CHAPTER-I

REVIEW OF LITERATURE

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1. Indole-3-acetic acid (IAA) :

a) <u>Introduction</u> :

Auxins are one of the most important groups of plant hormones because of their many-sided roles in plants. These substances were also the first growth factors identified as plant hormones.

Kogl, Erxleben and Haagen-Smit (1934) isolated a compound from human urine. It was named as hetero auxin. It is also called Indole-3-acetic acid (IAA, $C_{10}H_9O_2N$). IAA is the principal naturally occuring auxin in all higher plants.



A number of workers like Skoog (1954), Masuda <u>et al</u>. (1967) and Nooden (1968) have found that the action of auxin in regulating growth is associated with nucleic acid metabolism. The action of auxin on growth is very close to the gene level. The auxin is believed to release DNA template from the genes



Groundnut (<u>Arachis</u> <u>hypogaea</u> L.)

(mucleo histone) for mRNA synthesis. It would then cause the formation of an enzyme (protein) which would bring about insertion of new materials into the cell wall. This would result in increased wall plasticity and extension. It is also suggested that auxin may act at a membrane level, causing alterations in membrane properties and functions.

b) <u>Responses of Groundnut to IAA</u> :

i) Seed germinability :

Sanjeevalah <u>et al</u>. (1967) reported that there was highest germination rate in groundnut seeds when they were treated with IAA solution. Chellappa and Karivartharaju (1973) also observed that the presowing soaking treatment of groundnut seeds with IAA resulted in highest germination percentage in groundnut.

ii) <u>Vegetative growth</u> :

Chellappa and Karivartharaju (1973) studied the effects of presowing soaking treatments with phytohormones on the yield of groundnut. They observed increase in root length in groundnut seedlings when seeds were soaked with 5 ppm IAA solution before sowing. Murty and Venkateswarlu (1975) investigated the effect of auxin and auxin antagonists on groundnut plant. They observed that presowing soaking treatment with IAA solution resulted in increase in length of hypocotyl and cotyledonary stalk, erect orientation of the cotyledons and suppression of cotyledonary buds. Salim and Oryem-Origa (1981) observed highest rate of root growth (16.8 mm/day) after the application of IAA solution (5 x 10^{-10} M).

iii) <u>Reproductive growth</u> :

In 1973 Chellappa and Karivaratharaju studied the effects of presowing soaking treatments with phytohormones on the yield of groundnut. They observed increase in flower number in groundnut when seeds were soaked with 5 ppm IAA solution for 12 h before sowing. They further reported that presowing soaking treatment of groundnut seeds resulted in increase in the rate of pegformation. Manzava (1979) studied the effects of exogenous application of growth substances on the regulation of gynophore elongation and fructification in groundnut CV Spanish Bunch. He observed enhancement in fructification and inhibition of gynophore elongation after the application of IAA.

iv) <u>Yield</u> :

Sanjeevaiah <u>et al</u>. (1967) reported that IAA application was useful in obtaining maximum pod yield in groundnut cultivar H.G.S. Chellappa and Karivaratharaju (1973) also noticed that presowing soaking treatment with IAA resulted in highest pod yield. These workers further observed increase in 100 seed weight of groundnut when the seeds were given soaking treatment with IAA before sowing. Rao (1980) studied the effects of presowing soaking treatment with phytohormones on the yield and nutritive value in legumes. He observed increase in number and size of pods and pods and seed yields of groundnut when seeds were soaked in 10 ppm IAA solution and mixture of IAA and NAA solution before sowing.

v) <u>Oil Content</u> :

Sanjeevaiah <u>et al</u>. (1967) investigated influence of IAA application on groundnut CV H.G.8. They observed increase in oil content of groundnut seeds after the application of IAA. Chellappa and Karivaratharaju (1973) reported that presowing soaking treatment with IAA resulted in increase in oil content of groundnut seeds.

2. <u>Gibberellins</u> (GA) :

a) Introduction

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Gibberellins form one of the important groups of plant hormones. The history of the discovery of the gibberellins dates back to the 19th Century when the Japanese farmers noticed that in the rice fields, certain diseased plants grew abnormally thin and tall. The disease was named as Bakanse disease or foolish seeding disease. The abnormal growth in rice plants was due to the infection of a fungal strain <u>Gibberella fujikuroi</u>. Kurosawa (1926) applied filtrates of fungus culture to healthy rice seedlings. By conducting number of such experiments he concluded that the filtrate obtained from fungus culture was able to cause infection of Bakane disease to healthy seedlings of rice. Yabuta and Hayashi(1939) made successful attempts to isolate pure crystalline chemical which they named 'Gibberellin'. These outstanding works of Japanese workers remained unnoticed outside Japan for quite sometime due to World war II. After 1950 the study of Gibberellin was carried out outside the Japan. Uptil now about 75 gibberellins are isolated from various plant species. They are chemically known as gibberelic acids. The gibberellins are widely distributed in nature. They are present in algae, fungi, mosses, ferns, gymnosperms and angiosperms. They are concentrated in growth regions of the plants such as stem apex, young leaves and seeds. The gibberellic acid has gibbane ring skeleton.

Structure :



Now a days gibberellins have got great importance in agriculture. They are used to increase the yields of various crops and fruit plants, they are used to improve the qualities of fruits due to their ability to induce parthenocarpy in fruits, stem elongation, as well as they are useful in promoting flowering in long day plants and breaking the dormancy of buds of potato tubers and seeds of many plants.

- b) Responses of groundnut to GA :-
- i) Seed Germinability :

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Chellappa and Karivaratharaju (1973) reported that presowing soaking treatment with GA resulted in increase in germination percentage of groundmut seeds. Narasimha Reddy and Swamy (1976) investigated the influence of various growth-regulators on the germination of dormant groundnut seeds. They noticed that the effect of GA in breaking dormancy was very weak, although combination of GA and kinetin gave greater radicle length. Sengupta et al. (1979) have also studied the effect of growth regulators on seed germination in groundmut. They observed that application of 0.1 mg GA/lit. increased germination percentage in one week dormant and seed coat removed groundmut seeds. They also reported that inhibitory effect of chloromequat could be reversed by the application of 200 ppm GA 3. According to Joshi et al. (1978) GA is moderately effective in breaking dormancy in Trombey groundnut seeds.

ii) <u>Vegetative growth</u> :

Rabechault and Guenin (1967) studied the effect of GA on two groundmut varieties CV 28-204 (spanish type, early, semierect with non-dormant seeds) and CV 28-206 (virginia type, late, prostrate, dormant seeds). They observed that there was significant increase in length of main stem and branches mainly due to the elongatum of internodes after the application of GA treatment at the rate of two treatments per week. They also

noticed that this elongation effect was greater in young stages and was observed mainly in the main stem of CV 28-204 and in the branches of CV 28-206. Sreeramlu and Rao (1968) noticed that application of exogenous GA 3 (0.1 mg/lit.) increased growth of dormant embryonic axes. Chellappa and Karivarathraju (1973) reported that presowing soaking treatment of groundnut seeds with GA, induced root elongation in seedlings. Narasimha Reddy and Swamy (1976) reported that combination of GA 3 and kinetin gave greater radicle growth in groundnut CV TMV 3, then seeds treated with GA 3 alone. Suryanarayan (1977) has noticed that there was increase in plant height after the application of GA. Gardner (1983) also observed an increase in length of stem and petioles of greenhouse and field grown groundmut CV Florunner and Dixie Runner after the application of GA. Thus the above studies have clearly demonstrated that GA application promotes vegetative growth in groundnut.

iii) <u>Reproductive growth</u> :

Rabechault and Guenin (1967) reported that application of GA resulted in increase in number of flowers and gynophores (peg). Chellappa and Karivarathraju (1973) studied the effects of presowing treatment of GA on groundnut and reported that there was increase in number of flowers and pegs in GA treated groundnut. Gurubaksh Singh <u>et al</u>. (1978) noticed that there was increase in number of gynophores per plant in groundnuts after the application of 250 ppm GA in the form of foliar sprays. Manzava (1979) studied

the effects of exogenous application of growth regulators on the regulation of gynophore elongation and fructification in groundnut CV Spanish Bunch type. His findings revealed that GA promoted gynophore elongation and postponed fructification.

iv) <u>Yield</u> :

Chellappa and Karivaratharaju (1973) noticed that presowing soaking treatment of groundmut seeds with GA resulted in increase in pod yield and hundred seed weight. However, not much attention has been paid to the effect of GA post treatment in this respect.

v) <u>Oil Content</u> :

Chellappa and Karivaratharaju (1973) reported that there was increase in seed oil content in groundnut when seeds were treated with GA before sowing.

vi) Physiological changes

Vyas <u>et al</u>. (1965) studied the influence of pre-germination chemical treatment of GA on ascorbic acid content and ascorbic acid oxidase content in germinating groundmut seeds. They observed that ascorbic acid oxidase appeared after 3 days and it reached a maximum on the 10th day and then diminished, during germination of groundmut seeds pre treated with GA (30 mg/100 ml).

3. Cytokinins :

a) <u>Introduction</u> :

Miller (1954) was the first to isolate the first crystals of a cell-division inducing substance from autoclaved herring sperm DNA. Later Miller <u>et al</u>. (1956) found this substance to be very effective in causing cell division even in very low concentrations (1 part per billion) when auxin was also present in the medium. Since the substance had specific effect on cytokinesis, it was named Kinetin. The term cytokinin was proposed by Letham (1963). Skoog, Strong and Miller (1965) have defined Cytokinins as chemicals which, regardless of their other activities, promote cytokinesis in cells of various plant organs.

Kinetin has been found to be a derivative of the purine base adenine which bears furfuryl substituted at the 9th position which migrated to 6 position of the adenine ring during autoclaving of DNA. All the cytokinins have purine (adenine) ring with a side chain of \mathbb{N}^6 position (aminosubstituted adenine). Structure :



It resist decay in senescent and detached organs.

The cytokinins may accelerate or promote :

- i) Cell division and related DNA and RNA synthesis.
- ii) Cell enlargement in leaves (they may also inhibit it).
- iii) Leaf bud formation (eliminating the related polarity).
- iv) Root formation and root growth (inhibition occassionally).
 - v) Germination.
- vi) Breaking of dormancy.
- vii) Respiration.
- viii) Translocation of low molecular weight nitrogen compounds. They may also
 - ix) inhibit protein degradation and
 - x) influence leaf shape and pigments.

Cytokinin treatment has been shown to result in increase in the content of cellular RNA and protein content experiments with inhibitors have also demonstrated similar results kinetin promotes the synthesis of some enzymes and suppresses the activity of other enzymes (viz. the nucleic acid degrading enzymes, ribonuclease and deoxyribonuclease). Kinetin preserves protein in detached leaves by increasing RNA and protein synthesis. Recently it has been shown that transfer RNAs yield active cytokinins on acid hydrolysis or degradation of enzymes. These experiments tend to support the view that the cytokinins like gibberellins act at the level of genes concerned in the growth and differentiation of plants.

b) Responses of groundnut to Cytokinins :

i) Seed Germinability :

Ketring and Morgan (1971) studied the effects of growth regulators on germination of dormant virginia type groundmut. They observed that kinetin induced extensive germination. Narasimha Reddy and Swamy (1976) studied the effects of various growth regulators on the germination of dormant groundmut seeds. They noticed that kinetin was highly effective in breaking dormancy even in presence of seed coat. They also reported that inhibitory effects of ABA and seed coat were reversed by kinetin. Joshi et al. (1978) reported that kinetin $(10^{-5} \text{ M} \text{ and } 10^{-2} \text{ M})$ was more effective in breaking dormancy of Trombay groundmut seeds. Sengupta et al. (1979) noticed that dormancy of freshly harvested groundnut seeds was removed by kinetin. They also reported that kinetin could reverse the inhibitory effect of ABA on seed germination. However, it could not counteract the inhibitory effect of CCC on seed germination. Sengupta and Sharma (1986) reported that kinetin strongly promoted the germination of groundmut seeds.

ii) <u>Vegetative growth</u> :

Narasimha Reddy and Swamy (1976) studied the effects of various growth regulators on the germination of dormant groundmut seeds. They noticed that combination of GA 3 and kinetin gave greater radicle growth than the untreated control or kinetin alone.

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iii) <u>Reproductive growth</u> :

Ketring and Schubert (1979) investigated the effect of cytokinin containing plant extract "Cytex" on groundnut cultivars, florunner, Starr and Tamut-74. They observed no significant effects on groundnut reproductive potential after the application of 100 ppm. Kinetin at early flowering stage, late flowering and pegging stage or both.

iv) <u>Yield</u> :

Ketring and Schubert (1979) studied the effects of cytokinin containing plant extract "Cytex" on groundmut yield. They noticed that cytex was not effective in increasing the yield of groundnut.

v) Physiological changes :

Ketring and Morgan (1971) reported that kinetin could stimulate CO₂ production and ethylene production in germinating Virginia type groundnut. Narasimha Reddy and Swamy (1976) found that kinetin increased water uptake and mobility of food reserves in the cotyledons prior to translocation to the embryos. Sengupta and Sharma (1986) studied the effect of kinetin on glutamate dehydrogenase activity in germinating groundnut seeds. They observed that kinetin treatment promoted the germination and it was associated with an increase in GOT (Glutamate oxaloacetate) activity. They also found that kinetin treatment decreased GDH (Glutamate dehydrogenase) activity in dormant seeds.

4) Abscisic Acid (ABA) :

a) <u>Introduction</u>:

Abscisic acid is a naturally occuring growth inhibitor. There are several reports of accumulation of this inhibitor in plant tissues under the conditions of environmental stresses. It has a wide range of physiological effects such as inducing promotion of senescence and abscission of plant organs, induction of seed dormancy and retardation and inhibition of plant growth. Osborne (1955) for the first time found that senescent leaves contained diffusible abscission-accelerating substance Robinson et al. (1963) made successful attempts to isolate inhibitory substance and called it dormin. Addicott et al. (1964) isolated several abscission-accelerating substances from cotton plants which they named abscisin-I and abscisin-II. Abscisin-I was isolated from the burs of matu re cotton fruit, while abscisin-II was isolated from immature cotton fruits. Ohkuma et al. (1965) determined the chemical structure of abscisin-II. The structure was confirmed by Cornforth et al. (1965). They also showed that dormin and abscisin-II were identical. Afterwards in 1967, it was decided that abscisin-II and dormin should be named as abscisic acid (ABA).

Chemical structure



- b) Responses of Groundnut to ABA :
- i) Germinability :

Ketring and Morgan (1971) reported that ABA inhibited germination in seeds of dormant Virginia groundnuts. Allfrey and Northcote (1977) also reported similar observations. However, Sengupta <u>et al</u>. (1979) reported that application of ABA inhibited seed germination of the non-dormant cultivar but failed to show similar effect in the dormant cultivar. Sengupta and Sharma (1986) reported that ABA inhibited germination in groundnut seeds.

ii) Physiological changes :

Ketring and Morgan (1971) reported that ABA inhibited ethylene production but increased CO₂ production in germinating seeds of dormant Virginia type groundnuts. Allfrey and Northcote (1977) noticed that ABA treatment was effective in increasing activities of enzymes lipase, isocitrate lyase and amylase in germinating groundnut seeds. They also reported that there was increase in the process of starch breakdown in presence of ABA. These findings indicate favourable influence of ABA in metabolism of germinating groundnut seeds. Sengupta and Sharma (1986) reported that ABA induced dormancy in non-dormant seeds was accompanied by increase in GDH (glutamate dehydrogenase) and decrease in GOT (glutamate oxaloacetate transaminase) activity.

5. Ethylene

a) <u>Introduction</u> :

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It is said that the use of smoke to bring about floral initiation in pineapp le was actually discovered in 1893 when a carpenter working in a greenhouse in the Azores accidentally set fire to a pile of shavings. To the surprise of the grower who had thought his plants were ruined, they burst into flower instead of being damaged. By the 1920's, it was a recognized fact that pineapple could be forced to flower by smoke from fires (used during cold weather to prevent stoppage of growth) and that this effect was caused by the smoke's content of unsaturated gases such as ethylene. By the 1930's ethylene was shown to accelerate flowering in pineapple.

Ethylene is the only gaseous hormone which stimulates transverse or isodiametric growth. It's ability to induce fruit ripening was known for a long time but its recognition as a growth regulator came only recently. Ethylene has simplest chemical structure ($CH_2 = CH_2$) and is synthesized from methionine, β -alanine or isoamyl alcohol. It inhibits cell elongation in roots and induces short and wide cells. Several ethylene releasing substances like etheral or ethephon are available in market.

Structure :

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- b) Responses of groundnut to ethylene :
- i) <u>Germination</u> :

Ketring and Morgan (1971) studied the effect of ethephon on germination of groundnut seeds. They observed that ethephon increased germination of the more dormant basal seeds to a larger extent than that of the less dormant apical seeds.

Clark (1971) reported that contact with a 10^{-3} M solution of ethral (Ethephon) for 1-3 days was totally effective in inducing germination of dormant seeds of Virginia type groundnut CV NC 13 and Spanish type CV Starr. He also notic ed that there was requirement of 3 to 4 days ethephon treatment for inducing germination in freshly harvested seeds of CV NC 13 but after a storage for 1 month, a day treatment was sufficient. Bailey and Bear (1973) found that aqueous solution of ethephon $(1 \times 10^{-3} M)$ or a slurry in conjunction with theram dust as a presowing seed treatment was effective in breaking dormancy of groundnut seeds. Ketring (1975) studied the germination of NC-13 Virginia type groundnut seeds in the presence of inhibitors and ethylene. He noticed that when seeds were imbibed in cyclohexanide-6-methylpurene or 6-azauracil (protein and mucleic acid synthesis inhibitors) failed to germinate even after ethylene treatment. However, there was hundred percent germination in water imbibed seeds after ethylene treatment.

Ketring (1977) reported that in field trials treatment of groundmut seeds with 1% ethrel was effective in inducing more

than 95% germination. Joshi <u>et al</u>. (1978) noticed that ethephon was effective in breaking dormancy of Tromhay groundnut seeds. Gautreau (1980) also reported that powder or liquid formulations of ethephon could break dormancy in groundnut seeds. All the above observations indicate that ethylene has tremendous capacity of breaking dormancy in groundnut seeds.

ii) <u>Vegetative growth</u> :

Clark (1971) observed that continuous exposure to ethephon solution produced adverse effects in groundnut seedling. He noticed that such exposure caused retardation in secondary root development and some root tips became yellowish. Krishnamoorthy (1972) reported that when 15 days old seedlings were sprayed with solution of 125-500 mg ethrel (ethephon)/1 then there was inhibition of root nodule formation. Ziv <u>et al.</u> (1976) found that treatment of runner type groundnuts with ethrel (ethephon) gave plagiotropic orientation of branches and bushy habit. Thilsted and Santelmann (1977) found that application of ethephon at the rate of 1 lb/ac at the time of pegging stage was effective in reducing foliage growth.

iii) <u>Reproductive growth</u>

Ketring and Schubert (1978) studied effect of ethrel on growth, flowering and fruiting of groundnut. They found that use of ethrel at the rate 0.5 lb a.e/acre to 10 week old plants was effective in inhibition of late flowering and delaying in

fruit maturation. Manzava (1979) reported that ethephon inhibited gynophore elongation but enhanced fructification in groundnut. Krishnamoorthy (1972) reported that when 15 days old seedlings were sprayed with solution of 125-500 mg ethrel/1 there was inhibition of flowering.

iv) <u>Yield</u> :

Ketring (1978) found that application of ethrel to 4 week old groundmut plant was effective in reducing seed yields. However, there was no effect on seed yields when ethrel was applied to 10 week old groundmut plant.

v) Physiological changes :

Ziv <u>et al</u>. (1976) reported that there was increase in GA like substances and a decrease in growth inhibitors when groundnut plants were treated with ethrel.

6. Indole-3-butyric acid :

a) Introduction :

Indole-3-butyric acid (IBA) is a compound similar to IAA in many respects.

Structure :

СH₂- CH₂- CH₂- COOH

- b) Responses of groundmut to IBA :
- i) Seed germinability :

Mukherjee and Sen (1966) reported that there was increase in germination percentage of seeds of groundnut cultivars AH 25 and HG 8 when seeds were given presowing soaking treatment with 1 and 10 ppm IBA solution for 12 h and 24 h respectively.

ii) <u>Vegetative growth</u> :

Mukherjee and Sen (1966) studied the effects of presowing soaking treatment with IBA on growth of groundnut cultivars AH 25 and HG 8. They observed increase in plant height, number of branches, number of leaves in both varieties when seeds were pretreated with 1 and 10 ppm IBA for 12 h and 24 h respectively.

iii) <u>Yield</u> :

Mukherjee and Sen (1966) observed that there was increase in pod yield, fifty seed weight and seed size, in groundmut cultivar AH 25 (late, spreading) and HG 8 (early, bunch type) when the seeds were soaked in 1 and 10 ppm solution of IBA for 12 h and 24 h respectively before sowing.

7. <u>Napthyl acetic acid</u> (NAA) :

a) Introduction :

NAA is one of the first growth regulators to find important commercial uses in horticulture. As early as 1939 it was used in U.S.A. to prevent pre-harvest drop of apples. In 1942, it was applied to pineapples to induce synchronous flowering in Hawaii. NAA is also used for initiating adventitious root formation in cuttings. The most popular commercial formulation of NAA is 'Planofix'.

Structure -



b) <u>Responses of groundnut to NAA</u> :

i) <u>Vegetative growth</u> :

Rao and Rao (1970) studied the response of groundnut to NAA and respiratory inhibitor MH in dark. They grew seeds of groundnut CV TMV 2 for about one week in 10 ppm NAA or MH solution They observed that NAA was effective in inhibiting seedling growth more strongly than MH. Suryanarayan (1977) on the other hand noticed that there was increase in plant height when groundnut was sprayed with NAA solution.

Srinivasan and Gopal Krishnan (1977) reported that planofix an NAA formulation (40 ppm), when applied as foliar spray, 40 and 60 days after sowing to groundnut CV TMV-7 was effective in increasing nodule weight. They also reported that nodule number was the greatest with 20-40 ppm NAA. These findings indicate favourable influence of NAA on biological nitrogen fixation in groundnut.

ii) <u>Reproductive growth</u> :

Gurubaksh Singh <u>et al</u>. (1978) reported that a foliar spray of 5 ppm planofix (NAA) to groundmit (50 days after sowing) significantly increased the number of gynophores per plant.

iii) <u>Yield</u> :

Krishnamurthy (1967) studied the differential effects of hormones as foliar spray. He observed that 2 foliar sprays with NAA at concentration of 100 ppm at 24 and 9 days before harvesting groundnuts increased pod yields as compared with water sprayed controls. He also reported that the yield increase was associated with an increase in weight and number of pods and kernels per plant. Puttaswamy et al. (1976) reported that 2 foliar sprays of solution containing 15 ppm planofix (NAA) at the preflowering stage and at 15 days later increased yields of unshelled muts. Gopalkrishnan and Srinivasan (1975) reported that 2 sprays of 40 ppm NAA applied to groundnuts (40 and 80 days after sowing) were effective in increasing yield of unshelled muts and shelling percentage. Suryanarayan (1977) reported that NAA was effective in increasing pod yield of groundmut. Gurubaksha Singh et al. (1978) observed that a foliar spray (5 to 10 ppm planofix) was effective in increasing yield of unshelled nuts. Reddy (1978) studied the effect of NAA on growth and yield of various groundmut varieties. In field trials, NAA at the rate of 20-60 ppm was applied as foliar spray to three groundmut cultivar CV BH-8-18 (semispreading); CV S-230 (semispreading) and CV TMV-2 (bunch type). He observed that the highest yield was given by semispreading CV BH-8-18. He reported that average yields were higher with 40 ppm NAA than with its other concentration. He also reported that optimum NAA concentration was 40 ppm for CV BH 8-18 and S.230 while it was 60 ppm for TMV-2. These findings indicate a varietal difference in groundmut with respect to dose of this growth promoter. Rao (1980) reported that application of 50 ppm NAA increased yield components such as pod yield, shelling percentage and harvest index. All the above observations clearly indicate that groundnut yield can be positively manipulated by application NAA.

iv) Seed Oil Content : >

Gopalkrishnan and Srinivasan (1975) reported that foliar application of planofix (NAA) to groundnut increased seed oil content. Suryanarayan (1977) also observed increase in seed oil content of groundnut after the application of NAA.

v) Physiological changes :

Rao and Rao (1970) studied the metabolic changes in groundnut seeds induced by NAA. They reported that there was reduction in water uptake as well as fatty acid content of embryo axis when groundnut seeds CV TMV-2 were allowed to

germinate for about 1 week in 10 ppm NAA solution. Gopalkrishnan and Srinivasan (1975) reported that application of planofix (NAA) was effective in increasing Ca-translocation capacity. They also reported that NAA (at 40 ppm) when applied as foliar spray (40 and 60 days after sowing) to groundmut CV TMV-7, was effective in increasing total nitrogen and carbohydrate contents in the plant.

- 8. Ascorbic acid (AA) :
 - a) Introduction

Excellent work of Chinoy and co-workers (1969) has highlighted the key role of Ascorbic acid (Vitamin C) as a plant growth regulator in number of physiological processes such as germination, flowering and stress tolerance.



b) Responses of groundmut to ascorbic acid :

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i) Germinability :

Sreeramulu and Rao (1968) studied the seed germination in non-dormant bunch type groundmut (TMV-2). They observed that seed treatment with 0.35% ascorbic acid was effective in causing hundred percent germination.

ii) <u>Vegetative growth</u> :

Root nodule formation (Nodulation) :

Goswami and Garg (1978) studied the effect of Ascorbic acid on nodulation, nitrogen fixation and growth of groundmut. They noticed that number and weight of nodules were increased by 2-3 times with ascorbic acid treatments.

iii) Physiological changes :

Vyas and Patel (1968) studied the effect of growth regulators on amylase activity in groundmut seeds during germination. They found that amylase activity of seeds soaked in ascorbic acid increased during the first 10 days and then decreased. Vyas <u>et al</u>. (1969) reported that the rate of fatty acid metabolism in germinating groundmut seeds was higher after 6th day of germination in seeds which were treated with ascorbic acid (15 mg/100 ml solution). Goswami and Garg (1978) reported that root nitrogen and nitrogen content of different plant parts increased with ascorbic acid treatments.

9. 2,3.5-Tri iodo benzoic acid (TIBA) :

a) Introduction :

TIBA belongs to a group of substances which block the movement of endogenous hormones. These compounds can cause local accumulation of the hormones which, in turn, produce dramatic effects on growth and morphogenesis. Growth regulators of this type, by blocking auxin movement from terminal bud on a shoot,

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can cause the lateral buds to be released from dormancy and grow out into side shoots. This results in more bushy habit of growth that can be beneficial in some crop plants and ornamental plants. Through their effects in blocking gibberellin movement these compounds can also promote flowering in many crops. TIBA (2,3,5 triiodobenzoic acid) is such a well known transport inhibitor. Tomato plants sprayed with TIBA show spectacular effects. After application of TIBA not only side shoots are formed but all of them develop as inflorescence instead of vegetative shoots.

Structure :



b) Responses of groundmut to TIBA :

i) Germinability :

Mukherjee and Sen (1966) gave presowing soaking treatments of 10 ppm TIBA for 24 h to seeds of groundmut varieties AH-25 (late, spreading) and HG 8 (early, bunch type). They noticed that TIBA treatment produced favourable effects on the germination.

ii) <u>Vegetative growth</u> :

Mukherjee and Sen (1966) reported that presowing soaking treatment of 10 ppm TIBA for 24 h to groundnut seeds of varieties AH 25 (late, spreading) and HG-8 (early, bunch type) was effective in increasing plant height, number of branches and leaves. Murty and Venkateswarlu (1975) noticed that when groundnut seeds were soaked for 24 h in solution of TIBA (0-100 mg/1 water), there was inhibition of growth of tap root and lateral roots, downward shifting of the zone of lateral roots and formation of a spine like structures at the tip of the tap root. They also reported that application of TIBA at very low concentrations stimulated growth. However, Hartzook and Goldin (1970) observed opposite effects of Foliar application of TIBA. They reported that in groundnut CV Virginia bunch improved, Spanish and Valencia there was reduction of plant height when sprayed with 50 or 100 ppm TIBA. Bauman and Norden (1971) studied the effect of TIBA on vegetative growth. They reported that three applications of TIBA at the rate of 25 g/ha at 30, 40 and 50 days after sowing, were effective in reducing cotyledonary lateral branch length, main stem height and internode length.

iii) <u>Reproductive growth</u> :

Manzava (1979) reported that TIBA inhibited gynophore elongation in groundmut CV Spanish bunch type.

iv) <u>Yield</u> :

Mukherjee and Sen (1966) reported that presowing soaking treatment of 10 ppm TIBA for 24 h to groundnut seeds was effective in increasing the pod yield, shelling percentage and seed size and 50 seed weight in groundnut varieties HG-8 (early, bunch type) and AH-25 (late, spreading). On the other hand, Hartzook and Goldin (1970) observed that there was reduction in average pod weight of groundnut variety Virginia Bunch improved, when sprayed with 50 or 100 ppm TIBA.

v) <u>Oil Content</u> :

Mukherjee and Sen (1966) reported that presowing soaking treatment of groundnut seeds with 10 ppm TIBA for 24 h brought about an increase in seed oil content.

10. 2,4 dichlorophenoxy acetic acid (2,4-D) :

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a) <u>Introduction</u>

The chemical 2,4-dichlorophenoxy acetic acid is a white crystalline substance, very slightly soluble in water or in aliphatic oil, fairly soluble in aromatic oil (1 percent is benzene) and very soluble in ethyl alcohol and similar organic solvents. In early formulations polyethylene glycol was used to make the acid compatible with water in aqueous sprays. Now a days salts and esters and many other 2,4-D compounds are available.

In 1942 Zimmermann and Hitchcock discovered the differential herbicidal action of 2,4-D on grasses and broad leaves. <u>Structure</u> :



Mode of action of 2,4-D :

2,4-D applied to leaves involves penetration of the cuticle, absorption by living cells, migration to the vascular channels, translocation to regions of food utilization and finally a toxic action resulting in growth inhibition.

2,4-D penetrate, the cuticle and is taken up by the living parenchyma cells of the mesophyll. Here, by movement along the symplast (inter-connected system of living cells) the molecules of acid reach the phloem, and upon entry into the sieve tubes they move along with the assimilate stream from foliage to region of growth and reproduction where foods are being actively utilized (Crafts, 1961). In these regions of active metabolism the herbicide molecules are accumulated to toxic levels, and they induce cell division, cell enlargement, callus and tumor formation, tissue crushing, and if they are present in sufficient quantity, death.

b) Responses of groundmut to 2,4-Dichlorophenoxy acetic acid :

There are very few attempts to study responses of groundnut to 2,4-D application.

i) <u>Vegetative growth</u> :

Belova (1969) reported that application of 2,4-D (Sodium salt) at the rate of 0.01% was effective in retarding seedling growth. Suryanarayan (1977) reported that 2,4-D was effective in decreasing height of groundnut plants.

ii) Metabolic changes :

Belova (1969) reported that application of 2,4-D disrupted the metabolism of sugars and their flow from cotyledons to seedlings in groundmut plants.

11. Morphactins :

a) <u>Introduction</u> :

These are synthetic growth inhibitors derived from fluorenecarboxylic acid. They have a very pronounced effect on growth and development of plants. They show polyvalent action on the growth and development of plants. Morphactins are absorbed into the plants via leaf and root and are then transferred to different parts.

Morphactins are named as such because they are morphogenetically active. They inhibit the growth of almost all the parts of a plant. They inhibit the germination of seeds and growth of the seedlings. The growth of the shoot and the leaf lamina is checked. They counteract the apical dominance because of which the lateral buds grow to give the plant a bushy appearance. They inhibit the growth of rosette plants.

Morphactins in combination with 2,4-D have been found to act as excellent agents of weed control. They are given alongwith maleic hydrazide to supress growth of grasses in the lawns so that repeated mowing is not required.

Structure :



- b) Responses of groundnut to morphacting
- i) Vegetative growth :

Ketring (1977) reported that there was reduction in shoot fresh weight at the time of harvest in "Starr" Spanish type groundnut after the application of morphactin at early flowering stage.

ii) <u>Reproductive growth</u> :

Manzava and Flocker (1978) noticed that groundnut gynophores became geotropic when plants were subjected to 50 ppm morphactin application. However, the geotropic response was diminished at higher concentrations of morphactin. Manzava (1979) reported that application of morphactin to groundnut plants was effective in enhancing fructification. But, it was inhibitory to genophore elongation.

12. Maleic hydrazide (MH) :

a) <u>Introduction</u> :

Maleic hydrazide belongs to a group of compounds known as growth inhibitors. This is a group of miscellaneous compounds that differ from growth retardants in that, their effects are mainly on the apical, rather than the sub-apical meristem, and are not reversed by gibberellin. Maleic hydrazide is systemic growth inhibitor that interferes with cell division at the apex, thus inducing cessation of stem elongation and loss of apical dominance. It's primary effect seems to be on nucleic acid synthesis. It prevents sprouting in onion, potato and certain root crops. It suppresses suckering in tobacco and retards the growth of grasses along the roadways.

Structure :



b) Responses of groundmut to Maleic hydrazide (MH) :

i) <u>Germinability</u> :

Vaithialingam and Rao (1973) studied the effects of maleic hydrazide on seed germination of groundnut CV TMV-2. They observed that MH treatment reduced percentage germination of groundnut seeds to a minimum of 56% of the untreated control. These workers further reported that foliar application of 5000-2000 ppm MH-30 at 70, 80 or 90 days after sowing induced dormancy in the resulting seeds. The dormancy was greatest with 15000 ppm MH applied at 70 or 80 days after sowing. These workers further studied some physiological aspects of the resulting seeds. They reported that in general MH decreased weights of primary axes, cotyledons and embryos.

Krishnamurthy (1967) studied the effect of MH on the sprouting of kernels. He reported that 500 ppm MH as foliar spray 15 and 25 days before harvest was the most effective in reducing sprout numbers in CV Spanish Improved. Thus maleic hydrazide seems to be very promising growth retardant for inducing dormancy in groundnut seeds.

ii) <u>Vegetative growth</u> :

Vaithialingam and Rao (1973) studied the effects of presowing soaking of maleic hydrazide on groundnut. They soaked non-dormant seeds of groundnut CV TMV-2 in 0, 5000, 10000, 15000, 20000, 25000 or 30000 ppm MH for 24 h. They found that MH

treatment severely inhibited shoot and root growth, increased shoot:root ratio. Murthy and Venkateswarlu (1975) reported that MH inhibited growth of all organs and completely suppressed lateral root growth when seeds were soaked for 24 h in solution of MH (0-100 mg/1) before sowing. Suryanarayanan (1977) found that application of 100 ppm MH was effective in decreasing height of the groundmut plants.

iii) <u>Reproductive growth</u> :

Gurubaksh Singh <u>et al</u>. (1978) reported that a foliar application of 250 ppm MH to groundnuts (50 days after sowing), significantly increased the number of gynophores per plant.

iv) <u>Yield</u> :

Krishnamurthy (1967) studied the effects of hormones as foliar application on the yield of groundnut. He reported that application of two foliar sprays of MH at a concentration of 100 ppm at 24 and 9 days before harvesting groundnuts were effective in increasing pod yields, weight and number of pods and kernels per plant. Suryanarayan (1977) found that application of 100 ppm MH was effective in increasing number of total and filled pods. Gurubaksha Singh <u>et al</u>. (1978) reported that application of MH as foliar spray at the rate of 250 ppm (50 days after sowing) on groundmuts gave the highest yield. Rao (1980) found that application of 50 ppm MH was effective in increasing pod yields, shelling percentage and harvest index in groundmuts.

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v) <u>Oil content</u> :

Rao (1980) reported that foliar app lication of 50 ppm MH was effective in increasing oil content of groundmut seeds.

vi) Physiological changes :

Vyas et al. (1965) studied the influence of pre-germination treatment with MH on ascorbic acid content and ascorbic acid oxidase activity of groundnut. They observed that germinating groundnut seeds showed maximum ascorbic acid content on 6th day of germination when pretreated with 3×10^{-4} M Maleic hydrazide. They also reported that in MH pretreated germinating seeds, ascorbic acid oxidase appeared on 3rd day of germination, reached a maximum on the 10th day and then diminished. Vyas and Patel (1968) studied the effect of growth regulators on amylase activity of groundnut during germination. They found that MH treated seeds showed maximum anylase activity on 12-14 days. Rao and Rao (1970) studied the metabolic response of groundnut to respiratory inhibitor MH in dark. They found that when seeds of groundnut CV TMV-2, were allowed to germinate in solution of 10 ppm MH then there was reduction in water uptake. They also found that MH treatment reduced the respiratory activity of embryo axis to a greater extent and the fatty acid content of the embryo axis was reduced to 75% by MH. Vathialingam and Rao (1973) found that seed pretreatment with MH caused increase in the fresh and dry weights of cotyledons. Based on

these observations they came to conclusion that the utilization of stored reserves in the cotyledons had been inhibited by MH treatment. These workers further reported that MH treatment decreased catalase activity in seeds and increased sucrose content in cotyledons and primary axis of groundmit. They also reported that foliar application of 5000-30000 ppm MH-30 at 70, 80 or 90 days after sowing increased amino acid content of resulting groundmit seeds.

The obove observations clearly reveal marked influence of maleic hydrazide on metabolism of groundmut.

13. Succinic acid, 2,2, dimethyl hydrazide (SADH) :

a) Introduction :

Many compounds, including auxin and ethylene, will retard plant growth if used at a high enough concentration, but the term growth retardant is usually restricted to those which specifically inhibit cell division and cell expansion in the sub-apical region of the stem. This results in a plant having shortened internodes, but with leaf size, leaf number and apical dominance remaining relatively unaffected. The internode shortening can often be reversed by treating the plant with gibberellin, suggesting that growth retardants function by inhibiting gibberellin biosynthesis. Riddle <u>et al</u>. (1962) reported that applications of sprays of N-dimethyl amino succinamic acid to foliage retarded the growth of legumes, vine crops, potatoes and ornamental plants. This compound is also recognized by other names such as 'B-995', 'B-9', 'Alar', 'Kylar', 'B-Nine' and SADH.

Structure :



b) Responses of groundnut to SADH :

i) Germinability :

Bockelee-Morvan et al. (1975) reported that SADH (Alar-85) was effective in increasing seed germinability in groundnuts.

ii) <u>Vegetative growth</u> :

Baumann and Norden (1972) studied the effect of succinic acid 2,2,dimethylhydrazide (Kylar) on morphological characteristics and yield of groundmut. They found that application of Kylar at 1.12 and 1.68 Kg/ha was effective in shortening of main stem height and internode length. Bockelee-Morvan and Giller (1973) also reported that there was decrease in stem length of groundmit plants after the application of SADH in the form of spraying solution of strength 0.05%.

Brown et al. (1973) noticed that applications of SADH to groundnut were effective in reducing the height of the plants and length of the pods. Brown and Ethredge (1974) observed that SADH treatment was effective in reducing main stem length. Whittey and Gorbet (1974) reported that there was retardation of internode length after the application of SADH (Kylar). Wynne et al. (1974) found that use of SADH reduced fruit and seed size in groundnut variety NC-17 and fruit size in groundnut variety NC-5. Daughtry et al. (1975) used SADH (Alar-85) in the form of spray at the rate of 0.95 kg/ha to check the height of groundnut plants and observed that SADH application was effective in checking the height of the plants. Gorbet and Rhoads (1975) noticed that SADH (Kylar) used as foliar spray was effective in reducing stem length. Hammerton (1976) reported that SADH reduced main stem and branch inter nodes in several groundnut varieties. Santelmann and Thilsted (1977) noticed that SADH affected the rapidity of foliar growth when applied at early stage. Thilsted and Santelmanm (1977) reported that SADH significantly reduced the growth of groundnut CV Spanish. Wu and Santelmann (1977) found that SADH when applied at a rate of 1.1 or 2.2 kg/ha to 1 month old groundnut plants, reduced plant height compared with untreated plants when soil moisture content was

held at 12-15%. They also observed that SADH increased dry root weight. Hartzook (1978) reported that SADH when applied at conc. of 2000 to 4000 ppm 1,2 or 3 time at weekly intervals to groundnut at pod set and filling stage there was no effect on plant height. Walker <u>et al</u>. (1981) found that use of SADH was effective in reduction in plant weight of groundnut. Kvien and Littrell (1985) noticed that SADH effectively controlled excessive vine growth in groundnut plants. Thus all the above observations clearly indicate that the vegetative growth in groundnut can be controlled by SADH application.

iii) <u>Reproductive growth</u> :

Baumann and Norden (1972) reported that when SADH was applied to groundnut at various rates, it produced variable effects on peg formation. The rate of peg format ion was reduced at lower rate of SADH application and it was higher at higher rate of SADH application. Bockelee-Morvan and Giller (1973) studied th e action of growth regulators on groundnut. They found that SADH, when applied 45 days after sowing, was more effective in increasing number of pegs and pods per plant. Brown <u>et al</u>. (1973) applied SADH to the groundnut CV Starr at 60-90 days after sowing. They found that there was 4% reduction in length of gynophore and 4 to 10% reduction in pod length as compared to the untreated plants. Wynne <u>et al</u>. (1974) reported that SADH reduced fruit size and seed size in groundnut CV NC 17 and fruit size in CV NG 5. Daughtry <u>et al</u>. (1975) found that

there was decrease in pod weight, length and diameter and seed size of groundmut CV Starr, Tifspan and Florunner when SADH was applied a 6-10 weeks after sowing. However, they observed increase in pod length and weight when SADH was applied 12 weeks after sowing. Hammerton (1976) studied the effects of B-9 on growth and yield of groundmut. He found that in bunch type groundmut CV Valencia local, podding was more compact but mean seed dry weight and number of seeds/pod were slightly decreased by daminozide. In contrast to all the above observations, Mozingo and Steele (1983) reported that SADH (Kylar) was effective in improvement of seed size and pod uniformity in Virginia type groundmut cultivars.

iv) <u>Yield</u> :

Baumann and Norden (1972) studied the effect of SADH on yield of groundnut. They found that there was reduction in 100-seed weight and yield of groundnut after the application of SADH (Kylar) at the rate of 1.12 and 1.68 kg/ha. On the other hand Bockelee-Morvan and Giller (1973) noticed that application of SADH increased average yield of unshelled nuts and seed quality in groundnut. Brown <u>et al</u>. (1973) also noticed that there was increase in pod yields after the application of SADH, they further reported that there was no consistency in this increasing yield effect of SADH. Gorbet and Whitty (1973) studied the response of groundnuts to growth regulators. They found that when soil moisture was adequate, SADH increased

yields of unshelled groundnuts. Brown and Ethredge (1974) studied the effects of SADH on yield and other characteristics of groundnut cultivars. They observed increase in average pod yields of groundmut cultivars. Whittey and Gorbet (1974) reported that SADH was useful to increase the yields of ground-Wynne et al. (1974) studied the effects of spacing and a nut. growth regulator SADH (Kylar) on size and yield of fruit of virginia type groundnut cultivars. They found that the response to SADH varies with variety of groundnut. SADH reduced yield and fruit and seed size in NC-17. However, there was no effect on yield of cultivar NC-5, Bockelee-Morvan et al. (1975) reported that SADH was effective in increasing yields of unshelled groundnuts when applied 40 to 45 days after sowing. Gorbet and Rhoads (1975) found that SADH (Kylar) was useful to increase yields of groundnut pods. Hartzook (1978) studied the effect of SADH (Kylar-85) on groundnuts. He found that application of SADH at concentrations of 2000 or 4000 ppm to groundnut at pod set and filling stage was effective in increasing seed yield but average pod and seed weight were reduced. Bahat et al. (1979) reported that application of SADH was effective in increasing yields of groundnut pods. Halevy et al. (1979) noticed that there was increase in pod yield of groundnut after the application of 2% solution of SADH as foliar spray. Rao (1980) noticed that application of 250-2000 ppm SADH increased pod yields in groundnut. Walker et al. (1981) reported that pod

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yields were increased with the application of SADH (Kylar). Reddy and Patil (1981) observed that foliar application of 2000-4000 ppm SADH 60 days after sowing increased yields of unshelled nuts in groundnut CV Spanish improved. Mozingo and Steele (1983, 1984) reported that SADH significantly increased seed yields in groundnut CV Florigiant and NC-6.

Rao (1980) reported that SADH increased shelling percentage of groundmut. Witzenberger <u>et al</u>. (1985) studied yield, components of yield and quality responses of groundmut cultivar as influenced by photoperiod and growth regulator, SADH. They noticed that shelling percentage was increased by SADH and short day treatments in groundmut cultivars. It is obvious from the foregoing account that in spite of being a growth retardant, SADH has positive influence on various yield parameters of groundmut.

v) <u>Oil content</u> :

Rao (1980) studied the effect of growth regulators on growth and yield of irrigated groundnuts. He found that application of SADH at the rate of 250-2000 ppm was effective in increasing seed oil content and oil yield in groundnuts.

vi) <u>Physiological changes</u> :

There are few attempts to study infl uence of SADH on groundnut metabolism. Brittain (1968) reported that SADH (Alar) increased calcium content of stems and chlorophyll content of

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leaves. He also reported that at close spacing SADH increased the rate of net CO₂ assimilation. Worthington and Smith (1974) studied the alterations in fatty acid composition of groundmut oil induced by foliar application of SADH (Kylar). They reported that SADH when applied alone decreased the linoleic acid content. However, it was effective in increasing palmitic and oleic acid content. Giller and Bockelle-Morvan (1976) studied the effect of SADH on calcium movement and they concluded that SADH increased Ca translocation to the seeds. These observations and findings of Brittain (1968) indicate that SADH influences calcium mutrition in groundmut.

14. Chloro Choline Chloride (CCC) :

a) Introduction :

CCC is a choline derivative in which hydroxy group is replaced with a chlorine substituent. It's name is chlorocholine chloride (2 chloroethyl-trimethyl ammonium chloride) which is abbreviated as CCC. It is also known as Cycoel or Chlormequat.

Tolbert (1960) firstly reported the growth retarding activity of CCC on wheat. CCC is well known for its antigibberellin action. Although wheat and cucumber have been found to be most responsive species to CCC, its applications to other crops is also gaining an increasing attention.

Molecular weight of CCC is 158 daltons, it is completely soluble in water and highly hygroscopic. It remains persistent in soil for 3-4 weeks. It's use in solution culture is safe.

Structure :

$$\operatorname{ClCH}_2 \longrightarrow \operatorname{CH}_2 \longrightarrow \operatorname{N}^+_1 \longrightarrow \operatorname{CH}_3 \operatorname{CH}_3$$

b) Responses of groundnut to CCC :

i) Germinability :

Sengupta <u>et al</u>. (1979) studied the effect of various growth regulators on seed germination in groundnut. They observed that application of CCC inhibited seed germination of the non-dormant groundnut cultivar. They further reported that kinetin did not counteract the inhibitory effect of CCC.

ii) <u>Reproductive growth</u> :

Contradictory reports are available regarding the influence of CCC on reproductive parameters in groundnut. Manzava (1979) reported that CCC inhibited gynophore elongation in groundnuts. Gurubaksh Singh and Sharma (1982) observed that application of two foliar sprays of 100 ppm CCC to groundnuts (40 and 50 days after sowing) increased the number of gynophores and pods per plant.

iii) <u>Yield</u> :

Gupta (1975) investigated the effects of CCC on groundmut yield. He reported that 5000 ppm CCC treatment as foliar spray was effective in increasing yields of unshelled and shelled muts. Gurubaksh Singh <u>et al.</u> (1978) reported that a foliar spray of 50-100 ppm CCC to groundnuts (50 days after sowing) was effective in increasing yield of unshelled nuts. Rao (1980) observed that application of 250-1000 ppm CCC increased pod yields, shelling percentage and harvest index. Reddy and Patil (1981) studied the effect of growth retardants on the yield and yield attributes of groundmut. They found that a foliar application of 1000 to 2000 ppm CCC to groundmut (60 days after sowing) increased the yields of unshelled muts.

Gurubaksh Singh and Sharma (1982) observed that two foliar sprays of 100 ppm CCC to groundnut (40 and 50 days after sowing) were effective in increasing dry pod yield and hundred seed weight. All the above reports indicate that eventhough CCC is regarded as a growth retardant, it brings about yield enhancing effect in crops like groundnut.

iv) Seed Oil content :

Gupta (1975) reported that application of foliar sprays of 500 ppm CCC to groundmut (21, 35 and 49 days after sowing) resulted in increase in seed oil content. Rao (1980) also noticed that application of 250-1000 ppm CCC increased oil content in groundmut seeds.

Scope of Present Investigations :

It is evident from the foregoing account that groundnut gives positive response to number of growth regulators. In this respect both natural and synthetic growth regulators are equally effective. At the same time, it is noticed that yield studies are undertaken on a large scale and metabolic studies are paid very little attention. Hence we thought it worthwhile to investigate physiological responses of groundnut to presowing soaking treatment of a well known growth retardant CCC which is also regarded as antigibberellin by some workers. This study was also supplemented with study of responses of groundnut to kinetin pretreatments under identical conditions. Since drought is a major contraint on groundmut productivity, the physiological parameters relevant to drought resistance are mainly studied and comparatively little attention is paid to growth and yield response. These physiological parameters include fate of mineral mutrients, alterations in status of carbohydrates and organic acids, accumulation of proline and ascorbic acid, maintenance of SH groups, nucleic acids and nitrate reductase activity, stability of photosynthetic pigments and stomatal behaviour.