CHAPTER - 4

GENERAL DISCUSSION

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The present chapter deals with the discussions based on the results of the experimental work conducted on two tree species *viz., Dalbergia sissoo* Roxb. and *Albizzia lebbeck* Benth. in the light of reported work on similar aspects.

This research endeavour had two main directions of special emphasis, First : Selection of `elite' plants of *Dalbergia sissoo* Roxb. and *Albizzia lebbeck* Benth. on the basis of highest flavonoid (quercetin) synthesised / accumulated in their organs. Second : rapid multiplication of the `elite' plant through

a) Axillary bud sprouting,

b) adventitious bud induction,

c) plant regeneration from callus cultures.

(1) SELECTION OF `ELITE' *DALBERGIA SISSOO* ROXB. AND *ALBIZZIA LEBBECK* BENTH.

During the course of its metabolic activity plant sythesises certain chemical compounds which renders it

medicinally important. These compounds are classified as secondary metabolites. There are few reports indicating the medicinal importance of plant due to the presence of secondary metabolite *viz.*, flavonoid. Van Hoof and co-workers (1984) reported the presence of 3-methyl quercetin and 3-methyl kaempferol in African *Euphorbia* which might be responsible for antiviral properties exhibited by the species. Kiviranta and co-workers (1986) also reported the beneficial pharmacological effects of quercetin present in *Allium cepa.*

The chemical constituent isolated from selected *D.sissoo* and *A.lebbeck* stem-bark and leaves was a flavonol *viz.*, quercetin. Identification of the compound was done by paper chromatographic analysis. The presence of quercetin was confirmed when UV absorption spectra of the isolated compound was compared with that of the authentic sample. Quantitative estimation of quercetin revealed that it could not be quantified in stem-bark samples while in leaf samples it varied according to the localities in which trees were growing. This variation in the quantity of secondary metabolite in the organs of a single plant

might be due to the fact that their site of synthesis and accumulation are different (Waller and Nowacki, 1978). Barz and Koster (1981) supported the view that flavonols such as kaempferol and quercetin occur widely as glycosides in leaves and petals. In *D.sissoo,* Arboratum (1.25%) and Refinery township (0.65%) growing plant leaf samples accumulated highest and lowest quantity of quercetin respectively when calculated on percentage dry weight basis (Expt.1).

In *A. lebbeck* leaf samples, Subhanpura growing tree accumulated highest quercetin (1.02%) while that of Refinery township growing tree was lowest (0.62%) on dry weight basis (Expt.16). Similar quantitative variation in diosgenin contents in different populations of *Costus speciosus* has been reported by Banerjee and Sharma (1982). Hence, mature trees of *D.sissoo* and *A.lebbeck* which synthesised/accumulated highest quercetin content were selected as 'elite' plants. However, quercetin content of *A.lebbeck* plant was lower than that of *D.sissoo*.

(2) RAPID MULTIPLICATION OF THE 'ELITE' *DALBERGIA SISSOO* AND *ALBIZZIA LEBBECK* PLANTS THROUGH

a) Axillary bud sprouting

Axillary buds have proven to be useful for multiplying mature selected angiosperm trees such as *Crataeva nurvala* (Sharma and Padhya, 1996); *Dalbergia latifolia* (Raghava Swamy *et al.*, 1992). However, Maclean (1977) pointed out that as trees mature, morphogenic ability of their cells was repressed. Maturation is a complex process involving changes in both the nucleus and cytoplasm.

Mc Comb and Newton (1981) pointed out that there being a single apical bud per shoot, it would be ideal to use axillary buds as explants for multiplication of the species. Later in 1984, George and Sherrington reported that by axillary bud sprouting, numerous shoot-buds were produced which could be used as `Vegetative-Propagules'

Problems encountered during axillary shoot proliferation may include toxic exudation from the explant, death of explant, poor branching, poor shoot elongation, production of low quality shoots, hyperhydricity and general lack of growth. These responses can be manipulated by appropriate choice of growth regulator and by nutrient salts in the medium. Hence, in the present investigation (Expt 2), nodal segments with an axillary bud from 'elite' *D.sissoo* were cultured on Murashige and Skoog's (1962) medium containinng sucrose (3%) supplemented with Kn (1-8 µM/l). It was observed that Kn at 1-6 µM/l failed to induce axillary bud proliferation. Similar report of Kn treatment on axillary buds of *Lagerstroemia flos-reginae* was given by Paily and D'souza (1986). Maximum growth and development into a single shoot-bud in 80% of the cultures was observed at 2µM/l of Kn. However, at its 8µM/l level bud sprouted into 4±0.15 buds along with intervening callus at the base of explant. Synonymous effect of Kn on bud sprouting was reported by Lal *et al.,* (1988) in shoot-tip cultures of *Picrorhiza kurroa.*

Replacing Kn with BAP, cultured axillary bud showed sprouting, maximum being at 2µM/l level. However, these buds were thick and stunted structures in comparison to buds sprouted on Kn supplemented media. A report identical to the present observation was given by Bertrand and Lalonde(1985) when multiple shoots of *Elaeagnus angustifolia,* which failed to grow afterwords were produced on BAP supplemented media. In the present observation of *D.sissoo* shoots cultured on BAP supplemented media, out of the multiple shoots produced one shoot nearly always became dominant over others. Similar response was observed by Geneve *et al.*, (1990) in *Gymnocladus dioicus* shoot cultures.

Upon testing the effect of Kn in combination with BAP, it was observed that maximum number of buds sprouted (5±0.35) on medium supplemented with Kn and BAP each at 2µM/l. Identical synergestic effect of Kn in combination with BAP was observed by Gupta and co-workers in 1980 while working with *Tectona grandis* cultures. Anderson (1984) also reported effectiveness of more then one cytokinin supplemented media for shoot multiplication in

Corylus avellana cultures. Thus, the above mentioned medium was designated as multiplication medium for this species.

In *Albizzia lebbeck*, axillary buds cultured on MS medium containing sucrose (3%) with Kn (1-6µM/l) resulted in growth and development of a single shoot-bud, maximum being at 2µM/l (Expt.17). Replacing Kn with BAP there was sprouting of bud, maximum being at 4µM/l with an intervening calus phase. Thus, BAP proved superior to Kn in bud proliferation as was observed in *Prosopis juliflora* nodal explants (Nandwani and Ramawat, 1991). In *Prunus* shoot cultures Kn produced only single shoot while multiple shoots were produced in BAP supplemented media (Martinelli, 1985). Out of the cytokinins tested, Kn proved superior to BAP for axillary bud sprouting in *Prosopis cineraria* while in *Tecomella undulata*, BAP was more effective than Kn, producing about 15-25 shoot buds per explant (Arya *et al.*, 1989)

When combination of Kn with BAP was tested, it was observed that maximum number of buds sprouted (5±0.10) on MS medium containing sucrose (3%) with Kn (2µM/l) and BAP (4µM/l).

Thus, this medium was designated as `Multiplication medium' for *A.lebbeck*. In agreement with the above results, combination of Kn and BAP proved superior for multiple shoot formation in other tree species also eg. *Leucaena leucocephala* (Goyal *et al.*, 1985). Further, requirement of low levels of Kn and BAP for multiple shoot formation was also reported in *Eucalyptus citriodora* (Gupta *et al.*, 1981), Cytokinins at low concentrations stimulate the division of cytologically stable meristematic cells (Kibler and Neumann, 1980).

In Expt.3, auxins (IAA/NAA) when tested in combination with cytokinins of the multiplication medium in *D.sissoo* indicated the superiority of IAA over NAA. Bud sprouting was observed at lower levels of IAA while NAA failed to do so in any of the combinations tested. Gupta and co-workers (1980, 1981) also reported inhibitory effect of NAA when used in combination with Kn on shoot-bud sprouting in *Eucalyptus citriodora* and *Tectona grandis* cultures. Hilderbrandt and Harney (1983) reported that NAA strengthens apical dominance at the expense of shoot proliferation.

A.lebbeck axillary buds cultured on media supplemented with auxins (IAA/NAA) in combination with optimal levels of Kn and BAP induced sprouting. However, with a marginal difference in the number of buds sprouted (Expt.18). Between two auxins tested, IAA proved superior. Similarly in *Prosopis juliflora,* IAA (0.1mg/l) when used in combination with BAP (5mg/l) produced maximum number of shoots (7) per node (Nandwani and Ramawat, 1991).

It has been suggested that the requirement of growth regulator varies with the system and with the method of shoot multiplication (Bhojwani and Razdan, 1983). Bergmann and his co-workers (1985) reported that the tendency of axillary bud to develop into shoots depended upon the genotype and the number of previous subcultures.

In Expt.4, the relative merits of culture media for axillary bud sprouting in *D.sissoo* were evaluated. Out of the five known culture media *viz.*, MS, WB, B_5, SH and LM containing

sucrose (3%) supplemented with Kn and BAP each at 2μM/l, when tested showed that MS medium induced maximum sprouting followed by LM, SH, WB and B_5 media. This superiority of one medium over other might be due to concentrations of the major elements present in a particular medium.

Results of Expt.19 indicate that *A. lebbeck* axillary buds cultured on MS medium induced maximum sprouting followed by B_5, WB and then SH with least number of buds sprouting in LM medium. This superiority of MS medium over others might be due to its high nitrate contents in the form of potassium and ammonium salts. Analogous superiority of MS media over others for axillary bud sprouting was reported by Raghava Swamy and co-workers(1992).

Constituents of culture medium greatly influence the success of plant propagation of any species (George and Sherrington, 1984). In Expts.4 and 19, the levels of inorganic nitrogen/ vitamins /myo-inositol were altered as its absence/ half /

standard / double dose to find out their effects on axillary bud proliferation in *D.sissoo* and *A.lebbeck*. As mineral nutrition influences cellular differentiation, doubling or halving the concentration of inorganic nitrogen in the culture medium, there was a decline in the number of shoot buds sprouted. However, at the standard dose of inorganic nitrogen, highest number of shoot-buds sprouted indicating the necessity of inorganic nitrogen for axillary bud sprouting. In agreement with the above observation a report on axillary bud proliferation in 30 yr. old *D.sissoo* explants cultured on standard inorganic nitrogen of MS medium was published by Datta and co-workers (1983).

The vitamin levels as present in MS medium were found to be optimal for axillary bud sprouting in *D.sissoo* and *A.lebbeck*. Similarly, Roest and Bokelman (1976) reported increased shoot formation in *Chrysanthemum* pedicels when MS vitamins were present in the culture medium, due to their essential catalytic role in the metabolism of plant cells.

Inositol at 100mg/l, when supplemented to MS media was found to be optimal for sprouting of axillary bud in *D.sissoo* and *A.lebbeck*. Increasing its level to 200mg/l or decreasing it to 50mg/l was not beneficial for axillary shoot multiplication. Simillar report was given by Sharma (1990) that myo-inositol at the standard level induced maximum bud proliferation in *Crataeva nurvala* plant. Letham (1966) reported that myo-inositol interacts with cytokinin to promote cell division in carrot phloem explants. However, inositol has been reported to take part in the synthesis of phospholipids and cell wall pectins (Pollard *et al.*, 1961).

Sugars provide carbon skeleton for cell components and source of energy for all the metabolic activities (Simpkins and Street, 1970). Sucrose has been the main mobile carbohydrate as an energy source in the culture of tissues (Fowler, 1978). In the present studies (Expt.5 and 20) various carbohydrates *viz.*, glucose, fructose, sucrose, mannitol and starch were tested for axillary bud sprouting in *D.sissoo* and *A.lebbeck*. It was observed that maximum number of buds were induced in sucrose supplemented media followed by glucose and fructose. Mannitol

was poor energy source while starch was unsuitable for bud growth. Working on similar lines Bonga (1982) stated that generally the osmotic potential of the medium was controlled with sucrose, but other osmotic agents could be used. Contrary to the present observation, Chauvin and Salesses (1988) while working with *Castanea* shoot cultures reported stimulation of axillary shoot formation when sucrose was replaced by fructose. The superiority of sucrose might be due to more effective translocation of sucrose to meristems (Butcher and street, 1964). *Alnus* cultures grown on mannitol containing media died since it was not metabolised (Tremblay and Lalonde, 1984). In addition to it, they reported that for *Alnus glutinosa* shoot-bud cultures, sucrose was superior to fructose. Unsuitability of starch for proliferation of bud in both the trees under investigation might be due to inactivity / non-availability of amylase in the cultured cells.

After ascertaining the superiority of sucrose its various levels were tested for axillary bud sprouting in *D.sissoo* and *A.lebbeck*. It was observed that sucrose at 3% proved to be optimal for bud sprouting. With the increase or decrease in the

level of sucrose,there was subsequent decline in the number of buds sprouted. Mittal and co-workers (1989) reported that sucrose (3%) proved to be optimal for axillary bud proliferation in *Acacia auriculiformis*. Analogous report on axillary bud proliferation in *Prosopis cineraria* was given by Kackar and co-workers (1991). Dwara and his co-workers (1984) reported sucrose (3%) to be optimal for axillary bud proliferation in mature *Dalbergia sissoo* tree while Datta and his co-workers (1983) reported 2% sucrose level to be optimal in the same plant. Gamborg and co-workers (1974) stated that the efficacy of nitrate and ammonium ions depends on sucrose concentration.

Histological studies during axillary bud sprouting in *D.sissoo* were conducted (Expt.6) . It was observed that in response to cytokinin treatment there was cell division in the cells of quiescent meristem as a result of which fresh buds initiated from them. In agreement with the present observation Mantell *et al.*, 1985 reported that both axillary and apical buds contained quiescent or active meristems depending on the physiological state of the plant and when provided with nutrient formulations adequate

for normal growth, induced cell divisions in them. Hence, the rate of multiplication possible through axillary bud proliferation varies with the genotype and reflect the relative ability of the genotype to produce shoot-buds.

Seasonal changes in endogenous growth regulator type and concentration are known to occur in trees (Junttila, 1982; Van Staden and Davey, 1981). *D.sissoo* and *A.lebbeck* being deciduous trees show development of new foliage in the months of March-April and Sept-Oct. Axillary buds of both these trees were cultured on their respective multiplication media in different months throughout the year. It was observed that optimal bud sprouting occured in the months of March-April (Expt.7 and 21). According to Bonga (1987), the best season for bud initiation of most tree species was spring as it coincided with bud break. Barve (1989) reported that *Commiphora wightii* explants cultured in the months of April-June showed maximum proliferation. Besides, consideration should be made to avoid the period of rainy season to get rid of the risk of contamination.

b) Adventitious bud induction

Induction of adventitious buds from excised organs is another potential tissue culture technique applied for rapid multiplication of the species.

In Expt.22, adventitious buds were induced from excised leaflets of *A.lebbeck* in response to cytokinins while in *D.sissoo* cultured leaflets failed to show bud induction.

In *A.lebbeck,* leaflets cultured on MS medium containing sucrose (3%) supplemented with BAP (8μM/l) singly or in combination with Kn (2μM/l) induced adventitious buds. Maximum (6±0.25) buds were induced per leaflet in media supplemented with Kn (2μM/l) and BAP (8μM/l). Similarly, in *Populus* leaf midveins, shoot-buds were induced in presence of BAP (Lee Stadelman *et al.*, 1989). Kn and BAP when used together produced a synergestic effect resulting in an increase in the number of buds sprouted in *Annona squamosa* leaves (Nair *et al.*, 1984). Similarly, *Azadirachta indica* leaves when cultured on media supplemented with Kn and BAP induced optimal number of buds

due to the synergestic effect of two cytokinins (Ramesh and Padhya, 1990).

Further, in the same Expt. 22, when leaflets were cultured on media supplemented with $AdSO_4$ in combination with optimal levels of Kn and BAP, leaflets failed to show any enhancement in the number of buds induced. Sharma (1990) reported that cultured leaflets of *Crataeva nurvala* in response to this hormone treatment resulted in an increase in the number of buds induced per leaflet.

Histological studies (Expt.22) during adventitious bud induction revealed that in *A.lebbeck*, lower epidermal cells of the leaflet were disorganised and inner mesophyll cells possessed morphogenetic potential. In response to the hormone treatment, cell division occured, resulting in swelling and subsequent bursting of cells with the production of meristematic growth centres from parenchyma cells of the mesophyll. These meristematic growth centres organised themselves into shoot-buds. Cheah and Cheng (19৭৪) traced the origin of adventitious buds to mesophyll cells of

the Douglas fir cotyledon. Similarly, shoots on *Nicotiana tabacum* leaves were found to arise indirectly from nodules at the edges of the explant. The nodules were mainly formed by division of palisade mesophyll cells around the edge of explant (Attfield and Evans, 1991).

Results of Expt.23 showed that leaflets cultured on media supplemented with auxins (IAA/NAA)in combination with optimal dose of cytokinins [Kn(2µM/l) and BAP (8µM/l)] resulted in a decline in the number of buds sprouted when compared with the medium containing only cytokinins. However, between the auxins tested, IAA proved superior over NAA in induction of greater number of buds as was reported in foliar explants of *Duboisia myoporoides* (Kukreja *et al.*, 1986).

In Expt.24, effect of explant size and orientation on adventitious bud induction was studied in *A. lebbeck* leaflets and it was observed that intact leaflets possessed the capacity for shoot-bud regeneration. Identical response of only complete *Crataeva* leaflets possessing bud inducing capacity was reported by Sharma

(1990). While Lee Stadelman and co-workers (1989) reported that the size of explant was critical for adventitious bud induction from microcross sections of hybrid *Populus* leaf.

In the same Expt.24, leaflets when cultured on their abaxial side in contact with the medium produced adventitious shoot-buds in 80% cultures, while those cultured on their adaxial side in contact with the medium failed to show any response. Thus, it was evident that the orientation of leaflets played an important role in the induction of adventitious buds.
Azadirachta indica leaf discs induced equal number of shoot-buds when cultured on either abaxial or adaxial side (Ramesh and Padhya, 1990).

There are now several reports on plant regeneration via adventitious bud induction directly on the explant but none have mentioned the significant genetic abnormalities (Boulay, 1987). Although adventitious shoot formation directly from excised organs would definitly have been a better approach for clonal propagation but this does not imply that adventitious buds are always true to

type (Bhojwani and Razdan, 1983). Hence, only after tracing the origin of the bud, plants should be propagated by this technique.

c) Plant regeneration from callus cultures

Results recorded in Expt. 8 indicate that *D.sissoo* stem/ leaflet pieces cultured on Murashige and Skoog's (1962) medium containing sucrose (2%) failed to induce callus, thereby indicating that endogenous levels of phytohormones were not sufficient for induction of callus.

Explants cultured on medium supplemented with Kn/BAP alone at all the levels tested failed to show callus induction in both the explants. However, when explants were cultured on Kn in combination with IAA/NAA/2,4-D, it was observed that in IAA supplemented media, stem explants showed best response at Kn (1/2 µM/l) in combination with IAA (2µM/l). Similarly, leaflet explants cultured on various IAA levels showed best response at 2µM/l level but here the induced callus was compact. However, both explants cultured on media supplemented

with Kn in combination with NAA induced callus at a slower rate, when compared with IAA supplemented media.

Out of the various auxins tested, both explants cultured on 2,4-D supplemented media produced friable callus, maximum being at Kn (1µM/l) with 2,4-D(2µM/l).

For callus initiation from explants of 'elite' *A.lebbeck*, MS medium was employed (Expt.25).Stem and leaflet explants cultured on Kn and IAA supplemented media induced white and light yellow coloured compact callus respectively with slow growth. While explants cultured on media supplemented with Kn and NAA failed to show callus initiation. Explants cultured on 2,4-D supplemented media induced callus at a faster rate, maximum being at Kn(2µM/l) and 2,4-D(8µM/l) In agreement with the above observation in *Sandalwood* cultures, stem callus initiated on MS medium supplemented with Kn and 2,4-D (Lakshmi Sita *et al.*, 1979). This variation in phytohormone requirement by different explants could be explained on the basis that callus being heterogenous in its cell population, for initiating long term callus cultures, hormonal constitution of the culture medium was the critical factor (Yeoman, 1987). Jaiswal and Narayan (1985)

achieved callus induction from stem segments of *Ficus religiosa* on MS medium supplemented with 2,4-D (1 mg/l).

Sufficient quantity of biomass was required for conducting experimental work on callus tissues. Hence, in *D.sissoo* cultures nutritional and hormonal levels were standardised (Expt.9) for optimal biomass production of callus cultures. Cells in culture are not autotrophic and thus there is a requirement of exogenous supply of carbohydrates (Seabrook, 1980).

Experiments 9 and 26 were conducted to test the carbohydrate *viz.*, glucose / sucrose / mannitol (2%) requirement for optimal biomass production of *D.sissoo* and *A.lebbeck* callus cultures. It was observed that in *D.sissoo* cultures sucrose supplemented media induced maximum growth of stem and leaflet callus tissues, being 3450 ± 25 mg and 225 ± 05 mg; 4150 ± 65 mg and 275 ± 02 mg in terms of fresh and dry weights respectively. In *A.lebbeck* cultures, sucrose supplemented media induced maximum growth of stem and leaflet callus tissues, being 3150 ± 22 mg and 205 ± 03 mg; 3750 ± 15 mg and 245 ± 01 mg in terms of fresh and

dry weights respectively. Generally, disaccharide such as sucrose at 2-3% was the most suitable carbohydrate for plant tissue culture work.Rhodes and co-workers (1986) reported that sucrose was the major translocated carbohydrate in plants and that might be the reason indicating its support for highest biomass production.

In the same Expt.9, hormonal requirements for optimal biomass production of *D.sissoo* cultures were standardised and it was observed that 1µM/l of Kn in combination with 2µM/l of 2,4-D proved to be the optimal levels for biomass production. The values of stem and leaflet callus tissues were 3550 ± 25 mg and 235 ± 06 mg; 4000 ± 30 mg and 265 ± 02 mg in terms of fresh and dry weights respectively. In Expt. 26, *A.lebbeck* cultures produced optimal biomass on media supplemented with 2µM/l of Kn and 8µM/l of 2,4-D. Biomass values of stem and leaflet callus in this plant were 3300±10 mg and 220±02 mg; 3850±25 mg and 250±01 mg in terms of fresh and dry weights respectively. Thus, it was concluded that the type of explant from which the callus originated influences its growth and biomass.

The growth and chemical analysis of stem / leaflet callus tissues on standard medium followed a normal growth pattern in *D.sissoo* during four week culture period (Expt.10). Stem callus indicated the presence of quercetin at the end of four weeks. Finally, at the end of eight weeks, .007% of quercetin was quantified in the stem callus.

Leaflet callus of *D.sissoo* when analysed chemically indicated the presence of quercetin at the end of third week. Subsequently, at the end of four and eight weeks .02% and .05% quercetin was quantified.

In Expt.27, *A. lebbeck* stem/leaflet callus cultures showed that they followed a normal growth pattern. Chemical analysis of stem callus tissue indicated that at the end of four weeks, .005% quercetin was detected which failed to show any enhancement even after eight weeks. While leaflet callus accumulated 0.01% quercetin at the end of four weeks. Subsequently, at the end of eight weeks .04% quercetin was

detected. The amount of quercetin accumulated in stem and leaflet callus cultures of *A. lebbeck* was less than that observed in *D. sissoo.* The plant organs of *D.sissoo* and *A.lebbeck* produced quercetin (Expt.1 and 16) which was also found in their callus cultures. Hence, in agreement with the present observation, Butcher (1977) reported that tissue culture produced compounds were similar to those present in the plant from which cultures were initiated.

The morphogenic potential of callus cultures of stem / leaflet of *D. sissoo.* and *A. lebbeck* were studied. It was observed that in *D. sissoo* (Expt.11) stem/leaflet callus turned compact and nodular when subcultured on media supplemented with BAP (4µM/l) and NAA (0.5µM/l). Nodular callus when examined histologically revealed that it consisted of meristematic growth centres comprised of thin walled cells with prominent nuclei in them. These meristematic growth centres from leaflet callus when subcultured on medium supplemented with BAP (1µM/l) differentiated into shoot-buds.

In Expt.28 morphogenic potential of *A. lebbeck* callus cultures was examined. It was observed that stem / leaflet callus cultures turned nodular when subcultured on media supplemented with BAP (2µM/l) and IAA (0.4µM/l). This leaflet nodular callus when transferred to medium containing BAP (1µM/l) induced shoot-buds. According to Ross and Thorpe (1973) growth centres are the sites for organ formation in callus. Das and co-workers (1995) reported shoot-bud differentiation from hypocotyl and cotyledon callus cultures of *Dalbergia* spp. on MS medium supplemented with BAP and NAA. Lakshmi Sita and Raghava Swamy (1993) reported high frequency regeneration from *Dalbergia latifolia* leaf disc cultures on MS medium (3/4th major elements) supplemented with BAP and NAA. Upadhyay and Chandra (1983) reported shoot-bud organogenesis from hypocotyl callus of *A. lebbeck* cultured on media supplemented with BAP and IAA. Contrary to the present observation auxin suppresses shoot bud formation in callus cultures of Aegle marmelos (Arya *et al.*, 1981).

Elongation of shoot-buds

Shoot buds produced either by axillary bud proliferation or by callus cultures in *Dalbergia sissoo* and those produced by axillary bud proliferation, adventitious bud induction or by callus cultures in *Albizzia lebbeck* were tested for their shoot-bud elongation.

Individual bud of *D. sissoo* on isolation was excised from the cluster, cultured on MS medium containing 3% sucrose and supplemented with GA_3 (2µM/l) induced maximum elongation (8.0 ± 0.56 cm) in 90% cultures (Expt. 12). However, shoots thus produced showed premature leaf fall. This might be due to omission of cytokinins from the medium. Hence, in order to develop healthy foliage on these elongated shoots, $AdSO_4$ at 2 µ/l proved optimal for the same.In agreement with the present observation Paek *et al.*, (1987) reported that in *Brassica campestris* addition of $AdSO_4$ to medium containing Kn and IBA induced dark green leaves with healthy shoots.

In *A.lebbeck,* isolated shoots when cultured on GA_3 at 1µM/l showed maximum elongation (6±0.30 cm) of shoots (Expt.29). Kartha and co-workers (1974) were of the opinion that GA_3 promoted cell division which might have resulted in shoot elongation in potato cultures. While Wareing and Phillips (1982) reported that GA_3 in culture induces cell wall extension during cell division. Weiler and Spanier (1981) investigated the process in detail and reported that the action of GA_3 might be via its effect at the transcriptional level.

Plant formation

In Expt.13, well developed shoots of *D. sissoo* when transferred to MS medium with its concentration reduced to one half containing 1% sucrose supplemented with IBA (0.5µM/l) induced tap root in 80% of the cultures tested. In agreement with the present observation Patel and co-workers (1986) reported the essentiality of a diluted mineral fraction during rhizogenesis. A favourable effect of diluted mineral fraction on rooting can be explained by the reduction in nitrogen as these salts represent such a high proporation of the total solute concentration(Hyndman

et al., 1982; Driver and Suttle, 1987). Further, Trewaves (1983) suggested that the nitrate ion at low concentration could mimic the action of IAA and in any case supports its role.

In *A. lebbeck* shoots, roots were induced in half strength MS medium supplemented with IAA (0.5µM/l). Similarly in *Leucaena leucocephala* cultures, shoots developed roots on MS medium supplemented with IAA as reported by Dhawan and Bhojwani (1985).

Acclimatization and transfer of plants to field

Acclimatization process involves adaptation of plants to new environmental conditions such as lower relative humidity, higher light intensity, fluctuating temperatures and constant disease stress (Preece and Sutter, 1991).

The plantlets of *D. sissoo* and *A. lebbeck* were gradually acclimatized and finally transferred to field (Expt.14 and 31). In both the plants under investigation, during the process of acclimatization plantlets were covered with a glass beaker and

they were exposed to natural conditions intermittently which favoured their transplantation process. Antagonastic to this observation, *Glehnia littoralis* plantlets survived in pots without any covering (Hiraoka and Oyanagi, 1988). Authors attribute this characterestic to a well developed cuticle on the surface of leaf and a small ratio of surface area to fresh weight of leaf blade. Cheng (1978) reported the use of mist for better establishment of plantlets as it provides humidity around the plantlets during their transfer operation. Gradual acclimatization period with decreasing humidity was essential for the survival of these plants during their transfer from culture vessel to greenhouse and then to field conditions.

Ploidy of the regenerated plants from leaflet callus of *D.sissoo* (Expt.15) and *A. lebbeck* (Expt.32) were checked and it was found that *in vitro* regenerated plants showed a uniformly diploid number. Identical report was given by Zimmerman (1982) when micro⁀propagated *Alocasia* shoots derived from callus cultures failed to show any genotypic variation.