2. LITERATURE REVIEW

Some organic chemical substances of varied chemical nature affect growth and development of plant. Organic chemical substances other than nutrients which are active in low concentration in promoting, inhibiting or otherwise modifying growth and development may be called growth regulators (Moore 1974). These growth regulators may be (a) naturally occurring (b) synthetic growth promoters and (c) inhibitors or growth retardants. Literatures regarding effects of growth regulators on various physiological processes (viz- seed germination, seedling growth, chlorophyll development, metabolism and yield) are reviewed:

2.1.0. Plant Growth Hormone

(A) Growth regulators:
1) Gibberellic acid (GA$_3$)
2) GA$_4+7$
3) Kinetin (KN)
4) 6-benzyladenine (BA)
5) Salicylic acid (SA)

(B) Growth Retardants:
1) Chlоро choline chloride (CCC)
2) Maleic hydrazide (MH)

2.1.1 Gibberellic acid (GA$_3$)

The Gibberellins comprise a large family of diterpenoid acids which were initially discovered by Kurosawa (1926) as metabolites of Gibberella fujikuroi (Fusarium moniliforme) the causative fungus of the ‘Bakanae’ disease of Oryza sativa.

GA$_3$ fulfills the following reasonable criteria for endogenous plant hormones—(a) when applied exogenously in extreme small amounts they induce a wide range of plant growth responses,(b) they are present in most of all higher
There are circumstantial evidences that they move within the plant from site(s) of biosynthesis to site(s) of action.

The first gibberellin (GA) was structurally elucidated in the mid 1950’s (Curtis and Cross 1954, Stodola 1958). In 1938 Yabuta and Sumuki isolated Gibberellins A and Gibberellins B in the form of crystals from the fungus *G. fujikuroi* but soon after it was also isolated from the higher plants. Stodola *et al.* (1955) purified the active principle and called it gibberellins X while Curtis and Cross (1954) in UK independently isolated a similar chemically pure substance and named it gibberellic acid. Both this substances were found to be identical (Cross 1954) and contain a gibbane ring (Cross *et al.* 1961). All naturally occurring compounds which possess gibbane skeleton and requisite biological properties are given the generic name gibberellin. Gibberellins A of Yabuta and Sumiki (1938) was later found to be mixture of 3 gibberellins, GA₁, GA₂ and GA₃ (Takashashi *et al.* 1955 and 1967-69). The gibberellins are a large group of related compounds (more than 125 are known) that, unlike the auxins, are defined by their chemical structure rather than by their biological activity (Davies 2002).

![Structure of gibberellic acid (GA₃)](image)

**Fig:** Structure of gibberellic acid (GA₃)

Analytical study shows the empirical formula for GA₃ to be C₁₉H₂₂O₆ (Cross 1954).

The gibberellins are unique among plant growth regulators in their ability to stimulate growth in many intact plants. This growth effects are –1) phenotypic reversal of dwarfism in plants; 2) induction of stem elongation in rosette plants; 3) stimulation of flowering; 4) breaking of dormancy in shoots, tubers, buds and seed. In addition to these gross effects on whole plants gibberellins have been
shown to stimulate the de-novo synthesis and excretion of many enzymes for ex-α-amylase, β-glucanase and endopentosanase during the germination of cereals grain.

GA3 has been applied to break dormancy to accelerate vegetative growth and also to enhance flowering in plants. Brian and Hemming (1955) and Baijal et al. (1981) applied GA3 to break dormancy in potato. The first successful assay using dwarf corn was made by Phinney et al. (1960) and later on with dwarf pea (McComb 1964). That gibberellins are related with dormancy of plants have been shown by Kahn et al. (1957) and there is an increased accumulation of gibberellins when the seed emerge from the dormant condition (Naylor and Simpson 1967).

In many plants dormancy can be removed with GA3. Normally the level of endogenous gibberellins increases leading eventually to germination. The application of GA5 from outside can however promote germination in some seeds and tubers where dormancy is due to low level of endogenous gibberellins. When the seed imbibes water, free gibberellins are released from the bound form in the embryo, then move to the aleurone cells of the endosperm and induce the fresh synthesis of an enzyme namely α-amylase. This enzyme in turn hydrolyses the starch present in endosperm into sugar.

**Role of GA3 on Germination:**

Gibberellins have been applied to study a variety of responses of plant growth and development. GA3 has been applied to break dormancy of bud and tuber and germination of seeds, to accelerate vegetative growth and also for enhancing flowering in plants (Jones and Stoddart 1977, Thomas 1976 and Miyoshi and Mii 1995). Khan et al. (1956) were among the first to show that gibberellic acid stimulates the germination of Lactuca scariola and Lactuca sativa in the dark. Roberts (1963) reported that GA3 caused an increased in germination rate of dormant rice seeds. There are few reports on the effects of GA5 on the germination of rice seeds (Ellis et al. 1983). No comparative study on germination
of indica and japonica rice seeds after treatment with GA$_3$ and kinetin has been made.

Gibberellic acid is the most potent germination promoter and breaking seed dormancy in a wide range of crop species such as beans and peas (Sarma 1982), potato (Utheib et al. 1981, Haque 1987), lettuce (Bewley 1980, Kojima and Ooata 1980), (Sarma and Borooh 1986), onion (Loper and Waller 1982), radish (Pawar et al. 1977). Evenari (1949) reported that GA$_3$ substitutes for light in the promotion of germination of photoblastic seeds. Vyas and Garg (1971) observed that GA$_3$ was capable of not only bypassing the light requirement, but also to overcome some of the temperature block on germination.

GA$_3$ breaks dormancy of various kinds of seeds and buds (Frankland 1961, Paleg 1965). Its stimulatory effects on germination of seeds have also been reported by Amen (1968), Baskin (1971) and Juntilla (1970). GA$_3$ applied 5-35 days after anthesis was most effective in promoting pea seed germination (Sarma 1982). Certain weed species of Assam stimulation of germination by GA$_3$ was also reported by Sarma (1980). Deka and Das (1978) reported that lower concentration of GA$_3$ (50 mg/l) enhanced the germination of pea seeds. Chailakhyan et al. (1975) observed that 0.0001 per cent GA$_3$ solution was less effective and 0.01 per cent concentration adversely affected seed germination. The fresh harvested radish seed’s dormancy was broken by treating with GA$_3$ at 500 ppm by Negi et al. (1983). Effect of GA$_3$ was the sprouting of potato tubers immediately after harvest (Peterson 1967).

GA$_3$ stimulation of germination of photoblastic seeds of lettuce and tobacco in dark was reported by Sarma and Chakraborty (1975, 1981), Sarma and Barooah (1975, 1982, 1986), Sarma and Phukan (1977, 1981, a, b). Stimulation of germination and seedling growth of *Raphanus sativa* by GA$_3$ treatment was reported by Sarma and Sarma (1987). Kumar and Alka (1978) obtained 100 per cent germination in *Raphanus sativa* by treating with GA$_3$. At 200 ppm of GA$_3$ treatment the rate of germination was raised and improved seedling growth in *Cichorium intubus* L. (Srivastava et al. 1974). Sarma et al. (1976) reported that in
freshly harvested or stored seeds of wild oat (Avena fatua) dormancy was removed by GA$_3$. Pal et al. (1974) reported that application of lower concentration of GA$_3$ on Vitis vinifera seeds stimulation of germination was high.

The activity of GA$_3$ was gradual and regular. That GA$_3$ induces quicker germination compared with untreated seeds was reported by Leal et al. (1976) on the seeds of avocado. GA$_3$ 100 ppm increased germination of cotton seeds (Chandola et al. 1973). Application of GA$_3$ on seeds of Solanum laciniatum Act. and Solanum aviculave Frost. increased the percentage of germination to 80-90 (Porter and Gilmore 1976). Highest percentage of germination was recorded on Coreopsis tinctoria (92%), Centauree moschata (80%) and Phlox drummondii (94%) after treatment with 100 ppm of GA$_3$ (Jana and Dass 1984). Simpson (1990) reported positive effect of gibberellins on the germination of seeds of poaceae.

Ellis et al. (1983) observed germination of dormant seeds of grapes (Vitis spp.) soak in hydrogen peroxide for 24 hours was followed by treatment with 1000 ppm GA$_3$. Lewak and khan (1977) observed that the time of GA$_3$ application influenced the rate and the final percentage of germinated seeds. Uniform and enhanced germination of corn seed by GA$_3$ treatment has been reported by Ermilov (1961) and Murmotsov (1961). Bal et al. (1990) treated wild pear (Pyrus pashia Buch. Ham.) seeds for 36 hours with GA$_3$ at 50, 100, 150 and 200 ppm and maximum germination was obtained at 50 ppm (89.5 per cent). GA$_3$ soaking of seeds of Panicum maximum stimulated germination (Basra et al. 1990). Kitchen and Meyer (1991) reported that seed germination of Pentemous is enhanced by stratification and GA$_3$ application.

Paleg (1960 a, b) reported that GA$_3$ treatment on barley seeds increased germination and similar reports were filed by Srivastav et al. (1974). GA$_3$ soaked dormant seeds of Seramum indicum L. dormancy was completely broken (Ashri and Palevitch 1979). Five to nine years old seeds of tobacco were also germinated by GA$_3$ (Yamaguchi et al. 1983). Cavusoglu and Kabar (2007) studied the comparative effect of some growth regulators (GA$_3$, KN) on the germination of barley and radish seeds under high temperature stress. Endogenous hormones
have been proposed to play a significant role in potato tuber and bud dormancy regulation (Suttle 2004, Coleman et al. 2001).

**GA₃ on growth and development:**
Gibberellins regulate plant growth by affecting stem elongation and leaves show weak responses to GA. Gibberellic acid regulated the growth, flowering and fruit and yield quality in pineapple (A o KC 1995). GA₃ promoted shoot length, number of leaves and number of branches of ground nut seedlings (Chakraborty 2001). Sarma (2001) reported that the number of branches and leaves gradually increased by GA₃ from lower (10 ppm) to the optimum concentration (500 ppm) in coleus. The effects of GA₅ are the transformation of genetically dwarf plants into tall ones by greatly increasing stem elongations (Phinney 1965). Kato (1955) and Brian (1959) had reported that GA₃ application results in stem elongation. The elongation of the internodes of *Phaseolus vulgaris* was chiefly the results of increases in the rate of cell division by the effects of GA₃ (Greulach and Haesloop 1953). In cucumber elongation of radical was promoted by the application of GA₃ (Katsumi and Kawamura 1980). Hayasi *et al.* (1953) observed that the treatment with GA₃ causes of stimulation of cell enlargement. Haber and Luippold (1960) irradiated seeds to prevent mitosis and obtained large promotions which was the results of cell enlargement. That the elongation of internodes is the results of GA₅ induced cell division and cell elongation has been more or less established (Dure and Jensen 1957, Copper 1958 and Bradley and Crane 1957).

GA₃ promotes growth of wheat coleoptiles during the phase of elongation growth. Mukharjee and Dutta (1964) and Wittwer and Bukovac (1957) studied the effects of GA₃ on the stem elongation of different plants. GA₃ stimulated extension growth in lentil (Nitsan and Lang 1966). Sachs (1956) reported that GA₃ stimulated growth via cell division. Kumar and Baijal (1979) reported that the shoot length of potato was increased with GA₃ treatment.

Mulge *et al.* (1998) reported beneficial effect of GA₃ spray on shoot length and seedling growth in onion. Foliar spray of GA₃ on trees exhibited an
increase in shoot length, internodes and chlorophyll content (Naidu and Swamy 1995). In Trifolium alexandrium foliar treatments of GA$_3$ have an identical result (Agarawal et al. 1994). The length of seedlings of Shorea assamica treated with GA$_3$ was highest at 500 ppm and the number of leaves was highest at the same concentration (Dutta 2001). Similar effect of GA$_3$ on elongation of shoot growth of kesseru plant has been observed by Dhekial Phukan (2002) as well as in pea and soybean by Bora (2002). Barkataki (2002) has also observed the identical results in pineapple. Treatment with GA$_3$ caused an increase in plant height, internodes length, circumference of stem and number of branches in chrysanthemum (Meher et al. 1999). In cow pea 10 ppm of GA$_3$ increased the plant height significantly (Yadava and Sreenath 1975). Abdul et al. (1985) observed that GA$_3$ and NAA increased plant height and leaf number in cow pea.

Balraj et al. (2002) recorded that GA$_3$ at 100 ppm significantly increase plant height and number of branches of chilli. GA$_3$ affects a remarkable increase in height of shoots in black gram and green gram (Patel and Saxena 1994). Significant increase in vegetative growth and plant height as a result of foliar treatment of GA$_3$ of pterocarpus Santanilus L. was reported by Venkataramaiah and Swami (1981).


**GA$_3$ on chlorophyll development:**

GA$_3$ treated plant plays significant role in controlling chlorophyll contents of leaves. Malik et al. (1981) reported that Datura metal L. treated with GA$_3$ at 5 to 100 ppm at 70 and 80 days of plant age increased leaf content of chlorophyll a and chlorophyll b and carotenoids. Evidence of increase in chlorophyll a and chlorophyll b contents due to the activity of GA$_3$ was also reported by El-Tahawi

**GA₃ on metabolism:**

In addition to the changes in morphological and cell division that occur in early phages of the stolon tuber transition, tuber initiation and enlargement are accompanied by massive changes in the physiology and metabolism. The metabolic changes occurring in the early stages of germination are the result of the activity of various enzymes. Generally, enzymes breaking down starch, proteins, hemicelluloses, lipids and other storage materials, rise in activity fairly as germination proceeds. The yield and oil content of the nuts were also increased by GA₃.

A remarkable increase in carbohydrate content in response to GA₃ in seed pretreated and foliar sprayed plants of Vicia faba was observed by Gaber et al. (2002). Cao and Shannon (1997) examined the effect of GA₃ on growth, protein secretion, and starch accumulation in cultured maize (Zea mays L.) endosperm suspension cells. GA₃ (5 and 30µM) increased the fresh weight, dry weight and protein content of the cultured cells, but the effects of GA₃ at 50µM was not significantly different (Cao and Shannon 1997). GA₃ had a dramatic effect on the starch content.

Plant growth regulators play pivotal roles in the regulating of plant growth and development especially GA₃ (Cao and Chan 1995, Davies 1987) as well as in the interaction with other organisms. GA₃ play essential roles in the breakdown of reserve starch in germinating cereals grains (Jacobsen and Chandler1987). Accumulation of GA₃ in developing grains of wheat (Radley 1976), barely (Mounla 1978) and Phvbitis nil (Barendes et al. 1991) is well correlated with an
increase in the fresh weight during early development, suggesting a role of GA$_3$ in cell growth in these fast growing tissues. GA$_3$ was reported to cause a decrease in the starch content of isolated chloroplast (Kazama and Katsumi 1984) and sweet potato suspension-cultured cells (Sasaki and Kainuma 1984).

GA$_3$ stimulates germination via amylase production. Otherwise, one would expect amylase activity to increase prior to the onset of germination. Seeds of Himalaya barley (*Hordeum vulgare* L.) widely used in studies of GAs induced amylase synthesis, behave similarly. GA$_3$ has been reported to increase the activity of about 18 enzymes and to decrease the activities of 2 enzymes. GA$_3$ appears to regulate the activity of the enzyme rather than their synthesis.

**GA$_3$ on yield:**
GA$_3$ can enhances the productivity of crops as reported by Ray and Choudhary (1981) on different crops. Pandita *et al.* (1981) reported increase in the size of the tuber and total yield with GA$_3$ application at 25 ppm as a seed tuber dip treatment of potato cv. *Kufri Chandramukhi*. That GA$_3$ also increased the yield of Arabia coffee was reported by Opile (1974) in Kenya. Holmes and Lang (1978) on studying the effect of different concentrations of GA$_3$ on yield of potato reported that increased in tuber number of majestic and pent land crown following GA$_3$ treatment was associated with an increased stem number. Increase in sugarcane yield was also obtained with GA$_3$ application (Moore 1980). GA$_3$ also can enhance the growth and yield of chilli at different picking (Balraji *et al.* 2002).

**2.1.2 GA$_{4+7}$**
Gibberellins have been shown to play a decisive role in all phases of plant growth and development from germination of seeds. Some closely related gibberellins are difficult to separate and identify. GA$_4$ and GA$_7$ differ from GA$_3$ in the presence or absence of hydroxyl at the junction of rings C and D and there is also difference in regard to the presence or absence of double bond ring A.
Dennis and Nitsch (1966) positively identified GA₄ and GA₇ in immature endosperm of apple. Originally GA₄ and GA₇ were isolated from Gibberella fujikuroi mycellial filtrate (Takahashi et al. 1955) in which they are biosynthetically precursors of GA₃. GA₄ has been shown to improve fruit quality of apple, where GA₇ although structurally similar to GA₄, considerably is less effective where as mixtures of GA₄ + GA₇ are more effective in the induction of parthenocarpic fruit development in apple and other species (Varga 1966).

GA₃ has already found wide application in agriculture and horticulture for their multifarious responses. In the mean time other gibberellins including mixtures of one or two GA₄s such as GA₄ and GA₇ i.e. GA₄+7 has become commercially available.

Physiologically GA₄ has been reported to be more active than GA₃ in bringing about germination of lettuce seeds. It was found to be ten times more active than GA₃ (Poggi Pellegrin and Bulard 1976). Stanb et al. (1989) observed that treatment of GA₄+7 in cucumber seeds improved germination. Watermelon seed soaking by GA₄+7 (0.05 to 5mM) stimulated seed germination (Nerson et al. 1985).

The germination of light requiring seeds of Chenopodium album L. was induced by GA₄+7 (Karssen 1976). The inhibitory effect of high temperature on the germination of celery seeds can completely be eliminated by treating the seeds with GA₄+7.

The growth regulators are less effective at low temperature. If a constant temperature is maintained more GA₄+7 is required to promote germination.
(Biddington and Thomas 1978b). That a mixture of GA$_4$ and GA$_7$ are more
effective in promoting rapid germination of cucumber seed was reported by
Nelson and Sharples (1980). Infusion of GA$_{4+7}$ in the muskmelon seeds increased
the total germination at 16° C. GA$_{4+7}$ proved to be highly stimulatory at high day
time temperature alternating with night temperature of 20 to 25° C (Paletich and
Thomas 1974). Thermo-inhibition of celery seed germination is associated with
the accumulation of inhibitors, but such type of dormancy can be broken by
applying GA$_{4+7}$ in combinations with cytokinins (Biddington et al. 1978b).
Imbibitions of celery seeds in acidic solution of GA$_{4+7}$, before sowing greatly
alleviated the inhibitory effect of heat stress on germination and seedling
emergence (Paletich et al. 1978).

_Cucurbita pepo_ L. cv. HS-1 plant treated with GA$_3$ and GA$_{4+7}$ exhibited
marked stimulation of growth but effect of the GA$_{4+7}$ was more pronounced
(Krishnamurthy 1975). GA$_{4+7}$ also proved to be highly stimulatory on the
extension growth of hypocotyl section of bean _in vitro_ (Sarma and Phukan 1988).

GA$_{4+7}$ are also known to reduce the shoot length in some species. Brian
and Jackson (1982) reported that GA$_{4+7}$ reduced the length of apple shoot. GA$_{4+7}$
induced callus growth on the cut ends when applied. Dhalial _et al._ (1977) working
with grapes found that spraying of GA$_{4+7}$ caused an increase in branch length,
berry length and weight.

Gibberellins (GA$_1$, GA$_3$, GA$_{4+7}$, GA$_{20}$) were bringing about growth of the
excised lettuce hypocotyl sections. GA$_{4+7}$ proved to be the best one followed by
GA$_9$ (Nash _et al._ 1978). Hypocotyl and cotyledon growth were enhanced by
GA$_{4+7}$ when applied to the tender tomato seedlings (Aung and Byrne 1976).

Ripened seeds of Muscat grape (_Vitis vinifera_) were treated with GA$_3$ and
GA$_{4+7}$ at the concentrations of 0, 50, 100, 250, 500 and 1000 ppm of each. All
gibberellins except GA$_{4+7}$ at concentration beyond 50 ppm promoted germination
especially lower concentrations. The activity was evident with a narrow
concentration range for germination promotion of tuber. Wide variation in the
response of GA$_{4+7}$ on seedling growth was also observed (Pal _et al._ 1974). GA$_{4+7}$
stimulated germination of *Abies frasen* seeds in darkness but GA_{4+7} had no significant effect in the light (Henry and Blazich 1988).

GA_{4+7} as the major GA_{5} in developing apple seeds (Dennis and Nitsch 1966; Luckwill *et al* 1969) considered with the greater effectiveness of GA_{4+7} in induction of apple. Bland *et al* (1967) showed that 3-keto derivatives of GA_{4+7} are active in the cucumber bioassay but not in the dwarf pea and lettuce hypocotyls tests. GA_{4+7} effective in retarding leaf senescence at least at the concentration of 25 mg/l (Fletcher *et al* 1972) on dandelion leaves.

### 2.1.3 Cytokinins: (Kinetin)

Cytokinins have the unique ability to alleviate the effects of various inhibitors in seeds and other organs in a wide variety of plants (Khan and Tolbert 1965, Khan 1975). High temperature induced dormancy in lettuce seeds relieved by cytokinins (Smith *et al* 1968). Kinetin has been found to contain carbon, hydrogen, oxygen in the ratio C_{10} H_{9} N_{5} O (Miller1963). Since their discovery cytokinins have been shown to have effects on many other physiological and developmental processes, including leaf senescence, nutrient mobilization, apical dominance, the formation and activity of shoot apical meristems, floral development, the breaking of bud dormancy and seed germination (Kieber 2002).

![Chemical structure of 6-Furfurylaminopurine (kinetin)](image)

**Fig:** Chemical structure of 6-Furfurylaminopurine (kinetin)

A cell division causing substances was discovered by heating at high temperature and pressure (autoclaving) herring sperm DNA by Miller *et al* (1955) and this
substance caused cell-division in tobacco pith cell at low concentration. Later on this substance was identified as 6-furfuryl amino purine and named as ‘Kinetin’ from ‘kinesis’ to imply a substance that promotes cell-division (Miller et al. 1955).

Miller (1956) was the first to isolate two potent Cytokinin namely—kinetin and 6-benzyladenine (BA). According to Skoog et al. (1967) and Mothes (1964) the term cytokinin is universally used as generic names for substances which promote cell-division.

**KN on seed germination:**
Kinetin affects germination of seeds especially of lettuce seeds. That kinetin promotes the germination of seeds (Miller 1958) and even breaks seed dormancy (Khan 1966, 1971) has long been known. Sarma (2001) reported that GA₃ at 500 µg/ml and kinetin at 1.0 µg/ml proved to be optimal in inducing sprouting on dormant coleus tuber. Same combinations manifested in highest leaf numbers, more branches and better shoot growth in *Coleus parviflorus*. Kefferd et al. (1965) also observed the stimulation of germination of lettuce seeds by kinetin. Some of 79 per cent of kinetin treated seeds germinated at 30°C.

Dayal (1986) observed differential responses in seed germination under low temperature in presence of KN 100 mg/l in different tomato cultivars, while Wu et al. (1983) recorded no significant effect on germination when tomato seeds were soaked for 24 hrs in KN before sowing. Suttle (1998) observed that application of kinetin resulted in the termination of dormancy and enhanced sprouting of potato tuber and increased yield of potato. An increase in cytokinin content is the principal factors leading to the loss of dormancy (Suttle 2004).

Kinetin (0.1-50µg /Lt) proved highly stimulatory for germination of seeds of *Lactuca sativa* cv. Arctic king at temperatures above 27°C in continuous light or after short periods of illumination during early stage of imbibitions (Reynolds and Thompson 1973).
Kinetin can bring about germination in a wide variety of seeds showing various forms of dormancy (Lang 1965). Sengupta et al. (1979) studied the effect of kinetin on dormant and non-dormant types of peanut. In dormant peanut, kinetin removed the dormancy in the freshly harvested seeds. The germination percentage of *Oryzopsis hymenoids* seeds was highly dependent on the concentration of kinetin and was highest at 0.1µM (Tao et al. 1974). Cell enlargement after kinetin treatment has also been observed in tobacco pith cultures (Glasziou 1957) and in excised artichoke tissue (Adamson 1962).

A large number of derivatives of kinetin, where the furfuryl group is replaced by other grouping, also stimulate germination. Earlier it was thought that kinetin substitutes for red light in germination but later it was shown that the seeds were sensitized by kinetin so that a smaller dose of light would induce their germination. In *Pinus taeda* and *Taxodium distichum* (Biswas et al. 1972), in lettuce (Miller 1958) red light treatment promoted seed germination after dormancy was broken by chilling. In thermo-inhibited celery seeds, the accumulated germination inhibitor interacts with cytokinins (Biddington et al. 1980 and Thomas et al. 1986) and such type of dormancy can be broken by pre-sowing seed treatment with cytokinins. Kinetin stimulated germination of certain light requiring seeds in the dark (Miller 1956, Skinner et al. 1958). Far red light inhibited germination but this inhibitory effect was overcome by 100ppm kinetin alone or in combination with 250 ppm of GA₃. Kinetin application could be effective in alleviation of high temperature on barley seed germination (Cavusoglu and Kaber 2007). Of the pretreatments alone GA₃, KN, BA not only overcome the preventive effect of high temperature on barley seeds germination but also shortened the time required for germination by alleviating its germination-delaying effect (Cavusoglu and Kaber 2007).

Cytokinin has been shown to have stimulatory effects on the germination of seeds of a wide range of plant species (Jones and Stoddart 1977, Thomas 1997, Miyoshi and Mii1995). Kinetin showed a slightly promotive effect on the germination of intact seeds of cultivated rice (Roberts1963). Preliminary work
showed that $10^{-3}$ M kinetin was most effective in stimulating the germination of dehusked seeds of Indian and Japonica rice, irrespective of the cultivar and the time of harvest (Miyoshi and Mii 1997).

**KN on growth and development:**

External application of cytokinins has been shown to substitute for the physiological influence of roots on the growth of derooted oat (Jordan and Skoog 1971) and soybean seedlings (Holm and Key 1969). Cytokinins have been implicated in the control of the growth of wheat coleoptiles (Wright 1966). Cytokinins are involved in the process of radicle elongation (Haber and Luippold 1960, Pinfield and Stobbart 1972) and cotyledons expansion (Kursanov et al. 1964 and Ikuma and Thimann 1963). Webb and Dumbroff (1969) demonstrated the ability of cytokinins to substitute for chilling treatment to break dormancy and stimulate radicle and cotyledon growth on sugar maple seeds.

Kinetin not only promotes cell-division but also induces cell enlargement. Kinetin stimulates the germination of upper seed of *Xanthium* and dormant peanut seeds (Ketring et al. 1971), better performance on the growth and number of leaves of guava (Rao et al. 1977), and also highest germination at $5 \times 10^4$ M of groundnut seed (Narasimhareddy et al. 1975). The effectiveness of kinetin varies from plant to plant. Singh (1999) reported that kinetin at the optimal concentration (1.0 µg/ml) stimulated shoot growth of *M. depika* to 12.25 cm against 8 cm at the control while Dutta (2001) reported that kinetin stimulated better seedling growth and biomass production of *Shorea assamica* and the maximum height and number of leaves per seedling at 2.5 µg/ml of kinetin.

Cheema et al. (1987) reported that foliar spray at flowering and pod filling stages on mustard and *Gobhi sarson* improved the leaf area index, leaf chlorophyll content, seed weight and the seed yield.

The nodes are the sites of synthesis of gibberellins and cytokinin. In *Begonia* leaf discs kinetin stimulated shoot appeared sooner and in large quantities (Badizadegan et al. 1972).
Lactuca sativa requires red light for breaking seed dormancy but this effect was replaced by the application of kinetin. Similar effects were reported in the seeds of tobacco, carpet grass and Xanthium pennsylvanicum (Khan1964). Dormancy of Lemna minor can be interrupted by kinetin. Thompson and Kosar (1939) reported the stimulation of lettuce seed germination by some sulphur compound as well as by kinetin. Kinetin stimulated germination of certain seeds (Lang 1966, Miller 1956, Skinner et al. 1958) but it failed to completely replace the requirement of red light in Grand Rapids lettuce seeds, although it increased their sensitivity to red light (Miller 1956).

Kinetin is very effective in retarding senescence of excised leaves of diverse genera (Nooden and Leopold 1978). KN is also reported to induce re-greening of detached yellow tobacco leaves (Kursanov et al. 1964). Kinetin markedly retards senescence of attached leaves of some plants such as soybean (Lindoo and Nooden 1978), French bean (Fletcher and Adedipe 1972), rice (Ray et al. 1983), wheat (Mishra and Gaur 1985, Wittenbach 1977), and oat (Thimann et al. 1974). De-topping of tobacco (Mothes 1964) and bean plants (Venkatarayappa et al. 1984) induced re-greening of lower leaves, an effect also evoked by application of exogenously applied kinetin.

Haber and Tolbert (1959) reported that the effects of kinetin are highly temperature dependent. Tolbert (1959), Skiner and Shire (1959) and Smith et al. (1968) observed stimulation of germination as a result of application of kinetin at high temperatures where little or no effects were found at lower temperature.

Kinetin caused an increase in fresh weight of the epicotyls, increase in leaf expansion and increase in the elongation of stems and petioles of bean (Miller 1956). Lin (1985) studied the effects of GA, kinetin, ABA, and CCC on seed germination and growth of maize seedlings under salt stress, and found that only with 10 ppm kinetin leaf chlorophyll content was increased. Treatment of leaf discs cut from etiolated leaves, with kinetin caused significant cell enlargements (Miller 1956). In in vitro culture of Verticordia grandis shoot proliferation with kinetin was highest compared with BA (McComb et al. 1986). Saini et al. (1987)
recorded favourable effect on the growth and yield of mustard after kinetin spraying.

Nilovaskaya et al. (1985) observed increased photosynthetic activity of chloroplast and chlorophyll content of spring wheat when kinetin was applied as seed treatment. Cytokinines caused stimulation of chlorophyll synthesis and accelerated chloroplast differentiation in detached cotyledons in light (Parthier 1979). Nandi et al. (1989b) has indicated that the stimulus required for chlorophyll formation and cotyledon expansion in germination of lupin seeds would appear to be KN emanating from the axis. Cytokinins have been reported to be essential for chlorophyll synthesis in tobacco cell cultures (Stetler and Laetsch 1965, Seyer et al. 1975). Dushkova et al. (1981) reported that seed treatment with $10^{-6}$ M kinetin increased the chloroplast activity in maize plants grown with and without soil moisture stress. Kinetin sprayed plants generally had higher chlorophyll a and chlorophyll b contents both in unsalinized and salinized plants (Katz et al. 1978). Malibari (1993) reported that the values of chlorophyll a and chlorophyll b contents in plants treated with the combination of ABA and kinetin together were higher than unsprayed plants. It seems that kinetin is more effective in preventing chlorophyll degradation and enhances chlorophyll accumulation. Similarly, Singh et al. (1994) reported under different levels of salt stress decrease in chlorophyll a and chlorophyll b with kinetin treatment.

Sairam et al. (1991) reported that application of kinetin increased the photosynthesis, nitrate-reductase activity and increased grain yield in wheat. Takashima et al. (2000) reported an increase in yield of potato tubers as a result of kinetin applications.

Cytokinins cause changes in the protein and nucleic acid components of tissues which are the basis of affects of cytokinins on cell division as well as on growth and mobilization actions. Sekhon and Singh (1994) reported that kinetin was effective in increasing starch content during later stages of grain development in early as well as normal sowing of wheat. They also reported higher rate of protein synthesis on wheat.
2.1.4 6-benzyladenine (BA)

6-benzyladenine is one of the most important synthetic cytokinins which is very active in some assays. Sarma and Neog (1981) reported the highly stimulatory effect of 6-benzyladenine on the extension growth of hypocotyl segments. BA + GA$_3$ combination became more successful on the elongation of *Arbor vitae* and *Red pine* redicles in 100 ppm. BA + KN also show good result on elongation of *Arbor vitae* seedlings. High concentration of 6-benzyladenine enhanced axillary bud formation in the stem node of *Euphorbia lathyris* but the number of plantlets was less (Tideman and Hawker 1982). Suttle (2004) reported that dormancy of potato tuber was effectively broken with 6-benzyladenine at a concentration of 20 ppm used for 24 hours. Narasimhareddy (1975) reported that BA was found to be effective in breaking dormancy even in the presence of seed coat. The dormancy breaking effect of low temperature could be substituted for by treated with BA or GA$_3$ and germination could be ascribed to the effects of these treatments on the ability of the seeds to react to ethylene (Whitehead and Sutcliffe 1995).

Singh and Murti (1987) studied the effect of BA on seed germination and seedling growth of *Cassia fistula* L. Raja (1978) recorded stimulatory effect of BA on seed germination of Kalyansona wheat. In *Cucumis anguria* BA at 25 to 100 mg/l (Castro *et al.* 1987) and at 0.05, 0.5 or 5 µm in watermelon (Nelson *et al.* 1985) as pre-sowing seed treatment promoted germination. Yun-Kyong-Shin *et al.* (2011) studied the effect of BA and ultrasonic pre-treatments on *in vitro* germination and protocorm formation of *Calanthe* hybrids.

![Chemical structure of 6-benzyladenine (BA)](image)

**Fig:** Chemical structure of 6-benzyladenine (BA)
Strawberry plants developed into multiple shoots from axillary buds on Ms medium containing 0.02 or 0.2 mg/l BA. Ms medium containing 6-benzyladenine at 2mg/l produced multiple plantlets (Lee and Park 1980). Das et al. (1995) observed that benzyl amino purine (1.0-2.0µg/ml) in combination with NAA (0.5-1.0µg/ml) caused high frequency regeneration of shoot bud of Dalbergia species using hypocotyl and cotyledon explants. The growth promotion due to BA treatment may be a consequence of enhanced nucleic acid and protein metabolism which was observed by Fletcher and Osborne (1965). Burger et al. (1985) suggested that axillary buds elongate most rapidly on a medium containing 1 mg/1 BA and 0.1mg/1 NAA.

BA stimulation of chlorophyll synthesis has been observed by Mansoor et al. (1994). Borelli et al. (1996) also reported BA stimulation of chlorophyll in potato plant. Yokoyama et al. (1980) observed that BA treatment stimulated an increase in chlorophyll content of bean (Phaseolus vulgaris L.) leaves. They also observed that BA treatment at day 6 enhanced protein content and RNA without significant influence of DNA and chlorophyll content. Pretreatment of etiolated cucurbita cotyledons (4-10 days old) with BA increased the amount of chlorophyll produced in light (Fletcher et al. 1971).

Bang-Zhen et al. (2010) observed that BA treatment significantly increased the seed yield of the biofuel plant Jatropha curcas. Argall and Stewart (1984) observed positive effects of decapitation and BA on growth and yield of cow pea (Vigna unguiculata L. Walp).

2.1.5 Salicylic acid
Salicylic acid (SA) or ortho hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolics. The first commercial production of synthetic salicylic acid began in Germany in 1874 (Raskin 1992 a, b). Salicylic acid, the name comes from the Latin word for the willow tree, salix, from the bark of which it can be obtained. This colourless crystalline organic acid is widely used in organic synthesis and function as a plant hormone. It is derived from the
metabolism of salicin. SA is an organic acid biosynthesized from the amino acid phenylalanine.

![Chemical structure of salicylic acid](image)

**Fig:** Chemical structure of salicylic acid

The chemical formula of salicylic acid is $\text{C}_6\text{H}_4(\text{OH})\text{CO}_2\text{H}$, where the OH group is adjacent to the carboxyl group. Phenolics are usually defined as substances that possess an aromatic ring bearing a hydroxyl group or its functional derivate. Free salicylic acid is a crystalline powder that melts at 157-159°C. It is moderately soluble in water and very soluble in polar organic solvents.

In many plants, such as rice, crabgrass, barley, soybean, the levels of salicylic acid has been found to be approximately 1 µg g⁻¹ fresh weight. A survey of SA in leaves and the reproductive structures of non-thermogenic angiosperms confirmed the ubiquitous distribution of this compound in plants. Levels of SA varied substantially in the floral parts of seven nonthermogenic species and in the leaves of 27 non-thermogenic plants (Raskin *et al.* 1990).

In plants salicylic acid is synthesized by two possible pathways and two key enzymes are involved in SA biosynthesis and metabolism (Hayat and Ahmed 2007). Large amount of SA have been found in the soil samples taken from the rhizosphere of *Zea mays* and *Phaseolus aureus* (Pareek and Gaur 1973).

Salicylic acid plays an essential role in the regulation of different physiological processes, including plant growth and development, ion uptake and photosynthesis. Produced by plant roots some SA are essential for germination and plant development (Lynn and Chang 1990). SA induced potato tuberization *in vitro* at concentrations greater than $10^{-5}$ M (Koda *et al.* 1992). SA seems to be
involved in tuberization in yam plants (Koda and Kikuta 1991). SA promotes the biosynthesis of TA or JA and as a result, they exhibit tuber-inducing activity.

Salicylic acids perform important actions in growth and development processes of plants. It is a potent signaling molecule in plants and is involved in eliciting responses to biotic and abiotic stresses (Krankev et al. 2008). These actions include exercising a thermogenic effect (Ansari and Misra 2007), stimulating adventives root formation (King and Meyer 1983), reducing leaf shed (Ferrarse et al. 1996) and changing the quality and quantity of protein (Doares et al. 1995).

Singh and Srivastava (1987) studied the effect of salicylic acid on NADH glutamate synthetase activity in root and leaf tissue of maize seedlings. They reported that NADH glutamate synthetase activity was stimulated by 1 to 1000 M salicylic acid in the leaf and by 50 to 100 M salicylic acid in the root tissues of maize seedlings. Higher concentration of salicylic acid inhibited enzyme activity in both tissues. The stimulatory concentrations of SA protected both in vivo and in vitro decline in enzyme activity to some extent, which was more apparent in leaves and roots. They also suggested that SA increases glutamate synthetase activity by inducing synthesis of enzyme as well as by some kind of direct modulator of enzyme molecule. Kaur (2002) reported that salicylic acid has stimulatory effect on soybean (Glycine max L.) for the growth and development of plant. SA effects of providing resistance against different stress factors in plants (Yan and Lin 2008) by modifying the effects of abscisic acid and gibberellic acid (Apte and Laloraya 1982), and cytokinins (Ray et al. 1983). These observations and reports on many other physiological effects brought about that SA might be a new plant growth regulator (Hayat and Ahmad 2007).

Kumar et al. (1999) observed significant increase in pod weight, grain weight and harvest index of soybean by exogenous application of salicylic acid. Maximum pod weight and grain weight were observed at 50 ppm concentration. Miguel et al. (2003) observed that stem diameter and height of the plants increased by applying SA $10^{-10}$ and $10^{-8}$. 
The SA effect was not specific and it promoted flowering in combination with other plant regulators (gibberellins). Besides flowering SA also affected multiplication rate of anthocyanin and chlorophyll contents in Spirodella polyrrhiza (Khurana and Maheswari 1980). High concentration of SA retarded the growth of fronds. Singh et al. (2010) observed the positive correlation between chlorophyll content and total nitrogen in cucumber cotyledon. Increase in nitrogen content and chlorophyll content at lower concentrations of SA indicates that this play a regulatory role during the biosynthesis of active photosynthetic pigment.

The effects of SA on the alteration pathway respiration in slice and isolated mitochondria of dormant and dormancy breaking potato tubers was compared (Wen and Liang 1994). The involvement of the alterative pathway was enhanced by SA to a greater extent in dormancy breaking potato tubers. Application of SA to oil seed rape plants increased the concentration of glucosinoles in their leaves (Kiddle et al. 1994). Khan et al. (2003) found that spraying of SA and ASA (10^{-5} \text{ mol L}^{-1}) on the leaves led to an increase in the overall photosynthetic yield of soybean and corn. Pancheva et al. (1996) demonstrated that long term treatment (7 days) of barley seedlings with SA decreased the rate of photosynthesis. They also observed that to reduce leaf area (secondary leaf), root growth, as well as protein and chlorophyll (a + b) amount parallel to an increase in its concentrations in barley plants which were developed from barley seeds germinated in SA solutions of varying concentrations.

### 2.1.6 Growth Retardants

Plant growth retardants are known to be inhibitors of gibberellic acid biosynthesis and this may inhibit the seed germination. These synthetic organic chemicals cause a retardation of cell division and cell elongation by blocking specific sites in pathway of hormone biosynthesis without eliciting substantial growth malformations. The earliest group of retardants to be developed was the ammonium compounds. The most important retardants in agricultural terms are Chloromequat chloride (CCC) and mepaquat chloride. The application of growth
retardants, such as CCC, MH and AMO-1618 caused shortening of internodes and reduction in shoot height in treated plants and also suppressed mitotic activity of plants (Mitchell et al. 1949, Marth et al. 1953, Tolbert 1960, Riddell et al. 1962, Schoene and Hoffmann 1949, Sachs et al. 1960). Treatment with CCC reduces the GAs content of wheat seedlings (Smith 1982). Audus (1972) classified them as growth suppressors ‘that include at the one extreme such substances as Maleic hydrazide’ (MH) which can completely inhibit some phases of cell growth and thus produce considerable modification of plant form and at the other hand so called growth retardants whose action without gross formative effects. The compounds generally have very similar effects on plants though they have different chemical structures.

2.1.6.1 Chloro coline chloride (CCC)
Chloro coline chloride (CCC) belongs to quaternary ammonium compound groups of synthetic chemicals. Since its discovery in the 1960s, CCC has become one of the most widely utilized commercial plant growth regulators in the world, because it exhibits low toxicity and exerts its effects on many crop plants.

Growth retardants have been found to increase the salt tolerance of crops (Marth and Frank 1961). CCC retards stem extension without any physiological aberration. Application of this chemical neither suppresses the growth permanently nor causes any deleterious effect on the vigour of the treated plants.

\[
\text{CH}_3 \\
\| \\
\text{Cl. CH}_2. \text{CH}_2 - \text{N}^+ - \text{CH}_3, \text{Cl} \\
\| \\
\text{CH}_3
\]

**Fig:** Chemical structure of (2-Chloroethyl) trimethyl ammonium chloride (CCC)
Ever since the discovery of this growth retarding chemicals, there has been substantial evidence to demonstrate its potential to decrease the plant height by reducing the internal length and slowing down cell division or cell elongation.

**CCC on seed germination:**
CCC strongly inhibited the lettuce seed germination (Wittwer and Tolbert 1960). Zeevart (1966) found that treatment of *Pharbitis nil* with CCC both prior to and following anthesis resulted in a marked reduction of GAs level. CCC could suppress germination induced by gibberellins and red light (Cathey and Stuart 1961). A small but significant increase in germination of seeds treated with CCC at a concentration of 500 ppm was reported before planting (Michniewtez and Kenteer 1965). Kende *et al.* (1963) provided the first direct experimental evidence that plant growth retardants CCC and AMO-1618 were potent inhibitors of gibberellins synthesis in cultures of *F. moniliforme*. Majeed and Beno (2006) studied the role of CCC in breaking potato tuber dormancy. The effect of CCC on potato tuber was highly stimulatory in stolon formation, chlorophyll synthesis and tuberization (Majeed and Bano 2006).

Sengupta *et al.* (1979) studied the effect of CCC on seed germination of groundnut M-13 and observed a slower rate of seed germination when the seed were soaked with CCC after the completion of its dormancy period. The higher concentrations of CCC (5000 to 10,000 ppm) almost completely inhibited germination. On the other hand GA₃ caused germination to those seeds which remained ungerminated due to CCC treatment. The effect of GA₃ was more when lower concentration of CCC was used. Murti *et al.* (1979) reported that soaking of seed tubers of potato in 2000-6000 ppm CCC solution had no marked effect on plant growth.

**CCC on seedling growth:**
In many plant species CCC retarded the plant height. Thomas (1964), Hook *et al.*, (1973) experimentally proved that CCC influences the formation and
development of cells in plant stalk. Treatment with CCC reduced shoot and stolon growth and dry weight but promoted tuberisation. Chakraborty (2001) reported that CCC inhibited shoot length and exhibited increased leaf numbers, branches and yield in groundnuts in comparison to the control. Bora (2002) also observed similar results in pea and soya bean with CCC treatment. In field trial on a variety of rye and other plants elongation of four internodes was found to be reduced which was due to decreased cell extension and cell division. It was reported that the walls of parenchyma cells of CCC treated plants were thinner and those sclerenchyma cells were thicker compared to the cell walls of the control (Kosher et al. 1982). Retardation of shoot growth under the influence of CCC was reported by Murti et al. (1979), Brav and Singh (1985) and Volkova et al. (1985).

CCC retarded the stem elongation up to 500 µg/ml (Sharma 2005) in Brassica campesteris and this finding of reduction of stem length substantiates the earlier results of Deka (1988) in sweet pepper, Kumari et al. (1990) in sunflower, Chakraborty (2001) in groundnut, Sarma (2001) in Coleus, Bora (2002) in legumes and Sarkar (2005) in sweet potato. Nambair et al. (1976) observed decreased vine length, vine weight of sweet potato cv H-42 with the increase in number of tubers/plant. GA increased the plant height of soybeans cv Davis where CCC and SADH reduced length, but increased the number of leaves (Castro and Moraes 1980). The increasing CCC concentration decreased plant height (Misra and Malik 1980). Spring varieties of oil seeds rape (Brassica alba, B. Juncea, B. Compesteris) exhibited reduced plant height and internode length but increased thickness of stem on application with CCC (Sharma 2005).

Treatment of tomato plants with CCC reduced their growth in height and dry weight and increased the number of flowers formed in the first inflorescence (Abdul et al. 1978). Mohsin and Smith (1972) with beans found decrease in fresh and dry weight as a result of the application of $10^{-4}$ M to $10^{-3}$ M CCC on 7 day old seedlings. However, the leaves were observed to be thicker and greener. Combe (1970) found that the localized application of CCC, grape inflorescence increased fruit set, but inhibited shoot growth. The important growth attributes of groundnut
except the height were substantially increased as a result of CCC application (Chakraborty 2001). CCC showed inhibitory effect on elongation growth of sweet potato (Sarkar 2005). The rate of inhibition was increased with increasing CCC concentration. Identical results were also reported by Gowda and Gowda (1983) and Abdul et al. (1985) in different types of crops.

CCC was found to be useful in reducing the foliage and creating a better environment for seed formation and its growth (Pando and Srivastava 1985). Foliar spray of CCC in cotton plant temporarily arrested vegetative growth but development better root system. Dunberg and Elliasson (1972) reported that CCC at the concentrations of 300, 100, 10 and 1 ppm caused growth retardation in young seedlings of Picea abies when added to the nutrient medium.

CCC reduced the plant height of cow pea significantly and increased the branch number, leaf number and dry matter percentage. CCC shows down the growth of potato stem and stolon, accelerates tuberization, increases yield especially of earlier harvested and increased leaf area, chlorophyll contents and photosynthetic productivity (Nemchenko et al. 1981). Such response of potato to applied retardant was also recorded by Hruska et al. (1970), Popravko (1976), Islam (1984) and Haque (1987).

CCC inhibited auxin induced expansion growth of tuber slices of artichoke and the inhibitory effect of CCC was eliminated by GA₃ (Kamisaka and Masuda 1968). Nanda et al. (1968) observed that GA₃ and CCC affected stem growth by increasing the length of individual internodes and the number of internodes that respond to the treatment and not by increasing number of internodes produced on the plant. This is an indication that CCC interacts with GA₃ in control of elongation of hypocotyls of plants (Nwachuka and Lockhart 1964). Sarma and Deka (1977 b) reported antagonistic effects between GA₃ and CCC on the extension growth of bean hypocotyls segments.

CCC application at a higher concentration (2000 ppm) reduced internodes length and developed fewer nodes than control shoots. The number of nodes was probably decreased because of CCC caused terminal bud to become relatively
dormant. CCC retarded shoot growth of grapes as it decreased the internodes length but increased the number of leaves (Sedletskii et al. 1980).

Dale and Felipe (1968) obtained a redistributatation of growth between Phaseoleus vulgaris leaves (enlarged) and internodes (shortened) when treated with CCC. Lovelly (1968) concluded that competition for nutrients might be a factor controlling the extent of GA enlargement of potato leaves. CCC treatment increased the photosynthetic activity of leaves of Phaseolus vulgaris (Davely et al. 1970). CCC applied in barley and bean plants caused decreased in growth (Kirk Han et al. 1972). CCC treated barley seeds showed inhibited seedling growth during the initial stage of growth phase (Rutgar1965). CCC prevented the synthesis of gibberellins and it is proposed that their action on the whole plant is due to its effects of induction of tolerance of various stress condition. CCC reduced the soybeans plant height but increased the number of leaves. CCC treatment on wheat caused shorter and thicker stem, broader and green leaves, earlier and stronger tillering and more uniform growth of (Tolbert 1960 b).

Prasad and Prasad (1994) reported reduced plant height, leaf area and ball per plant with an increase in ball weight by CCC spraying in cotton. Arora et al. (1998) reported that CCC applied at 50 per cent flowering stage increased number of flowers, pods, pod set percentage, number of seeds, seed size and yield per plant along with higher harvest index in chick pea. Godha et al. (2000) reported reduced plant height and reversed the GA induced increased in fresh weight of flower in chrysanthemum with CCC application. Reduction of plant height with subsequent increase in branching and yield by the application of CCC was reported by Devi (2002).

**CCC on chlorophyll synthesis:**

CCC treatment improved the chlorophyll content of barley (Cheema et al. 1975), groundnut (Chakraborty 2001), coleus (Sarma 2001) and sweet potato (Sarkar 2005). CCC treatment increased appreciably chlorophyll a and chlorophyll b content of leaves (Virgin 1955) and total sugar content in Egyptian grapes during
storage. That CCC treatment improved the chlorophyll content of barley along with increase in yield was observed in CCC treated tomato (Emmerikh and Gordeeva 1982). In the tubers, CCC increased starch content by about 11 per cent compared to untreated control, whereas GA$_3$ decreased starch content by 13 per cent. In CCC treated crops, the higher chlorophyll content of the leaves with reduced stolon length promoted efficient sucrose supply to the tubers. Amutha and Rajendra (2001) observed increased reducing sugar in seedless grapes. Lynrah et al. (2002) observed increase in protein, carbohydrate and fat content as a result of CCC application.

Choudhary et al. (1976) and Haque (1987) reported higher yield of potato tuber by CCC treatment at 1000-2500 ppm. That foliar application of CCC increased the yield of sweet potato was reported by Fouly et al. (1971), Nambiaon et al. (1976), Chhonkar et al. (1977), Indira et al. (1980), Fatima et al. (1982), Sarma (1987) and Sarkar (2005). Highest seed cotton yield was obtained with 40 and 160 ppm CCC in variety of J-250 and Lohit. Increase in yield of potatoes after giving a foliar spray of 500-1500 ppm of CCC was also reported by Misra and Misra (1986). The effect of CCC on growth and grain yield of several crops has been reported during last thirty years (Lovett and Orchard 1977). In those plants where excessive vegetative growth causes low grain yield is due to poor carbon partitioning.

Srivastava et al. (2001) observed the number of pod/plant, grain/pod and 1000 grain weight significantly higher due to CCC application in chick pea. Bhattacharyya (1990) and Sarma (2001) also found similar effect on growth and yield of Coleus parviflorous.

2.1.6.2 Maleic hydrazide (MH)

Maleic hydrazide (MH) is a plant growth retardant which is formulated as dispersible granules and liquid formulation. The MH compound was first evaluated in 1976. Its main uses were for suppression of sprouting in stored
potatoes and bulb vegetables. The control of sucker on tobacco and of volunteer potatoes in crops and as a grass growth retardant in non-crop situation.

MH may readily enter through either the leaves or roots and be rapidly transported to regions of meristematic activity and shows; a) loss of apical dominance; b) expansion of leaves already formed, often to a size greater than controls; c) production of a noticeably darker green colour than the controls; d) increase in production of anthocyanin pigmentation; and e) some chlorosis. Subsequent behavior depends to a large extent upon the amount of MH given. At the lowest concentrations, 0.05 per cent axillary buds of seedlings begin to grow to very soon after treatment. At the highest concentrations, of MH (0.2%-0.4%) vegetative bud developments are slow to occur and may not occur at all.

![Structure of Maleic hydrazide (1, 2-Dihydro-3, 6-pyridazinedione)](image)

**Fig:** Structure of Maleic hydrazide (1, 2-Dihydro-3, 6-pyridazinedione)

Root as well as top growth is affected by MH. Roots were less affected than the tops. Seedlings are most sensitive to MH, but it can inhibit growth at any stage up to maturity without killing the plant. Generally, in the plants tested, a spray of 0.4 per cent concentration will cause all meristematic growth to cease (Aubrey *et al.* 1950).

Many plant species are likely to develop undesirable soft shoots when exposed to high temperatures following a long period in cold storage. Such development, the cost of cold storage facilities, might be avoided if nursery stock could be kept dormant by spraying nursery plants in fall or early spring. MH is effective as a low temperature spray on colorant nursery stock and may be a useful material for this purpose.
Since the discovery, MH has an effect on plant growth (Schoene and Hoffman 1949). In general, most reports agree that MH differs from most growth controlling chemicals in that it causes only inhibition of growth with no formative effects (Naylor and Davis 1950, Nickell 1952, Wittwer et al. 1950, Zuckel 1950a, b). Lange (1961) observed that MH has an inhibitory effect of germination on *Carica papaya*. MH treatment of potato tuber prevents sprouting and maintains the original high quality. According to Wittwer et al. (1950) sprouting was practically nil at higher concentration (2500 ppm) of MH. At lower concentration (500 ppm) of MH also significantly retarded sprouting of carrots and onions. The inhibitory effect of MH was observed in tomato by (Kumar 1979).

General effects of MH upon 11 species belonging to 5 distantly related families of plants have been determined. MH effects on germination and subsequent growth of rumex seeds were inhibitory. The presence of MH caused both the percentage of germination and subsequent primary root growth to be decreased. MH has remarkably similar effects on monocotyledons and dicotyledonous plants. Seedlings are most sensitive to MH; but it can inhibit growth at any stage up to maturity without killing the plant (Nooden 1969). Generally, in the plants tested, a spray of 0.4 per cent concentration will cause all meristematic growth to cease. Gibberellic acid and MH show significant effects on *Trifolium alexandrinum* seeds during germination (Bhatia et al. 1974). *Rosa dilecta* sprayed with 0.3 per cent solution of MH were effectively inhibited.

The effect of maleic hydrazide was first described by Schoene and Hoffman in 1949. Dilute solutions of MH inhibited growth of tomatoes and various grasses. Growth was inhibited for a few days to several months depending upon the concentration of the chemical. Stem extension of dwarf peas was inhibited by MH. At low doses MH broke apical dominance and developed side branches of peas (Brian and Hemming 1957). Knott (1978) found that growth in a *Pyracantha hedge* could be controlled by spraying with 0 to 0.5 per cent solutions. David and Wayne (1952) reported that the treatment of MH on cotton showed characteristic effects in vegetative growth. MH reduced growth in height,
number of nodes on main stem and length of vegetative branches. They also observed MH effects on vegetative and reproductive character in cotton plant. Nickell (1953) observed the effects of MH on normal and a typical growth of *Rumex acetose* (serrol). Chakraborty (1957) reported suppression of extensive growth loss of apical dominance and profuse branching in *Brassica* species by foliar spraying of 0.2 per cent MH. Mathur and Jadav (1975) reported that MH may inhibit growth by interfering with nucleic acid metabolism in surface sterilized colonies of *Spirodella polyrrhiza* and indirectly suggest a blocking action of MH on the biosynthesis of nucleic acid in plants. Tayal and Gopal (1976) reported that morphactin and MH both retarded germination and seedling growth of *Trigonella foenumgraecum* L. and had a synergistic effect in combination. MH also greatly suppressed cotyledon stage seedling growth and development of *Pinus resinosa* (Kozlowski 1985). Suryanarayan (1977) used MH (100-200 ppm) on monsoon and winter cultivations of peanut and observed decreased plant height. Venketeswarlu *et al.* (1984) reported that MH (50 ppm) enhanced pod yield and other yield attributes in groundnut. Singh *et al.* (1978) studied the effect of MH (250 ppm) on some yield contributing parameters in m-13 plants and observed enhanced number of gynophores per plants than control.

In addition to controlling sprouting of potato tubers, MH has been found to increase the food value and quality of tubers. MH treated tubers held at low temperatures accumulate less sugar and produce lighter coloured potato chips than the non-treated control. MH treated potato results in low contents of reducing sugar.

Application of MH too early in the growing season can cause a cracking of the bud end and in some cases an internal brown spot flaking on the apical end of the potato tuber. MH suppresses external and internal mode of action. Because of its internal mode of action, MH is the only chemical that will prevent tubers left in the field at harvest from growing into volunteer plants the next year (Nooden 1969). If allowed to grow, these volunteer plants serve as a source of leaf roll virus, which reduce yields and cause an internal discolouration. Inhibition
of volunteer growth of potatoes reduces the potential sources of leaf roll virus inoculum by as much as 98 per cent. There is no alternative sprout inhibitor that prevents volunteer potatoes, since all other inhibitors are applied after the tubers have been harvested and removed from the field.