Chapter - 5

5. DISCUSSION

A few of the plant growth regulators have been extensively used for promoting rooting on cuttings. In the present investigation cuttings of ***Dahlia***, ***Chrysanthemum*** **rose,** ***Mussaenda*** and **azalea** were used for experimentation. Indole-3 butyric Acid (IBA) Napthalene acetic acid (NAA) and their interaction with GA_3 and GA_{4+7}, 2-Chloroethyl Phosphonic acid (Ethrel) were applied at varying concentrations Commercial formulation of IBA and NAA, like Rootex and Rootone were also applied at varying concentrations to examine the rooting responses of the cuttings. The results are discussed below :

5.1 Effect of Indole-3-butyric acid (IBA)

IBA was applied at the concentrations of 0, 100, 250, 500, 1000 and 1500 μg/ml of which 1000 μg/ml stood as optimal in induction of highest number of roots per cutting. Aqueous solution of IBA was tried on the cuttings. In all the examined plant it has been observed that the number of roots declined after the 1000 μg/ml. For the experiment "Quick Dip Method" was followed.

In case of ***Dahlia*** the number of roots induced increased gradually from lower to higher concentration of 1000 μg/ml. After 1000 μg/ml the number of roots declined at 1500 μg/ml. Thus after 7 days of treatment the number of roots was counted as 0, 10, 11.8, 13.2, 13.6, 13 at the concentration 0, 100, 250 500, 1000, 1500 μg/ml. After 14, 21 and 28^{th} day at the optimal concentration of 1000 μg/ml the number of root was recorded as 16.8, 19.8, 23.6 against 5, 8.6 and 13.2 at control.

In case of *Chrysanthemum* the number of roots induced increased gradually from lower to higher concentration of 1000 μg/ml. After 7, 14, 21, 28 days at the optimal concentration of 1000 μg/ml the number of roots was 23.8, 46.6, 56.4, 72.2 against 0, 6, 13.8, 16.2 at control.

In rose no root was induced after 7, 14, 21 and 28 days. But thereafter number of roots induced increased gradually from lower concentration to higher concentration of 1000 μg/ml. At optimal concentration of 1000 μg/ml the number of roots was recorded as 20.2, 26.2, 31.6, 33.6 after 7, 14, 21, 28 days.

In *Mussaenda* after 7 days there was no induction of roots upto 1500 μg/ml concentration. At control also there was no induction of roots up to 42 days. After 14, 21 28 35, 42 days at the optimal concentration of 1000 μg/ml the number of root was 6.6., 12.4, 19.6, 21.6 24.6.

In Azalea there was no induction of roots upto 14 days. Induction of roots was evident only after 21 days. At control there was also no induction of roots upto 42 days. At the optimal concentration of 1000 μg/ml the number of roots was recorded as 9.8, 17.2, 20.8, 21.4 after 21, 28, 35 and 42 days.

From the performed experimental results it is found that all the concentrations of IBA proved stimulatory in *Dahlia, Chrysanthem,* rose *mussaenda* and azalea.

Among the endogenous growth regulators auxin seems to be the primary trigger of adventitious root formation (Haissig 1974, Gasper and Hoffmger 1989,

Torry 1986, Bellamine et al 1988), since it appears to be involved in cell division (Thimann 1941, Gasper and Hoffinger 1989, Blackesley 1994)

The superiority of IBA over other auxins on rooting on cuttings has been unequivocally demonstrated by several workers (Torrey 1976, Greenwood and Berlyn 1973, Singh 1950, Sen and Bose 1958, Jauhari 1960, Gregory and Samantarai 1950, Samantarai and Kabi 1954 and others) The results obtained on cuttings of *Litchi chinensis* Sonn. (Sen 1941), *Prunus salicina* Lindl. (Dikhsit 1956, 67), *Kigelia pinnata* D.C. (Singh 1956), *Hibiscus rosa-sinensis* L. and *Allamanda cathartica* L. (Shanmugavelu 1960) and *Psidium guajava* (Singh *et al.* 1962) establish the fact that IBA is equally effective on variety of plants and also on soft wood to 'hard wood' cuttings. The present finding is in conformity with those workers and confirmed that IBA induced rootings of soft cutting of *Dahlia* and *Chrysanthemum* and semi-hard wood of rose, *Mussaenda*, azalea and at lower concentrations.

The formation of root was found to take place at any time, under the application of appropriate doses of hormone treatment (Hartman 1976). The location of roots in cuttings varied, according to the species. Most cuttings under the influence of their endogenous auxins formed roots very close to the basal cut surface. The initiation and formation of roots followed the following patterns.

(1) Appearance of trichoblast and differentiation of cells into groups of recognisible root initials in *Dahlia*, *Chrysanthemum*, rose, *Mussaenda*, azalea.

(2) Initiation of groups of meristematic cells in *Dahlia, Chrysanthemum* rose, *Mussaenda*, in azalea in vascular bundles.

(3) Some cells behind the protective layer divided and formed callus in rose.

A number of workers used mungbean cuttings for the study of adventitious root formation and it has become a model system for studying the adventitious root formation (Friedman *et al.* 1982, Jarvis *et al.* 1983, Shyr and Kao 1985). Intense mitotic activity and metabolic changes appear to be an essential prerequisite for root formation (Torrey 1986) and is dependent on an array of endogenous physiological factors (Heuser and Hess 1972, Torry 1986). Exogenous application of different types of auxins (IBA, IAA, NAA, 2,4-D and NOA) exhibited a quite different and variable responses on root primordia development, first order root establishment, second order root and total root length per cutting of mungbean (*Vigna radiata* L. cv. 105,) (Nag *et al.* 1999, 2000). Among the PGRs, IBA was most effective for improving the development of root primordia and second order root formation. Wareing (1951) Larson (1962), Wort (1962) Haissig (1970a,b) also observed that root primordia formation in *Brittle willow* was governed by auxins.

5.2 Effect of α-Napthalene acetic acid (NAA)

NAA at the concentrations of 0, 100, 280, 500, 1000, 1500 μg/ml were applied to ***Dahlia Chrysanthemum*, rose, *Mussaenda*** and **azalea** cuttings as aqueous solution. All concentrations of NAA were found to be significantly suprior as

compared to control on induction of rooting. Out of the five concentrations applied, 1000 µg/ml stood as optimal in induction of highest number of roots per cuttings. Quick dip method was followed for the experiment.

In case of ***Dahlia*** number of roots induced increased gradually from lower to higher concentrations tried. The induction of roots increased from 100 µg/ml upto 1000 µg/ml of NAA and the later concentration emerged as optimal concentration. In *Dahlia* after 7 days the number of root was recorded as 0, 5.8, 7.4, 8.6, 10.8 and 9.6 at the concentration of 0, 100, 250, 500, 1000, 1500 µg/ml NAA. After 28 days of treatment the maximum number of roots was counted as 20.4 at the optimal concentration.

The same trend of induction of rooting was found in ***Chysanthemum.*** The number of roots induced increased gradually from lower concentration of 100 µg/ml to higher concentration of 1000 µg/ml. Thus after 7 days of treatment the number of roots was counted as 0, 13.4, 15.6, 21.8, 26.2, 25.3 at the concentrations of 0, 100, 250, 500, 1000, 1500 µg/ml. After 28 days at the optimal concentration of 1000 µg/ml the number of root was recorded as 46.8 against 16.2 at control.

In case of rose there was no induction of roots at control. After 7 days upto 250 µg/ml of NAA concentration there was no induction of roots. At the optimal concentration of 1000 µg/ml the maximum number of roots was recorded as 13.8 after 28 days.

In ***Mussaenda*** there was also no induction of roots at control upto 42 days. After 7 days there was also no induction of roots at 100, 250, 500, 1000, 1500 µg/

ml of NAA. At the optimal concentration of 1000 μg/ml the maximum number of roots was recorded as 12.8 after 42 days.

In **azalea** at control and 100 μg/ml concentration of NAA, there was no induction of roots upto 42 days. After 7 days there was also no induction of roots at the concentration of 250, 500, 1000, 1500 μg/ml of NAA. At the optimal concentration of 1000 μg/ml maximum number of roots was recorded as 15.2 after 42 days.

NAA treated cuttings formed roots very close to the basal cut surface. Root formation in cuttings after NAA treatment followed certain histological changes.

(1) Enlargement of certain cells in cut zone near the vascular bundles or ourside the vascular bundles followed by cell division and cellular differentiation in all the tried species viz ***Dahlia, Chrysanthemum,*** **rose,** ***Mussaenda*** **and azalea)**

(2) Initiation of groups of meristematic cells in all the species tried.

(3) Differentiation of cells into groups of recognisible root initials in all the species tried.

The present investigation corroborates the findings of Singh and Sharma (1954), Jauhari and Amarjit (1960a) and Bhombota (1959) who reported stimulation of rooting on cuttings obtained from deffenent types of plants. Sen and Bose (1962), however reported that NAA responded selectively in 3 type varieties tried. That NAA induced rooting on cuttings was also reported by Jauhari

(1960a), Jauhari and Kohli (1960), Singh *et al.* (1962) and Singh and Bhatnagar (1955).

Nag *et al* (1999, 2000) reported that IBA, NAA, 2, 4-D were effective in root primodia formation. They are less effective in establishing first order roots. Among the auxins IBA was most effective, followed by 2,4-D, NAA in the formation of root permodia. Hartmann (1976) reported that exogenous application of known growth substances greatly modify the rooting potential of stem cuttings. Among them auxin causes the primary trigger for adventitious root formation (Haissig 1971, Gasper and Hoffinger 1989, Torry 1986, Bellamine *et al* 1998) and auxin is involved in cell division (Thimann *et al.*1941, Gasper and Hoffinger 1989 Blackesley 1994). Growth and emergence of root primordia both outward and toward vascular tissues where conducting connections are made reported by Hartmann *et al.* (1990). Root initials develop naturally on plant parts which are still attached to the parent plant, although emergence prior to detachment can occur (Lovell and White 1986). Adventitious roots in herbaceous plants usually originate just out side the vascular bundles (Preistley and Swingle 1929). But in woody perennial plants, adventitious roots in stem cuttings usually originate from living parenchyma cells, and sometimes arise from vascular rays, cambium, phloem or pith (Lovell and White 1986, Ginzburg 1967).

5.3 Effect of Rootex

Rootex was applied at the concentrations of 0, 100, 250, 500, 1000, 1500 µg/ml as aqueous solution and of these five concentration used, 1000 µg/ml emerged as the optimal concentration.

In ***Dhalia*** there was promotion of root formation from 100 µg/ml to 1000 µg/ml. At 1000 µg/ml the number of roots were 8.8, 14, 21.2, 24 against 0, 4.2, 5.8, 7.6 at control after 7, 14, 21, 28 days. Maximum number of roots recorded was 24 at 1000 µg/ml after 28 days. With the progress of time the number of roots increased gradually. After 28 days the number of roots was recorded as 7.6, 14.2, 15.2, 22.2 24 and 23.4 at 0, 100, 250, 500, 1000 µg/ml of Rootex.

In ***Chrysanthemum*** also there was promotion of root induction from 100 µg/ml to 1000 µg/ml which declined at 1500 µg/ml. At the optimum concentragtion of 1000 µg/ml the number of roots was recorded as 24.8, 37.8, 50.8, 61.4 against 0, 6, 13.8, 16.2 at control after 7, 14, 21, 28 days. With the progress of time the number of roots increased gradually. After 28 days the number of roots was recorded as 16.2, 51.4, 55.6, 58.2 61.4, 58 at 0, 100, 250, 500 1000, 1500 µg/ml of Rootex. Maximum number of roots was recorded as 61.4 at 1000 µg/ml after 28 days.

In **rose** the promotion of root induction was evident from 100 to 1000 µg/ml and then it declined at 1500 µg/ml. At the optimal concentration 1000 µg/ml the number of roots was recorded as 11.4, 17, 24.2, 27.4 against control after 7, 14, 21, 28 days. There was no induction of roots upto 28 days. The number of roots increased gradually from first week to (4th) fourth week. Maximum number of roots was recorded as 27.4 at 1000 µg/ml after 28 days.

In ***Mussaenda*** cuttings the maximum number of roots (21) was recorded at optimal concentration of 1000 µg/ml after 42 days against no roots at control, In

Mussaenda root induction was observed after 21 days and with the progress of time the number of roots increased gradually. At optimal concentration, number of roots was recorded as 5.6, 12.6 15.4, 21 after 21, 28, 35, 42 days.

In **azalea** cuttings also maximum number of roots was recorded at 1000 µg/ml (optimal concentration) as 20.4 after 42 days. At 1000 µg/ml (optimal concentration) the number of roots was 6.2, 13, 17.4, 20.4 after 21, 28, 35, 42 days whereas there was no induction of roots up to 42 days at control. In Azalea induction of roots took place after 21 days and with the passes of time the number of roots increased gradually.

Rootex is a commercial formulation of IBA. Result of rootex application was also similar with IBA application. Rootex stood as the best bioregulator on induction of rooting on cuttings. This is in agreement with the findings of Jauhari and kohli (1960) and Singh *et al.* (1962) who recorded better results with IBA than any other growth regulators.

5.4 Effect of Rootone (commercial formulation of IBA and NAA)

Rootone was applied at the concentration 0, 100, 250, 500, 1000, 1500 µg/ml as aqueous solution. Out of these five concentrations 500 µg/ml and the 1000 µg/ml emerged as optimal concentrations depending on species.

Maximum number of root on *Dahlia* cutting was recorded at 500 µg/ml(optimal concentration) as 27.3 after 28 days. There was promotion of root

formation from 100 μg/ml to 1000 μg/ml. After 1000 μg/ml the number of roots slightly declined.

In ***Chrysanthemum*** maximum number of roots (48) was recorded at 500 μg/ml after 28 days. Induction of number or roots increased from 100 μg/ml to 1000 μg/ml. Then it slightly declined at 1500 μg/ml.

In **rose** cuttings, rootone at 1000 μg/ml proved to be optimal recording 18.0, 25.0, 20.3, 33.0 number of roots against no induction of roots at control after 7, 14, 21, 28 days of treatment.

The response of ***Mussaenda*** to the same range of concentrations of rootone was not so pronounced as was exhibited by ***Dahlia, Chrysanthemum*** **and rose.** Number of roots produced was also lesser than the other species tried. At the optimal concentration of 1000 μg/ml the number of roots was recorded as 9.3, 14.6, 15.0, 16.6 after 21, 28, 35, and 42 days as against no roots at the control.

In response to rootone **azalea** started produciing roots after 14 days of treatments. Rootone at 1000 μg/ml stood as the optimal concentration producing 6.3, 11.6, 15.6, 18.6, 21.0 number of roots after 14, 21, 28, 35, 42 days as against no roots at the control.

Rootone is a commercial formulation of IBA abd NAA. Results of rootone application was also identical with those of IBA and NAA. To some extent results obtained on rootone treatment was similar as interaction between IBA and NAA. In combination both IBA and NAA proved to be best root promoting combination

for plant cuttings

Our findings are in agreement with the findings of Stoutemyer (1954). The widespread use of auxins by nurserymen 'florists' and horticulturists indicates that these are valuable aids in induction of rootings on cuttiings. A very wide categories of auxins have been used for the rooting on cuttings, but the one most commonly used with great success is IBA (Sen and Bose 1959). IAA and NAA have also been used but with less uniform success. According to Hitchcock and Zimmerman (1940) different plant cuttings behave differently to different growth regulatory substances. NAA is also an excellent root promoter (Singh and Teotia 1951, Jauhari *et al.* 1960, Chattopadhyay 1960, Shanmugavelu 1961a,b, Singh and Bhatnagar 1975, Pandey *et al.* 1983, Ghosh *et al.* 1988).

The combination of IBA and NAA have given much better rooting on cuttings than the use of any one of these taken alone. Jauhari (1960) obtained 100 per cent success in *Curissa acandus* with IBA/NAA mixture. Leopold (1955) also reported increased number of roots after using NAA/IBA than the use of any one of them alone. Chandrasekhariah (1961) reported that combination induced better rooting than individual regulator of IBA and NAA.

5.5 Interactions

GA_3 and GA_{4+7} alone caused inhibition on rooting. But the combined effect of GA_3 + IBA, GA_3 + NAA, GA_{4+7} + IBA and GA_{4+7} + NAA revealed root promotion to some extent GA_3 and GA_{4+7} was applied at the same concentration of 50 µg/ ml, IBA and NAA was applied at 100, 250, 500, 1000, 1500 µg/ml.

5.5.1 Interaction between GA_3 and IBA

In *Dahlia* combination GA_3 50 + IBA 500 µg/ml stood as optimal and recorded number of roots was 10.0, 14.0, 19.6, 20.6 after 7, 14, 21, 28 days against control where recorded number was 0, 6.8, 9.0, 10.6 after 7, 14, 21, 28 days.

In *Chrysanthemum*, rose, *Mussaenda* and azalea the combination $GA_3$50 + IBA 1000 µg/ml stood as optimal and at $GA_3$50 + IBA 1500 µg/ml the number of roots produced per cutting was decreased. Recorded number of roots in optimal concentration in Chraysanthemum was 14.3, 16.6, 21.6, 25.3, in rose was 5.0, 10.6, 14.6, 15.6, in Mussaenda 0, 7.3, 12.3, 13.0, in Azalea 0, 0, 7.6, 10.0 after 7, 14, 21, 28 days against control.

In *Mussaenda* and *azalea* the produced root number was lower than the Dahlia, Chrysanthemum and rose and there was no response up to 14 days.

5.5.2 Interaction between GA3 and NAA

Out of five combinations used $GA_3$50 + NAA 500 µg/ml was optimal for *Dahlia*, *Chrysanthemum* and azalea. The recorded number of roots in the combination $GA_3$50 + NAA 500 µg/ml in *Dahlia* was 7.0, 13.3, 14.0, 17.3, in *Chrysanthemum* 7.0, 12.0, 13.3, 15.6 and in azalea 0, 5.3, 7.3, 9.6 after 7, 14, 21, 28 days.

The combination $GA_3$50 + NAA 250 µg/ml was optimal for rose cuttings and GA_3 50 + NAA 1000 µg/ml for *Mussaenda* cuttings. In optimal combination recorded number of roots was in rose 0, 5.6, 9.6, 10.6 and in *Mussaenda* 0, 5.0, 7.6, 9.6 after 7, 14, 21, 28 days.

5.5.3 Interaction between GA_{4+7} and IBA

The combination GA_{4+7} 50 + IBA 500 µg/ml stood as optimal in all the five species tried viz. *Dahlia, Chrysanthemum,* rose, *Mussaenda* and azalea. In optimal concentration at GA_{4+7} 50 + IBA 500 µg/ml the number of roots recorded was 0, 8.3, 12.3, 15.0 in *Dahlia,* 7.0, 13.3, 13.3, 15.3 in *Chrysanthemum* 0, 0, 5.6, 8.3 in rose, 0, 0, 5.6, 7.6, 8.6 in *Mussaenda,* 0, 0, 0, 5.3, 6.6 in azalea after 7, 14, 21, 28, 35 days.

5.5.4 Interaction between GA_{4+7} and NAA

Out of five combinations GA_{4+7} 50 + NAA 250 µg/ml and GA_{4+7} 50 + NAA 500 µg/ml stood as optimal in all the five species tried. Produced root number increased from the combination GA_{4+7} 50 + NAA 100 µg/ml to GA_{4+7}50 + NAA 500 µg/ml and then declined at GA_{4+7}50 + NAA 1000 µg/ml. At the optimal concentration the recorded root number was 0, 6.6, 9.6, 10.6 in *Dahlia,* 5.3, 8.3, 9.6, 15.6 in *Chrysanthemum*; 0, 0, 5.0, 8.3 in rose; 0, 0, 7.3, 8.0, 6.6 in *Mussaenda*; 0, 0, 0, 6.2, 7.3 in azalea after 7, 14, 21, 28, 35 days.

The combined effect of GA_3 and GA_{4+7} with IBA and NAA resulted in reducing the inhibitory effect of GA_3 and GA_{4+7} in the presence of IBA and NAA.

The present findings on interaction between GA_3 and IBA, GA_3 and NAA, GA_{4+7} and IBA, GA_{4+7} and NAA, are in conformity with the findings of Netien (1975), Spangeroberg and Gautheret (1964) who observed that the gibberellin alone does not stimulate general cell division but enhence the auxin effect on cell

division in the system. Nanda *et al.* (1967) also reported that GA_3 promoted rooting in certain species. leroux (1968) found that GA stimulated root formation in stem fragments of *Pisum sativum* L.

On anatomical studies it has been found that the combination of GA_3, GA_{4+7} with IBA and NAA stimulated cambial activity leading to cell division and cellular differentiation and resulted in forming groups of meristematic cells which produced root initials Haissig (1972) reported that GA treatment of intact brittle willow stems stimulated cambial activity leading to increase of xylem fiber production. The effect of GA on cambial activity very much depends on the type of system used. Wareing (1958) Shininger (1971) reported that when GA applied alone to isolated stem segments or decapitated *Xanthium* plants it promoted cambial division without accompanying xylem differentiation. In intact plants GA may increase xylem production and differentiation (Bardley and Crane, 1957; Ahuja and Doering, 1967; Bostrack and Struckmeyer, 1967; Skok, 1968) or it may enhence. 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).

The results presented by Wareing (1956) suggestive of GA as a co-factor of Indole-3-acetic acid for cambial activity of *Acer pseudoplantanus*. Application of GA stimulates the cells of cambium to divide, but in absence of added IAA the cells showed little vacuolation and lignification and no typical xylem differentiation took place. Exogenous auxin supply alone caused lignification without normal cambial activity. Indole-acetic acid in conjunction with GA induced normal cambium division and xylem differentiation.

5.5.5 Interaction between Ethrel and IBA

Ethrel was applied at the concentration of 50 µg/ml and IBA was applied at the concentrations of 100, 250, 500, 1000, 1500 µg/ml. The combination of ethrel 50 + IBA 500 µg/ml proved to be optimal in all the five species tried and recorded root number was in *Dahlia* 16.3, 30.6, 34.3, 36.3; in *Chrysanthemum* 23.3, 28.3, 33.3, 35.3, ; in rose 0, 19.3, 24.6, 30.3, 33.3; in *Mussaenda* 0, 8.3, 15.3, 22.6, 26.6; in azalea 0, 0, 8.3, 11.6, 14.6 after 7, 14, 21, 28 and 35 days.

5.5.6 Interaction between ethrel and NAA

Ethrel was applied at the concentration 50 µg/ml and NAA was applied at 100, 250, 500, 1000, 1500 µg/ml. Out of five combinations ethrel 50 + NAA 500 µg/ml stood as optimal and recorded root numbers was 10.6, 18.3, 25.3, 35.3 in *Dahlia*' 24.3, 29.6, 35.3, 38.3 in *Chrysanthemum* 6.3, 8.6, 13.3, 20.6 in rose; 0, 4.3, 8.3, 11.3 13.6 in *Mussaenda* and 0, 5.3, 9.6, 11.3, 13.6 in azalea after 7, 14, 21, 28, 35 days.

The present investigation showed that ethrel alone have no effect on cuttings of *Dahlia, Chrysanthemum,* rose, *Mussaenda,* and azalea. But the combined effect of ethrel with IBA and NAA was stimulatory to some extent on rooting on cuttings.

The present investigation is in conformity with the works of Malik and Srivastava (1982). They reported stimulatory effect of ethylene on rooting of cuttings of **Phaseolus.** Early reports of Zimmerman and Hitchcock (1933) showed ethylene to be stimulatory of root initiation. Kender *et al.* (1969) also reported

that ethylene treatment is neutral to root induction on blueberry stem cuttings. Read and Hoyster (1960) reported the promoting effect of ethylene on root cuttings. Roy *et al.* (1972) also reported that ethylene effect is neither promoting nor inhibiting in some fruit species.

On the other hand both IBA and NAA are stimulatory on rooting on cuttings. Recent works of Arteca (1990) report that auxins stimulate ethylene biosynthesis in many plant tissues and it has been suggested that auxin induced ethylene may be responsible for induction of adventitious root formation instead of auxin itself (Mudge 1989).