STUDIES IN SEED GERMINATION IN THE GENUS NICOTIANA

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Md. MOMTAZ HOSSAIN

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Studies in seed germination in the genus Nicotiana. Md. M. Hossain.

Summary

Light and temperature requirements of different seed samples of the genus Nicotiana obtained from various sources were investigated.

Freshly harvested tobacco seeds germinated in the presence of light at 20°C and at high temperature (30°C) light was not able to promote germination. Old seed samples showed a high percentage of germination in the dark and their germination became light dependent at 35°C. In a seed sample where high temperature reduced dark germination, low temperature pre-treatments along with KNO_3 solution increased dark germination slightly.

In all seed samples light promoted germination. In some cases light was absolutely necessary. Time of attainment of maximum light sensitivity was found to be 24 hrs or thereabouts. Promotion or inhibition of germination by red or far-red light depended upon seed batch and time of imbibition.

Gibberellic acid (GA_3) and a mixture of GA_{4+7} could induce dark germination in light sensitive tobacco seeds. Response of old and new seed samples to various concentrations of Gibberellins was variable. GA_{4+7} was found much more effective in inducing dark germination at low concentration (0.12 mg/ml). Kinetin at 10-30 p.p.m. failed to induce dark germination in typical photoblastic seeds.

Low temperature pre-treatments along with GA_3 at 10^{-3}M had some deleterious effects on seedlings.

Light requirement in tobacco seeds could not be overcome by seed coat treatments. But physically or chemically injured seeds germinated better in dark when treated with 10^{-5}M GA_3 than untreated seeds.

Certain chemical compounds including cyanides could stimulate dark germination in old seed samples. In freshly harvested seeds these chemicals failed to stimulate germination in dark.

N. tabacum seeds collected from the capsules of different stages of

ripeness showed mixed light sensitivity and degree of dormancy immediately after harvest. Some plants produced deeply dormant seeds which did not respond in germination tests.

Developing capsules or plants in which leaves were shaded with cinemoid filters of different spectral composition, produced seeds varying in their capacity to germinate. Plants in which leaves were covered with deep red cinemoid filters at time of seed maturation gave heavier seeds with typical light sensitivity. Seeds matured on plants under short-day (8 hrs) treatments after full flowering showed light dependency for their germination. Long-day seeds showed greater response to light at 30°C.

It appeared that light controlled dormancy in N. tabacum could possibly be manipulated by changing photoperiods and/or light quality around mother plants during seed maturation.

CONTENTS

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STUDIES IN SEED GERMINATION IN NICOTIANA

Introduction and review of the literature

The seed is a simple product of the development of the ovule after fertilization. Fertilization stimulates the growth of fruit, the embryo and that of the endosperm. But we have very little knowledge of the physiology of these early stages of development. However, most cultivated and wild plants are normally propagated from seed and, therefore, it constitutes an important plant part to the seed growers and modern seed technologists.

Nicotiana produces very small seeds with rough reticulate seed coats. Seed size may vary according to variety (Avery, 1933). In 1921 Kondo (Avery, 1933) reported that tobacco seed average 0.75 mm. long, 0.53 mm. broad and 0.47 mm. thick, a thousand weighing 0.08 gms. Tobacco seed coat consists of (i) epidermis, (ii) outer seed coat, (iii) inner seed coat (Avery, 1933). After maturity the outer thin wall forms the reticulate appearance of the seed. The inner wall is double layered and its cells are cutinized and slightly lignified. The endosperm consists of three to five layers of thick walled cells rich in aleurone and oil droplets (Avery, 1933).

A seed is judged by its germination capability and seedling vigour. A seed bears an embryonic plant, and germination is the resumption of growth of the embryo and its development into an independent seedling (Toole et al., 1956). Several biological activities occur before complete germination of the seed. The

exact order of the various physiological steps involved in the process of germination is not clear. However, in a favourable environment it is believed that during the process of germination mobilization of reserve food materials may be continued. This mobilization of the food materials largely depends upon the chemical nature of the reserve food materials in the seed.

It has been pointed out that small seed like tobacco contain very little food reserves (Kincaid, 1935; Wells, 1959), which limits the successful germination of the seed in the absence of certain additional factors such as light during germination.

From extensive studies (Ilin, 1934; McCormick, 1932; Giovannozzi-Sermanni et al., 1956; Abdoh & Naffissi, 1963) it is revealed that tobacco seed contains an abundance of protein, fat and a small amount of sugar but no starch is present in the seed (Wada, 1957). Analytical studies (Abdoh & Naffissi, 1963; Giovannossi-Sermanni et al 1956) showed that in mature tobacco seed a number of amino acids does exist. However, it is not clear how the reserve food materials could limit the successful germination in tobacco seed because germination could be prevented by other physiological blockages.

One of the important problems relating to seed germination is the failure of viable seed to germinate when all the conditions necessa for germination are fulfilled. This failure may be due to some externa factor such as moisture, gases, temperature, light, etc., or it may be due to internal factors within the seed itself. According to Crocker (1916) dormancy of seeds may be due to the inhibition

of one or more processes before, during or after germination. Due to lack of established criteria, the term "dormancy" in seed is misleading and confusing (Koller, 1955; Roberts, 1972).

Dormancy in a seed may be biologically and ecologically important for the seed itself in spreading or delaying germination until favourable conditions exist in nature (Koller, 1955; Baskin & Baskin, 1970, 1971 and 1972). The period of dormancy is very variable from plant to plant. Dormancy soon after harvest may be a special adaptation for the seed so that they can pass through a long unfavourable wetting and drying period during summer (Baskin & Baskin, 1972).

In nature we find two types of dormancy (i) Primary, (ii) Secondary or induced dormancy. Primary dormancy is very common in freshly harvested seeds and secondary dormancy may appear in the seed if they are held imbibed in a condition unfavourable for germination. However, these are general observations in this field of study. In some cases dormancy in the seeds produced by a single plant is very variable and confusing. As, for example, upper and lower seed of Xanthium Pennsylvanicum (Wareing & Foda, 1957) and wild oat may show different degree of dormancy (Thurston, 1953; Morgan & Berrie, 1970). The reason for such variation is not clear and it needs further investigation.

Causes of dormancy result from a number of complex sequences. Common causes are (1) seed coats impermeable to water and/or gases; (2) condition exists in the embryo itself; (3) presence of inhibitors in the embryo or surrounding tissues. A number of lilies, such as

Lilium auratum, Lilium japonicum, Lilium superbum, etc. (Barton, 1965) shows epicotyl dormancy. In those cases the root extension occurs at normal temperatures but shoots fail to grow. The partially germinated seeds could be given low temperature treatment for a short period of time to break the epicotyl dormancy. Other factors could be involved in inducing dormancy. These may be partly genetic and probably also to weather conditions or environment around the mother plants during or after maturation of seeds. However, we shall try to discuss it in brief in the relevant section.

Dormancy in Nicotiana seed has been studied by a number of authors. Burk (1957) reported that under normal conditions the seed of Nicotiana germinate immediately after harvest. But he pointed out that N. gossii and other species have an extended dormancy period. Moorthi & Moss (1969) tested the germination behaviour of about 40 species of tobacco seed and reported that some seeds showed a high percentage of germination after two months' storage. Some took four months. But in the variety "Dixie shade" dormancy extended year after year although they were viable. They have presumed that a germination inhibitor could be involved in dormant seed. But so far nothing about the nature of such an inhibitor or promotor in tobacco seed has been reported.

The seed coat sometimes is of considerable physiological importance because it may be the cause of dormancy. Seed coat dormancy has been explained in a number of ways. The factors associated with seed coat may be impermeability to water, and/or oxygen, or mechanical resistance to the embryo (Crocker, 1906), or it may prevent leaching

of inhibitor from the embryo or endosperm (Wareing & Foda, 1957). But recent studies in this direction showed that none of above factors may not be the cause of dormancy.

Born & Corns (1958) studied the seeds of some noxious weeds e.g. Fagopyrum tartaricum, and they came to the conclusion that dormancy in these seed may not be due to one single factor, because removal of seed coat brings about only a small increase in the percentage of germination. It has been found that though seed coats may be permeable to water (Crocker, 1906; Roberts, 1961) the seed may remain dormant.

Other factors may be involved in controlling germination of Striga lutea (Egley, 1972) and Stachys alpina (Pinfield et al., 1972). In the former species increasing the oxygen tension or CO_2 pressure did not improve germination though the seed coat is permeable to these gases. In these semi-parasites germination is dependent on the presen of root exudate from the host plant.

Now-a-days a number of studies has been made to establish a relationship between germination inhibitors, seed coat and dormancy. The presence of a germination inhibitor could form a physiological block which is the cause of dormancy in many seeds. Some water soluble growth inhibitors specific to the seed are found to occur in upper and lower seed of Xanthium (Wareing & Foda, 1957). During storage some changes occur in seed coat, and entrance of O_2 could break down the inhibitor in Xanthium.

Presence of inhibitors has been reported from many seeds, a few of them are oat (Black, 1959), Lovell peach (Lipe, 1966), Kidney

beans (Pusztai, 1972). Removal of the seed coat or removal of inhibitor may allow seed to germinate. Dormin (abscisic acid), a naturally occurring compound, is known to regulate dormancy in Rosa arvensis (Jackson, 1966) and in Lovell peach seed (Lipe, 1966). A similar compound is also reported from Prunus domestica (Lin and Boe, 1972). However, known inhibitors present in the seed are ammonia, hydrogen cyanide, ethylene, essential oils, mustard oil, alcoloids, unsaturated lactones (such as coumarin), phenolic acids (cinnamic, caffeic) and trypsin inhibitor (Evenari, 1949; Wareing, 1965; Pusztai, 1972). But whether these inhibitors influence the process of germination or inhibit the different subsequent phases of development is not clear. Very little is known about the presence of inhibitors in the seed of N. tabacum, but it is reported that some water extractable inhibitor could be extracted from old seeds of N. rustica (Scarascia, 1957).

Very recently it has been suggested that in Acer pseudoplatanus (Webb & Wareing, 1972), oxygen uptake, water uptake, mechanical resistance to the embryo and presence of germination inhibitor in the testa were not responsible for dormancy immediately post-harvest. The restriction of outward diffusion of a germination inhibitor, present in the embryo, through the testa constitutes the block which is the cause of this dormancy.

Germination of a wide range of species is governed by light. It may either promote or inhibit. The question of light requirement has been the subject of studies for many years. Caspary in 1860

was the first botanist who observed the beneficial influence of light in seed germination (Gardner, 1921). Photo-inhibition of seed germination was first reported in 1904 by Heinricher. Tobacco seed is known to be light sensitive, which was reported by Raciborski in 1900. Since then different authors have studied the light requirement in tobacco seed germination. But differences of opinion regarding the light sensitivity of different varieties of tobacco still exist.

Goodspeed in 1919 examined critically the light sensitivity of several types of tobacco seed and came to the conclusion that five representative types of N. tabacum and of five varieties of N. rustica germinated well in the dark. He, however, noticed that seeds from six to twelve years old showed a low percentage of germination in darkness.

Gardner (1921) found that some tobacco seed were light sensitive Johnson et al. (1930) made an extensive study and found that as a matter of fact tobacco seed does not require any "after-ripening" period. It can germinate as soon as it is ripe. They, however, passed the opinion that the relation between light and tobacco seed germination is complicated and not well understood, and, therefore, it is better to expose the seed to light during germination.

The photoblastism in Nicotiana attracted the attention of geneticists. As far back as in 1926 Honning stated that light requirement for the germination of tobacco seed is controlled by some genetic factors. He considered that light-needing character is dominant over light-indifferent. Later on he, however, came to

the conclusion that light requirement for germination in tobacco is not universal (Honning, 1928, 1930, 1946).

Kasperbauer (1968) actually confirmed the findings of Honning. He studied the seed of N. tabacum from self- and cross-pollinated plants. However, he found that all seeds were light-sensitive at the time of harvest. Seed become more and more light-indifferent during storage.

The basic idea of genetic control of light sensitivity in tobacco may be disputed because of the fact that development of dormancy determined by the environment in which the seed matures (Morgan & Berrie, 1970). Our knowledge relating to genotype, physiology and development is very limited and it needs further investigation.

Further investigations have been made in tobacco seed germination. It is believed that maximum and minimum values of light sensitivity depends upon strain and after-ripening of the seed (Isikawa, 1953). Light-requiring tobacco seeds are known to be stimulated by brief exposure to light (Kincaid, 1935; Igikawa, 1952).

Since the discovery of phytochrome it is believed that phytochrome controls germination in a number of seed. When the seed is illuminated at 660 nm phytochrome present in the seed is converted into alternative form (Pfr) which promotes germination. Exposure to longer wavelength (730 nm) converts the pigment into original form (Pr) which possibly could be thought to act in an inhibitory capacity rather than Pfr being promotive.

The observations of Flint and McAllister in 1935 and 1937 were

confirmed in lettuce seed (Borthwick, et al 1952). However, phytochrome mediated germination in different seed is very variable. A true reversib reaction may not be found in all seed lots, Some seed may not exhibit phytochrome controlled germination but none the less it is considered that light still plays a role in their germination (Borthwick, et al 1952). It has been observed that dark germination of certain varieties of tomato can be halted by far-red irradiation and it can again be promoted by red irradiation, depending upon variety (Mancinelli et al., 1966). Long or short exposure to far-red is required before this phenomenon is manifest. Here also germination can be said to be controlled by phytochrome.

In recent studies Taylorson (1972) observed that seeds of six weed species buried for six months in the field became light sensitive, whereas they were ungermineable before burial. He suggested that during burial there might be changes in phytochrome which are responsible for the seasonal variation in light-sensitive seeds. However, phytochrome mediated germination is very complicated and how changes in total phytochrome take place is not clear. It is also reported that to get full expression of phytochrome control of germination dehydration of the pigment is necessary in Amaranthus retroflexus (Taylorson & Hendricks, 1972).

In light sensitive seeds like Lamium amplexicaule (henbit) (Jones & Bailey, 1956), Nemophila (Black & Wareing, 1957) normal light may prevent the seeds from germinating.

In tobacco seed it is claimed that typical reversible phytochrome

action could be obtained only when the seeds are soaked in Kinetin (Ogawara & Ono, 1961). Kasperbauer (1968) proposed that the germination of light-requiring tobacco seed is controlled by phytochrome. However, he could not offer any explanation how the light requirement in certain tobacco seed is by-passed during storage. One of the possibilities he mentioned that during storage phytochrome in the light-indifferent N. tabacum seed become capable of attaining the biologically active form during rehydration without exposure to light. Very recently Holdsworth (1972) reported that he observed true phytochrome reversible reaction in "Golden Virginia" type tobacco during first day imbibition, when the period of imbibition is increased the seeds become indifferent to red and what he called deep red light. Isikawa (1961) has suggested that the phytochrome system needs repeated investigation because the interaction between light and temperature treatment are more complicated.

It has been suggested by some authors that in light-requiring seed germination may be prevented by some block of respiration (Evenari et al., 1955). This germination block could be overcome by light. Kipp in 1929 (Toole, et al., 1956) found that in the dark, respiration of tobacco seed decreased gradually after imbibition is completed. However, if the fully imbibed tobacco seeds are exposed to light respiration again increases. Powell (1958) also reported that aerobic conditions are required during imbibition of photoblastic tobacco seed in order to respond to light. The results of Powell and Kipp with tobacco seed do not agree with earlier report of Wieser

in 1927 (Evenari, 1965). Because he (Wieser) has demonstrated that tobacco seeds imbibed in an oxygen-free atmosphere for several hours (69 hours) when irradiated and transferred to air, germinated well.

In fact light is known to control many other biological processes such as flowering, photoperiodism, growth, etc., and in light-requiring germination process actual mechanism of photoreceptive systems and reactions involved are still obscure.

In a number of cases seed coat dormancy can be broken by physical and chemical treatments. A few of them are Prosopis (Khudairi, 1956), Oat (Black, 1959), Stachys alpina (Pinfield et al, 1972), Acer saccarum (Webb & Dumbroff, 1969), Asclepias syriaca (Oegema & Fletcher, 1972). In all cases seed coats play an important part in controlling germination. Removal, puncturing, chemical scarification, etc., can break this seed coat dormancy. In other cases germination inhibitors are found to occur within the seed when they are dormant. In all cases removal of inhibitors or changes in seed coat may permit seed to germinate (Wareing & Foda, 1957; Black, 1959). Some authors have suggested that chemical scarification may help to remove seed coat dormancy by removing inhibitors from the seed or seed coat (Koller & Negbi, 1959; Bhat & Dhar, 1971). In Acer ginnala (Dumbroff & Webb, 1970) and Asclepias syriaca (Oegema & Fletcher, 1972) stratification could remove seed coat dormancy. In some parasitic angiosperms a stimulant from the root exudates may break seed coat dormancy (Egley, 1972). However, dormancy in those seed may not be due to one single factor as light may be required after seed coat treatment in Cyperus inflexus

(Baskin & Baskin, 1971).

Different seeds have different ranges of temperature within which they can germinate. Seeds, particularly freshly harvested seeds, of many plants show a sharp inhibition of germination with the rise of temperature. This inhibition can be negated by light, alternate temperature, stratification, storage, etc.

The cardinal temperatures for the germination of Florida cigar wrapper tobacco seeds are approximately 10°, 24° and $34^{\circ}C$ (Kincaid, 1935). Powell (1958) reported that in Golden Harvest tobacco no germination occurred at $30^{\circ}C$ both in dark and light. It appears that temperature range varies from variety to variety.

Alternating temperature may have some influence in breaking dormancy. Responses of some seeds to light during germination could be altered by brief alternating temperatures (Harrington, 1923; Berrie, 1966). In Poa pratensis (Toole & Borthwick, 1970) certain temperature alteratio could substitute light requirement. Kincaid (1935) found that daily alteration of moderately high temperature could improve tobacco seed germination. In some seeds, e.g. Digitaria (Toole & Toole, 1941) and Polygonum amphibium (Justice, 1944) low or alternating temperature may be a pre-requisite condition before any treatment. It appears that alternating temperature is more favourable than that of constant temperature in promoting germination. But Alcorm & Kurtz in 1959 (Evenari, 1965) reported that Carnegia gigantia germinate better at constant temperature than at alternating temperature if the seeds are irradiated.

In another approach cold treatments or stratification have been found very effective in breaking dormancy. Stratification is known to increase germination in total darkness in Pinus taeda and Pinus strobus, and stratified seed responded to phytochrome activation (Toole et al., 1962).

Elsholtzia seed require certain period of cold treatment after irradiation (Isikawa & Ishikawa, 1960). Birch seeds are known to show photoperiodic response at lower temperature. But chilled seed did not require light for germination (Black & Wareing, 1955). It is also found that cold treatment sometimes bring about germination at high temperature.

Now-a-days it has been demonstrated that some germination regulating substances are produced during stratification. A period of stratification increased the cytokinin level Acer saccharum (Webb et al., 1973; Staden et al., 1972). In wheat stratification may increase RNA ase activity in the seed. It appears that stratification brings about changes in growth hormone for the removal of dormancy (Webb et al., 1973) or dormancy in unstratified seed may be controlled by inhibitor-promotor complex in Ambrosia artemissiifolia (Willemsen & Rice, 1972) and in Asclepias syriaca (Oegema & Fletcher, 1972).

It has been reported that the light requirement in lettuce could be replaced by treating the seeds with Sulphur-containing compounds (Thompson & Kosar, 1939). Roberts (1963a & 1964) has demonstrated that germination of dormant rice seed could be stimulated by some

nitrogenous compounds and a number of respiratory inhibitors could also bring about a marked stimulatory effect on the breaking of dormancy of rice and other seeds. Very recently Hendricks & Taylorson (1972) have tested a number of chemicals including some respiratory inhibitors. They have claimed that almost all of these chemicals stimulated dark germination in lettuce. But Baskin & Baskin (1971) found that no chemical compounds could substitute for a light requirement in dormant Cyperus inflexus seed.

Some nitrogenous compounds are known to be effective promotors of germination of photoblastic seed (Toole et al., 1956). But there is a lot of contradiction regarding the dark germinability of tobacco seed in presence of particular chemical compounds. In a local Japanese variety "Bright yellow" nitrogenous compounds are reported to intensify the effect of GA to a great extent when mixed together (Hashimoto, 1958). The author suggested that nitrogenous compounds, as with GA or light, in some way were involved in the completion of some necessary physiological processes.

Yamaki et al (1961) proposed that dark germination in tobacco seed is induced by two different ways (i) the seeds need both GA or GA-like substances and other compounds, and (ii) need only GA or GA-like substances. In the first case an acidic medium is favourable. He also claimed that ammonium malate or tartarate alone could induce dark germination in tobacco and only glutamic acid at 4.7 to 5.5 pH ranges was effective in bringing about dark germination.

Inorganic phosphate along with GA_3 could induce dark germination

in tobacco seed (Hashimoto & Yamaki, 1962). The authors assumed that phosphate may help in the uptake of carboxylic acid in the seed. However, discriminating behaviour of phosphate promoting only GA_3 induced germination is quite interesting and it needs further explanation.

From available information in literature it is quite clear that gibberellin can overcome induced or natural or even the onset of dormancy in oat (Black & Naylor, 1959; Hay, 1968) and other seeds. It can negate thermodormancy in lettuce and Lepidium virginicum seed (Kahn, 1960; Toole & Cathey, 1961). GA_3 could also reduce critical day-length for germination in Begonia evansiana (Nagao et al., 1959), Guayule (Hammond, 1959), and overcome chilling requirement in peach (Gray, 1958). There are evidences that GA-like substances are found to occur in developing apricot fruit (Jackson, 1966) or level of GA-like substances markedly increased during germination in Persea americana (Leshem et al., 1973). However, GA cannot overcome dormancy in intact seeds of plum (Lin & Boe, 1972), or the effect of prolonged far-red irradiation in lettuce (Burdett, 1972), and the action of GA_3 in Ledum seed may be partly reduced at lower temperature (Junttila, 1972).

Ogawara & Ono (1961) demonstrated that GA_3 was very effective in inducing dark germination in tobacco when the concentration is comparatively high. The rate of germination increased with increased concentration of GA_3. GA_3 is also known to be synergistic with light or when used in combination with other chemicals such as KNO_3 and kinetin. Kinetin or KNO_3 alone failed to bring about germination

in dark (Ogawara & Ono, 1961). But Hashimoto (1961) while working with N. tabacum var. "Bright yellow" did not agree with the observation of Ogawara and Ono and Kinetin has been shown to be effective in inducing dark germination in tobacco seed.

Other chemicals are also known to break dormancy and few of them are thiourea in peach (Tukey & Carlson, 1945), in lettuce (Thompson & Kosar, 1939), malonic acid, succinic acid (Simmonds & Simpson, 1972), and ethylene in lettuce (Negim et al., 1972).

From all this information it appears that light requirement of positively photoblastic seed could be replaced by treating the seeds with certain chemicals. But in a number of studies it appears doubtful if they could fully substitute for light requirement.

From the works of different authors it is revealed that conditions under which seed are produced may have some influence in their subsequent germinability. Environmental factors around the mother plant such as photoperiodic pattern, colour of light, temperature, seasonal variations, etc., may influence the development of seed. These factors influence so greatly that some plants with the same genetical constituent when grown in different environmental conditions appear to be distantly related.

Morley (1958) found that the degree of dormancy in subterranean clover was highly heritable. But this author has agreed that many other factors including environmental factors affect parent plant in the production of dormant seed in clover which needs further investigation.

Seasonal variation or place of origin of seed may have some

influence on the degree of dormancy. McWilliams et al.,(1968) found that seeds from northern population of U.S.A. showed a higher percentage of germination at 20°C than that of Southern populations. Allessio (1969) pointed out that variability of degree of dormancy in Bistort seed lots collected from same site in different seasons may be due to environmental pre-conditioning via mother plant. It has also been claimed that seeds from drier parts showed greater dormancy in Amaranthus retroflexus (McWilliams et al., 1968).

It is also important to note that light along with temperature, moisture or humidity constitute important factors in the environment of a developing plant. It is evident that some environmental factors could alter in a number of seeds their degree of dormancy, impermeability and quality.

It has also been reported by a number of authors that mother plants grown under different photoperiods and temperature regimes produced seeds of variable germination quality. In "Grand Rapid" lettuce quality seed could be obtained when seeds are matured under high temperature and short photoperiod. Seed matured under high temperature or continuous light increased temperature tolerance of seed germination both in dark and light (Koller, 1962). In Rosa, when mother plants are subjected to high temperature and light a marked reduction in dormancy could be obtained as compared with field grown control (von Abrams & Hand, 1956).

Photoperiod during the maturation of seed may be an important factor in determining the degree of dormancy. Lona in 1947 (Barton)

has shown that in Chenopodium amaranticolor grown in long-day condition produced dormant seed whereas seed harvested under short-day were non-dormant. In Ononis secula, seed produced on short-day treated plants have seed coats which are more permeable to water than seeds produced on long-day treated plants (Evenari et al., 1966).

Karssen (1970) has investigated the influence of short-day, long day and short-day with an interruption of one hour red light on the induction of dormancy in Chenopodium album. The seed collected from plants which were grown under long-day or short-day with one hour red light showed a higher degree of dormancy after harvest. But plants which received short-day conditions during the last period of their life cycle produced non-dormant seed. He presumed that the different effect of photoperiod on the induction of dormancy is partly regulated by phytochrome and partly by photosynthetic activity of the plants.

It appears that photoperiod, light quality and temperature play an important role in the process of seed development. The role of light around the seed environment may be important from an ecological point of view. As for example germination of light sensitive seed may be inhibited by light passing through green vegetation due to R/FR ratio of the light (Cumming, 1963). Further investigation to know more about the influence of different spectra during maturation or germination may have some ecological importance in shaded areas.

It appears from the review of literature that in certain tobacco seed, germination is blocked by a light requirement. The gibberellin induced dark germination in tobacco is quite interesting because

replacement of light requirement by other chemicals is doubtful.

We have realised that the causes of dormancy and method of breaking it are of both physical and practical value. If the causes of dormancy are known it could be possible to give proper treatment to break it which could assure prompt and uniform germination in order to produce uniform seedlings.

During our investigation we have tried to test the uniformity of light sensitivity of different type of tobacco seed received from various commercial sources. Light-sensitivity was found very variable in different seed lots at different temperatures and during different stages of dark imbibition.

Considering all the contradiction and confusion regarding the dark induction of germination by chemicals, we have used a number of methods to look at whether these chemicals could really replace light requirement in tobacco seed. Some results have been discussed.

During our investigation it has been decided that development of seed on parent plant under certain light conditions and their subsequent germinability might be important. Physiology of light-sensitive tobacco seed harvested at different stages of development may also be important in understanding the variability of dormancy encountered in seed lots. Although the basic principles or mechanism of light requirement in tobacco seed germination is not clear, some results have been reported and discussed in this present investigation.

Materials and Methods

Ten types of tobacco seeds, 3 cvs. of Nicotiana affinis and 7 cvs. of N. tabacum obtained from "Thompson & Morgan (Ipswich), Ltd" and "John Macffee, Seedsman, Paisley" were tested for their light sensitivity. Freshly harvested seeds of Nicotiana tabacum grown during 1970 and 1971 in Glasgow Botanic Garden and Garscube Botanical Research Institute garden were also used in our experiments. Immediately after collection all the seed lots were stored at 4°C until required.

The seeds were placed on 4.25 cm petri dishes containing Whatman's seed test paper 0.4 mm thick moistened with 1 ml of deionised water or test solution. Seeding was always done under green safe light in the dark room. Four replicates of 50 seeds each, unless otherwise mentioned, were placed in light tight aluminium cans to get complete darkness immediately after sowing.

The germination tests were carried out in temperature-controlled incubators. The incubating temperatures used in different experiments were: 10°, 20°, 24°, 30° and 35°C. In some cases one half of the duplicate was held in the darkness in the cans at a definitely controlled temperature and the seeds of the other half were exposed to different light levels (short or long) after certain desired periods of dark incubation. In a number of cases the seeds were exposed to red light alone or red followed by far-red in the sequence depicted

in the relevant section. In most of the cases the seeds were taken out from darkness and after light treatment they were put into the light-tight cans and returned to original temperatures for final germination.

Mechanical abrasion was tried by rubbing the seeds with fine sandpaper. In other cases the seeds were pricked with the help of a sharp needle under microscope after 24 hours dark imbibition. Certain chemicals such as H_2SO_4, H_2O_2, sodium hypochlorite (Domestos), ethyl alcohol, petroleum ether, acetone, were used as chemical means of scarification in breaking dormancy. Duration and other details have been mentioned in the appropriate section.

Several organic and inorganic compounds such as GA_3, GA_{4+7}, Kinetin, thiourea, coumarin, KNO_3, $NaNO_3$, $NaNO_2$, Hydroxyl ammonium chloride, Methyl hydrazine sulphate, Hydrazinium sulphate, Potassium ferricyanide, Potassium ferrocyanide, & potassium azide, were tested in order to investigate if they could fully replace light requirement in different batches of light sensitive tobacco seeds. The stock solutions of the above chemicals were prepared before use. In some cases the stock solutions were kept at $4^{\circ}C$ in a refrigerator and used for experiments within two weeks. Necessary dilutions were made from them immediately before use. All chemical solutions to be tested were added to the seed test paper at the beginning of incubation. The seed test papers soaked with deionised water were taken as control.

In another experiment the seedlings were raised from the seeds

of Nicotiana tabaccum cv. Montcalno. Small seedlings were planted in the plastic pots containing a mixture of peat and soil. The plants were grown in a thermostatically controlled growth cabinet. Temperatur in the growth cabinet varied from 18° - 22°C. The plants were watered frequently and fed with commercial "Vitafeed" nutrient solution twice in a week.

The plants in the growing room were receiving artificial light from warm white alone or natural and warm white fluorescent tubes throughout.

In one experiment the plants were grown under a mixture of natural and warm white fluorescent light 18 hours in a day. In that case capsules of tobacco plants were harvested at different stages of development after anthesis and the seeds were used for studying germinating behaviour in dark and light. Diagrams showing the position of the capsules on the inflorescences were drawn and description of the colour of the capsules, calyx, were noted down.

In all other experiments, plants in the growth chamber received 18 hours warm white fluorescent light from the very beginning and all treatments given to the mother plants were started after full flowering. The flower and fruit bearing plants which were receiving short-day treatment, were taken to the growth cabinet at 9.00 a.m. and returned to the dark room at 5.00 p.m. manually every day.

In other cases six cinemoid sheets of different colours, viz. green, dark deep blue, deep red, light blue, light red, and yellow were chosen. They transmitted light of different spectral composition

In one experiment some small boxes approximately 1.5 by 1.5 cm wide and about 2 cm high were built up out of deep blue and deep red cinemoid sheets. Full details and design of capping the capsules at different stages of development with these boxes will be reported in the relevant section. Capsules open to white light throughout and those covered with aluminium foil were taken as control. A study was undertaken to look at the germination behaviour of the seeds collected from all the treated capsules.

In another experiment only the leaves of 6-flowering plants were covered with 6-differently coloured cinemoid sheets, but the inflorescences bearing the flowers remained exposed to white light. Only a limited number of developing capsules was left on the inflorescences. The capsules collected for seed tests were fully mature, dry, brown in colour and had started cracking at the top. The capsules from all experiments were removed from the plants, dried for a few days in the laboratory then they were stored by capsules in glass vials at $5^{\circ}C$ in a refrigerator until required for tests. The germination tests were carried out as quickly as possible from the date of harvesting.

In all experiments, counts of percentage of germination were made with the aid of a binocular microscope at the end of seven days (unless otherwise mentioned). All tests were repeated at least twice. In the preliminary survey of germination the emergence of radicle was taken as the criterion of successful germination.

Light sources

(1) Red light was obtained from a 12V 100W tungsten halogen lamp designed to operate with the condenser optics of a 5 cm x 5 cm slide projector. This projector was provided with a 20 cm water filter to remove infra-red radiation emitted by the lamp that was not absorbed by the heat absorbing filter of the condenser unit. The light thus obtained was passed through an interference filter made by Barr & Stroud. The characteristics of the filter were as follows:-

Maximum transmission 656 nm, band width 8 nm, percentage transmission at peak wavelength 60, second order transmission removed by optical filter. This gave a radiant flux of 475$\mu Jcm^{-2}sec^{-1}$ at seed level. The radiant energy was measured by a Kipp & Zonen compensated thermopile.

(2) Far-red light was generated by using the same unit as in (1) but replacing the red filter with another interference filter having the following characteristics:-

Peak transmission 730 nm, band width 10 nm, 70% transmission at peak wavelength. This gave radiant flux of 172$\mu Jcm^{-2}sec^{-1}$ at seed level.

(3) White light was obtained from either of the following:-

(i) 1.5 ft, 13 watt WWX fluorescent operating at 250V

(ii) 2 ft, 20 watt WWX fluorescent operating at 250V

In (i) the light source was always situated 8 cms from the petri dishes. This gave radiant energy at a value of 612$\mu Jcm^{-2}sec^{-1}$ at seed level, and in (ii) irradiation 10 cms from the light source was 516$\mu Jcm^{-2}sec^{-1}$ at seed level.

The radiant output of these lamps was measured shortly after switching on by means of a Kipp & Zonen compensated thermopile. The temperature of the tube envelope and the lamp housing was not substantially above ambient and, therefore, the amount of long wave radiation emitted was low. This was tested by measuring the differen between thermopile readings with the thermopile 15 cms from the tube envelope, with and without a 10 cms water filter. There was no difference in these readings. After the lamp had been on for some length of time the tube envelope and housing temperature rose and the thermopile readings became higher. The increase in measured radiation is due to the lamp and housing beginning to act as a "black' body emitter. However, the luminous flux remained the same.

Part I: Preliminary survey of temperature and light requirement in the germination of seed of N. tabacum

For successful germination seed should be placed in a favourable environment. Water, temperature, a supply of oxygen and in some cases light around the seed bed are known to influence the process of germination. Temperature and light constitute important factors in regulating tobacco seed germination. It has been reported that tobacco seed can germinate within a limited range of temperature (Avery, 1933; Kincaid, 1935; Powell, 1958). But with other seeds it has been stated that optimal temperature requirement for different seed samples may vary and the temperature requirement also largely depends upon previous treatment of the seeds, their age (Went, 1953), and temperature conditions under which they were matured (Koller, 1962).

Photoblastism in N. tabacum seed is well known and very complicate (Gardner, 1921; Johnson et al., 1930). It has been demonstrated that alternating temperature could overcome light requirement in positively photoblastic seed such as lettuce, Poa (Harrington, 1923; Siegel, 1950; Berrie, 1966; and Toole et al., 1970). According to Evenari (1965), Alcorn and Kurtz in 1950 reported that alternating temperature was not effective in inducing dark germination in light sensitive Carnegiea seed.

Taking all this information into account an investigation was made to have some knowledge of the temperature and light requirement of freshly harvested and old seed samples of N. tabacum. In this

section the effect of a single temperature shift on tobacco seed germination in absence of light has also been reported.

Materials and Methods

Three different seed samples of *N. tabacum* were tested. These are an old seed sample (Batch I) obtained from the Department of Botany; a freshly harvested seed sample (BG71) grown in the Botanic Garden during 1971; and a light indifferent *N. tabacum* cv. *virginica* purchased from a commercial source.

Germination tests were carried out in 4.5 cm. petri dishes containing Whatman's seed test papers moistened with 1 ml. deionised water or test solution. Light tight cans provided complete darkness where it was necessary. Germination counts were made after a suitable interval from the onset of imbibition. The source of illumination was white fluorescent light. (2ft. 20 watt 250V WWX.) Aluminium cans containing dark imbibed seeds were transferred manually from one incubator to another during single temperature shift experiments. Seeds germinated were counted with the aid of a binocular microscope.

TABLE I: Percentage germination in N. tabacum (Batch I) at 8°, 20° and 30°C irradiated at different light levels

Light conditions	Temperature	Percentage germination after 4d	6d	7d	30d
Continuous dark (Exposed to safe light)	8°C	00	00	00	50.5
	20°C	00	08	41.75	-
	30°C	07	54	62	-
2 minutes illumination once after 24 hours dark imbibition	8°C	00	00	00	42
	20°C	00	38	50.5	-
	30°C	58	64.5	63.5	-
4 hours white light once after 24 hours dark imbibition	8°C	00	00	00	44
	20°C	00	39	52.75	-
	30°C	62	60.5	61	-
Left at room temperature for 4 hours once after 24 hours in darkness	8°C	00	00	00	55
	20°C	00	5.1	30	-
	30°C	0.25	56	55	-
Dark control (never exposed to green light)	20°C			00	
	30°C			9.5	

Experiment I: To demonstrate the effect of temperature and different light levels (short or long) on N. tabacum seed germination

A study was undertaken to determine the temperature range within which seed can germinate and the light requirement for old seed samples of N. tabacum (Batch I). A typical experimental procedure was as follows

(1) 4 replicates of 50 seed each per treatment kept continuously in dark at 8°, 20° and 30°C.

(2) 2 minutes light was given once after 24 hours dark imbibition at 8°, 20° and 30°C.

(3) 4 hours light treatment once after 24 hours dark imbibition at 8°, 20° and 30°C.

(4) Left for 4 hours without exposure to light at room temperature once after 24 hours dark imbibition at 8°, 20° and 30°C.

After irradiation treatments the seeds were returned to original temperature for final germination. In addition four replicates of 50 seeds were kept at 20° and 30°C in complete darkness for seven days. Germination counts were made on the 4th and subsequent days under a green light without observable effect on lettuce seed germination. The results indicate that light was necessary for germination in this batch of seed at all temperatures tested.

Experiment II: To demonstrate the temperature range and light requirement of freshly harvested N. tabacum seed soaked in H_2O and KNO_3 at 20° and 30°C.

Freshly harvested seeds grown in Botanic Garden during 1971 (BG71) were used in this experiment. The seeds were irradiated in white fluorescent light for 1 minute, 10 minutes and 1 hour of time once after 24 hours dark imbibition. The results shown in Table II indicate that freshly harvested seeds appear to be inhibited by high temperature and light is ineffective in promoting germination at higher temperature even in presence of KNO_3 (0.02M) solution.

TABLE II: Percentage germination after 7 days in N. tabacum (BG71) at 20° and 30°C in dark and light

Period of illumination	Germination media	Temperatures	
		20°C	30°C
1 minute	H_2O	04	00
	KNO_3	02	0.6
10 minutes	H_2O	30	00
	KNO_3	22	02
1 hour	H_2O	76	02
	KNO_3	86	06
Dark control	H_2O	00	00
	KNO_3	00	00

TABLE III: Percentage germination of tobacco seed (Batch I) exposed to 15° or 20°C after a period of imbibition at 30°C and then returned to 30°C

(A)

Duration of exposure to 28°C	Duration at 30°C before exposure to 15°C: 4 hr	16 hrs	24 hrs	48 hrs
4 hours	0.5	0.5	1.0	2.0
16 hours	00	00	1.0	00
24 hours	00	0.5	1.0	00
32 hours	00	0.5	00	0.5

(B)

Duration of exposure to 20°C	Duration at 30°C before exposure to 20°C: 4 hr	16 hrs	24 hrs	48 hrs
4 hours	6.5	1.0	3.5	2.5
16 hours	0.5	1.5	2.0	2.5
24 hours	0.5	1.0	5.5	3.0
32 hours	0.5	0.5	2.5	3.5

Continuous at 30°C in dark = 2.5%

Continuous at 20°C in dark = 1.0%

Continuous at 15°C in dark = 1.0%

Experiment III: To demonstrate the effect of single temperature shift on N. tabacum (Batch I) germination in absence of light

Two separate experiments were conducted to investigate the effect of single temperature shift between 15° and 30°C and 20° and 30°C on N. tabacum (Batch I) seed germination in absence of light. Dispensed seeds were left at 30°C in complete darkness for 4, 16, 24 and 48 hours and after these periods of time at 30°C they were exposed to 15° or 20°C for 4, 16, 24 and 32 hours and returned to 30°C for final germination counts. Results of two experiments indicate that in this batch of tobacco seed dark germination cannot be induced by exposure to low or high temperature treatment.

TABLE IV: Percentage germination of N. tabacum cv. virginica incubated at 35°C after initial exposure to 12°C in presence of KNO_3 solution in darkness

(A)

Germination media	Hours of treatment at 12°C before transferred at 35°C: Cont. at 35°C	Cont. at 12°C	Hours: 1	2	4	8	16	24	32	40	48	56	64	72	χ^2 (1df) control (35°) vs. 8 hrs
H_2O	6	81	7	15	12	20	31	29	34	26	31	25	34	38	8.65
KNO_3	12	-	8	9	20	37	35	51	24	20	23	20	50	81	16.88

(B)

Germination medium	Hours at 4°C before transferred to 35°C: Continuous at 35°C	1 hr	4 hrs	24 hrs	48 hrs	96 hrs
KNO_3	00	00	1.5	1.5	7.0	0.5

Experiment IV: To show the effect of initial exposure to low temperature on N. tabacum cv. virginica seed germination in complete darkness

The seed sample N. tabacum cv. virginica used in this experiment was found light indifferent at lower temperature but at 35°C germination became light dependent. An experiment was designed to see if this high temperature induced light sensitivity in this batch of seed could be negated by exposing the seeds to lower temperatures (4° or 12°C) from the very beginning for 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, 64 and 72 hours and then transferred at 35°C for final germination. In some experiments the germination medium was freshly prepared KNO_3 (0.02M) solution and deionised water was taken as control.

It appears from the results that this batch of seed high constant temperature (35°C) reduced germination percentage but initial low temperature treatments have some beneficial effect. The results are shown in Table IV (A & B).

Discussion

In nature germination of seed may be inhibited or delayed due to various environmental factors around the seed lying in the soil (Crocker, 1916; Baskin & Baskin, 1971; Taylorson, 1970). As for example lettuce seed is found sensitive to temperature and light and with the rise of temperature seed may fail to germinate in the field even when other conditions are favourable. As the optimal temperature requirement for seed germination may vary from species to species, it was thought necessary to find a favourable temperature range within which tobacco seed could germinate before going in detail studies in physiology of germination.

Kincaid (1935) established that minimal, optimal and maximal temperature for florida cigar-wrapper tobacco seeds are 10°, 24° and 34°C respectively. But previous workers in this line of research have shown that maximum temperature within which tobacco seed could germinate lies between 32° and 40°C (Kincaid, 1935). Powells (1958) has reported that "Golden Harvest" tobacco seed failed to germinate at 30°C even in presence of light.

In our preliminary survey of N. tabacum (Batch I) seed germination (Table I), it appeared that at lower temperature (8°C) germination was not prevented but extremely delayed. No germination was obtained at 20°C on the fourth day both in dark and light, whereas at 30°C exposure of seeds for two minutes and four hours in white fluorescent light once after 24 hours dark imbibition brought

about 58 and 62% germination respectively. Seeds left at room temperature in complete darkness for four hours during the treatment showed similar percentage of germination as obtained with dark control. Therefore, four hour light was effective agent not the exposure to a different temperature. All germination counts on the fourth day of imbibition were made with the help of a binocular microscope provided with green safe filter in the darkroom and the seeds were returned to three incubating temperatures for further counts. On the sixth day increased percentage of germination was observed at all temperatures and in all treatments, including dark control once examined under safe light, and on the seventh day of counting germination percentage at 20° and 30°C and dark control reached more or less the same level, whereas percentage of germination at 20° and 30°C never exceeded 9.5% when seeds were held in continuous darkness for seven days. This could possibly be due to the fact that the green safe light which was biologically safe for lettuce seed germination might be in some way stimulating for tobacco seed germination.

To compare the light sensitivity and temperature tolerance of freshly harvested N. tabacum seed with that of Batch I seed we conducte an experiment with BG71 seeds at 20° and 30°C, under different light level (short or long) and germination media. In this batch of seed light appeared to be necessary for germination at 20°C and with the increased length of period of illumination increased germination percentage was obtained (see Table II). But at 30°C there was very little germination either in light or in darkness. It has been

claimed that presence of nitrate solution in germination media reduced light requirement in post-harvest tobacco seed (Kerr, 1955), but in our experiment with freshly harvested BG71 seed it was clear that irradiation in presence of KNO_3 (0.02M) solution in the germination media at 30°C was found not better than that in deionised water.

However, it appeared from the preliminary observation that in the seed samples of BG71 and Batch I light undoubtedly promoted germination in comparison with dark control. Two minutes white light once after 24 hours dark imbibition was quite sufficient to bring about higher percentage of germination in Batch I at 30°C and continued presence of light was not required, but freshly harvested seeds (BG71) failed to germinate at 30°C both in dark and light. This could be due to age of the seed or storage condition.

Temperature fluctuation is natural in field condition and, therefore, successful germination of particular species may be confined to a particular geographical region, or it may largely depend upon seasonal variations (Koller, 1955). Another important factor which is associated with temperature in seed germination is light. At certain temperature range seed may not germinate unless they are exposed to light. In lettuce seed germination it has been demonstrated that high temperature imposed dormancy may be overcome by exposing the seeds for a short period of time at lower temperature (Berrie, 1966).

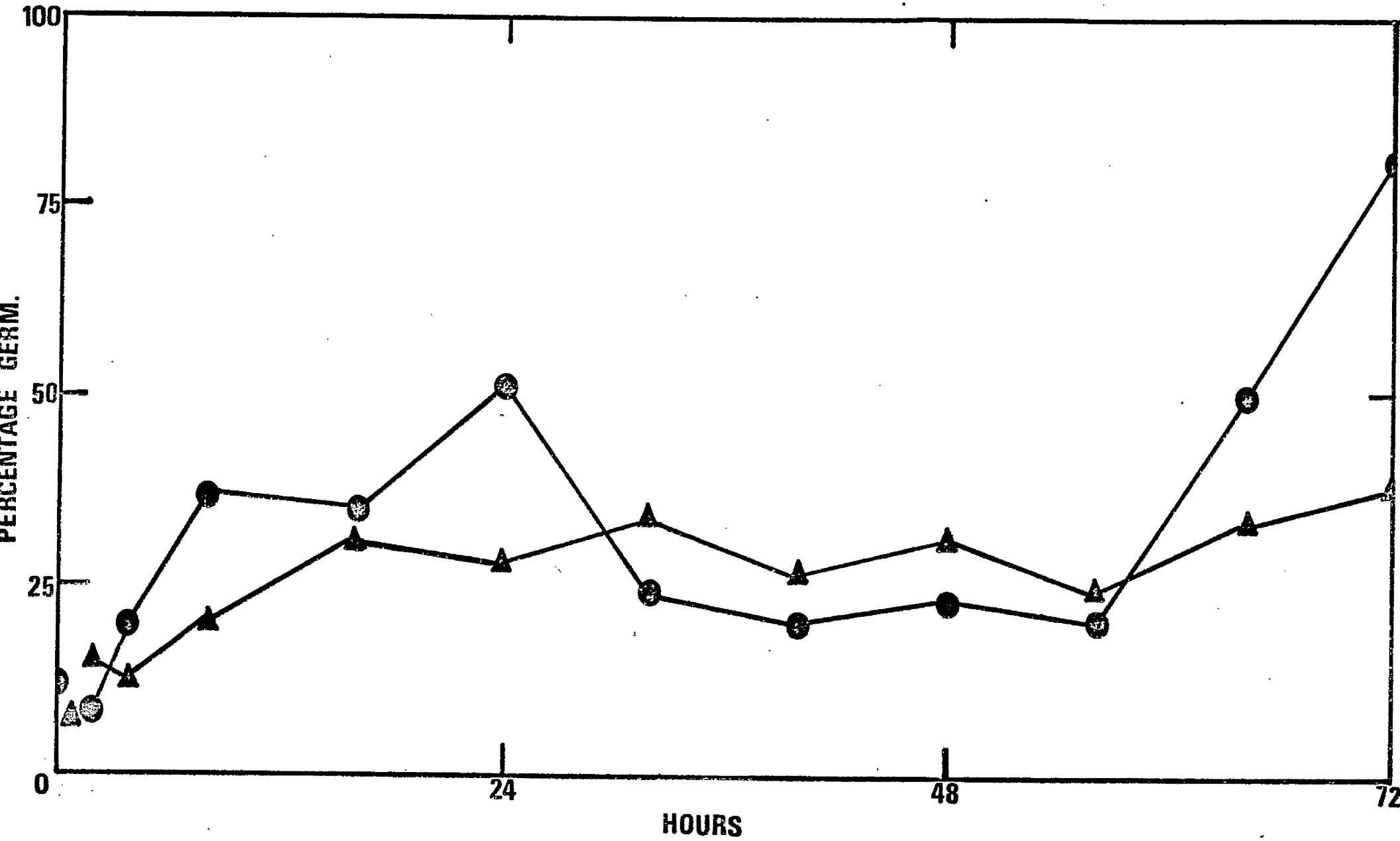
In our single temperature shift treatments with Batch I seed sample (see table III A & B), it appeared that no temperature condition

Figure 1. Effects of potassium nitrate (0.02M) and low temperature pre-treatments on the dark germination of N. tabacum cv. virginica at 35°C

Potassium nitrate (●) and water (▲)

Abscissa: Hours at 12°C before transferred to 35°C

Ordinate: Percentage germination


100
75
50
25
0
PERCENTAGE GERM.
24
48
72
HOURS

including temperature alternation treatment could replace light requirement. But in another batch of N. tabacum cv. virginica where 35°C causes a decrease in germination that can be overcome by light, an exposure to low temperature (12°C) prior to being placed at 35°C had some statistically significant effect on the level of dark germination (see Table IVA). But very low (4°C) initial temperature treatment in the same batch of seed appeared to be without any marked effect (Table IVB). It was clear that the longer the period of exposure at lower temperature in presence of KNO_3 before transfer to 35°C for final germination more and more seed could escape light requirement (See Figure I). Markedly high percentage of germination was obtained after 72 hours initial low temperature (12°C) treatment along with KNO_3.

Summary of Part I:

(1) Light and temperature requirement in tobacco seed (Batch I and BG71) germination was investigated.

(2) Very few seed germinated in total darkness at 8°, 20° and 30°C constant temperature. Short exposure of seed to white light once after 24 hours dark imbibition was found triggering and continued presence of light was not necessary. Germination of Batch I seed at 8°C was delayed but not prevented.

(3) Two seed samples N. tabacum (Batch I) and newly harvested (BG71) showed different degrees of light and temperature sensitivity at 20°C and 30°C. Freshly harvested tobacco seeds were found to

have a comparatively narrow temperature range for germination and with the rise of temperature there was no more germination either in light or in darkness.

(4) A single temperature shift between 15° and 30°C or 20° and 30°C in absence of light was not effective in promoting germination in N. tabacum (Batch I).

(5) In seed samples N. tabacum cv. virginica where higher temperature reduces dark germination, an initial low temperature treatment had some beneficial effect on dark germination.

Part II: Study of light sensitivity of different tobacco seed samples at various temperatures

Light sensitivity in seed was first described by Caspary in 1860. Since then inhibition or enhancement of germination by light has been confirmed in many seeds. Light requirement in tobacco seed germination is a problem to the growers. In some tobacco a small percentage of germination occurs in the absence of light as compared with dark control, but many representative types of N. tabacum could show high percentage of germination in complete darkness (Goodspeed, 1919).

Since the discovery of photo-reversibility of seed germination (Flint & McAllister, 1935) many authors have confirmed true red, and far-red reversible reaction in a number of species including N. tabacum (Toole, 1961; Borthwick et al., 1952, Kasperbauer, 1968 and Holdsworth, 1972), but this phenomenon of red and far-red reversible reaction is variable among different seed lots we have tested. It has been reported that a very small amount of daylight is effective in stimulating tobacco seed germination (Kincaid, 1935). We have tried to determine the sensitivity of our samples of seed. In some cases attempts have been made to determine the attainment of maximum sensitivity to light in different batches of seeds.

Materials and Methods

We have carried out experiments with the following seed samples (1) 10 types of commercial tobacco seed (7 cvs. of N. tabacum and 3 cvs. of N. affinis), (2) an old seed sample (Batch I), and (3) a freshly harvested seed sample from Garscube (GB71).

In all experiments seeds were scattered on Whatman's seed test paper in 4.5 cm petri dishes moistened with 1 ml deionised water. Four replicates of 50 seeds each (unless otherwise mentioned) per treatment were used. Soon after light treatments the seeds were put back into light tight cans and returned to the original temperature for final germination. A stopwatch and two camera shutters were used to record exact times of exposure. Handlings of seeds immediately before or after irradiation were performed in complete darkness. Incubating temperatures were 20°, 30° and 35°C obtained from thermostatically controlled incubators.

The sources of irradiations were:

(1) White fluorescent light (1.5 ft 13 watt 250V WWX; giving radiation 612μJ $cm^{-2}sec^{-1}$ at seed level)

(2) Red light (giving radiation 475μJ $cm^{-2}sec^{-1}$ at seed level)

(3) Far-red light (giving radiation 172μJ $cm^{-2}sec^{-1}$ at seed level).

Table V: Percentage germination of 10 types of tobacco seed in dark and light at 20° and 30°C after 7 days

	Name and type of tobacco	20°C Dark	20°C Light	30°C Dark	30°C Light
1.	N. affinis cv. Daylight	54.5	86.6**	45	92**
2.	N. affinis cv. Lime green	33.5	66.5**	36	76.5**
3.	N. affinis cv. Sensation mixed	39	77.5**	50	81.5**
4.	N. tabacum cv. Montealne	31	49*	17	50**
5.	N. tabacum cv. Sandare crimson Bedder	25	46**	3.6	25.6**
6.	N. tabacum cv. Sandare crimson king	38	77**	31.5	71**
7.	N. tabacum cv. Sandare Knapton Scarlet	27	38	27	42**
8.	N. tabacum cv. virginica	75	93.5	82	93.5
9.	N. tabacum cv. Virginia No. 25	81.5	86	70	88
10.	N. tabacum cv. Virginian	44.6	97.5	50	54

$x^2 > 3.841 < 6.635$ * $p < 0.05 > 0.01$

$x^2 > 6.635$ ** $p < 0.01$

Results

Experiment V: To show light and dark germinability of 10 types of commercial tobacco seeds at 20° and 30°C

7 cvs. of N. tabacum and 3 cvs. of N. affinis seed samples were tested for their light and dark germinability. Water imbibed seeds were held at 20° and 30°C for 24 hours and then they were irradiated in white fluorescent light for 30 minutes. The mean germination percentage is shown in Table V.

Table VI. Percentage germination of four types tobacco [illegible] ination after seven days at 24°, 30° and 35°C when irradiated at different [illegible] n period in complete darkness

Hours of dark Imbibition before irradiation	24°C	30°C				35°C			
	N. tabacum cv. Montcalne	N. tabacum cv. Montcalne	N. tabacum Batch I	N. affi	abacum virginica	N. tabacum cv. Montcalne	N. tabacum	N. affinis	N. tabacum cv. virginica
1 hour	41	19	2.5	45	82	00	-	48.5	69
2 hours	53	20	10.5	58	85	00	-	48.5	55
4 hours	51	30	36.25	51	84	02	-	48.5	75
8 hours	53	31	51.75	53	84	04	-	47.5	85
16 hours	49	48	64.25	50	88	08	-	54	82
24 hours	53	50	56.5	52	90	22	-	49.5	77
32 hours	71	50	67.5	53	86	16	-	46	72
40 hours	56	52	50.5	54	89	20	-	57	64
48 hours	61	55	58.5	52	88	19	-	49.5	51.5
56 hours	53	49	55.5	53	88	14	-	53	40
64 hours	55	50	30	33	83	-	-	40.5	39.5
72 hours	57	51	6.0	43	85	08	-	37.5	11.5
Dark control	43	19	1.5	43	81	00	-	45	12.5

Experiment VI: Response of different seed samples of Nicotiana to light during different imbibition period in darkness at various temperatures regime

In order to determine time of attainment of maximum sensitivity to light in N. tabacum (Batch I), N. affinis, N. tabacum cv. Montcalne and N. tabacum cv. virginica seed samples, four replicates of 50 imbibed seed each per treatment were placed at 24°, 30° and 35°C in complete darkness. Then the seeds were exposed to white fluorescent light for two minutes once after 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, 64 and 72 hours of dark imbibition. Final germination was allowed at original temperature after light treatment. Average percentage of germination is shown in the Table below (see Table VI).

Table VII: Germination percentage of N. tabacum (Batch I) at 30°C after red and far-red light treatments for various length of period of time after seven days

A. Red light

Seconds irrad. in Red	Doses of light (R)		Av.% Germ.
		$mJcm^{-2}$	
5	2.37	"	49.5
10	4.75	"	44.5
15	7.12	"	59.0
20	9.50	"	55.5
25	11.87	"	54.5
30	14.25	"	60
35	16.62	"	62.5
40	19.00	"	64
45	21.37	"	62
50	23.75	"	68
55	26.12	"	64.5
60	28.50	"	64.5
Dark control	00		5.0

B. Far-red light

Seconds irrad. in FR	Doses of light		Av.% Germ.
1	1/2	μJcm^{-2}	40
6	1.03	$mJcm^{-2}$	33
12	2.06	"	49
18	3.09	"	47
24	4.13	"	45.5
30	5.16	"	51.5
36	6.19	"	50.0
42	7.22	"	43.5
48	8.26	"	49
54	9.29	"	53.5
60	10.32	"	56
66	11.35	"	42.5
72	12.38	"	53
78	13.41	"	50.5
84	14.44	"	45.5
90	15.48	"	57
Dark control	00		1.5

Experiment VII: Effect of length of period of Red (R) and Far-red (FR) irradiation on germination of N. tabacum at 30°C

The old seed sample of N. tabacum (Batch I) was used in this experiment throughout in order to investigate the characteristic red and far-red light sensitivity. Four replicates of 50 imbibed seeds each per treatment were held at 30°C in light tight cans for 24 hours and then the seeds were exposed to Red and Far-red light sources for various length of period of time shown in Table VII.

During our observation it has been found that after 24 hours dark imbibition at 30°C both red and far-red light were promotive. Results are tabulated opposite.

Table VIII. Percentage germination after 7 days at 30°C in seeds exposed to low doses of red and far-red light after 24 hours dark imbibition

(i) shutter A

Nominal Exposure to R & FR in Seconds	Doses of Red Light		Av.% Germ. (R)	Doses of Far-Red		Av.% Germ. (FR)
0.04	19.0	μJcm^{-2}	34.5	6.88	μJcm^{-2}	19
0.02	9.50	"	24	3.44	"	22
0.01	4.75	"	29	1.72	"	14
0.004	1.90	"	29	0.68	"	16
0.002	0.95	"	24	0.34	"	9.0
0.001	0.475	"	22	0.172	"	1.0

Dark control : 1.5%

Table VIII (Continued)

(ii) shutter B

Nominal Seconds irrad. in R & FR	Doses of Red light		Av.% germ. (R)	Doses of Far-red		Av.% germ. (FR)
0.04	19.0	μJcm-2	24	6.88	μJcm^{-2}	17
0.02	9.50	"	25	3.44	"	22
0.013	6.17	"	29	2.23	"	16
0.01	4.75	"	26	1.72	"	18
0.005	2.37	"	14	0.86	"	13

Dark control : 00%

Experiment VIII: To demonstrate the effect of low doses of red and far-red light on N. tabacum seed germination

The experiments were carried out with N. tabacum (Batch I) seed sample. After 24 hours dark imbibition at 30°C the seeds were exposed to low doses of red and far-red with the help of two camera shutters. In one shutter exposure time could be adjusted from 1/25th to 1/1000th of a second, and in the other from 1/25th to 1/200th of a second. After the treatment the seeds were returned to original temperature for final germination. All operations were performed in complete darkness. From the results it appeared that small amounts of red and far-red light promoted germination in comparison with dark control. The results are shown opposite.

Table IX. Percentage germination at 30°C after red and far-red light treatment at four hours dark imbibition in N. tabacum (Batch I)

Seconds irrad. in R & FR	Doses of Red light		Av.% germ. (R)	Doses of FR light		Av.% germ. (FR)
1	0.47	$mJcm^{-2}$	1.0	0.17	$mJcm^{-2}$	1.0
4	1.90	"	9.0	0.68	"	3.0
8	3.80	"	17	1.37	"	2.0
12	5.70	"	27	2.06	"	7.0
16	7.60	"	38	2.75	"	5.0
20	9.50	"	37	3.44	"	4.0
24	11.40	"	48	4.12	"	5.0
32	15.20	"	45	5.50	"	2.0
40	19.0	"	59	6.88	"	5.0
48	22.80	"	55	8.26	"	2.0
56	26.60	"	61	9.62	"	3.0
64	30.40	"	60	11.00	"	3.0
72	34.20	"	59	12.38	"	7.0
80	38.00	"	69	13.76	"	5.0
92	43.70	"	70	15.82	"	7.0
Dark control	00		2.0	00		4.0

Experiment IX: To show red and far-red light sensitivity of *N. tabacum* at four hours of dark imbibition

An experiment was designed to see if both red and far-red light were promotive when the same seed sample (Batch I) was irradiated after only four hours of dark imbibition at 30°C. The specific length of period of exposure to red and far-red light sources is shown in the Table. The results showed that red and far-red light sensitivities differed unlike that of 24 hours imbibed seed observed in the experiment Nos. VII and VIII. Far-red light at four hours treatment appeared *not* to be promotive while red light still promoted germination. The results are tabulated below (see Table IX).

Table X. Percentage germination at four hours treatment showing R FR relationship in N. tabacum.

Red and Far-red light two minutes each (total doses of red light 57.00mJcm^{-2}; Far-red 20.64mJcm^{-2}) at seed level

Sequences of R, FR irrad.	Percentage germination
Dark control	3.0
R(2m)	57
R-FR (2m - 2m)	4.0
R-FR-R (2m - 2m - 2m)	51

Experiment X: To demonstrate red, far-red photoreversibility at four hours treatment in N. tabacum

To examine red and far-red photoreversibility at four hours dark imbibition, N. tabacum Batch I seeds were used throughout. Due to lack of sufficient seeds, 100 seeds (4 replicates of 25) for each treatment were incubated at 30°C. After the appropriate dark imbibition (four hours) the seeds were exposed to red alone or to red followed by far-red in the sequences shown in Table IX. Seeds kept imbibed in continuous darkness were taken as control. From the results it appears that the action of red light could be reversed completely by far-red at four hours imbibition

Table XI. Percentage germination of freshly harvested N. tabacum seed irradiated with white, red and far-red light at 20°C. White fluorescent light for ten minutes; Red and far-red light for five minutes in each case. R-FR indicate Red light followed by Far-red.

Irradiations	1st day	7th day
Dark control		00
White light	78	92
Red(R)	87	97
Far-red(FR)	5.0	1.5
R-FR	3.0	3.0
R-FR-R	63	83

Experiment XI: To demonstrate the response of freshly harvested seeds of N. tabacum to red and far-red photo-reversibility at 20°C

In order to compare the reversible red, far-red photo-reaction in freshly harvested seeds, a seed sample from Garscube Botanical Research Institute grown during 1971 (GC71) were used in this experiment. Incubating temperature was chosen only 20°C because at 30°C germination did not occur both in dark and light. Seeds were irradiated on the first and seventh days of dark imbibition in white, red and far-red light in the sequences depicted in the Table. Final counts were made after seven days from the time of illumination. From the experimental results it appeared that in freshly harvested seeds action of red light could be completely reversed by far-red no matter the duration of imbibition. The mean percentage of germination can be seen in Table XI.

Discussion

According to Crocker (Borthwick, 1965) and Isikawa, 1962, Kinzel first studied the light sensitivity of about 964 kinds of seed and came to the conclusion that light promoted germination in 70% of the species he studied and only 3% seeds were indifferent to light. From the studies of different workers (Goodspeed, 1919; Gardner, 1921; Johnson *et al.*, 1930; Kincaid, 1935; Isikawa, 1952) we have gathered diverse data and opinions regarding light requirement and sensitivity of tobacco seed. We have studied light and dark germinability of ten types of commercial tobacco seeds and results indicated that in almost all types of tobacco seed light promoted germination at 20° and 30°C. Some seed samples, e.g. *N. tabacum* cv. virginica, *N. tabacum* cv. virginia No. 25, and *N. tabacum* cv. virginian were not statistically significant in respect to light promotion of germination. But 3 cvs. of *N. affinis* and 3 cvs. of *N. tabacum* showed highly significant light promotion of germination at 20° and 30°C. It has also been observed that most of the seed samples showed considerably high percentage of germination in complete darkness (see Table V). In previous experiments with Batch I (see Experiment I) and freshly harvested BG71 (see Experiment II) seed we have demonstrated that in complete darkness no germination occurred at 20° and 30°C. Freshly harvested seeds responded to light within a limited range of temperature because at 30°C light was found no more efficient. Although there is a complex interrelationship between light and

Figure 2. Light sensitivity of *N. tabacum* (Batch I) seed at various stages of dark imbibition at 30°C

Abscissa: Hours of dark imbibition before white light ($73.44 mJcm^{-2}$)

Ordinate: Percentage germination

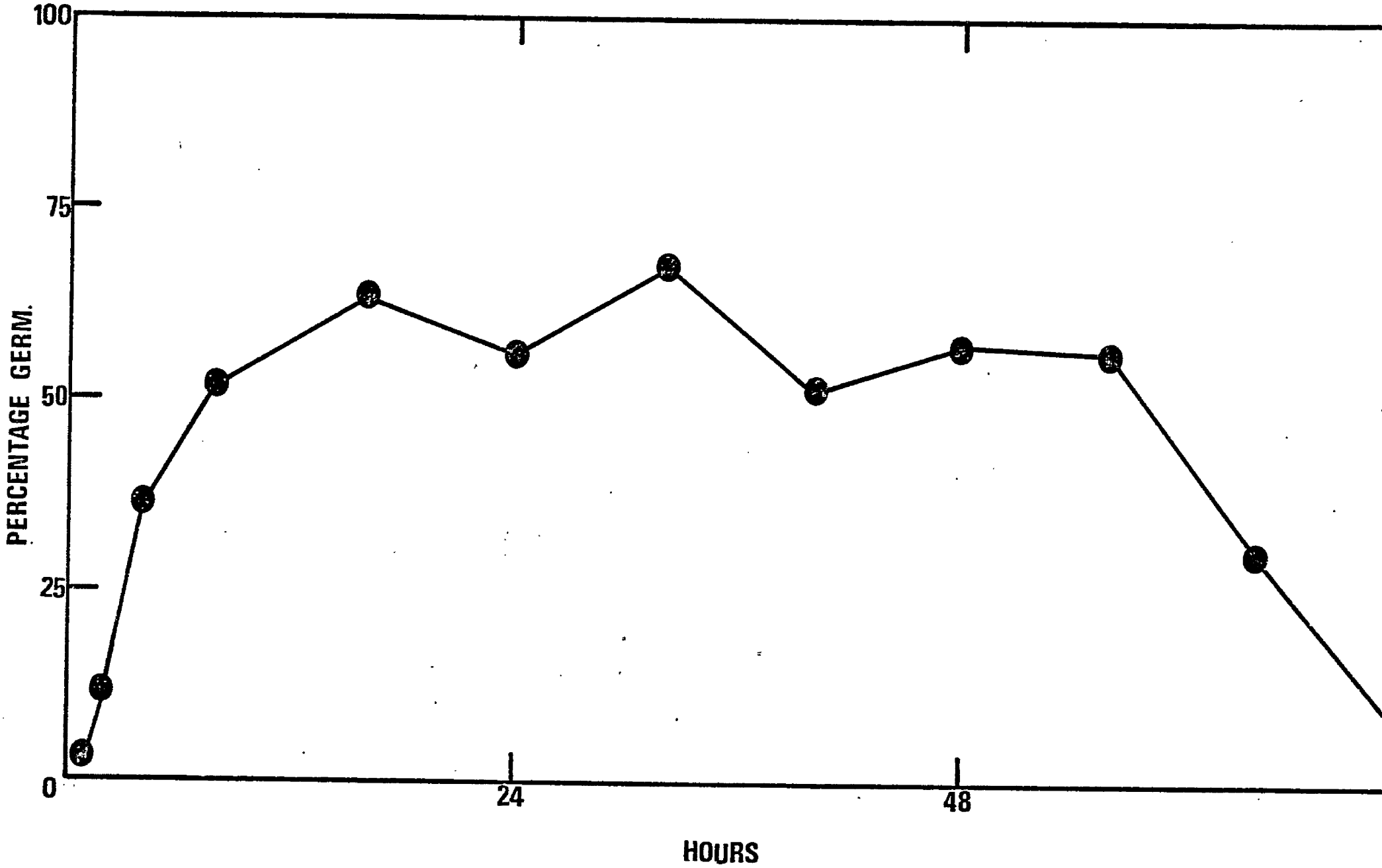

100
75
50
25
0
PERCENTAGE GERM.
24
48
HOURS

Figure 3. Light sensitivity of N. tabacum cv. montcalne at different stages of dark imbibition at 24°, 30° and 35°C

24°C(■), 30°C(●) and 35°C(▲)

Abscissa: Hours of dark imbibition before white light ($73.44 mJ cm^{-2}$)

Ordinate: Percentage germination

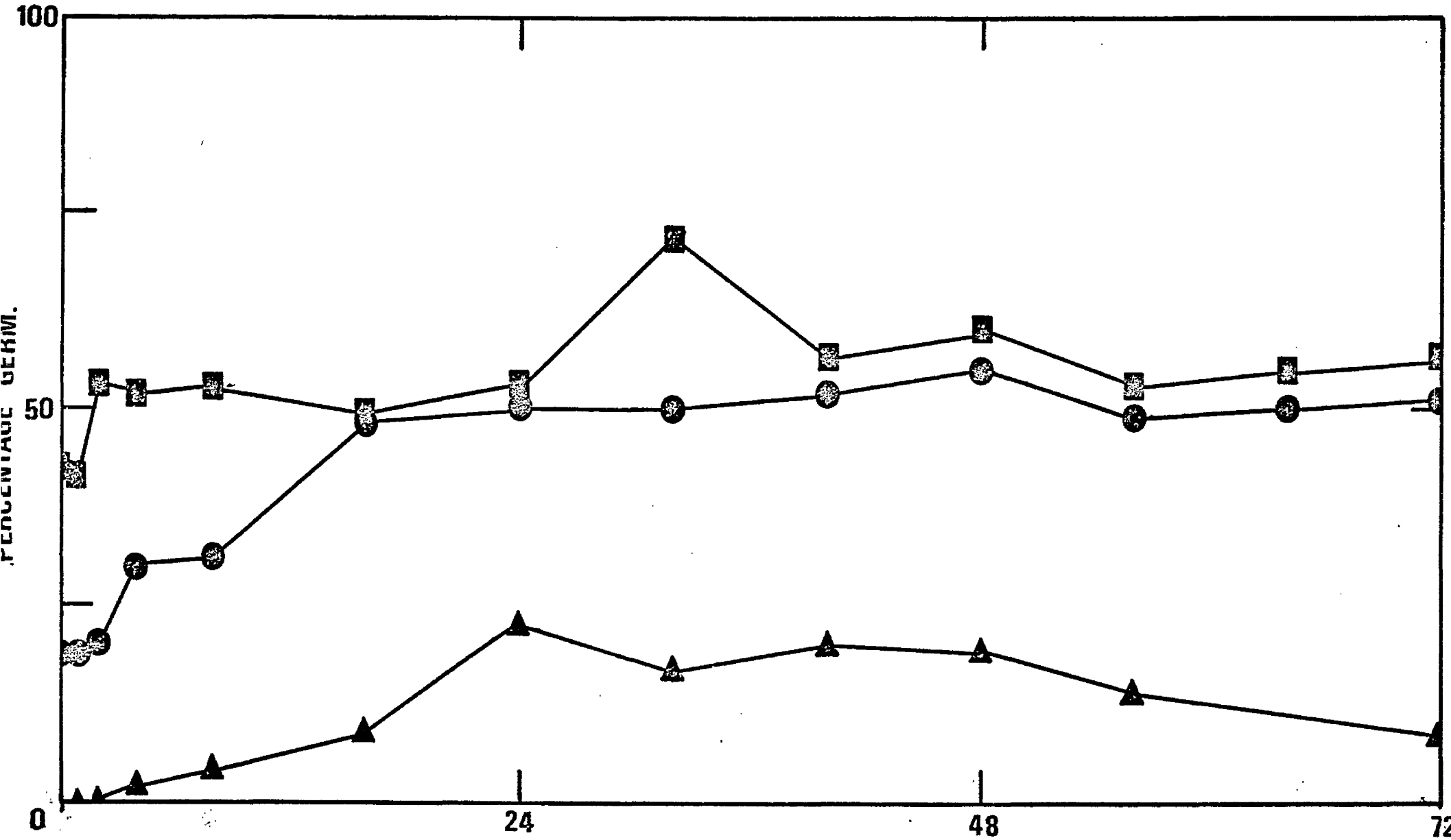

100
50
0
PERCENTAGE GERM.
24
48
72
HOURS

Figure 4. Light sensitivity of N. affinis at 30° and 35°C at various stages of dark imbibition

30°C (●) and 35°C (▲)

Abscissa: Hours after start of imbibition in dark before white light ($73.44 mJcm^{-2}$)

Ordinate: Percentage germination

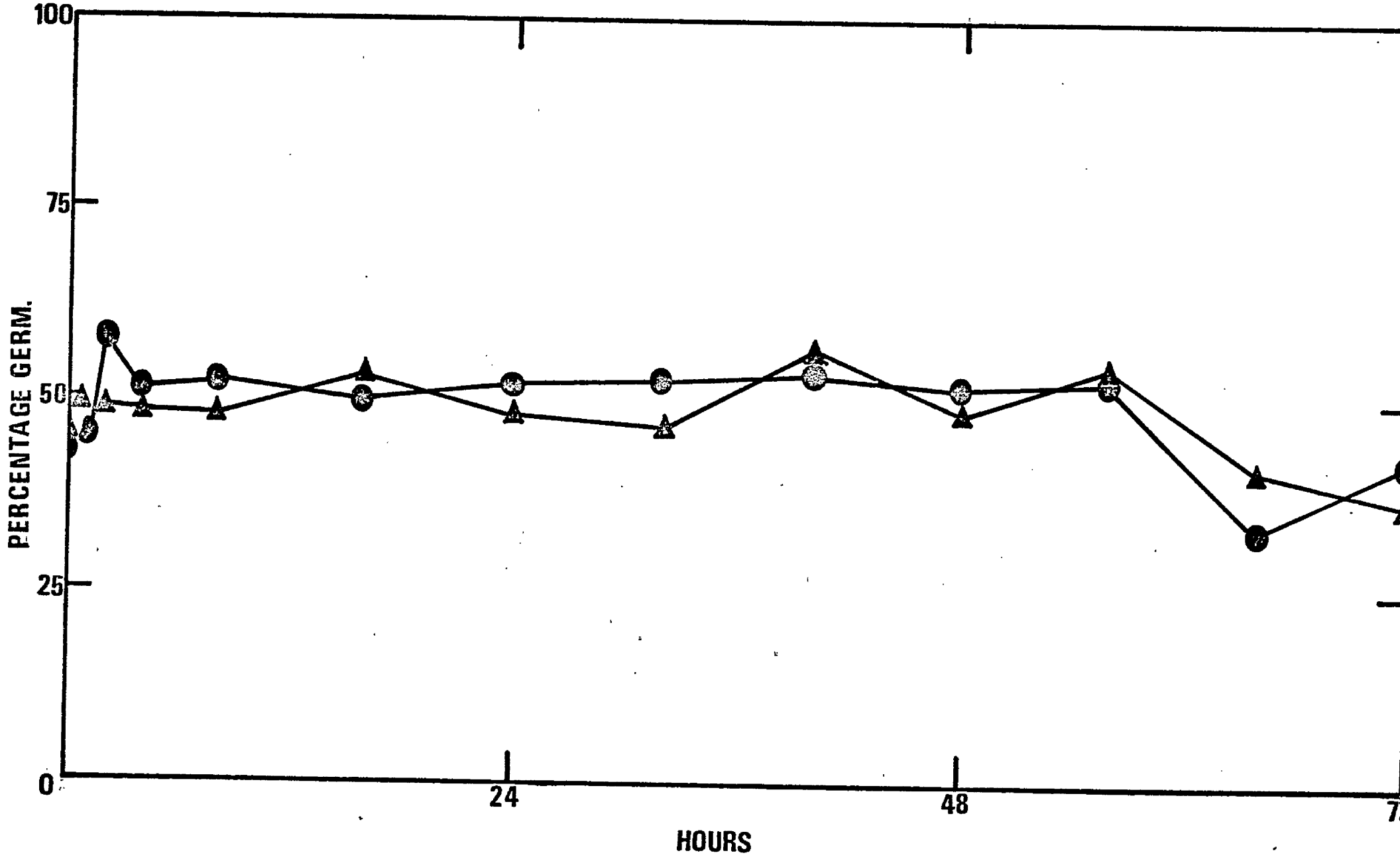
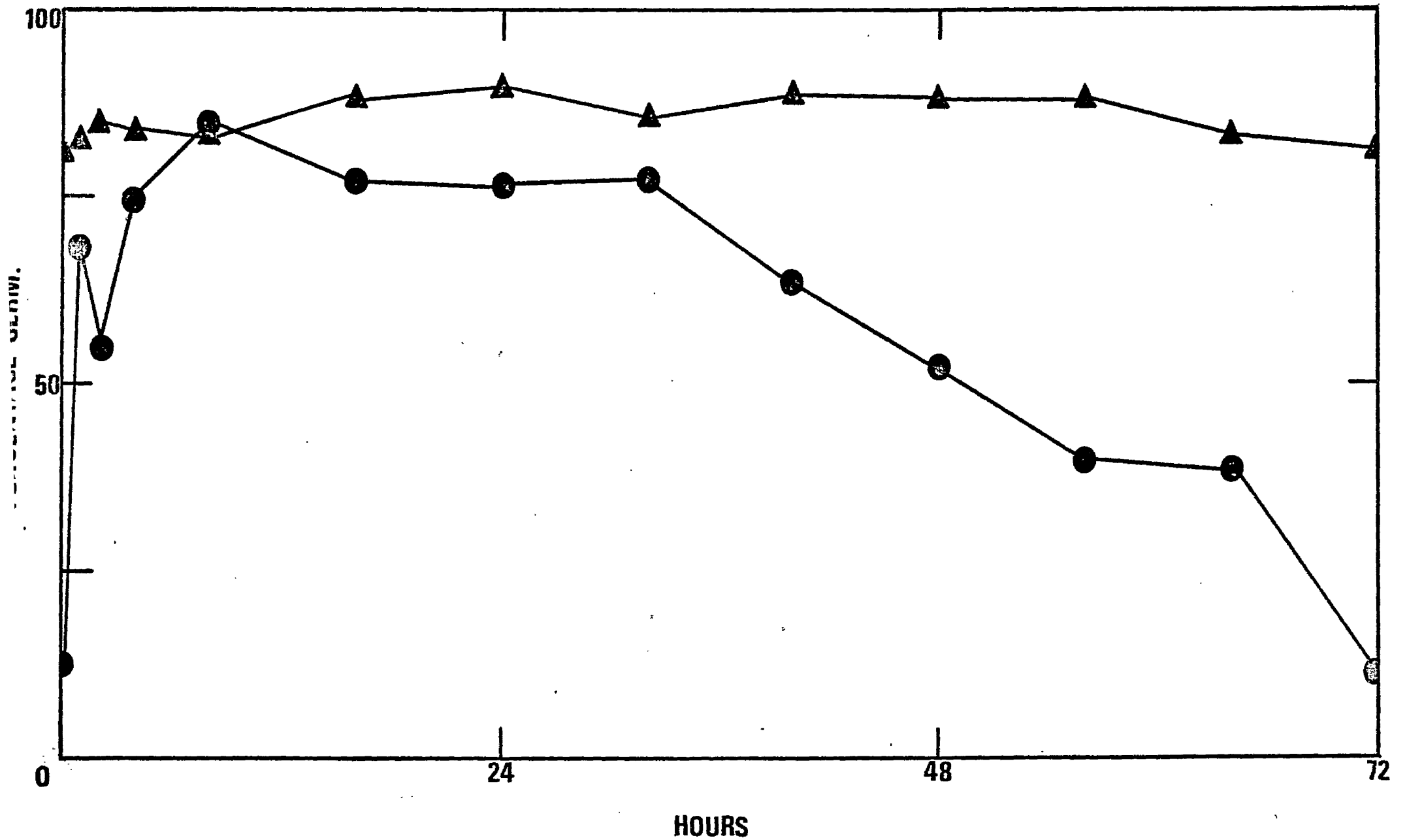

100
75
50
25
0
PERCENTAGE GERM.
24
48
HOURS

Figure 5. Light sensitivity shown by N. tabacum cv. virginica at 30° and 35°C with the lapse of time of dark imbibition

30°C (▲) and 35°C (●)

Abscissa: Hours of dark imbibition before white light ($74.44 mJ cm^{-2}$).

Ordinate: Percentage germination

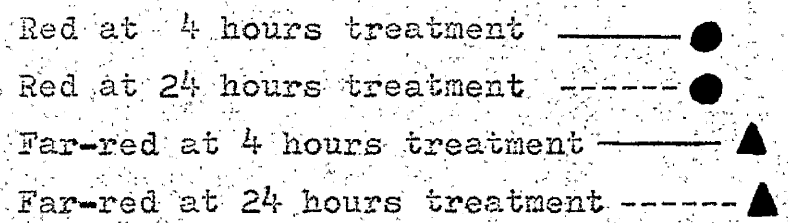

100
50
0
24
48
72
HOURS

temperature in photoblastic seed (Berrie, 1966) it appeared that effective range of light largely depend upon batches of tobacco seed. We have tried to determine time of attainment of maximum light sensitivity in four seed lots and experiments were conducted at 24°, 30° and 35°C (see Table VI). At 30°C light sensitivity of Batch I seed lot of N. tabacum (see Figure 2) and N. tabacum cv. Montcalne increased wuth the increased period of imbibition and time of attainment of maximum sensitivity to light was 24 hours or thereabouts (See Figure 3). But in two other seed lots, N. affinis (see Figure 4) and N. tabacum cv. virginica (see Figure 5) there was a little increase in percentage of germination at 30°C in comparison with dark control.

When the seeds of three samples of N. tabacum cv. Montcalne, N. affinis and N. tabacum cv. virginica were incubated at 35°C light sensitivity gradually increased with increased time of imbibition in the dark. But after 72 hours dark imbibition at 35°C irradiation was found to be without any effect and percentage of germination came to dark control level. Seeds of N. tabacum cv. virginica and N. affinis became sensitive to light immediately after soaking whereas N. tabacum cv. Montcalne seed were stimulated by light after several hours of dark imbibition. In all seed lots we obtained reduced percentage of germination at 35°C both in dark and light.

From our observation it is clear that light sensitivity not only changes with time of imbitibion but also with sharp rise of temperature. Isikawa (1952) and Kincaid (1935) only studied the effect of soaking

Figure 6. Red and far-red light sensitivity of N. tabacum (Batch I) after 4 and 24 hours dark imbibition



Abscissa: Seconds of irradiation in red and far-red light after 4 and 24 hours dark imbibition

Ordinate: Percentage germination

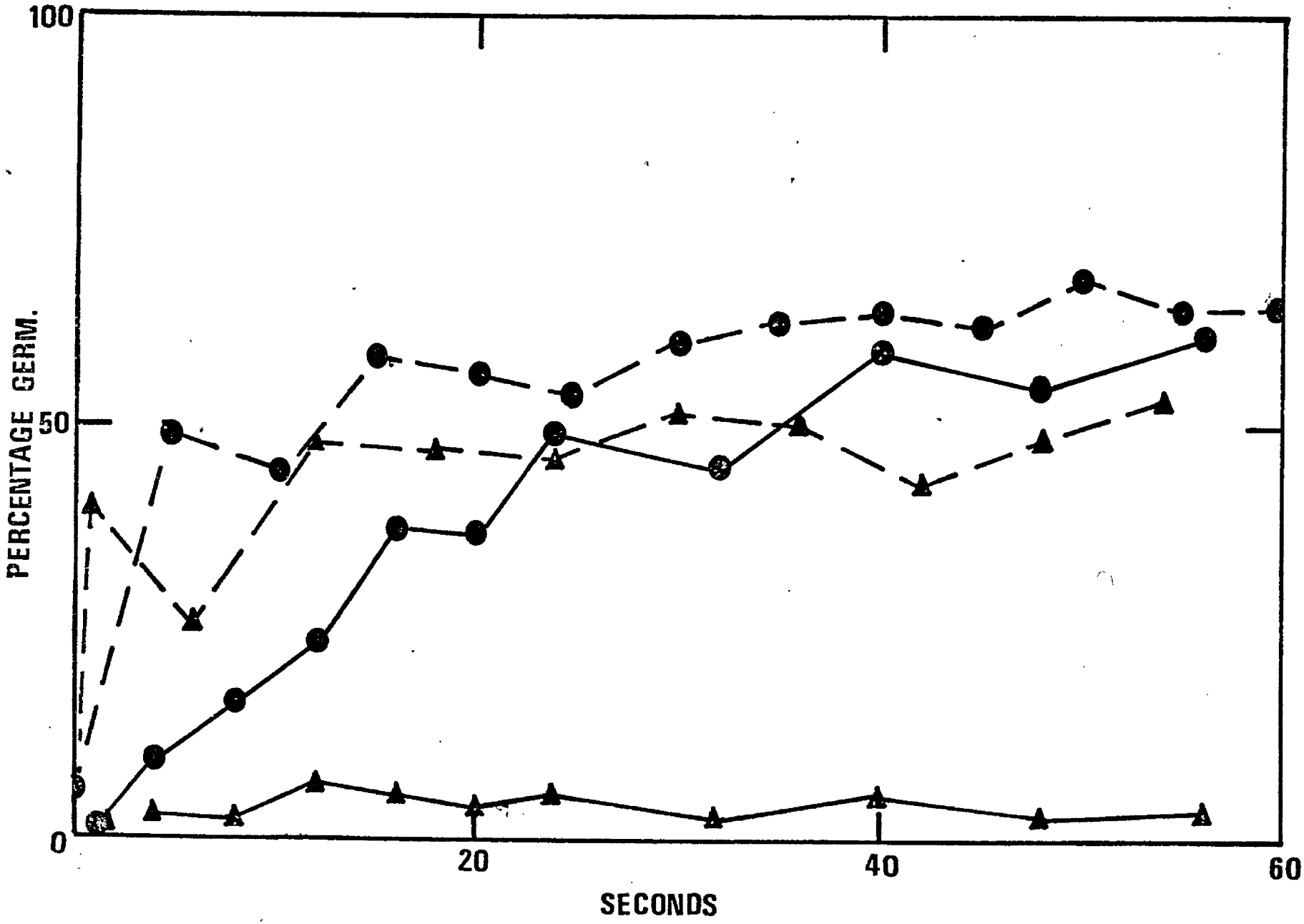
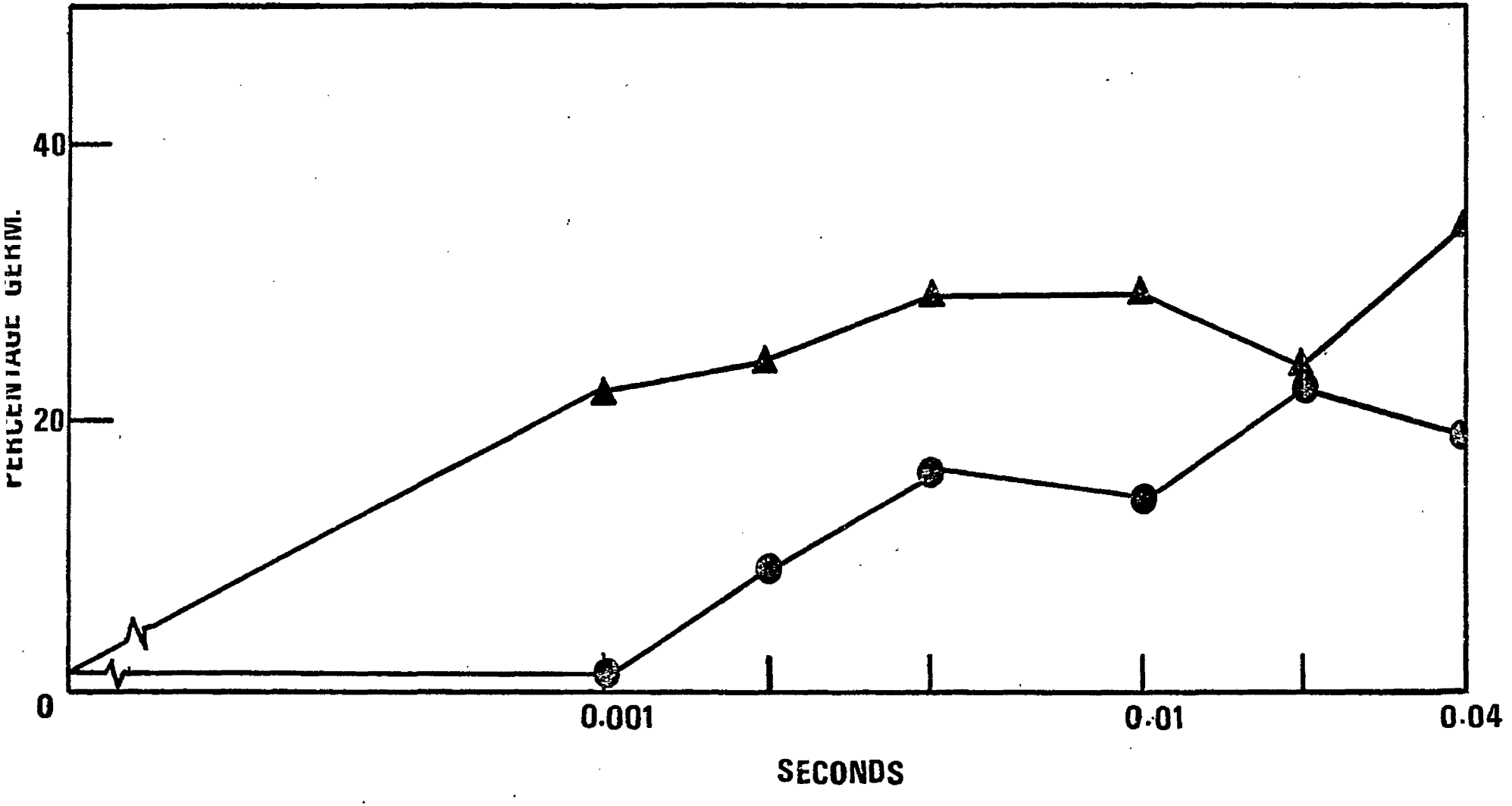

100
50
0
PERCENTAGE GERM.
20
40
60
SECONDS

Figure 7. Promotion of germination in N. tabacum (Batch I) at 30°C by small dosage of red and far-red light at 24 hours treatments (Menial shutter A)

Red (▲) and far-red (●)

Abscissa: Seconds irradiated after 24 hours dark imbibition

Ordinate: Percentage germination


40
20
0
0.001
0.01
0.04
SECONDS

on light sensitiveness in tobacco seed, but in our opinion influence of incubating temperature and seed lots could be taken into account in this sort of study.

It has been suggested that germination of tobacco seed is controlle by phytochrome. In our experimentation with Batch I seed lot (see Experiment VII), it appeared that after 24 hours dark imbibition at 30°C both red and far-red light was equally promotive (see Figure 6). Even 1 sec. to 1/1000th of a second red and 1/200th of a second far-red (see Experiment VIII, Figure 7) irradiation could stimulate germination above that of dark control (1.5%). Photoreversibility of red and far-red in Batch I and freshly harvested seeds (GC71) was also worked out. True red / far-red reaction could be obtained in Batch I seed only after four hours dark imbibition at 30°C (see Table X). But freshly harvested seed showed the reversible red, far-red photoreaction even when irradiated on the seventh day of dark imbibition at 20°C (see Table XI). This result, however, does not agree with that of Holdsworth (1972).

During our experiment with different seed lots we did not get uniform light sensitivity. This could be due to the interaction between light and other variables. Because germination is a complex process and a series of changes may take place within the seed after rehydration Light requirement may be due to a block somewhere in this pathway (Toole, 1961). As for example, high temperature imposed dormancy in lettuce seed could be negated by exposing the seed for a short period of time at low temperature or light (Berrie, 1966).

Red and far-red light sensitivity was not uniform in all seed lots of tobacco we have tested. In Artemisia monosperma (Koller et al., 1964) and Bidens pilosa (Valio, et al 1972) some confusing light sensitivity has been observed where entire visible spectra may influence germination. In those seeds it has been proposed that phytochrome seems to be either absent or masked by different pigments which may control germination (Koller, 1964). However, a detailed action spectra of tobacco seed germination is not known. From our observations it appeared that promotion or inhibition influence of red and far-red light on tobacco seed germination is also the functions of incubating temperature, time of imbibition and seed lots. It seems that phytochrom control of germination in photoblastic seed is very complicated and it needs further investigation.

Butler et al., 1964 (Siegelman, 1969) and Pratt & Briggs, 1966 (Mohr, 1969) believed that at the photostationary state when irradiated with 660 nm phytochrome exists in the ratio 81/19 Pfr/Pr. When irradiated with 730 nm the Pfr/Pr ratio is 1/99.

If Pfr is the form of phytochrome which is active in promoting germination then depending on the sensitivity of the seed to active phytochrome it may appear to respond positively to far-red irradiation Tobacco after 24 hour imbibition may be an instance of a system which shows extreme sensitivity to low levels of Pfr. At 4 hour imbibition the system is not as sensitive and far-red irradiation does not produce enough Pfr at that time to promote germination.

Another possibility is that at 24 hour Pfr is immediately on formation utilised in the mediation of germination. If this is occurring then even under far-red irradiation, the existing Pr would all be converted. However, the responsiveness of the seed to very low levels of far-red irradiation does not substantiate the second possibility.

The promotion of germination of seed of Batch I by red or far-red irradiation (Fig. 6) never exceeds 70% but this batch does not appear to have a high germination capacity since the maximum percentage germination in other experiments (Fig. 2 and Table I) never rose above this value. The viability of the non-germinated seeds was not tested but it is reasonable to consider that the old batch of seed might have lost some of its viability in storage.

The patterns of response to red and far-red irradiation in

Fig. 6, therefore, most probably respresent the fulfilment of the phytochrome mediated response though it should be noted that at 24 hours imbibition the far-red treated never quite germinate to the extent of red irradiated seed. Perhaps the photostationary state arrived at under far-red does not provide enough Pfr to saturate the system but almost fulfils it.

Summary of Part II

1. Light and dark germinability of ten types of commercial tobacco seed was worked out. Light promoted germination in almost all seed lots at 20° and 30°C. Seed samples N. tabacum cv. virginica, N. tabacum cv. virginia No. 25, and N. tabacum cv. virginian were found not statistically significant in respect to light promotion of germination and considerably high percentage of germination was obtained in darkness at 20° and 30°C.

2. Light sensitivity at various stages of imbibition in different seed samples appeared to be conditioned by type of seed, length of period of imbibition and incubating temperatures. In four seed samples, viz. N. tabacum (Batch I), N. affinis, N. tabacum cv. Montcalne and N. tabacum cv. virginica, time of attainment of maximum sensitivity to light was found to be 24 hours or thereabouts.

3. Small dose of red (1/1000th of a second) and far-red (1/200th of a second) stimulated germination in N. tabacum (Batch I) after 24 hours dark imbibition at 30°C. But in the same batch of seed action of red light could completely be reversed by far-red at four hours treatment.

4. Freshly harvested N. tabacum (GC71) seed showed red, far-red photoreversibility when irradiated on the first and seventh day

of dark imbibition at 20°C.

5. It is observed that red and far-red light sensitivity or the reversibility between the promotive action of red light and inhibitive action of far-red light largely depend upon batch of seed and length of period of dark imbibition.

Part III: Effects of plant hormones on the breaking of light controlled dormancy in N. tabacum

Some hormones, particularly gibberellins and kinetins, are known to be involved in breaking dormancy in seeds (Toole, 1961; Roberts, 1963; Wood & Paleg, 1972). In lettuce seed (Miller, 1958) kinetin has been shown much more effective in promoting germination when there is in addition an exposure to a low dose of light.

A study was made to examine the responses of our light sensitive tobacco seed samples to gibberellin and kinetin at various germination conditions.

Materials and Methods

The data presented in this section were obtained by using three seed samples 1) *N. tabacum* (Batch I), 2) B671, and 3) G071.

Germination tests were carried out on seed test papers in small petri dishes with requisite amount of water or test solution as mentioned in Materials and Methods section. Incubating temperatures were 20° and 30°C. Light (two minutes) treatments were always given after 24 hours dark imbibition. Four replicates of 50 seeds each (unless otherwise mentioned) were taken for each treatment. Percentage of germination was recorded after seven days from onset of imbibition.

Two samples of gibberellins, GA_3 and a mixture of GA_{4+7}, were used in our experiments. Fresh stock solution was diluted with deionised water to prepare the concentrations to be tested. Kinetin Batch No. 53126, received from Koch-light Laboratory, England, was used in our studies.

Irradiation source was white fluorescent light (2ft., 250V, 20 watt WWX, giving radiation 516$\mu J sec^{-1} cm^{-2}$ at seed level).

Table XII. Percentage germination after 7 days at 20° and 30°C in N. tabacum

a) Old seed samples (Batch I)

Conc. of GA_3	30°C Dark	30°C Light
00	1.0	40.5
10^{-4}M	34	62
10^{-3}M	56.5	68

b) Freshly harvested seed (GC71)

Conc. of GA_3	20°C Dark	20°C Light	30°C Dark	30°C Light
00	00	61	00	00
10^{-5}M	1.0	76	0.5	00
10^{-4}M	4.0	85	2.0	3.0
10^{-3}M	95	92	95	94

c) Values of χ^2 of dark and light

Conc. of GA_3	20°C New seed	30°C Old seed
00	60.01	36.65
10^{-5}M	73.05	-
10^{-4}M	73.71	8.166
10^{-3}M	0.048	1.06

Results

Experiment XII: To show the effects of concentrations of GA_3 on germination of N. tabacum seed samples

The seed samples used in the experiments were of different ages. Germination of Batch I was light-dependent at 30°C, but in the case of GC71 (newly harvested) light appeared to be ineffective in promoting germination at 30°C.

The experiments were conducted to determine the responses of the above seed samples to GA_3 both in dark and light. Incubating temperatures tested were 20° and 30°C.

Experimental results and values of χ^2 shown in the Table (see Table XIIc) indicated that light and low concentrations of GA_3 together significantly increased percentage of germination, both in new (GC71) and old (Batch I) seed samples at 20° and 30°C respectively.

Experiment XIII: To demonstrate the relative activity of GA_3 and GA_4 + 7 in inducing dark germination in N. tabacum

BG71 seed sample was used in this experiment. The experiments were conducted to determine the comparative effectiveness of GA_3 and GA_4 + 7 in promoting dark germination in freshly harvested seeds. Three replicates of 50 seeds each per treatment were used. Average percentage of germination is shown below. The results indicated that GA_4 + 7 was much more effective than GA_3 at lower concentrations.

Table XIII. Percentage germination after 7 days in dark induced by GA_3 and GA_4 + 7 in N. tabacum at 20° and 30°C

Conc. of Gibberellin mg/ml	Gibberellins	20°C	χ^2 (1df) GA_3 vs. GA_4+7	30°C	χ^2 (1df) GA_3 vs. GA_4+7
0.5	GA_3	92	215.06	87	64.104
	GA_4 + 7	7.0		43	
0.25	GA_3	53	51.84	65	31.77
	GA_4 + 7	91		92	
0.12	GA_3	17	159.26	22	156.31
	GA_4 + 7	90		93	
Water control		00		00	

Table XIV. Germination percentage of freshly harvested N. tabacum seeds at 20° and 30°C after low temperature treatment along with GA_3

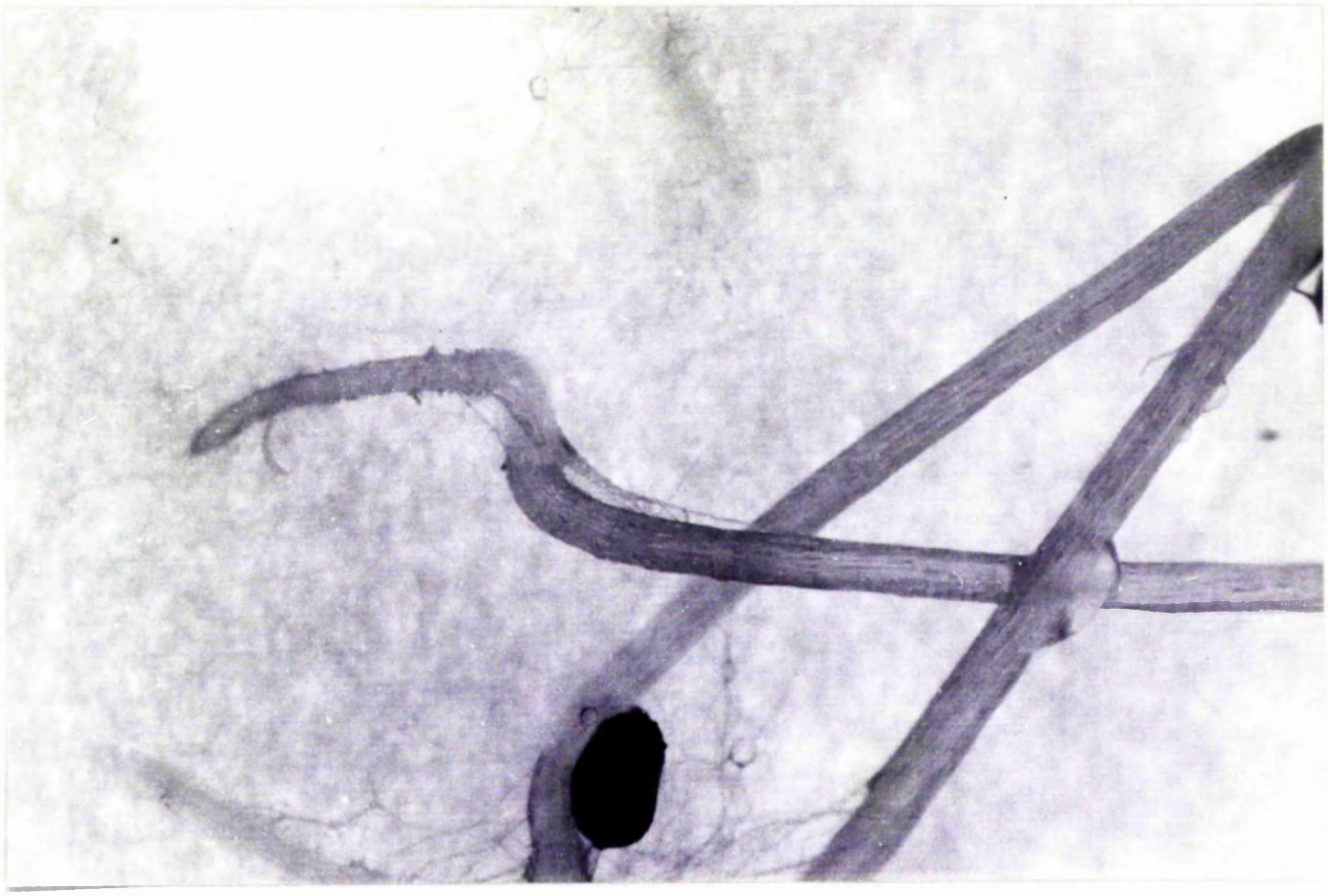
Days at 4°C	20°C				30°C			
	H_2O	$GA_3 10^{-3}$M	$GA_3 10^{-4}$M	$GA_3 10^{-5}$M	H_2O	$GA_3 10^{-3}$M	$GA_3 10^{-4}$M	$GA_3 10^{-5}$M
5 days	00	97*	11	02	00	82*	02	00
10 days	00	94*	10	01	00	78*	08	1.5
20 days	00	91*	11	05	00	77.3*	08	0.5

*Abnormal seedlings shown in the photograph

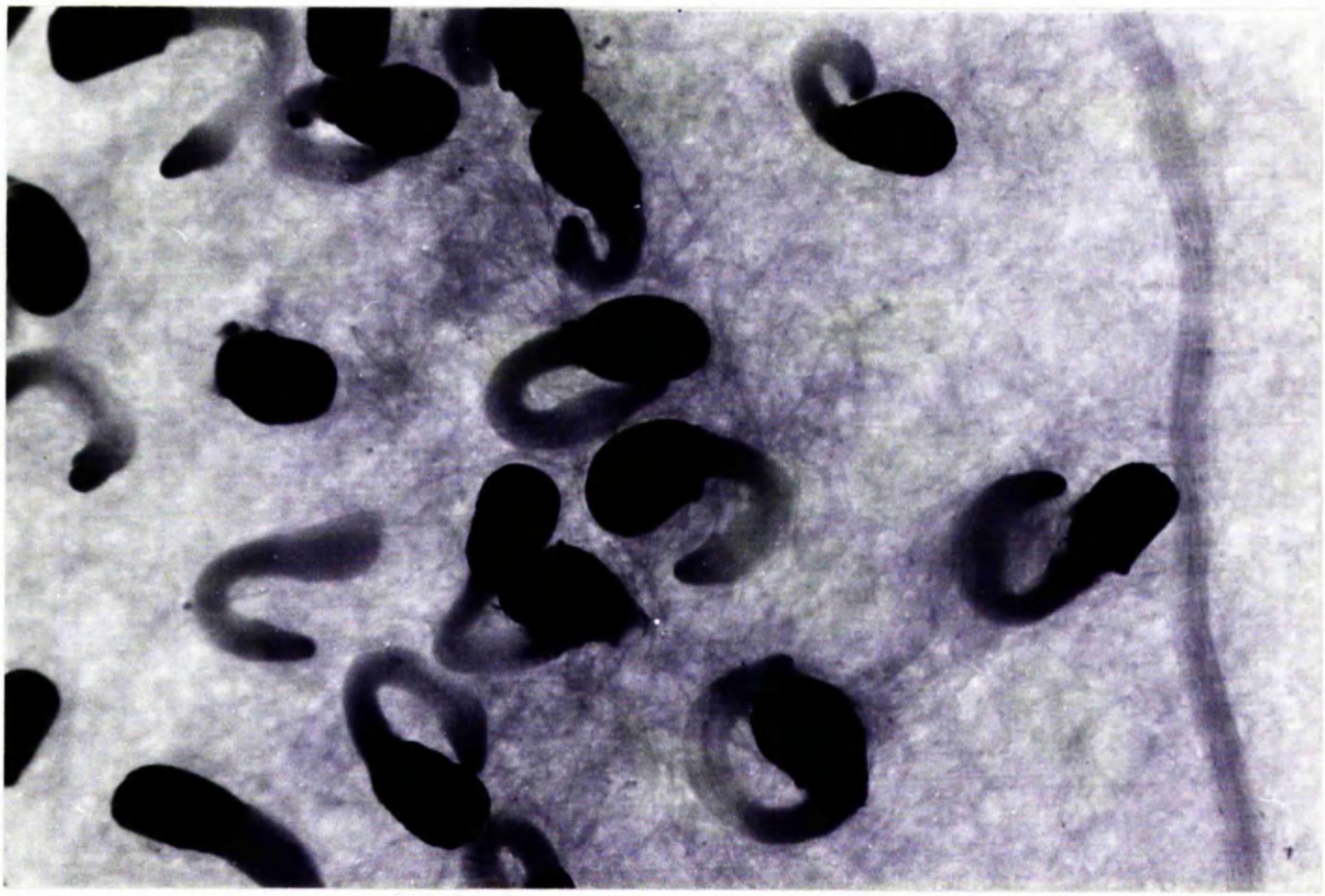
PLATE 1. Shows the deleterious effect of high concentration of GA_3 together with low temperature pre-treatment on tobacco seedlings

A. H_2O control

B. Treated



A



B

Experiment XIV. To show the effects of low temperature pre-treatments in presence of GA_3 on N. tabacum seed germination

Stratification or cold treatment sometimes brings about germination of post-harvest dormant seeds at higher temperature. Gibberellins are known to replace cold requirement in number of seeds (Gray, 1958; Staden et al., 1972). The purpose of this study was to see the interaction between GA_3 and low temperature ($4^{\circ}C$) in the germination of tobacco seed.

Light requiring newly harvested BG71 seeds imbibed in different concentrations of GA_3 were exposed to $4^{\circ}C$ for 5, 10, and 20 days in complete darkness, and then they were transferred to $20^{\circ}C$ and $30^{\circ}C$ for final germination.

A seed was considered germinated when the radicle had protruded. However, in certain treatments (asterisked in Table XIV) the radicles did not develop normally (See photograph).

Experiment XV. To demonstrate the effects of Kinetin on N. tabacum seed germination

An investigation was made to see the dark germination inducing capacity of kinetin in freshly harvested seed sample BG71. The concentration of kinetin used in the germination medium were 10, 20 and 30 ppm. Incubating temperature was 20°C. Three replicates of 50 seeds were used per treatment.

Results shown below (see Table XV) indicated that kinetin was ineffective in inducing dark germination and higher concentration (30 ppm) retarded germination even in presence of light.

Table XV. Effects of kinetin in N. tabacum (BG71) at 20°C in dark and light

Conc. of kinetin	Dark	Light
00	1.0	79
10 ppm	1.0	73
20 ppm	0.5	75
30 ppm	0.5	21

Discussion

It has been demonstrated that exogenous application of GA_3 induces germination of N. tabacum in dark (Ogawara & Ono, 1961). From our experimental results it appeared that low concentrations of GA_3 failed to bring about appreciable dark germination in freshly harvested seed sample (GC71) at 20° or 30°C (see Table XIIb). Whereas the same concentrations of GA_3 could induce considerably higher percentage of germination in an old seed sample (see Table XIIa). It has been observed that high concentration of GA_3(10^{-3}M) always induced high percentage of germination in dark in two seed samples at all temperatures tested. The new seed sample (GC71), however, did not respond to 10^{-4}M or 10^{-5}M solution of GA_3 in the dark as well or in the light at 30°C. This lack of response to light and low concentration of GA_3, alone or together, could be due to the fact that at elevated temperature, higher concentration of GA_3 or higher doses of light might be required to break thermodormancy in newly harvested tobacco seeds.

In a study of comparative effectiveness of GA_3 and GA_4 + 7 it has been observed that considerably lower concentration of GA_4 + 7 could induce dark germination both at 20° and 30°C in freshly harvested seeds. In higher concentration (0.5 mg/ml) reduced percentage of germination was obtained at 20° and 30°C. Whereas the 10^{-3}M (0.346 mg/ml) GA_3 was required to get full expression of germination at the temperatures tested. It appeared that dark inducing capacity of

of GA_3 and GA_4 + 7 largely depended upon concentrations of the hormones and incubating temperature. Statistical analysis showed that GA_4 + 7 was found much more effective than GA_3 (see Table XIII).

It has been found that stratification or cold treatments (4°C) given to the seeds soaked in high concentration of GA_3(10^{-3}M) before being transferred to 20°C or 30°C for final germination produced abnormal seedlings showing characteristic coilings of the radicle. Lower concentrations of GA_3 were found ineffective in inducing dark germination (see Table XIV). The reason for the abnormal seedlings at high concentration of GA_3 after low temperature treatment is not known. It could be due to unknown changes within the seed during stratification.

The reports on the induction of dark germination in tobacco seed by kinetin are very conflicting. The chemical was found stimulatory only in presence of light (Ogawara & Ono, 1961) but according to Hashimoto (1961) kinetin was very effective in promoting dark germination in certain Japanese tobacco. Our observations with freshly harvested BG71 seed (see Table XV) confirmed the results of Ogawara and Ono.

Summary of Part III

1. The response of seeds to different concentrations of GA_3 varies with old (Batch I) and new (BG71) seed samples.

2. In a comparative study of effectiveness of GA_3 and a mixture of GA_4 + 7 showed that latter was much more effective in inducing the dark germination of seeds at low concentrations.

3. Low temperature ($4^{\circ}C$) pre-treatments given to the newly harvested seeds along with higher concentration of $GA_3(10^{-3}M)$ appeared to have a deleterious effect on seedlings.

4. Kinetin was found almost ineffective in causing germination in BG71 seed in absence of light.

Part IV: Effects of physical and chemical scarification on the light controlled germination in *N. tabacum*

Seed coats or other coverings affect germination in many ways (Crocker, 1906, 1916; Harrington, 1923). They also play an important role in controlling dormancy in a number of seeds (Roberts, 1961; Webb & Wareing, 1972). It appears that seed germination of many species may be improved by cutting, puncturing or chemically treating the seed coats. As, for example, in lettuce, removal of seed coats may eliminate light requirement for germination at higher temperature (Evenari & Newmann, 1952; Ikuma & Thimann, 1958). A study was undertaken to see if the seed coats of *N. tabacum* are in any way involved in controlling germination. Some common methods such as washing, abrasion pricking and chemical means of scarifications were tried.

Materials and Methods

Freshly harvested seed sample BG71 was used in this section throughout. Germination tests were carried out in 4.5 cm petri dishes on Whatman's seed test paper to which was added 1 ml of deionised water or test solution. Timing of treatments was controlled with the help of a stopwatch. Where details differ in this section mention will be made at the appropriate place.

Four replicates of 50 seeds each per treatment were used. Incubating temperatures were 20° and $30^{\circ}C$. In some cases seeds were irradiated in white fluorescent light (1.5ft 250V 13 watt WWX) for 15 minutes once after 24 hours dark imbibition. Intact seeds were considered as control.

Table XVI. Percentage germination after 12 days in *N. tabacum* seeds washed with various organic solvents

Treatments	20°C		30°C	
	Dark	Light	Dark	Light
Untreated control	00	85	00	00
Petroleum ether	00	78	00	00
Ethyl alcohol	00	86	00	00
Acetone	01	80	00	00

Results

Experiment XVI. Effect of irrigating with various organic solvents on tobacco seed germination

Before giving details of other methods of scarification it was decided to determine if washing the seeds with petroleum ether, ethyl alcohol or acetone could improve germination in dark and light at optimal (20°C) and supra-optimal (30°C) temperatures. The object was that the above chemicals could probably dissolve out impermeable waxes and fats from the seed coats.

50 seeds were taken in a watch glass and 5 ml of the desired solvent was poured over them. The seeds were then stirred frequently by means of a camel hair brush and the duration of treatment was ten minutes. After the treatments the seeds were taken out of the solvent and put on seed test paper in petri dishes. One ml deionised water was added to the test paper after the evaporation of the solvents. Half of the duplicates of the treated seeds were irradiated in white light for 15 minutes after 24 hours dark imbibition. The results indicated (see Table XVI) that exposing seeds to solvent before sowing failed to improve germination in dark.

Experiment XVII. To demonstrate the effects of abrasion on germination in N. tabacum

The data reported in this experiment were secured with freshly harvested BG71 seed sample. Germination tests were carried out to examine if mechanical abrasion could give better germination in dark.

50 seeds were rubbed against sandpaper until the brown seed coats were removed from almost all seeds. Every possible precaution was taken so that the seed would not be damaged during this operation. The abraded seeds were scattered on seed test paper in petri dishes moistened with 1 ml water or GA_3 solution. The seeds were never exposed to light after imbibition. Intact seeds were taken as control. The results shown in Table XVII indicate some beneficial effects of abrasion.

Table XVII. Percentage germination of abraded N. tabacum seed samples after 8 days at 24° and 30°C in presence and absence of GA_3

Con. of GA_3 in Mol.	24°C		30°C	
	Intact	Abraded	Intact	Abraded
00	1.0	00	00	00
10^{-5}M	2.0	54	00	41
10^{-4}M	2.0	42	00	31

Experiment XVIII. To show the effect of pricking and concentration of GA_3 on seed germination

It was assumed that lack of response shown by freshly harvested intact seeds to low concentration of GA_3 (see Table XVIII) was due to a seed coat and/or endosperm effect since on the destruction of these tissues the concentrations of GA_3 used promoted dark germination.

In this experiment seeds were soaked in the low concentrations of GA_3 (10^{-4}M and 10^{-5}M) for 24 hours in complete darkness at 30°C then the seeds were pricked one by one with a mounted needle using a binocular microscope. This operation was carried out as quickly as possible and did not exceed 3 minutes. In all cases intact seeds were taken as control and they were exposed to the illuminator of microscope for 3 minutes in order to make sure that light from the illuminator had no effect. The results are tabulated below.

Table XVIII. Percentage germination of pricked and non-pricked seeds after 7 days at 30°C

Germination medium	Dark (Intact)	Exposed to Illuminator (Intact)	Pricked
H_2O	00	3.0	1.5
$GA_3 10^{-5}$M	1.0	4.5	61
$GA_3 10^{-4}$M	3.5	6.5	75
$GA_3 10^{-3}$M	90	93	-

Table XIX. Percentage germination after 7 days at 30°C after scarification with H_2SO_4

Germination media	30°C			
	Intact	Scarified (one min.)	Intact	Scarified (two mins.)
H_2O	00	00	1.0	00
$GA_3 10^{-5}M$	6.0	34	2.0	00
$GA_3 10^{-4}M$	00	43	5.5	00
$GA_3 10^{-3}M$	74	42	80	00

Experiment XIX: To demonstrate the effect of chemical scarification on newly harvested N. tabacum seed

Light requiring BG71 seeds have a seed coat/endosperm restriction in germination which can be removed by physical means (see Tables XVII and XVIII). It was decided to see if chemical scarification could replace the pricking or abrasion treatments.

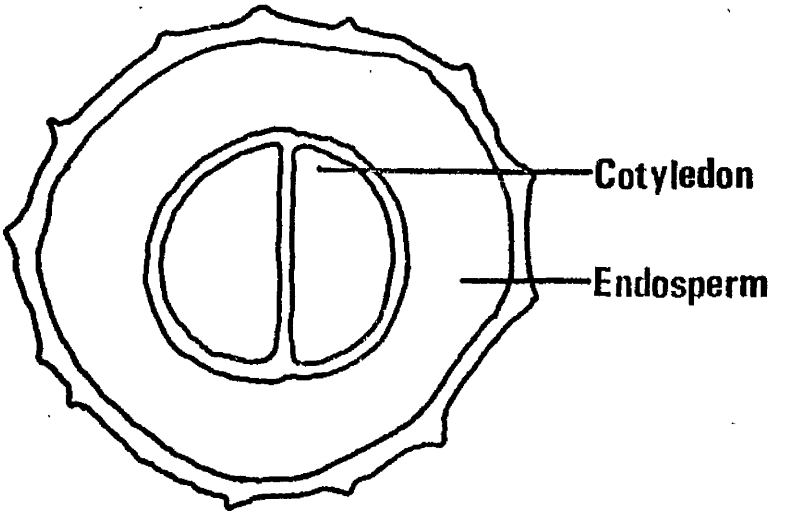
Sulphuric acid was used as a chemical scarifier. One ml of concentrated H_2SO_4 was taken in a 250 ml beaker and 50 seeds were scattered on the liquid. The beaker containing the seed in acid was shaken for one or two minutes and then the acid was diluted by adding 250 ml tap water. Whole liquid was filtered through fine muslin cloth. The seeds on the muslin were washed three times with tap water in order to get rid of the acid. The scarified seeds were placed in test condition.

The results (see Table XIX) showed that acid scarification improved dark germination at $30^{\circ}C$ only in presence of GA_3 and long exposure of seed to acid was found damaging to the embryo.

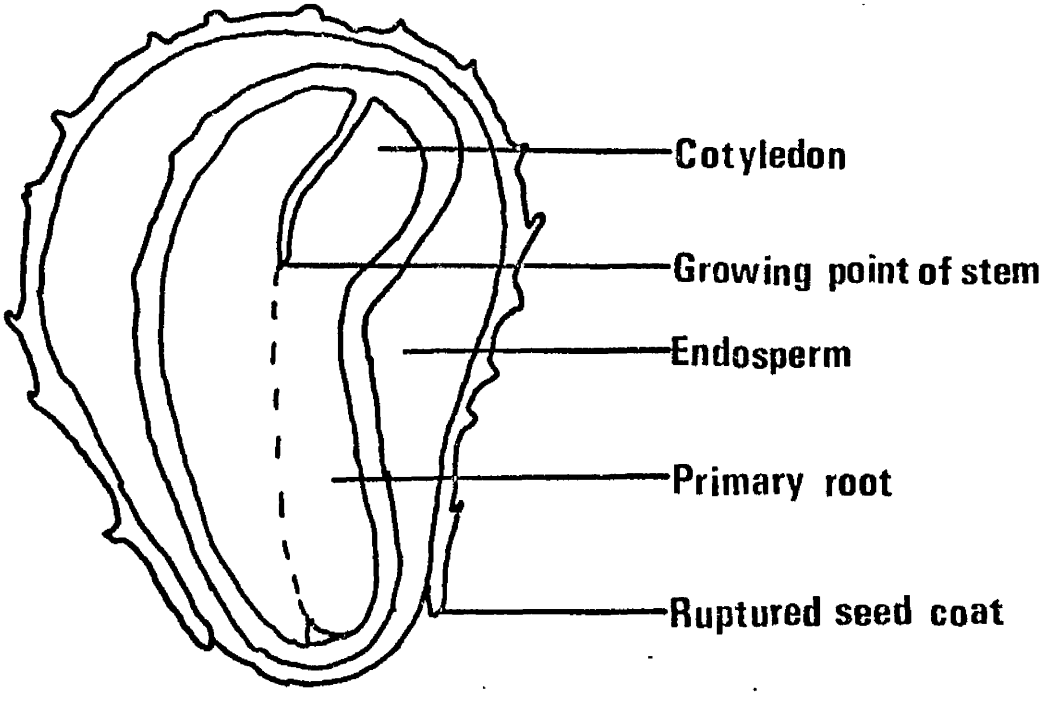
Figure 8.

A. Transverse section through dormant seed at cotyledonary level (X70) - After Avery (1933)

B. Longitudinal-median section through seed just starting to germinate, showing ruptured seed coats (X50) - After Avery (1933)


Cotyledon
Endosperm

A


Cotyledon
Growing point of stem
Endosperm
Primary root
Ruptured seed coat

B

Discussion

Scarification is a treatment which may render the seed coat permeable to water and/or gases or it could remove mechanical restraint. Freshly harvested seeds (BG71) required light for germination. Light is known 1) to activate the release of enzymes for weakening the seed integuments (Ikuma & Thimann, 1958), or 2) to activate or suppress inhibitor in the seed (Bonner & Galston, 1952). From the anatomical studies of Avery (1933) it appeared that the seed coat structure of N. tabacum is complex and some of the characteristic features, it was thought, might be involved in controlling dark germination (see Figure 8).

In a review article Evenari (1949) pointed out that a kind of germination inhibitor could be present in the seed coat of N. rustica. Similar suggestion of presence of inhibitor in N. tabacum was given by Krishnamorthi & Moss (1969). It is reported that washing seeds with various chemicals may remove inhibitor from the dormant seeds of Atropa belladona (Bhat & Dhar, 1971) and Luzula spicata (Amen, 1967).

Our experiment with newly harvested seeds (see Experiment XVI and Table XVI) showed that mere washing of the seeds with petroleum ether, ethyl alcohol or acetone was not effective in replacing the light requirement, although it was observed that the chemicals were not injurious to the embryo.

In Experiment XVII it has been demonstrated that abraded seeds

failed to germinate in complete darkness at 24° and 30°C when soaked in water. But when the seeds were imbibed in low concentration of GA_3 (10^{-4}M and 10^{-5}M) slightly improved germination was obtained both at 24° and 30°C in complete darkness. The above concentrations of GA_3 failed to induce dark germination in intact seeds both at 24° and 30°C. GA_3 at 10^{-3}M was effective (see Table XIX). Does the abrasion of external allow the entrance of an effective amount of GA_3 when the solution is weak or does injury result in a changed metabolism which can make better use of GA_3 in germination?

If the seed coverings of N. tabacum constitute a barrier for successful germination in dark, then pricking or chemical scarification might facilitate the entry of water, gases or GA_3 into the embryo or endosperm. An examination of data presented in Tables XVIII and XIX show that pricking of the seed coat or acid scarification give good dark germination at supra-optimal temperature (30°C) only in presence of GA_3.

Other agents of chemical scarification we used were 1) a commercial liquid containing sodium hypochlorite, and 2) H_2O_2. Scarification with both the chemicals at a range of exposures did not eliminate the light requirement for germination at 20° or 30°C even in presence of GA_3 (low concentration). It has been observed that tobacco seed can stand fairly high concentrations of sodium hypochlorite without any damage to the viability. But high concentration of H_2O_2 bleached and killed the seeds and other concentrations were not effective.

Gibberellins stimulate germination in many seeds. It has been confirmed that it failed to have any effect when coat structure is

intact, e.g. Rosaceous seeds (Frankland, 1961). In Trollius seeds prolonged soaking in gibberellin solution was necessary for germination (Kallio & Piiroinen, 1959). Dark germination responses of scarified seeds of N. tabacum to low concentration of GA_3 at high temperature ($30^{\circ}C$) was striking. Why physically or chemically injured seed germinated better in presence of GA_3 only is not clear. It was likely that GA_3 penetrated into the seeds when scarified, since scarified seeds not exposed to GA_3 did not germinate. It is also believed that injury to the cells causes the liberation of a hormone-like substance in the damaged area (Curtis & Clark, 1950). It could be possible that wound stimuli during pricking or abrasion might in some way influence the synthesis of some metabolite(s) within the seed and other hormone(s) besides metabolite(s) is(are) also necessary for radicle elongation in tobacco seed germination.

Summary of Part IV

(1) Irrigating with organic solvents, abrasion, pricking and chemical scarification did not eliminate light requirement in light sensitive N. tabacum seed.

(2) Physically or chemically injured seeds germinated in darkness in the presence of low concentrations of GA_3 (10^{-4}M and 10^{-5}M). The above concentrations were found ineffective in inducing dark germination in intact seeds.

(3) Chemical scarification with sodium hypochlorite and hydrogen peroxide over a number of different times of treatments failed to replace light requirement even in the presence of GA_3 (low concentrations).

Part V. Responses of different tobacco seed samples to various chemical treatments

A number of organic and inorganic compounds are known to stimulate germination in different seeds (Thompson & Kosar, 1938, 1939; Roberts, 1963, 1964; Baskin & Baskin, 1971; Hendricks & Taylorson, 1972; Taylorson & Hendricks, 1973). Several attempts have been made to overcome light requirement in photoblastic seeds during germination by treating the seeds with various chemicals (Hashimoto, 1958; Yamaki & Takahashi, 1961; Baskin & Baskin, 1971). The last authors concluded that no chemical compounds could substitute for light requirement in Cyperus inflexus seeds.

Roberts (1964) demonstrated that some respiratory inhibitors, e.g. Cyanides had marked stimulatory effect on the breaking of rice seed dormancy. Very recently Hendricks & Taylorson (1972) have claimed that a number of inorganic salts, including cyanides, could induce dark germination in lettuce.

In the literature there is a lot of contradiction regarding the dark germinability of tobacco seeds. The object of this study is to examine the dark germination inducing capacity of various chemicals including cyanides in different tobacco seed samples.

Materials and Methods

Three seed samples (1) BG70 (grown in Botanic Garden during 1970), (2) BG71 and (3) N. tabacum cv. montcalne, were used in our studies. Germination tests were carried out according to the standard procedure mentioned elsewhere. The chemicals tested were sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), Hydroxyl ammonium chloride ($NH_2OH.HCl$) Methyl hydrazine sulphate ($CH_3NH.NH_2H_2SO_4$), Hydrazinium sulphate ($NH_2NH_2.H_2SO_4$), Potassium ferricyanide ($K_3Fe(CN)_6$), Potassium ferrocyanide $K_4Fe(CN)_6.3H_2O$, Potassium azide (KN_3), and Potassium cyanide (KCN). The seeds were soaked in 10^{-2}M, 10^{-3}M and 10^{-4}M solutions of each compound from the beginning. Four replicates of 50 seeds each (unless otherwise mentioned) per treatment were used. The incubating temperatures were 20° and 30°C. In some cases imbibed seeds were exposed to white light for 5 minutes once after 24 hours dark imbibition. Counting was made after eight days from the date of imbibition. The emergence of radicle was taken as seed germination.

Source of light (white fluorescent tube, 1.5ft. 250V 23 watt WWX; giving radiation 612μJcm^{-2}sec^{-1} at seed level).

RESULTS

TABLE XX. Response of N. tabacum (BG70A*) to various chemicals at 20° and 30°C in dark and light

*Second seed lot obtained from Botanic Garden

Chemical treatments	20°C						30°C					
	10^{-2}M		10^{-3}M		10^{-4}M		10^{-2}M		10^{-3}M		10^{-4}M	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
Sodium nitrate $NaNO_3$	58	92	53.5	94	58.5	90	45.5	94.5	40	95	30.5	94.5
Sodium nitrite $NaNO_2$	72.5	94	66	93.5	56	96	42.5	93.5	40	90.5	35.5	94
Hydroxylammonium chloride	60.5	74	84	88	63.5	95.5	65	79.3	71.5	90.5	46.5	93
Methyl hydrazine sulphate	29	88.5	47.5	88	45	96	56	88	59	93	46.5	96.5
Hydrazinium sulphate	33	61	62	96	60	93	68	89	55	95.5	38.5	95
Potassium ferricyanide	60.5	93	84	96	64.5	95	39	93.5	63.5	90.5	44	94
Potassium ferrocyanide	56	93.5	85.5	98	73	93	37.5	90.5	55	93	46.5	95
H_2O (control)	Dark 51	Light 92					Dark 36	Light 92				

Table XXI. Percentage germination in BG70 tobacco seed sample treated with various chemicals in complete darkness. Counting was made after eight days.

Chemicals	20°C			30°C		
	10^{-2}M	10^{-3}M	10^{-4}M	10^{-2}M	10^{-3}M	10^{-4}M
Hydroxylammonium chloride	13	90	90	13.5	38.5	26.5
Hydrazinium sulphate	-	-	-	36	24	8
Methyl hydrazine sulphate	39	68	71	26.5	25.5	18
Potassium ferricyanide	74	72	75	12.5	12.5	15
Potassium ferrocyanide	75	79	83	35	21	24.5
Sodium nitrite $NaNO_2$	81	94	78	66	30	20
Sodium nitrate $NaNO_3$	-	-	-	32	31	23
Potassium cyanide KCN	73	80	83	14	22	43
Potassium azide	00	00	52	00	00	51
H_2O control		68			12	

Table XXII. Percentage germination in N. tabacum cv. Montcalne treated with various chemicals in complete darkness. Four replicates of 50 seeds each per treatment.

Chemicals	20°C			30°C		
	10^{-2}M	10^{-3}M	10^{-4}M	10^{-2}M	10^{-3}M	10^{-4}M
Sodium nitrate $NaNO_3$	33.5	30.5	34.5	21	24	20
Sodium nitrite $NaNO_2$	36	24	29	31	26.5	25
Hydroxylammonium chloride	00	22.5	29.5	00	11.5	17.5
Methyl hydrazine sulphate	14	17.5	20	16	14.5	21
Hydrazinium sulphate	13	17.5	22	02	8.5	16.5
Potassium ferricyanide	33.5	36	35	15	21.5	12
Potassium ferrocyanide	34	37	33.5	18.5	21.5	19
H_2O control		28			21	

Table XXIII. Response of BG70A and BG71 seeds to various chemicals at 20° and 30°C in dark and light

A. BG70A (4 replicates of 50 seeds each

Chemicals (10^{-3}M)	20°C Dark	20°C Light	30°C Dark	30°C Light
Sodium nitrite $NaNO_2$	81	90.5	47	92
Sodium nitrate $NaNO_3$	80	85	35	86.5
Hydroxylammonium chloride	91	87	76	94
Hydrazinium sulphate	81	90	70	96
Methyl hydrazine sulphate	79	93	56.5	96
Potassium ferricyanide	91	93	66.5	97.5
Potassium ferrocyanide	88	90	56.5	91.5
H_2O control	85	98	41	98

B. BG71 (2 replicates of 50 seeds each)

Chemicals (10^{-3}M)	20°C Dark	20°C Light	30°C Dark	30°C Light
Sodium nitrite $NaNO_2$	00	76.5	00	2.5
Sodium nitrate $NaNO_3$	00	77.5	00	00
Hydroxylammonium chloride	00	78	00	00
Hydrazinium sulphate	04	80.5	00	1.5
Methyl hydrazine sulphate	01	85	00	0.5
Potassium ferricyanide	01	86.5	00	00
Potassium ferrocyanide	02	88	00	1.5
H_2O control	01	87	00	00

Results and Discussion

It has been found from our experiments with different seed lots that seeds harvested in Glasgow Botanic Gardens during 1970 responded to several chemicals (see Tables XX, XXI and XXIIIA). It appeared that certain chemical compounds, not all, including cyanides induced dark germination as compared with water control. Optimal concentration of the chemicals seemed to be 10^{-3}M for germination. In all concentrations hydroxyl ammonium chloride and hydrazinium sulphate retarded radicle elongation and root hair development. The inhibiting effect was permanent because the seedlings failed to grow in water even when they were removed from the chemical solutions. Stimulatory effects of lower concentration of cyanides and potassium azide in tobacco seed germination agree with the results reported by Roberts (1964) on rice seed germination. Seedlings in cyanide and $NaNO_3$ solutions looked normal but higher concentrations of the chemicals were found toxic. No marked increase in percentage of germination was obtained with N. tabacum cv. montcalne sample. Highest percentage of dark germination was 37% at 20°C and 31.2% at 30°C (see Table XXII) in comparison with H_2O control (28% at 20°C and 21% at 30°C).

In experiment with BG71 seed we used only one concentration (10^{-3}M) of each chemical for want of sufficient seeds (see Table XXVB) It has been observed that newly harvested BG71 seed sample did not respond to any chemicals at 20° or 30°C. It has also been found that

light treatments increased percentage germination in this batch and other seed samples in presence or absence of chemical solutions tested.

It is clear from our experiments with different seed samples that certain chemical compounds (at 10^{-3}M conc.) could induce dark germination in BG70, BG70A and N. tabacum cv. Montcalne, but none of the chemicals tested at that concentration could replace light requirement in BG71 seed sample (see Table XXIIIB). Therefore, one cannot generalise that a particular chemical compound at certain concentration can or cannot induce dark germination in tobacco. This could be cause of discrepancy among Hashimoto and Ogaware and Ono which has been mentioned elsewhere.

It has been observed that older seed lots which showed high percentages of germination in uninterrupted darkness could readily respond to the exogenous application of stimulatory chemicals. It could be that with the age of the seed, certain chemical conversions occur within the seeds which lead them to respond to the chemicals in the germination medium. Hendricks & Taylorson (1972) suggested that dark induction of germination in lettuce seed by cyanides and other compounds may be due to the involvement of cytochrome and electron transport system during respiration of seeds in presence of chemicals. But the accumulation of knowledge relating to the physiology of respiration in seed germination is very scanty and it needs further investigation. At this moment we cannot offer exact explanation regarding the actual mechanism of stimulation given by the chemicals in the process of tobacco seed germination.

Summary of Part V

(1) Dark germination of old N. tabacum seed samples was further stimulated by certain chemicals including cyanides.

(2) In newly harvested seeds light requirement cannot be replaced by chemical treatments.

(3) Hydroxylammonium chloride and Hydrazinium sulphate at all concentrations had toxic effect on radicle.

(4) Response of old seed samples to the chemicals could be due to their age.

Part VI. Germination behaviour of N. tabacum seeds collected from capsules of different stages of ripeness

The germination capability of seeds harvested prior to maturity is unclear (Harlan & Pope, 1922; Nutman, 1941; Grabe, 1956). It is believed that several complex changes take place during growth and development of seed on the mother plant during maturation. Recently it has been demonstrated that the level of dormancy in wild oat is dependent on the degree of maturation (Morgan & Berrie, 1970).

In our studies, N. tabacum seeds harvested at different stages of maturity were tested for their light sensitivity and degree of dormancy.

Figure 9. Diagrammatic drawings of plants 1-6 used for studies in the relationship between degree of maturity and degree of dormancy in N. tabacum

9.1, plant 1; 9.2, plant 2; 9.3, plant 3;

9.4, plant 4; 9.5, plant 5; 9.6, plant 6.

Figure 9.1

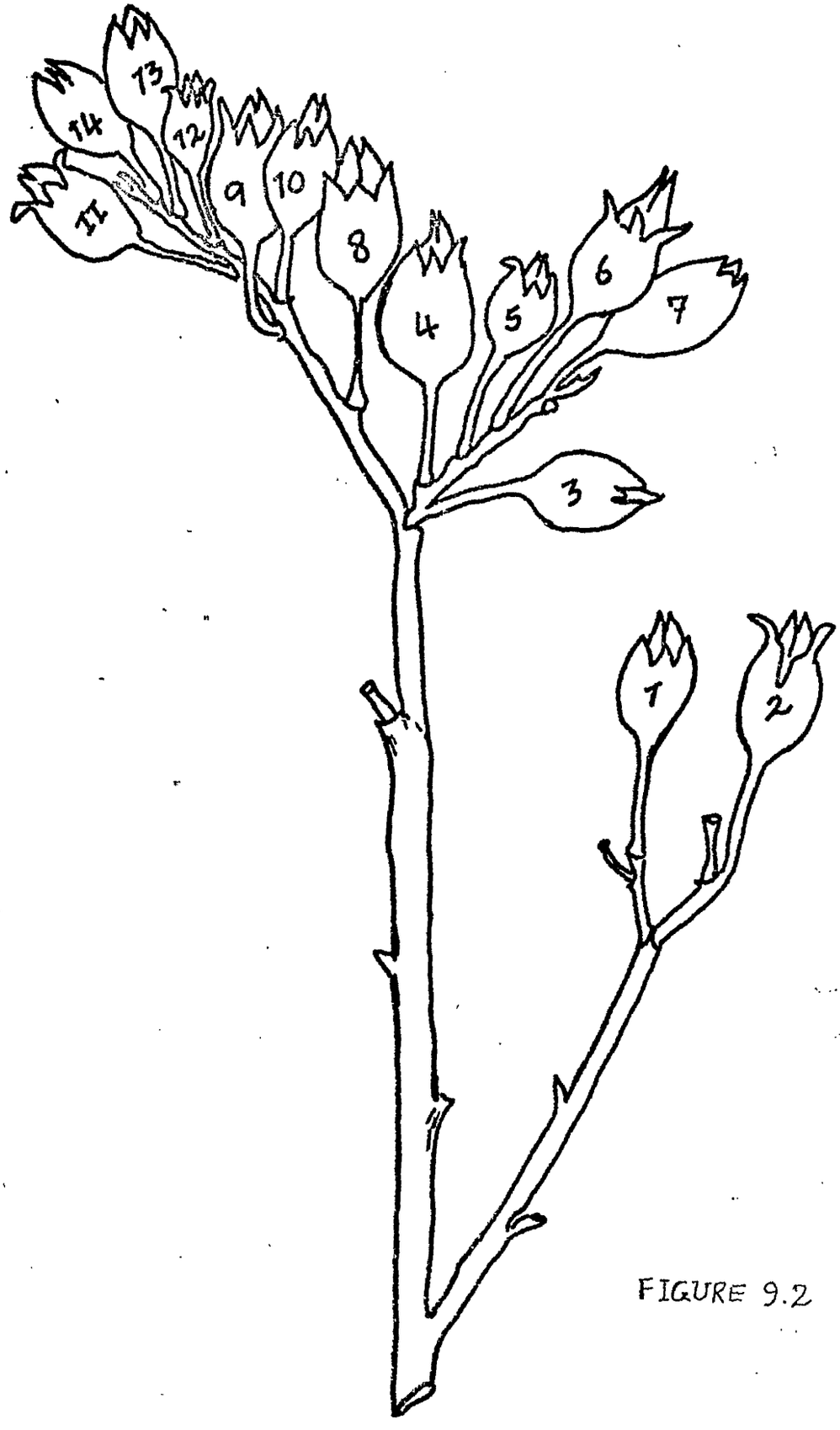


FIGURE 9.2

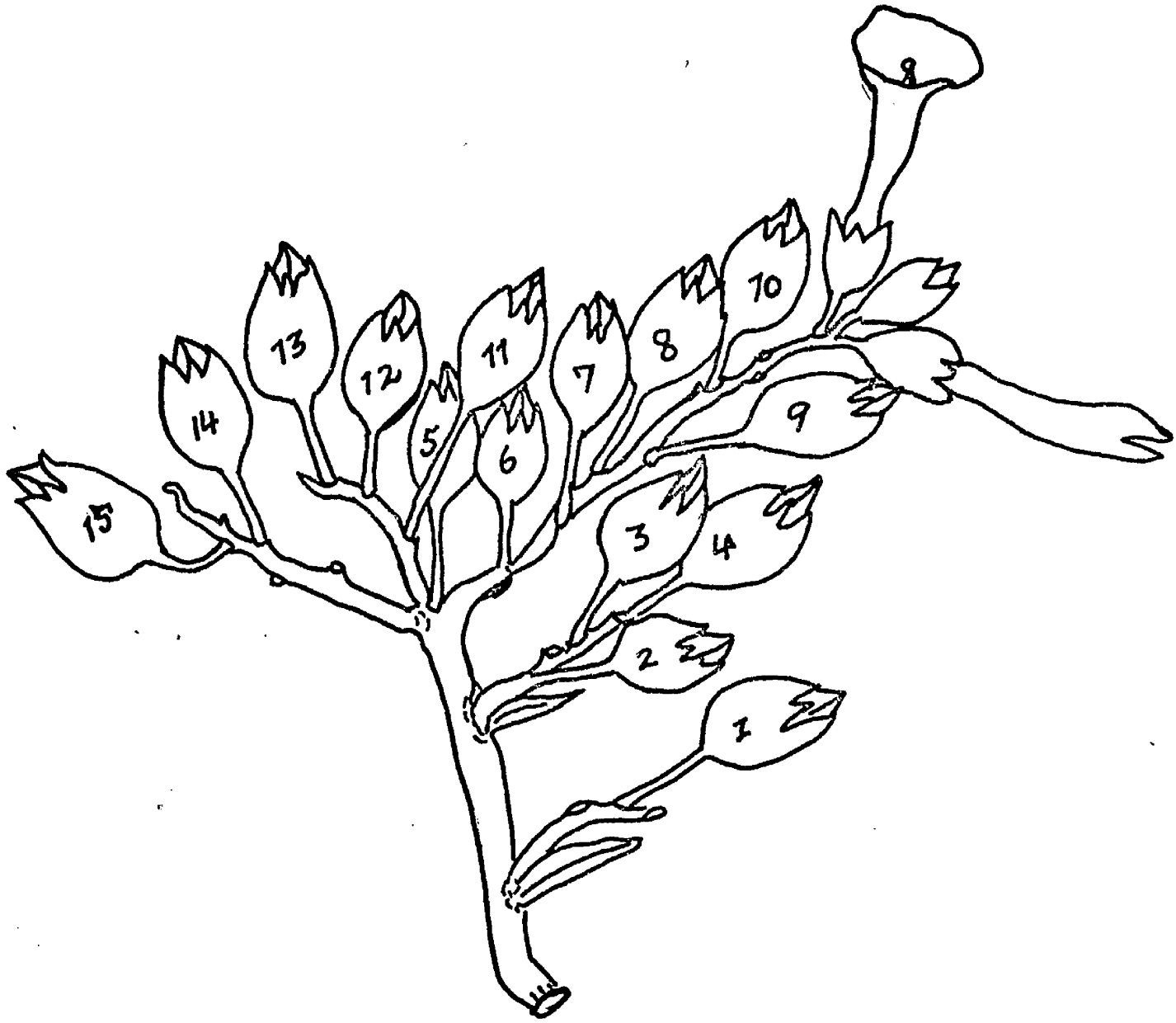


Figure 9.3

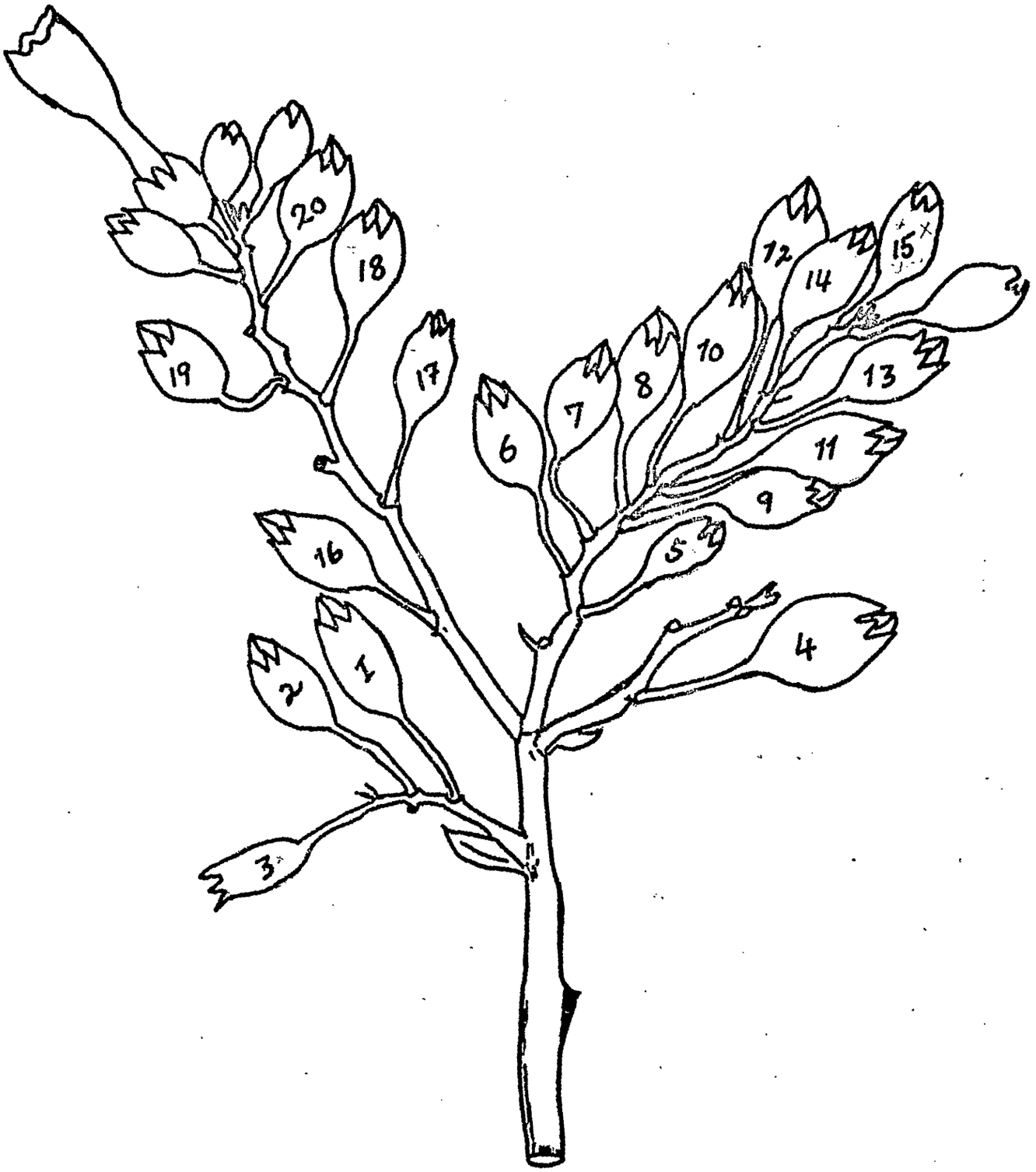


Figure 9.4

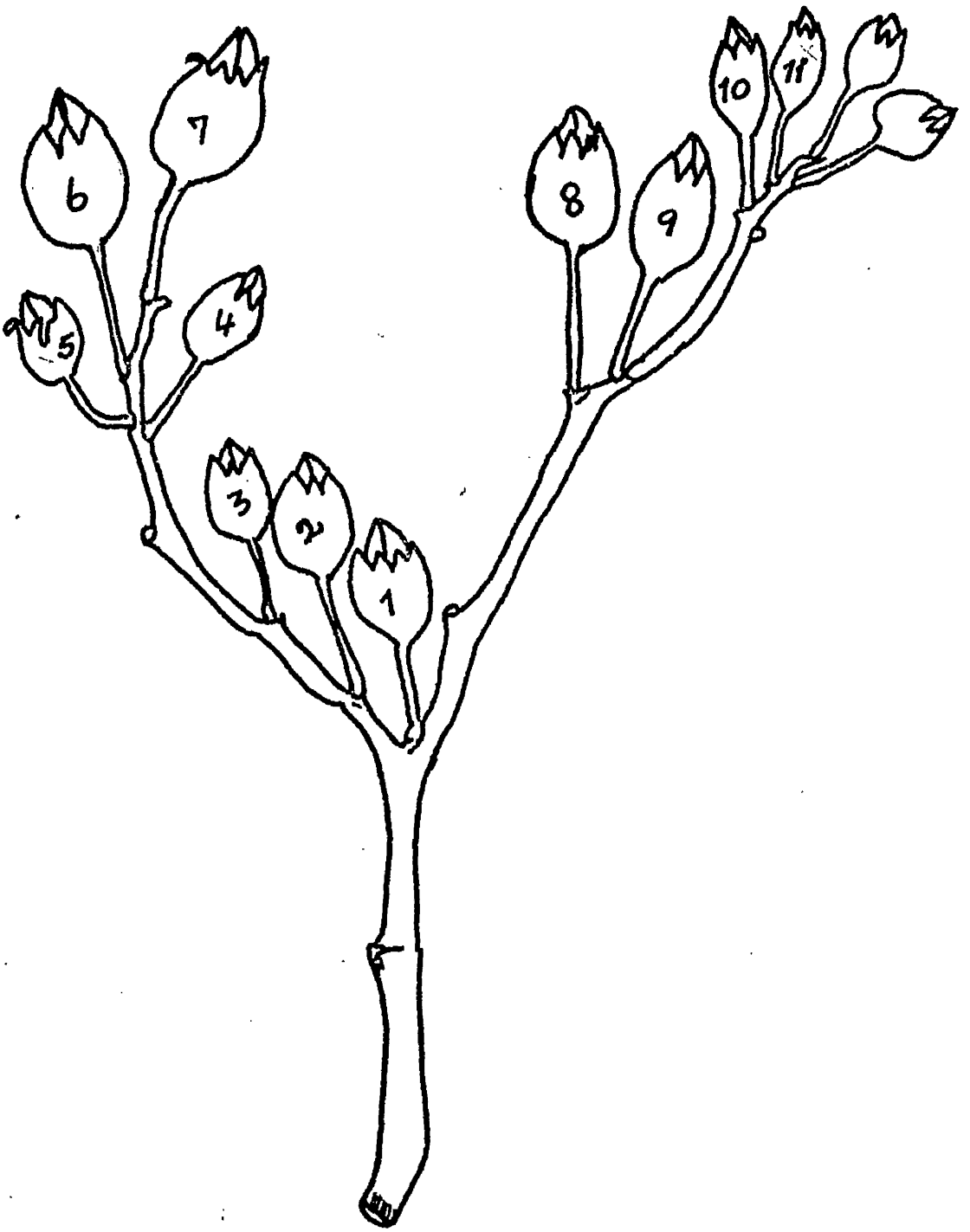


Figure 9.5

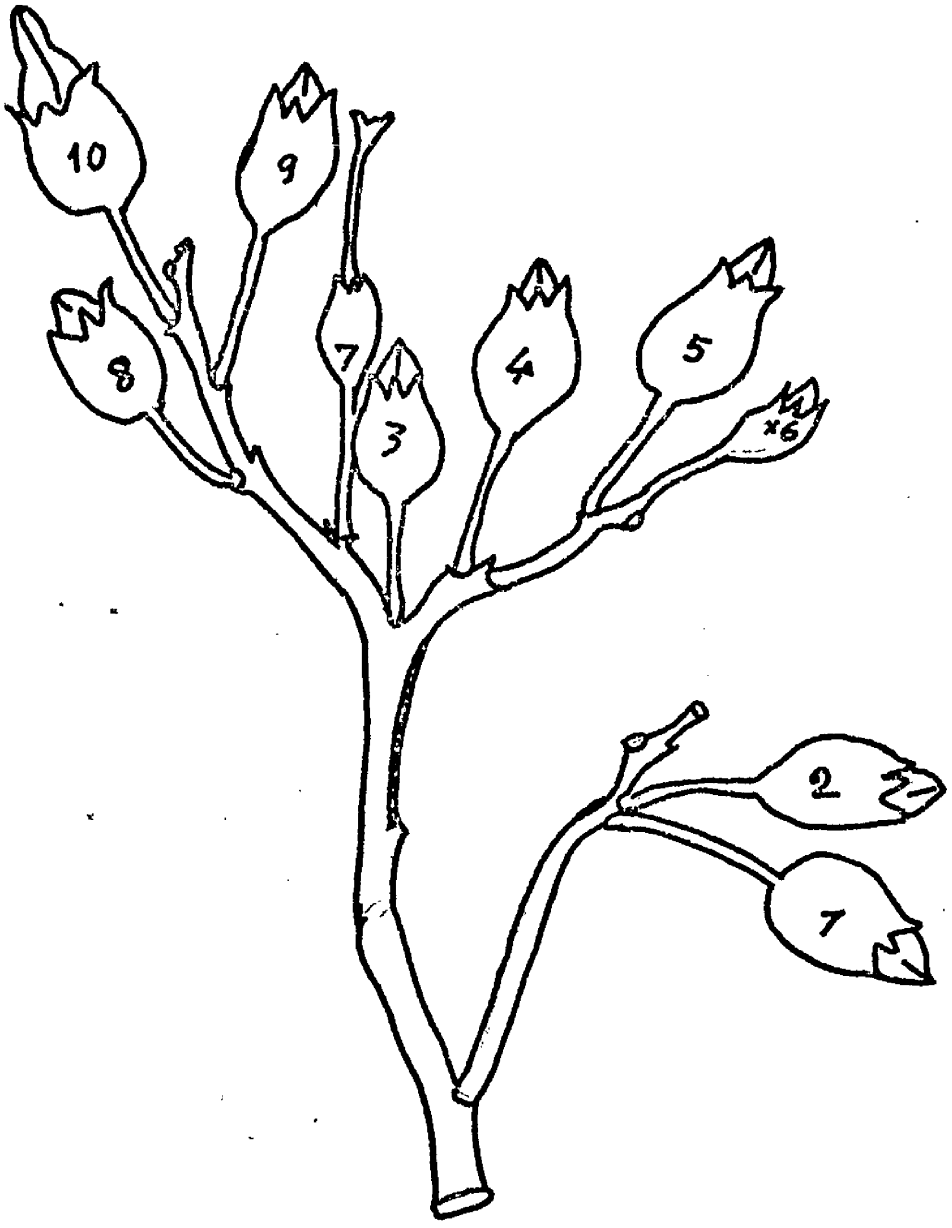


Fig. 9.6

Materials and Methods

The seedlings of N. tabacum cv. Montcalne were raised and six healthy plants grown in a thermostatically controlled growth cabinet. The plants were receiving 18 hours photoperiods from fluorescent tubes (5 ft. 250V 80 watt WWX) throughout. Air temperature was maintained at 18°C. Capsules were of different stages of ripeness at the time of harvest. Free hand diagrams of each detached infructescence showing the position of the capsules were drawn (see Fig. 9). Descriptions of each capsule of six different plants were recorded separately. Numbering of the capsules shown in the diagrams was made according to approximate age of the capsule, e.g. capsule number 1 was always older than capsule number 2, and so on.

The seeds collected from each capsule were tested for their capacity to germinate both in dark and light at 20°C. Germination tests were carried out as before. The capsules omitted in the tests were found shrunken and insufficient seeds were available for the tests. At least two replicates of 50 seeds each were used per treatment. White light treatments (1.5 ft. 250V. 13 watt WWX giving radiation 612μJsec^{-1}cm^{-2} at seed level) for 30 minutes was given to the seed once after 24 hours dark imbibition. Germination counts were made after eight days from the date of illumination.

RESULTS

Table XXIV. Plant No. 1. Date of harvest 11.4.72

(See Fig. 9.1)

Capsule Nos.	Colour of Capsule	Colour of Seeds	Av. percentage germination Dark	Av. percentage germination Light
1(a)	Deep brown	Brown	00	67.2
1(b)	Deep brown	Brown	04.0	51.1
2	Deep brown	Brown	00	45.3
3	Deep brown	Brown	00	55
4	Brown	Brown	28	79
5*	Brown	Brown	--	--
6	Light brown	Yellow	00	8.5
7	Green	White	00	00
8	Deep brown	Brown	8.0	45
9	Deep brown	Brown	00	70
10	Brown	Brown	00	5.7
11	Green	Yellowish	00	00
12*	Green	White	--	--
14	Green	White	00	00

*Shrunken and produced a few seeds.

Response of white seeds to GA_3 in dark

Caps. No.	Conc. of GA_3 00	10^{-4}	10^{-3}
14	00	00	00

Table XXV. Plant No. 2. Date of harvest 21.4.72

(Aec Fig. 9.2)

Capsule Nos.	Colour of Capsule	Colour of Seed	Av. percentage germination Dark	Av. percentage germination Light
1	Deep brown	Brown	00	84
2	Deep brown	Brown	00	92
3	Deep brown	Brown	00	50
4	Brown	Light brown	00	31
5	Brown	Light brown	00	80
6	Brown	Brown	00	13
7	Green	Yellow	00	4.0
8	Brown	Light brown	00	50
9	Brown	Brown	00	13
10	Brown	Brown	00	21
11	Brown	Brown	00	8.0
12	Brown	Brown	00	40
13	Green	Yellowish	00	2.8
14	Green	Whitish	00	5.7

Table XXVIa. Plant No. 3. Date of harvest 4.5.72

(See Fig. 9.3)

Capsule Nos.	Colour of Capsule	Colour of Seed	Av. percentage germination Dark	Av. percentage germination Light
1	Deep brown	Brown	00	00
2	Deep brown	Brown	00	00
3	Brown	Brown	00	00
4	Brown	Brown	00	00
5	Brown	Brown	00	00
6	Brown	Brown	00	00
7	Brown	Brown	00	00
8	Brown	Brown	00	00
9	Brown	Brown	00	00
10	Green	Yellow	00	00
11	Brown	Brown	00	00
12	Brown	Brown	00	00
13	Brown	Brown	00	00
14	Light Brown	Yellow	00	00
15	Light Brown	Yellow	00	00

Table XXVIb. Response of seeds of PL-3 to GA_3. Two replicates of 50 seeds each

Capsule Nos.	Colour of		Concentration of GA_3					
			00		10^{-4}M		10^{-3}M	
	Capsule	Seed	D	L	D	L	D	L
1	Deep brown	Brown	00	00	2.0	5.0	84	96
2	Deep brown	Brown	00	1.0	1.0	3.0	96	93
14	Light brown	Yellow	00	3.0	4.0	6.0	97	98

Table XXVII. Plant No. 4. Date of harvest 18.5.72

(See Fig. 9.4)

Capsule Nos.	Colour of Capsule	Colour of Seed	Av. percentage germination Dark	Av. percentage germination Light
1	Brown	Brown	00	00
2	Brown	Brown	00	4.0
3	Brown	Shrunken	--	--
4	Brown	Brown	00	4.0
5	Brown	Shrunken	--	--
6	Brown	Shrunken	--	--
7	Brown	Brown	00	00
8	Brown	Brown	00	00
9	Brown	Brown	00	00
10	Brown	Brown	00	00
11	Brown	Brown	00	00
12	Brown	Brown	00	00
13	Brown	Brown	00	00
14	Brown	Shrunken	--	--
15	Green	White	--	--
16	Brown	Brown	00	00
17	Brown	Shrunken	--	--
18	Brown	No seed	--	--
19	Brown	Brown	00	2.0
20	Brown	Yellow	00	00

Table XXVIII. Plant No. 5. Date of harvest 25.5.72

(See Fig. 9.5)

Capsule Nos.	Colour of Capsule	Colour of Seed	Percentage germination Dark	Percentage germination Light
1	Brown	Brown	00	19
2	Brown	Brown	4.0	92
6	Brown	Light brown	00	41
7	Brown	Brown	00	9.0
8	Brown	Brown	00	55
9	Brown	Brown	00	42
10	Brown	Brown	00	46

Table XXIX. Plant No. 6. Date of harvest 8.6.72

(See Fig. 9.6)

Capsule Nos.	Colour of Capsule	Colour of Seed	Percentage germination Dark	Percentage germination Light
1	Brown	Brown	2.0	92
2	Brown	Brown	1.0	84
3	Deep brown	Brown	00	83
4	Brown	Brown	00	66
5	Deep brown	Brown	00	60
8	Brown	Yellow	00	60
9	Light brown	Yellow	00	84
10	Brown	Brown	00	37

Results and Discussion

Light sensitive seed and seed with a chilling requirement may contain certain germination inhibitors (Wareing, 1965). It has been found that light negates or abolishes the inhibition caused by these inhibitors. In birch seeds the endogenous inhibitor appears not to be destroyed by low intensity of light and prolonged irradiation is required for high percentage of germination (Wareing, 1965). Wareing & Foda (1957) also demonstrated that gradual accumulation of inhibitors may take place during the later stages of Xanthium seed development. However, there is evidence that dormancy of wild oat may be determined by the stages of ripeness at which the seeds are harvested (Quail & Carter, 1969). In oat the degree of dormancy may also depend on position and number of caryopsis on the spikelet (Morgan & Berrie, 1970).

No information exists regarding the light sensitivity of N. tabacum seeds produced in different capsules on the same mother plant. Our germination studies with N. tabacum seeds from six individual plants showed that light was absolutely necessary for germination in the seeds collected from plants 1 (see Table XXIV), 2 (Table XXV), 5 (see Table XXVIII) and 6 (Table XXIX). It was observed that in plants 3 (see Table XXVI) and 4 (see Table XXVII), 30 minutes white light once after 24 hours dark imbibition had no or little effect on germination, although the seeds gave high percentage of germination

in darkness when they were soaked at 10^{-3}M solution of GA_3 (See Table XXVIb). The reasons for nonresponsiveness of the seeds to light is not clear. This could be due to plant or other unknown environmental effects.

Colour of capsule or seed may indicate ripeness or full growth but germination capability of the seeds obtained from them may vary Only a few capsules produced a limited number of seed which can germinate in darkness (see Tables XXIV, XXVIII and XXIX). In most of the cases brown seed germinated well after light treatments but white seed did not show any sign of germination both in light and GA_3(10^{-3}M) (Table XXIV).

From a study of the data presented in Tables XXIV, XXV, XXVIa and b, XXVII, XXVIII and XXIX, it appeared that N. tabacum seeds produced by a single plant may have mixed light sensitivity or degree of dormancy. Different light and temperature treatments may be required in some seeds to break the post harvest dormancy. This may have some biological importance for the survival of the species. But how the inception of such variable light sensitivity or dormancy occurs in the seed is not clear.

Summary of Part VI

(1) Germination behaviour of *N. tabacum* seeds collected from capsules of different stages of ripeness was investigated immediately after harvest.

(2) All seeds showed light requirement for germination. Some plants produced deeply dormant seeds which did not respond in germination tests soon after harvest.

(3) Position and degree of maturity of capsules on plants could be involved in the light sensitivity and degree of dormancy.

(4) Seeds from six test plants showed mixed light sensitivity. Production of mixed light sensitive seeds by a single plant is not clear, but see elsewhere (in Discussion).

Part VII. Effects of light quality during maturation of *N. tabacum* seed on germination

Light is involved in a number of distinct physiological processes in plants, e.g. photosynthesis, photoperiodism and dormancy. The leaves of the plants have been suggested as the principal site of radiation absorption which could be regulating induction or inhibition of flowering (Withrow & Biebel, 1936; Withrow & Withrow, 1940; Borthwick & Parker, 1940). Wareing (1954) postulated that a growth inhibitor which is involved in dormancy in woody species is produced by the leaves during long dark period and formation of this inhibitor could be negated by exposing the leaves to continuous light.

However, in a number of plants it has been demonstrated that different photoperiods (short or long) or other controlled environment under which parent plants are grown may have some influence on subsequent germination quality of the seeds (Koller, 1962; Karssen, 1970; Datta *et al.*, 1972).

An investigation was made of the nature and variability of light sensitivity or dormancy in *N. tabacum* seeds collected from capsules or mother plants exposed to light of different spectral composition preceding full maturity of the seeds.

Figure 10. Shows the details of capping with deep red and blue cinemoid filters at different stages of seed development

A - H = Anthesis to harvest

R = Deep red filter

B = Deep blue filter

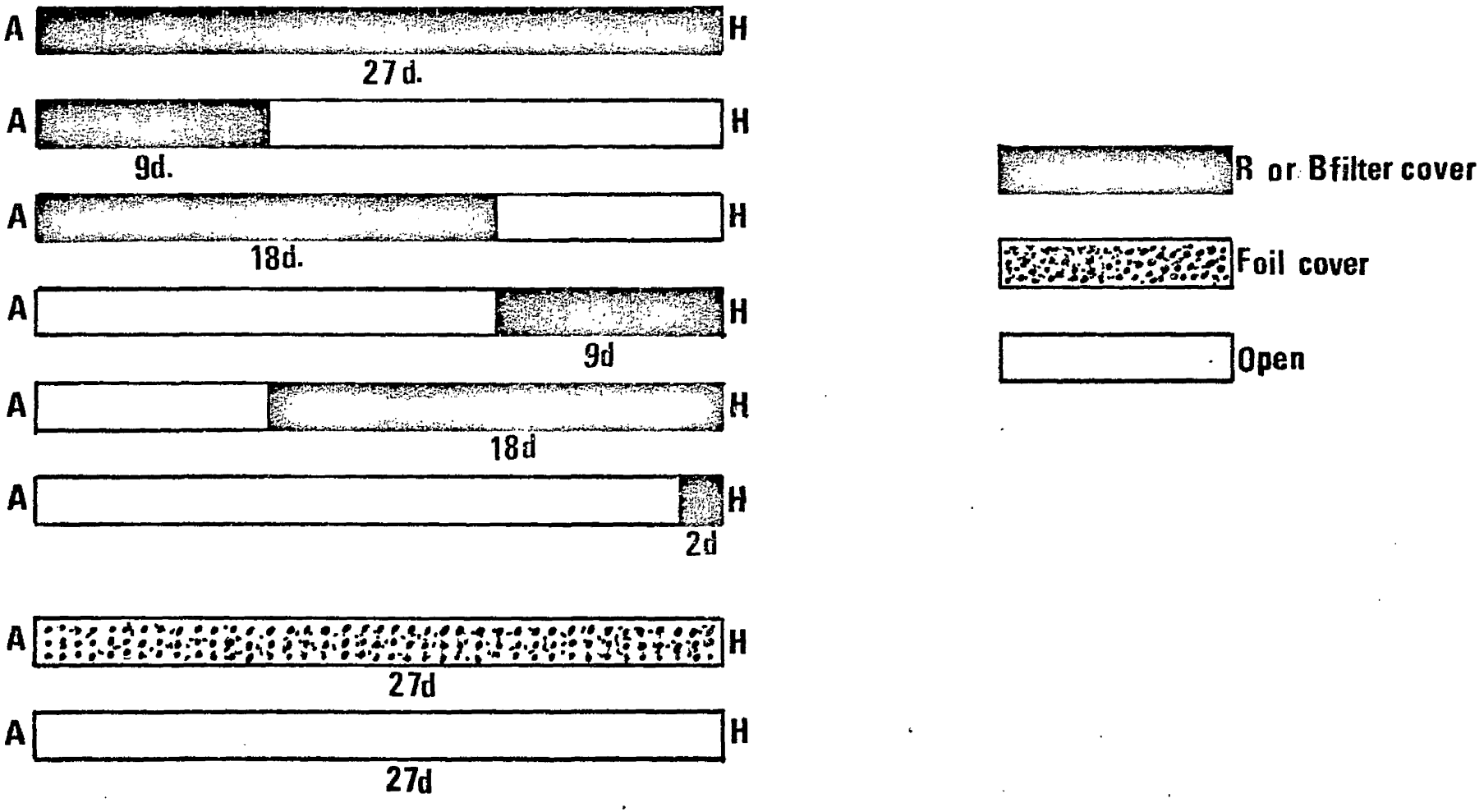

A
H
27 d.
9d.
18d.
9d
18d
2d
27d
27d
R or B filter cover
Foil cover
Open

Figure 10

Materials and Methods

The seeds used in this section were obtained from the following treatments given to the mother plants (N. tabacum cv. Montcalne) or developing capsules. All plants under treatments received artificial white photosynthetic light (18 hours) from fluorescent tubes in the growth cabinet. Other details of growing conditions have been mentioned in Part VI and General Materials and Methods section.

I. Capping: Four healthy plants were selectedfor treatment. Treatments were carried out from anthesis to harvest, a period of 27 days.

Small rectangular boxes (approx. 1.5 x 1.5 cm wide and 2 cm high) made of deep red and blue cinemoid filters were used to cover the capsules for various lengths of time at different stages of seed development within this period. Reference was made to capsules either open, or uncovered, or dark, covered with foil soon after anthesis. A typical experimental design is illustrated in Fig. 10. Only 14 capsules received treatments per plant soon after anthesis and the remaining flowers or developing capsules were removed. The capsules were harvested 27 days after anthesis.

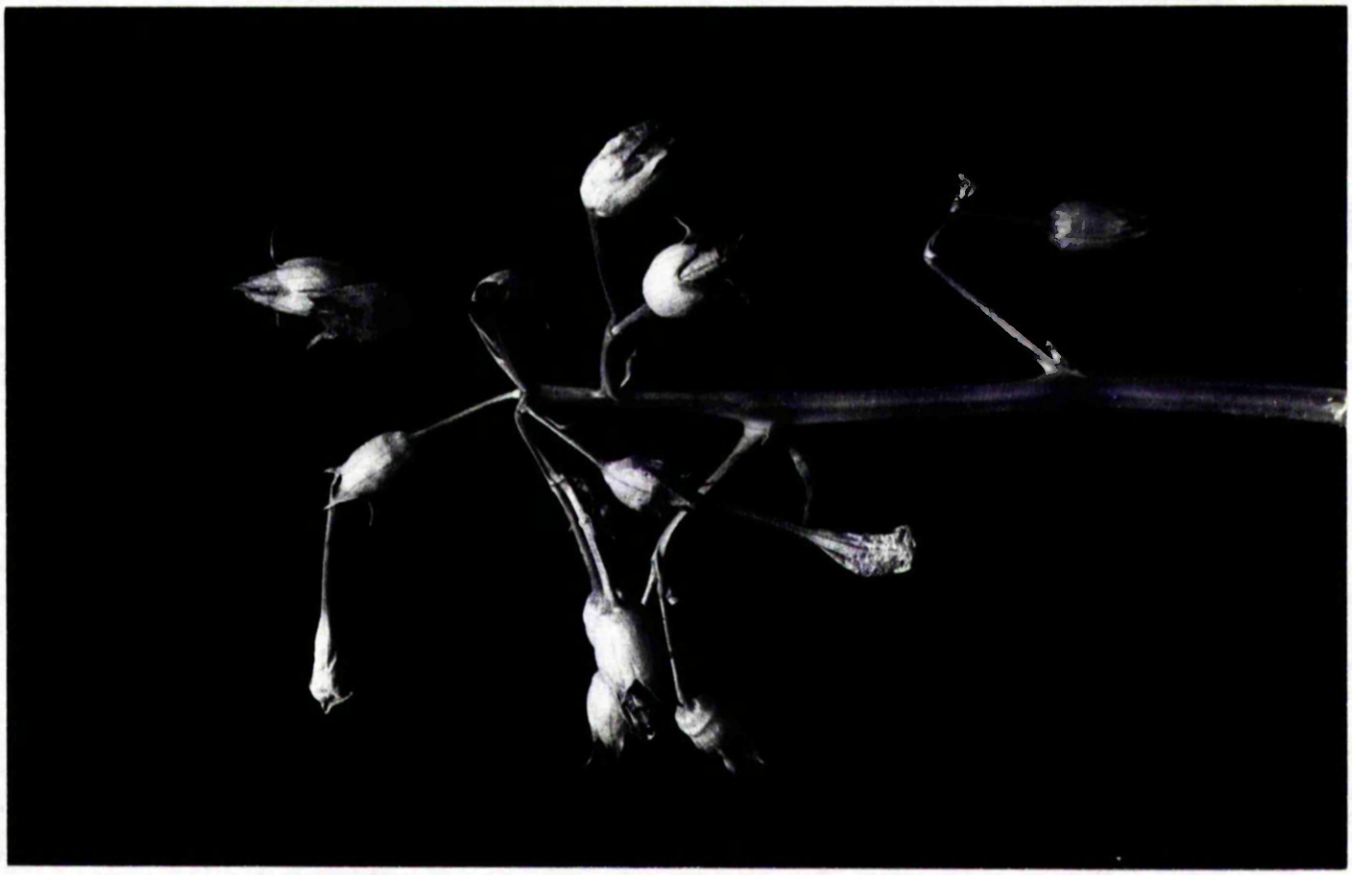
II. Leaf Covers: In this treatment the leaves alone were covered with variously coloured cinemoid filters soon after the plants showed sign of flowering. Inflorescences of the plants were left exposed to "white" light. Only a limited number of capsules

PLATE 2. Shows the photographs of mature plant heads in which leaves were covered with cinemoid filters

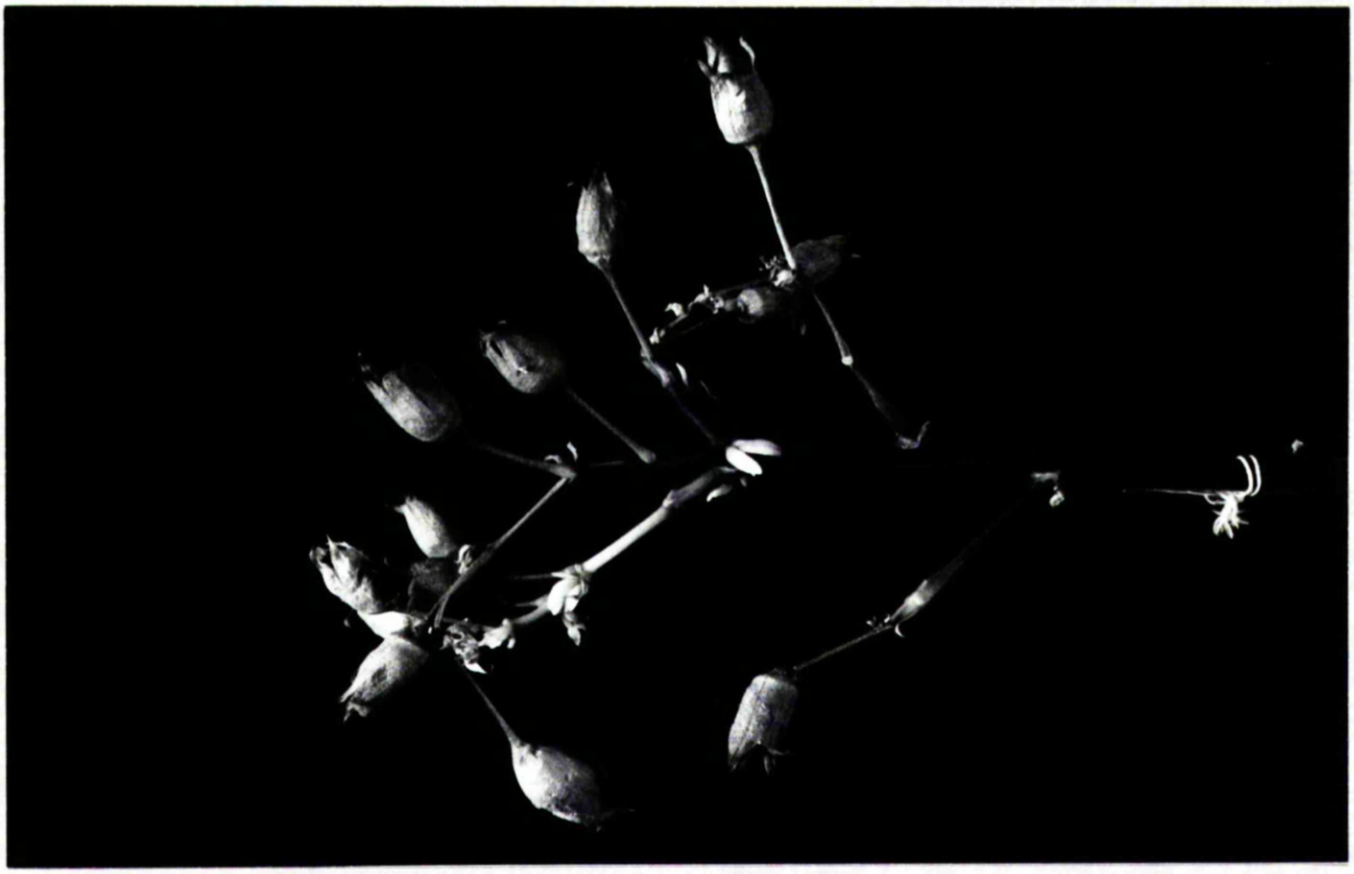
1. Light red	2. Deep red	3. Deep blue
4. Yellow	5. Green	6. Light blue

1

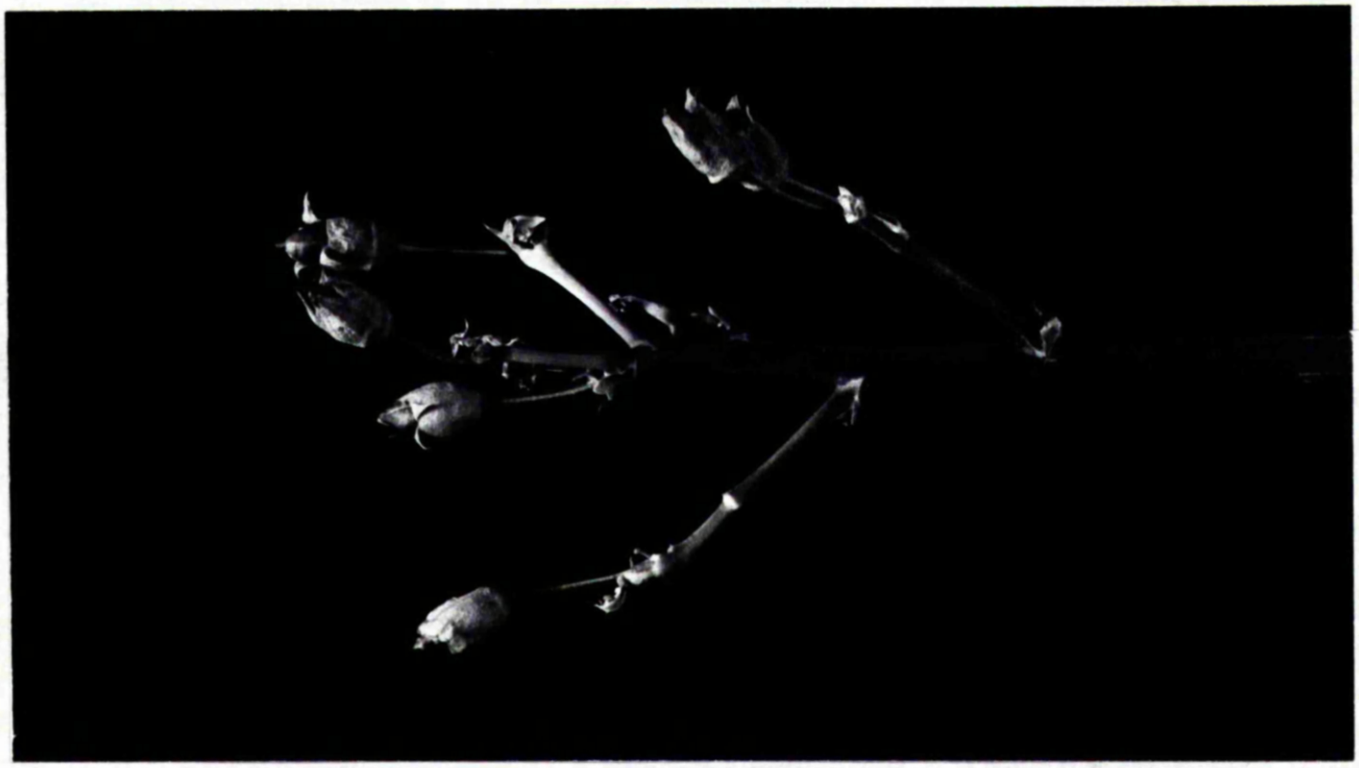
2



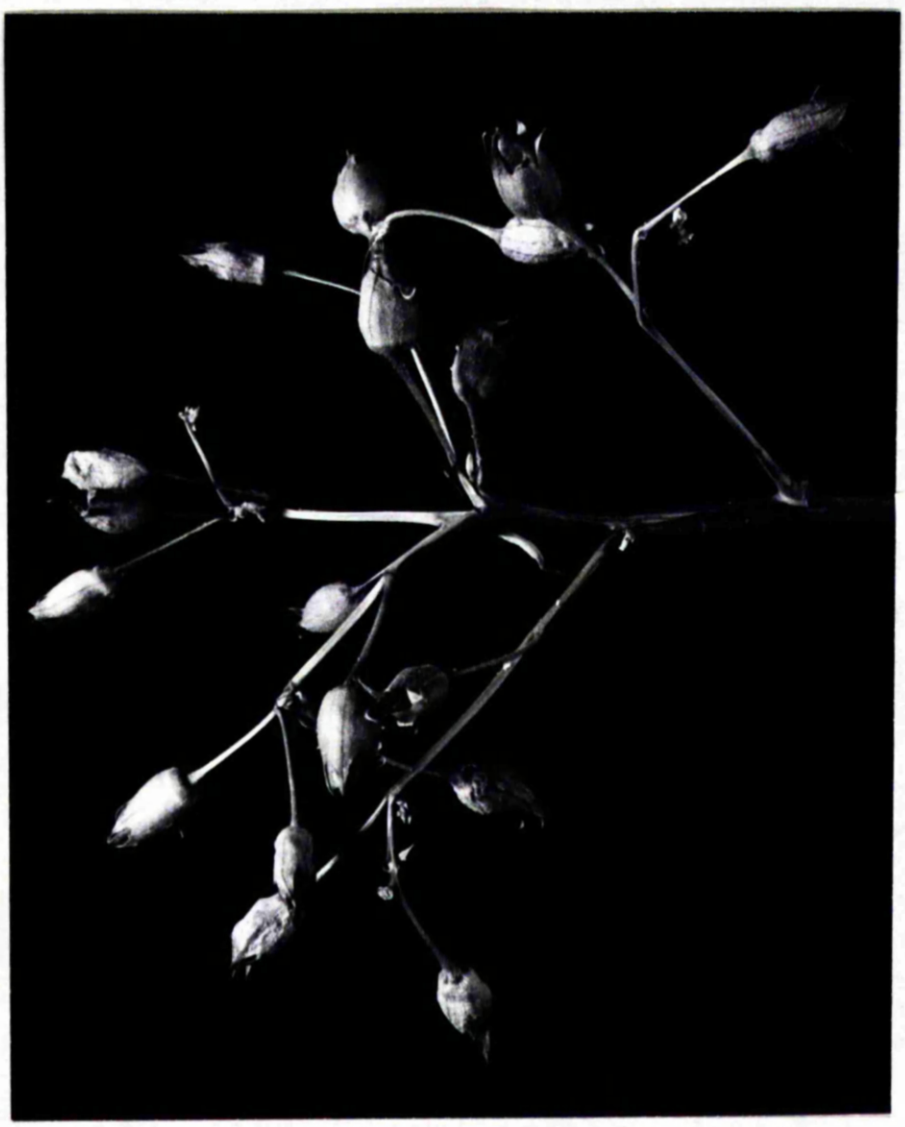
3



4



5



6

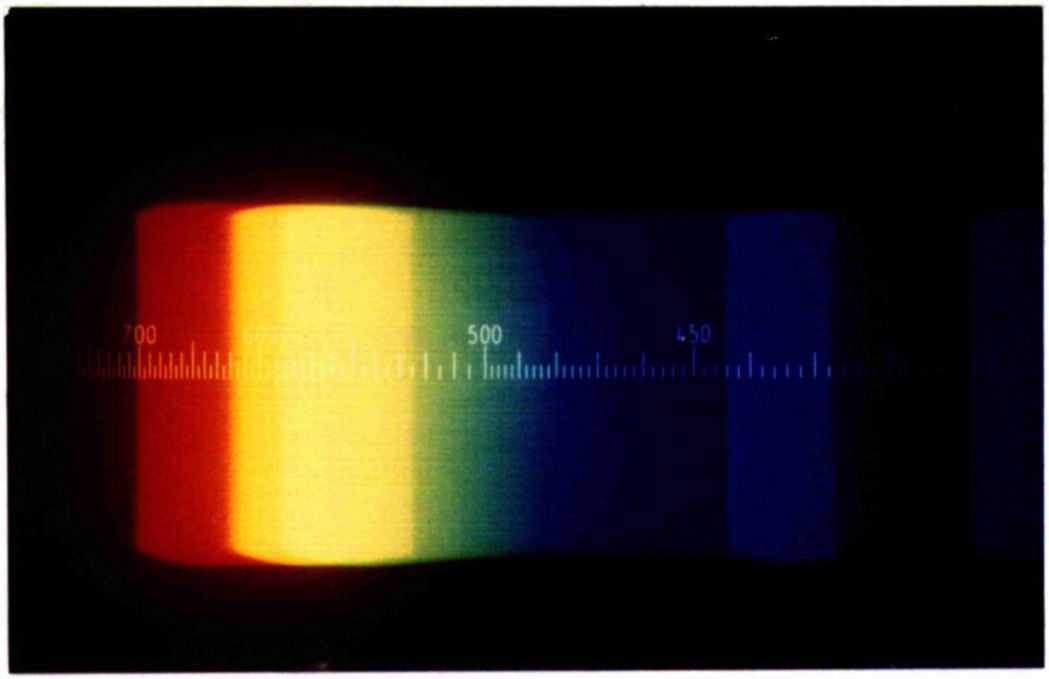
(18) was allowed to attain full maturity. The capsules harvested were brown in colour and had started cracking at the tops. Plant heads showing the position and number of capsules developed can be seen in the photographs. (See Plate 2

III. Photoperiod: In this experiment mother plants were given either short day (8 hours) treatments from flowering to harvest or 18 hours photoperiod throughout.

Soon after harvests the capsules were left at room temperature for a few days and then were put in sealed glass bottles and subsequent stored at 4^{o}C until required for tests. Germination tests were carried out as quickly as possible. The standard germination procedure described elsewhere (see Page 21) was employed. Incubating temperatures were 20^{o}, 24^{o} and 30^{o}C. Light treatments (612μJ.cm^{-2}sec^{-1} at seed level) were given for 15 minutes once after 24 hours dark imbibition.

PLATE 3. Shows the spectra transmitted through cinemoid filters

1. Control
2. Dark red
3. Deep blue
4. Yellow
5. Green
6. Light blue
7. Light red



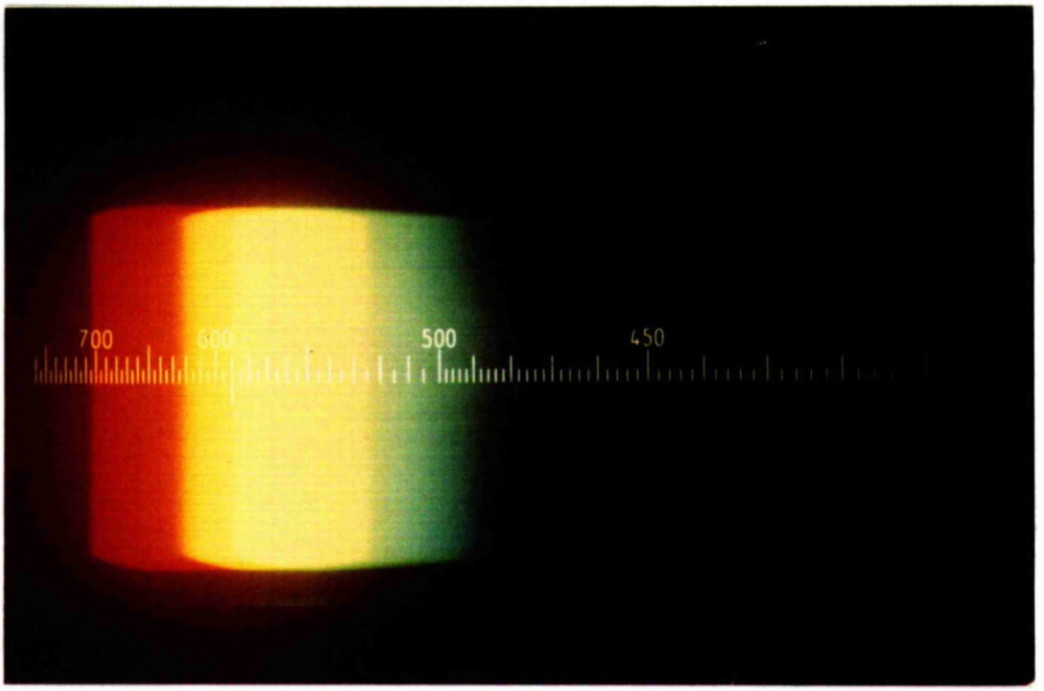
1

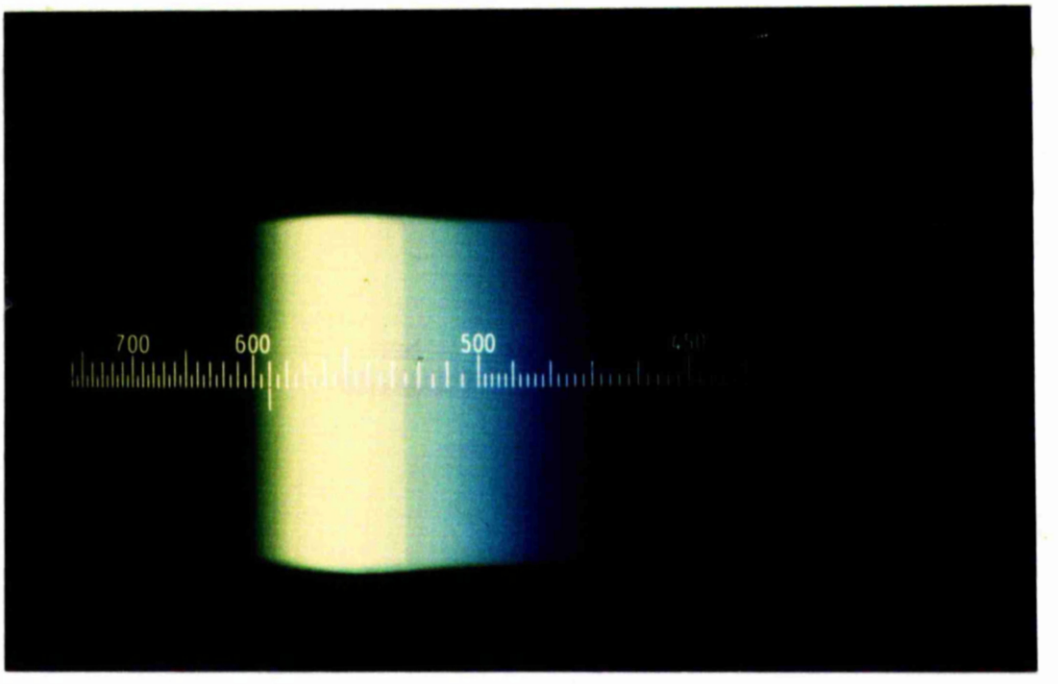
NOTE

Due to some technical difficulties prints of Nos. 2 and 3 were not available. Transparency showed the following:

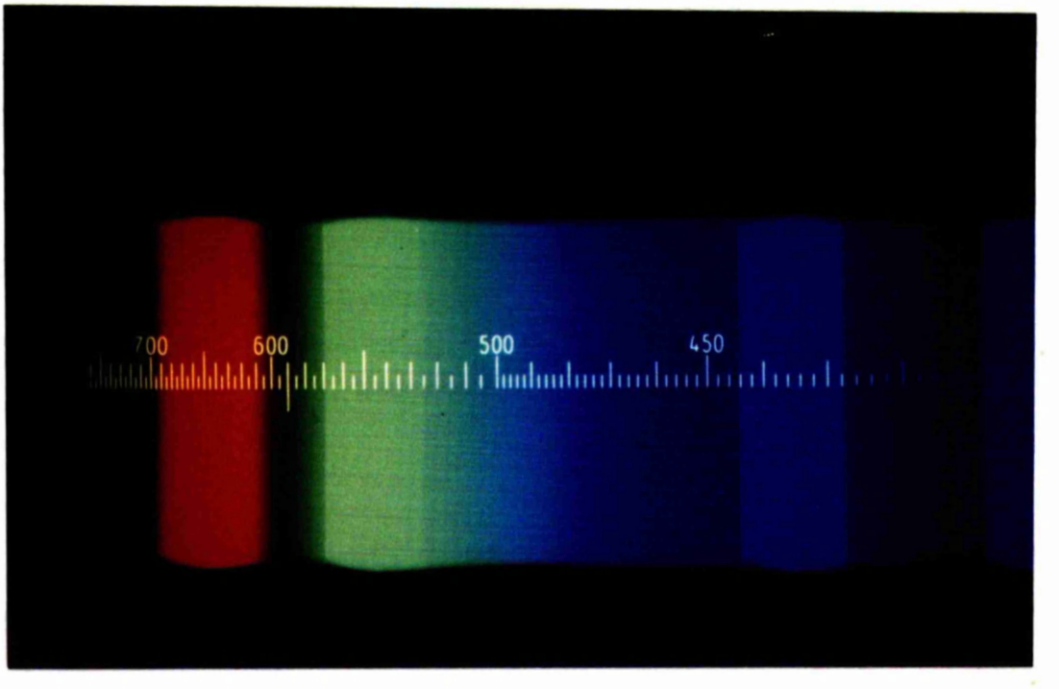
No. 2. Deep blue, extends from 430-480μ.

No. 3. Deep red, extends from 590-700μ.

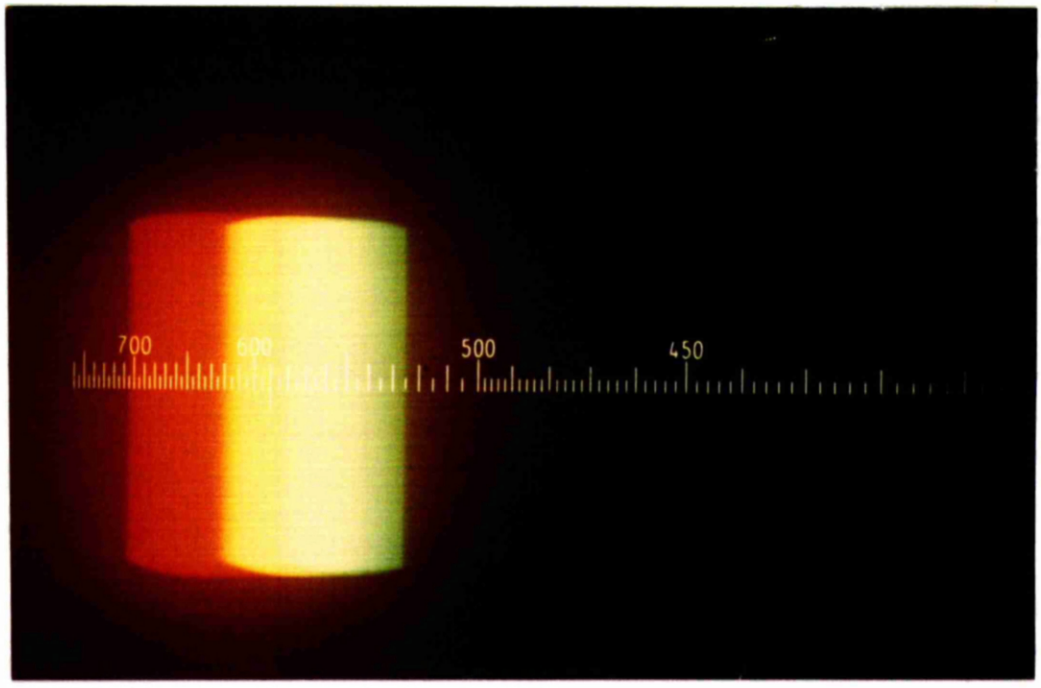

700
600
500
450



5



6


700
600
500
450

Transmission spectra of cinemoid filters were as follows:

Deep red : 590 - 680mμ(intense at red region)

Light red : 530 - 690mμ(intense at green and red region)

Deep blue : 420 - 530mμ(intense at blue and green)

Light blue : 430 - 700mμ(intense at blue, yellow and red)

Yellow : 460 - 690mμ(intense at yellow and red)

Green : 460 - 590mμ(intense at blue, yellow and green)

See photograph of spectra.(Plate 3)

RESULTS

Table XXXa. Germination percentage of N. tabacum seeds at 20°, 24° and 30°C collected from capsules covered with Deep red and Deep blue cinemoid boxes

Colour of Boxes	Treatments	Irradiation	20°C Plant numbers				24°C Plant numbers				30°C Plant numbers			
			1	2	3	4	1	2	3	4	1	2	3	4
Red	9 days from the beginning	Dark	4.0	5.0	25	16	00	00	1.0	00	00	00	3.0	00
		Light	44	80	94	94	8.0	18	40	36	00	4.0	29	00
Red	18 days from the beginning	Dark	2.0	1.0	9.0	1.0	00	00	1.0	-	00	00	00	00
		Light	74	77	89	95	31	6.0	39	-	2.0	3.0	25	00
Red	18 days at the end	Dark	2.0	00	13	00	00	00	1.0	00	00	00	2.0	00
		Light	00	85	90	90	37	2.0	82	17	00	00	71	1.0
Red	9 days at the end	Dark	4.0	00	2.0	2.0	00	00	00	00	00	00	00	00
		Light	62	68	92	96	42	1.0	68	25	00	00	51	00
Red	2 days at the end	Dark	00	00	10	00	00	00	1.0	00	00	00	00	00
		Light	78	68	96	80	16	00	88	5.0	00	00	23	1.0
Red	27 days continuous	Dark	00	00	6.0	00	00	00	00	00	00	00	00	00
		Light	82	51	94	97	30	1.0	45	12	00	1.0	4.0	1.0
Open control	27 days continuous	Dark	8.0	2.0	22	14	00	00	2.0	00	00	00	00	00
		Light	72	67	91	71	33	10	77	27	22	7.0	44	1.0
Foil cover control	27 days continuous	Dark	4.0	7.0	00	20	00	00	1.0	12	00	2.0	1.0	00
		Light	100	97	97	98	70	17	82	98	36	49	35	28

Table XXXb

Colour of Boxes	Treatments	Irradiation	20°C Plant numbers				24°C Plant numbers				30°C Plant numbers			
			1	2	3	4	1	2	3	4	1	2	3	4
Blue	9 days from the beginning	Dark	2.0	1.0	44	32	00	00	17	00	00	00	6.0	00
		Light	94	43	97	94	43	00	91	24	18	00	41	00
Blue	18 days from the beginning	Dark	14	00	54	4.0	00	00	2.0	00	00	00	4.0	00
		Light	94	93	96	97	73	19	60	13	24	1.0	64	00
Blue	18 days at the end	Dark	00	00	1.0	00	00	00	2.0	00	00	00	00	-
		Light	98	83	97	95	65	24	76	15	6.0	2.0	26	-
Blue	9 days at the end	Dark	10	1.0	1.0	4.0	00	00	00	00	00	00	00	00
		Light	92	94	91	97	80	31	26	56	22	3.0	12	00
Blue	2 days at the end	Dark	00	1.0	25	3.0	00	00	00	00	00	00	6.0	00
		Light	93	81	97	97	19	11	70	54	00	00	39	00
Blue	27 days continuous	Dark	00	00	10	00	00	-	4.0	00	00	00	1.0	00
		Light	100	97	100	94	44	-	96	58	18	00	60	00
Open control	27 days continuous	Dark	8.0	2.0	22	14	00	00	2.0	00	00	00	00	00
		Light	72	67	91	71	33	10	77	27	22	7.0	44	1.0
Foil cover control	27 days continuous	Dark	4.0	7.0	00	20	00	00	1.0	12	00	2.0	1.0	00
		Light	100	97	97	98	70	17	82	98	36	49	35	28

Table XXXc. Values of X^2 of treated vs. control capsules in the results presented in Tables XXXa and XXXb. Four plots per treatment. 100 seeds from each plant.

Treatments	Irradiation	20°C				24°C				30°C			
		X^2(1df) Red vs. open	X^2(1df) Red vs. foil	X^2(1df) Blue vs. open	X^2(1df) Blue vs. foil	X^2(1df) Red vs. open	X^2(1df) Red vs. foil	X^2(1df) Blue vs. open	X^2(1df) Blue vs. foil	X^2(1df) Red vs. open	X^2(1df) Red vs. foil	X^2(1df) Blue vs. open	X^2(1df) Blue vs. foil
9 days from the beginning	Dark	0.1894	4.9589	10.3253	24.2840	11.8075	136.9476	0.6411	59.6180	18.1358	94.4314	2.0290	51.6232
	Light	0.8445	75.7576	5.4222	56.8889								
18 days from the beginning	Dark	19.9271	7.7922	5.8382	17.2354	30.5590	186.1857	1.7024	52.3550	21.3969	100.6105	1.7335	20.8816
	Light	8.8664	48.9759	61.6099	5.3294								
18 days at the end	Dark	17.0545	5.9047	45.77	29.2869	0.4414	83.2180	5.6326	38.3748	0.0335	36.2131	17.1269	86.0291
	Light	-	-	48.8343	10.7882								
9 days at the end	Dark	28.6764	14.2592	15.7356	5.0860	0.6615	85.8098	10.8235	28.0102	5.0157	105.7250	13.3199	86.6341
	Light	2.0636	68.5571	50.5268	9.9524								
2 days at the end	Dark	24.8848	11.3371	4.2519	0.0721	8.2948	125.2710	0.2610	64.0215	25.5814	113.8793	12.6238	85.9872
	Light	3.1994	63.8394	40.9772	15.1579								
27 days continuous	Dark	32.9082	17.7121	24.8848	11.3371	0.9737	162.2585	13.2556	24.4506	64.2222	162.1486	0.1298	30.2179
	Light	3.8693	61.5057	86.7052	0.0601								

Irradiation	20°C X^2(1df) open vs. foil	24°C X^2(1df) open vs. foil	30°C X^2(1df) open vs. foil
Dark	3.233	2.045	--
Light	89.341	72.087	34.140

X^2(1df) = 3.841 for P = 0.05

X^2(1df) = 6.635 for P = 0.01

All values in excess of these the treatments are considered to differ significantly.

Table XXXI. Germination percentage of N. tabacum seeds matured on different plants in which leaves were covered with coloured filters during ripening of the seeds

Batch I:

Colour of filter	Number of capsules developed	Total wt. in gms	Av. wt. each capsule gms	Germination % at 20°C			
				Oldest capsule		Topmost capsule	
				Dark	Light	Dark	Light
Deep blue (420-530mμ)	12	2.27	0.18	49.5	89.5	36.5	62
Light blue (430-700mμ)	18	2.59	0.14	0.5	85.5	1.0	55
Green (460-590mμ)	6	0.70	0.11	13	94	29	94.5
Yellow (460-690mμ)	13	2.52	0.19	7.0	75	35	83.5
Light red (530-690mμ)	16	4.37	0.27	19	83	12.5	46.5
Deep red (590-680mμ)	10	2.79	0.28	2.0	82	3.5	69.5
Open control	6	0.50	0.08	8.0	79	6.0	81

Table XXXI (continued)

Colour of filter	Irradiation	20°C Capsule Nos.			24°C Capsule Nos.			30°C Capsule Nos.		
		1	2	3	1	2	3	1	2	3
Deep blue (420-530mμ)	Dark	5.0	91	89	00	42	98	00	41	48
	Light	97	99	99	84	100	97	4.0	98	95
Green (460-590mμ)	Dark	83	30	94	42	25	94	5.0	3.0	60
	Light	100	100	98	99	96	98	65	77	97
Deep red (590-680mμ)	Dark	00	2.0	13	00	00	6.0	00	00	1.0
	Light	80	68	98	82	63	72	9.0	9.0	15
Open control	Dark	14	32	67	11	23	80	4.0	4.0	55
	Light	89	93	94	75	94	91	51	72	94

Table XXXII. Germination percentage of N. tabacum seeds at 20°, 24° and 30°C matured on plants under short and long day treatments.

100 seeds from each capsule per treatment.

Temperatures	Capsules	Short day treatments (8 hrs)						Long day treatments (18 hrs)					
		PL-1		PL-2		PL-3		PL-1		PL-2		PL-3	
		D	L	D	L	D	L	D	L	D	L	D	L
20°C	Lower	11	94	00	93	10	98	74	99	78	96	9.0	96
	Middle	94	100	00	73	11	99	72	95	25	95	32	100
	Upper	96	95	3.0	89	7.0	96	85	96	38	99	35	96
24°C	Lower	-	-	00	92	4.0	95	32	92	89	96	14	98
	Middle	96	99	00	87	4.0	96	71	94	15	96	13	96
	Upper	-	-	00	85	6.0	99	79	91	39	100	33	99
30°C	Lower	00	68	00	19	-	-	4	94	72	99	00	43
	Middle	64	82	1.0	44	-	-	00	82	00	67	1.0	68
	Upper	64	92	00	25	-	-	20	100	29	94	2.0	52

Results and Discussion

We have studied the effects of light of different spectral composition transmitted through cinemoid filters on N. tabacum seeds while ripening on parent plants. This study was thought to be useful from an ecological point of view, because it might be that photoperiod or light quality during seed maturation could have some effect on seeds and their subsequent germinability.

In all experiments where capsules were covered with deep red and blue boxes at various stages of seed development, light sensitivity of N. tabacum seed varied remarkably among seed lots matured on different plants. In all seeds light promoted germination at 20° and 24°C. At higher temperature (30°C) in some cases germination was reduced to nil both in dark and light. In a few plants dark germination was obtained at 20°C in seeds matured under deep red and deep blue box covers. Dark germination of seeds obtained from blue box treated capsule of plant No. 3 at 20°C was higher (54%; see Table XXXc) than those obtained from red covered - 25%, open - 22% and foil covered - 00% (see Table XXXa).

The seeds obtained from the capsules kept covered with aluminium foil soon after anthesis showed very little germination in dark in all plants except plant No. 4 (20% at 20°C; see Table XXXa). But it appeared that they showed a significant response (Table XXXc) of light promoted germination at all temperatures (particularly at 24° and 30°C) tested in comparison with the seeds obtained from open,

deep-red and deep blue (box treated) capsules (Tables XXXa and XXXb). It has been observed that red or blue cover treatments given to the capsules for only 9 - 18 days from the date of anthesis have some influence on dark germinability of seeds matured on plant No. 3 (Tables XXXa and XXXb). Seeds from all other plants and treatments showed little germination in dark. However, χ^2 tests (see Table XXXc) indicate that a significant difference in light and dark germination exists between the seeds of treated and open capsules.

Our observation with cinemoid filters have shown that mother plants in which leaves were covered with filters at the time of formation and maturation of seeds produced seeds of variable germination quality. Seeds matured on plants with green and blue filters showed considerably higher percentage of germination in darkness at 20°C in comparison with open control. Plants with red filters produced typical light sensitive seeds and very few seeds germinated in darkness at all temperatures tested. This variable degree of dark germinability between the seeds matured on plants with filter covers is not clear. It has been suggested that production of inhibitor(s) or promotor(s) by parent plants affects dormancy in seed (Morley, 1958; Morgan & Berrie, 1970). If leaves are regarded as the principal sites of synthesis of such inhibitor(s) or promotor(s) (Wareing, 1954) in that case leaves of tobacco plants covered with cinemoid filters transmitting different spectral zones could possibly be involved in controlling light and dark germinability of Nicotiana seeds.

Lona (Austin, 1972) and Karssen (1970) established that photo -

periods in which seeds are matured on the parent plants affected dormancy in Chenopodium. In our experiments with tobacco plants short day (8 hours) treatment was given to the mother plants only after full flowering and plants which flowered and fruited under 18 hours photoperiods throughout were treated as control (long day). It has been observed that all six plants under observation produced seeds in which light promoted germination. But three plants under longer photoperiods produced seeds with considerably higher dark germinability even at 24° and 30°C (Table XXXII). In all short day treated plants except plant No.1 (see Table XXXII) the seeds showed little germination in dark. It also appeared that long day seeds gave better response to light at 30°C. Dark germinability also varied among the seeds of lower, middle and upper capsules in treated plants (see Table XXXII).

During our studies it has been found that tobacco plants produced seeds with differences in degree of light sensitivity and dark germinability. We have demonstrated that seeds obtained from dark (foil covered), blue and red box treated capsules could show different degrees of light sensitivity even when they were produced on the same plant. In nature one can assume that colour or different levels of light around the developing seed could possibly play an important part in the induction of light sensitivity in typical photoblastic seeds.

A genetic heterogeneity in tobacco plants which is complex (Honing, 1930; Kasperbauer, 1968) may have some influence on our results. However, there is a possibility that we can manipulate

light controlled dormancy in N. tabacum by subjecting the mother plants to particular types of incident light at time of seed maturation.

Summary of Part VII

(1) *N. tabacum* capsules exposed under cinemoid filter covers at various stages of development produced seeds of variable germination quality and light sensitivity.

(2) Mother plants in which leaves were receiving filtered light of different spectral composition at time of maturation of seeds had some influence on light and dark germinability. The plants with red filters produced fat capsules and heavier seeds (see Table XXXI) with typical light sensitivity.

(3) Seeds matured on plants under short day (8 hours) treatments only after full flowering showed typical light dependency in comparison with that of seeds matured under long days (18 hours).

(4) It appeared that light controlled germination in *N. tabacum* seed could possibly be manipulated by exposing the mother plant at time of seed ripening to photoperiods and/or particular spectral bands.

General Discussion

In cultivated tobacco light sensitivity varies between races (Goodspeed, 1919; Johnson et al., 1930; Kasperbauer, 1968). Variation in germination as a result of cultural conditions has been ascribed to burying seeds to varying depths. Light and temperature effects on seed germination are not well understood. Light may be involved in establishing processes essential for seed germination, such as polysome formation in lettuce (Rosemary & Villiers, 1972) and inactivating inhibitors (Floris et al., 1972). Therefore, germination would not be likely to occur in the absence of light in positively photoblastic seeds.

During our preliminary survey of optimal temperature and light requirement in tobacco seed germination it has been found that in all seed samples light stimulated germination within a limited range of temperature above which it had no effect. Seeds obtained from some commercial sources showed greater tolerance to high temperature for their dark germination. The results agree with the known behaviour of many species in which temperature and light interact (Berrie, 1966; Wareing, 1969). It has been demonstrated that light requirement at higher temperature (35°C) in older seeds could be negated partially by treating the seeds with KNO_3 solution and low temperature (12°C) for a certain length of time before being transferred to 35°C for final germination. In typical light requiring seeds (Batch I) dark germination could not be obtained by single

temperature shift. A single temperature shift is known to interact with the form of phytochrome induced by red light (Taylorson & Hendricks, 1972). It is not clear how KNO_3, together with low temperature pre-treatment, could induce dark germination at higher temperature in N. tabacum cv. virginicum. It is stated that nitrates could replace after ripening of seeds which require stratification (Stokes, 1953, 1965). However, it has been observed that comparatively low temperature ($4^{\circ}C$) pre-treatment along with KNO_3 did not bring about any change in dark germination in seeds transferred to $35^{\circ}C$.

From our observation it appeared that light sensitive seed samples of N. tabacum responded differently to light at different temperatures with light requiring response decreasing with increased temperature and time of imbibition. It has also been observed that red and far-red light sensitivity of tobacco seeds (Batch I) changes with time of imbibition at $30^{\circ}C$. Small doses of red and far-red light were found promotive after 24 hours dark imbibition at $30^{\circ}C$, but at four hours treatments, far-red light appeared to be non-promotive. In freshly harvested seeds, however, true red, far-red reversible reaction could be obtained even after several days in darkness at $20^{\circ}C$. True reversible red, far-red reaction could depend upon germination conditions and seed samples.

Seed coats and endosperm and not the embryo itself, may be involved in seed dormancy. Although in most cases seed coverings do not prevent water uptake (Roberts, 1961; Barton et al., 1971) puncturing, cutting and scarification can improve germination in many

seeds. In our studies physical and chemical scarification failed to eliminate light requirement in typical light requiring tobacco seeds. The results were different from those of other workers in lettuce (Ikuma & Thimann, 1958) and wild oat (Cumming & Hay, 1958; Hart, 1966) where rupturing the seed coats affected light sensitivity.

Gibberellin and kinetin are known to stimulate germination in a number of seeds (Kahn, 1960; Frankland & Wareing, 1960; Roberts, 1963) but negative results with GA and kinetin have been reported on unscarified seed of some Rosaceae (Frankland, 1961) and in Luzula (Amen, 1967). Induction of dark germination in tobacco seed by chemicals is not well understood from the works of different authors (Hashimoto, 1958, 1961; Hashimoto & Yamaki, 1962; Takahashi et al., 1962). In our studies it appeared that in typical light dependent seeds dark germination cannot be induced by soaking the seeds with KNO_3, kinetin and thiourea which are known to stimulate dark germination in other species. Only cyanides and some of the chemical compounds reported in Part V could increase dark germination in older seed samples. Typical light requiring seeds (BG71) did not respond to the chemicals and light was found stimulatory both in their presence or absence. It has been observed that the optimal concentration of gibberellin for dark germination varied among the seed samples. Physically or chemically injured seeds germinated better in presence of low concentrations of GA_3. In a comparative study, a mixture of GA_{4+7} was found much more effective at low concentration in promoting dark germination than GA_3. Chemical changes within the seeds with

their ages could possibly be responsible for variable responses of tobacco seed lots to different chemicals.

Apart from genetical, environmental or physiological factors could play an important role during maturation of seeds (Barton, 1965) The environmental factors such as temperature and photoperiods around the mother plant have some influence on seed quality (Koller, 1962; Robertson et al., 1962; Grantlipp & Ballard, 1963; Karssen, 1970). We exposed the developing capsules of N. tabacum under filters transmitting light of different spectral composition at various stages of seed setting and ripening. The results and statistical analysis indicated that light sensitivity of seeds obtained from the capsules kept open or covered with aluminium foil was quite different from those matured under coloured filters (deep red and deep blue) on the same plant. From our observation it could be presumed that in nature all the capsules developing on the same plant may not receive the same quality light due to mutual shading. Furthermore there is a wide possibility that light quality transmitted through green leaves of neighbouring plants around the developing seeds or capsules may also influence seed quality.

The leaves are the organs which receive the photoperiodic stimulu and transmission of growth inhibitor from leaves to buds may be involved in dormancy in Betula pubescens (Eagles & Wareing, 1963). Karssen (1970) presumed that induction of dormancy by different photoperiods in Chenopodium was partly regulated by phytochrome and partly by photosynthetic activity of plants. During our observations

it appeared that plants in which leaves were covered with cinemoid filters and were receiving light of different spectral composition at time of seed setting produced seeds of different degree of light sensitivity and dormancy. The plants under deep red filter (590-680mμ produced seeds of greater light dependency for their germination than those under deep blue (420-530mμ) and green (460-590mμ) filters. Similar results were obtained with two batches of plants. Production of heavier seeds on plants with red filters could be due to increased photosynthetic activity. (See Plate 2)

Plants under short and long day treatments after full flowering produced seeds of variable light and dark germination quality. Seeds produced on long day plants showed greater light sensitivity and greater tolerance of high temperature for their germination. Germination studies in N. tabacum seeds collected from capsules of different stages of ripeness showed mixed light sensitivity and degree of dormancy. Some plants produced deeply dormant seeds. From our studies it is not clearly understood how seed from a single plant attain a varying degree of light sensitivity or dormancy. Very little is known about the time of the inception of light sensitivity in N. tabacum seed when attached to the mother plant.

From our studies it appeared that light controlled germination in N. tabacum seed, apart from genetics, could be determined by complex environmental factors around the mother plants.

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