

Chapter - 2

2. LITERATURE REVIEW

The ability of many plants and plants parts to form roots from cuttings under the proper conditions is important in the propagation of many species. Several difficult-to-root species of plants respond to the application of synthetic bioregulators. Several others however failed to respond adequately or proved to be recalcitrant to such treatments. There is a wide range of adventitious root forming ability in plants which may range from very easy-to-root to those which will not root at all (Davis and Haissig 1994). Jauhari (1960), Haissig (1970), Schreiber (1973) and Larsen (1982) showed a variety of patterns in rooting responses to different plant cuttings. The same growth substance showed fluctuating potentiality in its rooting ability to different species of plants and the same plant showed difference to its reaction to different growth substances under different environmental conditions. The action of growth regulators on rooting on cuttings depends on the age and nature of the stock materials. Cuttings rooted more readily with hormonal application when taken from shoot of old trees (Sen 1941, Bhombota 1959, Jauhari 1960). Woody plants in general require a higher concentration of hormones than the herbaceous stem.

2.1 Auxin

Auxin mechanism of induction of growth of roots in stem is still inadequately understood although voluminous works had already been done. Cutting do not

produce roots in same magnitude even if they are subjected to treatments with similar auxin.

Thimann and Koepfli (1935), Zimmerman and Wilcoxon (1935) demonstrated that two synthetic auxins, Indole-3-butyric acid (IBA) and Napthalene acetic acid (NAA) were more effective than the naturally occurring IAA for rooting. Since then there have been numerous reports indicating that auxin is involved in the initiation of adventitious roots and that division of root initials is dependent either upon exogenous or endogenous auxin. Experimental evidence from studies with transgenic plant tissues have shown that transfer of the Ri (root-inducing) plasmid into plant tissues increased their sensitivity to auxin (Shen *et al* 1988, Maurel *et al.* 1991; Blakesley and Chaldecott 1993). Indole-butyric-acid (IBA) and Napthalene acetic acid (NAA) are the most commonly used auxins on a commercial basis (Blazich 1989).

Benefits of auxin treatments - Although auxins are not effective in all plant species there are several direct benefits of using auxins :

1. A higher percentage of cuttings produce roots.
2. Root initiation is typically quicker.
3. The number and quantity of roots per cutting is increased.
4. Uniformity of rooting along the length of the cutting is increased (Blazich 1989).

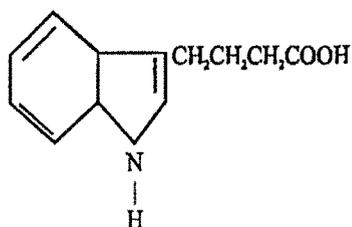
Auxin application enhances nucleic acid synthesis in root initials. It is generally suggested that the physiological basis of adventitious root formation is

associated with the actual auxin level in the tissue or a balance between auxin and other plant constituents (Malik 1999).

The involvement of nucleic acid in protein in auxin induced rooting has been clearly demonstrated by various workers (Nanda *et al.* 1973a, Nanda *et al.*, 1973b, 1974 ; Dhaliwal *et al.* 1974 ; Bhattacharya *et al.* 1974). It was suggested that some new RNAs that were induced in etiolated stem segments of *Populus nigra* cultured in glucose + IAA were suppressed by actinomycin-D, suggest involvement in root initiation. These RNAs had low molecular weight therefore may be either mRNA and tRNA type (Nanda *et al.*, 1973c ; Bhattacharya *et al.*, 1974). It was suggested that IAA or its oxidation products probably act as trigger at trascriptional level and nutrition as source of carbon to regulate the translation in synthesis of proteins that are required for differentiation of cambial activity into root primordia and their development.

The widespread use of auxins by nurserymen, culturists and horticulturists indicates that these are valuable aids in induction of rooting on cuttings (Stoutemyer 1954). A very wide category of auxins have been used for the rooting on cuttings, but both IBA and NAA are typically the principal auxins used for rooting on cuttings. Sen and Bose (1959) demonstrated that the one most commonly used with great success auxin is IBA. Although the search for new auxins which have a stimulatory effect on rooting continues, IBA and NAA are still the most commonly used auxins on a commercial basis (Blazich 1989).

2.1.1. Indole-3-butyric acid (IBA) :



Amongst auxins, Indole-3-butyric acid is more effective than others in case of induction of roots. The success of this compound may be due to its auxin activity being relatively immobile and also resistant to destruction by enzyme. IBA generally produces a more fibrous root system than others. It has been suggested that a high ratio of carbohydrates to nitrogen compounds in plant tissue, as well as optimum supply of auxin, particularly, IBA contributes to best rooting on cuttings. Chemically IBA with an even number of carbon atoms in the side chain of indole ring is more effective for initiating rooting on cuttings.

Synthetic IBA has been widely used for making the stem cutting of plant species to root (Torrey 1976, Greenwood and Berlyn, 1973). Sinha *et al.* (1962) studied the rooting effect of different auxins on *Psidium guajava* and found that the treatment of IBA followed closely by IAA proved superior as compared to others. Samantarai and Sinha (1957) studied the rooting effect on *Ipomoea batatas* and observed that roots induced by IBA showed a highly increased number of vascular strand in relation to its concentration and if IBA is followed with the supply of sugar or nitrogen, it induces greater number of roots and higher number of strands. Similar results with IBA were reported by Sen and Bose (1959), Jauhari (1960), Gregory and Samantarai (1950), Samantarai and Kabi (1954), and Lingaraj

and Chandrasekhariah (1961). Sen (1941) recorded the rooting of two-year-old litchi (*Litchi chinensis* Sonn) cuttings by treatment with 50 to 100 ppm IBA. A stronger solution induced more number of roots as compared to lower concentrations.

Singh (1956) reported vegetative propagation of *Kigelia pinnata* DC. by inducing rooting on hard wood cutting with 20 ppm IBA for 12 hr treatment. Dikshit (1956, 57) carried out experiments on the regeneration of stem cutting of *Prunus salicina* Lindl by treatment with IBA. The important varieties e.g. 'Early round' and Howe' which were difficult to propagate by cutting induced to rooting with 30 ppm IBA. He also recorded positive correlation between the leaf area and root growth.

Shanmugavelu (1960) showed the soak method with 2 to 200 ppm of IBA was the most promising for the rooting in the cutting of *Hibiscus rosa-sinensis* and *Allamandu cathartion* L. Jauhari and Kohil (1960) studied the effect of growth regulators on the propagation of peach (*Prunus* sp var. Sharbati) by stem cuttings. The basal end of the cuttings were dipped for 24 hr in solution of 20 to 100 ppm of growth regulators and subsequently planted in sand in pots. IBA was more effective than others and the best response was obtained in treatment with 40 to 60 ppm of IBA.

Singh (1963) noted a marked improvement in rooting as well as sprouting of sweet lime cuttings (*Citrus limattioides* Tanka) by quick dip application of 200 ppm IBA. The higher concentration 4000 ppm was slightly inferior to 200 ppm.

Chauhan and Singh (1971) observed that application of IBA to etiolated basal end part under intermittent mist helped root initiation on stem cuttings of fruit plants. Treatment of cuttings with IBA improved rooting percentages and also stimulated initiation of adventitious roots (Breen and Mraoka 1973, Schreiber 1973). Sarma (1980) observed the response of IBA on *Litchi*, *Olea* and *Psidium* and found that IBA at 1000 ppm was optimal to *Litchi* and *Olea* stem cuttings, while 2000 ppm of the same was best for *Psidium*.

Ghosh et al. (1983) studied the effect of IBA on stem cuttings of *Pomegranate*. In soft wood cuttings, maximum rooting success (83 %) was recorded under IBA at 5000 ppm as compared to control with only 44.4 per cent. At 5000 ppm of IBA semi-hard wood cuttings showed 77.7 per cent rooting. Highest rooting percentage of 83.3 per cent was recorded in hard wood cuttings with 5000 ppm IBA. IBA alone has been reported to enhance the rooting in cuttings of *Pyrus pyrifolia* (Singh *et al.* 1987). That IBA enhances the rooting on apple was reported by Howard (1968). Reighard *et al.* (1990) reported that IBA is best for promoting the rooting potentiality on stem cuttings on *Prunus*. Efficiency of IBA in relation to rooting of *Coleus blumei* was studied by Das (1993) and reported that 60 ppm IBA was optimal for inducing rooting.

Halder *et al.* (2002) studied the effect of IBA with different ornamental plant cuttings. The ornamental plant under study showed significant variation in respect of all the parameters, after 15, 25, 35, 45 and 55 days. *Ixora (Ixora chinensis)* performed the best in respect of highest number leaves and roots per

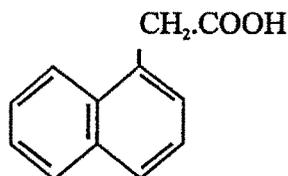
cutting, longest root and fresh and dry weight of roots per cuttings. Poinsettia (*poinsettia pulcherima*) also produced highest number of roots per cutting.

Mohammad *et al.* (2002) studied the axillary shoot proliferation, culture multiplication and rooting of the in vitro generated and proliferated of IBA was found to be the best among three auxins NAA, IBA, IAA, tested. The highest percentage.

Jasmine *et al* (2003) studied the effect of growth regulators on cuttings of coleus Forskohlii with basal, middle and terminal cutting treated with NAA and IBA at the concentration of 500 ppm and 1000 ppm and a combination of NAA and IBA at the concentration of 500 + 500 ppm and 1000 + 1000 ppm by quick dip method. Then the cuttings were planted in the rooting media after 30 days. The results revealed terminal cuttings treated with IBA 500 ppm produces higher number of roots, length of root, shoot length and survival percentage (90.03%)

Panncerselvam *et al.* (2003) studied the effect of growth regulators and planting media on rooting of cuttings in *Nathopodytes nimmoniana* mabberly. They found that treated with 200 ppm IBA in talc form in soft wood cutting, 3000 ppm IBA talc form in semihard wood cutting and 4000 ppm IBA talc form in hard wood cuttings resistered maximum sprouting, rooting, root length, shoot length, numebr of roots per cuttings and survivial percentage.

2.1.2. α -Naphthalene acetic acid (NAA) :



Activities of the Naphthalene acids on rooting were observed by Zimmerman *et al.* (1939). Naphthalene acetic acid lacking the indole ring but retaining the acetic side chain is also biologically active. NAA also has a positive effect on rooting of cuttings but with less uniform success than IBA being quite a strong auxin (Singh *et al.* 1961).

Singh and Teotia (1951) studied the effect of NAA on mango varieties and noted that 10 per cent NAA in 'Langra' varieties induced 20 per cent and 100 per cent in Deshri varieties. Thimann *et al.* (1950) reported that NAA at low concentration (1-100 ppm) can induce roots on woody cuttings.

Hundred per cent rooting with 3 per cent NAA was reported by Singh and Sarma (1954) in the cuttings from *Eriobotrya japonica* (Thamb) Lind. var 'Golden yellow'. Singh (1957) reported that cuttings of *Coleus blumei* rooted more satisfactorily after soaking for 22 hr. in NAA. NAA at 100 ppm responded more positively.

Jauhari *et al.* (1960) showed that treatment with high concentration of NAA ensured 50 per cent success in *Eriobotrya japonica* and 90 per cent success was obtained with NAA treatment in *Jasminium* soft wood cuttings (Shing and

Bhatnagar 1955, Samantarai 1950). Singh (1957) and Prasad (1962) also reported similar stimulation of rootings by NAA.

Shanmugavelu (1961 a b) treated 12.5 cm thick hardwood cutting of one year old *Hibiscus rosa-sinensis* L. with IAA, IBA and NAA by soak, quick dip and dust method and obtained 85-95 per cent rooting after soak treatment with NAA for 24 hr.

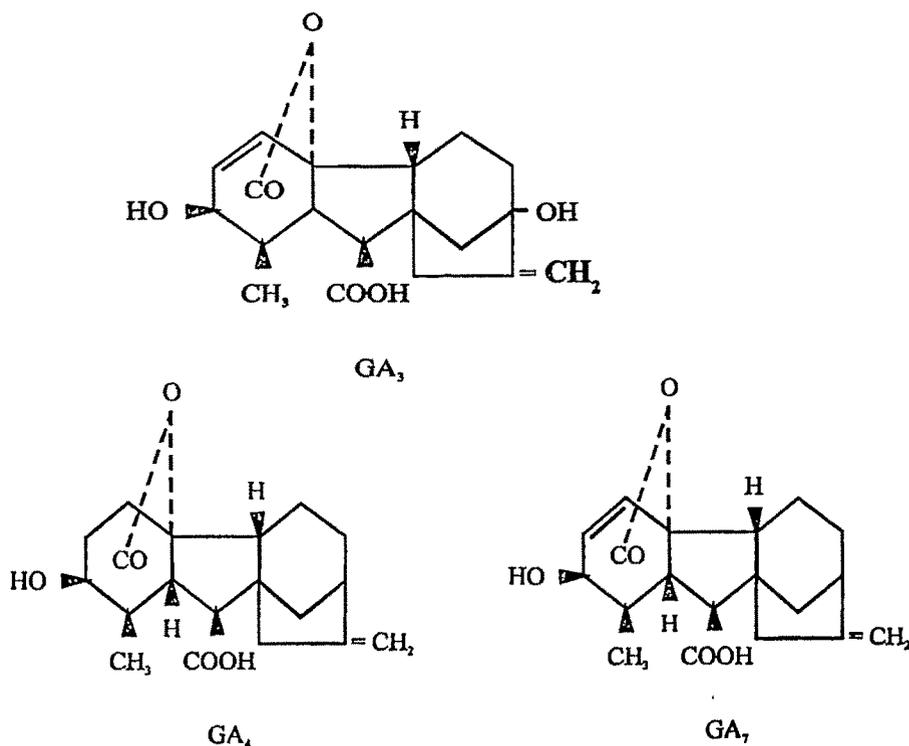
Rooting on apple stem cuttings was also induced by NAA. Howard and Nahlawi (1969) reported that NAA 0.1 to 1 ppm was more suitable for rooting. Chattopadhyay (1960) reported vegetative propagation of some essential oil yielding plants introduced at Nungpo, Darjeeling and reported that *Lavandula* sp. responded more favourably to NAA. Chattopadhyay (1959) reported that NAA also induced high rate of rooting in *Ipecac* cuttings. Singh and Bhatnagar (1975) reported 90 per cent of rootings with 500 ppm NAA in hard wood stem cuttings of *Jasminum grandiflorum*. Pandey *et al.* (1983) observed the rooting behaviour of walnut using auxins and found that NAA alone is effective in inducing rooting on stem cuttings.

Ghosh *et al.* (1988) while working on effect of NAA on adventitious root formation in stem cuttings of *Punica granatum* L observed 38.8 per cent rooting on soft-wood cuttings and 22.2 per cent rootings on semi-hard wood cuttings at 1000 ppm. Higher concentrations of NAA proved inhibitory on rootings.

The combination of IBA and NAA with phenoxy compounds resulted in much better rooting on cuttings than the use of any one of these taken alone. Much progress has been achieved in regular vigour and general structure of the root system by using appropriate mixture of these compounds. In many cases, a great number of roots resulted from the mixture of IAA/IBA and IAA/NAA in equal portions than from either compounds acting alone (Singh 1962). Jauhari (1960) obtained 100 per cent success in *Curissa acandus* after using NAA/IBA than the use of any one alone. Lingraj and Chandrasekhariah (1961) applied IAA, IBA and NAA individually and in combinations for inducing rooting in cuttings of *Antirrhinum majus* L and observed that combinations induced better rooting than individual regulators. Gregory and Samantarai (1950) showed that in consequence of the application of synthetic hormones hydrolysis of nitrogenous and carbohydrate materials took place and translocation of hydrolysed products occurred from the cut ends towards the growing regions. Roots were found scattered all over the underground portions of cuttings, as a result of auxin treatment, but in the untreated controls roots were confined to the basal region only (Bhombota 1959). If the cell walls are more or less of gel condition or are thick enough, higher concentration of auxin are necessary to bring about the initiation of roots, whereas in the more delicate types, lower concentrations are very much promising for rooting. At higher concentrations, these hormones proved toxic to cuttings (Samantarai and Kabi 1954, Sengupta and Chattopadhyay 1954, Singh and Bhatnagar 1955, Singh 1962). Of the auxins used IBA was more effective than NAA in increasing rooting (Hurov 1967, Monsour 1968, Chauhan and Singh 1971). Both IBA and NAA treatment

caused increased rooting over control but higher concentrations of NAA are toxic to cuttings. Cuttings treated with NAA produced generally thick fleshy and unbranched root system while those receiving IBA produced thin, finely divided fibrous root system (Bower *et al.* 1975).

2.2 Gibberellins



Gibberellins (GA₃), a group of naturally occurring plant growth promoting substances, whose presence in the fungus *Fusarium heterosporum* was first discovered by Kurosawa in 1926 and named gibberellin for these compound, was given by Yabuta (1935). West and Phiney (1956) discovered GA as a natural product in higher plants. Gibberellins are a large family of diterpenoid acid having a gibbane ring skeleton with 4 to 5 systems, possessing one or more carboxyl group and

were shown to cause a wide range of often spectacular growth responses when applied to intact plants (Jones and MacMillan 1984).

Since the first gibberellin (GA) was structurally elucidated in the mid-1950s (Curtis and Cross 1954, Stodala *et al.* 1955) GAs have been subjected to extensive investigation and at the same time there have been radical advances in the analytical sciences. Such an untiring efforts on the part of the scientists yielded very encouraging results and now at least (79) naturally occurring GAs (GA₁-GA₇₉) have been characterized (Takahashi *et al.* 1990).

Gibberellins are now classified as endogenous plant hormone for the following properties : (a) when applied exogenously in extremely small amounts they induce wide range of plant growth responses, (b) they are present in most and probably in all higher plants. (c) there is circumstantial evidence that they move within the plants from site(s) of biosynthesis to site(s) of action (MacMillan 1974).

Gibberellins act similarly to IAA and promote cell elongation (Brian and Hemming 1955) and induce RNA and protein synthesis (Varner 1964).

2.2.1. Role of Gibberellins

The most commonly used and active gibberellins are GA₃ (Gibberellic acid) GA₄ and GA₇ . Gibberellins have long been known for its stimulatory effect on germination. Bewly and Black (1982) reported the stimulatory effect on germination of both dormant and non-dormant seeds of species. In the past, gibberellins were

considered to have little or no effect on root growth (Cleland 1969) but a careful survey of recent literature reveals many conflicting reports (Burstrom 1960). Most reports indicate that gibberellin inhibits root growth (Stowe and Yamaki 1957) or had no effect (Blakely *et al.* 1972) but a few indicate to the contrary (Richardson 1957).

Exogenous applications of gibberellins have been shown to inhibit adventitious root formation in many species, with the inhibition becoming greater with increasing concentrations of GA₃ from 1 µm and higher. There have also been reports, although a lesser number, which show that under specific conditions gibberellins enhance root formation (Batten and Goodwin 1978 ; Hartmann *et al.* 1990, Hansen 1987).

The responsiveness of a given plant to GA is affected by a number of factors. A complicating factor which is encountered when evaluating the effects of gibberellins on rooting is that GA₃ is the only gibberellin that has been extensively studied with respect to rooting and only a limited number of studies have been conducted with other gibberellins (Hansen 1987).

Haissig (1972) also reported that GA treatment of intact Brittle willow stems stimulated cambial activity leading to increased xylem fiber production. In intact plants gibberellic acid may increase xylem production and differentiation (Bardly and Crane, 1957, Ahuja and Doering 1967 ; Bostrack and Struckmeyer 1967, Skok 1968) or it may enhance differentiation without any effect on cambial

activity (Okunda 1959) or it may only slightly enhance division with no effect on differentiation (Morey and Cronshaw 1968).

Haissig (1972) reported a well designed experimental system for the determination of the gibberellin effect on rooting using the Brittle willow (*Salix fragilis* L.). In that species the third node had no root primordia, node four showed initiation stages and node five was the position at which subsequent developmental stages were found. He applied GA via the roots at stages ranging from 1 to 100 μ M. GA at the concentration 1 μ m gave reduction in the number of primordium initiated in node three, but the number of cells per primordium was unaffected. At higher levels of GA the number of primordia in node there was again reduced and the remaining primordia also had fewer cells. The effect of GA was most pronounced on the internodes which were older at the time of GA application where primordial initiation was not a factor. He concluded that the role of GA was not to prevent meristematic activity but rather to bring about the loss of primordia via an attrition of cells.

In callus derived from Jerusalem artichoke GA stimulates root formation in the dark and either inhibits or has no effect in the light (Spanjersberg and Gartheret 1964). Gibberellin alone does not stimulate general cell division but enhances the auxin effect on cell division in this system (Netien 1975, Spangersberg and Gautheret 1964). Murashige (1964) however, found that GA inhibited root formation in tobacco callus (*Nicotiana tubacum*, cv. Wisconsin).

Gergale *et al.* (1971) examined the effect of auxins and gibberellins on rooting in callus derived from *Morus alba* and *Populus nigra*. They also found that GA prevented root initiation. Julliard (1964) found that GA strongly stimulated rhizogenesis in cuttings of *Vitis vinifera*. L in the absence of buds and in dark. Leroux (1968) found that GA stimulated root formation in stem fragments of *Pisum sativum* L. and in this case light was inhibitory to the response but only at low GA concentrations.

The role of GA in the production and differentiation of xylem has been studied in a variety of experimental systems which differ in the degree of complexity or organisation from whole plants to callus pieces. Roberts *et al* (1966) concluded that GA enhanced cell expansion when applied to disbudded stem segments of willow (*Salix fragilis* L.) and that there was only a slight stimulation of xylem differentiation.

Wareing, (1958) found that in disbudded stem segments of sycamore the addition of GA induced cambial these newly formed cells failed to differentiate and again auxin was needed to induce normal differentiation. Other workers have also reported that GA treatment inhibits root formation Nanda and Jain (1972) reported this effect in epiphyllous buds of *Bryophyllum tubiflorum* while Brian *et al* (1960) and Reinert and Besemer (1967) reported it in stem cuttings.

Brian *et al* (1960) reported that GA inhibited rooting on stem cuttings of pea (*Pisum sativum*, several cultivars) and bean (*Phaseolus vulgaris*) while not inhibiting elongation growth when GA was applied basally. If, however, the

gibberellin was applied apically to the cutting then extension growth was promoted and there was no effect on rooting. Debudding presented the promotion of extension growth but not the inhibition of rooting clearly. Extension growth and rooting have different requirements. They concluded that the role of GA in the prevention of rooting was to prevent the early cell divisions leading to the establishment of a root primordium. This effect is very much similar to the gibberellin inhibition of bud development (both lateral and adventitious) and to the interpretation of act effect on nodulation. Other studies on cutting of '*Acer rubrum* and *Eucalyptus camaldulensis* Dehn. Show that auxin is required for rooting and that GA and kinetin both inhibit when applied basally regardless of the presence or absence of auxin (Bachelard and Stowe, 1963).

Jansen (1967) showed that the inhibition of rooting in tomato cuttings was GA dose dependent and that the first three days after excision were the most critical period for GA inhibition of rooting.

Fullenberg (1969) using pea epicotyl found that GA inhibited rooting only if applied during the first 24-72 hours after excision. Again the similarities to GA effects on bud development are obvious. GA also inhibits rooting in *Populus nigra* cuttings under otherwise favourable conditions (Nanda *et al.* 1968). Pecket (1960) working with excised roots from two varieties of peas, found that gibberellic acid strongly enhanced the development of root hairs.

Butcher and street (1960) , working with excised tomato roots, found that low concentrations of gibberellic acid (and NAA) enhanced the growth of the

main axis by both increased cell division and cell elongation and also increased the number and length of lateral roots. However, higher concentrations of GA enhanced the loss of meristematic activity and inhibited growth.

It has been found that the GA stimulation of plant growth in dwarf seedlings is associated with increase in auxin content. Further, GA suppresses the activity of Indole-acetic-acid oxidase, the enzyme responsible for IAA degradation. Based on these observations, it was suggested that GA affects plant processes through auxins and the auxins replace gibberellins. It was found that application of GA to barley aleurone layers causes a rapid increase in α -amylase activity, while auxins do not have such effect.

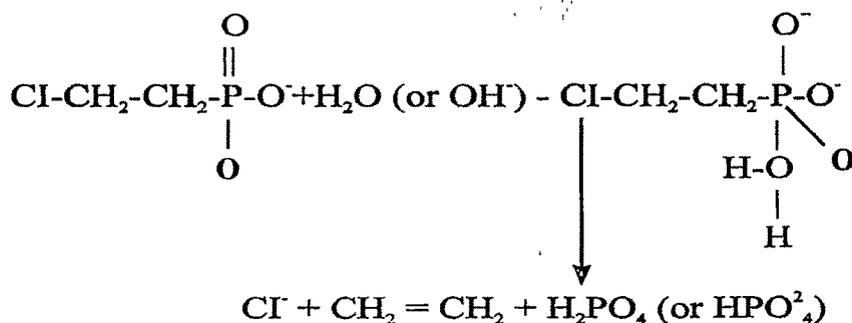
Ethylene

In the 1930s scientists at the Boyce Thompson Institute for Plant Research discovered that ethylene and ethylene analogs stimulate adventitious root formation (Zimmerman *et al.* 1939 ; Zimmerman and Hitchcock, 1933). This discovery was made prior to research by Thimann and Went (1934) which showed that auxin had a promotive effect on rooting. Auxins have also been shown to stimulate ethylene biosynthesis in many plant tissues (Arteca 1990), and it has been suggested that auxin induced ethylene may induce adventitious root formation instead of auxin itself (Mudge 1989). There are a number of reports in the literature on the effects of ethylene on rooting with some showing a stimulation, inhibition, or no effect and sometimes within the same genus and species (eg. *Vigna radiata*) (Mudge 1989). Some of the reactions of plants to ethylene are not only of theoretical

interest but could be useful in agriculture and horticulture. Ethylene as a gas only used under certain circumstances more or less closed systems like glass houses or incubation chambers, limiting the usefulness of the hormone itself to a very few applications. Interest in ethylene biochemistry and physiology occurred in the late 1950s and this has been a very active and productive field of research since then. Pratt and Goesehl (1969) remarked that "with very sensitive instruments and very careful technique it has become possible to show that ethylene is an endogenous growth regulator in plants".

Beginning in the 1960s, there were several efforts in the agrochemical industries to develop liquid or solid ethylene compounds for easier handling. These compounds degrade in presence of water, releasing ethylene (Draber, 1977). The development of such compounds still is only example of a rational design of the plant growth regulators or pesticides with a new mode of action.

2-chlorophenoxy acetic acid (CEPA) patented as "Ethrel" and given trivial name "Ethepon" is the best known compounds of this group.



It releases ethylene above pH4 in aqueous solution is relatively stable below pH4. The possibility of stabilization under certain conditions is of course important

for a commercial plant growth regulator, as such compound is stored for sometime before use. The degradation of 2-chloroethyl phosphonic acid to ethylene is a base catalysed elimination reaction (Cooke and Randall 1968, Sterry 1969).

Several esters and other derivatives of 2-chlorethyl-phosphonic acid are described in the patent literature (Draber 1977). Most probably all the compounds are connected to 2-chloroethyl phosphonic acid in the cell. Theoretically an improved performance could be achieved by the derivatives by a better penetration into the cell or by a different time course of ethylene evolution (Larsen 1982). However, these derivatives were generally not superior to ethephon itself.

With regards to the role of ethrel in root formation there were conflicting reports. Read and Hoysler (1969) reported the stimulatory effect of ethrel. While Cucumis and Fiorino (1969), Read and Hoysler (1971) and Nell (1971) observed inhibitory effect of ethrel on root cuttings. In *Phaseolus* rooting of cuttings was enhanced through ethylene application (Malik *et al.* 1982). Kender *et al.* (1969) working on blueberry stem cuttings observed that ethrel treatment is neutral to root induction. Neither promoting nor inhibiting effect of ethylene was reported in some fruit species by Roy *et al.* (1972). Anderson (1975) emphasized the importance of the medium for dissolving ethephon and its concentrations. Observations on etiolated *Vigna radiata* L. seedlings showed that ethrel is not directly involved in adventitious root formation (Batten and Mullins 1978).