CHAPTER II
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Seeds of many species are known to be photosensitive and very considerably in their response to light with respect to their normal germination. Some seeds have an absolute requirement for light for germination. In darkness these seeds show a very poor germination or some many not germinate at all. These are termed as positively photoblastic. In other seeds, exposure to light is inhibitory to germination, i.e. these are negatively photoblastic. Still in others germination is affected by an alternation of light and dark periods. Tobacco seeds are positively photoblastic seeds and show a marked differentiation in germination when placed in light as well as in darkness.

Many works have been done to study the role of light and dark in germination (Ewenari, 1951; Crocker, 1957 & others). The quantitative aspects of the subject have been developed since 1951. Promotion of seed germination by visible radiation of Acanthostachys was noted as early 1960 and inhibition of seed germination of Acanthostachys was reported in 1903. The quantitative effects of light on Ruscex seeds have been reported by Isikawa and Fujii (1961), who found reciprocity between intensity and duration of light exposure.

Flint and McAlister (1937) made a detailed study on the stimulation of germination of leltuce seed by light, and they found that red light was most effective in breaking dormancy. The effects of red (R) light and far-red (FR)
light on seed dormancy have since been found to be reversible (Borthwick et al., 1954). In this low energy reversible R/FR mechanism, the pigment 'phytochrome' participates. However, the studies on the molecular mechanism of phytochrome action in the seed germination have proceeded in very slowly, and at present there is no agreement about the mechanism of action of phytochrome in seed germination (Mohr, 1972).

In contrast to the stimulatory effects of light on germination, numerous species are only inhibited by light (Mayer and Poljakoff-Mayfer, 1963).

The location of the light-sensitive system in the seed is not well known; in lettuce, the removal of seed coat can relieve the light requirement (Svenari, 1957), which seems to imply that the pigment system resides in the seed coat. In Citrullus, the light inhibiting action is localized in the radicle and not in the cotyledon (Koller et al., 1963); which Nakata and Thimann (1959) investigated that the effective site for the light action is present in the cotyledon.

Although R/FR mechanism has been found to have been to operate in most light-sensitive seeds investigated, it is probably not the only mechanism involved. Svenari (1965) and Mohr (1960) reported those possible mechanism and other aspects of germination of photoblastic seeds.

A series of attempts have been made to provide the seeds a condition in dark which would replace light effect for their germination. Similar effect have been observed
for other seeds also. Thus seeds of Strigalutea, peaches, Gladiolus, various tree seeds such as Piceca, Larix as well as oaks have been shown by various investigators to be effe­cted by thiourea (Brown and Edwards, 1945, Tuckey and Carlson, 1945, Shiere, 1945, Thomson, 1945, Döblner, 1932 ).

Haber and Luippold (1950 ) showed that thiourea can also reverse the gamma ray induced dormancy in lettuce seeds. Thiourea can also overcome the high temperature inhibition caused by ceremarin on lettuce seed germination (Thompson and Horn 1944). Later more detailed work showed the stimulatory effect of thiourea in the dark at 26°C (Poljakoff-Mayber, 1952; Mayer and Poljakoff-Mayber 1963; Mayor and Zacks, 1958) at and at unspecified temperatures (Mayer, 1956).

Thompson and Kosar (1930) reported that the highest percentage of germination of Grand Rapids lettuce was recorded with 0.5% thiourca. These led the scientists to treat the seeds with different growth promoters and inhibitors on the germination, behaviour of different photoblastic seeds. A brief review of works done so far in different parts of the world on the problem are given in this chapter. Our review covers only those chemicals which are used in our experiments including their interactions.

**THIOUREA**

Thiourea, a sulphur-nitrogen compound, has long been known as a compound of marked biological activity.
In plants thiourea has long been known for its dormancy breaking action. This was described by Denny et al. (193) for potato tubers. Steinbauer (1939) envisaged that the rest period of Jerusalem artichokes was shortened by treatment with 5% thiourea. Later works extended this to a study of the dormancy breaking action of lettuce seeds (Thompson and Horn, 1941 and Thompson and Kosar, 1939). In conformity with these findings Mayer (1956) reported that the light requirement of light sensitive lettuce seed (Grand Rapids) can be replaced by thiourea. Similarly, Garman and Burton (1946a,b) reported that thiourea stimulates the germination of lettuce seeds. Amen (1968) found that gibberellic acid (GA₃), thiourea and KNO₃ promoted germination photoblastic seeds such as tobacco and lettuce. Poljakoff-Mayber et al. (1958) found that in dark, increase of thiourea concentration parallelly increases the lettuce seed germination. However, Mayer and Poljakoff-Mayber (1963) observed that prolonged period of treatment of seeds with high concentration of thiourea causes an apparent inhibition of germination, because the imirgence of root is prevented. Studies over a range of concentrations showed that both Urea and thiourea promoted Lettuce seed germination to some degree at some concentrations (Kefford, Zwar, and Bruce, 1956, Bruce, Zwar and Kefford, 1965) and these effects were missed previously by using too high or too low a concentration. A logical explanation of some
some of these effects has been made possible by the discovery of specific, sharply defined temperature limits to germination of many seeds (Thompson, 1970). Usually, there is a clear optimal period of treatment at a given concentration; prolongation of the period of treatment beyond this optimum causes a decrease in the stimulatory effect.

Thiourea breaks the secondary dormancy induced by high temperature. Thiourea may substitute for low temperature treatment in the promotion of after-ripening (Johnson, 1946) in many seeds. That thiourea also prevents thermodormancy at high temperature has been reported by Thompson and Hora (1944). However, it seems that this resistance is able to promote germination only in relatively dormant seeds and not in those which are truly dormant (Vegis, 1964).

Mayer (1966) has shown that thiourea is not metabolised in lettuce seeds during germination despite its stimulatory action. Thiourea enters the seeds at almost the same rate as water, considerable amounts taken up by 72 hours old seedings. Germination is stimulated over a wide range, 0.14-1.66x10^-2.

Negbi et al. (1968) coded that germination of Grand Rapids lettuce seeds, promoted by redlight, GA3 or thiourea depends upon the occurrence of certain processes which proceed in darkness independently of any of the factors. Sarma and Gohain (1972) reported increased percentage of germination
of lettuce seeds in the dark which was enhanced by light. Sarma and Chakraborty (1975) reported that thiourea promoted germination of tobacco seeds (CV. Motihari) to the extent of 84% in light as against 66% in dark.

POTASSIUM NITRATE (KNO$_3$)

Potassium nitrate, an inorganic nitrogen compound, is known as the germination stimulating compound since the work of Lehmann (1909) and Grassner (1915 a, b). It was established from the findings of these workers that nitrate especially KNO$_3$, and related compounds replace the light requirement in many positively photoblastic seeds, i.e. bring about germination in dark (Svenari, 1956; Toole et al., 1956).

An extensive study of nitrate effect on germination of Lepidium Virganicum at a temperature of 20°C there was no germination at all in darkness, but irradiation R brought about 30% germination. But when irradiated with R in the presence of KNO$_3$, full germination was obtained. Thus, an interaction between photomechanism and KNO$_3$ in germination was established.

Maier (1933) had reported for Poa (grass) that nitrate increases the light sensitivity and decreases the light requirement. As on the other hand, KNO$_3$ brings about germination in darkness, the block to germination can be
removed without operating the photomechanism. In their own way Qaasner (1915 a-b) and Leggatt (1946) had also come to this conclusion.

Grace (1940) conducted experiments on *Marquis* wheat using KNO$_3$ and reported that this chemical have stimulatory activity on germination and could accelerate the phenomenon. Hay (1958) found that KNO$_3$ breaks both natural and induced dormancy. Potassium nitrate was reported to improve delayed germination of Vieland Oats (Schwendimann and Shands, 1943) and to enhance the stimulation of germination of dormant hard seeds (Frankland, 1961).

Ogawara and Ono (1961) studied the effect of KNO$_3$ on the germination of light sensitive tobacco seeds. They found that combination of KNO$_3$ with gibberellic acid or Kinetin was synergistic in light. Takahashi (1961) showed that KNO$_3$, sulphur chloride, etc. enhance the stimulatory effect on germination of various seeds.

Nutile (1945) found that if the substratum was moistened with 0.3% KNO$_3$ solution after being removed from the dark and then tested in light, all seeds germinated. Similar results obtained if the seeds were prechilled 3 days at $4^\circ$C before testing in light. If KNO$_3$ is added to the medium in dark and tests extended several days in darkness, the ungerminated seeds still failed to germinate. KNO$_3$ is uneffective in breaking dormancy in dark and only does so in the presence of light (Nutile, 1945).
Mayer and Poljakoff-Mayber (1963) who applied Knop's solution observed that the chemical responsible for promoting germination in Knop's solution was $\text{KNO}_3$. This was in conformity with Hesse (1924) who recorded stimulation of germination in *Veronica longifolia*, many species of Hypericum and *Asplenium* etc. by $\text{KNO}_3$. Since then its stimulatory effect in germination has been established particularly during after-ripening (Stebnauer and Frank, 1954) and in light-sensitive seeds (Stebnauer and Grigsby 1957).

Mayer and Poljakoff-Mayber (1963) found that as with light, $\text{KNO}_3$ stimulation exhibits interaction with temperature. The germination of *Eragrostis curvula* was stimulated between 15 - 30°C in dark by 0.2% $\text{KNO}_3$ solution. At high temperature there is no effect. In contrast Tools (1938) recorded promotion of germination of *Polygonum* only at alternating temperature.

Further experiments were conducted which prove that "Black seeded simpson" lettuce seeds made light-sensitive by coumarin responded to prechilling, and effect of thiourea, may then went into deeper dormancy which could be overcome by the action of $\text{KNO}_3$ on soil by the presence of light. This is somewhat similar to naturally dormant Grand Rapids lettuce seed.

An interesting interaction has also been reported between light and $\text{KNO}_3$ by Sarma and Barman (1973). They reported that $\text{KNO}_3$ stimulated as high as 80% germination.
in light as against 12% in dark when seeds were treated with the same concentration of $\text{KNO}_3$ (250 ppm).

GIBBERELLIC ACID ($\text{GA}_3$)

Gibberellic acid ($\text{GA}_3$), a naturally occurring plant growth promoting substance, has long been known for its stimulatory effect on germination particularly of positively photoblastic seeds placed in dark. Lana (1956) and Kohn et al. (1956) 1957 were among the first to demonstrate that Gibberellic acid ($\text{GA}_3$) is a potent germination stimulator of *Lactuca scariola* and *Lactuca Sativa* in the dark. Evenari (1949), Lana (1956), Kahn et al. (1956, 1957) and Hashimoto (1958) reported that $\text{GA}_3$ substitutes for light in the promotion of germination of photoblastic seeds. The effects of red-light (R) and Gibberellic on germination are found to be additive when R is given a few hours after the start of imbibition in germination (Evenari et al. 1958; Kahn, 1960 b). When seeds are exposed to R for 20 hours or more after imbibition, the effect may be greater than additive, pointing that gibberelin either increases the R sensitivity or prevents it from decreasing with long duration of imbibition Kahn, 1960 b). Short FR (flared) fails to prevent promotion caused by gibberelin or has only negligible effect (Kahn et al. 1957, Kahn, 1960 b; Evenari et al.; 1958, V.K.Toole and Cathey, 1959; Jkuma and Thimann, 1960). Prolonged irradiation by R FR delays the gibberelin effect but doesnot prevent it permanently (Kahn, 1960 b).
Desensitization of the seeds to gibberellin treatment is reported to be caused by high doses of MR (Ikuma and Thimann, 1960) in negatively photoblastic seeds of *Phacelia tenacetifolia*. Gibberellic acid counteracts the inhibitor effect of blue, red and even white light, but not that of FR (Bollin, 1959).

In lettuce achenes also, the application of gibberelins have been found to stimulate germination in dark (Kahn, Gross and Smith, 1957), but considerable variations have been reported in the response of different varieties of lettuce (Skinner et al., 1961) and in the activity of different varieties of gibberelins (Toole and Cathey, 1961; Brian et al., 1962). Application of gibberelins have little effect in reversing high temperature inhibition of the germination of lettuce achenes exposed to light (Babor and Luippold, 1960 b; Kahn, 1960 b; Reynolds and Thompson, 1971).

Gibberelins not only promote the dark germination in lettuce seeds, but also have been found to prevent the development of thermodormancy when applied in high temperature treatment, and of dark osmotic inhibition when used either simultaneously with the inhibiting substance or as pretreatment (Kahn et al., 1956, 1957, Kahn, 1960b, Poljakoff-Mayber et al., 1958a, Evenari et al., 1958; V.K.Toole and Lathey, 1959).
Gibberelin and heat treatment has long been known to counteract each other and that the counterbalancing effect of gibberelin decreases with longer heat treatment. Poljakoff-Mayber et al. (1966), Evenari (1957 B) established that the combined effects of R in breaking the not yet fully established thermodynamic, and of gibberellin are slightly greater than additive. Gibberellin also prevents (or reverses) the inhibition of germination caused by coumarin (Mayer, 1959).

Thus, gibberellin not only allows the germination to bypass the light requirement but also overcomes some of the temperature blocks to germination. Black and Naylor (1959) reported that applied gibberellin prevented the onset of dormancy during maturation of seeds.

Stimulation of germination by \( \text{GA}_3 \) has also been found in many of the photoblastic seeds as well as in many light indifferent seeds (Nagao et al., 1959; Fujii and Ishikawa, 1961; Fujii and Ishikawa and Nagakawa, 1960; but it is not a universal feature (Leisorovitz and Poljakoff-Mayber, 1954, Nekrasova, 1960).

As in lettuce seeds, Toole and Galy (1959) reported identical effect of gibberellin on the germination of *Lepidium Virginicum*. The positively photoblastic seeds of *Sedum Kamtschaticum* germinate in dark after a short application of gibberellin high concentration (200 - 1000 ppm). But continuous contact with the compound at a concentration of 100 ppm inhibits germination.
The stimulatory effect of gibberellin on the germination was substantiated by Phinney and West (1960). They extracted gibberellin like substances from seeds of Phaseolus, lettuce and many other seeds and found that they either failed to germinate or the percentage of germination was very low. In the negatively photoblastic seeds of Phaseolus tanacetifolia, \( \text{GA}_3 \) counteracts the inhibitory effect of blue, red and even white light, but not that of FR (Rollin, 1959). FR does not reverse the stimulation of germination produced by gibberellin and the time curves of sensitivity to gibberellin and FR are parallel (Fujii et al., 1960). Percentage of germination of the non-stratified seeds of Douglas fir was enhanced by the treatment with gibberellin both in light and dark but the final percentage of germination was not effected (Richardson, 1959). Stratified and non-stratified seeds of Baldy carpus (Biswas et al., 1972) are also stimulated by gibberellin. Dark germination in a strain of Arabidopsis which has an absolute light requirement for germination is greatly stimulated by gibberellin (Kribben, 1957). In the seeds of Carnegiea gigantea which do not germinate in darkness (only 0.16%) gibberellin alone has only a slight effect (only 9% germination), whereas gibberellin and FR promote up to 42% (FR alone about 23%) (Alcorn and Kurtz, 1952).

In the positively photoblastic seeds of tobacco, the dark germination is found to be stimulated by gibberellin alone. But gibberellin in conjunction with potassium or
ammonium nitrate stimulates dark germination of tobacco seeds considerably more although both of the nitrogenous compounds are nearly ineffective (Ogawara and Ona, 1947; Hashimoto, 1958). Of the four gibberellins (GA - GA₄) tested, GA₃ was the most, GA₂ was the least effective (Hashimoto and Yamaki, 1960). Germination of tobacco seeds has been even suggested as a bioassay for GA₃ (Ogawara and Ona, 1957).

Frankland (1961) tested GA₃, Kinetin, KNO₃ and thiourea on germination of dormant hazel seeds. He observed that GA₃ soaked embryos resulted in the highest percentage germination, followed by Kinetin, thiourea and KNO₃ solution. Reports are available on the effect of gibberellic Acid (GA₃) to show that it promotes the germination of seeds of several species (Baskin and Baskin, 1971), and it substituted for the after ripening requirements (Miller, 1958).

A very interesting effect of gibberellin was reported by Bunsow and V. Bredow (1958 a, b) in Kalanchoe blossfeldiana. They observed that gibberellin did not promote germination in dark but brought about a decrease in the critical day length necessary for full germination and increased the germination percentage in continuous light. Light and gibberellin interacted to produce an additive effect. A similar case was reported by Nagao et al. (1959) for the long day germinator Begonia evansiana, where
gibberellin reduced the critical day length from 12 - 24 hours to as little as 1 minute.

Thus, a number of reports are available to show that Gibberellin Acid promotes the germination of seeds. It is found to have removed the dormancy of various kinds of seeds and buds (Frankland, 1961; Paleg, 1965; Lang, 1970). Amen (1968), Baskin and Baskin (1971) and Juntilla (1969; 1970 a, b, 1972) have reported the stimulatory effect of Gibberelin. That GA₃ can substitute for light in promoting germination of positively photoblastic seeds has also been substantiated by the findings of Toole and Cathey (1959), Skinner et al. (1958), Haber and Luippold (1960), Webb and Dumbroff (1969), Thompson (1969), Wareing and Saunders (1971), Sarma and Barman (1973), and Sarma and Chakraborty (1975).

Webb and Dumbroff (1969), Morris (1968), Naylor and Simpson (1961), Goo and Tusihasl (1958), Gray (1958) showed that by applying gibberelin to some freshly harvested seeds, or seeds requiring dry storage or chilling could be made to germinate.

The far-red (FR) inhibition of lettuce seed (var. Grand Rapids) germination has been eliminated by GA₃ and thiourea (Negbi et al. 1968). A number of species of plants (e.g. Avena Fatsia, Sinapis arvensis, Gentiana nivalis, etc.) whose germination is not affected by light have also been shown to be promoted by GA₃ (Kallio and Piironen, 1959; Corns, 1960).
Another growth substance which has emerged prominently in recent years is kinetin. A number of investigators have made it clear that kinetin has a stimulatory effect in the germination of seeds. Thompson and Kočař (1939) reported the stimulation of lettuce seed germination by some sulphur compounds as well as by kinetin.

Miller (1956) after making an extensive study on lettuce seed germination concluded that kinetin can replace the requirements for red light when treated in darkness. Sverni et al. (1958) also corroborated that kinetin replaces the requirements for light in positively photoblastic seeds. Weiss (1960) reported that at 20°C kinetin was ineffective in darkness. Miller (1956) and Skinner et al. (1956, 1957) demonstrated that kinetin and a number of other substituted amino- and thio-purines promoted germination in the dark at a temperature between 25 - 30°C and in light at a temperature above 30°C. But later on, Miller (1958) envisaged that kinetin has no effect on lettuce seed germination in complete darkness. At least a short pinch of light is necessary for the stimulatory action to take place. This has also been substantiated by Ikuma and Thimann (1960, 1963) by treating seeds with mixtures of gibberellin and kinetin.

Bewley, Nagbi and Black (1968) reported that very short preliminary light treatments during imbibition prepared the fruit for subsequent stimulations by low concentrations of kinetin.
In contrast, Haber and Tolbert (1950) were of the opinion that Kinetin only stimulates germination in darkness and presented no interaction with light.

Haber and Tolbert (1959), Haber and Luippold (1960) Skinner and shieve (1959) and Smith et al (1968) reported that the effects of Kinetin on the stimulation of germination are highly temperature dependent. Reynolds and Thompson (1971) using continuous temperature gradients have shown that the main effect of application of Kinetin is to raise the level at which temperature becomes inhibitory to germination.

Hoque and Croix (1970) noted that Kinetin at 50 mg/l caused rapid and complete germination of excised Russian alive seeds while GA and Thiowrea were without any effect.

Reynolds and Thompson (1971) reported that lettuce seed germination is characterised by inhibition at high temperatures which can be clearly defined for any particular variety (Thompson,1973). Kinetin (0.1mg/l) 10 mg/l) strongly promoted germination at temperature above 27°C in continuous light or after short periods of illumination during the early stages of inhibition. In total darkness, however, Kinetin treatment resulted in only minor promotive effect (Reynold and Thompson, 1973).

The promotive effects of light exposure at the start of imbibition were first reported by Evenari and Neumann (1953) and were confirmed by Reynolds and Thompson (1973) who also showed that addition of Kinetin greatly enhanced the promotion.
Pre-soaking in several varieties of lettuce seeds in various 6-(Substituted) amino- and Thio-purines has been found to increase their rate of germination (Miller 1956, Kinner, Claybrook, 1957, Skinner, Claybrook, Tolbert and Shive, 1958). Recently it has been also reported that these purines has been also reported that these purine derivatives are synergistic with gibberellin in inducing these biological responses whereas gibberellin alone was moderately active in stimulating germination. The mode of biochemical action of these purine derivatives and gibberellins (Kahn, Goss, 1953) in affecting the rate of germination is not yet fully understood although it has been observed that seed activation of white light is augmented by pretreatment of the seed with various 6-(Substituted) purine solutions (Skinner, Claybrook, Tolbert and Shive, 1957).

Miller (1958) demonstrated that the maximal purine activation is somewhat dependent on the presence of light. The action spectrum for the influence of light on germination is not necessarily the same in the presence of 6-(Substituted) purines, as in the absence (Borthwick, Hendericks, Parker, Toole, V.K. Toole, Vivian, 1958); far-red light on germination of seeds is said to be initiated after pretreatment with kinetin, even though the far-red light is less effective than red light.

Skinner and Shive (1959) studied the minimum contact time required for effective activation as initiated by pre-soaking lettuce seed in 6-(substituted) Benzalmine, or
a mixture (synergistic) of benzalazine-purine and gibberellin. The effect of the presence or absence of light on the rate of germination of pre-treated lettuce seeds was alone determined.

The inhibition of lettuce seed germination by several naturally occurring growth inhibitors is reversed by Kinetin and red light (Khan and Träbert, 1956b, Khan 1967, 1968, 1969). Kinetin and red light also breaks the dormancy in Xanthium seed, presumably by counteracting the inhibitor present in the red (Khan, 1966, Wasing and Foda, 1957).

The effect of Kinetin in breaking the dormancy of seeds is probably only another aspect of their stimulatory effect on cell enlargement, as indicated by the work of Haber and Luippold (1969b) on gamma irradiated lettuce seeds. Mitosis was delayed more than cell expansion, and it was possible to show that Kinetin induced germination preceded mitotic activity and was apparently the result of root, cell enlargement. The latter point is in despite, however, as Ikuma and Thimann (1968) believe that the site of Cytokinins action on seed germination is the cotyledon.

Khan (1968) showed that lettuce seeds are relatively insensitive to cytokinin alone, but when the seed has been inhibited by ABA, Cytokinin application results in marked enhancement of germination. Khan (1971) postulated that breaking of dormancy by cytokinin is restricted to relatively few species of plants and then only under some conditions.
COUMARIN

The design of regulating systems might reasonably include not only means by which growth can be stimulated but also means by which growth can be restrained. Hormonal systems which suppress growth include auxins at high concentrations, and ethylene, there are instances too when gibberellins and cytokinins suppress growth. The plant has at hand a wide range of secondary plant substances, which accumulate in the plant and have no apparent role in the metabolic sequence. In a wide range of growth phenomena, these secondary plant chemicals serve as inhibitors in the plant. Among the secondary plant chemicals that act as inhibitors are phenolic acids, lactones and related flaviniones. Coumarin is one of much unsaturated lactones which inhibits physiological functions. The "Blastocholine" (germination inhibitor) properties of coumarin were first demonstrated by Schreiner, Reed and Skimov (1909).

The interest in coumarin as the inhibitor in the growth of plants was focussed due partly to its apparently universal occurrence in the plant world and partly to its physiological properties. The views expressed by different workers on the possible mechanism of actions of these inhibitors are rather conflicting (Torrey, 1956). Its selective phytoecidal properties had been studied by Audus and Quarstel (1947) and Goodwin and Taves (1950). However, Thimann and Bonner (1949) reported stimulatory effect of coumarin on germination and a synergistic effect of coumarin and IAA on the growth of oat coleoptile sections.
The inhibition of germination of seeds in a number of plant species has been reported Mayer and Evenari (1953) and Misra and Patnaik (1959). It has become evident from these studies that the inhibitory concentration is different for different species and even differs in different varieties of the same species. Because of its wide spread distribution in plants and due to its strong inhibitory action, coumarin is considered to be one of the substances which may function as a natural germination inhibitor. This has been frequently itself in seeds at inhibitory concentrations has been proved only in few instances. Lerner et al. (1959) could prove the existence of coumarin in such inhibitory level in the seeds of Trigonella arabica.

Coumarin and its derivatives should probably be thought as inhibitor rather than as toxic substances. The effect of growth of roots have been shown to be reversed by coumarin (Audus, 1948). It also had the same effect on seed germination (Nutile, 1945).

The inhibition caused by coumarin on germination and the elimination of induced dormancy by other chemical compounds and light have been reported (Mayer, 1959; Khan, 1967, 1968, 1969; Khan and Tolbert 1965 a,b; 1966 and Berrie et al. 1963).

Mayer (1958) studied the action of ascorbic and oxidase in germinating lettuce seed and its inhibitor. The germination stimulating Thiourea and germination inhibiting coumarin affected the ascorbic acid oxidase present in the germinating seeds.
Mayor and Poljakoff-Mayber (1963) reported coumarin as an inhibitor of seed germination. Yet at low concentrations coumarin also has a stimulatory effect on germination (Neumann 1959, Mayer and Poljakoff-Mayber, 1961).

Khan and Tolbert (1965a) reported that coumarin and other germination inhibitors participate in a photo-reversible phytochrome system during lettuce seed germination. Evenari (1965) reported that non-photoblastic seeds were rendered photoblastic by coumarin. Nutile (1943) showed that coumarin induces dormancy in non-dormant lettuce seeds. Other experiments carried out by Nutile (1945) on "Grand Rapids" lettuce seed and "Black seeded simpson" had shown that non-dormant seeds could be made dormant by coumarin treatment. This imposed dormancy could be overcome by exposing seeds to high humidity and light and then again be thrown back into dormant state by coumarin.

Having once established the fact that this inhibiting property of coumarin can also induce dormancy in non-dormant lettuce seeds, it was thought desirable to see if coumarin treated seed responded in germination, to various conditions in a manner of naturally dormant lettuce seed (Nutile, 1945). Nutile in 1943/44 also showed that in lettuce seed light can counteract the strong inhibitory action of coumarin on germination. Klein (1956) and Evenari (1957b) also demonstrated the distinct interaction between coumarin and light.

According to Goodwin and Taves (1950), the substitution at various position on the coumarin nucleus may
effect the physiological activity of the molecule by altering its rate of penetration through the membranes, its solubility, its activity with specific substances etc. The fact that many of the compounds treated induce inhibitions within the first 3-4 hours after application indicates that these compounds readily penetrate.

Yegis (1964) reported that by supplying inhibiting substances like coumarin, which naturally occur in plants, to non-resting potato tubers, it is possible to induce dormancy. Coumarin has been widely used for germination studies as an inhibitor. It is able to induce light sensitivity in many varieties of lettuce seeds which are not photoplastic. This indicates decreased growth activity.

Coumarin or other naturally occurring growth inhibitors alone or in combination with other growth substances may regulate germination and growth. L.H. Toole, V.K. Toole, Borthwick and Hendericks (1955a) reported for Lepidium virginicum that coumarin in low concentration in conjunction with red light (R) promoted germination above that of R controls in water, slightly higher concentrations were known to be inhibitory to germination.

Toole, Hendricks, Borthwick and Yode (1957) held the view that coumarin and other compounds of δ-unsaturated lactone type act as specific poison of the reaction associated with controlling reversible photoreaction.
Klein (1956) held the view that the effect of coumarin on lettuce seed is not altered by red light (R). R only raises the percentage of germination of positively photoblastic seeds, it accelerates the rate of germination as well. This view, however, is not inconformity with Nutile (1945) who demonstrated the elimination of coumarin effect by exposure to light.

Nutile (1943, 1914, 1946), Evenari (1952, 1957) reported that the aphotoblastic seed of lettuce (Var. "Profress") become photoblastic when treated with coumarin.

Mayer (1953) came to conclusion that any explanation of coumarin action on germination has to take into account the degree of penetration of the compound into the seed, and the fact that coumarin can be destroyed enzymatically by the seeds.

Evenari (1952) observed that the combined effect of R and coumarin is temperature dependent. He observed coumarin inhibition of germination of lettuce seeds Var. "Grand Rapids" in the dark completely (at 14°C, 80mg/l). But in the light the germination percentage was 86. At 30°C 10mg/l were enough to reduce germination to zero in darkness, whereas 7% still germinated in light.

Coumarin, thus, can be considered as growth inhibiting substance which is confirmed with regard to the effect of coumarin on cell division in most cell layers in roots (Sevensson, 1971, 1972).
CCC is recognised as a plant growth retardant. The term growth retardant is used for a diverse group of chemicals which reduce stem elongation without causing malformation (Cathey, 1964). These chemicals exhibit adverse effects on cell elongation and cell division stem tissues and regulate plant height. These substances were reviewed by Cathey (1964) and later on by Lang (1970). All the retardants now known are synthetic compounds, although the definition does not exclude naturally occurring ones. The group includes several compounds which are well recognised gibberellin antagonists.

Tolbert in 1960, reported a group of quarternary ammonium compounds of which chloro-choline-chloride, abbreviated to CCC (2-chloro-ethyl) trimethyl ammonium chloride is one of most active growth retardants.

Light sensitive lettuce seeds can be stimulated to germinate in the dark if treated with GA3 (Ikuma and Thimann, 1960; Lenna, 1956). Similarly Kinetin (Miller, 1956) is effective in promoting germination of these seeds. It can be argued that exogenously applied growth regulators supply the requirement that is necessary and normally met with when the synthesis of these compounds triggered by exposure to light. Any treatment of lettuce seed which is induced by exposure to light to germinate, by compounds thought to prevent the synthesis of gibberellin, or Kinetin, should prevent germination.
The dwarfing compounds CCC (Chlornequat), B-995 and phosfon D, Phosfons, are thought to prevent the synthesis of gibberellin (Baldev and Lang; Lang and agatep, 1965; Denais et al, 1965; Harada and Lang, 1965; Ryugs and Sachs, 1969) and it is proposed that their action on the whole plant is due to this. It would be expected that it would not respond to light, though it should germinate if given an exogenous supply of gibberellins.

Among a number of growth retardants that have been reported, CCC is well known as the dwarfing compound based on the study of their effect on growth, development and metabolism of plants; Wittwer and Tolbert, 1960a,b; Lockhart, 1962; Paleg et al 1965; Halevy, 1967; Tongnoi et al 1965.

Wittwer and Tolbert (1960) applied chlornequant (CCC) to grand Rapids lettuce seed and observed a marked reduction in germination percentage and that this reduction was prevented either by irradiating the seeds with red light or by applying gibberellin. That the red light was effective in promoting the germination of treatment lettuce seed suggested that either gibberellin synthesis is not inhibited by red light treatment or that chlornequate is not effective in preventing \( \text{GA}_3 \) synthesis in this system.

Michniewez and Lamparska (1965) have investigated the effect of CCC on vitamin C metabolism of bean plants. Gibberellic acid induced sugar release in barley endosperm is not inhibited by growth retardants (Paleg et al, 1965). Khan and Faust (1967) have shown that CCC inhibited \( \alpha \)-amylase production.
of germinating barley seed. The work of El-Fouly (1966) shows that amylase activity is significantly increased by CCC treatment during the development of wheat plants.

Saxena et al (1968) have shown that CCC did not behave as growth retardants when used in low concentrations. Germination of peanut and wheat was not inhibited at 20 ppm. On the other hand in pea nut it was inhibited by 2,000 ppm CCC (Research Progress Report, No. VIII). Experiments done with barley endosperm Paleg (1965) showed that Cycocel did not interfere with gibberellic acid action. Thus Cycocel would be an inhibitor of gibberellin synthesis, but not of gibberellin action. The growth retardants were not analogous to any known growth substance (Talbert, 1961), but they apparently gave a competitive interaction which is distinguishable from independent effects with the naturally occurring growth substances. Lockhart (1968) considered growth retardants as anti-metabolites rather than anti-gibberellin or anti-auxin.

Stuart and Cathay (1961) first demonstrated that application of growth retardants phosphon D, B995 and CCC caused suppression of vegetative growth and prompt initiation of flower buds in Rhododendron. Kim (1968) reported that CCC inhibited the growth of Chlorella.

Kende et al (1966) showed that AMO 1918 and CCC inhibited the biosynthesis of gibberellin in the fungus Fusarium moniliforme and suggested that gibberellin synthesized in higher plants and in the fungus followed similar pathways. From various sources it has been concluded that
that the action of growth retardants on plants is caused by their inhibitory effect on biosynthesis of endogenous gibberellin.

Though CCC has been reported to inhibit gibberellin biosynthesis (Ninnemann, Zeccaart, Kende and Lang, 1964; Harada and Lang, 1965; Zeccaart, Kende, 1966; Zeccaart, 1966), certain effects of this compound are not completely explained by this phenomenon (Kurish and Muir, 1963; Sachs and Wohlers, 1964; Cleland, 1965; Carlisle, Eilis and Mc Veigh, 1968; Cleland and Briggs, 1969; Berry and Smith, 1970).

Pharies et al. (1967) found that the effects of growth retardants could be prevented or at least decreased by simultaneous application of GA. This is consistent with the hypothesis that growth retardants act through inhibition of gibberellin biosynthesis (Cathey, 1964; Lang, 1970).

Ross and Bradbeer (1971a,b) working with hazel seeds have shown that stratified seed synthesize GA and that this can be prevented by treatment with dwarfing compounds. Cleland and Zeccaart (1970) and Zeccaart (1966) have shown that growth of retardants can reduce the level of endogenous GA₃ in higher plants.

Ounberg and Eliasson (1972) envisaged the counteracting effects of growth retardants and GA. The application of GA could restore to some extent normal growth of Norway spruce seedling treated with dwarfing compounds CCC, and AMO 1613 (4 hydroxyl-5 isopropyl-2 methyl-phenyl trimethyl ammonium chloride,1-piperidine carboxylate ).
Recent investigations have added much to our knowledge of the action of CCC on gibberellin biosynthesis or counteracting effects of both the compounds. That CCC induces inhibition of gibberellin biosynthesis was reported by several workers (Lang, 1970; Baldev et al. 1965; Ross and Bradbear, 1971a,b; Dennis et al. 1965; Harada and Lang, 1965; Ryugo and Saches, 1969; Cathey, 1964). On the contrary, some investigators reported that gibberellin content either increased as a result of CCC application (Reid and Crosier, 1970; Bristow and Simmonds, 1968) or there was no change in gibberellin content (Fontes et al., 1970).

Berrle and Robertson (1973) suggested that growth retardants may not act primarily on the synthesis of GA$_3$, though it may be that its effect is equal to the reduction of GA level. However, there are reports that GA levels may also be increased, or that the nature of GA's produced is altered when plants are treated with growth retardants (Dennis et al. 1968; Reid and Cary, 1967; Reid and Crosier, 1970).

Berrle and Robertson (1973), Wittwer and Tolbert (1980) and Knypl (1967a) observed inhibition of germination of lettuce seeds and Kale seeds by CCC and other related dwarfing compounds. This inhibition was prevented either by irradiating seeds with red light or by applying gibberellin and Kinetin.
Thiourea has long been known for its dormancy breaking action. The study of the interaction of thiourea with other compounds has brought to light new aspects including its synergistic or antagonists effects on germination.

Both gibberellin acid and thiourea promote the germination of lettuce and tobacco seeds, so their interaction is expected to enhance their individual efforts.

Thiourea proved to be a potent germination stimulating compound. This apparently affects germination through various metabolic paths as well as through its possible interaction with or effect on growth stimulator in the seeds. This has been substantiated by several workers investigating the interaction of thiourea with other compounds (Mayer and Poljakoff-Mayber, 1968; Mayer et al., 1958a, b; Poljakoff-Mayber Mayer and Zacks, 1958a, b; 1959).

Four concentrations of GA₃ (25, 50, 100 and 150 ppm) and three concentrations of thiourea (2, 500, 5,000, and 10,000 ppm) were tested for their influence on the germination of peach seeds by Singh et al. (1966). These treatments were given in combination with three chilling treatments of 4°, 8° and 10°C and four stratification period of 7, 14, 21 and 28 days. GA₃ at 50 ppm resulted in higher percentage germination of peach
seeds as compared with its other concentrations. The medium level (5,000 ppm) of thiourea proved to be more effective than either of the lower (2,500 ppm) or the higher level (10,000 ppm) in increasing the germination percentage of peach seeds. Combination of both the compounds resulted in still higher percentage of germination.

ii) \( \text{GA}_3 \) and \( \text{KNO}_3 \):

Nitrates have long been known as powerful agents in germination particularly during after ripening (Steinbauer, 1954) and in light-sensitive seeds (Steinbauer, 1957).

Nitrates act synergistically with gibberellic acid (Hashimoto, 1958) and with Kinetin in inducing germination.

Most frequently gibberellic acid stimulates the dark germination of light sensitive seeds, especially in the presence of \( \text{KNO}_3 \). Germination of tobacco seeds has been suggested as a bioassay for gibberellic acid (Koller, Mayer, Poljakoff-Mayber and Klein, 1962).

That \( \text{GA}_3 \) stimulates the dark germination of photoblastic seeds in combination with \( \text{KNO}_3 \) was substantiated by Mayer and Poljakoff-Mayber (1963).

iii) \( \text{GA}_3 \) and Kinetin:

Both gibberellic acid (\( \text{GA}_3 \)) and Kinetin are reported to be potent germination stimulators of photoblastic
seeds. They act synergistically with light in promoting germination and also bring about higher percentage (over control) of germination in dark, break dormancy and reverse effects of cycocel etc.

Interaction between the effects of Kinetin, gibberellins were found by Skinner et al. (1958), Skinner and Shieve (1958) and Skinner et al. (1961), and the inhibition induced by ABA may be reversed by application of kinetin (Aspinal et al., 1967; Khan 1967; Sankhla and Sankhla 1968; Reynolds and Thompson, 1971).

Skinner and Shieve (1958) reported that the rate of lettuce seed (cv. Early curved simpson) presoaked in a mixture of one of the many 6-(Substituted) aminopurine derivatives and gibberellin was seen to be synergistically stimulated under both light and dark.

The combined effect of \( \text{GA}_3 \) and Kinetin was found to be synergistic in promoting tobacco seed germination in light and dark (Ogawara, 1961).

The promotive effects of kinetin on lettuce seed germination at higher temperature was due to the raising of the upper temperature limit (cut off point) to a higher value (Reynolds and Thompson, 1971).

GA in conjunction with kinetin can reverse ABA inhibition of germination in lettuce seeds and excised embryos of non-dormant Fraxinus. These and similar observations
have led to the emergence of the hypothesis that in some seeds, GA is the primary stimulus of germination. ABA blocks the GA-mediated germination response and the role of the cytokinins is to antagonize the action of the inhibitor, thereby allowing GA to function (Khan, 1971; Khan and Hett, 1969).

iv) GA$_3$ and Coumarin:

Brian (1958) suggested that gibberellin acid might alleviate the inhibitory action of coumarin. Kato (1958) reported that the inhibition caused by coumarin on pea stem sections was reduced by gibberellin.

Phillips (1961, 1962) examined the possibility that dormancy and growth are controlled, in part, by an interaction between endogenous inhibitor and gibberellin acid. He demonstrated that the antagonistic effect of two naturally occurring inhibitors, coumarin and narigenin, upon dormancy breaking or growth stimulation by gibberellin acid. Mayer (1959, Phillips (1961) first demonstrated that narigenin is able to induce a light requirement for germination of "Great lakes" lettuce seeds. This effect of narigenin, like a similar effect of coumarin induced interaction could not be reversed by treatment with gibberellin acid.

Gover and Tomer (1971) worked on the effects of sesselin and coumarin on growth, with special reference to 

mam cucumber radicle. Their work resulted in the inhibition of radicle growth by coumarin.
Svensson (1978) suggested that coumarin induced interaction could not be counteracted by IAA, CO₂, Kinetin, gibberellic acid and cycocel.

v) GA₃ and CCC:

The action of gibberellins and growth retardants in altering the growth of plants are mutually antagonistic. But, there is no apparent structural similarity between CCC and gibberellin antagonists. These substances are not analogous of any known naturally occurring substance (Tolbert, 1961), but they show competitive interaction with naturally occurring growth substances (Lockhart, 1961).

Wittwer and Tolbert (1960) and Cthey and Stuart (1961) reported that CCC reduces internode length and therefore decreases plant height and also that the primary effect of CCC is an inhibition of cell division (Zeevaart 1966), Gibberelin on the other hand promotes cell growth.

Pharis et al (1967) found that the effects of growth retardants could be prevented or at least decreased by simultaneous application of GA. This is in consistency with the hypothesis that growth retardants act through inhibition of gibberellin biosynthesis (Calhey, 1964; Lang, 1970). Dunberg and Aåliasson (1970) also reported such interactions.

Cleland and Zeevaart (1970) and Zeevaart (1966) have shown that growth retardants can reduce the level of endogenous GA₃ in higher plants.
Barrie and Robertson (1973), Wittwer and Tolbert (1969) and Knypl (1967a) envisaged inhibition of germination of lettuce and Kale seeds by CCC and other related compounds. This inhibition was eliminated either by irradiating seeds with red light or by applying gibberellin and Kinetin.