The propagation of plants by stem cuttings was practiced in pre-historic ages. A reference of propagation of "rudraksha" belonging to the genus Eleocarpus (Family: TILIACEAE) and of "Pipal" belonging to the genus Ficus religiosa (Family: MORACEAE) by means of stem cuttings is found in "Shiva and other puranas". In the Punjab, 500 years ago, Guru Nanak, the founder of the Sikh religion, brushed his teeth with a twig of "ber" (Zizyphus iujuba) and planted it in the soil at Sultanpur Lodhi. The legend has it that it grew into a full-fledged tree which is now regarded as sacred by the Sikhs (Fig.1). Rose plantation
through stem cuttings is popular in India since the advent of Mugal period.

The first scientific report on rooting of stem cuttings was, however, published by Duhamel du Monceau in the year 1758. A considerable amount of work on different aspects of rooting stem cuttings has since been done. Thimann & Behnke-Rogers (1950) showed that the rooting of stem cuttings of many plant species was stimulated by synthetic growth regulators. The results of researches carried out since then, have demonstrated that the rooting ability of stem cuttings of different plant species varies considerably. While some root easily, others do not root even with the application of synthetic auxins.

A considerable amount of literature dealing with different aspects of rooting of stem cuttings has accumulated now. As a detailed discussion of all the literature is beyond the scope of this work, a brief review of the more recent work on the following aspects is presented here:

A - FACTORS AFFECTING ROOTING
B - EFFECTS OF SYNTHETIC GROWTH REGULATORS ON ROOTING
C - BIOCHEMICAL CHANGES ACCOMPANYING ROOTING
D - ANTIMETABOLITES AND ROOTING
E - ANATOMICAL CHANGES AND ROOTING
F - FORMATION AND DIFFERENTIATION OF CALLUS
A - FACTORS AFFECTING ROOTING

The rooting of stem cuttings is influenced both by external and internal factors. Excellent review on the subject have appeared from time to time (Allen and McComb, 1955; Haissig, 1965; Dore, 1965; Fernqvist, 1966; Hyun, 1967).

(1) EXTERNAL FACTORS
(a) Season

Mirov (1944), Nanda (1967), Nanda et al. (1968, 1969, 1970, 1971a, b) have shown that the season in which stem cuttings root profusely varies with the plant species. Yin and Liu (1948) found that stem cuttings of tung trees rooted profusely immediately after the dormant period but poorly in March, June and November. Klein (1953) considered that seasonal variation in the rooting response of stem cuttings was determined by differences in the amount of nutrients. Tyce (1957) reported that stem cuttings of Salix fragilis rooted poorly in December but profusely in March to July. He ascribed this change in rooting response to changes in the levels of endogenous growth substances. Shapiro (1957) reported that roots appeared on the whole length of cuttings of Populus nigra in winter, but only at the basal part when these were taken from the trees during active growth in spring. Lanphear and Meahi (1963) found that spring season
was the best for rooting stem cuttings of selected evergreen plants. Chailakhyan and Nekrasova (1962) found that the initiation and development of roots was more on cuttings taken from trees during the rest period in summer than during the active period. Wareing and Smith (1963) found that the rooting of actively growing soft wood cuttings of *Populus robusta* was profuse in June and July but of dormant hard wood cuttings in autumn. Marcavillaca and Montaldi (1963) and Morishita (1964) reported June and July to be the best months for rooting cuttings of *Eucalyptus rostrata*. Kostovic (1964) found that cuttings of conifers rooted better in winter or early spring than in other seasons. Farmer (1966) reported more rooting on cuttings of *Populus deltoides* in February than in December, January or early March. Fadl and Hartmann (1967) demonstrated that seasonal effects determined the formation of adventitious roots on stem cuttings of pear. Vieitez (1968) and Nanda and Anand (1970) demonstrated rhythmical changes in the rooting of stem cuttings of *Salix atrocinera* and *Populus nigra*, respectively with phases of profuse and scarce rooting alternating with each other. Nanda (1971) worked on the rooting response of 156 forest tree species and considers that the seasonal changes in the rooting response were correlated with the changes in the cambial activity during the annual growth cycle which were determined by the
prevailing temperature and light conditions.

(b) Temperature

Leopold (1960) reported profuse rooting of cuttings at high temperatures, while Wareing and Smith (1962) reported stimulation of rooting of *Populus* cuttings by chilling prior to planting. Heide (1965) reported that high temperatures suppressed the formation of buds, antagonised the effect of kinins but enhanced the effect of auxins on the production of roots. Nanda (1968) observed that while in *Salmalia malabarica* rooting on stem cuttings did not differ at 25 and 35°C, in *Dalbergia sissoo* it was more at 35°C but in *Bryophyllum tubiflorum* at 25°C. The stem cuttings of these species did not root at 15°C.

(c) Light

Galston (1948) showed that the roots were produced on stem tips of *Asparagus* when these were cultured in a medium containing IAA and were kept in the dark but did not when these were maintained in light. Stem tips sub-cultured in the dark for several months, lost the ability to root even when these were subsequently treated with IAA. However, roots were produced when the tips were exposed to light prior to auxin treatment. The literature on the effect of light in inhibiting the production of roots has
been reviewed by Galston (1949), Torrey (1952) and Shapiro (1957). Galston and Baker (1953) and Herman and Hess (1964) showed that the stem cuttings of peas, beans and *Hibiscus* taken from seedlings grown in the dark rooted better than those grown in the light. Frolich (1961) found that avocado cuttings which ordinarily did not root, rooted when they were etiolated. Arnold and Astraon (1961) found that the number of roots produced on hypocotyls of *Impatiens balsamina* was not affected by light. However, Turetskaya and Kopf (1964) reported that stem cuttings of bean seedlings rooted when these were planted in the light, the conditions under which they were raised. Nanda et al. (1971) reported that etiolated stem segments of *Populus nigra* did not root in water or in auxin alone but rooted in glucose and the number of roots increased when IAA was added to glucose. Kawase (1965) reported inhibition of rooting when bean hypocotyls were etiolated but promotion of rooting of centrifuged willow cuttings by etiolation.

Light may affect rooting by virtue of its intensity, quality and duration.

(i) **Intensity:** Heide (1965) observed that high intensity light promoted the initiation but inhibited the elongation of roots on *Begonia* leaf cuttings. Ermakov et al (1965) reported that the percentage of cuttings of pine that rooted,
decreased while the number of roots increased with the decreasing intensity of light. Nanda (1968) showed that both the number and length of roots in *Bryophyllum tubiflorum* decreased with the decreasing intensity of light from 1,000 to 100 ft.c. However, at 15,000 ft.c. the number of roots increased but their length decreased. In *Delbergia sissoo* and *Salmalia malabarica*, both the number and length of roots increased with the decreasing intensity of light. Auxins inhibited rooting at all light intensities.

(ii) **Light quality:** Galston and Hand (1949) reported that light inhibited the initiation as well as growth of roots. Galston and Baker (1953) found that fewer roots were produced on stem cuttings of peas under red light than in the dark. Fletcher et al. (1965), however, reported that a large number of roots were produced on *Phaseolus vulgaris* when exposed to red light and the least when these were exposed to blue or far red light. Humphries (1961) reported that light from fluorescent tubes had no effect on the rooting of dwarf bean hypocotyls and considered that the formation of roots was controlled by the red, far-red system. He observed that the rooting of bean hypocotyl segments was influenced favourably by incandescent light under short photoperiods but unfavourably under long photoperiods.
Nanda (1968) observed that more roots were produced on stem cuttings of Dalbergia sissoo in white, less in red and the least in blue light, although the number of rooted cuttings and the length of roots did not differ much with the quality of light. Auxins stimulated rooting in red and blue lights. In Bryophyllum tubiflorum, however, red and blue lights were more effective than white light and rooting was stimulated by lower concentrations of auxins under these light conditions while in Salmalia malabarica 100 mg/l of IAA and IBA were most effective in blue, less in red and the least in white light.

(iii) Duration: The production of roots is markedly influenced by the day length to which both the stock plants and the cuttings taken from them, are subjected. (Selin, 1956; Shapiro, 1957; Mitsch, 1957; Piringer, 1961; Lanphear and Meahl, 1963 and Nanda, 1971). Bachlard and Stowe (1963) showed that the effect of photoperiod on the stock plant was more pronounced than on the cuttings. Mitsch (1957), Piringer (1961) and Joiner and Dicky (1963) found that long days enhanced the production of roots on stem cuttings. Lanphear and Meahl (1963) found that photoperiod affected the rooting of cuttings of some conifers but not of others. Wareing and Smith (1963) found that rooting of Populus robusta was more profuse under long than under short days. Heide (1965) observed
that continuous illumination promoted the number of roots on *Begonia* leaf cuttings, while short days inhibited it. Stoutemyer et al. (1947) found that the cuttings taken from *Gordonia axillaries* grown under 16-hour photoperiod, rooted better than those taken from plants grown under natural day length. Bala (1965) found that hypocotylar as well as epicotylar cuttings of *Impatiens balsamina* rooted better under long than under short days. Nanda (1967) reported that short days promoted while long days inhibited the rooting of stem cuttings of *Bryophyllum tubiflorum*, *Dalbergia sissoo* and *Salmalia malabarica*.

(d) Humidity and Aeration

Hurov (1961), working on hard-wood leafy cuttings, found that both humidity and aeration helped the cuttings to root. Okinura (1962) reported that the best condition for the rooting of pine cutting was when the air and water contents of soil were more or less equal. Marcavillaca and Montaldi (1964) found that high humidity promoted the development of roots on the leaves of *Eucalyptus rostrata*. Geister (1965) reported that proper aeration was necessary for the growth of roots.

(e) Rooting Medium

Hitchcock (1928) reported that 1:1 mixture of peat and sand was better for rooting of stem cuttings
than either alone. However, Hsu and Hendricks (1958) reported that a mixture of 1 : 1 peat and builders sand was more favourable for this purpose. On the other hand, Müller et al. (1962), working with *Salix viminalis*, reported that distilled water was more favourable for rooting than tap water. Similarly, Pantos (1963) found that the cuttings of Italian Robusta Poplar rooted better as water culture than as sand culture. 1 : 1 mixture of sand and triturated lava; coarse sand and perlite were found as suitable media for rooting stem cuttings by Marcavillaca and Montaldi (1963), Farmer (1963) and Schrieber (1963), respectively.

Mergen and Simpson (1964) reported that sand/vermiculite was the best medium for propagating plants from Pinus needle fascicles. Mamedov (1964) found that river sand, sand peat, sand/conifer soil and perlite were suitable for the rapid development of roots and aerial parts on cuttings of woody species. Ermakov and Grevin (1965) reported that seaside sand was the best of the various tried media for the initiation and development of roots.

(f) **Acidity (pH)**

Small (1923) reported that the addition of acetic acid to the medium promoted root growth. Smith (1926)
reported that favourable pH for rooting Coleus cuttings ranged from 4.0 to 9.2. Dore (1965) postulated that a slight acidity may not affect rooting but may be unsuitable for the growth of micro-organisms and may, therefore, protect the cut surface. Cormack (1965) and Cormack and Lemay (1966) reported that the emergence and subsequent growth of adventitious roots on cuttings of balsam poplar decreased with the increasing alkalinity of the medium.

(g) **Nutrients**

Both organic and inorganic nutrients affect the rooting response of stem cuttings.

(i) **Carbohydrates**: Kraus and Kraybill (1918); Knight (1926); Carlson (1929) and Durham (1934) reported that an external supply of carbohydrates increased rooting considerably. However, Klein (1953) found no correlation between the sugar content and the rooting of grape cuttings. Nanda et al. (1971) and Nanda and Jain (1971) reported that rooting of 2.5 cm stem segments of Populus nigra and Salix tetrasperma was limited primarily by nutritional factors. Rooting did not take place on segments without leaves in water, IAA, or IBA, but occurred in glucose. However, rooting occurred even in water on segments on which the leaves were left intact. They concluded that rooting was
determined by a proper balance between nutritional and regulatory factors and may not occur even when the concentration of one of these is very high if that of the other is very low.

(ii) Nitrogenous compounds: Went (1938) and Doak (1939) found that some amino acids increased the rooting capacity of cuttings. Went and Thimann (1937) found that bioten and adenine were the most effective nitrogenous compounds that were concerned in rooting. van Overbeek et al. (1945) noted that organic forms of nitrogen were better for rooting than inorganic forms. However, they reported that neither of them was as effective as arginine. Bachelard and Stowe (1963) found that nitrogenous compounds along with sucrose enhanced the ability of stem cuttings to root. Bala (1965) reported that while amino acids at low concentrations enhanced but at higher concentrations inhibited the rooting of stem cuttings of Impatiens balsamina, tryptophan enhanced it at all concentrations. Marin (1965) reported that nitrogenous fertilizers at low concentrations promoted but at higher concentrations inhibited the production of roots. The promotion of root formation at low concentrations of nitrogenous fertilizers has also been reported earlier by a number of workers (Pearse, 1943, 1946; van Overbeek et al., 1946).
(iii) Boron: Hemberg (1951), Weiser (1959) and Sen and Bose (1959) reported that boron affected the rooting of stem cuttings.

(II) INTERNAL FACTORS

(a) Age of Stock Plants

Stoutemyer (1937, 1938), working with apple and Winters and Muzik (1963) with black pepper, reported that the age of the mother plant from which cuttings were taken was not important in rooting. However, Thimann and Delisle (1942) reported that pine and oak cuttings from younger plants rooted more easily than from the older ones. The literature on the subject has been adequately reviewed by Allen and McComb (1955) and Gorter (1961).

(b) Juvenility

Mirov (1938) reported that the cuttings of white pine could be rooted easily up to the age of 10 years without any auxin treatment, but the capacity to root decreased with the age of the tree. Stem cuttings taken from juvenile trees rooted better than those from the older ones (Sax, 1962). Lacok and Niznauska (1963) observed that 1- and 15-year old trees of Poplars species differed both biochemically and morphologically. Hashimoto et al. (1963) propagated cuttings of Pinus and Cryptomeria obtained from trees of varying ages and found that roots were produced on
100% cuttings of *Pinus* taken from 1- to 5- and 10-year old trees, as compared to that on 63% cuttings taken from 11-year old *Cryptomeria* trees. Ruggeri (1963) found that cuttings taken from 1-year old *Eucalyptus rostrata* rooted easily, while those from mature plants did not root at all. Attempts to root stem cuttings taken from mature *Pseudotsuga taxifolia* failed, although seedling cuttings rooted fairly well (Wheat, 1966). The percentage of rooted cuttings taken from 1, 2 and 3 year old trees of *Pinus strobus* was found to be 31, 18 and 9 respectively by Doran (1957) and Thimann and Delsile (1963). Yim (1962) found that rooting occurred on 86, 43, 28 and 16% cuttings of *Pinus rigida* taken from 1, 2, 10 and 20 year old trees, respectively.

Grace (1939) observed that rooting occurred on 48% of the cuttings taken from the upper portion of a cone bearing Norway spruce as compared to 86% of those taken from the lower portion. de Muckadill (1954) made cuttings from graded locations on the tree and found that the new plants raised from cuttings taken from the base rooted more profusely than from the upper part.

(c) **Age of the Tissue**

The physiological age of the tissue is also important in rooting. Rea (1932) noted that mature cuttings of
cotton wood rooted better than the younger ones. Weirszyłowsky (1937) reported that the seedling cuttings of *Pyrus* rooted better than those from the clones. Gibbins (1937) also found that young branches of *Coffea* rooted better than the older ones. Went and Thimann (1937) observed that the apical fragment of a shoot-rooted better than the basal one. These findings were supported by Chandler (1959) and Conover and Joiner (1963).

Mirow (1944) found that the cuttings from the lower branches of the main stem of white pine rooted better than those from the upper ones. Grace (1939), Stoutemyer (1944) concluded that in some trees the auxins were more effective in rooting on cuttings from the lower than the upper branches. Similarly Edgerton (1944) while working with red maple, observed that the cuttings from the lower half of the crown rooted more readily than from the upper half. Yin and Liu (1948) observed that the cuttings from the basal portion of tung tree rooted more profusely than those from the upper regions. Purohit and Nanda (1964) also obtained similar results in * Bryophyllum tubiflorum*. Ruggeri (1963) found that the root formation in *Eucalyptus rostrata* was more on the basal than upper cuttings. Sax (1962) in his review concluded that the terminal branches were more difficult to root than the lateral ones. Woycicki and Terpinski (1937), on the other hand, observed better
rooting of internodal cuttings of *Dahlia* and *Pelargonium* than of the nodal ones.

(d) **Size of Stock Plants and of Cuttings**

Edgerton (1944) obtained more roots on cuttings from larger than from smaller trees. Deubar and Ferrar (1940) found that the 4 inch or longer cuttings of Norway spruce rooted better than the shorter ones. Nishimura et al. (1944), however, found that short cuttings of *Guayule* from later season growth also rooted easily. Ting et al. (1963) found that the length of cuttings did not affect the rooting of *Pyracantha*. Ching (1963) observed that 15 cm long cuttings of *Populus* rooted better than the smaller ones and 1 cm thick ones better than the thicker ones. However, Alonzo and Sancko (1964) suggested that the position of the shoot from which cuttings were taken, was more important in rooting than their diameter.

(e) **Morpho-physiological Status of Mother Plant**

Morpho-physiological status which is determined by the nature of the buds and the leaves on the plant, also influences the rooting response of stem cuttings.

(i) **Vegetative buds:** Gorter (1957) found that stem cuttings with vegetative buds rooted better than those without them.
even with auxin application. Tyce (1957) found that stem cuttings of *Salix fragilis* rooted more profusely in March to July when the vegetative buds were actively growing than in other months. Richardson (1958) also found a close correlation between the initiation and growth of roots in spring and the presence of at least one physiologically non-dormant bud in *Acer saccharinum*. Gorter (1961) found that isolated pea stem cuttings did not react to auxin application in the absence of buds. Wareing (1963) noted that debudded cuttings of *Populus robusta* did not root. Rooting did not occur even when the buds were in dormant state. Kawase (1964) reported that the root forming ability of willow cuttings was due to the presence of axillary buds, if they were kept intact after centrifugation.

(ii) Reproductive buds: Boremann (1939) worked with 376 plant species and concluded that the blooming of the stock plant lowers the rooting capacity of cuttings taken from it. Dore (1953) and Sneddon (1962) also found that the flowering branches of plants were difficult to root. Selim (1956), however, observed that the flowering of day neutral tomato, had no influence on the rootability of its cuttings. Gorter (1956) obtained similar results in day-neutral plant, *Coleus rhenaltianus*. He found that the nodes of the
vegetative plants rooted better than those of the flowering ones and the middle nodes more than the upper and lower ones. Resende (1946) considered that the inability of stem cuttings taken from the plant at flowering stage to root can be ascribed to utilization of auxin towards the developing reproductive organs. However, Gorter (1961) attributed this to the smaller leaf area of the cuttings from the flowering than from the vegetative branches.

(iii) **Leaf:** Gorter (1956) found that the rooting response of *Coleus* cuttings was determined by the area of the leaves at the nodes, if separate nodes were propagated. Sen and Basu (1960) reported that in *Justica nendarussa* cuttings, the leaves did not affect the number of roots but affected their development significantly. Wareing (1962) observed that in *Populus robusta* the mature leaves of *Populus robusta* played a significant role in the rooting of soft wood cuttings.

(f) **Hormones and Rooting Co-factors**

Hormones and rooting co-factors have been reported to influence the rooting response of stem cuttings.

(i) **Auxins and other indole compounds:** Wareing (1951) stated that the initiation of cambial activity depends
upon the production of free auxins by developing buds. Larson (1962) and Wort (1962) correlated auxins with cambial activity. Digby and Wareing (1966) also found that the division of cambial cells and their differentiation into xylem was stimulated by IAA in wooden shoots of *Robinia pseudocacia*.

Vieitez et al. (1964, 1966, 1967) observed an increase in the content of auxins in easy-to-root as compared to difficult-to-root species. Katsumi et al. (1969) suggested that in rooting of cuttings of cucumber hypocotyls, auxin was one of the factors supplied by cotyledons. Vieitez et al. (1964) made a hormonal survey on easy-to-root and difficult-to-root tree species and reported qualitatively and quantitatively higher indolic hormonal content in the cuttings of easy rooting species than in the difficult-to-root ones.

Haissig (1970a,b) observed that the formation of root primordia in brittle willow was governed by auxin supply.

(ii) Cytokinins and gibberellins: Giesbuhler and Skoog (1957) showed that it was the relative levels of kinins and auxins that determined whether the tissue differentiated into root or shoot; the low value of kinin/auxin favoured rooting, while the high value favoured the formation of buds. Endogenous growth substances other than indole derivatives
which are known to affect the production of adventitious roots are cytokinins and gibberellins (Thimann, 1963; Miller, 1961). The hypocotyls of detached stems of Phaseolus vulgaris in culture solution produced adventitious roots sooner than the petioles of detached leaves, both exuding auxin, probably IAA, and cytokinins into the solution prior to root formation (Wheeler, 1971).

(iii) Rhizocaline, growth factors and rooting co-factors: The idea of an internal factor or hormone which controls rooting was first advanced by Vanderlack (1925). Bouillenne and Went (1933) reported the occurrence of an acidic, heat stable factor with low molecular weight concerned in the formation of roots and named it 'rhizocaline'. Hess (1962) and Kawase (1964) supported the existence of such substances. Some substances other than 'rhizocaline' were also shown to be necessary for the formation of roots (Torrey, 1950; Libbert, 1956a,b). Bouillenne, Bouillenne and Walrand (1955) postulated that the initiation and development of adventitious roots depends on 3 factors: (1) A non-specific translocatable factor that has ortho diphenolic groups and is translocated from the leaves; (2) a specific mobile factor (auxin) that is present in concentrations within physiological limits and (3) a specific enzyme of phenol oxidase type that is located in the cells of certain tissues.
They believe that these factors accumulate at the site of auxin application and cause the initiation of roots. Hess (1962) obtained the alcoholic extracts from the easy-to-root and the difficult-to-root stem cuttings of juvenile Hedra helix, red flowering Hibiscus and easy-to-root Chrysanthemum and obtained four zones of root initiating substances with Rf values at 0.1, 0.3, 0.6 and 0.8 and called these as co-factors 1, 2, 3 and 4, respectively. Co-factors 1 and 2 were basic in nature while co-factor 3 was acidic and co-factor 4 a neutral substance. All these were thermostable and their activity in root initiation could not be replaced by any of the known nutrients or growth substances. The content of these co-factors in the extracts from the difficult-to-root plant species was lower than that in the easy-to-root species. Girouard (1969) separated Hess's co-factor 4 into 5 major and a number of minor fractions. Co-factor 2 was identified as chlorogenic acid and co-factor 3 was found to consist of 3 components, chlorogenic acid, iso-chlorogenic acid and an unknown promoter.

Fadl and Hartmann (1967) and Fadl (1967) isolated a rooting co-factor from pear cuttings treated with IBA with intact buds and this was identified as a complex of indole and phenolic compounds. This co-factor could not be isolated from cuttings which were either dormant or
without buds even when these were treated with IBA.

The investigations of many workers have lent support to the concept of rooting co-factors (Herman and Hess, 1963; Zimmerman, 1964; Challenger et al., 1964; Richards, 1964; Nelson and Pepper, 1965; Kawase, 1964, 1965; Heuser, 1966; Stoltz and Hess, 1966; Lanphear and Meahl, 1966; Girouard and Hess, 1966; Girouard, 1967a,b, 1969).

Many workers have suggested extractable growth substances present in stem cuttings along with the inhibitors (Fraunman, 1953, 1959; Allen, 1960; Ogasawara, 1961; Ogasawara and Kondo, 1962). Hess (1962) found that only small differences occurred between the growth substances of easy-rooting juvenile and difficult-rooting mature cuttings.

Bachelard and Stowe (1963) found that the rooting of the cuttings of *Acer* and *Eucalyptus* was correlated with the formation of anthocyanin.

B - EFFECTS OF SYNTHETIC GROWTH REGULATORS ON ROOTING

(a) Auxins

Auxins have been used for rooting stem cuttings of many plant species (Zimmerman and Wilcoxon, 1935; Thimann and Koepfli, 1935; Thimann and Delisle, 1942; Shapiro, 1957; Bachelard and Stowe, 1963; Wheat, 1964; Sen and Bose, 1959, 1965; Farmer, 1966; Nanda and Kochhar, 1968 and Nanda
et al., 1968, 1970, 1971a, b). The relative effectiveness of different auxins in rooting stem cuttings of many plant species has been reported by Thimann and Behnke-Rogers (1950), Leopold (1960) and Nanda (1970, 1971a, b). The optimum concentration of an auxin required for rooting stem cuttings varies with the plant species. In general, IBA and NAA are more effective in rooting than other auxins because of their powerful activity and slow destruction. IAA is less effective as it is readily oxidized. Phenoxy acids are highly effective in inducing root primordia but are injurious to the growth of leaves. A mixture of auxins is more effective in rooting than the individual ones (Evans, 1952; van Onsem, 1953; Nanda and Kochhar, 1968).

There are many species which do not root even with the application of auxins (Tyce, 1957; Hess, 1963 and Nanda, 1971). In fact, NAA is toxic to rooting tung tree cuttings (Yin and Liu, 1948) and IBA ineffective in rooting stem cuttings of Pittosporium sp. and of Pinus taeda. The concentration of an auxin that is favourable for root growth may not be favourable for shoot growth (Lanphere and Meahl, 1963). Chandler (1967) found that IBA and 2,4-D facilitated the rooting of Larix cuttings. Hartmann and Kester (1968) considered that due to low solubility of auxins as such, these should be provided in the form of salts. Nanda et al. (1967) found that both photoperiod and auxin,
had a profound effect on rooting stem cuttings of *Bryophyllum tubiflorum*. Short day requirement for rooting of this species could be replaced by treatment with IAA or IBA. Nanda (1971) reported that auxins play multifarious role. They are concerned in the division of meristematic cells, in their elongation, in differentiating the cambial initials into root primordia and above all in the mobilization of reserve food materials caused by enhancing the activity of hydrolysing enzymes and passing the mobilized sugars to the site of root initiation. He considers that the effect of exogenously applied auxins on rooting of stem cuttings is mediated primarily through their effect on mobilization of starch caused by enhanced activity of hydrolysing enzymes. Shibata (1971) found auxin sensitive and auxin non-sensitive phases in the rooting of stem cuttings of *Azukia* cuttings and explained on this basis the effect of higher concentrations of auxins in promoting the production of adventitious roots and the ineffectiveness of low concentration to this effect.

(b) **Anti-auxins**

Muir and Hansch (1951) reported that tri-iodobenzoic acid (TIBA) played a dual role, as an auxin and an anti-auxin. Kaindl (1957) found that TIBA inhibited the formation as well as growth of roots. Niedergang-Kamien and Leopold
(1957) stated that TIBA behaved as a weak auxin and a SH reagent. Audus and Thresh (1956) demonstrated that TIBA reduced the level of endogenous IAA in pea roots and inhibited the secretion of auxin from the tissues (Hertal and Leopold, 1963; Christie and Leopold, 1965). Aberg (1957) considered that TIBA, at low concentrations, acted synergistically with the supra-optimal concentrations of endogenous auxin to bring about increased manifestations. Blackmann and Sargent (1959) concluded that the uptake of TIBA and auxin was similar although the two differed from each other in their physiological action. Panigrahi and Audus (1966) stated that TIBA reduced apical dominance and the elongation of intact plants. It decreased the concentration of auxin (Pilet, 1963, 1967), the amount of mobile auxins (Winter, 1967) and their diffusions and transport (Hay, 1956; Zzar and Rijivan, 1956).

(c) Gibberellins

Gibberellic acid (GA₃) has been reported to inhibit rooting in many plants (Brian et al., 1957, 1960; Bachelard and Stowe, 1963; Schraudolf and Reinert, 1959; Mitsuhashi et al., 1969). Bachelard and Stowe (1963) reported that the inhibitory effect of GA₃ could not be reversed by auxin application. Libbert and Krelle (1966) reported that GA₃ inhibited the formation of roots on hypocotyls of Phaseolus.
vulgaris and Pharbitis purpurea. Schott and Schraudorf (1967) reported that GA$_3$ totally suppressed the formation of adventitious roots on Begonia leaf discs. Nanda et al. (1968) reported that GA$_3$ inhibited the rooting of Populus nigra cuttings and ascribed this effect to the stimulation of shoot growth. However, Mitsuhashi et al. (1969), while working with Azukia, reported inhibition of root formation without any corresponding increase in shoot growth. Heide (1969) proposed that GA$_3$ acted by blocking the organised cell division for root initiation. On the other hand, GA$_3$ promoted the rooting of stem cuttings of Bryophyllum tubiflorum and Ipomoea fistulosa (Nanda et al., 1967, 1972; Anand et al., 1972), and pea cuttings (Erikson, 1971).

(d) Growth Retardants

Cathey and Piringer (1961) showed that retardants enhanced the development of root system in plants. Low concentrations of cycocel (CCC) stimulated root initiation in Phaseolus vulgaris and Pharbitis purpurea (Libbert and Krelle, 1966) and in tomato (Mishra and Pradhan, 1968), while AMO 1618 inhibited it in Chrysanthemum (Cathey and Marth, 1960). Connell and Struckmeyer (1969) reported that B 995 caused coil-like growth of roots in Zinnia elegans but inhibited the division and enlargement of cells.
Morphactins suppressed rooting completely on *Begonia* leaf discs (Schott and Schraudolf, 1967) and inhibited it in dwarf pea seedlings (Tognoni et al., 1967) and on epiphyllous buds of *Bryophyllum tubiflorum* (Nanda et al., 1968). However, Besemer et al. (1969) reported that root formation on isolated leaves of *Cichorium* was not inhibited by morphactin. Schneider (1970) reported that morphactin promoted the formation of roots on bean hypocotyl cylinders but inhibited the extension of root primordia. The retardation of root growth by morphactin was also observed in auxin supplied explants of *Vitis vinifera* (Julliard, 1966; Alleweldt and Bourguin, 1967 and Alleweldt, 1969). Krelle (1967) also found that morphactin in concentrations up to $10^{-8}$M promoted but in higher concentrations inhibited the production of roots in *Phaseolus* and *Helianthus*. In *Coleus* an initial inhibition of root formation by $10^{-5}$M morphactin was followed by the promotive effect later. Vogt (1968) also reported strong multiplication of adventitious root primordia and stimulation of root extension growth up to a concentration of $10^{-6}$M morphactin but inhibition at higher concentrations in *Coleus*. The promotive effect could be counteracted by IAA and increased with the increasing concentration. Pieniagek and Saniewski (1968) reported that morphactin could not induce rooting and suppressed the
growth of adventitious roots induced by NAA on stem cuttings of *Malus sylvestris*. Morphactins stimulate induction of cell division but further organisation is histologically disturbed so that extension of roots is retarded (Saniewski et al., 1968; Ziegler et al., 1969). Kochhar et al. (1971) found that morphactin inhibited completely the formation of new root primordia on stem cuttings of *Salix tetrasperma* but allowed the existing root primordia to develop into roots. While the production of primary roots was inhibited, that of the secondary roots was enhanced by morphactin.

(f) **Kinin**

De Ropp (1956) reported that kinetin inhibited rooting. Englebrecht and Mothes (1961) found that kinetin in combination with NAA, enhanced both the initiation and growth of roots. Bachelard and Stowe (1963) found that kinetin inhibited the rooting of stem cuttings of *Acer rubrum* when applied at the site of root formation but stimulated it when applied to the leaves. Heide (1965) on the basis of his work with *Begonia* leaf cuttings, concluded that auxins and kinins showed interaction effects on rooting. Libbert (1956) and Hess (1961) found that the increase in the number of roots caused by kinetin was more on cuttings without than with leaves and postulated the existence of an unknown factor in leaves that makes the cuttings to root.
(g) Phenols

Gortner and Kent (1958) showed that many phenols affected the activity of indoleacetic acid oxidase from pineapple in vitro; ortho and para dihydric phenols being inhibitors, while monohydric phenols, particularly p-coumaric acid, activators. This has led to the concept that dihydric phenols act as synergists for indole-acetic-acid (IAA) but monohydric phenols decrease IAA-induced growth. Some phenolic compounds are synthesized within the plant for a brief period during a particular stage of growth (Mann et al., 1963; El Basyouni and Towers, 1964; Hadwiger et al., 1965) or during the differentiation of a particular tissue (Commoner and Zucker, 1953; Zenk, 1965).

Goodwin (1963) considered that phenolic compounds act to cause feedback inhibition and activation of enzyme activity which involved rapid interaction between micro- and macromolecules. Gantzer (1960) attributed that phenolic compounds acts as an analogues of growth hormones because of the structural similarities in their configurations due to the presence of indole rings and acidic group and it is further supported by IAA oxidase concept. Towers (1964) examined the role of phenols as substrates in the respiratory activity of higher plants. The oxidation of $^{14}$C labelled catechins by tea plant cuttings was studied by Zaprometov (1959), who could account for 73 to 82 percent of the
absorbed activity of $^{14}O_2$ after 30 hours. The fact that the quinones, coenzyme Q and plastoquinone are known to be involved in mitochondrial oxidations and in photosynthesis, was thought to suggest their reversible reduction to phenols (Wagner and Folkers, 1964). Finkle (1967) put forth an hypothesis about the mode of action of phenolic acids in plants. He considers that they inhibit an enzyme that oxidatively destroys IAA and thereby its hormonal action. According to a supplementary hypothesis put forth by him both the phenolic compounds and the indole compounds possibly had some significant structural relationship. Folkers (1967) suggested phenols as actual precursors of coenzyme Q.

(h) **Vitamins**

Went et al. (1939) found that vitamin $B_1$ acted as a limiting factor in rooting *Pisum* and *Citrus* cuttings. Sen and Bose (1958) reported that the effect of growth hormones increased in the presence of thiamine and glutamic acid. Trione and Avellaneda (1963) showed that the rooting of cuttings when yeast was used along with IBA was enhanced. Pantos et al. (1964) found that the exogenously applied vitamins increased the initiation of roots but suppressed their growth.
C - BIOCHEMICAL CHANGES ACCOMPANYING ROOTING

(a) Carbohydrates

Baucer (1942) reported that 'rhizocaline' caused the formation of roots and also a decrease in the content of starch. Gall (1949) reported that 2,4-D also caused the digestion of starch. van Overbeek et al. (1946) demonstrated that leaves of red Hibiscus supplied sugars and nitrogenous materials for rooting stem cuttings. Langston (1954) found that the beneficial effect of leaves was accentuated by spraying the foliage with sugar solution. Mahestede and Haber (1959) reported that a high C/N ratio was necessary for the rapid formation of roots. Frieberg and Clark (1955) studied the effect of 2,4-D on changes in nitrogen fraction, proteolytic enzymes and water relations of soybean and concluded that an adequate supply of carbohydrates and proteins is necessary to satisfy energy and tissue building requirements. Herman and Hess (1967) found that the etiolated cuttings rooted better and had substantially higher level of sucrose, glucose, fructose and proteins. Wort and Stowe (1953), Wort (1954), Satoo (1956) and Nanda et al. (1967) reported a decrease in the starch content of cuttings soon after planting. Bachelard and Stowe (1963) showed that sucrose enhanced the rooting ability of Eucalyptus cuttings. Nanda et al. (1969, 1970) showed that the seasonal changes in the rooting response of
stem cuttings of *Bryophyllum tubiflorum* and *Dalbergia sissoo* were closely related to the disappearance of starch, the low rooting in winter months corresponding with high and high rooting with low content of starch. These changes were caused by seasonal changes in the activity of hydrolyzing enzymes.

(b) Nitrogen, Amino Acids and Proteins

Hyun (1967) found that the nitrogen content of the cuttings of *Pinus rigida* and *Populus alba* increased rapidly with the growing season till early June, part being higher in the upper than in the lower part (Hyun, 1968). Locock and Niznanska (1963) reported an increase while Bala (1965) reported a decrease in the content of amino acids during rooting of stem cuttings. Woodstock and Skoog (1960) demonstrated that there was a relationship between growth rates and nucleic acid content in the roots of inbred lines of corn.

Warmke and Warmke (1950) found an increase in the free, bound and neutral fractions of auxin during rooting. Jalouzot (1971) studied changes in the contents of nucleic acids and proteins during the initiation of adventitious roots in *Cicer arietinum* and found that an early phase of incorporation of precursors into RNA was essential for their initiation and a stable mRNA factor synthesised soon after
the stimulus of cuttings for activation.

D - ANTIMETABOLITES AND ROOTING

The literature on the effect of antimetabolites on rooting is rather scanty. Torrey (1953) observed that DNP, an uncoupler of aerobic respiration from the phosphorylation system at a concentration of $10^{-4}$M and pH 5.0, completely stopped the differentiation of primary vascular tissue and root elongation. The secondary wall thickening and lignification of protoxylem was also initiated by IAA. He suggested that the action of IAA, iodo acetic acid, and 2,4-dinitrophenol, centres round cellular processes associated with carbohydrate metabolism.

Krul (1968) reported that 2,4-dinitrophenol increased the number of root primordia on hypocotyls in the darkness and more so in the presence of IAA. Chloramphenicol and puromycin decreased rooting only slightly but cycloheximide and ethionine decreased it by 96 and 88%, respectively. Nanda et al. (1971) showed that IAA promoted the formation of roots but GA$_3$ that of shoot but cycloheximide inhibited both in epiphyllous buds of *Bryophyllum tubiflorum*.

It has been established that actinomycine suppresses the synthesis of DNA dependent RNA; cycloheximide the synthesis of cytoplasmic proteins and chloramphenicol
the synthesis of mitochondrial ribosomes. Tadashi et al. (1971) reported that 2-thiouracil suppressed the synthesis of uracil and 5-bromodeoxyuridine the synthesis of thymadin. They consider that the effect of these base analogues on the formation of adventitious roots is due to their participation in the synthesis of nucleic acids.

E - ANATOMICAL CHANGES AND ROOTING

(a) Stem Anatomy and Rooting

Wellensick (1952) showed that the lateral shoots from sphaeroblasts exhibited the juvenile morphological characters. According to Garner (1953) the continuity of sclerenchymatous ring in stem increases with a fall in the propagational capacity of a plant. Giampi and Gellini (1958) found that sclerenchymatous tissue was absent in the stem of olive whose cuttings rooted profusely. According to Beakbane (1961) readily rooting sphaeroblast shoots have fewer fibres and sclereids in the primary phloem than the shy-rooting normal shoots from the same apple clone. She also observed that shoots of shy-rooting varieties were frequently characterised by a high degree of sclerification in the tissue of primary phloem. But Metcalf (1961) showed that in Pittosporum and Erica species, the presence of periphloic sclerenchyma was not the sole cause of prevention of easy rooting. Nanda (1971)
has shown a close relationship of the distribution of sclerenchymatous tissue with the rooting response of stem cuttings of many forest tree species.

(b) **Cambial Activity**

Cambium being a lateral meristem, is responsible for radial growth in woody species and its activity is correlated with active extension growth of the shoot (Wareing, 1958; Larson, 1962; Went, 1962). Kozlowski (1962) suggested that higher cambial activity coincided with the period of higher photosynthetic production. Alvin (1956) found higher cambial activity in June and July and a positive correlation between temperature and cambial activity. Clark and Gibbs (1957) also found that cambium activity depended upon temperature and not on water. Wareing and Roberts (1956) studied the photoperiodic control of cambium activity in *Robinia pseudocacia*. According to Wareing (1951), initiation of cambial activity appears to depend upon the production of free auxins by the expanding buds. Larson (1962) and Wort (1962) also demonstrated the importance of auxins in cambial activity. Wareing et al. (1964) found that the endogenous hormones had a significant role to play in cambial activity. Bardley and Crane (1957) and Wareing (1958) showed that gibberellic acid stimulated cambial activity. Digby and Wareing (1966)
also found a relationship between the level of endogenous hormones and seasonal changes in cambial activity. They also found that IAA stimulated cambial activity but the cambial derivatives remained undifferentiated and that high ratio of IAA/GA₃ favoured xylem formation, whereas low IAA/GA₃ favoured the production of phloem.

(c) Autolysis and Xylem Differentiation

According to Scheidrake (1971) auxin is formed by autolysing cells and that in higher plants, it is normally produced as a result of the death of cells (Scheidrake and Northcote, 1968a, b, c). The differentiation of xylem cells involved autolysis and this creates a gradient of auxins from xylem to phloem across the cambium resulting in different hormonal environments on different sides of the cambium (Scheidrake, 1971). Both sucrose and auxins are necessary for organised cell differentiation, a high sucrose to low auxin ratio leads to excessive production of phloem but high auxin to low sucrose ratio stimulates xylem differentiation (Wetmore and Rier, 1963; Jeffs and Northcote, 1967; Rier and Beslow, 1967). Wodzicki (1971) found that in *Pinus sylvestris* (1) the variation in radial diameter of tracheids was probably dependent on seasonal changes in the rate of growth during the phase of radial enlargement, (2) the daily rate of cell wall formation
determined the final cell wall thickness of tracheids only at the beginning and the end of the season, (3) both rates were affected by temperature, (4) the seasonal changes in cell wall thickness were dependent mostly upon seasonal changes in the duration of the maturation period, (5) the changes in the duration of the maturation period which brought about transition from early to late wood, were determined mostly by the delay in the onset of autolysis of cytoplasm which terminates the phase of tracheid maturation. This process, unlike the xylem production from cambium and the termination of radial enlargement, was not affected by seasonal variation in temperature. He further stated that auxin and environmental factors, such as precipitation and temperature, were not specific for xylem differentiation but affected wood differentiation when they become limiting or exceed the limit of tolerance. They did not determine the differentiation of annual rings into early and late wood. The final thickness of the wall of the tracheidal cells was closely related to the duration of the maturation phase over most of the season, a relation which determines the transition of thin-walled tracheids of early wood to thick-walled tracheids of the late wood. (Wodzicki, 1960b, 1971; Wodzicki and Pede, 1963; Skene, 1969). Wodzicki (1971) further showed that the factors responsible for
inducing or inhibiting the onset of final stage of maturation, involved the autolysis of protoplasm. The rate of termination of the maturation phase was not related to the weather conditions, unlike the rates of the production of cambial xylem and the completion of the phase of radial enlargement. Gordon and Larson (1968) and Larson (1969) conducted direct studies on seasonal changes in the supply of carbohydrate to the stem. There is hardly any direct evidence to show that auxin induces xylem differentiation from either procambium or cambium. Jacobs and Morrow (1957) presented data correlating the differentiation of the primary xylem in Coleus with the production of auxin in the leaf. Wangermann (1967) demonstrated that exogenous auxin could replace the leaf in its xylogenic effect in Coleus stems. However, in neither of these cases is it clear whether auxin is required for the induction of xylem differentiation, or for the maturation of xylem, or both. Young (1954) found that exogenous IAA could not substitute for the developing leaf in the induction of primary xylem differentiation from the procambium in Lupinus albus.

Studies of differentiation of secondary xylem in response to growth regulators are complicated by the involvement of cambium. It has often been assumed that the induction of cambial division and xylem differentiation in response to a growth regulator demonstrated a requirement
for the growth regulator in xylogenesis. Since the induction of cambial division is a pre-requisite to secondary xylem differentiation, it can not be assumed that a factor which induces the division is directly necessary for differentiation. Auxin induces cambial division in developing stems (Snow, 1935) and reactivates the cambium after a dormant period (Gouventak, 1941). The differentiation of the cambial derivatives into xylem in response to auxin may (Dvorak, 1961; Gouventak, 1941; Palser, 1942; Snow, 1935) or may not (Reim, 1935; Jost, 1939; Soding, 1936, 1940) occur. Auxin was fully effective in the induction of cambial division in those cases where it failed to induce normal xylem differentiation.

Auxin alone may not promote cambial division but will promote xylem differentiation if cambial division is invoked by gibberellic acid (Wareing, 1958). There is apparently some difference in the response to gibberellic acid depending on the type of system used. Studies which deal with the application of GA₃ alone to stem segments, show that the effect of GA₃ is to promote cambial division without accompanying xylem differentiation (Wareing, 1958). Studies dealing with intact plants show that the effect of GA₃ in that situation is to increase the production of xylem and the resulting cells differentiate (Ahuja and Doering, 1967; Bostrack and Struckmeyer, 1967; Bradley and
Crane, 1957; Kiermayer, 1959 and Skok, 1968), or there is no effect on cambial division but differentiation is enhanced (Okunda, 1959), or there is only slight enhancement of division and no effect on differentiation (Morey and Cronshaw, 1968).

The effect of auxins, gibberellins, and cytokinins on xylem differentiation has been recently reviewed by Roberts (1969). Shininger (1971) has shown that cambial division continued in decapitated Xanthium plants without concomitant xylem fibre differentiation. The application of IAA to these plants did not affect the production of cambial derivatives or induce xylem fibre differentiation. When NAA was applied either to the second internode or to the stump of a lateral shoot, xylem fibre differentiation was induced in the newly formed cambial derivatives on the xylem side of the cambium in the stem, but when it was applied unilaterally, xylem fibre differentiation was restricted to that side of the stem in the first internode and hypocotyl. NAA also enhanced the production of cambial derivatives. Gibberellic acid enhanced the production of cambial derivatives but did not affect the differentiation of xylem fibres. Similar numbers of cambial derivatives were produced in some NAA treated plants in which xylem fibre differentiation was induced and in GA3 treated plants
which did not differentiate xylem. When NAA was applied 72 hours after decapitation, the oldest of the cambial derivatives on the xylem side failed to develop into fibres although younger cells did. He concluded that auxin had its direct effect on the induction of xylem differentiation rather than on the induction of divisions prerequisite to differentiation. He attributed that the lack of response to applied IAA was due to the presence of peroxidases and polyphenyloxidases at the cut surface of Xanthium stems. Peroxidases have been shown to be capable of destroying IAA and other indole auxins (Tang and Bonner, 1947).

(d) Origin of Roots

Struckmeyer (1951) working with Solanum lycopersicum and Peperomia sp., showed that cell division begins in one or a group of undifferentiated cells and after a series of unregulated divisions, meristematic activity starts and results in a root primordium. According to Satoo (1956) "wound roots" in coniferous cuttings originate from (1) cambial and phloem regions; (2) leaf and branch traces; (3) bud meristem and bud traces and (4) irregularly arranged patches of parenchyma and callus tissue. However, in species which do not root spontaneously, all the roots originate from callus tissue, which primarily arises by
the division of cambium and phloem parenchyma cells (Snyder, 1954; Buck, 1954). Vogl and Kemner (1962) considered that roots were formed from interfascicular cambium which is not inhibited by winter rest. Ruggeri (1963) found that root formation occurred only on young cuttings and originated from the cambium which is often accompanied by callus formation. Bala (1965), working with Impatiens balsamina, found that in hypocotyl as well as epicotyl roots arose from the intra-stellar cells. While in the former, they arose from the cells opposite the protoxylem elements, in the latter they arose from the inter fascicular region. Nanda (1965, 1970) reported that roots originate from the region of dilatations between the vascular bundles by the activity of cambial cells.

Mairo (1965) demonstrated that the position of adventitious roots was determined by blocks of starch storing tissue that replaced some of the phloem and xylem of outwardly deviating vascular bundles immediately below the nodes. Gorgenyi Meszaros (1965) showed that the development of roots in both woody and herbaceous plants may begin independent of callus. According to Girouard (1967a,b) the formation of adventitious roots in Hedera helix starts near the phloem ray parenchyma of internodes with and without wound wood and in callus near the basal end of the cuttings.
FORMATION AND DIFFERENTIATION OF CALLUS

Shippy (1930) and Leopold (1960) found that low temperature promoted callus formation on stem cuttings. Bloomberg (1964) found that callus on poplar cuttings increased with an increase in the temperature provided the moisture content was high. Heide (1964) found that temperature exerted a pronounced effect on the regeneration ability of tissues.

Mamedov (1964) found that perlite was very suitable for quick development of callus on stem cuttings. Sinnott (1960) reported that a higher concentration of auxin favours callus formation.

Ruggeri (1963) found that in *Eucalyptus rostrata*, the degree of callus was more on the basal than on the upper cuttings. Bala (1965) found that callus was formed largely from the cortex and the pith, although cambial cells, xylem and phloem parenchyma were also capable of proliferation. She stated that roots originated from the finger-like protuberances of callus. The excessive callus, however, impeded root development (Reines and McAlpine, 1959). Ruggeri (1963) found that callus formation was accompanied by cambial activity. The meristematic cells in the pith and later in the cambium and phloem contributed to the formation of callus (Mergen and Simpson, 1964). Gorgenyl Meszaros (1965) stated that callus formation was
independent of rooting in stem cuttings. Girouard (1967a,b) obtained callus at the basal end of the cuttings which produced adventitious roots subsequently.