ORGANOGENETIC DIFFERENTIATION IN TISSUE CULTURES OF *DALBERGIA LANCEOLARIA*

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TISSUE culture technique is becoming an important tool for the rapid clonal multiplication of selected plants. Although herbaceous angiosperms have been extensively studied through tissue culture and morphogenetic techniques[1], trees have received rather scant attention. The specialized growth habits of trees pose difficult problems for tissue culturists as they are slow growing coupled with long dormancy and their calli are very hard to differentiate. Since the majority of the trees are not amenable to vegetative propagation by rooting of excised branches and grafting, tissue culture is attracting considerable attention for obtaining genetically pure elite populations under the *in vitro* conditions. Sporadic differentiation of leafy shoots and/or roots from the tissue culture of various tree species is now well known[2–8]. In the majority of these cases, the explants used had been seeds, seedlings or juvenile plant parts. The success with the explants from mature selected trees has been limited only to a few cases[9–14].

The present investigations on *Dalbergia lanceolaria* Linn.—a hardwood tree of great commercial importance were undertaken with a view to initiating callus cultures from different vegetative organs and thereafter to explore the potentialities of the calli for organogenesis.

Fresh seeds of *D. lanceolaria* were surface sterilized with 0.1% mercuric chloride for 5 min, followed by repeated washings with sterile distilled water, and inoculated on Murashige and Skoog's (MS) medium[15]. The seeds germinated after 5–6 days and seedlings attained a height of 8–10 cm after 4–5 weeks. Segments of root, hypocotyl, cotyledon, stem and leaf were excised and cultured on MS medium supplemented with naphthaleneacetic acid (NAA), indoleacetic acid (IAA), indolebutyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), kinetin (K), casein hydrolysate (CH), yeast extract (YE) in different concentrations and combinations. The young leaves and stem pieces from a mature tree growing in the university botanic gardens, were surface sterilized with 0.2% mercuric chloride for 8–10 min. and planted likewise on variously supplemented MS medium. The media were sterilized at 15 psi for 15–20 min. The cultures were maintained at 25 ± 2°C and exposed to 12 h light (3,500 lux): 12 hr dark cycle.

The various explants callused on MS medium supplemented with 2,4-D or NAA (each 1–4 mg/l)—the latter was more effective. The calli were slow growing. Incorporation of 1 mg/l K together with NAA (4 mg/l) considerably enhanced callusing. The addition of YE further enhanced the growth rate. Maximum proliferation occurred on MS + NAA (4 mg/l) + K (1 mg/l) + YE (600 mg/l). Callusing of excised cotyledon explants started at the cut ends (figure 1A) and within 5 weeks the entire explant turned into a mass of greenish yellow compact callus. The growth rate enhanced in subsequent subcultures although the green colour tended to disappear.

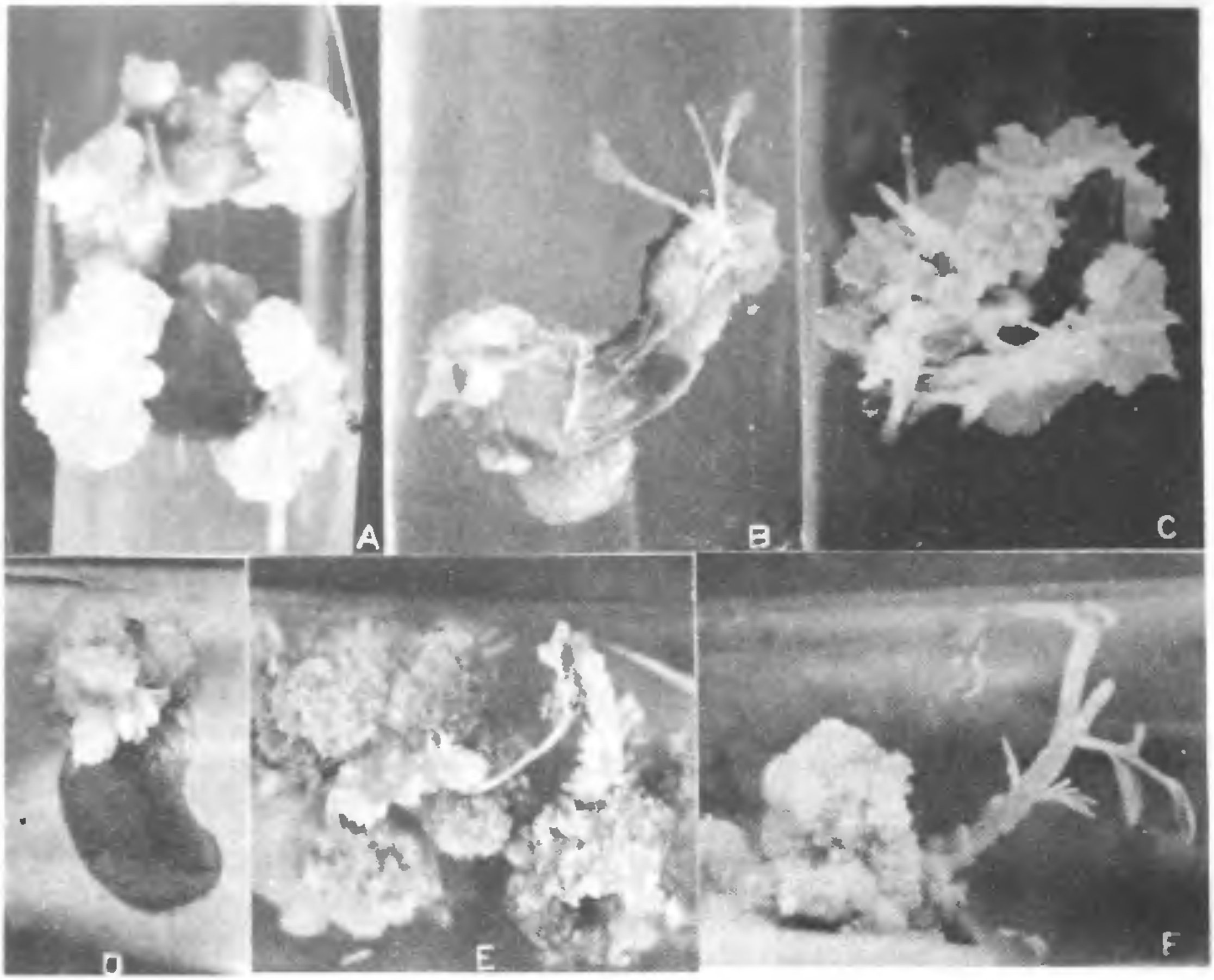
The different explants regenerated directly numerous roots on MS medium containing either of the two auxins IBA and IAA (1–4 mg/l, the rooting being more pronounced on IBA supplemented medium. Rooting occurred either at the cut ends or along the entire surface after 10–12 days of planting (figure 1B). The roots were white, unbranched and bore no root hairs.

Two week-old calli of seedling origin exhibited vascular differentiation in the form of tracheidal cells which had reticulate or spiral thickenings on their walls.

Organogensis was observed in the form of roots or shoots in all the calli except root callus which exhibited rhizogenesis only. Numerous roots differentiated from the callus on MS + NAA (4 mg/l) + K (1 mg/l) after 3 weeks of culturing (figure 1C). Addition of YE (600 mg/l) in the above medium considerably enhanced the number of differentiated roots which were white, unbranched and mostly without root hairs. 80% of callus cultures exhibited rhizogenesis on this medium.

On MS + BAP (1–4 mg/l), greenish yellow callus was formed which differentiated numerous shoot buds after 4–6 weeks (figures 1D, E). These buds grew well and developed into 5–6 cm. long shoots bearing 6–10 nodes after 8–10 weeks. Figure 1F shows 8-week-old cotyledon callus bearing a normal green shoot with many leaves. Shoot differentiation occurred in 40% of cotyledon and leaf callus cultures. Hypocotyl and stem calli exhibited the highest percentage (nearly 55%) of shoot differentiation. Rooting of these shoots was obtained on MS + IBA (2–4 mg/l). Attempts are underway to have a large scale regeneration of plantlets for transplantation to the soil.

Callus of the leaf and stem segments taken from mature trees could be subcultured and maintained for



Figures 1A–F. Tissue culture of *Dalbergia lanceolaria*. **A.** Two cotyledon explants callused at the cut ends on MS + NAA (4 mg/l) + K (1 mg/l) + YE (600 mg/l) after 2-weeks of culture. **B.** A leaf segment regenerating roots on MS + IBA (4 mg/l). **C.** Differentiation of roots from 3-week-old cotyledon callus. **D–E.** Cotyledon callus differentiating shoot buds on MS + BAP (2 mg/l). **F.** One of the shoots from cotyledon callus has further developed leaves × 2.

several months with no sign of organ differentation under any of the tested treatments.

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RESPONSE TO EXOGENOUS PROLACTIN DURING GONADAL PHOTOSTIMULATION IN BLACKHEADED BUNTING

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PROLACTIN (PRL) has been reported to inhibit or to have no effect on photoperiodic gonadal responses in a number of migratory avian species[1-3]. The different findings may be attributed to the fact that either different workers have utilized different kinds of migrants or exogenous PRL has been administered during different phases of the reproductive cycle of species investigated. In view of the conflicting results, the present study was conducted with a strong avian migrant, the blackheaded bunting (*Emberiza melanocephala*).

The blackheaded bunting is a migratory emberizid finch (family – Emberizidae), which uses annual photoperiods to time its seasonal responses[4, 5]. It arrives at Varanasi (India; 25° 18'N, 83° 01'E) during the commencement of winter (September/October), and returns to its breeding grounds (West Asia and East Europe) during late spring or early summer (March/April); it covers nearly a distance of 7,000 km each way[6].

Adult males were allowed to acclimate to the laboratory conditions for a fortnight, divided into groups ($n = 5$ or 6), and exposed to short photoperiod (8L:16D) for eight weeks so that they would become photosensitive. Laparotomy at 4-week intervals during this pretreatment indicated that the buntings had maintained minimal testicular size (combined testicular weight, CTW = ca. 5 mg). With such photosensitive birds two experiments were performed. Exp. 1: five groups were exposed to daily long photoperiod (15L:9D). Beginning on day 0, prolactin (ovine) solution, 100 μg in 0.1 ml of 0.9% NaCl, was administered daily to each bird for 15 days by subcutaneous injections. The controls received 0.1 ml injections of 0.9% NaCl. Birds received, respectively, PRL and NaCl injections either between 1000–1030 hr (group Ia and Ib) or between 1930–2000 hr (group IIa and IIb). Group III, on the other hand, did not receive any injection and served as sham control. After the last day of injection, all the birds were laparotomized and returned to outdoor aviary. Exp. 2: this experiment was performed to determine if PRL could cause involution once the testes were photostimulated. Birds received injections only during noon hours (1200–1230 hr). Two groups were exposed to 15L:9D and after laparotomy on day 15, one group was given 10 consecutive daily injections of 100 μg PRL in 0.1 ml saline, beginning on day 16 and other group received 0.1 ml of physiological saline as control. On day 26, all birds were laparotomized and returned to outdoor aviary.

Testicular growth at each laparotomy was assessed as combined testicular weight (CTW) by comparing the size *in situ* with a standard series of fixed gonads of known weights. The errors inherent in this method is less than (±) 20%. Food and water were provided *ad libitum*. Light provided during the experimental period was at an intensity of about 300 lux at perch level, and light 'on' coincided with the pretreatment commencing on 0600 hr. Statistical analysis was done by Student's t test.

Data from the experiments are presented in figure 1A, B. In photosensitive bird testes were stimulated ($P < 0.001$), only in those birds, received NaCl injections or uninjected. But daily PRL injections either early or late caused inhibition of the photoperiodic testicular development (figure 1A); it also caused gonadal involution ($P < 0.001$) in photostimulated individuals receiving injections only during the noon hours (figure 1B). Thus, regardless the time of injection and gonadal status prolactin exerts an inhibitory effect on photostimulation in blackheaded bunting (*E. melanocephala*).

In general, our data are in agreement with those of other workers who suggested the antigonadal action of PRL on avian gonad[3, 11]. They indicate that PRL inhibits the photoperiodic induction of gonadal growth which could be due to suppression of FSH[7-9]. Although PRL injections given early during the day appeared to be slightly more effective (figure 1A), present findings do not suggest that gonadal inhibition (may be partially) in buntings depends upon the time of injection. Several findings, however, have shown a