	0.778	tryptophanyl-tRNA synthetase putative expressed	AT2G25840.2
CUST_2427_PI390587928	1.865	0.660	1.812	0.477	1.533	0.504	1.252	0.386	expressed protein	
CUST_18081_PI390587928	1.475	0.319	1.227	0.342	1.392	0.448	1.346	0.266	expressed protein	
CUST_34001_PI390587928	1.506	0.452	1.582	0.317	1.476	0.454	1.375	0.328	mRNA binding protein precursor putative expressed	AT3G63140.1
CUST_18112_PI390587928	1.582	0.746	1.534	0.644	1.639	0.785	1.299	0.593	-	
CUST_426_PI390587928	1.200	0.540	1.434	0.541	1.411	0.567	1.274	0.508	ribonucleoside-diphosphate reductase large subunit putative expressed	AT2G21790.1
CUST_31036_PI390587928	1.742	0.592	1.632	0.382	1.535	0.496	1.283	0.354	50S ribosomal protein L12-1 chloroplast precursor putative expressed	AT3G27850.1
CUST_39328_PI390587928	1.251	0.802	1.141	0.699	1.259	0.855	1.259	0.869	protease Do-like 1 chloroplast precursor putative expressed	AT3G27925.1
CUST_33096_PI390587928	1.164	0.745	1.275	0.675	1.380	0.777	1.167	0.718	anthocyanidin 53-O-glucosyltransferase putative expressed	AT3G16520.2
CUST_33904_PI390587928	1.373	0.729	1.323	0.843	1.410	0.860	1.311	0.732	pathogenesis-related 10 protein PR10-1 putative	
CUST_847_PI390587928	1.503	0.548	1.674	0.389	2.023	0.410	1.678	0.397	expressed protein	AT1G22540.1
CUST_42078_PI390587928	1.228	0.625	1.300	0.547	1.541	0.909	1.409	0.691	-	
CUST_38152_PI390587928	1.169	0.816	1.319	0.832	1.407	0.760	1.327	0.819	eukaryotic peptide chain release factor subunit 1-1 putative expressed	AT3G26618.1
CUST_30971_PI390587928	1.195	0.859	1.112	0.673	1.234	0.784	1.129	0.717	maf-like protein CV_0124 putative expressed	AT5G66550.1
CUST_32542_PI390587928	1.284	0.784	1.170	0.646	1.371	0.828	1.220	0.708	-	AT3G60250.1
CUST_13890_PI390587928	1.388	0.536	1.359	0.630	1.484	0.597	1.342	0.480	-	
CUST_29622_PI390587928	1.612	0.510	1.717	0.471	1.781	0.458	1.475	0.371	potassium transporter 10 putative expressed	AT5G14880.1
CUST_35809_PI390587928	1.244	0.686	1.258	0.590	1.219	0.566	1.050	0.537	anaphase-promoting complex subunit 11 putative expressed	AT3G05870.1
CUST_9825_PI390587928	1.319	0.785	1.255	0.755	1.670	0.737	1.393	0.678	expressed protein	AT4G35250.1
CUST_4599_PI390587928	1.445	0.592	1.635	0.503	1.688	0.461	1.779	0.314	expressed protein	AT5G23890.1
CUST_18744_PI390587928	1.359	0.571	1.453	0.504	1.996	0.729	1.750	0.453	protein binding protein putative expressed	
CUST_15291_PI390587928	1.524	0.490	1.687	0.289	1.855	0.600	1.557	0.357	peroxisomal-coenzyme A synthetase putative expressed	AT1G30520.1
CUST_35702_PI390587928	1.366	0.603	1.502	0.659	1.524	0.629	1.437	0.585	GAMYB-binding protein putative expressed	AT3G61600.2
CUST_26534_PI390587928	1.223	0.757	1.316	0.621	1.361	0.834	1.230	0.644	ERD1 protein chloroplast precursor putative expressed	AT5G51070.1
CUST_11351_PI390587928	1.729	0.586	1.344	0.491	1.207	0.605	1.337	0.495	MAR binding filament-like protein 1 putative expressed	AT3G16000.1
CUST_33255_PI390587928	1.074	0.665	1.153	0.619	1.274	0.750	1.211	0.751	-	
CUST_10004_PI390587928	1.061	0.636	1.107	0.644	1.249	0.719	1.191	0.617	tRNA modification GTPase trmE putative expressed	
CUST_13256_PI390587928	1.483	0.731	1.324	0.642	1.395	0.769	1.478	0.650	peptidyl-prolyl cis-trans isomerase CYP37 chloroplast precursor putative expressed	AT3G15520.1
CUST_34154_PI390587928	1.630	0.514	1.885	0.553	1.443	0.453	1.726	0.616	expressed protein	
CUST_26225_PI390587928	2.596	0.628	1.753	0.502	3.426	0.628	2.564	0.481	expressed protein	AT5G23110.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_37437_PI390587928	1.453	0.602	1.238	0.398	1.563	0.625	1.453	0.552	GRF zinc finger family protein expressed	
CUST_28316_PI390587928	1.498	0.808	1.380	0.694	1.533	0.862	1.482	0.717	ubiquitin-conjugating enzyme E2-21 kDa 1 putative expressed	AT1G63800.1
CUST_30028_PI390587928	1.666	0.931	1.526	0.721	1.441	0.776	1.307	0.732	complex interacting protein 9 putative expressed	AT2G24020.1
CUST_38289_PI390587928	1.487	0.628	1.419	0.502	1.195	0.604	1.256	0.649	alpha-amylase/trypsin inhibitor putative expressed	AT4G11650.1
CUST_6865_PI390587928	1.616	0.398	1.366	0.274	1.905	0.641	1.390	0.350	-	
CUST_5082_PI390587928	1.126	0.702	1.296	0.871	1.327	0.807	1.232	0.750	DNA binding protein putative expressed	
CUST_6088_PI390587928	1.372	0.759	1.313	0.640	1.310	0.713	1.107	0.603	histidyl-tRNA synthetase putative expressed	AT3G46100.1
CUST_13538_PI390587928	1.314	0.827	1.137	0.785	1.296	0.838	1.365	0.755	-	
CUST_1903_PI390587928	1.265	0.726	1.253	0.542	1.281	0.770	1.335	0.687	expressed protein	AT5G54290.1
CUST_15784_PI390587928	1.308	0.560	1.250	0.798	1.211	0.709	1.111	0.631	-	AT4G19003.1
CUST_14576_PI390587928	6.781	0.487	4.592	0.548	3.339	0.208	2.469	0.135	aquaporin PIP2.7 putative expressed	AT4G35100.1
CUST_3947_PI390587928	1.394	0.533	1.422	0.459	1.721	0.725	1.894	0.721	expressed protein	
CUST_15831_PI390587928	1.342	0.800	1.275	0.706	1.254	0.752	1.066	0.549	nucleic acid binding protein putative expressed	AT1G30680.1
CUST_3963_PI390587928	1.453	0.524	1.978	0.913	1.855	0.583	1.465	0.592	nodulin-like protein putative expressed	AT4G34950.1
CUST_2755_PI390587928	1.755	0.333	2.049	0.192	1.919	0.196	1.587	0.119	seven in absentia protein family protein expressed	AT5G37930.1
CUST_21344_PI390587928	1.764	0.547	1.946	0.837	1.767	0.731	2.292	0.696	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor putative expressed	AT5G10290.1
CUST_7764_PI390587928	1.244	0.604	1.247	0.632	1.197	0.601	1.199	0.649	ATP synthase B chain chloroplast precursor putative expressed	AT4G32260.1
CUST_38773_PI390587928	1.654	0.687	1.487	0.674	1.422	0.675	1.350	0.711	RWP-RK domain containing protein expressed	
CUST_21360_PI390587928	1.923	0.697	1.685	0.476	1.740	0.541	1.514	0.377	GTP-binding nuclear protein Ran-A1 putative expressed	AT5G20020.1
CUST_32556_PI390587928	2.004	0.551	1.926	0.455	1.695	0.449	1.298	0.298	protein kinase Kelch repeat:Kelch putative expressed	AT1G80440.1
CUST_13229_PI390587928	1.558	0.707	1.401	0.580	1.480	0.701	1.347	0.608	aspartyl/glutamyl-tRNA amidotransferase subunit B putative expressed	AT1G48520.1
CUST_9839_PI390587928	1.224	0.842	1.357	0.837	1.228	0.789	1.296	0.851	palmitoyltransferase pfa3 putative expressed	AT5G04270.1
CUST_37612_PI390587928	1.159	0.775	1.212	0.793	1.205	0.651	1.099	0.754	histone-lysine N-methyltransferase H3 lysine-9 specific SUVH1 putative expressed	AT5G04940.2
CUST_36373_PI390587928	1.170	0.728	1.295	0.802	1.333	0.840	1.295	0.775	cytochrome P450 89A2 putative expressed	AT1G64950.1
CUST_8647_PI390587928	1.373	0.935	1.141	0.823	1.328	0.887	1.220	0.790	harpin-induced protein putative	AT3G44380.1
CUST_39656_PI390587928	1.306	0.836	1.212	0.705	1.400	0.761	1.278	0.727	seed maturation protein PM23 putative expressed	AT2G14910.1
CUST_40362_PI390587928	1.668	0.797	1.588	0.720	1.374	0.783	1.282	0.647	expressed protein	AT2G31040.1
CUST_32433_PI390587928	1.328	0.478	1.428	0.454	1.957	0.646	1.487	0.412	-	
CUST_24520_PI390587928	1.514	0.671	1.591	0.659	1.429	0.621	1.253	0.582	TPR Domain containing protein expressed	AT1G02910.1
CUST_9716_PI390587928	1.989	0.496	1.927	0.359	1.707	0.475	1.742	0.368	carbonic anhydrase chloroplast precursor putative expressed	AT5G14740.5
CUST_10934_PI390587928	2.547	0.554	2.478	0.265	2.002	0.201	1.434	0.115	purple acid phosphatase precursor putative expressed	AT1G52940.1
CUST_20938_PI390587928	1.599	0.614	1.451	0.415	1.532	0.605	1.447	0.512	APE1 putative expressed	AT5G38660.1
CUST_28151_PI390587928	1.402	0.601	1.247	0.514	1.553	0.740	1.338	0.590	apospory-associated protein C putative expressed	AT5G66530.1
CUST_18449_PI390587928	1.413	0.594	1.335	0.435	1.637	0.691	1.588	0.543	-	
CUST_7099_PI390587928	1.587	0.611	1.367	0.350	1.699	0.506	1.635	0.373	serine carboxypeptidase 1 precursor putative expressed	AT5G09640.1
CUST_322_PI390587928	1.459	0.731	1.263	0.703	1.413	0.633	1.112	0.534	-	
CUST_2153_PI390587928	1.544	0.628	1.373	0.439	1.531	0.620	1.181	0.484	-	
CUST_35967_PI390587928	1.132	0.551	1.330	0.542	1.477	0.621	1.407	0.556	leucine-rich repeat receptor protein kinase EXS precursor putative expressed	AT2G34930.1
CUST_10034_PI390587928	1.143	0.799	1.250	0.730	1.318	0.649	1.481	0.619	ATP binding protein putative expressed	AT4G32300.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_23003_PI390587928	1.313	0.750	1.338	0.800	1.344	0.756	1.266	0.722	tesmin/TSO1-like CXC domain containing protein expressed	AT4G29000.1
CUST_18295_PI390587928	1.243	0.775	1.218	0.781	1.171	0.669	1.015	0.641	sister-chromatide cohesion protein putative expressed	AT2G47980.1
CUST_13877_PI390587928	1.316	0.733	1.203	0.693	1.489	0.725	1.296	0.583	polyadenylate-binding protein 2 putative expressed	
CUST_27868_PI390587928	2.005	0.702	1.341	0.429	1.180	0.538	1.286	0.496	-	
CUST_10455_PI390587928	1.170	0.795	1.180	0.790	1.377	0.870	1.162	0.779	fiber protein Fb2 putative expressed	AT3G05700.1
CUST_2621_PI390587928	1.232	0.795	1.190	0.848	1.168	0.860	1.205	0.823	cleavage stimulation factor 64 putative expressed	AT1G71800.1
CUST_27698_PI390587928	1.112	0.702	1.188	0.657	1.079	0.634	1.057	0.625	aminopeptidase N putative expressed	AT1G63770.4
CUST_29975_PI390587928	1.444	0.759	1.200	0.541	1.230	0.574	1.188	0.610	expressed protein	AT5G10200.1
CUST_36696_PI390587928	1.487	0.740	1.375	0.506	1.477	0.648	1.412	0.538	peptidyl-prolyl cis-trans isomerase CYP37 chloroplast precursor putative expressed	AT3G15520.1
CUST_33948_PI390587928	1.319	0.893	1.210	0.815	1.207	0.866	1.184	0.721	pentatricopeptide repeat protein PPR986-12 putative expressed	AT3G63370.1
CUST_30712_PI390587928	1.564	0.554	1.820	0.438	1.984	0.621	1.689	0.374	Mg-chelatase subunit XANTHA-F putative expressed	AT5G13630.1
CUST_4712_PI390587928	1.424	0.821	1.208	0.814	1.310	0.710	1.396	0.783	NOL1/NOP2/sun family protein expressed	AT5G66180.1
CUST_13584_PI390587928	1.561	0.710	1.341	0.589	1.291	0.704	1.173	0.455	RNA binding protein putative expressed	AT4G37510.1
CUST_34482_PI390587928	1.154	0.782	1.260	0.867	1.178	0.764	1.216	0.817	-	AT1G62020.1
CUST_26553_PI390587928	1.958	0.423	2.009	0.254	1.882	0.401	1.497	0.335	haemolysin-III related family protein expressed	AT5G20270.1
CUST_29836_PI390587928	1.845	0.499	1.515	0.441	1.727	0.322	1.474	0.270	-	
CUST_18438_PI390587928	1.594	0.561	1.343	0.434	1.565	0.674	1.559	0.541	serine/threonine-protein kinase receptor precursor putative expressed	AT1G11350.1
CUST_40546_PI390587928	1.489	0.773	1.582	0.654	1.509	0.824	1.244	0.711	rf1 protein mitochondrial precursor putative expressed	AT1G09900.1
CUST_39856_PI390587928	1.616	0.525	1.622	0.561	1.490	0.383	1.705	0.433	40S ribosomal protein S5 putative expressed	ATCG00900.1
CUST_3148_PI390587928	1.212	0.618	1.248	0.538	1.327	0.600	1.194	0.571	F-box protein interaction domain containing protein expressed	
CUST_25695_PI390587928	1.169	0.691	1.164	0.699	1.517	0.725	1.265	0.618	-	
CUST_18331_PI390587928	1.865	0.183	1.493	0.158	2.041	0.139	2.284	0.118	-	
CUST_22273_PI390587928	1.466	0.732	1.498	0.665	1.338	0.602	1.166	0.544	sugar transporter family protein putative expressed	AT5G59250.1
CUST_39717_PI390587928	1.405	0.563	1.446	0.419	1.433	0.512	1.208	0.473	ATA15 protein putative expressed	AT1G66330.2
CUST_40423_PI390587928	1.452	0.735	1.365	0.620	1.571	0.623	1.386	0.750	protein binding protein putative expressed	AT3G47990.1
CUST_22087_PI390587928	1.233	0.554	1.238	0.571	1.309	0.608	1.162	0.483	calreticulin-3 precursor putative expressed	AT1G08450.2
CUST_24348_PI390587928	1.316	0.598	1.037	0.492	1.448	0.668	1.352	0.514	-	
CUST_8554_PI390587928	1.163	0.813	1.264	0.773	1.355	0.814	1.206	0.865	F-box domain containing protein expressed	
CUST_2928_PI390587928	1.280	0.643	1.270	0.502	1.306	0.752	1.283	0.681	proline synthetase co-transcribed bacterial homolog protein putative expressed	AT4G26860.1
CUST_32138_PI390587928	1.135	0.529	1.250	0.552	1.160	0.535	1.125	0.448	phototropin-1 putative expressed	AT5G58140.1
CUST_26999_PI390587928	1.685	0.633	1.591	0.598	1.440	0.552	1.208	0.499	-	
CUST_34959_PI390587928	1.399	0.771	1.364	0.806	1.526	0.784	1.422	0.784	-	AT1G71440.1
CUST_40705_PI390587928	1.185	0.742	1.357	0.704	1.349	0.763	1.143	0.653	-	
CUST_30988_PI390587928	1.723	0.646	1.778	0.523	1.925	0.602	1.822	0.541	MYB59 putative expressed	AT5G59780.3
CUST_8991_PI390587928	1.252	0.819	1.296	0.703	1.112	0.663	1.098	0.576	serine/threonine-protein kinase receptor precursor putative	AT4G23130.2
CUST_38792_PI390587928	2.378	0.226	2.160	0.161	2.083	0.229	1.618	0.131	retrotransposon protein putative unclassified	
CUST_13938_PI390587928	1.091	0.741	1.130	0.665	1.097	0.710	1.181	0.801	beta-13-galactosyltransferase sqv-2 putative expressed	AT5G53340.1
CUST_12699_PI390587928	1.310	0.559	1.227	0.347	1.271	0.560	1.278	0.459	2-hydroxy-3-oxopropionate reductase putative expressed	AT1G17650.1
CUST_9874_PI390587928	1.289	0.835	1.321	0.784	1.403	0.685	1.293	0.598	TLD family protein expressed	AT5G39590.1
CUST_39691_PI390587928	1.612	0.763	1.397	0.709	1.374	0.732	1.293	0.595	SET domain containing protein expressed	AT5G14260.2

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_26800_PI390587928	1.083	0.636	1.227	0.402	1.618	0.522	1.524	0.457	expressed protein	AT2G31560.2
CUST_35751_PI390587928	1.529	0.606	1.420	0.514	1.478	0.629	1.258	0.358	chlorophyll a-b binding protein chloroplast precursor putative expressed	AT3G61470.1
CUST_16393_PI390587928	1.273	0.717	1.249	0.561	1.350	0.699	1.187	0.596	tRNA pseudouridine synthase A putative expressed	AT3G06950.1
CUST_511_PI390587928	1.381	0.694	1.345	0.638	1.506	0.681	1.303	0.548	-	
CUST_5578_PI390587928	1.190	0.847	1.196	0.765	1.219	0.858	1.174	0.830	transglutaminase-like superfamily protein expressed	AT5G49570.1
CUST_278_PI390587928	1.313	0.523	1.362	0.534	1.500	0.705	1.392	0.532	glycyl-tRNA synthetase 2 chloroplast/mitochondrial precursor putative expressed	
CUST_24167_PI390587928	1.168	0.811	1.231	0.713	1.140	0.754	1.094	0.675	HEC/Ndc80p family protein expressed	AT3G54630.1
CUST_21419_PI390587928	1.300	0.544	1.240	0.728	1.545	0.683	1.628	0.647	expressed protein	
CUST_9612_PI390587928	1.518	0.516	1.416	0.288	1.592	0.334	1.443	0.307	phospholipid hydroperoxide glutathione peroxidase putative expressed	AT4G11600.1
CUST_35379_PI390587928	1.372	0.612	1.448	0.576	1.304	0.629	1.106	0.466	expressed protein	AT2G38780.1
CUST_13491_PI390587928	1.243	0.601	1.447	0.729	1.362	0.674	1.287	0.520	-	AT2G48160.1
CUST_11261_PI390587928	1.604	0.596	1.448	0.401	1.359	0.563	1.266	0.456	NHL repeat protein putative expressed	AT1G56500.1
CUST_4417_PI390587928	1.612	0.612	1.377	0.431	1.613	0.631	1.451	0.414	4-methyl-5-thiazole monophosphate biosynthesis protein putative expressed	AT4G34020.2
CUST_33212_PI390587928	1.579	0.780	1.412	0.887	1.400	0.735	1.299	0.686	expressed protein	AT1G44920.1
CUST_9272_PI390587928	1.320	0.827	1.282	0.672	1.366	0.849	1.220	0.722	MYND finger family protein expressed	
CUST_35863_PI390587928	1.466	0.889	1.679	0.946	1.586	0.851	1.486	0.867	expressed protein	
CUST_26135_PI390587928	1.775	0.538	1.337	0.340	1.599	0.636	1.488	0.364	pentatricopeptide repeat protein PPR986-12 putative expressed	AT1G25360.1
CUST_22148_PI390587928	1.260	0.745	1.194	0.616	1.296	0.711	1.239	0.625	-	
CUST_35117_PI390587928	1.263	0.715	1.322	0.652	1.385	0.697	1.131	0.698	-	
CUST_22739_PI390587928	1.329	0.609	1.334	0.551	1.601	0.801	1.458	0.612	nucleoporin putative expressed	
CUST_14795_PI390587928	1.662	0.563	1.515	0.551	1.442	0.644	1.296	0.478	MAR binding filament-like protein 1 putative expressed	AT3G16000.1
CUST_4140_PI390587928	1.407	0.691	1.568	0.705	1.663	0.703	1.349	0.595	coronatine-insensitive protein 1 putative expressed	AT2G39940.1
CUST_35724_PI390587928	1.443	0.519	1.634	0.433	1.413	0.685	1.421	0.509	glyceraldehyde-3-phosphate dehydrogenase A chloroplast precursor putative expressed	AT1G12900.1
CUST_7967_PI390587928	1.489	0.665	1.560	0.567	1.549	0.763	1.636	0.677	resistance protein LR10 putative expressed	
CUST_32806_PI390587928	1.064	0.734	1.185	0.768	1.184	0.764	1.124	0.791	-	
CUST_10212_PI390587928	1.086	0.829	1.135	0.751	1.187	0.846	1.145	0.867	glycogen operon protein glgX putative expressed	AT4G09020.1
CUST_7828_PI390587928	1.185	0.632	1.164	0.684	1.357	0.851	1.432	0.742	-	
CUST_31583_PI390587928	6.297	0.343	3.561	0.144	4.759	0.090	2.541	0.043	thiol protease SEN102 precursor putative expressed	AT2G34080.1
CUST_11234_PI390587928	1.662	0.670	1.189	0.399	1.460	0.693	1.433	0.553	ATP-dependent peptidase putative	AT1G35340.1
CUST_29094_PI390587928	1.390	0.885	1.210	0.629	1.187	0.706	1.143	0.701	expp1 protein precursor putative expressed	AT3G44150.1
CUST_10716_PI390587928	2.805	0.487	1.706	0.260	1.920	0.443	1.618	0.240	3-oxoacyl-synthase I chloroplast precursor putative expressed	AT5G46290.2
CUST_29716_PI390587928	1.304	0.590	1.148	0.411	1.505	0.553	1.286	0.447	OTU-like cysteine protease family protein expressed	AT3G57810.3
CUST_17312_PI390587928	1.232	0.654	1.287	0.616	1.278	0.608	1.126	0.529	haloacid dehalogenase-like hydrolase domain-containing protein 1A putative expressed	AT4G21470.1
CUST_39689_PI390587928	1.626	0.651	1.195	0.568	1.579	0.587	1.766	0.470	SET domain containing protein expressed	AT5G14260.2
CUST_15369_PI390587928	1.210	0.662	1.212	0.508	1.197	0.632	1.228	0.625	expressed protein	AT5G16810.1
CUST_24553_PI390587928	1.551	0.609	1.403	0.516	1.804	0.747	1.674	0.542	repressor of RNA polymerase III transcription MAF1 putative expressed	AT5G13240.1
CUST_25591_PI390587928	1.322	0.881	1.154	0.797	1.340	0.863	1.270	0.798	-	
CUST_23361_PI390587928	1.367	0.722	1.330	0.688	1.623	0.682	1.493	0.731	armadillo/beta-catenin-like repeat family protein expressed	AT4G34940.1
CUST_2542_PI390587928	1.226	0.774	1.263	0.801	1.251	0.880	1.161	0.796	alpha-N-arabinofuranosidase 1 precursor putative expressed	AT3G10740.1
CUST_6327_PI390587928	1.603	0.765	1.607	0.662	1.360	0.730	1.393	0.808	JD1 putative expressed	AT1G80950.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_15199_PI390587928	1.211	0.895	1.138	0.769	1.322	0.844	1.293	0.803	ybaK/prolyl-tRNA synthetase associated region putative expressed	AT1G44835.1
CUST_36330_PI390587928	1.138	0.603	1.429	0.707	1.499	0.877	1.206	0.699	AP2 domain-containing protein putative expressed	
CUST_41511_PI390587928	1.264	0.831	1.399	0.769	1.502	0.841	1.570	0.787	DNA binding protein putative expressed	AT2G45850.2
CUST_41325_PI390587928	1.430	0.854	1.359	0.726	1.388	0.761	1.212	0.667	dnaJ domain containing protein expressed	AT5G23040.1
CUST_11259_PI390587928	1.429	0.818	1.471	0.771	1.292	0.721	1.259	0.686	chaperone protein dnaJ putative expressed	AT1G80030.2
CUST_20864_PI390587928	1.139	0.868	1.161	0.801	1.158	0.827	1.121	0.788	-	
CUST_10690_PI390587928	1.356	0.637	1.189	0.532	1.708	0.892	1.483	0.548	-	
CUST_7103_PI390587928	1.199	0.844	1.113	0.730	1.238	0.834	1.150	0.810	expressed protein	
CUST_23504_PI390587928	1.454	0.500	1.643	0.393	1.582	0.561	1.420	0.426	fructose-bisphosphate aldolase chloroplast precursor putative expressed	AT4G38970.1
CUST_34685_PI390587928	1.294	0.739	1.280	0.748	1.276	0.691	1.208	0.702	protein dimerization putative expressed	AT1G79740.1
CUST_4698_PI390587928	1.422	0.710	1.244	0.573	1.300	0.804	1.382	0.709	expressed protein	AT4G22890.5
CUST_11573_PI390587928	1.592	0.756	1.535	0.499	1.406	0.652	1.203	0.472	esterase/lipase/thioesterase family active site protein putative expressed	AT5G17670.1
CUST_33710_PI390587928	1.673	0.522	1.464	0.313	2.335	0.310	2.256	0.217	tonoplast dicarboxylate transporter putative expressed	AT5G47560.1
CUST_12595_PI390587928	1.156	0.719	1.159	0.697	1.284	0.794	1.235	0.711	transposon protein putative unclassified	AT4G15415.1
CUST_35738_PI390587928	1.698	0.254	1.472	0.194	1.602	0.196	1.611	0.070	chlorophyll a-b binding protein 2 chloroplast precursor putative expressed	AT2G34430.1
CUST_18843_PI390587928	1.409	0.687	1.567	0.740	1.570	0.803	1.543	0.715	SET domain containing protein expressed	
CUST_33508_PI390587928	1.226	0.828	1.376	0.798	1.172	0.678	1.206	0.825	ras-related protein ARA-4 putative expressed	AT5G47520.1
CUST_35770_PI390587928	1.424	0.332	1.415	0.298	1.480	0.316	1.591	0.172	chlorophyll a-b binding protein 2 chloroplast precursor putative expressed	AT2G34420.1
CUST_19881_PI390587928	1.422	0.766	1.411	0.577	1.362	0.543	1.325	0.373	dnaJ protein homolog 1 putative expressed	
CUST_41957_PI390587928	1.367	0.637	1.361	0.509	1.353	0.639	1.165	0.604	naphthoate synthase putative expressed	AT1G60550.1
CUST_22832_PI390587928	1.378	0.679	1.414	0.577	1.508	0.702	1.276	0.537	ribose-phosphate pyrophosphokinase 1 putative expressed	AT2G35390.2
CUST_34562_PI390587928	1.172	0.765	1.134	0.707	1.084	0.700	1.213	0.791	transmembrane 9 superfamily protein member 2 precursor putative expressed	AT5G25100.1
CUST_39602_PI390587928	1.508	0.564	1.454	0.413	1.464	0.499	1.117	0.442	expressed protein	
CUST_4606_PI390587928	1.321	0.923	1.317	0.845	1.290	0.851	1.208	0.829	tyrosyl-tRNA synthetase putative expressed	AT3G02660.1
CUST_30389_PI390587928	1.238	0.734	1.291	0.743	1.344	0.770	1.143	0.684	-	
CUST_19556_PI390587928	1.225	0.701	1.314	0.713	1.421	0.883	1.314	0.714	3-5 exonuclease eri-1 putative expressed	
CUST_29746_PI390587928	1.389	0.705	1.240	0.676	1.324	0.651	1.261	0.605	-	
CUST_10103_PI390587928	1.207	0.586	1.302	0.531	1.466	0.729	1.304	0.565	nucleoporin putative expressed	
CUST_16134_PI390587928	1.219	0.363	1.177	0.383	1.419	0.566	1.230	0.367	-	
CUST_28554_PI390587928	1.323	0.883	1.129	0.686	1.272	0.759	1.118	0.670	thioredoxin X chloroplast precursor putative expressed	AT1G50320.1
CUST_26324_PI390587928	1.217	0.787	1.369	0.754	1.325	0.711	1.234	0.635	hexose carrier protein HEX6 putative expressed	AT5G26340.1
CUST_14377_PI390587928	1.200	0.804	1.200	0.733	1.408	0.758	1.304	0.819	BGGP Beta-1-3-galactosyl-O-glycosyl-glycoprotein putative expressed	AT5G39990.1
CUST_19386_PI390587928	1.479	0.714	1.475	0.751	1.432	0.582	1.292	0.507	EMB1381 putative expressed	AT2G31340.1
CUST_21129_PI390587928	1.385	0.771	1.166	0.748	1.176	0.751	1.274	0.627	U2 small nuclear ribonucleoprotein A putative expressed	AT1G09760.1
CUST_30313_PI390587928	1.734	0.809	1.371	0.677	1.483	0.843	1.263	0.730	zinc finger protein 7 putative expressed	AT1G24625.1
CUST_32373_PI390587928	1.230	0.904	1.182	0.768	1.181	0.838	1.116	0.809	FK506 binding protein putative expressed	AT4G19830.1
CUST_33395_PI390587928	1.661	0.443	1.567	0.366	2.013	0.352	1.530	0.333	FLP1 putative expressed	
CUST_13046_PI390587928	1.283	0.650	1.208	0.478	1.381	0.532	1.209	0.519	conserved hypothetical protein	AT3G50120.1
CUST_22821_PI390587928	2.900	0.214	2.373	0.222	2.120	0.252	1.691	0.215	-	
CUST_19093_PI390587928	1.321	0.774	1.153	0.790	1.156	0.796	1.226	0.837	actin-depolymerizing factor 6 putative expressed	AT5G52360.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_15930_PI390587928	1.222	0.672	1.295	0.688	1.311	0.736	1.252	0.728	expressed protein	
CUST_41807_PI390587928	1.380	0.789	1.146	0.854	1.169	0.729	1.228	0.694	-	
CUST_25934_PI390587928	1.639	0.517	1.983	0.490	1.573	0.410	1.314	0.392	histone H3 putative expressed	
CUST_3907_PI390587928	3.116	0.285	2.505	0.086	3.095	0.079	1.884	0.050	O-methyltransferase ZRP4 putative expressed	AT4G35160.1
CUST_7693_PI390587928	1.471	0.827	1.400	0.707	1.564	0.786	1.472	0.750	apospory-associated protein C putative expressed	AT5G66530.1
CUST_27994_PI390587928	1.461	0.553	1.847	0.416	1.603	0.637	1.547	0.480	ribulose bisphosphate carboxylase/oxygenase activase chloroplast precursor putative expressed	AT2G39730.1
CUST_10581_PI390587928	1.594	0.597	1.476	0.715	1.501	0.700	1.269	0.464	expressed protein	
CUST_15605_PI390587928	1.158	0.700	1.343	0.663	1.489	0.812	1.269	0.695	actin-6 putative expressed	AT5G56180.1
CUST_6516_PI390587928	1.178	0.740	1.251	0.749	1.376	0.826	1.219	0.750	kelch motif family protein expressed	AT1G22040.1
CUST_29581_PI390587928	1.430	0.768	1.351	0.643	1.286	0.616	1.169	0.599	expressed protein	AT3G60660.1
CUST_7538_PI390587928	1.269	0.830	1.308	0.762	1.397	0.687	1.215	0.588	auxin-repressed protein putative expressed	
CUST_19422_PI390587928	1.122	0.697	1.109	0.772	1.111	0.685	1.243	0.710	expressed protein	AT2G26770.2
CUST_20616_PI390587928	1.245	0.816	1.335	0.856	1.445	0.788	1.285	0.660	zinc finger C2H2 type family protein expressed	AT5G52010.1
CUST_24386_PI390587928	1.490	0.802	1.216	0.601	1.503	0.871	1.652	0.732	peptidase M48 Ste24p putative expressed	AT3G27110.2
CUST_3567_PI390587928	1.163	0.776	1.163	0.789	1.152	0.708	1.106	0.685	-	
CUST_33570_PI390587928	1.390	0.851	1.302	0.604	1.328	0.505	1.206	0.514	CENP-E like kinetochore protein putative expressed	AT2G30500.1
CUST_18687_PI390587928	1.441	0.774	1.504	0.774	1.516	0.694	1.311	0.770	-	
CUST_7384_PI390587928	2.060	0.370	1.713	0.229	1.815	0.353	1.608	0.202	cytochrome P450 87A3 putative expressed	AT1G12740.1
CUST_41344_PI390587928	1.655	0.347	1.992	0.386	2.625	0.358	2.671	0.403	-	
CUST_29256_PI390587928	1.192	0.762	1.183	0.928	1.328	0.884	1.269	0.848	-	
CUST_22017_PI390587928	1.141	0.727	1.093	0.641	1.333	0.673	1.256	0.685	expressed protein	
CUST_6223_PI390587928	1.761	0.660	1.498	0.520	1.570	0.569	1.380	0.500	cytochrome c oxidase polypeptide Vc putative expressed	AT5G40382.1
CUST_4466_PI390587928	1.338	0.766	1.402	0.839	1.389	0.839	1.519	0.916	chromatin complex subunit A101 putative expressed	AT5G66750.1
CUST_13385_PI390587928	1.257	0.837	1.237	0.766	1.543	0.943	1.276	0.683	expressed protein	
CUST_10724_PI390587928	1.764	0.688	1.949	0.662	1.581	0.804	1.552	0.621	sigma factor sigB regulation protein rsbQ putative expressed	AT3G24420.1
CUST_26961_PI390587928	1.176	0.773	1.218	0.854	1.242	0.878	1.174	0.718	-	
CUST_18239_PI390587928	1.250	0.623	1.290	0.608	1.471	0.717	1.351	0.601	transport inhibitor response 1 protein putative expressed	AT3G26810.1
CUST_31956_PI390587928	1.502	0.793	1.305	0.822	1.276	0.789	1.233	0.743	membrane protein putative expressed	AT1G54520.1
CUST_8160_PI390587928	1.301	0.861	1.262	0.757	1.282	0.747	1.247	0.842	novel plant SNARE 11 putative expressed	AT3G17440.1
CUST_9166_PI390587928	1.335	0.748	1.359	0.656	1.622	0.744	1.581	0.752	F-box protein family AtFBL4 putative expressed	AT5G23340.1
CUST_11592_PI390587928	1.211	0.848	1.355	0.902	1.239	0.713	1.216	0.789	expressed protein	AT5G57460.1
CUST_14_PI390587928	1.294	0.696	1.299	0.660	1.568	0.744	1.449	0.649	transposon protein putative unclassified	
CUST_32978_PI390587928	1.195	0.746	1.212	0.630	1.148	0.653	1.193	0.738	HEAT repeat family protein expressed	AT5G14790.1
CUST_3742_PI390587928	1.186	0.734	1.256	0.685	1.298	0.663	1.153	0.852	glyoxylate reductase putative expressed	AT1G79870.1
CUST_15897_PI390587928	1.509	0.497	1.547	0.369	1.954	0.504	1.502	0.362	-	
CUST_26061_PI390587928	1.777	0.486	1.685	0.692	1.489	0.690	1.785	0.573	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor putative expressed	
CUST_20419_PI390587928	1.287	0.843	1.231	0.842	1.324	0.827	1.305	0.806	CCR4-NOT transcription complex subunit 3 putative expressed	
CUST_19745_PI390587928	1.429	0.782	1.377	0.732	1.259	0.633	1.109	0.557	acetyl-coenzyme A carboxylase putative expressed	AT1G36160.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_2411_PI390587928	2.290	0.700	1.867	0.721	3.326	0.560	2.644	0.684	C-4 methylsterol oxidase putative expressed	AT4G12110.1
CUST_2884_PI390587928	2.125	0.889	1.510	0.637	2.191	0.876	1.856	0.804	-	
CUST_15790_PI390587928	1.380	0.637	1.467	0.725	1.285	0.636	1.355	0.466	receptor-like protein kinase 5 precursor putative expressed	AT1G28440.1
CUST_410_PI390587928	1.539	0.761	1.433	0.692	1.339	0.650	1.302	0.628	expressed protein	
CUST_36533_PI390587928	1.711	0.822	1.423	0.657	1.393	0.714	1.325	0.528	BSD domain containing protein expressed	
CUST_23580_PI390587928	1.173	0.683	1.136	0.698	1.137	0.729	1.115	0.680	polyamine ABC transporter periplasmic polyamine-binding protein putative expressed	AT1G31410.1
CUST_22372_PI390587928	1.456	0.685	1.870	0.834	1.250	0.641	1.411	0.678	cytokinin-O-glucosyltransferase 2 putative expressed	AT2G28080.1
CUST_19452_PI390587928	1.333	0.763	1.239	0.610	1.303	0.705	1.282	0.717	ubiquitin-conjugating enzyme E2-17 kDa putative expressed	AT3G08690.1
CUST_2575_PI390587928	1.149	0.783	1.184	0.930	1.248	0.853	1.162	0.765	-	
CUST_12043_PI390587928	1.140	0.679	1.425	0.586	1.511	0.707	1.455	0.650	retrotransposon protein putative unclassified expressed	ATMG00860.1
CUST_1414_PI390587928	1.277	0.809	1.330	0.798	1.380	0.714	1.254	0.664	-	
CUST_16534_PI390587928	1.509	0.381	1.471	0.311	1.507	0.434	1.669	0.215	chlorophyll a-b binding protein chloroplast precursor putative expressed	AT3G27690.1
CUST_26034_PI390587928	1.247	0.825	1.395	0.841	1.299	0.769	1.133	0.803	-	
CUST_14320_PI390587928	1.338	0.518	1.251	0.456	1.617	0.675	1.516	0.501	serine/threonine protein kinase putative expressed	AT4G38830.1
CUST_7461_PI390587928	1.354	0.636	1.364	0.509	1.527	0.746	1.217	0.747	expressed protein	
CUST_26050_PI390587928	1.339	0.856	1.208	0.790	1.346	0.817	1.319	0.763	myrosinase precursor putative expressed	
CUST_27072_PI390587928	1.547	0.273	1.367	0.295	1.855	0.240	1.422	0.185	histone H1 putative expressed	
CUST_10924_PI390587928	1.362	0.561	1.430	0.462	1.360	0.630	1.413	0.450	peptidyl-prolyl cis-trans isomerase NIMA-interacting 4 putative expressed	AT1G26550.1
CUST_10940_PI390587928	1.665	0.545	1.348	0.550	1.646	0.539	1.727	0.557	-	
CUST_31061_PI390587928	1.385	0.790	1.434	0.726	1.436	0.767	1.228	0.778	F-box only protein 9 putative expressed	AT1G21760.1
CUST_41251_PI390587928	1.518	0.688	1.590	0.475	1.709	0.615	1.235	0.473	6-phosphogluconolactonase putative expressed	AT1G13700.1
CUST_25362_PI390587928	1.285	0.747	1.285	0.833	1.381	0.858	1.227	0.809	-	
CUST_36101_PI390587928	1.363	0.625	1.472	0.722	1.491	0.745	1.468	0.576	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor putative expressed	AT5G10290.1
CUST_1681_PI390587928	1.147	0.748	1.196	0.765	1.355	0.768	1.266	0.695	expressed protein	
CUST_30948_PI390587928	1.904	0.419	2.011	0.256	1.832	0.294	1.389	0.210	protein ABIL1 putative expressed	AT2G46225.1
CUST_15033_PI390587928	1.307	0.846	1.396	0.824	1.193	0.697	1.098	0.804	gb protein putative expressed	AT5G18250.1
CUST_20790_PI390587928	1.235	0.722	1.329	0.814	1.191	0.702	1.081	0.596	tRNA 2phosphotransferase putative expressed	AT2G45330.2
CUST_20805_PI390587928	4.571	0.035	8.585	0.057	6.692	0.118	6.297	0.137	cytokinin-O-glucosyltransferase 2 putative expressed	AT3G02100.1
CUST_4187_PI390587928	1.320	0.630	1.291	0.594	1.577	0.746	1.381	0.611	-	
CUST_26836_PI390587928	1.200	0.844	1.108	0.781	1.106	0.720	1.186	0.835	ras-related protein RHN1 putative expressed	AT5G45130.1
CUST_34019_PI390587928	1.367	0.674	1.369	0.559	1.354	0.659	1.184	0.576	glycyl-tRNA synthetase 1 mitochondrial precursor putative expressed	AT1G29880.1
CUST_19945_PI390587928	1.159	0.725	1.142	0.776	1.152	0.714	1.076	0.697	membrane-associated 30 kDa protein chloroplast precursor putative expressed	AT1G65260.1
CUST_3617_PI390587928	1.303	0.716	1.349	0.609	1.556	0.682	1.472	0.687	coronatine-insensitive protein 1 putative expressed	AT2G39940.1
CUST_24452_PI390587928	1.636	0.464	1.828	0.435	1.748	0.453	1.519	0.413	expressed protein	AT1G75100.1
CUST_3633_PI390587928	1.286	0.625	1.049	0.409	1.371	0.508	1.349	0.555	receptor-like kinase ARK1AS putative expressed	AT5G38280.1
CUST_21720_PI390587928	1.793	0.560	1.577	0.388	1.652	0.496	1.350	0.281	plastid-specific 30S ribosomal protein 2 chloroplast precursor putative expressed	AT2G35410.1
CUST_31677_PI390587928	1.561	0.828	1.439	0.757	1.528	0.789	1.496	0.779	OsWRKY78 - Superfamily of rice TFs having WRKY and zinc finger domains expressed	AT4G26640.2
CUST_15788_PI390587928	1.180	0.623	1.170	0.516	1.238	0.548	1.343	0.652	SAM-dependent methyltransferase putative expressed	AT2G26680.1
CUST_5708_PI390587928	1.337	0.828	1.314	0.846	1.282	0.910	1.231	0.762	expressed protein	AT2G46915.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_35509_PI390587928	2.880	0.342	2.768	0.262	2.153	0.301	2.037	0.250	phosphoglucomutase/phosphomannomutase family protein putative expressed	AT1G70820.1
CUST_3510_PI390587928	1.697	0.716	1.413	0.699	1.368	0.558	1.290	0.599	-	
CUST_40489_PI390587928	1.870	0.514	1.451	0.449	1.980	0.407	1.565	0.376	-	
CUST_7311_PI390587928	1.153	0.910	1.272	0.817	1.268	0.866	1.134	0.788	expressed protein	AT1G27510.1
CUST_21364_PI390587928	1.442	0.736	1.446	0.619	1.324	0.694	1.241	0.619	SOUL heme-binding protein expressed	AT5G20140.1
CUST_33109_PI390587928	1.250	0.864	1.193	0.797	1.227	0.824	1.197	0.769	oxysterol binding protein putative expressed	AT1G13170.1
CUST_29656_PI390587928	1.371	0.684	1.451	0.419	2.008	0.697	1.453	0.517	expressed protein	AT1G02070.1
CUST_15006_PI390587928	1.331	0.683	1.196	0.603	1.459	0.792	1.263	0.631	-	
CUST_22993_PI390587928	1.183	0.646	1.338	0.659	1.190	0.617	1.177	0.556	-	
CUST_13856_PI390587928	1.662	0.518	1.631	0.370	1.541	0.483	1.354	0.331	elongation factor TS family protein expressed	AT4G29060.1
CUST_20017_PI390587928	1.410	0.770	1.483	0.729	1.298	0.770	1.258	0.728	calcineurin B-like protein 10 putative expressed	
CUST_12711_PI390587928	1.693	0.238	2.031	0.148	3.129	0.290	3.346	0.423	peptide transporter PTR2 putative expressed	AT1G32450.1
CUST_9062_PI390587928	1.587	0.638	1.534	0.565	1.481	0.636	1.336	0.536	expressed protein	AT5G28500.1
CUST_1865_PI390587928	1.285	0.766	1.374	0.773	1.516	0.642	1.401	0.542	calcium binding atopy-related autoantigen 1 putative expressed	AT4G32060.1
CUST_6314_PI390587928	1.205	0.631	1.153	0.571	1.258	0.694	1.165	0.587	expressed protein	AT1G78420.1
CUST_4100_PI390587928	1.676	0.660	1.280	0.454	1.374	0.595	1.443	0.582	glutamine amidotransferase subunit pdxT putative expressed	AT5G60540.1
CUST_33927_PI390587928	1.215	0.670	1.086	0.578	1.370	0.685	1.241	0.701	-	
CUST_32719_PI390587928	1.361	0.796	1.219	0.746	1.441	0.775	1.371	0.772	10-deacetylbaccatin III 10-O-acetyltransferase putative expressed	
CUST_39197_PI390587928	1.837	0.637	1.997	0.451	2.107	0.507	1.382	0.168	OsGrx_C6 - glutaredoxin subgroup III	AT4G15700.1
CUST_763_PI390587928	1.354	0.641	1.752	0.686	1.703	0.750	1.491	0.790	major Facilitator Superfamily protein expressed	AT5G45275.1
CUST_13238_PI390587928	1.382	0.554	1.287	0.483	1.319	0.579	1.310	0.477	-	
CUST_16723_PI390587928	1.274	0.682	1.258	0.626	1.278	0.753	1.174	0.580	vegetative storage protein putative expressed	AT3G57430.1
CUST_40339_PI390587928	1.657	0.942	1.303	0.640	1.428	0.731	1.394	0.693	cell division protein ftsY putative expressed	AT2G45770.1
CUST_11497_PI390587928	1.366	0.452	1.415	0.458	1.538	0.545	1.407	0.428	hhH-GPD superfamily base excision DNA repair protein expressed	
CUST_33634_PI390587928	1.245	0.759	1.301	0.897	1.318	0.740	1.297	0.764	-	
CUST_32395_PI390587928	1.309	0.590	1.112	0.551	1.561	0.571	1.381	0.462	universal stress protein family protein expressed	AT3G17020.1
CUST_36413_PI390587928	1.426	0.485	1.477	0.374	1.536	0.542	1.297	0.382	leaf protein putative expressed	AT5G25630.1
CUST_5877_PI390587928	1.316	0.924	1.124	0.825	1.136	0.786	1.130	0.887	signal transducer putative expressed	AT5G54200.1
CUST_31420_PI390587928	1.455	0.579	1.526	0.649	1.514	0.528	1.604	0.522	expressed protein	
CUST_640_PI390587928	1.319	0.493	1.274	0.296	1.699	0.478	1.564	0.362	tubulin alpha-3 chain putative expressed	AT4G14960.2
CUST_22066_PI390587928	1.663	0.411	1.838	0.426	1.925	0.341	1.932	0.337	expressed protein	
CUST_15361_PI390587928	1.396	0.767	1.138	0.619	1.349	0.677	1.455	0.694	acid phosphatase putative expressed	
CUST_33278_PI390587928	1.294	0.733	1.375	0.610	1.564	0.719	1.368	0.593	ATP binding protein putative expressed	
CUST_14153_PI390587928	2.495	0.625	2.546	0.660	1.461	0.572	1.806	0.592	EBNA1 putative	
CUST_23135_PI390587928	1.462	0.753	1.374	0.701	1.418	0.700	1.309	0.654	bile acid sodium symporter putative expressed	
CUST_20372_PI390587928	1.486	0.631	1.284	0.420	1.405	0.635	1.359	0.537	cytochrome P450 97B2 putative expressed	AT3G53130.1
CUST_5568_PI390587928	1.225	0.559	1.466	0.495	1.400	0.418	1.375	0.445	phosphoethanolamine N-methyltransferase putative expressed	AT3G18000.1
CUST_35369_PI390587928	1.405	0.605	1.279	0.520	1.430	0.639	1.278	0.419	expressed protein	
CUST_37142_PI390587928	1.426	0.818	1.380	0.710	1.435	0.761	1.317	0.719	peroxin Pex14 putative expressed	
CUST_19023_PI390587928	1.258	0.632	1.334	0.501	1.317	0.565	1.208	0.463	nodulin-like protein putative expressed	AT1G80530.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_26450_PI390587928	1.343	0.617	1.563	0.457	1.483	0.672	1.344	0.531	ferredoxin--NADP reductase leaf isozyme chloroplast precursor putative expressed	AT5G66190.1
CUST_27456_PI390587928	1.194	0.739	1.253	0.803	1.251	0.679	1.155	0.812	-	AT1G21790.1
CUST_4967_PI390587928	1.584	0.720	1.303	0.563	1.269	0.778	1.164	0.550	polynucleotide phosphorylase putative expressed	AT3G03710.1
CUST_27472_PI390587928	1.152	0.517	1.222	0.530	1.408	0.745	1.310	0.543	rab GDP dissociation inhibitor alpha putative expressed	AT2G44100.2
CUST_36438_PI390587928	2.274	0.615	2.298	0.525	1.769	0.486	1.433	0.289	serine-rich protein putative expressed	
CUST_12678_PI390587928	2.995	0.361	2.586	0.236	2.133	0.092	2.542	0.047	-	
CUST_16712_PI390587928	1.412	0.489	1.285	0.317	1.479	0.549	1.330	0.315	-	
CUST_9076_PI390587928	1.419	0.588	1.641	0.639	1.690	0.681	1.467	0.517	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor putative expressed	AT5G65240.1
CUST_8101_PI390587928	1.351	0.818	1.453	0.718	1.551	0.887	1.450	0.729	-	
CUST_35667_PI390587928	1.455	0.824	1.251	0.628	1.289	0.767	1.211	0.573	50S ribosomal protein L10 putative expressed	AT5G13510.1
CUST_40848_PI390587928	2.714	0.663	1.417	0.373	2.933	0.465	2.878	0.298	caffeic acid 3-O-methyltransferase putative expressed	AT5G54160.1
CUST_9714_PI390587928	1.363	0.876	1.242	0.694	1.431	0.851	1.385	0.795	lycopene beta cyclase chloroplast precursor putative expressed	AT3G10230.2
CUST_7973_PI390587928	1.548	0.714	1.360	0.687	1.350	0.808	1.296	0.741	-	AT3G56160.1
CUST_34336_PI390587928	1.188	0.730	1.229	0.738	1.279	0.727	1.160	0.654	cyclin-T1 putative expressed	AT5G45190.2
CUST_7818_PI390587928	1.845	0.526	1.731	0.486	1.728	0.538	1.386	0.342	-	
CUST_3677_PI390587928	1.181	0.802	1.181	0.873	1.284	0.847	1.303	0.899	recessive suppressor of secretory defect putative expressed	AT3G51830.1
CUST_11101_PI390587928	1.765	0.778	1.570	0.637	1.911	0.777	1.750	0.677	ubiquinone biosynthesis protein ubiB putative expressed	AT1G71810.1
CUST_27291_PI390587928	1.397	0.685	1.307	0.632	1.288	0.584	1.113	0.509	CDK5RAP1-like protein putative expressed	AT4G36390.1
CUST_19362_PI390587928	1.468	0.660	1.456	0.491	1.572	0.551	1.404	0.534	ubiquinone biosynthesis protein ubiB putative expressed	AT1G71810.1
CUST_32286_PI390587928	1.567	0.583	1.619	0.492	1.383	0.455	1.566	0.477	-	
CUST_37295_PI390587928	1.313	0.866	1.246	0.836	1.370	0.842	1.290	0.818	cysteine synthase putative expressed	AT4G14880.2
CUST_22920_PI390587928	1.189	0.700	1.216	0.574	1.326	0.788	1.193	0.732	relA-SpoT like protein RSH4 putative expressed	AT3G17470.1
CUST_11979_PI390587928	1.313	0.819	1.248	0.814	1.182	0.744	1.194	0.742	expressed protein	AT2G02880.1
CUST_4072_PI390587928	1.205	0.604	1.415	0.710	1.160	0.700	1.253	0.709	OsFtsH1 - Oryza sativa FtsH protease homologue of AtFtsH1/5 expressed	AT5G42270.1
CUST_31078_PI390587928	1.300	0.554	1.345	0.518	1.258	0.482	1.219	0.474	expressed protein	
CUST_25379_PI390587928	1.305	0.678	1.354	0.618	1.421	0.667	1.337	0.655	ubiquitin-conjugating enzyme E2-17 kDa putative expressed	AT3G08690.1
CUST_1884_PI390587928	1.365	0.667	1.352	0.608	1.381	0.610	1.265	0.584	mov34/MPN/PAD-1 family protein expressed	AT1G80210.2
CUST_28158_PI390587928	1.163	0.771	1.373	0.755	1.529	0.821	1.440	0.860	disulfide oxidoreductase/ monooxygenase putative expressed	AT1G63370.1
CUST_5094_PI390587928	1.470	0.737	1.087	0.602	1.564	0.745	1.614	0.755	armadillo/beta-catenin-like repeat family protein expressed	AT5G66200.1
CUST_2346_PI390587928	1.618	0.679	1.318	0.569	1.797	0.819	1.570	0.631	coatomer subunit beta putative expressed	AT1G79990.5
CUST_14805_PI390587928	1.114	0.778	1.159	0.721	1.437	0.788	1.217	0.788	expressed protein	AT2G44090.2
CUST_8984_PI390587928	1.263	0.736	1.248	0.713	1.530	0.835	1.616	0.811	enolase 1 putative expressed	
CUST_33185_PI390587928	2.027	0.340	1.664	0.228	2.072	0.266	1.512	0.187	-	AT5G39210.1
CUST_2192_PI390587928	1.428	0.742	1.480	0.720	1.413	0.854	1.167	0.647	-	
CUST_952_PI390587928	1.870	0.744	1.886	0.694	1.507	0.708	1.172	0.464	acid phosphatase putative expressed	AT5G15070.1
CUST_29057_PI390587928	1.503	0.521	1.323	0.298	1.621	0.355	1.488	0.254	rhodanese family protein putative expressed	AT4G24750.1
CUST_8238_PI390587928	1.421	0.916	1.226	0.713	1.263	0.737	1.180	0.827	WRKY transcription factor 3 putative expressed	AT2G03340.1
CUST_10664_PI390587928	1.224	0.529	1.217	0.412	1.385	0.547	1.137	0.364	expressed protein	AT1G28760.1
CUST_15518_PI390587928	1.279	0.685	1.235	0.650	1.365	0.680	1.199	0.568	expressed protein	AT5G06560.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_39093_PI390587928	1.146	0.683	1.245	0.771	1.167	0.780	1.138	0.646	tRNA uridine 5-carboxymethylaminomethyl modification enzyme gidA putative expressed	AT2G13440.1
CUST_38102_PI390587928	1.791	0.514	1.390	0.352	2.015	0.668	1.788	0.425	cysteine synthase putative expressed	AT3G59760.2
CUST_27970_PI390587928	1.536	0.918	1.311	0.705	1.451	0.799	1.141	0.717	-	
CUST_8691_PI390587928	1.251	0.764	1.242	0.787	1.375	0.802	1.208	0.716	MAK16-like protein RBM13 putative expressed	AT5G23860.1
CUST_6949_PI390587928	1.538	0.786	1.898	0.634	1.811	0.613	1.344	0.486	expressed protein	
CUST_2474_PI390587928	1.853	0.741	1.624	0.707	1.335	0.520	1.311	0.443	lactoylglutathione lyase putative expressed	AT1G67280.2
CUST_31757_PI390587928	2.097	0.563	1.863	0.289	2.146	0.680	1.724	0.416	expressed protein	AT1G74880.1
CUST_26592_PI390587928	1.377	0.515	1.435	0.383	1.538	0.349	1.443	0.330	NB-ARC domain containing protein expressed	
CUST_42279_PI390587928	1.157	0.657	1.177	0.572	1.312	0.651	1.135	0.584	-	
CUST_40081_PI390587928	1.229	0.727	1.208	0.705	1.339	0.722	1.210	0.718	splicing factor arginine/serine-rich 7 putative expressed	
CUST_34382_PI390587928	3.650	0.464	2.535	0.146	3.118	0.263	2.429	0.103	plant-specific domain TIGR01568 family protein	
CUST_1143_PI390587928	1.000	0.698	1.419	0.940	1.197	0.719	1.218	0.773	lectin-like receptor kinase 7 putative expressed	AT2G37710.1
CUST_11254_PI390587928	1.239	0.762	1.231	0.528	1.399	0.743	1.305	0.764	zinc finger protein putative expressed	AT2G02960.3
CUST_17269_PI390587928	1.400	0.758	1.213	0.752	1.357	0.832	1.367	0.772	casein kinase II subunit beta-4 putative expressed	AT2G44680.2
CUST_5634_PI390587928	1.272	0.671	1.272	0.640	1.274	0.790	1.172	0.524	expressed protein	AT1G28760.1
CUST_30395_PI390587928	1.688	0.464	1.601	0.287	1.701	0.382	1.540	0.346	microtubule-associated protein MAP65-1a putative expressed	AT1G14690.2
CUST_17285_PI390587928	1.540	0.798	1.448	0.718	1.315	0.626	1.468	0.763	DNA binding protein putative expressed	AT5G52660.2
CUST_12292_PI390587928	1.222	0.684	1.156	0.760	1.483	0.868	1.336	0.825	-	
CUST_7205_PI390587928	1.766	0.310	1.471	0.169	1.642	0.308	1.649	0.147	CMV 1a interacting protein 1 putative expressed	
CUST_36457_PI390587928	1.304	0.795	1.107	0.796	1.286	0.804	1.268	0.725	-	
CUST_19562_PI390587928	1.445	0.513	1.330	0.389	1.290	0.521	1.129	0.468	thiamine biosynthesis protein thiC putative expressed	AT2G29630.1
CUST_28528_PI390587928	1.634	0.609	1.486	0.459	1.580	0.547	1.372	0.391	CIPK-like protein 1 putative expressed	AT5G21326.1
CUST_20082_PI390587928	1.389	0.739	1.355	0.722	1.666	0.885	1.367	0.733	40S ribosomal protein S14 putative expressed	
CUST_26330_PI390587928	1.479	0.495	1.554	0.483	1.508	0.477	1.294	0.373	zinc finger C3HC4 type family protein expressed	AT2G45530.1
CUST_35281_PI390587928	1.453	0.670	1.289	0.682	1.467	0.856	1.432	0.769	nuclear pore protein 84 / 107 containing protein expressed	AT3G14120.2
CUST_19423_PI390587928	2.790	0.415	2.312	0.207	1.756	0.221	1.957	0.194	-	
CUST_12744_PI390587928	1.183	0.521	1.397	0.353	1.257	0.537	1.246	0.434	protein tyrosine/serine/threonine phosphatase putative expressed	AT3G01510.1
CUST_9095_PI390587928	1.199	0.845	1.354	0.813	1.315	0.855	1.235	0.858	ATP binding protein putative expressed	AT2G31010.1
CUST_6347_PI390587928	1.561	0.518	1.194	0.365	1.712	0.706	1.559	0.369	senescence-associated protein DIN1 putative expressed	AT2G17850.1
CUST_30910_PI390587928	1.132	0.802	1.194	0.690	1.131	0.745	1.063	0.759	seed specific protein Bn15D14A putative expressed	AT3G23090.1
CUST_1929_PI390587928	2.075	0.555	1.696	0.616	1.521	0.366	1.900	0.250	-	
CUST_36148_PI390587928	2.080	0.461	1.914	0.337	2.395	0.577	2.073	0.444	XI-I putative expressed	AT4G33200.1
CUST_30724_PI390587928	1.406	0.599	1.312	0.520	1.519	0.710	1.393	0.581	-	
CUST_5746_PI390587928	1.226	0.809	1.165	0.671	1.168	0.675	1.054	0.572	expressed protein	
CUST_19130_PI390587928	1.371	0.612	1.203	0.545	1.367	0.567	1.203	0.458	disulfide oxidoreductase/ monooxygenase/ oxidoreductase putative expressed	AT4G28720.1
CUST_32831_PI390587928	1.122	0.717	1.183	0.813	1.211	0.782	1.086	0.738	expressed protein	AT3G06180.1
CUST_36041_PI390587928	1.154	0.704	1.394	0.650	1.327	0.637	1.356	0.674	OsMPK17-1 - putative MAPK based on amino acid sequence homology expressed	AT3G18040.1
CUST_14479_PI390587928	2.165	0.725	1.826	0.615	1.545	0.656	1.416	0.375	indole-3-acetate beta-glucosyltransferase putative	
CUST_24902_PI390587928	1.618	0.707	1.346	0.398	1.471	0.512	1.259	0.467	expressed protein	AT2G21960.1
CUST_28672_PI390587928	1.329	0.800	1.156	0.739	1.258	0.702	1.182	0.704	selenium-binding protein putative expressed	AT4G14030.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_18513_PI390587928	1.552	0.752	1.367	0.587	1.547	0.685	1.225	0.514	structural constituent of ribosome putative expressed	AT3G20230.1
CUST_28703_PI390587928	1.387	0.617	1.419	0.420	1.317	0.634	1.158	0.513	APE1 putative expressed	AT5G38660.1
CUST_37654_PI390587928	1.610	0.574	1.474	0.391	1.487	0.501	1.369	0.364	-	AT1G20810.1
CUST_25940_PI390587928	2.069	0.536	1.792	0.607	1.770	0.357	1.902	0.235	bifunctional aspartokinase/homoserine dehydrogenase 2 chloroplast precursor putative expressed	AT1G31230.1
CUST_12769_PI390587928	1.673	0.810	1.548	0.812	1.539	0.742	1.533	0.685	expressed protein	
CUST_6661_PI390587928	1.461	0.450	1.651	0.428	1.473	0.425	1.452	0.305	CBS domain containing protein expressed	
CUST_20729_PI390587928	1.450	0.620	1.463	0.443	1.468	0.713	1.513	0.578	protein tyrosine/serine/threonine phosphatase putative expressed	AT3G01510.1
CUST_21263_PI390587928	1.139	0.718	1.112	0.591	1.193	0.692	1.147	0.643	stress regulated protein putative expressed	AT5G27290.1
CUST_8922_PI390587928	1.184	0.627	1.170	0.645	1.249	0.700	1.082	0.542	-	
CUST_14372_PI390587928	1.255	0.725	1.243	0.727	1.155	0.757	1.058	0.727	augmenter of liver regeneration putative expressed	AT1G49880.1
CUST_4765_PI390587928	1.259	0.570	1.321	0.486	1.576	0.638	1.271	0.516	PTI1-like kinase putative expressed	AT3G17410.1
CUST_40249_PI390587928	1.353	0.747	1.292	0.597	1.363	0.623	1.280	0.645	zinc finger protein 511 putative expressed	AT5G14140.1
CUST_38367_PI390587928	1.380	0.689	1.334	0.616	1.605	0.847	1.504	0.619	-	
CUST_36137_PI390587928	1.204	0.784	1.249	0.775	1.332	0.727	1.146	0.765	expressed protein	AT5G40740.1
CUST_24423_PI390587928	1.155	0.900	1.135	0.890	1.183	0.833	1.136	0.820	-	
CUST_10246_PI390587928	1.447	0.461	1.318	0.345	1.722	0.619	1.645	0.481	copper methylamine oxidase precursor putative expressed	AT2G42490.1
CUST_5144_PI390587928	1.336	0.704	1.284	0.712	1.347	0.665	1.317	0.810	histone H1 putative expressed	AT2G30620.1
CUST_10262_PI390587928	1.237	0.681	1.321	0.683	1.265	0.779	1.220	0.760	expressed protein	AT5G03900.2
CUST_15271_PI390587928	1.302	0.853	1.235	0.777	1.279	0.784	1.229	0.797	expressed protein	AT5G45170.1
CUST_37175_PI390587928	1.287	0.677	1.336	0.623	1.374	0.708	1.216	0.641	expressed protein	AT5G55660.1
CUST_2179_PI390587928	1.688	0.677	1.520	0.716	1.455	0.646	1.475	0.760	expressed protein	AT3G02420.1
CUST_34427_PI390587928	1.595	0.727	1.341	0.751	1.641	0.689	1.520	0.686	expressed protein	AT2G36835.1
CUST_17299_PI390587928	1.454	0.466	1.431	0.402	1.628	0.448	1.289	0.328	chlorophyllide a oxygenase chloroplast precursor putative expressed	AT1G44446.1
CUST_19103_PI390587928	1.073	0.848	1.258	0.880	1.182	0.800	1.138	0.867	nodulin-like protein putative expressed	AT1G74780.1
CUST_26328_PI390587928	1.429	0.529	1.443	0.395	1.378	0.495	1.145	0.269	-	AT1G16080.1
CUST_10714_PI390587928	1.414	0.792	1.494	0.727	1.650	0.720	1.431	0.615	TLD family protein expressed	
CUST_41009_PI390587928	1.333	0.739	1.401	0.625	1.225	0.721	1.281	0.634	expressed protein	AT5G19540.1
CUST_11736_PI390587928	1.465	0.485	1.653	0.413	2.245	0.713	1.873	0.493	MYB59 putative expressed	AT3G46130.1
CUST_9326_PI390587928	1.482	0.606	1.501	0.395	1.358	0.615	1.417	0.520	4-nitrophenylphosphatase putative expressed	AT5G36790.2
CUST_25727_PI390587928	1.463	0.781	1.383	0.784	1.513	0.754	1.387	0.707	nucleic acid binding protein putative expressed	AT2G27790.1
CUST_33112_PI390587928	1.227	0.832	1.284	0.822	1.231	0.739	1.217	0.718	snurportin-1 putative expressed	AT4G24880.1
CUST_4147_PI390587928	1.491	0.565	1.464	0.433	1.391	0.544	1.346	0.497	ATA15 protein putative expressed	AT1G66330.2
CUST_4738_PI390587928	1.422	0.391	1.353	0.425	1.370	0.311	1.304	0.340	expressed protein	
CUST_39004_PI390587928	1.382	0.569	1.555	0.536	2.135	0.316	1.967	0.231	cation transporter HKT6 putative expressed	AT4G10310.1
CUST_28872_PI390587928	1.307	0.394	1.590	0.398	1.475	0.549	1.432	0.267	Mg-chelatase subunit XANTHA-F putative expressed	AT5G13630.2
CUST_10219_PI390587928	1.558	0.803	1.368	0.718	1.569	0.623	1.558	0.717	expressed protein	
CUST_25450_PI390587928	3.851	0.442	3.642	0.339	4.607	0.516	2.264	0.303	-	
CUST_30398_PI390587928	1.252	0.584	1.360	0.613	1.575	0.716	1.372	0.605	AP2 domain-containing protein putative expressed	AT1G78080.1
CUST_19751_PI390587928	1.298	0.797	1.185	0.572	1.194	0.666	1.150	0.672	-	AT2G34860.2

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_31653_PI390587928	1.418	0.638	1.263	0.562	1.346	0.645	1.228	0.523	hemimethylated DNA binding domain containing protein	AT2G03390.1
CUST_25264_PI390587928	1.678	0.525	1.739	0.498	1.663	0.363	1.455	0.258	bifunctional aspartokinase/homoserine dehydrogenase 2 chloroplast precursor putative expressed	AT1G31230.1
CUST_39929_PI390587928	1.655	0.848	1.342	0.744	1.512	0.826	1.452	0.700	NADH kinase putative expressed	AT1G78590.1
CUST_30461_PI390587928	1.542	0.766	1.536	0.567	1.317	0.658	1.264	0.466	chlorophyll a-b binding protein 8 chloroplast precursor putative expressed	AT1G61520.1
CUST_25768_PI390587928	1.423	0.848	1.343	0.754	1.349	0.756	1.308	0.791	protein ariadne-1 putative expressed	AT1G65430.1
CUST_15121_PI390587928	1.380	0.716	1.311	0.658	1.299	0.686	1.310	0.609	glutathione reductase chloroplast precursor putative expressed	AT3G54660.1
CUST_21324_PI390587928	1.421	0.476	1.402	0.439	1.712	0.594	1.503	0.463	-	
CUST_7744_PI390587928	1.386	0.588	1.528	0.556	1.534	0.630	1.355	0.524	CP5 putative expressed	AT1G64720.1
CUST_26333_PI390587928	1.550	0.405	1.500	0.251	1.508	0.383	1.262	0.218	nitric-oxide synthase putative expressed	AT3G47450.2
CUST_37561_PI390587928	1.803	0.360	1.354	0.207	3.032	0.368	2.107	0.128	GATA transcription factor 9 putative expressed	AT3G60530.1
CUST_31328_PI390587928	1.914	0.877	1.790	0.827	1.811	0.906	1.642	0.676	glutamyl-tRNA reductase chloroplast precursor putative expressed	AT1G58290.1
CUST_40279_PI390587928	1.335	0.576	1.403	0.483	1.435	0.579	1.280	0.424	-	
CUST_36400_PI390587928	1.210	0.690	1.304	0.679	1.393	0.662	1.214	0.700	splicing factor arginine/serine-rich 7 putative expressed	AT2G24590.1
CUST_32973_PI390587928	1.328	0.667	1.366	0.651	1.494	0.606	1.444	0.531	-	
CUST_25002_PI390587928	1.297	0.824	1.259	0.820	1.416	0.847	1.343	0.796	tRNA modification GTPase trmE putative expressed	
CUST_22814_PI390587928	1.394	0.771	1.222	0.832	1.188	0.713	1.297	0.683	glycogen synthase putative expressed	AT4G18240.1
CUST_10867_PI390587928	1.604	0.370	1.377	0.310	2.014	0.459	1.663	0.363	ribosomal RNA apurinic site specific lyase putative expressed	
CUST_28270_PI390587928	1.394	0.603	1.395	0.608	1.294	0.572	1.162	0.382	glycyl-tRNA synthetase 2 chloroplast/mitochondrial precursor putative expressed	AT3G48110.1
CUST_6227_PI390587928	1.733	0.708	1.573	0.475	1.432	0.592	1.549	0.530	protease Do-like 1 chloroplast precursor putative expressed	AT5G39830.2
CUST_30013_PI390587928	1.286	0.445	1.250	0.228	1.484	0.589	1.374	0.303	AMP binding protein putative expressed	AT1G30520.1
CUST_10697_PI390587928	1.731	0.574	1.663	0.472	1.296	0.509	1.093	0.336	zinc finger C3HC4 type family protein expressed	
CUST_16961_PI390587928	1.333	0.889	1.293	0.685	1.242	0.757	1.103	0.659	expressed protein	AT2G39670.1
CUST_30620_PI390587928	1.446	0.582	1.403	0.554	1.418	0.763	1.204	0.403	-	
CUST_40017_PI390587928	1.271	0.752	1.204	0.593	1.467	0.810	1.273	0.716	expressed protein	AT4G29100.1
CUST_3885_PI390587928	1.412	0.372	1.505	0.389	1.731	0.412	1.380	0.317	S-adenosylmethionine-dependent methyltransferase/ methyltransferase/ thiopurine S-methyltransferase putative expressed	AT2G43940.1
CUST_34909_PI390587928	1.367	0.520	1.472	0.363	1.413	0.477	1.402	0.402	fructose-16-bisphosphatase chloroplast precursor putative expressed	AT5G64380.1
CUST_1485_PI390587928	1.194	0.775	1.159	0.673	1.107	0.708	1.162	0.715	-	
CUST_32509_PI390587928	1.193	0.813	1.112	0.762	1.372	0.891	1.315	0.911	calcium-dependent protein kinase isoform AK1 putative expressed	AT5G04870.1
CUST_25804_PI390587928	1.516	0.719	1.786	0.534	1.322	0.711	1.519	0.634	peroxidase 66 precursor putative expressed	
CUST_36985_PI390587928	1.595	0.698	1.693	0.674	1.461	0.657	1.152	0.707	indole-3-acetic acid-amido synthetase GH3.5 putative expressed	AT2G46370.2
CUST_707_PI390587928	1.331	0.787	1.331	0.801	1.312	0.672	1.165	0.610	expressed protein	AT3G54500.2
CUST_4768_PI390587928	1.168	0.720	1.224	0.870	1.257	0.704	1.150	0.790	F-box domain containing protein expressed	AT3G10240.1
CUST_33547_PI390587928	1.196	0.799	1.303	0.723	1.285	0.818	1.147	0.761	FPA putative expressed	AT2G43410.4
CUST_723_PI390587928	1.146	0.652	1.196	0.659	1.305	0.716	1.187	0.695	methyl-CpG binding domain containing protein expressed	
CUST_25618_PI390587928	1.537	0.726	1.560	0.711	1.421	0.714	1.060	0.456	transposon protein putative unclassified expressed	
CUST_39609_PI390587928	1.172	0.689	1.212	0.763	1.344	0.787	1.236	0.667	jmjC domain containing protein expressed	
CUST_13230_PI390587928	1.434	0.781	1.195	0.547	1.347	0.654	1.178	0.602	aspartyl/glutamyl-tRNA amidotransferase subunit B putative expressed	AT1G48520.1
CUST_17519_PI390587928	1.195	0.767	1.225	0.697	1.213	0.742	1.058	0.688	expressed protein	

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_11287_PI390587928	1.467	0.835	1.415	0.873	1.447	0.913	1.347	0.763	expressed protein	
CUST_20471_PI390587928	1.263	0.610	1.307	0.669	1.539	0.714	1.424	0.570	-	
CUST_9670_PI390587928	1.430	0.606	1.327	0.633	1.494	0.594	1.497	0.558	myrosinase precursor putative expressed	
CUST_19579_PI390587928	1.204	0.725	1.173	0.729	1.253	0.794	1.208	0.706	3-5 exonuclease eri-1 putative expressed	AT3G15140.1
CUST_9515_PI390587928	1.473	0.366	1.498	0.291	1.716	0.222	1.324	0.194	cytochrome P450 72A1 putative expressed	AT2G26710.1
CUST_18620_PI390587928	1.214	0.361	1.935	0.433	1.155	0.248	1.361	0.389	conserved hypothetical protein	
CUST_28104_PI390587928	1.204	0.749	1.286	0.787	1.215	0.789	1.162	0.830	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase chloroplast precursor putative expressed	AT2G26930.1
CUST_41230_PI390587928	1.316	0.809	1.443	0.783	1.287	0.720	1.152	0.677	glycogen synthase putative expressed	AT4G18240.1
CUST_4522_PI390587928	1.451	0.458	1.519	0.335	1.507	0.512	1.589	0.335	oxygen-evolving enhancer protein 3-1 chloroplast precursor putative expressed	AT4G21280.2
CUST_21929_PI390587928	1.218	0.806	1.232	0.677	1.374	0.792	1.195	0.685	ubiquitinating enzyme putative expressed	AT3G17000.1
CUST_5358_PI390587928	2.364	0.527	1.784	0.318	1.730	0.448	1.612	0.274	FAD dependent oxidoreductase putative expressed	AT4G30720.1
CUST_21184_PI390587928	1.338	0.820	1.136	0.768	1.323	0.763	1.313	0.836	complex 1 protein containing protein expressed	AT2G39725.2
CUST_38001_PI390587928	1.321	0.621	1.227	0.652	1.275	0.710	1.083	0.547	protein kinase putative expressed	AT3G57120.1
CUST_4027_PI390587928	1.414	0.535	1.349	0.440	1.366	0.564	1.173	0.341	glycyl-tRNA synthetase 2 chloroplast/mitochondrial precursor putative expressed	AT3G48110.1
CUST_30587_PI390587928	1.423	0.606	1.527	0.554	1.720	0.700	1.560	0.616	lectin-like protein putative expressed	AT4G19840.1
CUST_21652_PI390587928	1.168	0.457	1.353	0.557	1.635	0.397	1.501	0.494	-	
CUST_16959_PI390587928	1.334	0.705	1.589	0.616	1.441	0.646	1.324	0.590	electron transporter/ heat shock protein binding protein putative expressed	AT2G42750.1
CUST_22690_PI390587928	1.246	0.559	1.416	0.559	1.538	0.756	1.363	0.575	nucleoporin putative expressed	
CUST_28145_PI390587928	1.376	0.564	1.355	0.499	1.301	0.719	1.250	0.545	ribulose bisphosphate carboxylase small chain C chloroplast precursor putative expressed	AT1G67090.1
CUST_33886_PI390587928	1.584	0.700	1.502	0.538	1.456	0.660	1.194	0.418	peptidase S16 lon N-terminal putative expressed	
CUST_29960_PI390587928	3.973	0.749	3.040	0.650	1.510	0.294	3.822	0.510	formate dehydrogenase 2 mitochondrial precursor putative expressed	AT5G14780.1
CUST_29167_PI390587928	1.323	0.860	1.256	0.867	1.374	0.845	1.360	0.817	NUP155 putative expressed	AT1G14850.1
CUST_10790_PI390587928	1.251	0.799	1.280	0.947	1.232	0.806	1.170	0.832	RING zinc finger protein-like putative expressed	AT3G29270.2
CUST_10588_PI390587928	1.189	0.750	1.318	0.845	1.150	0.741	1.232	0.670	carboxylic ester hydrolase putative expressed	AT3G09405.1
CUST_37750_PI390587928	1.189	0.839	1.235	0.779	1.251	0.729	1.161	0.759	-	
CUST_4511_PI390587928	1.418	0.784	1.190	0.603	1.169	0.671	1.086	0.492	uncharacterized low-complexity proteins putative expressed	
CUST_7763_PI390587928	1.455	0.704	1.376	0.666	1.227	0.636	1.201	0.690	expressed protein	AT3G48070.2
CUST_22365_PI390587928	2.020	0.575	1.377	0.485	1.518	0.471	1.471	0.346	valyl-tRNA synthetase putative expressed	AT5G16715.1
CUST_23620_PI390587928	1.508	0.704	1.403	0.582	1.356	0.634	1.297	0.556	permeases of the major facilitator superfamily putative expressed	AT5G52970.1
CUST_27405_PI390587928	1.336	0.526	1.399	0.563	1.441	0.706	1.428	0.481	expressed protein	
CUST_31347_PI390587928	1.271	0.734	1.398	0.650	1.314	0.669	1.075	0.546	nuc-1 negative regulatory protein preg putative expressed	AT2G44740.1
CUST_30139_PI390587928	1.634	0.898	1.338	0.562	1.380	0.793	1.299	0.590	expressed protein	
CUST_22195_PI390587928	1.276	0.866	1.421	0.789	1.287	0.746	1.222	0.663	salt tolerance-like protein putative expressed	AT4G38960.1
CUST_15521_PI390587928	1.263	0.671	1.358	0.659	1.370	0.809	1.244	0.520	geranylgeranyl hydrogenase putative expressed	AT1G74470.1
CUST_31208_PI390587928	1.329	0.538	1.396	0.427	1.546	0.426	1.664	0.314	-	
CUST_25509_PI390587928	2.693	0.505	3.009	0.411	2.154	0.429	1.942	0.465	phosphoglucomutase/phosphomannomutase family protein putative expressed	AT1G70820.1
CUST_21050_PI390587928	1.318	0.621	1.307	0.538	1.323	0.657	1.289	0.579	triosephosphate isomerase chloroplast precursor putative expressed	AT2G21170.1
CUST_7470_PI390587928	1.489	0.579	1.450	0.465	1.347	0.492	1.157	0.367	expressed protein	AT1G16080.1
CUST_40206_PI390587928	1.159	0.472	1.247	0.413	1.566	0.395	1.444	0.433	transfactor-like protein putative expressed	AT3G13040.2

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_29326_PI390587928	1.220	0.690	1.263	0.544	1.418	0.716	1.297	0.690	zinc finger CCCH type domain-containing protein ZFN-like 2 putative expressed	AT3G02830.1
CUST_16964_PI390587928	1.352	0.574	1.434	0.578	1.515	0.675	1.257	0.503	-	
CUST_9545_PI390587928	1.466	0.569	1.818	0.422	1.594	0.651	1.550	0.508	ribulose bisphosphate carboxylase/oxygenase activase chloroplast precursor putative expressed	AT2G39730.1
CUST_37085_PI390587928	1.333	0.678	1.228	0.447	1.242	0.688	1.210	0.585	thylakoid membrane phosphoprotein 14 kda chloroplast precursor putative expressed	AT1G52220.1
CUST_20937_PI390587928	1.548	0.598	1.511	0.441	1.527	0.600	1.388	0.500	APE1 putative expressed	AT5G38660.1
CUST_37116_PI390587928	2.331	0.394	2.359	0.243	2.140	0.208	2.080	0.078	1-aminocyclopropane-1-carboxylate oxidase 1 putative expressed	AT1G05010.1
CUST_26984_PI390587928	1.234	0.739	1.448	0.828	1.391	0.883	1.380	0.697	PHD zinc finger protein putative expressed	
CUST_24754_PI390587928	1.300	0.730	1.285	0.766	1.284	0.734	1.204	0.665	peptide transporter PTR2-B putative expressed	AT1G22540.1
CUST_11816_PI390587928	1.807	0.660	1.545	0.534	1.510	0.452	1.394	0.404	-	
CUST_9391_PI390587928	1.800	0.714	1.647	0.583	1.621	0.648	1.592	0.701	naringenin2-oxoglutarate 3-dioxygenase putative expressed	AT4G25310.1
CUST_26798_PI390587928	1.600	0.871	1.598	0.587	1.919	0.503	1.558	0.371	-	
CUST_31979_PI390587928	1.150	0.855	1.171	0.690	1.498	0.872	1.381	0.899	hydroxyacylglutathione hydrolase putative expressed	AT3G10850.1
CUST_135_PI390587928	1.267	0.632	1.302	0.676	1.663	0.696	1.527	0.493	expressed protein	
CUST_31995_PI390587928	1.584	0.629	1.806	0.617	1.792	0.835	1.606	0.822	pirin-like protein putative expressed	AT2G43120.1
CUST_12870_PI390587928	1.167	0.746	1.190	0.745	1.212	0.824	1.241	0.757	-	
CUST_18885_PI390587928	1.219	0.774	1.187	0.675	1.406	0.713	1.255	0.706	-	
CUST_30787_PI390587928	3.021	0.314	2.926	0.299	1.516	0.142	3.102	0.178	formate dehydrogenase 2 mitochondrial precursor putative expressed	AT5G14780.1
CUST_4787_PI390587928	1.177	0.724	1.154	0.800	1.140	0.754	1.214	0.821	-	
CUST_12901_PI390587928	1.414	0.639	1.528	0.630	1.443	0.643	1.360	0.608	-	
CUST_33006_PI390587928	1.242	0.835	1.177	0.784	1.355	0.885	1.298	0.784	-	
CUST_27867_PI390587928	1.151	0.746	1.149	0.774	1.311	0.651	1.342	0.576	3-hydroxy-3-methylglutaryl-coenzyme A reductase 3 putative expressed	AT1G76490.1
CUST_18139_PI390587928	1.396	0.831	1.247	0.635	1.398	0.776	1.321	0.634	FKBP-type peptidyl-prolyl cis-trans isomerase 2 chloroplast precursor putative expressed	AT2G43560.1
CUST_27883_PI390587928	2.158	0.390	1.795	0.315	1.726	0.282	1.689	0.211	geranylgeranyl hydrogenase putative expressed	AT1G74470.1
CUST_21682_PI390587928	1.388	0.404	1.226	0.350	1.243	0.381	1.230	0.215	chlorophyll a-b binding protein chloroplast precursor putative expressed	AT2G05070.1
CUST_16989_PI390587928	1.494	0.455	1.684	0.381	1.326	0.331	2.199	0.405	N-acetyl-gamma-glutamyl-phosphate reductase chloroplast precursor putative expressed	AT2G19940.2
CUST_19768_PI390587928	2.039	0.402	1.693	0.308	2.371	0.305	2.003	0.295	FLP1 putative expressed	AT5G24860.1
CUST_6925_PI390587928	1.815	0.497	1.802	0.338	1.758	0.425	1.716	0.321	expressed protein	AT3G56650.1
CUST_5686_PI390587928	1.435	0.807	1.352	0.746	1.373	0.740	1.263	0.645	mTERF family protein expressed	AT4G02990.1
CUST_29990_PI390587928	1.326	0.852	1.233	0.748	1.203	0.889	1.235	0.767	expressed protein	
CUST_16362_PI390587928	1.542	0.452	1.477	0.495	1.642	0.557	1.391	0.510	expressed protein	
CUST_30494_PI390587928	1.307	0.890	1.225	0.835	1.255	0.805	1.159	0.712	expressed protein	
CUST_1746_PI390587928	1.152	0.721	1.337	0.791	1.213	0.735	1.198	0.703	DNA binding protein putative expressed	AT4G35040.1
CUST_21357_PI390587928	1.441	0.578	1.646	0.466	1.730	0.468	1.794	0.325	-	ATCG00490.1
CUST_42255_PI390587928	1.779	0.833	1.360	0.571	1.429	0.672	1.442	0.627	-	AT5G52970.1
CUST_30541_PI390587928	1.392	0.686	1.326	0.609	1.422	0.790	1.214	0.555	-	
CUST_40498_PI390587928	1.816	0.600	1.527	0.373	1.612	0.519	1.459	0.389	50S ribosomal protein L12-1 chloroplast precursor putative expressed	
CUST_34326_PI390587928	1.265	0.650	1.408	0.577	1.412	0.624	1.183	0.642	sialin putative expressed	AT2G29650.1
CUST_42271_PI390587928	1.428	0.769	1.183	0.577	1.434	0.701	1.185	0.507	expressed protein	AT3G17668.1
CUST_15488_PI390587928	2.002	0.526	1.668	0.668	1.628	0.562	1.981	0.491	-	ATCG01010.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_6399_PI390587928	1.314	0.657	1.283	0.446	1.400	0.589	1.309	0.491	GMN10 putative expressed	AT5G22830.1
CUST_5408_PI390587928	1.456	0.732	1.206	0.525	1.286	0.717	1.148	0.499	metalloendopeptidase putative expressed	AT5G05740.2
CUST_9411_PI390587928	1.507	0.678	1.421	0.454	1.521	0.674	1.370	0.536	wound and phytochrome signaling involved receptor like kinase putative expressed	
CUST_23464_PI390587928	1.434	0.802	1.408	0.647	1.215	0.665	1.232	0.650	embryogenesis-associated protein EMB8 putative expressed	AT3G50790.1
CUST_38663_PI390587928	1.327	0.809	1.133	0.727	1.298	0.790	1.237	0.806	aldose reductase putative expressed	AT2G37790.1
CUST_25019_PI390587928	1.484	0.592	1.335	0.418	1.314	0.564	1.405	0.517	APE1 putative expressed	AT5G38660.1
CUST_13912_PI390587928	1.151	0.535	1.389	0.522	1.250	0.459	1.753	0.592	-	
CUST_30823_PI390587928	1.192	0.782	1.376	0.707	1.364	0.695	1.220	0.678	expressed protein	AT5G47860.1
CUST_21080_PI390587928	1.584	0.737	1.380	0.536	1.301	0.648	1.128	0.482	30S ribosomal protein S5 putative expressed	AT2G33800.1
CUST_35055_PI390587928	1.218	0.526	1.425	0.716	1.155	0.538	1.235	0.594	ubiquitin-protein ligase putative expressed	AT5G01720.1
CUST_13726_PI390587928	1.645	0.615	1.449	0.543	1.471	0.622	1.218	0.397	protein kinase domain containing protein expressed	AT3G50380.1
CUST_6292_PI390587928	1.218	0.732	1.210	0.744	1.289	0.746	1.194	0.654	ubiquitin ligase SINAT3 putative expressed	AT3G61790.1
CUST_28925_PI390587928	1.148	0.820	1.218	0.744	1.283	0.795	1.187	0.776	DNA binding protein putative expressed	AT2G03500.1
CUST_5301_PI390587928	1.160	0.788	1.413	0.771	1.410	0.808	1.251	0.835	RING zinc finger protein putative expressed	AT3G06140.1
CUST_37332_PI390587928	1.918	0.680	1.535	0.581	1.939	0.831	1.741	0.583	-	
CUST_36124_PI390587928	1.390	0.778	1.257	0.859	1.440	0.811	1.403	0.803	tubulin-specific chaperone E putative expressed	AT1G71440.1
CUST_30700_PI390587928	2.875	0.338	3.828	0.125	5.744	0.312	4.423	0.191	flavonoid 3-monooxygenase putative expressed	AT2G45550.1
CUST_21989_PI390587928	1.319	0.772	1.158	0.633	1.468	0.764	1.427	0.744	expressed protein	
CUST_8383_PI390587928	1.618	0.549	1.637	0.388	1.553	0.529	1.390	0.413	expressed protein	AT4G01935.1
CUST_6153_PI390587928	1.518	0.662	1.523	0.444	1.321	0.484	1.292	0.446	3-deoxy-manno-octulosonate cytidylyltransferase putative expressed	AT1G53000.1
CUST_15833_PI390587928	1.881	0.604	1.542	0.558	1.862	0.734	1.929	0.593	mitochondrial inner membrane protease subunit 2 putative expressed	AT3G08980.1
CUST_7766_PI390587928	1.260	0.759	1.237	0.600	1.366	0.684	1.262	0.770	endo-13;14-beta-D-glucanase precursor putative expressed	AT3G23570.1
CUST_12411_PI390587928	1.600	0.503	1.517	0.473	1.500	0.500	1.241	0.362	coproporphyrinogen III oxidase chloroplast precursor putative expressed	AT1G03475.1
CUST_28616_PI390587928	3.915	0.483	3.323	0.291	5.455	0.451	2.945	0.385	-	
CUST_6014_PI390587928	1.312	0.742	1.371	0.634	1.455	0.680	1.315	0.685	pterin-4-alpha-carbinolamine dehydratase putative expressed	AT1G29810.1
CUST_31366_PI390587928	1.652	0.691	1.538	0.840	1.263	0.698	1.520	0.667	threonyl-tRNA synthetase putative expressed	AT2G04842.1
CUST_29670_PI390587928	1.248	0.887	1.217	0.698	1.177	0.805	1.156	0.798	aspartyl aminopeptidase putative expressed	AT5G04710.1
CUST_22431_PI390587928	1.284	0.732	1.147	0.652	1.298	0.672	1.154	0.493	-	
CUST_35873_PI390587928	1.356	0.835	1.275	0.793	1.429	0.730	1.350	0.764	seed maturation protein putative expressed	
CUST_36895_PI390587928	1.545	0.498	1.570	0.334	1.629	0.465	1.424	0.369	signal recognition particle 54 kDa protein chloroplast precursor putative expressed	AT5G03940.1
CUST_3672_PI390587928	1.646	0.570	1.580	0.409	1.362	0.416	1.307	0.394	expressed protein	AT2G04790.2
CUST_24506_PI390587928	1.389	0.711	1.277	0.654	1.226	0.659	1.150	0.518	expressed protein	AT1G73170.2
CUST_29988_PI390587928	1.152	0.815	1.226	0.787	1.257	0.656	1.116	0.697	F-box domain containing protein	
CUST_4709_PI390587928	1.132	0.817	1.186	0.874	1.267	0.845	1.166	0.738	expressed protein	AT5G12920.2
CUST_35718_PI390587928	1.211	0.840	1.278	0.808	1.363	0.874	1.219	0.896	-	
CUST_8479_PI390587928	1.432	0.787	1.268	0.657	1.405	0.810	1.285	0.654	expressed protein	AT5G43822.1
CUST_11367_PI390587928	1.497	0.596	1.395	0.526	1.610	0.677	1.365	0.470	RNA polymerase sigma factor rpoD putative expressed	AT1G64860.1
CUST_2511_PI390587928	1.187	0.874	1.221	0.799	1.189	0.780	1.087	0.808	cyclic nucleotide-gated ion channel 14 putative expressed	
CUST_8526_PI390587928	1.455	0.868	1.302	0.748	1.145	0.699	1.144	0.604	GTP-binding protein hflX putative expressed	AT5G57960.1
CUST_2309_PI390587928	1.372	0.802	1.219	0.851	1.291	0.963	1.439	0.842	SWIRM domain containing protein expressed	AT1G21700.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		*+ RS*		mock		*+ RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_27619_PI390587928	1.157	0.708	1.110	0.613	1.192	0.794	1.099	0.735	thioesterase superfamily member 2 putative expressed	AT1G04290.1
CUST_35564_PI390587928	1.327	0.828	1.188	0.752	1.319	0.906	1.248	0.770	tRNA uridine 5-carboxymethylaminomethyl modification enzyme gidA putative expressed	AT2G13440.1
CUST_10206_PI390587928	1.613	0.692	1.361	0.507	1.417	0.647	1.275	0.408	RNA binding protein putative expressed	AT1G76050.2
CUST_9580_PI390587928	1.575	0.577	1.757	0.471	1.983	0.565	2.057	0.681	aquaporin PIP1.1 putative expressed	AT4G23400.1
CUST_36586_PI390587928	1.261	0.590	1.252	0.551	1.316	0.624	1.194	0.505	polycomb protein EZ3 putative expressed	AT4G02020.1
CUST_21994_PI390587928	1.078	0.843	1.312	0.785	1.143	0.726	1.242	0.826	-	AT5G52660.2
CUST_26443_PI390587928	1.321	0.685	1.294	0.607	1.449	0.804	1.304	0.770	sucrose phosphate synthase putative expressed	AT2G35840.2
CUST_324_PI390587928	1.596	0.758	1.357	0.737	1.558	0.651	1.176	0.534	-	
CUST_3970_PI390587928	1.616	0.220	1.863	0.260	2.379	0.306	3.444	0.388	peptide transporter PTR2 putative expressed	AT4G21680.1
CUST_7180_PI390587928	1.235	0.824	1.230	0.695	1.425	0.809	1.257	0.759	phosphatase DCR2 putative expressed	AT5G63140.1
CUST_34186_PI390587928	1.377	0.787	1.381	0.690	1.264	0.642	1.162	0.563	carboxylic ester hydrolase putative expressed	
CUST_8186_PI390587928	1.178	0.848	1.233	0.764	1.247	0.774	1.293	0.832	expressed protein	AT3G27460.1
CUST_20817_PI390587928	1.276	0.595	1.324	0.562	1.470	0.630	1.175	0.456	-	
CUST_4246_PI390587928	1.179	0.804	1.245	0.645	1.645	0.760	1.604	0.826	CESA2 - cellulose synthase expressed	AT5G05170.1
CUST_16130_PI390587928	1.262	0.615	1.258	0.545	1.228	0.559	1.338	0.446	cellulase containing protein expressed	
CUST_14964_PI390587928	1.804	0.692	1.730	0.758	1.217	0.727	1.136	0.461	acid phosphatase putative expressed	AT3G01310.2
CUST_10504_PI390587928	1.968	0.598	1.958	0.539	2.091	0.362	1.297	0.415	MADS-box transcription factor 8 putative expressed	AT4G22950.1
CUST_18190_PI390587928	1.145	0.767	1.245	0.765	1.187	0.717	1.096	0.756	-	AT1G69340.1
CUST_18205_PI390587928	1.743	0.590	2.006	0.516	1.723	0.708	1.161	0.375	HLS1 putative expressed	AT4G37580.1
CUST_31098_PI390587928	1.226	0.800	1.263	0.768	1.377	0.695	1.407	0.860	nucleic acid binding protein putative expressed	AT1G09660.2
CUST_9116_PI390587928	1.237	0.889	1.190	0.856	1.172	0.890	1.087	0.771	OsFtsH7 - Oryza sativa FtsH protease homologue of AtFtsH7 expressed	AT3G47060.1
CUST_18781_PI390587928	1.204	0.723	1.171	0.728	1.314	0.808	1.293	0.808	-	
CUST_6758_PI390587928	1.395	0.722	1.292	0.707	1.479	0.908	1.330	0.691	homogentisic acid geranylgeranyl transferase putative expressed	
CUST_23823_PI390587928	1.230	0.599	1.241	0.567	1.465	0.734	1.258	0.535	serine/threonine-protein kinase NAK putative expressed	AT1G20650.1
CUST_21376_PI390587928	1.498	0.651	1.313	0.572	1.571	0.523	1.562	0.502	omega-6 fatty acid desaturase endoplasmic reticulum isozyme 2 putative expressed	
CUST_5566_PI390587928	2.492	0.570	1.457	0.186	2.106	0.302	1.586	0.138	expressed protein	
CUST_22461_PI390587928	1.355	0.472	1.384	0.406	1.402	0.552	1.409	0.419	-	
CUST_7657_PI390587928	1.324	0.729	1.303	0.684	1.838	0.684	1.450	0.663	vacuolar cation/proton exchanger 1a putative expressed	
CUST_33673_PI390587928	1.263	0.785	1.221	0.795	1.105	0.755	1.130	0.764	tic22 putative expressed	AT4G33350.1
CUST_19339_PI390587928	1.309	0.904	1.114	0.882	1.187	0.842	1.329	0.894	RXW8 putative expressed	AT1G58520.1
CUST_9934_PI390587928	1.114	0.602	1.230	0.702	1.261	0.551	1.329	0.688	-	
CUST_32465_PI390587928	1.441	0.692	1.522	0.574	1.498	0.767	1.270	0.590	OsMPK20-4 - putative MAPK based on amino acid sequence homology expressed	AT2G42880.1
CUST_26123_PI390587928	1.357	0.831	1.151	0.605	1.431	0.732	1.331	0.534	expressed protein	AT2G33180.1
CUST_36313_PI390587928	1.129	0.730	1.458	0.847	1.260	0.725	1.179	0.708	transposon protein putative unclassified expressed	AT3G09880.1
CUST_5087_PI390587928	1.489	0.420	1.448	0.321	1.953	0.431	1.602	0.361	-	
CUST_39581_PI390587928	1.150	0.777	1.336	0.624	1.390	0.805	1.215	0.706	-	
CUST_10796_PI390587928	1.509	0.865	1.326	0.800	1.410	0.687	1.163	0.660	Leucine Rich Repeat family protein expressed	
CUST_14830_PI390587928	1.186	0.707	1.201	0.586	1.313	0.716	1.185	0.704	-	AT4G13150.1
CUST_4399_PI390587928	1.252	0.702	1.156	0.731	1.711	0.755	1.481	0.678	-	
CUST_2962_PI390587928	1.357	0.659	1.262	0.608	1.458	0.719	1.412	0.645	expressed protein	AT2G21960.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		+ *RS*		**mock**		+ *RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_34977_PI390587928	1.607	0.465	2.329	0.415	2.292	0.630	2.085	0.653	beta-galactosidase precursor putative expressed	AT2G16730.1
CUST_17289_PI390587928	1.275	0.740	1.311	0.671	1.327	0.614	1.221	0.593	expressed protein	
CUST_25249_PI390587928	1.284	0.847	1.310	0.904	1.363	0.801	1.191	0.805	-	
CUST_86_PI390587928	1.571	0.252	3.302	0.439	1.980	0.101	2.862	0.185	CBS domain containing protein expressed	
CUST_5011_PI390587928	1.547	0.834	1.641	0.737	1.753	0.874	1.289	0.576	MTN3 putative expressed	AT3G48740.1
CUST_35269_PI390587928	1.269	0.464	1.201	0.288	2.179	0.481	1.854	0.555	CBL-interacting serine/threonine-protein kinase 15 putative expressed	AT4G14580.1
CUST_16719_PI390587928	1.303	0.833	1.300	0.783	1.267	0.778	1.198	0.777	chloroplast outer envelope protein 86 putative expressed	AT5G20300.3
CUST_775_PI390587928	1.263	0.874	1.205	0.818	1.330	0.720	1.134	0.625	RING finger protein 13 putative expressed	AT1G71980.1
CUST_14978_PI390587928	1.349	0.932	1.061	0.635	1.155	0.689	1.216	0.765	polygalacturonase putative expressed	AT3G62110.1
CUST_35876_PI390587928	1.537	0.650	1.403	0.502	1.381	0.601	1.319	0.624	JD1 putative expressed	AT1G80950.1
CUST_17757_PI390587928	1.887	0.646	2.021	0.589	1.650	0.464	1.886	0.531	dehydration responsive element binding protein putative expressed	AT4G06746.1
CUST_8108_PI390587928	1.666	0.456	1.373	0.254	1.604	0.294	1.235	0.199	expressed protein	AT1G35910.1
CUST_11556_PI390587928	1.395	0.767	1.236	0.711	1.312	0.712	1.364	0.739	pRGR1 putative expressed	AT5G23550.1
CUST_16565_PI390587928	1.402	0.607	1.430	0.633	1.741	0.596	1.309	0.545	aspartic proteinase nepenthesin-2 precursor putative expressed	AT3G12700.1
CUST_39708_PI390587928	1.430	0.425	1.360	0.412	1.484	0.368	1.400	0.259	SET domain containing protein expressed	AT5G14260.2
CUST_39570_PI390587928	1.887	0.508	1.689	0.291	1.732	0.356	1.458	0.233	FK506 binding protein putative expressed	AT4G26555.1
CUST_513_PI390587928	1.030	0.505	1.230	0.640	1.300	0.649	1.197	0.523	exosome component 10 putative expressed	
CUST_5813_PI390587928	1.557	0.636	1.139	0.477	1.726	0.788	1.341	0.475	-	
CUST_19725_PI390587928	4.006	0.420	3.096	0.120	2.799	0.257	3.009	0.138	-	
CUST_327_PI390587928	1.519	0.485	1.388	0.375	1.495	0.506	1.340	0.341	expressed protein	AT2G47910.1
CUST_16272_PI390587928	1.440	0.865	1.371	0.705	1.396	0.746	1.336	0.699	plastid-lipid-associated protein 2 chloroplast precursor putative expressed	AT2G46910.1
CUST_36652_PI390587928	1.324	0.592	1.479	0.556	1.653	0.676	1.269	0.422	copine-8 putative expressed	
CUST_12285_PI390587928	1.525	0.625	1.398	0.638	1.279	0.623	1.189	0.493	gtk16 protein putative expressed	AT1G01760.1
CUST_36466_PI390587928	1.650	0.645	1.472	0.528	1.809	0.512	1.604	0.406	-	AT4G32060.2
CUST_75_PI390587928	1.296	0.652	1.355	0.711	1.665	0.671	1.620	0.628	-	
CUST_4249_PI390587928	1.223	0.530	1.296	0.395	1.341	0.448	1.208	0.430	DNA-directed RNA polymerase II 23 kDa polypeptide putative expressed	AT3G57080.1
CUST_19385_PI390587928	1.153	0.806	1.127	0.756	1.151	0.801	1.224	0.868	dnaJ homolog subfamily C member 8 putative expressed	AT5G22080.1
CUST_35290_PI390587928	1.326	0.680	1.314	0.694	1.442	0.704	1.176	0.623	-	
CUST_22352_PI390587928	1.170	0.676	1.271	0.767	1.359	0.802	1.179	0.721	-	
CUST_21921_PI390587928	1.527	0.551	1.290	0.429	1.304	0.587	1.341	0.422	PAP fibrillin family protein expressed	AT1G51110.1
CUST_5350_PI390587928	1.173	0.743	1.297	0.644	1.240	0.809	1.182	0.756	expressed protein	AT5G03900.2
CUST_36950_PI390587928	1.722	0.450	1.489	0.315	1.469	0.362	1.254	0.279	pentatricopeptide repeat protein PPR1106-17 putative expressed	AT4G18750.1
CUST_35151_PI390587928	1.138	0.892	1.131	0.803	1.307	0.914	1.201	0.986	phosphatidylinositol transfer protein alpha isoform putative expressed	
CUST_40099_PI390587928	1.270	0.673	1.331	0.563	1.291	0.642	1.150	0.602	D-xylose-proton symporter putative expressed	AT1G05030.1
CUST_7985_PI390587928	1.222	0.721	1.189	0.586	1.432	0.682	1.303	0.658	minor histocompatibility antigen H13 putative expressed	AT2G03120.1
CUST_29266_PI390587928	1.560	0.731	1.328	0.812	1.507	0.710	1.618	0.694	-	
CUST_1193_PI390587928	1.438	0.692	1.350	0.665	1.498	0.703	1.253	0.567	expressed protein	
CUST_33799_PI390587928	1.381	0.716	1.266	0.564	1.356	0.614	1.265	0.618	expressed protein	
CUST_22650_PI390587928	1.043	0.766	1.096	0.746	1.082	0.696	1.134	0.780	-	AT2G16405.1
CUST_5616_PI390587928	1.509	0.357	1.491	0.319	1.579	0.348	1.743	0.200	chlorophyll a-b binding protein 2 chloroplast precursor putative expressed	AT2G34430.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_8884_PI390587928	1.427	0.684	1.308	0.563	1.296	0.637	1.144	0.538	-	
CUST_26955_PI390587928	1.545	0.691	1.160	0.601	1.244	0.721	1.396	0.559	sterol-4-alpha-carboxylate 3-dehydrogenase decarboxylating putative expressed	AT2G26260.1
CUST_24539_PI390587928	1.487	0.697	1.607	0.702	1.745	0.811	1.693	0.712	MYND finger family protein expressed	
CUST_37493_PI390587928	1.349	0.802	1.218	0.588	1.555	0.713	1.490	0.673	S-adenosylmethionine-dependent methyltransferase putative expressed	AT1G78140.1
CUST_2730_PI390587928	1.610	0.678	1.169	0.490	1.654	0.783	1.667	0.529	structural molecule putative expressed	AT5G09820.2
CUST_3736_PI390587928	1.377	0.820	1.386	0.690	1.162	0.840	1.180	0.688	14-3-3-like protein A putative expressed	AT1G78300.1
CUST_19405_PI390587928	1.234	0.586	1.270	0.567	1.389	0.768	1.198	0.574	TPR Domain containing protein expressed	
CUST_35767_PI390587928	1.270	0.898	1.293	0.686	1.243	0.814	1.244	0.707	rubredoxin family protein expressed	AT5G17170.1
CUST_17648_PI390587928	1.279	0.705	1.249	0.529	1.378	0.771	1.344	0.681	expressed protein	AT5G54290.1
CUST_32298_PI390587928	1.215	0.710	1.219	0.704	1.441	0.801	1.436	0.676	-	
CUST_27838_PI390587928	1.281	0.780	1.219	0.691	1.210	0.773	1.256	0.641	expressed protein	
CUST_19894_PI390587928	1.490	0.512	1.419	0.328	1.334	0.508	1.358	0.391	phosphoglycerate mutase putative expressed	AT5G22620.2
CUST_9814_PI390587928	1.415	0.794	1.210	0.739	1.299	0.754	1.278	0.745	RING zinc finger protein-like putative expressed	AT3G29270.2
CUST_18686_PI390587928	1.454	0.584	1.261	0.568	1.505	0.710	1.485	0.596	aspartic proteinase nepenthesin-2 precursor putative expressed	AT5G22850.1
CUST_22171_PI390587928	1.328	0.861	1.472	0.949	1.147	0.703	1.231	0.777	protein kinase APK1A chloroplast precursor putative expressed	AT3G09830.2
CUST_37401_PI390587928	1.493	0.796	1.383	0.636	1.408	0.832	1.269	0.659	expressed protein	AT1G64770.1
CUST_27684_PI390587928	1.285	0.873	1.304	0.699	1.327	0.870	1.279	0.838	ferredoxin-thioredoxin reductase catalytic chain chloroplast precursor putative expressed	AT2G04700.1
CUST_4635_PI390587928	1.522	0.475	1.294	0.287	1.425	0.408	1.424	0.361	chaperone protein dnaJ 10 putative expressed	AT1G77020.1
CUST_6191_PI390587928	1.247	0.663	1.292	0.640	1.441	0.648	1.280	0.574	-	
CUST_7197_PI390587928	1.448	0.816	1.216	0.734	1.173	0.720	1.240	0.758	SHL1 putative expressed	AT4G22140.2
CUST_11868_PI390587928	1.342	0.608	1.260	0.597	1.357	0.631	1.227	0.511	-	
CUST_9458_PI390587928	1.493	0.738	1.256	0.474	1.741	0.648	1.471	0.478	IN2-1 protein putative expressed	AT3G55040.1
CUST_31014_PI390587928	1.590	0.442	1.607	0.292	1.465	0.420	1.348	0.309	NADH dehydrogenase I subunit N putative expressed	AT5G58260.1
CUST_41220_PI390587928	1.701	0.500	1.744	0.446	1.527	0.381	1.189	0.281	protein ABIL1 putative expressed	AT2G46225.2
CUST_24900_PI390587928	1.122	0.716	1.119	0.792	1.214	0.797	1.189	0.719	OsPDIL1-4 - Oryza sativa protein disulfide isomerase expressed	AT3G54960.2
CUST_17169_PI390587928	1.665	0.632	1.529	0.526	1.563	0.591	1.459	0.491	phototropin-1 putative expressed	AT5G58140.1
CUST_38300_PI390587928	1.391	0.619	1.319	0.603	1.432	0.628	1.314	0.465	protein kinase PVPK-1 putative expressed	AT2G44830.1
CUST_11729_PI390587928	1.203	0.544	1.280	0.482	1.642	0.427	1.601	0.367	-	
CUST_4295_PI390587928	1.605	0.545	1.569	0.369	1.327	0.495	1.210	0.387	deoxyribodipyrimidine photolyase family protein expressed	AT4G25290.1
CUST_26928_PI390587928	1.263	0.672	1.170	0.686	1.251	0.711	1.147	0.599	-	
CUST_35351_PI390587928	1.400	0.769	1.307	0.559	1.385	0.692	1.195	0.661	expressed protein	AT3G07310.1
CUST_8920_PI390587928	1.163	0.633	1.144	0.646	1.205	0.690	1.103	0.573	expressed protein	
CUST_25752_PI390587928	1.356	0.729	1.270	0.693	1.380	0.801	1.384	0.721	expressed protein	
CUST_11015_PI390587928	1.339	0.594	1.256	0.543	1.418	0.657	1.216	0.475	-	
CUST_4156_PI390587928	1.411	0.582	1.333	0.467	1.557	0.457	1.817	0.466	aldose reductase putative expressed	AT2G37790.1
CUST_12037_PI390587928	1.496	0.699	1.503	0.746	1.659	0.836	1.345	0.545	-	
CUST_20557_PI390587928	1.381	0.661	1.284	0.615	1.445	0.700	1.375	0.642	expressed protein	AT2G21960.1
CUST_35756_PI390587928	1.424	0.584	1.637	0.534	1.630	0.489	1.756	0.311	strictosidine synthase precursor putative expressed	AT5G22020.1
CUST_35026_PI390587928	1.537	0.756	1.529	0.760	1.353	0.553	1.321	0.550	mitochondrial carrier-like protein putative expressed	AT1G72820.1
CUST_12458_PI390587928	1.535	0.386	1.361	0.366	2.448	0.434	2.438	0.325	-	

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	2 days				4 days				Hit description	Top TAIR9 protein hit
	Mock		+ *RS*		mock		+ *RS*			
	C	-K	C	-K	C	-K	C	-K		
CUST_16965_PI390587928	1.682	0.449	1.536	0.453	1.760	0.673	1.719	0.527	-	
CUST_38869_PI390587928	1.549	0.750	1.263	0.792	1.586	0.848	1.406	0.751	RAB5-interacting protein isoform a putative expressed	AT5G59410.1
CUST_12288_PI390587928	1.595	0.730	1.351	0.607	1.963	0.870	1.635	0.634	FIP1 putative expressed	AT1G28200.1
CUST_3889_PI390587928	3.496	0.262	2.726	0.280	1.718	0.136	3.056	0.108	cyanate hydratase putative expressed	
CUST_12319_PI390587928	1.385	0.625	1.319	0.669	1.357	0.669	1.440	0.578	aspartic proteinase nepenthesin-1 precursor putative expressed	AT2G03200.1
CUST_19574_PI390587928	1.478	0.752	1.134	0.588	1.552	0.749	1.505	0.686	3-5 exonuclease eri-1 putative expressed	
CUST_12335_PI390587928	1.306	0.702	1.347	0.728	1.462	0.755	1.239	0.608	protein tyrosine phosphatase putative expressed	AT3G52180.1
CUST_36485_PI390587928	1.124	0.787	1.133	0.810	1.135	0.745	1.175	0.776	-	
CUST_8961_PI390587928	1.433	0.818	1.250	0.673	1.380	0.743	1.363	0.724	agenet domain containing protein expressed	AT5G52070.1
CUST_32731_PI390587928	1.508	0.898	1.317	0.670	1.333	0.760	1.214	0.626	chloroplast 30S ribosomal protein S10 putative expressed	AT3G13120.1
CUST_42231_PI390587928	1.409	0.553	1.227	0.443	1.382	0.604	1.366	0.518	retrotransposon protein putative unclassified	AT4G38180.1
CUST_27348_PI390587928	1.567	0.554	1.416	0.410	1.471	0.540	1.208	0.362	expressed protein	AT3G29290.1
CUST_17190_PI390587928	1.290	0.943	1.248	0.826	1.282	0.806	1.137	0.778	expressed protein	
CUST_2574_PI390587928	1.308	0.595	1.372	0.753	1.375	0.812	1.238	0.519	expressed protein	AT1G23170.1
CUST_23424_PI390587928	1.222	0.814	1.175	0.749	1.266	0.852	1.226	0.747	expressed protein	
CUST_40335_PI390587928	1.390	0.672	1.439	0.663	1.654	0.742	1.467	0.633	-	
CUST_17035_PI390587928	2.663	0.172	2.228	0.132	2.365	0.090	1.899	0.072	amino acid carrier putative expressed	AT1G44100.1
CUST_6391_PI390587928	1.451	0.661	1.388	0.538	1.175	0.569	1.201	0.553	calmodulin binding protein putative expressed	AT1G74690.1
CUST_19079_PI390587928	1.347	0.613	1.248	0.556	1.260	0.609	1.276	0.513	ATP binding protein putative expressed	AT5G35170.1
CUST_1227_PI390587928	1.607	0.739	1.662	0.662	1.567	0.609	1.521	0.675	seed maturation protein PM23 putative expressed	AT2G14910.1
CUST_27071_PI390587928	1.625	0.715	1.622	0.790	1.894	0.857	1.571	0.687	histone H1 putative expressed	
CUST_32843_PI390587928	1.411	0.738	1.377	0.668	1.339	0.755	1.166	0.588	-	
CUST_24914_PI390587928	1.298	0.822	1.240	0.773	1.434	0.843	1.263	0.717	-	
CUST_41825_PI390587928	1.447	0.668	1.194	0.472	1.526	0.838	1.379	0.574	-	
CUST_23691_PI390587928	1.495	0.647	1.537	0.677	1.392	0.669	1.323	0.564	NAC domain-containing protein 18 putative expressed	
CUST_38921_PI390587928	1.710	0.673	1.392	0.640	1.318	0.581	1.449	0.701	expressed protein	AT3G02420.1
CUST_10567_PI390587928	1.527	0.387	1.599	0.338	1.504	0.462	1.736	0.240	-	
CUST_41655_PI390587928	1.074	0.534	1.319	0.614	1.669	0.469	1.663	0.564	protein kinase putative expressed	
CUST_20773_PI390587928	1.262	0.747	1.321	0.651	1.511	0.676	1.163	0.683	jacalin-like lectin domain containing protein expressed	
CUST_10614_PI390587928	1.138	0.663	1.187	0.654	1.174	0.715	1.113	0.610	expressed protein	
CUST_20804_PI390587928	1.124	0.822	1.231	0.913	1.206	0.901	1.160	0.862	expressed protein	
CUST_25596_PI390587928	1.201	0.849	1.105	0.807	1.148	0.861	1.218	0.869	calcium-binding mitochondrial protein Anon-60Da putative expressed	AT1G65540.1
CUST_16661_PI390587928	1.453	0.611	1.364	0.518	1.673	0.672	1.370	0.711	-	
CUST_10428_PI390587928	1.967	0.549	1.577	0.348	1.652	0.488	1.587	0.295	OsAPx8 - Thylakoid-bound Ascorbate Peroxidase encoding gene expressed	AT1G77490.1
CUST_33572_PI390587928	1.564	0.673	1.452	0.525	1.606	0.760	1.260	0.481	mTERF-like protein putative expressed	AT2G36000.1
CUST_20634_PI390587928	1.669	0.449	1.133	0.275	1.274	0.269	1.297	0.232	AP2 domain transcription factor putative expressed	AT2G41710.1
CUST_35833_PI390587928	1.319	0.835	1.138	0.791	1.146	0.668	1.140	0.620	-	AT1G11290.1
CUST_8594_PI390587928	1.167	0.824	1.215	0.914	1.021	0.780	1.251	0.958	-	
CUST_7603_PI390587928	1.388	0.495	1.566	0.406	1.331	0.634	1.346	0.474	glyceraldehyde-3-phosphate dehydrogenase A chloroplast precursor putative expressed	AT1G12900.3
CUST_2610_PI390587928	1.186	0.793	1.187	0.792	1.196	0.655	1.428	0.674	ATP binding protein putative expressed	AT4G32300.1

Table S4-2 Genes downregulated in response to K-starvation (Continued).

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	Mock		*+ RS*		**mock**		*+ RS*			
	C	**-K**	**C**	**-K**	**C**	**-K**	**C**	**-K**		
CUST_27920_PI390587928	1.424	0.649	1.389	0.701	1.431	0.676	1.104	0.516	ABC-type Co2+ transport system permease component putative expressed	AT5G52780.1

Table S4-3 Genes with altered expression in response to *Rhynchosporium secalis*.
Expression values relative to the medium for all data, of genes with altered expression in response to *R. secalis* (-K) plants with p-values < 0.005 corrected for false discoveries using Bonferroni multiple testing correction. Results for control (C) and K-starved (-K) detached leaf segments mock inoculation with water (mock) or inoculated with *R. secalis* (+ *RS*), two and four days post inoculation are shown.

SCRI_Hv35_44K_v1 probe	**2 days**				**4 days**				**Hit description**	**Top TAIR9 protein hit**
	mock		+ R. secalis		mock		+ R. secalis			
	C	-K	C	-K	C	-K	C	-K		
CUST_10450_PI390587928	0.866205	0.876973	1.204222	1.548257	0.496386	0.585586	1.063852	1.151802	blue copper protein precursor putative expressed	AT1G22480.1
CUST_13374_PI390587928	0.743486	0.520523	1.427603	1.340865	0.900762	0.499093	1.755946	1.051077	cucumisin precursor putative expressed	AT4G10540.1
CUST_17083_PI390587928	0.83888	0.407153	2.022993	2.477771	0.758858	0.387414	2.228917	0.997355	germin-like protein subfamily 1 member 11 precursor putative expressed	AT5G39110.1
CUST_17976_PI390587928	0.282497	0.113025	1.436617	2.71792	0.060155	0.093383	3.85107	2.742784	elongation factor 1-alpha putative expressed	AT5G60390.2
CUST_18174_PI390587928	0.904766	0.331968	1.901651	2.110275	0.862087	0.376867	2.719532	0.907433	germin-like protein subfamily 1 member 8 precursor putative	
CUST_18626_PI390587928	1.372593	0.243828	3.567727	1.654798	1.146354	0.216371	4.286512	0.438514	L-ascorbate oxidase precursor putative expressed	AT4G39830.1
CUST_19062_PI390587928	0.840218	0.921074	1.140276	1.373771	0.822658	0.922362	1.025981	1.229395	vacuolar sorting receptor 7 precursor putative expressed	AT1G30900.1
CUST_21330_PI390587928	0.752346	0.663686	1.260356	1.760995	0.450479	0.600348	1.101926	1.645373	blue copper protein precursor putative expressed	AT2G31050.1
CUST_21975_PI390587928	0.782343	0.433263	1.714526	1.273674	0.560677	0.629267	1.688013	0.906035	electron transporter putative expressed	
CUST_24709_PI390587928	0.630195	1.444811	0.902833	2.657249	0.43782	0.991013	0.744144	2.839921	cytokinin-O-glucosyltransferase 3 putative expressed	AT2G36790.1
CUST_25260_PI390587928	0.254655	1.080757	0.709211	3.235116	0.179631	1.480901	0.979576	2.994828	germin-like protein subfamily 1 member 7 precursor putative expressed	
CUST_2743_PI390587928	1.258444	0.606263	1.728606	1.463757	0.526171	0.487334	0.971283	1.460069	-	
CUST_27822_PI390587928	1.146428	0.276799	3.519706	1.927374	1.35195	0.267418	3.972169	0.751509	peroxidase 1 precursor putative expressed	AT2G18140.1
CUST_31717_PI390587928	0.711015	1.127725	1.128872	2.837142	0.586185	0.913062	1.001395	2.402245	endochitinase PR4 precursor putative expressed	AT3G54420.1
CUST_35250_PI390587928	0.076099	0.034405	1.583113	3.262655	0.01	0.014503	4.335918	2.552905	60S ribosomal protein L7-1 putative expressed	AT2G44120.2
CUST_36018_PI390587928	0.892114	0.796421	1.466077	1.537158	0.831305	0.768703	1.29645	1.036812	expressed protein	
CUST_37089_PI390587928	0.540049	0.562084	1.302992	2.539836	0.362934	0.472228	1.216219	1.957425	blue copper protein precursor putative expressed	AT3G27200.1
CUST_37091_PI390587928	0.602406	0.598453	1.177203	2.233511	0.392143	0.434253	1.269264	2.173245	blue copper protein precursor putative expressed	AT2G32300.1
CUST_37839_PI390587928	0.749864	0.90849	1.136403	2.100184	0.382622	0.645062	1.063824	1.557924	ATPUP3 putative expressed	AT1G28220.1
CUST_40183_PI390587928	0.202429	0.139274	1.43698	2.422063	0.035111	0.03538	4.218044	2.290781	40S ribosomal protein S27a putative expressed	AT2G47110.1
CUST_4540_PI390587928	0.827997	0.472021	1.916665	2.152302	0.811148	0.439844	1.958907	0.998882	germin-like protein subfamily 1 member 11 precursor putative expressed	AT5G39110.1
CUST_4542_PI390587928	0.919602	0.437717	1.915403	1.672079	0.986564	0.383572	2.256806	0.799991	germin-like protein subfamily 1 member 11 precursor putative expressed	AT5G39110.1
CUST_4545_PI390587928	1.045891	0.27514	2.678953	1.980312	1.125858	0.309295	3.022123	0.66859	germin-like protein subfamily 1 member 11 precursor putative expressed	AT5G39110.1
CUST_4546_PI390587928	0.919134	0.425064	1.465306	1.600915	0.910521	0.372457	1.937557	0.747652	germin-like protein subfamily 1 member 11 precursor putative expressed	AT5G39110.1
CUST_6518_PI390587928	1.162385	0.508537	1.797887	1.581312	0.877073	0.424096	1.755898	0.947338	uclacyanin-2 precursor putative expressed	AT2G32300.1
CUST_7429_PI390587928	0.736172	0.622878	1.424142	1.634614	0.601739	0.550908	1.824046	1.444416	40S ribosomal protein S3a putative expressed	AT4G34670.1
CUST_756_PI390587928	0.177416	0.207797	1.014496	2.457848	0.043797	0.053558	2.910917	2.005241	-	AT5G45775.2
CUST_7871_PI390587928	0.803011	0.944161	0.913712	1.278153	0.829879	1.054379	0.967487	1.231616	GDP-mannose 35-epimerase 1 putative expressed	AT5G28840.1

References

Abe, H., Shimoda, T., Ohnishi, J., Kugimiya, S., Narusaka, M., Seo, S., Narusaka, Y., Tsuda, S. & Kobayashi, M. 2009. Jasmonate-dependent plant defense restricts thrips performance and preference. BMC Plant Biology, 9**:** 97.

Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D. & Shinozaki, K. 1997. Role of *Arabidopsis* MYC and MYB homologs in drought and abscisic acid regulated gene expression. The Plant Cell, 9**:** 1859-1868.

Able, A.J. 2003. Role of reactive oxygen species in the response of barley to necrotrophic pathogens. Protoplasma, 221**:** 137-143.

Agrios, G. 2005. Plant Pathology, London, Academic Press.

Allen, D.M. & Lyon, A.J.E. 1978. The influence of infection by *Rhynchosporium secalis* on growth regulators of barley. Physiological Plant Pathology, 12**:** 159-166.

Altman, A. & Levin, N. 1993. Interactions of polyamines and nitrogen nutrition in plants. Physiologia Plantarum, 89**:** 653-658.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. Basic local alignment search tool. Journal of Molecular Biology, 215**:** 403-410.

Amtmann, A. & Armengaud, P. 2009. Effects of N, P, K and S on metabolism: new knowledge gained from multi-level analysis. Current Opinion in Plant Biology, 12**:** 275 - 283.

Amtmann, A., Armengaud, P. & Volkov, V. 2004. Potassium nutrition and salt stress. In: BLATT, M.R. (ed.) Membrane transport in plants. Oxford: Blackwell.

Amtmann, A., Hammond, J.P., Armengaud, P. & White, P.J. 2006. Nutrient sensing and signalling in plants: potassium and phosphorus. Advances in Botanical Research. Academic Press.

Amtmann, A., Jelitto, T.C. & Sanders, D. 1999. K+-Selective inward-rectifying channels and apoplastic pH in barley roots. Plant Physiology, 120**:** 331-338.

Amtmann, A., Troufflard, S. & Armengaud, P. 2008. The effect of potassium nutrition on pest and disease resistance in plants. Physiologia Plantarum, 133**:** 682-691.

Anderson, J.A., Huprikar, S.S., Kochian, L.V., Lucas, W.J. & Gaber, R.F. 1992. Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*. Proceedings of the National Academy of Sciences of the United States of America, 89**:** 3736-3740.

Andresen, I., Becker, W., Schlüter, K., Burges, J., Parthier, B. & Apel, K. 1992. The identification of leaf thionin as one of the main jasmonate-induced proteins of barley (*Hordeum vulgare*). Plant Molecular Biology, 19**:** 193-204.

Armengaud, P., Breitling, R. & Amtmann, A. 2004. The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. Plant Physiology, 136**:** 2556-2576.

Armengaud, P., Breitling, R. & Amtmann, A. 2010. Coronatine-insensitive 1 (COI1) mediates transcriptional responses of *Arabidopsis thaliana* to external potassium supply. Molecular Plant, 3**:** 390 - 405.

Armengaud, P., Sulpice, R., Miller, A.J., Stitt, M., Amtmann, A. & Gibon, Y. 2009. Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in *Arabidopsis* roots. Plant Physiology, 150**:** 772-785.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *beta vulgaris*. Plant Physiology, 24**:** 1-15.

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. & Sherlock, G. 2000. Gene ontology: tool for the unification of biology. Nature Genetics, 25**:** 25-29.

Ashraf, M., Ahmad, A. & Mcneilly, T. 2001. Growth and photosynthetic characteristics in pearl millet under water stress and different potassium supply. Photosynthetica, 39**:** 389-394.

Avanci, N.C., Luche, D.D., Goldman, G.H. & Goldman, M.H.S. 2010. Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. Genetics and Molecular Research, 9**:** 484-505.

Ayesu-Offei, E. & Clare, B. 1970. Processes in the infection of barley leaves by *Rhynchosporium secalis*. Australian Journal of Biological Sciences, 23**:** 300-308.

Ayres, P.G. & Jones, P. 1975. Increased transpiration and the accumulation of root absorbed 86Rb in barley leaves infected by *Rhynchosporium secalis* (leaf blotch). Physiological Plant Pathology, 7**:** 49-58.

Ayres, P.G. & Owen, H. 1971. Resistance of barley varieties to establishment of subcuticular mycelia by *Rhynchosporium secalis*. Transactions of the British Mycological Society, 57**:** 233-240.

Bachmann, A., Hause, B., Maucher, H., Garbe, E., Vörös, K., Weichert, H., Wasternack, C. & Feussner, I. 2002. Jasmonate-induced lipid peroxidation in barley leaves initiated by distinct 13-LOX forms of chloroplasts. Biological Chemistry, 383**:** 1645-1657.

Bailey, J.S. & Laidlaw, A.S. 1998. Growth and development of white clover *(Trifolium repens L.)* as influenced by P and K nutrition. Annals of Botany, 81**:** 783-786.

Balbi, V. & Devoto, A. 2008. Jasmonate signalling network in *Arabidopsis thaliana*: crucial regulatory nodes and new physiological scenarios. New Phytologist, 177**:** 301-318.

Bari, R. & Jones, J. 2009. Role of plant hormones in plant defence responses. Plant Molecular Biology, 69**:** 473-488.

Bate, N.J., Orr, J., Ni, W., Meromi, A., Nadler-Hassar, T., Doerner, P.W., Dixon, R.A., Lamb, C.J. & Elkind, Y. 1994. Quantitative relationship between phenylalanine ammonia-lyase levels and phenylpropanoid accumulation in transgenic tobacco identifies a rate-determining step in natural product synthesis. Proceedings of the National Academy of Sciences of the United States of America, 91**:** 7608-7612.

Bednarz, C.W. & Oosterhuis, D.M. 1999. Physiological changes associated with potassium deficiency in cotton, Philadelphia, NJ, USA, Taylor & Francis.

Bednarz, C.W., Oosterhuis, D.M. & Evans, R.D. 1998. Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. Environmental and Experimental Botany, 39**:** 131-139.

Bell, E., Creelman, R.A. & Mullet, J.E. 1995. A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America, 92**:** 8675-8679.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 57**:** 289-300.

Berger, S., Bell, E., Sadka, A. & Mullet, J.E. 1995. *Arabidopsis thaliana* Atvsp is homologous to soybean VspA and VspB, genes encoding vegetative storage protein acid phosphatases, and is regulated similarly by methyl jasmonate, wounding, sugars, light and phosphate. Plant Molecular Biology, 27**:** 933-942.

Berrocal-Lobo, M., Molina, A. & Solano, R. 2002. Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. The Plant Journal, 29**:** 23-32.

Blatt, M.R. 1988. Potassium-dependent, bipolar gating of K+ channels in guard cells. Journal of Membrane Biology, 102**:** 235-246.

Blatt, M.R. 2000. Cellular signalling and volume control in stomatal movements in plants. Annual Review of Cell and Developmental Biology, 16**:** 221-241.

Blevins, D.G., Barnett, N.M. & Frost, W.B. 1978. Role of potassium and malate in nitrate uptake and translocation by wheat seedlings. Plant Physiology, 62**:** 784-788.

Bonferroni, C.E. 1936. Teoria statistica delle classi e calcolo delle probabilità. Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze, 8**:** 3-62.

Boscari, A., Clément, M., Volkov, V., Golldack, D., Hybiak, J., Miller, A.J., Amtmann, A. & Fricke, W. 2009. Potassium channels in barley: cloning, functional characterization and expression analyses in relation to leaf growth and development. Plant, Cell & Environment, 32**:** 1761-1777.

Boter, M., Ruíz-Rivero, O., Abdeen, A. & Prat, S. 2004. Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. Genes & Development, 18**:** 1577-1591.

Böttcher, C. & Pollmann, S. 2009. Plant oxylipins: Plant responses to 12-oxo-phytodienoic acid are governed by its specific structural and functional properties. FEBS Journal, 276**:** 4693-4704.

Boyle, E.I., Weng, S., Gollub, J., Jin, H., Botstein, D., Cherry, J.M. & Sherlock, G. 2004. GO::TermFinder—open source software for accessing Gene Ontology information and finding significantly enriched Gene Ontology terms associated with a list of genes. Bioinformatics, 20**:** 3710-3715.

Breitling, R., Amtmann, A. & Herzyk, P. 2004a. Iterative Group Analysis (iGA): A simple tool to enhance sensitivity and facilitate interpretation of microarray experiments. BMC Bioinformatics, 5**:** 34.

Breitling, R., Armengaud, P., Amtmann, A. & Herzyk, P. 2004b. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. Febs Letters, 573**:** 83-92.

Brennan, R.F. & Jayasena, K.W. 2007. Increasing applications of potassium fertiliser to barley crops grown on deficient sandy soils increased grain yields while decreasing some foliar diseases. Australian Journal of Agricultural Research, 58**:** 680-689

Broadley, M.R., Bowen, H.C., Cotterill, H.L., Hammond, J.P., Meacham, M.C., Mead, A. & White, P.J. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. Journal of Experimental Botany, 55**:** 321-336.

Brown, W.E. & Ryan, C.A. 1984. Isolation and characterization of a wound-induced trypsin inhibitor from alfalfa leaves. Biochemistry, 23**:** 3418-3422.

Browse, J. 2009. Jasmonate passes muster: A receptor and targets for the defense hormone. Annual Review of Plant Biology, 60**:** 183-205.

Büschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., Van Daelen, R., Van Der Lee, T., Diergaarde, P., Groenendijk, J., Töpsch, S., Vos, P., Salamini, F. & Schulze-Lefert, P. 1997. The barley Mlo gene: A novel control element of plant pathogen resistance. Cell, 88**:** 695-705.

Cakmak, I. 1994. Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorus-deficient leaves. Journal of Experimental Botany, 45**:** 1259-1266.

Cakmak, I. 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. Journal of Plant Nutrition and Soil Science, 168**:** 521-530.

Cao, H., Glazebrook, J., Clarke, J.D., Volko, S. & Dong, X. 1997. The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell, 88**:** 57-63.

Cao, S., Su, L. & Fang, Y. 2006. Evidence for involvement of jasmonic acid in the induction of leaf senescence by potassium deficiency in *Arabidopsis*. Canadian Journal of Botany, 84**:** 328-333.

Carisse, K.X., Burnett, P.A., Tewari, J.P., Chen, M.H., Turkington, T.K. & Helm, J.H. 2000. Histopathological study of barley cultivars resistant and susceptible to *Rhynchosporium secalis*. Phytopathology, 90**:** 94-102.

Carver, T.L.W., Ingerson-Morris, S.M., Thomas, B.J. & Gay, A.P. 1994. Light-mediated delay of primary haustorium formation by *Erysiphe graminis* f.sp. *avenae*. Physiological and Molecular Plant Pathology, 45**:** 59-79.

Carver, T.L.W., Kunoh, H., Thomas, B.J. & Nicholson, R.L. 1999. Release and visualization of the extracellular matrix of conidia of *Blumeria graminis*. Mycological Research, 103**:** 547-560.

Casaretto, J.A., Zúñiga, G.E. & Corcuera, L.J. 2004. Abscisic acid and jasmonic acid affect proteinase inhibitor activities in barley leaves. Journal of Plant Physiology, 161**:** 389-396.

Chakraborty, S. & Newton, A.C. 2011. Climate change, plant diseases and food security: an overview. Plant Pathology, 60**:** 2-14.

Chaudhry, B., Müller-Uri, F., Cameron-Mills, V., Gough, S., Simpson, D., Skriver, K. & Mundy, J. 1994. The barley 60 kDa jasmonate-induced protein (JIP60) is a novel ribosome-inactivating protein. The Plant Journal, 6**:** 815-824.

Chen, H., Mccaig, B.C., Melotto, M., He, S.Y. & Howe, G.A. 2004. Regulation of plant arginase by wounding, jasmonate, and the phytotoxin Coronatine. Journal of Biological Chemistry, 279**:** 45998-46007.

Chen, H., Wilkerson, C.G., Kuchar, J.A., Phinney, B.S. & Howe, G.A. 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. Proceedings of the National Academy of Sciences of the United States of America, 102**:** 19237-19242.

Chen, M.-S. 2008. Inducible direct plant defense against insect herbivores: A review. Insect Science, 15**:** 101-114.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G. & Thompson, J.D. 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research, 31**:** 3497-3500.

Chico, J.M., Chini, A., Fonseca, S. & Solano, R. 2008. JAZ repressors set the rhythm in jasmonate signaling. Current Opinion in Plant Biology, 11**:** 486-494.

Chini, A., Fonseca, S., Fernandez, G., Adie, B., Chico, J.M., Lorenzo, O., Garcia-Casado, G., Lopez-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L. & Solano, R. 2007. The JAZ family of repressors is the missing link in jasmonate signalling. Nature, 448**:** 666-671.

Chojecki, J. 1986. Identification and characterisation of a cDNA clone for cytosolic glyceraldehyde-3-phosphate dehydrogenase in barley. Carlsberg Research Communications, 51**:** 203-210.

Christou, P. & Twyman, R.M. 2004. The potential of genetically enhanced plants to address food insecurity. Nutrition Research Reviews, 17**:** 23-42.

Cipollini, D., Enright, S., Traw, M.B. & Bergelson, J. 2004. Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. Molecular Ecology, 13**:** 1643-1653.

Coghlan, S.E. & Walters, D.R. 1990. Polyamine metabolism in ‘green-islands’ on powdery mildew-infected barley leaves: possible interactions with senescence. New Phytologist, 116**:** 417-424.

Coram, T., Huang, X., Zhan, G., Settles, M. & Chen, X. 2010. Meta-analysis of transcripts associated with race-specific resistance to stripe rust in wheat demonstrates common induction of blue copper-binding protein, heat-stress transcription factor, pathogen-induced WIR1A protein, and ent-kaurene synthase transcripts. Functional and Integrative Genomics, 10**:** 383-392.

Cowley, T. & Walters, D. 2005. Local and systemic effects of oxylipins on powdery mildew infection in barley. Pest Management Science, 61**:** 572-576.

Cross, J.M., Von Korff, M., Altmann, T., Bartzetko, L., Sulpice, R., Gibon, Y., Palacios, N. & Stitt, M. 2006. Variation of enzyme activities and metabolite levels in 24 *Arabidopsis* accessions growing in carbon-limited conditions. Plant Physiology, 142**:** 1574-1588.

Cruz Castillo, M., Martínez, C., Buchala, A., Métraux, J.-P. & León, J. 2004. Gene-specific involvement of β-oxidation in wound-activated responses in *Arabidopsis*. Plant Physiology, 135**:** 85-94.

Dangl, J.L. & Jones, J.D.G. 2001. Plant pathogens and integrated defence responses to infection. Nature, 411**:** 826-833.

Davidson, R.M., Reeves, P.A., Manosalva, P.M. & Leach, J.E. 2009. Germins: A diverse protein family important for crop improvement. Plant Science, 177**:** 499-510.

Davis, H. & Fitt, B.D.L. 1992. Seasonal changes in primary and secondary inoculum during epidemics of leaf blotch (*Rhynchosporium secalis*) on winter barley. Annals of Applied Biology, 121**:** 39-49.

De Wit, P.J.G.M., Callow, J.A., Andrews, J.H. & Tommerup, I.C. 1995. Fungal avirulence genes and plant resistance genes: Unraveling the molecular basis of gene-for-gene interactions. Advances in Botanical Research. Academic Press.

Deeken, R., Geiger, D., Fromm, J., Koroleva, O., Ache, P., Langenfeld-Heyser, R., Sauer, N., May, S. & Hedrich, R. 2002. Loss of the AKT2/3 potassium channel affects sugar loading into the phloem of *Arabidopsis*. Planta, 216**:** 334-344.

Degl'innocenti, E., Hafsi, C., Guidi, L. & Navari-Izzo, F. 2009. The effect of salinity on photosynthetic activity in potassium-deficient barley species. Journal of Plant Physiology, 166**:** 1968-1981.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E. & Ryals, J. 1994. A central role of salicylic acid in plant disease resistance. Science, 266**:** 1247-1250.

Delker, C., Stenzel, I., Hause, B., Miersch, O., Feussner, I. & Wasternack, C. 2006. Jasmonate biosynthesis in *Arabidopsis thaliana* - enzymes, products, regulation. Plant Biology, 8**:** 297-306.

Delker, C., Zolman, B.K., Miersch, O. & Wasternack, C. 2007. Jasmonate biosynthesis in *Arabidopsis thaliana* requires peroxisomal β-oxidation enzymes - additional proof by properties of *pex6* and *aim1*. Phytochemistry, 68**:** 1642-1650.

Devoto, A., Nieto-Rostro, M., Xie, D., Ellis, C., Harmston, R., Patrick, E., Davis, J., Sherratt, L., Coleman, M. & Turner, J.G. 2002. COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in *Arabidopsis*, Oxford, UK, Blackwell.

Devoto, A. & Turner, J.G. 2005. Jasmonate-regulated *Arabidopsis* stress signalling network. Physiologia Plantarum, 123**:** 161 - 172.

Dewdney, J., Reuber, T.L., Wildermuth, M.C., Devoto, A., Cui, J., Stutius, L.M., Drummond, E.P. & Ausubel, F.M. 2000. Three unique mutants of *Arabidopsis* identify eds loci required for limiting growth of a biotrophic fungal pathogen. The Plant Journal, 24**:** 205-218.

Di Cera, E. 2006. A structural perspective on enzymes activated by monovalent cations. Journal of Biological Chemistry, 281**:** 1305-1308.

Dixon, R.A. & Paiva, N.L. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell, 7**:** 1085-1097.

Dolan, L. & Davies, J. 2004. Cell expansion in roots. Current Opinion in Plant Biology, 7**:** 33-39.

Dombrecht, B., Xue, G.P., Sprague, S.J., Kirkegaard, J.A., Ross, J.J., Reid, J.B., Fitt, G.P., Sewelam, N., Schenk, P.M., Manners, J.M. & Kazan, K. 2007. MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. The Plant Cell, 19**:** 2225-2245.

Drew, M.C. 1975. Comparison of the effects of a localised supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot in barley. New Phytologist, 75**:** 479-490.

Dumas, B., Freyssinet, G. & Pallett, K.E. 1995. Tissue-specific expression of germin-Like oxalate oxidase during development and fungal infection of barley seedlings. Plant Physiology, 107**:** 1091-1096.

Dunaeva, M., Goebel, C., Wasternack, C., Parthier, B. & Goerschen, E. 1999. The jasmonate-induced 60 kDa protein of barley exhibits N-glycosidase activity in vivo. Febs Letters, 452**:** 263-266.

Edwards, H.H. 2002. Development of primary germ tubes by conidia of *Blumeria graminis* f.sp. *hordei* on leaf epidermal cells of *Hordeum vulgare*. Canadian Journal of Botany, 80**:** 1121-1125.

Egilla, J., Davies, F. & Boutton, T. 2005. Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations. Photosynthetica, 43**:** 135-140.

Egilla, J.N., Davies, F.T. & Drew, M.C. 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. *Leprechaun*: Plant growth, leaf macro- and micronutrient content and root longevity. Plant and Soil, 229**:** 213-224.

El Dessougi, H., Claassen, N. & Steingrobe, B. 2002. Potassium efficiency mechanisms of wheat, barley, and sugar beet grown on a K fixing soil under controlled conditions. Journal of Plant Nutrition and Soil Science, 165**:** 732-737.

Ellis, C., Karafyllidis, I. & Turner, J.G. 2002. Constitutive activation of jasmonate signaling in an Arabidopsis mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. Molecular Plant-Microbe Interactions, 15**:** 1025-1030.

Ellis, C. & Turner, J.G. 2001. The *Arabidopsis* mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. The Plant Cell, 13**:** 1025-1033.

Epstein, E., Rains, D.W. & Elzam, O.E. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. Proceedings of the National Academy of Sciences of the United States of America, 49**:** 684-692.

Evans, H.J. 1963. Effect of potassium and other univalent cations on activity of pyruvate kinase in *Pisum sativum*. Plant Physiology, 38**:** 397-402.

Evans, H.J. & Sorger, G.J. 1966. Role of mineral elements with emphasis on the univalent cations. Annual Review of Plant Physiology, 17**:** 47-76.

Fao. http://faostat.fao.org [Online].

Fao. 2002. World agriculture: towards 2015/2030 [Online].

Farmer, E.E., Johnson, R.R. & Ryan, C.A. 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiology, 98**:** 995-1002.

Farmer, E.E. & Ryan, C.A. 1990. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proceedings of the National Academy of Sciences of the United States of America, 87**:** 7713-7716.

Farmer, E.E. & Ryan, C.A. 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. The Plant Cell, 4**:** 129-134.

Feys, B.J.F., Benedetti, C.E., Penfold, C.N. & Turner, J.G. 1994. *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. The Plant Cell, 6**:** 751-759.

Fischer, R.A. & Hsiao, T.C. 1968. Stomatal opening in isolated epidermal strips of *Vicia faba*. II. Responses to KCl concentration and the role of potassium absorption. Plant Physiology, 43**:** 1953-1958.

Fotopoulos, V., Gilbert, M.J., Pittman, J.K., Marvier, A.C., Buchanan, A.J., Sauer, N., Hall, J.L. & Williams, L.E. 2003. The monosaccharide transporter gene, *AtSTP4*, and the cell-wall invertase, *At*β*fruct1*, are induced in *Arabidopsis* during infection with the fungal biotroph *Erysiphe cichoracearum*. Plant Physiology, 132**:** 821-829.

Fry, W. 2008. *Phytophthora infestans*: the plant (and R gene) destroyer. Molecular Plant Pathology, 9**:** 385-402.

Fujimoto, S.Y., Ohta, M., Usui, A., Shinshi, H. & Ohme-Takagi, M. 2000. *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC Box–mediated gene expression. The Plant Cell, 12**:** 393-404.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. & Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science, 261**:** 754-756.

Gatehouse, J.A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytologist, 156**:** 145-169.

Gierth, M., Maser, P. & Schroeder, J.I. 2005. The potassium transporter AtHAK5 functions in K^+ deprivation-induced high-affinity K^+ uptake and AKT1 K^+ channel contribution to K^+ uptake kinetics in *Arabidopsis* roots. Plant Physiology, 137**:** 1105-1114.

Glawe, D.A. 2008. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. Annual Review of Phytopathology, 46**:** 27-51.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology, 43**:** 205-227.

Görschen, E., Dunaeva, M., Hause, B., Reeh, I., Wasternack, C. & Parthier, B. 1997. Expression of the ribosome-inactivating protein JIP60 from barley in transgenic tobacco leads to an abnormal phenotype and alterations on the level of translation. Planta, 202**:** 470-478.

Gregory, F.G. & Sen, P.K. 1937. Physiological studies in plant nutrition: VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency Annals of Botany, 1**:** 521-561.

Grewal, J. & Singh, S. 1980. Effect of potassium nutrition on frost damage and yield of potato plants on alluvial soils of the Punjab (India). Plant and Soil, 57**:** 105-110.

Guerineau, F., Benjdia, M. & Zhou, D.X. 2003. A jasmonate-responsive element within the *A. thaliana* vsp1 promoter. Journal of Experimental Botany, 54**:** 1153-1162.

Habgood, R.M. 1971. The transmission of *Rhynchosporium secalis* by infected barley seed. Plant Pathology, 20**:** 80-81.

Hafsi, C., Romero-Puertas, M., Del Río, L., Sandalio, L. & Abdelly, C. 2010. Differential antioxidative response in barley leaves subjected to the interactive effects of salinity and potassium deprivation. Plant and Soil, 334**:** 449-460.

Hahn, M., Jungling, S. & Knogge, W. 1993. Cultivar-specific elicitation of barley defense reactions by the phytotoxic peptide NIP1 from *Rhynchosporium secalis*. Molecular Plant-Microbe Interactions, 6**:** 745-754.

Halim, V.A., Altmann, S., Ellinger, D., Eschen-Lippold, L., Miersch, O., Scheel, D. & Rosahl, S. 2009. PAMP-induced defense responses in potato require both salicylic acid and jasmonic acid. The Plant Journal, 57**:** 230-242.

Halitschke, R. & Baldwin, I. 2004. Jasmonates and related compounds in plant-insect interactions. Journal of Plant Growth Regulation, 23**:** 238-245.

Hamberg, M. & Fahlstadius, P. 1990. Allene oxide cyclase: A new enzyme in plant lipid metabolism. Archives of Biochemistry and Biophysics, 276**:** 518-526.

Hammond, J.P., Bennett, M.J., Bowen, H.C., Broadley, M.R., Eastwood, D.C., May, S.T., Rahn, C., Swarup, R., Woolaway, K.E. & White, P.J. 2003. Changes in gene expression in

Arabidopsis shoots during phosphate starvation and the potential for developing smart plants. Plant Physiology, 132**:** 578-596.

Hampton, C.R., Bowen, H.C., Broadley, M.R., Hammond, J.P., Mead, A., Payne, K.A., Pritchard, J. & White, P.J. 2004. Cesium toxicity in *Arabidopsis*. Plant Physiology, 136**:** 3824-3837.

Harms, K., Ramirez, I. & Peña-Cortés, H. 1998. Inhibition of wound-induced accumulation of allene oxide synthase transcripts in flax leaves by aspirin and salicylic acid. Plant Physiology, 118**:** 1057-1065.

He, Y. & Gan, S. 2001. Identical promoter elements are involved in regulation of the *OPR1* gene by senescence and jasmonic acid in *Arabidopsis*. Plant Molecular Biology, 47**:** 595-605.

Helal, H. & Mengel, K. 1979. Nitrogen metabolism of young barley plants as affected by NaCl-salinity and potassium. Plant and Soil, 51**:** 457-462.

Helal, M., Koch, K. & Mengel, K. 1975. Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. Physiologia Plantarum, 35**:** 310-313.

Hermans, C., Hammond, J.P., White, P.J. & Verbruggen, N. 2006. How do plants respond to nutrient shortage by biomass allocation? Trends in Plant Science, 11**:** 610-617.

HGCA 2008. The encyclopaedia of cereal diseases http://www.hgca.com/cde.

Himmelbach, A., Liu, L., Zierold, U., Altschmied, L., Maucher, H., Beier, F., Müller, D., Hensel, G., Heise, A., Schützendübel, A., Kumlehn, J. & Schweizer, P. 2010. Promoters of the barley germin-like GER4 gene cluster enable strong transgene expression in response to pathogen attack. The Plant Cell, 22**:** 937-952.

Hoefgen, R. & Nikiforova, V.J. 2008. Metabolomics integrated with transcriptomics: assessing systems response to sulfur-deficiency stress. Physiologia Plantarum, 132**:** 190-198.

Høgh-Jensen, H. & Pedersen, M.B. 2003. Morphological plasticity by crop plants and their potassium use efficiency. Journal of Plant Nutrition, 26**:** 969-984.

Horie, T., Costa, A., Kim, T.H., Han, M.J., Horie, R., Leung, H.-Y., Miyao, A., Hirochika, H., An, G. & Schroeder, J.I. 2007. Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+-starved roots for growth. EMBO Journal, 26**:** 3003-3014.

Howe, G.A. & Jander, G. 2008. Plant Immunity to Insect Herbivores. Annual Review of Plant Biology, 59**:** 41-66.

Howe, G.A., Lightner, J., Browse, J. & Ryan, C.A. 1996. An Octadecanoid Pathway Mutant (JL5) of Tomato Is Compromised in Signaling for Defense against Insect Attack. The Plant Cell Online, 8**:** 2067-2077.

Howe, G.A. & Schilmiller, A.L. 2002. Oxylipin metabolism in response to stress. Current Opinion in Plant Biology, 5**:** 230 - 236.

Huber, S.C. 1984. Biochemical basis for effects of K-deficiency on assimilate export rate and accumulation of soluble sugars in soybean leaves. Plant Physiology, 76**:** 424-430.

Humphries, E.C. 1951. The Absorption of Ions by Excised Root Systems: II. Observations on roots of barley grown in solutions deficient in phosphorus, nitrogen, or potassium. Journal of Experimental Botany, 2**:** 344-379.

Humphry, M., Consonni, C. & Panstruga, R. 2006. mlo-based powdery mildew immunity: silver bullet or simply non-host resistance? Molecular Plant Pathology, 7**:** 605-610.

Hurkman, W.J. & Tanaka, C.K. 1996. Germin gene expression is induced in wheat leaves by powdery mildew infection. Plant Physiology, 111**:** 735-739.

Hwang, B.K. & Heitefuss, R. 1986. Sugar composition and acid invertase activity in spring barley plants in relation to adult-plant resistance to powdery mildew. Physiology and Biochemistry, 76**:** 365-369.

Hyun, Y., Choi, S., Hwang, H.-J., Yu, J., Nam, S.-J., Ko, J., Park, J.-Y., Seo, Y.S., Kim, E.Y., Ryu, S.B., Kim, W.T., Lee, Y.-H., Kang, H. & Lee, I. 2008. Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. Developmental Cell, 14**:** 183-192.

Imas, P. & Magen, H. 2000. Potash facts in brief. Bern, Switzerland and Haryana, India: International Potash institute.

Ishiguro, N., Miya, M. & Nishida, M. 2001. Complete mitochondrial DNA sequence of ayu Plecoglossus altivelis. Fisheries Science, 67**:** 474-481.

Jansen, C., Korell, M., Eckey, C., Biedenkopf, D. & Kogel, K.-H. 2005. Identification and transcriptional analysis of powdery mildew-induced barley genes. Plant Science, 168**:** 373-380.

Jenkyn, J.F. & Griffiths, E. 1976. Some effects of nutrition on *Rhynchosporium secalis*. Transactions of the British Mycological Society, 66**:** 329-332.

Jenkyn, J.F. & Griffiths, E. 1978. Relationships between the severity of leaf blotch (*Rhynchosporium secalis*) and the water-soluble carbohydrate and nitrogen contents of barley plants. Annals of Applied Biology, 90**:** 35-44.

Jensen, B. & Munk, L. 1997. Nitrogen-induced changes in colony density and spore production of *Erysiphe graminis f.sp. hordei* on seedlings of six spring barley cultivars. Plant Pathology, 46**:** 191-202.

Johnson, L.E.B., Bushnell, W.R. & Zeyen, R.J. 1981. Defense patterns in nonhost higher plant species agains two powdery mildew fungi I. Monocotyledonous species. Canadian Journal of Botany, 60**:** 1068-1083.

Jones, J.D.G. & Dangl, J.L. 2006. The plant immune system. Nature, 444**:** 323-329.

Jones, P. & Ayres, P.G. 1974. Rhynchosporium leaf blotch of barley studied during the subcuticular phase by electron microscopy. Physiological Plant Pathology, 4**:** 229-230, IN17-IN21, 231-233.

Jordan-Meille, L. & Pellerin, S. 2008. Shoot and root growth of hydroponic maize (*Zea mays L.*) as influenced by K-deficiency. Plant and Soil, 304**:** 157-168.

Jørgensen, I.H. 1992. Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. Euphytica, 63**:** 141-152.

Jørgensen, J.H. 1977. Spectrum of resistance conferred by MLO powdery mildew resistance genes in barley. Euphytica, 26**:** 55-62.

Jørgensen, J.H. & Wolfe, M. 1994. Genetics of powdery mildew resistance in barley. Critical Reviews in Plant Sciences, 13**:** 97 - 119.

Jung, C., Lyou, S., Yeu, S., Kim, M., Rhee, S., Kim, M., Lee, J., Choi, Y. & Cheong, J.-J. 2007. Microarray-based screening of jasmonate-responsive genes in *Arabidopsis thaliana*. Plant Cell Reports, 26**:** 1053-1063.

Jung, J.-Y., Shin, R. & Schachtman, D.P. 2009. Ethylene mediates response and tolerance to potassium deprivation in *Arabidopsis*. Plant Cell, 21**:** 607-621.

Kanai, S., Ohkura, K., Adu-Gyamfi, J.J., Mohapatra, P.K., Nguyen, N.T., Saneoka, H. & Fujita, K. 2007. Depression of sink activity precedes the inhibition of biomass production in tomato plants subjected to potassium deficiency stress. Journal of Experimental Botany, 58**:** 2917-2928.

Kang, J.G., Pyo, Y.J., Cho, J.W. & Cho, M.H. 2004. Comparative proteome analysis of differentially expressed proteins induced by K-deficiency in *Arabidopsis thaliana*. PROTEOMICS, 4**:** 3549-3559.

Karley, A.J. & White, P.J. 2009. Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Current Opinion in Plant Biology, 12**:** 291-298.

Kazan, K. & Manners, J.M. 2008. Jasmonate signaling: Toward an integrated view. Plant Physiology, 146**:** 1459-1468.

Kervinen, T., Peltonen, S., Utriainen, M., Kangasjärvi, J., Teeri, T.H. & Karjalainen, R. 1997. Cloning and characterization of cDNA clones encoding phenylalanine ammonia-lyase in barley. Plant Science, 123**:** 143-150.

Kim, S.-R., Choi, J.-L., Costa, M.A. & An, G. 1992. Identification of G-Box sequence as an essential element for methyl jasmonate response of potato proteinase inhibitor II promoter. Plant Physiology, 99**:** 627-631.

Kim, S.R., Kim, Y. & An, G. 1993. Identification of methyl jasmonate and salicylic acid response elements from the nopaline synthase (nos) promoter. Plant Physiology, 103**:** 97-103.

Kjemtrup, S., Nimchuk, Z. & Dangl, J.L. 2000. Effector proteins of phytopathogenic bacteria: bifunctional signals in virulence and host recognition. Current Opinion in Microbiology, 3**:** 73-78.

Kramell, R., Miersch, O., Atzorn, R., Parthier, B. & Wasternack, C. 2000. Octadecanoid-derived alteration of gene expression and the "oxylipin signature" in stressed barley leaves. Implications for different signaling pathways. Plant Physiology, 123**:** 177-188.

Kuć, J. 1982. Induced immunity to plant disease. BioScience, 32**:** 854-860.

Large, E.C. 1940. The advance of the fungi, Johnathan Cape, London.

Last, F.T. 1962. Effects of nutrition on the incidence of barley powdery mildew. Plant Pathology, 11**:** 133-136.

Laudert, D., Schaller, F. & Weiler, E.W. 2000. Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. Planta, 211**:** 163-165.

Laudert, D. & Weiler, E.W. 1998. Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. The Plant Journal, 15**:** 675-684.

Laurie-Berry, N., Joardar, V., Street, I.H. & Kunkel, B.N. 2006. The *Arabidopsis thaliana* JASMONATE INSENSITIVE 1 gene Is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. Molecular Plant-Microbe Interactions, 19**:** 789-800.

Lawton, K., Weymann, K., Friedrich, L., Vernooij, B., Uknes, S. & Ryals, J. 1995. Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. Molecular Plant Microbe Interactions, 8**:** 863-870.

Lehnackers, H. & Knogge, W. 1989. Cytological studies on the infection of barley cultivars with known resistance genotypes by *Rhynchosporium secalis.* Canadian Journal of Botany, 68**:** 1953-1961.

Leigh, R.A., Chater, M., Storey, R. & Johnston, A.E. 1986. Accumulation and subcellular distribution of cations in relation to the growth of potassium-deficient barley. Plant, Cell and Environment, 9**:** 595-604.

Leigh, R.A. & Wyn Jones, R.G. 1984. A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytologist, 97**:** 1-13.

Léon-Kloosterziel, K.M., Verhagen, B.W.M., Keurentjes, J.J.B., Van Pelt, J.A., Rep, M., Van Loon, L.C. & Pieterse, C.M.J. 2005. Colonization of the *Arabidopsis* rhizosphere by fluorescent *Pseudomonas* spp. activates a root-specific, ethylene-responsive *PR-5* gene in the vascular bundle. Plant Molecular Biology, 57**:** 731-748.

León, J., Rojo, E. & Sánchez-Serrano, J.J. 2001. Wound signalling in plants. Journal of Experimental Botany, 52**:** 1-9.

Leopold, J.L., Hause, B., Lehmann, J., Graner, A., Parthier, B. & Wasternack, C. 1996. Isolation, characterization and expression of a cDNA coding for a jasmonate-inducible protein of 37 kDa in barley leaves. Plant, Cell and Environment, 19**:** 675-684.

Li, C., Schilmiller, A.L., Liu, G., Lee, G.I., Jayanty, S., Sageman, C., Vrebalov, J., Giovannoni, J.J., Yagi, K., Kobayashi, Y. & Howe, G.A. 2005. Role of β-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. The Plant Cell, 17**:** 971-986.

Li, J., Brader, G. & Palva, E.T. 2004a. The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. The Plant Cell, 16**:** 319-331.

Li, L., Zhao, Y., Mccaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E. & Howe, G.A. 2004b. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. The Plant Cell, 16**:** 126-143.

Linsell, K.J., Keiper, F.J., Forgan, A. & Oldach, K.H. 2010. New insights into the infection process of *Rhynchosporium secalis* in barley using GFP. Fungal Genetics and Biology, 48**:** 124-131.

Loake, G. & Grant, M. 2007. Salicylic acid in plant defence - the players and protagonists. Current Opinion in Plant Biology, 10**:** 466 - 472.

Lorenzo, O., Chico, J.M., Sánchez-Serrano, J.J. & Solano, R. 2004. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. The Plant Cell, 16**:** 1938-1950.

Lorenzo, O. & Solano, R. 2005. Molecular players regulating the jasmonate signalling network. Current Opinion in Plant Biology, 8**:** 532-540.

Lütz, C., Steiger, A. & Godde, D. 1992. Influence of air pollutants and nutrient deficiency on D-1 protein content and photosynthesis in young spruce trees. Physiologia Plantarum, 85**:** 611-617.

Lyngs Jørgensen, H.J., De Neergaard, E. & Smedegaard-Petersen, V. 1993. Histological examination of the interaction between *Rhynchosporium secalis* and susceptible and resistant cultivars of barley. Physiological and Molecular Plant Pathology, 42**:** 345-358.

Maathuis, F.J.M., Filatov, V., Herzyk, P., Krijger, G.C., Axelsen, K.B., Chen, S., Green, B.J., Li, Y., Madagan, K.L., Sánchez-Fernández, R., Forde, B.G., Palmgren, M.G., Rea, P.A., Williams, L.E., Sanders, D. & Amtmann, A. 2003. Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. The Plant Journal, 35**:** 675-692.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. London: Academic Press.

Mason, H.S., Dewald, D.B. & Mullet, J.E. 1993. Identification of a methyl jasmonate-responsive domain in the soybean vspB promoter. The Plant Cell, 5**:** 241-251.

Maucher, H., Hause, B., Feussner, I., Ziegler, J. & Wasternack, C. 2000. Allene oxide synthases of barley (*Hordeum vulgare cv. Salome*): tissue specific regulation in seedling development. The Plant Journal, 21**:** 199-213.

Maucher, H., Stenzel, I., Miersch, O., Stein, N., Prasad, M., Zierold, U., Schweizer, P., Dorer, C., Hause, B. & Wasternack, C. 2004. The allene oxide cyclase of barley (*Hordeum vulgare L.*) - cloning and organ-specific expression. Phytochemistry, 65**:** 801-811.

Mayer, K.F.X., Martis, M., Hedley, P.E., Šimková, H., Liu, H., Morris, J.A., Steuernagel, B., Taudien, S., Roessner, S., Gundlach, H., Kubaláková, M., Suchánková, P., Murat, F., Felder, M., Nussbaumer, T., Graner, A., Salse, J., Endo, T., Sakai, H., Tanaka, T., Itoh, T., Sato, K., Platzer, M., Matsumoto, T., Scholz, U., Doležel, J., Waugh, R. & Stein, N. 2011. Unlocking the barley genome by chromosomal and comparative genomics. The Plant Cell, 23**:** 1249-1263.

Mcconn, M., Creelman, R.A., Bell, E., Mullet, J.E. & Browse, J. 1997. Jasmonate is essential for insect defense in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 94**:** 5473-5477.

Mcdowell, J.M. & Dangl, J.L. 2000. Signal transduction in the plant immune response. Trends in Biochemical Sciences, 25**:** 79-82.

Mejlhede, N., Kyjovska, Z., Backes, G., Burhenne, K., Rasmussen, S.K. & Jahoor, A. 2006. EcoTILLING for the identification of allelic variation in the powdery mildew resistance genes *mlo* and *Mla* of barley. Plant Breeding, 125**:** 461-467.

Melotto, M., Mecey, C., Niu, Y., Chung, H.S., Katsir, L., Yao, J., Zeng, W., Thines, B., Staswick, P., Browse, J., Howe, G.A. & He, S.Y. 2008. A critical role of two positively charged amino acids in the Jas motif of *Arabidopsis* JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. The Plant Journal, 55**:** 979-988.

Memelink, J. 2009. Regulation of gene expression by jasmonate hormones. Phytochemistry, 70**:** 1560-1570.

Mendgen, K. & Nass, P. 1988. The activity of powdery mildew haustoria after feeding the host cells with different sugars, as measured with a potentiometric cyanine dye. Planta, 174**:** 283-288.

Mengel, K. & Arneke, W.-W. 1982. Effect of potassium on the water potential, the pressure potential, the osmotic potential and cell elongation in leaves of *Phaseolus vulgaris*. Physiologia Plantarum, 54**:** 402-408.

Mengel, K. & Haeder, H.-E. 1977. Effect of potassium supply on the rate of phloem sap exudation and the composition of phloem sap of *Ricinus communis*. Plant Physiology, 59**:** 282-284.

Mengel, K., Kirkby, E.A., Kosegarten, H. & Appel, T. (eds.) 2001. Principles of plant nutrition, Dordrecht: Kluwer.

Menke, F.L.H., Champion, A., Kijne, J.W. & Memelink, J. 1999. A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene *Str* interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. EMBO Journal, 18**:** 4455-4463.

Miles, P.W. 1999. Aphid saliva. Biological Reviews, 74**:** 41-85.

Molina-Cano, J.L., Simiand, J.P., Sopena, A., Pérez-Vendrell, A.M., Dorsch, S., Rubiales, D., Swanston, J.S. & Jahoor, A. 2003. Mildew-resistant mutants induced in North American two- and six-rowed malting barley cultivars. Theoretical and Applied Genetics, 107**:** 1278-1287.

Morcuende, R., Bari, R., Gibon, Y., Zheng, W., Pant, B.D., Bläsing, O., Usadel, B., Czechowski, T., Udvardi, M.K., Stitt, M. & Scheible, W.-R. 2007. Genome-wide reprogramming of metabolism and regulatory networks of *Arabidopsis* in response to phosphorus. Plant, Cell & Environment, 30**:** 85-112.

Mori, H. 2000. Direct determination of nitrate using reductase in a flow system. Journal of health science, 46**:** 385-388.

Mosblech, A., Feussner, I. & Heilmann, I. 2009. Oxylipins: structurally diverse metabolites from fatty acid oxidation. Plant Physiology and Biochemistry, 47**:** 511 - 517.

Mouline, K., Véry, A.-A., Gaymard, F., Boucherez, J., Pilot, G., Devic, M., Bouchez, D., Thibaud, J.-B. & Sentenac, H. 2002. Pollen tube development and competitive ability are impaired by disruption of a Shaker K^+ channel in *Arabidopsis*. Genes & Development, 16**:** 339-350.

Müller-Uri, F., Cameron-Mills, V. & Mundy, J. 2002. The barley Jip23b gene. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression, 1576**:** 231-235.

Mur, L.A., Kenton, P., Atzorn, R., Miersch, O. & Wasternack, C. 2006. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiology, 140**:** 249 - 262.

Newton, A., Flavell, A., George, T., Leat, P., Mullholland, B., Ramsay, L., Revoredo-Giha, C., Russell, J., Steffenson, B., Swanston, J., Thomas, W., Waugh, R., White, P. & Bingham, I. 2011. Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. Food Security, 3**:** 141-178.

Newton, A.C., Hackett, C.A. & Guy, D.C. 1998. Diversity and complexity of *Erysiphe graminis f.sp. hordei* collected from barley cultivar mixtures or barley plots treated with a resistance elicitor. European Journal of Plant Pathology, 104**:** 925-931.

Newton, A.C., Searle, J., Guy, D.C., Hackett, C.A. & Cooke, D.E.L. 2001. Variability in pathotype, aggressiveness, RAPD profile, and rDNA ITS1 sequences of UK isolates of *Rhynchosporium secalis*, Stuttgart, Germany, Ulmer.

Nibbe, M., Hilpert, B., Wasternack, C., Miersch, O. & Apel, K. 2002. Cell death and salicylate- and jasmonate-dependent stress responses in *Arabidopsis* are controlled by single *cet* genes. Planta, 216**:** 120-128.

Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N. & Ohashi, Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco Leaves. Plant and Cell Physiology, 39**:** 500-507.

Nimchuk, Z., Eulgem, T., Holt Iii, B.F. & Dangl, J.L. 2003. Recognition and response in the plant immune system. Annual Review of Genetics, 37**:** 579-609.

Nitsos, R.E. & Evans, H.J. 1969. Effects of univalent cations on the activity of particulate starch synthetase. Plant Physiology, 44**:** 1260-1266.

Norton, G., Pappusamy, A., Yusof, F., Pujade-Renaud, V., Perkins, M., Griffiths, D. & Jones, H. 2007. Characterisation of recombinant *Hevea brasiliensis* allene oxide synthase: Effects of cycloxygenase inhibitors, lipoxygenase inhibitors and salicylates on enzyme activity. Plant Physiology and Biochemistry, 45**:** 129-138.

Nunes-Nesi, A., Carrari, F., Gibon, Y., Sulpice, R., Lytovchenko, A., Fisahn, J., Graham, J., Ratcliffe, R.G., Sweetlove, L. & Fernie, A.R. 2007. Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. The Plant Journal, 50**:** 1093-1106.

Oerke, E.C. 2006. Crop losses to pests. The Journal of Agricultural Science, 144**:** 31-43.

Oerke, E.C. & Dehne, H.W. 2004. Safeguarding production - losses in major crops and the role of crop protection. Crop Protection, 23**:** 275-285.

Ohme-Takagi, M. & Shinshi, H. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. The Plant Cell, 7**:** 173-182.

Olutiola, P.O. & Ayres, P.G. 1973. Utilization of carbohydrates by *Rhynchosporium secalis.* I. Growth and sporulation on glucose, galactose, and galacturonic acid. Physiologia Plantarum, 29**:** 92-96.

Ozturk, Z.N., Talamé, V., Deyholos, M., Michalowski, C.B., Galbraith, D.W., Gozukirmizi, N., Tuberosa, R. & Bohnert, H.J. 2002. Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. Plant Molecular Biology, 48**:** 551-573.

Padmanabhan, S.Y. 1973. The great bengal famine. Annual Review of Phytopathology, 11**:** 11-24.

Panstruga, R. & Schulze-Lefert, P. 2002. Live and let live: insights into powdery mildew disease and resistance. Molecular Plant Pathology, 3**:** 495-502.

Paschold, A., Halitschke, R. & Baldwin, I.T. 2007. Co(i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. The Plant Journal, 51**:** 79-91.

Pena-Cortés, H., Albrecht, T., Prat, S., Weiler, E.W. & Willmitzer, L. 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. Planta, 191**:** 123-128.

Peña-Cortés, H., Liu, X., Serrano, J.S., Schmid, R. & Willmitzer, L. 1992. Factors affecting gene expression of patatin and proteinase-inhibitor-II gene families in detached potato leaves. Planta, 186**:** 495-502.

Penninckx, I., Eggermont, K., Terras, F., Thomma, B., Samblanx, G., Buchala, A., Metraux, J.P., Manners, J.M. & Broekaert, W.F. 1996. Pathogen-Induced Systemic Activation of a Plant Defensin Gene in Arabidopsis Follows a Salicylic Acid-Independent Pathway. The Plant Cell Online, 8**:** 2309-2323.

Peoples, T.R. & Koch, D.W. 1979. Role of potassium in carbon dioxide assimilation in *Medicago sativa L.* Plant Physiology, 63**:** 878-881.

Perrenoud, S. 1990. Potassium and plant health, Horgen, Switzerland., IPI research topic No. 3. International Potash Institute.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H.B., Lacy, M., Austin, M.J., Parker, J.E., Sharma, S.B., Klessig, D.F., Martienssen, R., Mattsson, O., Jensen, A.B. & Mundy, J. 2000. *Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance. Cell, 103**:** 1111-1120.

Pieterse, C.M.J., Leon-Reyes, A., Van Der Ent, S. & Van Wees, S.C.M. 2009. Networking by small-molecule hormones in plant immunity. Nature Chemical Biology, 5**:** 308-316.

Piffanelli, P., Zhou, F., Casais, C., Orme, J., Jarosch, B., Schaffrath, U., Collins, N.C., Panstruga, R. & Schulze-Lefert, P. 2002. The barley MLO modulator of defense and cell death Is responsive to biotic and abiotic stress stimuli. Plant Physiology, 129**:** 1076-1085.

Reinbothe, C., Springer, A., Samol, I. & Reinbothe, S. 2009. Plant oxylipins: role of jasmonic acid during programmed cell death, defence and leaf senescence. FEBS Journal, 276**:** 4666-4681.

Reinbothe, S., Reinbothe, C., Lehmann, J., Becker, W., Apel, K. & Parthier, B. 1994. JIP60, a methyl jasmonate-induced ribosome-inactivating protein involved in plant stress reactions. Proceedings of the National Academy of Sciences, 91**:** 7012-7016.

Reymond, P., Weber, H., Damond, M. & Farmer, E.E. 2000. Differential Gene Expression in Response to Mechanical Wounding and Insect Feeding in Arabidopsis. The Plant Cell Online, 12**:** 707-720.

Richards, F.J. & Berner, E. 1954. Physiological studies in plant nutrition. Annals of Botany, 18**:** 15-33.

Richards, F.J. & Coleman, R.G. 1952. Occurrence of putrescine in potassium-deficient barley. Nature, 170**:** 460-460.

Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S. & Knogge, W. 1995. The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the Rrs1 resistance genotype. The EMBO journal, 14**:** 10.

Rojo, E., Solano, R. & Sánchez-Serrano, J. 2003. Interactions between signaling compounds involved in plant defense. Journal of Plant Growth Regulation, 22**:** 82-98.

Ross, A.F. 1961. Localized acquired resistance to plant virus infection in hypersensitive hosts. Virology, 14**:** 329-39.

Rouster, J., Leah, R., Mundy, J. & Cameron-Mills, V. 1997. Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain. The Plant Journal, 11**:** 513-523.

Rozen, S. & Skaletsky, H.J. 2000. Primer3 on the WWW for general users and for biologist programmers. . In: KRAWETZ, S. & MISENER, S. (eds.) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, NJ, USA: Humana Press.

Rufty, T.W., Jr., Jackson, W.A. & Raper, C.D., Jr. 1981. Nitrate reduction in roots as affected by the presence of potassium and by flux of nitrate through the roots. Plant Physiology, 68**:** 605-609.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y. & Hunt, M.D. 1996. Systemic acquired resistance. Plant Cell, 8**:** 1809-1819.

Schachtman, D.P. & Shin, R. 2007. Nutrient sensing and signaling: NPKS. Annual Review of Plant Biology, 58**:** 47-69.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. & Manners, J.M. 2000. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proceedings of the National Academy of Sciences of the United States of America, 97**:** 11655-11660.

Schilmiller, A.L., Koo, A.J.K. & Howe, G.A. 2007. Functional diversification of acyl-coenzyme A oxidases in jasmonic acid biosynthesis and action. Plant Physiology, 143**:** 812-824.

Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M. & Waner, D. 2001. Guard cell signal transduction. . Annual Review of Plant Physiology and Plant Molecular Biology, 52**:** 627-658.

Schulte, D., Close, T.J., Graner, A., Langridge, P., Matsumoto, T., Muehlbauer, G., Sato, K., Schulman, A.H., Waugh, R., Wise, R.P. & Stein, N. 2009. The international barley sequencing consortium - At the threshold of efficient access to the barley genome. Plant Physiology, 149**:** 142-147.

Schweizer, P., Gees, R. & Mosinger, E. 1993. Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare L.*) with the Powdery Mildew *Erysiphe graminis* f.sp. *hordei*. Plant Physiology, 102**:** 503-511.

Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J.M., Gaymard, F. & Grignon, C. 1992. Cloning and expression in yeast of a plant potassium ion transport system. Science, 256**:** 663-665.

Shah, J., Tsui, F. & Klessig, D.F. 1997. Characterization of a Salicylic Acid-Insensitive Mutant (sai1) of Arabidopsis thaliana, Identified in a Selective Screen Utilizing the SA-Inducible Expression of the tms2 Gene. Molecular Plant-Microbe Interactions, 10**:** 69-78.

Shin, R. & Schachtman, D.P. 2004. Hydrogen peroxide mediates plant root cell response to nutrient deprivation. Proceedings of the National Academy of Sciences of the United States of America, 101**:** 8827-8832.

Shipton, W.A., Boyd, W.J.R. & Ali, S.M. 1974. Scald of barley. Review of plant pathology, 11**:** 839-861.

Shoji, T., Ogawa, T. & Hashimoto, T. 2008. Jasmonate-induced nicotine formation in tobacco is mediated by tobacco *COI1* and *JAZ* Genes. Plant and Cell Physiology, 49**:** 1003-1012.

Shoresh, M., Yedidia, I. & Chet, I. 2005. Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. Phytopathology, 95**:** 76-84.

Song, W.C., Baertschi, S.W., Boeglin, W.E., Harris, T.M. & Brash, A.R. 1993. Formation of epoxyalcohols by a purified allene oxide synthase. Implications for the mechanism of allene oxide synthesis. Journal of Biological Chemistry, 268**:** 6293-6298.

Sreenivasulu, N., Graner, A. & Wobus, U. 2008. Barley genomics: An overview. International Journal of Plant Genomics, 486258.

Stamp, P. & Geisler, G. 1980. Effect of potassium deficiency on C3 and C4 cereals. Journal of Experimental Botany, 31**:** 371-377.

Staswick, P.E. 2008. JAZing up jasmonate signaling. Trends in Plant Science, 13**:** 66-71.

Staswick, P.E., Su, W. & Howell, S.H. 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proceedings of the National Academy of Sciences of the United States of America, 89**:** 6837-6840.

Staswick, P.E. & Tiryaki, I. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. The Plant Cell, 16**:** 2117-2127.

Steiner-Lange, S., Fischer, A., Boettcher, A., Rouhara, I., Liedgens, H., Schmelzer, E. & Knogge, W. 2003. Differential defense reactions in leaf tissues of barley in response to infection by *Rhynchosporium secalis* and to treatment with a fungal avirulence gene product. Molecular Plant-Microbe Interactions, 16**:** 893-902.

Stintzi, A. & Browse, J. 2000. The *Arabidopsis* male-sterile mutant, opr3, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proceedings of the National Academy of Sciences of the United States of America, 97**:** 10625-10630.

Stintzi, A., Weber, H., Reymond, P., Browse, J. & Farmer, E.E. 2001. Plant defense in the absence of jasmonic acid: The role of cyclopentenones. Proceedings of the National Academy of Sciences of the United States of America, 98**:** 12837-12842.

Stone, S.L. & Callis, J. 2007. Ubiquitin ligases mediate growth and development by promoting protein death. Current Opinion in Plant Biology, 10**:** 624-632.

Strange, R.N. & Scott, P.R. 2005. Plant disease: A threat to global food security. Annual Review of Phytopathology, 43**:** 83-116.

Strassner, J., Schaller, F., Frick, U.B., Howe, G.A., Weiler, E.W., Amrhein, N., Macheroux, P. & Schaller, A. 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. The Plant Journal, 32**:** 585-601.

Stushnoff, C., Ducreux, L.J.M., Hancock, R.D., Hedley, P.E., Holm, D.G., Mcdougall, G.J., Mcnicol, J.W., Morris, J., Morris, W.L., Sungurtas, J.A., Verrall, S.R., Zuber, T. & Taylor, M.A. 2010. Flavonoid profiling and transcriptome analysis reveals new gene–metabolite correlations in tubers of Solanum tuberosum L. Journal of Experimental Botany, 61**:** 1225-1238.

Sutton, P.N., Henry, M.J. & Hall, J.L. 1999. Glucose, and not sucrose, is transported from wheat to wheat powdery mildew. Planta, 208**:** 426-430.

Swarbrick, P.J., Schulze-Lefert, P. & Scholes, J.D. 2006. Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. Plant, Cell and Environment, 29**:** 1061-1076.

Taiz, L. & Zeiger, E. 1998. Plant physiology, Sunderland, MA, USA, Sinauer Associates Inc.

Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., Takamiya, K.-I., Shibata, D., Kobayashi, Y. & Ohta, H. 2005. 12-oxo-phytodienoic acid Triggers expression of a distinctset of genes and plays a role in wound-Induced gene expression in *Arabidopsis*. Plant Physiology, 139**:** 1268-1283.

Tewari, R.K., Kumar, P. & Sharma, P.N. 2007. Oxidative stress and antioxidant responses in young leaves of mulberry plants grown under nitrogen, phosphorus or potassium deficiency. Journal of Integrative Plant Biology, 49**:** 313-322.

Theodoulou, F.L., Job, K., Slocombe, S.P., Footitt, S., Holdsworth, M., Baker, A., Larson, T.R. & Graham, I.A. 2005. Jasmonic Acid Levels Are Reduced in COMATOSE ATP-Binding Cassette Transporter Mutants. Implications for Transport of Jasmonate Precursors into Peroxisomes. Plant Physiology, 137**:** 835-840.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A. & Browse, J. 2007. JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. Nature, 448**:** 661-665.

Thirugnanasambandam, A., Wright, K.M., Atkins, S.D., Whisson, S.C. & Newton, A.C. 2011. Infection of Rrs1 barley by an incompatible race of the fungus *Rhynchosporium secalis* expressing the green fluorescent protein. Plant Pathology**:** 513-521.

Ton, J., Van Pelt, J.A., Van Loon, L.C. & Pieterse, C.M.J. 2002. Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. Molecular Plant-Microbe Interactions, 15**:** 27-34.

Tosa, Y., Akiyama, T. & Ogura, H. 1989. Cytological aspects of interactions between formae speciales of *Erysiphe graminis* and genera of gramineous plants, and their evolutionary implications. Canadian Journal of Botany, 68**:** 1249 - 1253.

Troufflard, S., Mullen, W., Larson, T., Graham, I., Crozier, A., Amtmann, A. & Armengaud, P. 2010. Potassium deficiency induces the biosynthesis of oxylipins and glucosinolates in *Arabidopsis thaliana*. BMC Plant Biology, 10**:** 172.

Tschoep, H., Gibon, Y., Carillo, P., Armengaud, P., Szecowka, M., Nunes-Nesi, A., Fernie, A.R., Koehl, K. & Stitt, M. 2009. Adjustment of growth and central metabolism to a mild but sustained nitrogen-limitation in *Arabidopsis*. Plant, Cell & Environment, 32**:** 300-318.

Turner, J.G., Ellis, C. & Devoto, A. 2002. The jasmonate signal pathway. The Plant Cell, 14**:** S153-S164.

Vallad, G.E. & Goodman, R.M. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Science, 44**:** 1920-1934.

Van't Slot, K.a.E., Gierlich, A. & Knogge, W. 2007. A single binding site mediates resistance- and disease-associated activities of the effector protein NIP1 from the barley pathogen *Rhynchosporium secalis*. Plant Physiology, 144**:** 1654-1666.

Van Loon, L.C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. European Journal of Plant Pathology, 103**:** 753-765.

Van Mechelen, J., Schuurink, R., Smits, M., Graner, A., Douma, A., Sedee, N., Schmitt, N. & Valk, B. 1999. Molecular characterization of two lipoxygenases from barley. Plant Molecular Biology, 39**:** 1283-1298.

Van Oosten, V.R., Bodenhausen, N., Reymond, P., Van Pelt, J.A., Van Loon, L.C., Dicke, M. & Pieterse, C.M.J. 2008. Differential effectiveness of microbially induced resistance against herbivorous insects in *Arabidopsis*. Molecular Plant-Microbe Interactions, 21**:** 919-930.

Van Wees, S.C.M., De Swart, E.a.M., Van Pelt, J.A., Van Loon, L.C. & Pieterse, C.M.J. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the United States of America, 97**:** 8711-8716.

Van Wees, S.C.M., Van Der Ent, S. & Pieterse, C.M.J. 2008. Plant immune responses triggered by beneficial microbes. Current Opinion in Plant Biology, 11**:** 443-448.

Vellosillo, T., Martinez, M., Lopez, M.A., Vicente, J., Cascon, T., Dolan, L., Hamberg, M. & Castresana, C. 2007. Oxylipins Produced by the 9-Lipoxygenase Pathway in Arabidopsis Regulate Lateral Root Development and Defense Responses through a Specific Signaling Cascade. Plant Cell**:** tpc.106.046052.

Vick, B.A. & Zimmerman, D.C. 1984. Biosynthesis of jasmonic acid by several plant species. Plant Physiology, 75**:** 458-461.

Walker, D.J., Black, C.R. & Miller, A.J. 1998. The role of cytosolic potassium and pH in the growth of barley roots. Plant Physiology, 118**:** 957-964.

Walling, L.L. 2000. The myriad plant responses to herbivores. Journal of Plant Growth Regulation, 19**:** 195-216.

Walters, D., Cowley, T. & Mitchell, A. 2002. Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. Journal of Experimental Botany, 53**:** 747-756.

Walters, D. & Heil, M. 2007. Costs and trade-offs associated with induced resistance. Physiological and Molecular Plant Pathology, 71**:** 3-17.

Walters, D., Walsh, D., Newton, A. & Lyon, G. 2005. Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. Phytopathology, 95**:** 1368-1373.

Wang, T.-B., Gassmann, W., Rubio, F., Schroeder, J.I. & Glass, A.D.M. 1998. Rapid up-regulation of *HKT1,* a high-affinity potassium transporter gene, in roots of barley and wheat following withdrawal of potassium. Plant Physiology, 118**:** 651-659.

Wang, Z., Dai, L., Jiang, Z., Peng, W., Zhang, L., Wang, G. & Xie, D. 2005. GmCOI1, a soybean F-Box protein gene, shows ability to mediate jasmonate-regulated Plant defense and fertility in *Arabidopsis*. Molecular Plant-Microbe Interactions, 18**:** 1285-1295.

Wasternack, C. 2007. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. Annals of Botany, 100**:** 681 - 697.

Wasternack, C. & Kombrink, E. 2010. Jasmonates: Structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chemical Biology, 5**:** 63-77.

Wasternack, C., Stenzel, I., Hause, B., Hause, G., Kutter, C., Maucher, H., Neumerkel, J., Feussner, I. & Miersch, O. 2006. The wound response in tomato - Role of jasmonic acid. Journal of Plant Physiology, 163**:** 297-306.

Weidhase, R.A., Kramell, H.-M., Lehmann, J., Liebisch, H.-W., Lerbs, W. & Parthier, B. 1987. Methyljasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. Plant Science, 51**:** 177-186.

Weiskorn, C., Krämer, M., Ordon, F. & Friedt, W. 2002. Induced resistance in barley (*Hordeum vulgare L*.) against

Rhynchosporium secalis and Barley Yellow Dwarf Virus (BYDV). Induced Resistance in Plants against Insects and Diseases, 25**:** 149-153.

White, P. & Karley, A. 2010. Potassium. Cell biology of metals and nutrients. Berlin, Heidelberg, Germany: Springer-Verlag.

White, P.J. 1993. Relationship between the development and growth of rye (*Secale cereale L*.) and the potassium concentration in solution. Annals of Botany, 72**:** 349-358.

White, P.J. & Brown, P.H. 2010. Plant nutrition for sustainable development and global health. Annals of Botany, 105**:** 1073-1080.

White, R.F. 1979. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology, 99**:** 410-412.

Wicker, T., Schlagenhauf, E., Graner, A., Close, T., Keller, B. & Stein, N. 2006. 454 sequencing put to the test using the complex genome of barley. BMC Genomics, 7**:** 275.

Wicker, T., Taudien, S., Houben, A., Keller, B., Graner, A., Platzer, M. & Stein, N. 2009. A whole-genome snapshot of 454 sequences exposes the composition of the barley genome and provides evidence for parallel evolution of genome size in wheat and barley. The Plant Journal, 59**:** 712-722.

Wiese, J., Bagy, M.M.K. & Schubert, S. 2003. Soil properties, but not plant nutrients (N, P, K) interact with chemically induced resistance against powdery mildew in barley. Journal of Plant Nutrition and Soil Science, 166**:** 379-384.

Wolter, M., Hollricher, K., Salamini, F. & Schulze-Lefert, P. 1993. The *mlo* resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defence mimic phenotype. Molecular and General Genetics, 239**:** 122-128.

Xi, K., Xue, A.G., Burnett, P.A., Helm, J.H. & Turkington, T.K. 2000. Quantitative resistance of barley cultivars to *Rhynchosporium secalis*. Canadian Journal of plant pathology, 22**:** 217-223.

Xie, D.-X., Feys, B.F., James, S., Nieto-Rostro, M. & Turner, J.G. 1998. COI1: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science, 280**:** 1091-1094.

Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W.L., Ma, H., Peng, W., Huang, D. & Xie, D. 2002. The SCFCOI1 ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. The Plant Cell, 14**:** 1919-1935.

Yan, Y., Stolz, S., Chételat, A., Reymond, P., Pagni, M., Dubugnon, L. & Farmer, E.E. 2007. A downstream mediator in the growth repression limb of the jasmonate pathway. The Plant Cell, 19**:** 2470-2483.

Zaffarano, P.L., Mcdonald, B.A., Zala, M. & Linde, C.C. 2006. Global hierarchical gene diversity analysis suggests the fertile crescent Is not the center of Origin of the barley scald pathogen *Rhynchosporium secalis*. Phytopathology, 96**:** 941-950.

Zerbe, P., Weiler, E.W. & Schaller, F. 2007. Preparative enzymatic solid phase synthesis of cis(+)-12-oxo-phytodienoic acid - physical interaction of AOS and AOC is not necessary. Phytochemistry, 68**:** 229-236.

Zhan, J., Fitt, B.D.L., Pinnschmidt, H.O., Oxley, S.J.P. & Newton, A.C. 2008. Resistance, epidemiology and sustainable management of *Rhynchosporium secalis* populations on barley. Plant Pathology, 57**:** 1-14.

Zhang, Z., Collinge, D.B. & Thordal-Christensen, H. 1995. Germin-like oxalate oxidase, a H_2O_2-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. The Plant Journal, 8**:** 139-145.

Zhang, Z., Henderson, C., Perfect, E., Carver, T.L.W., Thomas, B.J., Skamnioti, P. & Gurr, S.J. 2005. Of genes and genomes, needles and haystacks: *Blumeria graminis* and functionality. Molecular Plant Pathology, 6**:** 561-575.

Zhao, D., Oosterhuis, D.M. & Bednarz, C.W. 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. Photosynthetica, 39**:** 103-109.

Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Hamberg, M., Grimm, R., Ganal, M. & Wasternack, C. 2000. Molecular cloning of allene oxide cyclase. Journal of Biological Chemistry, 275**:** 19132-19138.

Zien, C.A., Wang, C., Wang, X. & Welti, R. 2001. In vivo substrates and the contribution of the common phospholipase D, PLDα, to wound-induced metabolism of lipids in *Arabidopsis*. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1530**:** 236-248.

Zimmermann, G., Baumlein, H., Mock, H.P., Himmelbach, A. & Schweizer, P. 2006. The multigene family encoding germin-like proteins of barley. Regulation and function in basal host resistance. Plant Physiology, 142**:** 181 - 192.