SUMMARY

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Results of the experiments conducted on two leguminous trees *viz.*, *Dalbergia sissoo* Roxb. and *Albizzia lebbeck* Benth. under investigation have been summarised in this chapter.

Stem-bark and leaf samples of the two plants under investigation were collected from different localities in and around Baroda. Chemical analysis of the samples showed the presence of a medicinally important flavonoid *viz.*, quercetin. UV absorption spectra of the isolated and authentic samples revealed the development of their superimposable spectra. Further confirmation of the presence of quercetin was done by gas chromatographic analysis. Quercetin was detected in the stem-bark sample but it could not be quantified while in leaf samples it varied according to the localities.

`Elite' plants of *D.sissoo* and *A.lebbeck* were selected on the basis of highest quercetin synthesised/ accumulated in their leaf sample. In *D.sissoo* leaf sample highest amount of quercetin was 1.25% while in *A.lebbeck* it was 1.02% when calculated on dry weight basis.

Mature, selected `elite' *D.sissoo* and *A.lebbeck* were used to obtain various explants for their rapid multiplication and propagation by tissue culture techniques.

Axillary bud sprouting was induced by culturing them on Murashige and Skoog's medium containing 3% sucrose supplemented with cytokinins. In *D.sissoo*, axillary buds cultured on medium supplemented with Kn(2μM/l) and BAP(2μM/l) induced highest shoot-bud production (5±0.35) while in *A.lebbeck*, cultured axillary bud sprouted into maximum number of shoot-buds (5±0.10) on medium supplemented with Kn(2μM/l) and BAP (4μM/l). This indicates synergestic effect of two cytokinins on bud sprouting. Thus, the respective media for *D.sissoo* and *A.lebbeck* were designated as their `multiplication medium' for axillary bud sprouting. Supplementing auxins *viz.*, IAA/ NAA (0.5-2.0μM/l) to the medium containing optimal cytokinins failed to enhance the number of shoot-buds induced and there was callusing from the cut ends.

Effect of various known media *viz.*, MS, B_5, WB, SH and LM containing 3% sucrose and Kn (2µM/l) with BAP (2µM/l), when evaluated for axillary bud sprouting in *D.sissoo* indicated the superiority of MS medium over others. Highest, five buds sprouted per axillary bud in MS media followed by WB and B_5 media producing two shoot-buds while SH amd LM media produced three and four shoot-buds respectively. In *A.lebbeck,* cultured axillary bud showed maximum five buds sprouting in MS medium followed by four buds in B_5, three in SH & WB and two in LM media.

In *D.sissoo* and *A.lebbeck,* out of the carbohydrates tested, sucrose (3%) proved to be optimal for axillary bud sprouting.

Histological studies during axillary bud sprouting revealed that in response to cytokinin treatment,cell division takes place in the cells of quiescent meristem resulting in initiation of fresh buds from the leaf axil.

Dalbergia sissoo and *Albizzia lebbeck* are deciduous trees in which fresh sprouts are produced twice in a year. Axillary buds cultured on multiplication medium in the months of March-April produced optimal number of shoot-buds in both the tree species under investigation.

Another potential method for rapid propagation of the plant is by adventitious bud induction. These adventitious buds are produced in any other position than the leaf axil or apex of a branch. In *A.lebbeck* adventitious buds were induced from leaflet explants under *in vitro* conditions.

Cytokinins, Kn (2μM/l) and BAP (8μM/l) induced optimal number of shoot-buds from an excised leaflet. Anatomical studies during adventitious bud induction revealed that there was bursting of the lower epidermal cells producing meristematic growth center from parenchyma cells of the mesophyll. These growth centres organised themselves into shoot-buds. Supplementing auxins *viz.*, IAA / NAA to the medium containing

optimal cytokinins failed to enhance the number of buds induced per leaflet.

Leaflet explants cultured on their abaxial side in contact with the medium produced optimal number of shoot-buds. Moreover, intact leaflet produced greater number of adventitious buds than its pieces.

Callus cultures of stem/leaflet explants of *D.sissoo* and *A.lebbeck* were established on MS media containing sucrose (2%) supplemented with Kn and 2,4-D. In *D.sissoo*, histological studies during callus induction from stem and leaflet explants indicated that cortical and spongy parenchyma cells respectively were the sites of origin of callus. Optimal biomass production in *D.sissoo* stem and leaflet callus cultures *viz.*,3550±25mg and 235±06mg; 4000±30mg and 265±02mg in terms of fresh and dry weights respectively was achieved on MS media containing sucrose (2%) supplemented with Kn (1µM/l) and 2,4-D(2µM/l).

In *A.lebbeck,* optimal biomass production of stem and leaflet callus cultures *viz.*, 3300±10mg and 220±02mg; 3850±25mg and 250±01mg in terms of fresh and dry weights respectively was achieved on media supplemented with Kn(2 µM/l) and 2,4-D(8µM/l). These media for optimal biomass production in *D.sissoo* and *A.lebbeck* callus cultures were designated as their `standard media' respectively.

Stem/ leaflet callus tissues of *D.sissoo* and *A.lebbeck* followed a normal growth pattern. Chemical analysis of these callus tissues revealed that leaflet callus tissue accumulated/ synthesized more quercetin than stem callus tissue. In *D.sissoo,* stem callus accumulated .007% while it was .05% for leaflet callus. Similarly in *A.lebbeck,* stem callus showed the presence of quercetin which could not be quantified while in leaflet callus, 0.04% quercetin was quantified.

Callus cultures of *D.sissoo* and *A.lebbeck* turned compact and noduler when subcultured on MS media supplemented with BAP (4µM/l) and NAA (0.5µM/l); BAP (2µM/l) and IAA

(0.4μM/l) respectively. Histological analysis of this nodular callus revealed the presence of meristematic growth centres which differentiated into shoot-buds.

Shoot-buds produced by any of the tissue culture technique were elongated into well developed shoots on media supplemented with GA_3(2μM/l) in *D.sissoo* and with GA_3(1μM/l) in *A.lebbeck* cultures.

Isolated shoots were rooted on half strength MS media with sucrose (1%) supplemented with IBA (0.5μM/l) in *D.sissoo* and with IAA (0.5μM/l) in *A.lebbeck*.

These plantlets of *D.sissoo* and *A.lebbeck* were gradually acclimatized and finally transferred to environmental conditions. Ploidy of the regenerated plants produced from callus cultures revealed that they were diploid (2n = 20) and (2n = 26) in *D.sissoo* and *A.lebbeck* plants respectively.

The highlights of the investigation conducted on *Dalbergia sissoo* and *Albizzia lebbeck* are as follows :

1) Stem-bark and leaf samples collected from different localities showed the presence of quercetin in all of them,

2) on the basis of highest quercetin content in leaf samples, mature *D.sissoo* and *A.lebbeck* were selected as `elite' plants.

3) In order to multiply and propagate plants through tissue culture teachniques, axillary bud sprouting was achieved on Murashige and Skoog's medium containing 3% sucrose supplemented with Kn(2µM/l) and BAP (2µM/l) for *D.sissoo* and Kn (2µM/l) with BAP (4µM/l) for *A.lebbeck,*

4) histological studies during axillary bud sprouting in *D.sissoo* revealed that meristematic activity of quiescent meristems in response to cytokinin treatment resulted in axillary bud sprouting,

5) ideal months for rapid multiplication of both the plants were March and April.

6) Another method for rapid multiplication of `elite' trees was through induction of adventitious buds from excised leaflets in *A.lebbeck.* MS medium containing sucrose (3%) with Kn (2µM/l) and BAP (8µM/l) induced optimal number of buds per explant.

7) Callus cultures of *D.sissoo* and *A.lebbeck* were produced on MS media containing sucrose (2%) supplemented with Kn (1µM/l) and 2,4-D (2µM/l) while in *A.lebbeck*, suitable hormone composition was Kn (2µM/l) and 2,4-D (8µM/l),

8) shoot-buds differentiated from morphogenic stem/leaflet callus tissues of both these plants,

9) shoot-buds produced by any of the tissue culture technique in both these plants were elongated on GA_3 supplemented media,

10) shoots produced roots on half strength MS media containing 1% sucrose and supplemented with IBA (0.5µM/l) in *D.sissoo* or with IAA (0.5µM/l) in *A.lebbeck,*

11) gradual acclimatization of *in vitro* produced plants to natural environment was achieved in both these plants,

12) plants produced by callus cultures revealed the presence of uniformly diploid chromosome number.

It is evident from the results that both the plants propagated by tissue culture techniques were transferred to natural environment, which need to be further evaluated for their field performance.