It is proposed to review the existing literature on the subject under the following four heads:

A - FACTORS AFFECTING ROOTING

B - EFFECT OF EXOGENOUSLY APPLIED GROWTH REGULATORY SUBSTANCES ON ROOTING

C - ANTIMETABOLITES AND ROOTING

D - BIOCHEMICAL CHANGES ASSOCIATED WITH ROOTING

A - FACTORS AFFECTING ROOTING


I - EXTERNAL FACTORS

Seasonal changes in rooting caused by annual changes in light, temperature and humidity that affect morphological status of cuttings.

A number of reports are now available in literature to show that the rooting potential of stem cuttings varies with the season (Hitchcock and Zimmerman, 1930; Jimenez, 1937;

II - INTERNAL FACTORS

Some of the internal factors that affect rooting are as follows:

1. Role of leaves

Leaves beneficial to rooting as source of nutrition

As early as 1882, Sacha proposed that root initiation in
cuttings was stimulated by substances which moved from the leaves and buds and accumulated at the base. Loeb (1917) and van der Lek (1924) showed that the removal of leaves or the girdling of stem, drastically reduced root initiation. Went (1929, 1934, 1935) postulated that the leaves supplied nutrients as well as specific root-forming substance 'rhizocaline' that was also present in the buds and the cotyledons (Bouillenne and Went, 1933). Calma and Richey (1930) and Gortor (1957) found that the extent of root production was determined by the area of leaves that were left on the cuttings. Langstan (1954) reported that leaves helped in rooting by supplying sugars. However, Sen and Basu (1960) found that in Justicia gendarussa, leaves did not affect the number but enhanced the elongation of roots. Bachelard and Stowe (1963), Purchit and Nanda (1964) and Nanda et al. (1971) also reported that leaves had a beneficial effect on rooting stem cuttings. Nanda and Dhallwal (1974) showed that the cotyledons and apex served as sources of auxin and nutrition. In contrast to this, Kawase (1964) found that leafless cuttings rooted better than the leafy ones, but both rooted equally well, if centrifuged. He attributed this promotion to increased concentration of ethylene that resulted from centrifugation (Kawase, 1971).

(2) **Nutritional status and rooting**

Another important factor in determining the rooting response of cuttings is their nutritional status.
Many workers have shown that a high carbohydrate content was essential for the rooting of cuttings (Kraus and Kraybill, 1918; Knight, 1926; Carlson, 1929; Durham, 1934). Negishi and Satoo (1956) reported that starch content of cuttings started decreasing after planting. The decrease continued till the middle of May, when it fell down to nearly half its initial level. The reduction in the content of starch was considered to be due to the growth of buds. Pearse (1943), Sen and Basu (1960), Hyun (1967) considered that rooting was favoured by a high C/N ratio. Lacock and Nizaska (1963) showed that the younger cuttings of Populus deltoides contained more extractable substances and cellulose than the older ones. They also showed that the content of starch and cellulose was more near the base than towards the apex of cuttings. Faustov and Agfanov (1969) attributed differences in rooting ability of stem cuttings to differences in carbohydrate and nitrogen metabolisms.

Hermann and Hess (1967) found that etiolated cuttings that rooted better, had substantially higher levels of sucrose, glucose, fructose and proteins but lower levels of starch than the green ones. Went and Thimann (1937) demonstrated that several sugars promoted the initiation of roots in pea test. However, Klein (1953) did not find any relationship between sugar content and rooting of vine cuttings. Raines and Baping (1960) found that although stem cuttings of Pinus elliottii and Pinus taeda exhibited seasonal changes in the
content of carbohydrates, these were not related to their rooting response. Ali (1966) and Ali and Westwood (1966) found slightly higher starch and sugar contents in the adult than in the juvenile stem of Pyrus, although the juvenile cuttings tended to root more readily.

**Nitrogen plays a significant role in rooting**

Of the major elements, nitrogen seems to play a significant role in the rooting of stem cuttings. While a high level of nitrogen is generally detrimental to rooting, medium and low levels stimulate particularly the rooting of soft wood cuttings (Preston et al., 1953). Mahlstede and Hader (1959) found that high C/N ratio was necessary for rapid root formation. Went and Thimann (1937) found biotin and adenine to be the most effective amongst the nitrogenous compounds for rooting.

**Role of amino acids in rooting**

Various amino acids have been reported necessary for rooting (Went and Thimann, 1937; Sen and Bose, 1958; Trione and Avellaneda, 1963). Doak (1939) considered that rooting of Rhododendron cuttings was limited due to lack of amino acids. Stem cuttings rooted with the application of NAA only in the presence of ammonium sulphate and arginine. Doak (1941) and van Overbeek and Gregory (1945) reported that organic nitrogen was utilized more readily than inorganic nitrogen. Gregory and Samaanrai (1950) and Sen and Bose (1958) reported promotion of rooting by asparagine and glutamic acid, and Trione and Avellaneda (1963) by threonine and
glutamic acid. Schrader (1924) noted that a high carbohydrate content favoured root production but if the nitrogen content decreased below a certain level root production decreased in spite of the high content of carbohydrates. **Auxin-nutrition balance necessary for optimal rooting**

Nanda et al. (1971 b, 1972 c) and Nanda and Jain (1971) found that a proper balance between auxin and nutrition was necessary for optimal production of roots. Nanda and Jain (1972 b) also found that sucrose was more effective than ribose or glucose in rooting etiolated stem segments of *Populus nigra*. Starch also induced rooting in combination with auxins but it was first hydrolysed by enzymes that leached out of the segments.

**B - EFFECT OF EXOGENOUSLY APPLIED GROWTH REGULATORY SUBSTANCES ON ROOTING**

**Auxins**

**Auxins promote rooting**

Since the time Went and Thimann (1937) identified auxin as the root forming substance, synthetic auxins have been extensively used to promote rooting on cuttings. Auxins like indole-butyric acid (IBA), indole-propionic acid (IPA) and naphthalene acetic acid (NAA) have been widely used for this purpose (Zimmerman and Wilcoxon, 1935; Cooper, 1935; Leibach and Fischnick, 1936; Thimann and Koepfli, 1935; Biale and Halma, 1937; Thimann and Delisle, 1942; Shapiro, 1957; Sen and Bose, 1959, 1963; Sen et al., 1961; Kurosowa and Miyake, 1962; Bachelard and Stowe, 1963; Wheat, 1964; Buczek, 1965;

**Efficacy of auxins varies**

Auxins vary considerably in their effectiveness in rooting stem cuttings. In general, IBA and NAA are more effective because of their powerful activity and slow destruction. IAA is less effective as it is readily oxidized. Phenoxycetic acids are highly effective in inducing root primordia but are injurious to the growth of leaves. The relative effectiveness of different auxins in rooting stem cuttings of various plant species was studied by a number of workers (Pearse, 1939; Mitchell and Rice, 1942; Audus, 1959; Nanda et al., 1968 a, 1970, 1971 a; Nanda and Anand, 1970; Nanda and Jain, 1971) and it was found that the optimum concentration for rooting varied with the plant species and also with the nature of the auxin. A mixture of auxins is more effective than each alone (Hitchcock and Zimmerman, 1940, 1942; Evans, 1951; van Onsem, 1953).

**Some plant species obstinate to root even with auxin application**

Auxins in general promote rooting at low but inhibit it at high concentrations. There are, however, many species
which do not root even with the application of auxins (Pearse, 1939; Hatcher and Garner, 1947; Tyce, 1957; Hess, 1963; Nanda, 1970). Haissig (1965, 1974 a) suggests that IAA can induce rooting in predisposed cells but cannot induce their predisposition. Thus, difficult-to-root cuttings apparently lose the predisposition during development or the predisposition does not manifest itself during propagation.

The concentration of an auxin that is favourable for root growth may not be favourable for shoot growth (Thimann, 1935; Skoog, 1944; Lanphear and Meahl, 1963). Auxins affect rooting by stimulating cambial activity

Avery et al. (1937), Tincker (1937), Soding (1938) and Gouwentak (1941) considered that exogenously-applied IAA affected rooting by stimulating cambial activity. According to Wareing (1951), the initiation of cambial activity is dependent on the production of free auxins. Digby and Wareing (1966) found that in woody shoots of Robinia pseudocacia, the division of cambial cells and their differentiation into xylem was stimulated by the application of IAA.

Effectiveness of auxin varies with season

Nanda (1970) found that the effectiveness of auxins varied with the season. An auxin may stimulate rooting of plant species in one season but may inhibit it in another season. Again the maximum rooting of the same plant species may be caused by different auxins in two different seasons.
Auxins play multifarious roles

Nanda (1970) has also pointed out that auxins play multifarious roles. These are concerned with the division and elongation of meristematic cells, differentiating cambial initials into root primordia and in the mobilization of reserve food materials by enhancing the activity of hydrolysing enzymes. They consider that the effect of exogenously-applied auxins on rooting stem cuttings is mediated primarily through their effect on mobilization of starch caused by enhanced activity of hydrolysing enzymes provided the divisional activity of the cambium continues.

Stages involved in root formation

The formation of adventitious roots involves the
(i) the division of cells into root initials, (ii) the differentiation of initials into root primordia and (iii) their development into root tissues (Nanda, 1970; Haissig, 1974a).

Roots originate from meristematic zones of the stem

Struckmeyer (1951) reported that root primordia were formed by the divisions initiated in one or more groups of meristematic cells. Kraus et al. (1936), Snyder (1954) and Buck (1954) showed that the cambial, callus or even phloem parenchyma cells divided to produce root primordia. Nanda (1969, 1970) and Nanda and Anand (1970) reported that the roots that were produced by the division of cambial cells traversed through the space between vascular elements.
Auxin needed for division of first initial

Halssig (1974a) described three distinct phases in root initiation. In the first phase, there is the appearance of a root initial. It then divides to form a cluster of meristematic cells (primordia formation) which then differentiates into root tissues. Halssig (1970b, 1972) considers that the endogenous or applied IAA affects the division of the first initial cell which incorporates more of applied IAA than the cells in the developing primordia.

C - ANT IMETABOLITES AND ROOTING

Metabolic inhibitors inhibit root formation

The literature on the effect of antimetabolites on rooting is rather meagre. Fellenberg (1966) reported that actinomycin-D, 5-bromouracil and chloramphenicol inhibited root formation and differentiation of tracheids in the etiolated pea hypocotyls. Knypl (1966) reported that the inhibitors of the syntheses of RNA and proteins inhibited the IAA-induced growth of sunflower hypocotyl sections and also the production and subsequent development of roots on etiolated maize seedling sections. He concluded that the auxin-mediated growth phenomena were dependent on RNA and protein syntheses and suggested that IAA induced the synthesis of m-RNA.

The cells of root primordia synthesize RNA (Halssig, 1971), DNA and proteins (Molner and LaCroix, 1972b). Thus, root primordium initiation and development can be blocked by
substances which interfere with or modify DNA, RNA or protein synthesis (Antal et al., 1971; Fellenberg, 1965, 1966; Gillet, 1965; Hohn, 1965; Kamin, 1967; Knypl, 1966; Melicher, 1964; Mitsushashi et al., 1969; Jain and Nanda, 1972; Nanda and Jain, 1972 a; Nanda et al., 1971 a; Hugle, 1971) or by uncouplers or inhibitors of oxidative phosphorylation (Krul, 1968; Wirth, 1960), which restrict the energy supply for the synthesis of macromolecules.

If RNA synthesis is blocked with actinomycin-D (Fellenberg, 1966; Knypl, 1966) or protein synthesis is inhibited by chloramphenicol or puromycin (Fellenberg, 1966; Kamin, 1967; Knypl, 1966; Nanda and Jain, 1972 a) the number of roots per cutting is drastically reduced even in the presence of IAA. Fellenberg (1966) found that, although the number of roots produced per pea cutting fell markedly after either actinomycin-D or chloramphenicol treatment, the number of cells in root primordia was not affected. These results are contrary to those of Kamin (1967) who found that chloramphenicol reduced rooting by inhibiting the development of pea root primordia. Nanda et al. (1973 b) studied the effect of 5-FU, 5-FdU, actinomycin-D and cycloheximide on rooting etiolated stem segments of *Populus nigra* and showed that these antimetabolites decreased rooting and the degree of inhibition was more marked in glucose than in glucose + IAA. Similar results were obtained with etiolated stem segments of *Salix tetrasperma* (Bhattacharya et al., 1975d).
Stimulatory effect of antimetabolites on rooting

While inhibition of physiological processes like cell-elongation, cell enlargement and cell-wall extensibility has been reported by a number of workers (Lin and Key, 1966; Macdonald and Ellis, 1969; Key, 1966; Nooden, 1968; Courney et al., 1967; Gientka and Cherry, 1968; Ingle et al., 1968; Kaufman et al., 1968), a stimulatory effect of cycloheximide in low concentrations in rooting hypocotyl cuttings of Impatiens balsamina has been reported from this laboratory more recently (Nanda et al., 1979b). It has also been shown that isoperoxidases and IAA-oxidases are involved in cycloheximide caused promotion (Dhaliwal et al., 1974). Cycloheximide has also been shown to stimulate the rooting of hypocotyl cuttings of Phaseolus mungo (Gupta et al., 1975a). The lower concentrations of actinomycin-D and even of puromycin, 5-Fudr, 5-FU, 5-BU, 8-Aza-cytidine and acrydine orange have also been reported to stimulate rooting of hypocotyl cuttings of Phaseolus mungo (Arora et al., 1975).

Inhibitory effect of purine and pyrimidine analogues on rooting

2-thiouracil has been reported to inhibit the initiation as well as the development of root primordia (Fellenberg, 1966; Höhn, 1955; Melichar, 1964; Guillet, 1965; Knypl, 1966; Mitsuhashi et al., 1969). While Höhn (1955) and Mitsuhashi et al. (1969) reported that this inhibitory effect could be overcome by uracil, others did not find this
reversal. Guillet (1965) considered that the deleterious effect of 2-thiouracil was probably due to its ability to chelate copper ions. However, Fellenberg (1966) found that the depression in the initiation of root primordia occurred in a manner similar to the one caused by 2-4-dinitrophenol and cysteine indicating thereby of its pronounced side effect on "oxidative" processes. 5-bromouracil also inhibited the initiation and development of root primordia even in the presence of IAA. The effect could be reversed by thymine. It was also observed that 5-bromouracil was not always inhibitory if applied at the start of the experiment or during 36 to 120 hours (Fellenberg, 1967). In fact, its application during this period often stimulated rooting.

Some base analogues stimulate rooting.

Fellenberg (1966) found that 8-azaguanine decreased the production of roots on stem cuttings which could not be reversed by guanine. Ginzburg (1966) reported stimulation in the initiation of root primordia by both 8-azaguanine and 8-azaadenine. Meilichar (1964) also reported that 5-bromouracil stimulated rooting.

It appears that IAA affects rooting by causing quantitative and/or qualitative changes in the synthesis of proteins during an early part of regeneration period (Fellenberg, 1965; Kaminak, 1967) after an initial lag (Mitsuhashi et al., 1969; Moore and Lovell, 1972; Nanda and Jain 1972 a). The inhibitors of nucleic acid synthesis
may lengthen the lag phase and thereby enhance rooting in 
some species (Anzai et al., 1971).

**Effect of metabolic inhibitors on other growth manifestations**

The inhibitory effect of metabolic inhibitors on cell 
elongation, wall loosening and other manifestations of growth 
has been reported by many workers (Noeden and Thimmann, 1963, 
1965, 1966; Key, 1964; Key and Ingle, 1964; Key and Shannon, 
1964; Bonner, 1965; Masuda, 1966, 1968, 1969; Cleland, 1967, 
1968 a,b,c, 1970; Cleland et al., 1968; Evans and Ray, 1969; 
Bopp and Capesius, 1971). In contrast to this, Nanda et al. 
(1973 d) reported that cycloheximide (an inhibitor of pro-
etin synthesis) stimulated the GA₃-caused differentiation of 
leaves and floral buds in Impatiens balsamina. Goren et al. 
(1969) also reported earlier the promotion of flower forma-
tion and fruit set in citrus by antimetabolites of nucleic 
acid and proteins.

D - BIOCHEMICAL CHANGES ASSOCIATED WITH ROOTING

The work on changes in the metabolism of carbohydrates, 
nitrogen, nucleic acids, proteins including enzymes that 
take place during the initiation and development of roots 
is rather meagre.

(s) **Carbohydrates and hydrolysing enzymes**

A number of workers have shown that root formation is 
positively correlated with the carbohydrate content of stem 
cuttings (Reid, 1924 a,b; Schrader, 1924; Huan and Cornell, 
1951; Thorpe and Murashige, 1968; Molner and LaCroix, 1972a).
However, Nanda (1970) and Nanda et al. (1971 a) reported that the poor rooting response of stem cuttings of many species during winter months corresponded with high, and profuse rooting with low content of starch. The treatment of cuttings with IBA which increased the level of soluble carbohydrates but decreased that of starch, enhanced rooting.

Even starch utilized during rooting

It has been shown that starch content of stem cuttings decreased rapidly during root initiation (Schrader, 1924; Smith et al., 1940; Negishi and Satoo, 1956) and the decrease was faster in auxin-treated (in which rooting is also enhanced) than in control cuttings (Nanda et al., 1967, 1969, 1970; Bala et al., 1969; Nanda, 1970). Molnar and LaCroix (1972 a) reported that starch from rays behind the root initials and also from the endodermis, phloem and pith cells disappeared during the development of root primordia.

Sugar content of cuttings increases initially but decreases subsequently

Nanda (1970) showed that the free sugar content of stem cuttings was low initially but increased after 2-4 days to decrease subsequently, indicating that starch was hydrolysed and the sugars produced were utilized in the development of root primordia. Earlier, Stuart and Marth (1937) demonstrated that free sugar pool declined in the cuttings which did not contain starch. The exogenously supplied free sugars were effective in the absence of starch (Hausor, 1942) or under conditions of general insufficiency of carbohydrates (Nanda and Jain, 1971, 1972 b; Nanda et al., 1971 s,b).
Nanda, 1970). However, simple sugars either did not affect or inhibited rooting if fed to a system self sufficient in carbohydrates (Lovell et al., 1971, 1972; Moore et al., 1972).

(b) Involvement of nucleic acids and proteins in auxin effects of rooting

Applied IAA increased RNA synthesis

Heissig (1971) studied the effect of IAA on RNA synthesis in the cells of root primordia at different stages of development by autoradiographic investigation of uridine-2-14C incorporation. It was demonstrated that limiting the supply of IAA to root primordia reduced the synthesis of RNA only during the initial stage. The increase in RNA content by applied IAA was most pronounced, not during initiation, but during the early development of primordia. The results are suggestive that some factor in addition to IAA apparently triggers maximum RNA synthesis during root initiation.

Fellenberg (1967, 1969 a,b, 1970) considered that IAA and synthetic auxins enhance root initiation by stimulating RNA synthesis through derepression of the genes (cf. Jain and Nanda, 1972). More recently Jalouzet (1971) reported that an early phase of incorporation of precursors into RNA was essential for the initiation of roots in *Cicer arietinum*. He considers that a stable m-RNA factor that is synthesized soon after this stimulation, maintains this activation.

Nanda et al. (1973 b) and Arora et al. (1975) have also adduced evidence to show that the contents of nucleic acids and proteins increase manyfolds prior to the initiation and development of root primordia, and it is the size of the...
nucleotide and amino acids pools which determine the magnitude of rooting.

**Involvement of new RNA species in rooting**

Bottger and Lüdemann (1964) showed that the initiation and development of root primordia may require mRNA synthesis. They found that $^{32}P$ supplied to the cuttings during root regeneration rapidly accumulated in a fraction that was probably mRNA. The specific activity of the $^{32}P$-mRNA fraction increased rapidly from the start of the experiment until roots broke forth from the cuttings, but decreased as root growth proceeded (cf. Moore and Lovell, 1972). Key (1969) did not find similar results, although he showed that IAA treatment could result in increased mRNA synthesis in non-regenerating systems.

More recently Nanda and Bhattacharya (1973) have shown that auxins induce the production of some new species of mRNA or t-RNAs during the initiation and development of roots.

**Macromolecules other than nucleic acids associated with rooting**

Brown et al. (1950) observed that changes in the kind of amino acids helped in the rooting of bean cuttings. LaCock and Nizanksa (1963) working with *Populus deltoides*, observed that there was an increase in the content of free aminoacids and a decrease in the sugar content during rooting. Bala (1965) found a decrease in the amino-acid content of *Impatiens balsamina* cuttings.

Basu et al. (1968) using chromatographic technique showed that new proteins were synthesized during root initiation in
regenerating mango cuttings. It was also shown that in these proteins certain amino acids were preferentially incorporated. Roychoudhury (1971) showed that chloramphenicol, an inhibitor of protein synthesis, reduced rooting by preventing the incorporation of amino acids into newly synthesized proteins. The levels of DNA and RNA rose significantly during regeneration and this rise was accompanied by enhanced protein synthesis. The analysis of protein-bound amino acids in regenerating semihard wood cuttings of Justicia gendarussa showed that the pattern of incorporation of amino acids into proteins was significantly altered by auxin (Ghosh, 1971).

(c) Changes in the activity and isoenzyme patterns of enzymes in relation to rooting

Auxin in the presence of optimum nutrition is reported to regulate the synthesis of many enzymes viz., acid phosphatase (Palmer, 1970) cellulase (Maclauchlan, 1968), hemicellulase, B-glucanase (Stuart, 1938), peroxidase (Glassiou et al., 1968 and Galston et al., 1968). Key and Ingle (1964) consider that this regulatory effect of auxin on the synthesis of enzymes is mediated through regulation of the synthesis of m-RNAs. The production of specific m-RNAs should lead to the de novo synthesis of specific enzymes (cf. Jain and Nanda, 1972). The activities of several enzymes such as peroxidase (E.C. 1.11.1.7) (Chandra et al., 1971), and starch-hydrolysing enzymes (Molner and LaCroix, 1972 a; Nanda and Jain, 1972 b; Nanda et al., 1970) increase at the base of the cuttings or in the root primordia.
Auxins also increase the activity of hydrolysing enzymes

Auxins increase the activity of hydrolysing enzymes (Brakke and Nickell, 1952; Wort and Cowie, 1953; Venis, 1964; Nanda et al., 1967; Nanda and Anand, 1970) and such are able to increase rooting in winter months but only if the cambial activity continues. Nanda and Jain (1972 b) showed that etiolated stem segments of Populus nigra produced roots even when cultured in a medium containing starch as carbon source. This was brought about by hydrolysis of starch into sugars by the leaching out of the enzymes. Bhattacharya et al. (1976) reported that while some isoenzymes of - and B-amylases were associated with root initiation, others were associated with root development and still others with the suppression of roots. Molner and LaCroix (1972 a) reported the presence of amylases in the tissues surrounding root primordia and not in the primordia themselves.

Activity of hydrolysing enzymes increases during rooting

It has been shown that there is a close correlation between the disappearance of starch during rooting with an increase in the activity of hydrolysing enzymes (Nanda et al., 1967, 1969, 1970; Bala et al., 1969; Nanda, 1970; Nanda and Anand, 1970; Bhattacharya et al., 1976). The activity was high during April-August when rooting was profuse, decreased in October when rooting decreased and could not even be detected in December when rooting was poor. The poor rooting in winter months was, therefore, ascribed to
the low temperature that depressed the activity of hydrolysing enzymes (see Nanda, 1970; Nanda and Anand, 1970).

**Peroxidase activity increases with rooting**

Changes in the activity and isoenzyme pattern of peroxidase concomitant with tissue differentiation, have been reported by many workers (Siegel and Galston, 1967; Galston and Davies, 1969; Gordon and Allridge, 1971). Melner and LaCroix (1972 a) showed that peroxidase was the first enzyme whose activity increased with the initiation and development of roots in *Hydrangea* cuttings.

**Peroxidase isoenzymes associated with different phases of rooting**

Nanda and co-workers have reported that while some peroxidases are associated with root initiation, others are associated with root development and still others with their suppression (Nanda et al., 1973 a; Gurumurti and Nanda, 1974; Dhillwal et al., 1974). The association of some peroxidase isoenzymes with rooting has also been reported by Chandra et al. (1971).

**Some peroxidases act as IAA-oxidase**


**IAA-oxidase present in all plants**

The presence of IAA-oxidase was first demonstrated by Thimann (1934) who observed that tissue extracts during the
course of extraction inactivated the endogenous IAA. Larsen (1936, 1940) attributed it to the presence of a thermolabile enzyme. Goldacre (1949) named it IAA-oxidase. Since the extraction of IAA-oxidase by Tang and Bonner (1947) from pea homogenates, its presence has been shown in many plants (see Pilet and Gasper, 1968). In fact, it is stated that it may be present in almost all plants although in some cases its activity may be due to the present of powerful inhibitors (Pilet and Gasper, 1968).

### IAA-oxidase and rooting

Studies of changes in IAA-oxidase activity in relation to root initiation are rather scanty, although phenolics are known to synergise the effect of auxins by affecting IAA-oxidase activity. Monophenols are considered to act as co-factors and thus increase the activity of IAA-oxidase while polyphenols inhibit IAA-oxidase activity (Hare, 1964).

Galston (1967) considers that the regulation of IAA-oxidase activity by phenolics and indoles controls the level of endogenous auxin and thereby plant growth.

### High IAA-oxidase activity observed in rooting hypocotyl cuttings

Nanda et al. (1973) found that IAA-oxidase activity in stem segments of *Populus nigra* decreased at 24 hour in IAA + glucose but increased considerably in cultures containing antimetabolites. These antimetabolites inhibited the production of roots as well. It was also demonstrated that while some isoenzymes of IAA-oxidase were associated with root initiation, others were associated with root development.
and still others with the suppression of roots. In contrast to this, Dhalwal et al. (1974) reported a high IAA-oxidase activity in rooting hypocotyl cuttings of Impatiens balsamina and Frenkel and Hess (1974) in Phaseolus vulgaris. The intensification of isoenzymes in IAA and catechol, in which the cuttings rooted was also reported by Frenkel and Hess (1974).

Role of IAA-oxidation products in IAA-caused responses
Role of IAA-oxidase controversial

While Galston and Davies (1969) consider that IAA-oxidase has a detoxifying role, others consider that the physiological responses characteristic of IAA are due to its oxidation products (Meudt, 1967, 1971; Tuli and Moyer, 1969; Ockerse et al., 1970; Hoyle, 1974), although Haissig (1974) considers that the available evidence (Tomaszowski, 1959; Stenlid, 1963; Tomaszowski and Thimann, 1966) tends to refute the IAA-oxidation growth control theory as it relates to root initiation.

Biological activity of unidentified intermediates of IAA-oxidation

Meudt (1967) fractionated the oxidation reaction mixture at regular intervals, made their spectral analysis and studied the biological activity of each fraction by Avena first internode test of Nitsch and Nitsch (1959). He found that some fractions exhibited 400 times more activity than IAA, whereas others were low in activity. Prolonged incubation of IAA with Horse radish peroxidase and co-factors, however, rendered the secondary reaction
products ultimately inactive. He, thus, concludes that oxidation of IAA leads to the formation of biologically active compounds if the prevailing conditions prevent the formation of secondary oxidation products. Gurumurti et al. (1974) showed that metabisulphate which caused the oxidation of IAA also enhanced the formation of roots on hypocotyl cuttings of *Phaseolus mungo*.

**Oxidation products bind to macromolecules**

Meudt and Galston (1962b), Kefford et al. (1963), Galston et al. (1964) and Meudt (1967) consider that the primary oxidation products bind to the macromolecules and are thereby spared of transformation into the secondary oxidation products and thus cause physiological responses. Basu and Tuli (1972) demonstrated that 3-methylene oxindole which binds itself to protein fraction is an intermediate active oxidation product.