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# PRODUCTION OF SYNTHETIC SEEDS IN *DALBERGIA LATIFOLIA* ROXB.

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Bud break was obtained in *Dalbergia latifolia* Roxb. within 6-7 days after the incubation of the explants. Optimum callus induction was obtained in 2, 4-D : BAP (3.0 : 0.5 mg/l) within a week while the most effective combination for callus growth was BAP : NAA ( 1.0 : 0.8 mg/l). Somatic embryos from callus were obtained with 2, 4-D (2.0 mg/l) supplemented in modified MS medium after 1-2 sub-cultures. For maturity of these embryos hormonal combination of 2, 4-D : ABA (1.0 : 1.5 mg/l) was used with modified MS medium and incubated at 30°C ± 1°C in the culture room. The somatic embryos thus obtained were then encapsulated into 2% sodium alginate complexed with calcium chloride. These encapsulated embryos or synthetic seeds retained their viability when stored in an airtight container at 4°C for 1-2 months.

**Keywords** : *Dalbergia latifolia*, Somatic embryogenesis, Synthetic seeds.

Indian Rosewood (*Dalbergia latifolia* Roxb.) is the most important timber yielding plant of India. Most of the forest trees are propagated through seeds. For forestation seeds of superior genotypes are produced in seed orchards. It takes approximately 20-30 years to produce large quantities of seeds. Somatic embryogenesis has the potential to reduce this time dramatically. Synthetic seeds consist of somatic embryos surrounded by a protective coating. The first report of encapsulation was by Kitto and Janick[1] who successfully germinated carrot somatic embryos coated in a wafer disc made of 2.5% polyoxyethylene. This coating provides necessary protection from dehydration during storage, handling and mechanical planting to the embryos. Millions of somatic embryos can be produced in the laboratory throughout the year[2]. This technology is also very important for propagation of genetically engineered trees. The present investigation was, therefore, undertaken with an aim to reduce the time required to produce large number of superior seeds of *D. latifolia*.

In the present study, apical and axillary buds, (0.5-1.0 cm long), were used as explants from 1-2 year old *D. latifolia* plants. The explants were collected in distilled water containing 4-5 drops of Tween-20. They were washed and kept in citric acid (100 mg/l) solution for 25-30 min followed by 3-4 times washing. After completion of these steps the explants were inoculated aseptically into the Murashige and Skoog[3] medium for bud break. The explants were first inoculated in the medium supplemented with BAP : IBA : KIN (1.0 mg/l) each and incubated at 25°C±2 in the culture room. After bud break, the explants were transferred into fresh MS medium for callus induction. 2, 4-D alone (1.0, 2.0, 3.0, 4.0 mg/l) and with BAP (0.2 : 0.5, 1.0 : 0.5, 2.0 : 0.5, 3.0 : 0.5 mg/l) was used for callus induction. Calli were sub-cultured into fresh medium supplemented with BAP (1.0, 2.0 mg/l) and in combination with KIN (1.0 : 0.5, 2.0 : 0.5, 2.5 : 0.5 mg/l)and NAA (0.5 : 1.0 mg/l) for callus growth. Now the healthy calli (21 days old) were transferred into modified MS medium supplemented with 2, 4-D (2.0 mg/l) for somatic embryogenesis, incubated at 25°C±2. Premature somatic embryos were obtained after 4-5 sub-culturing at the interval of 14 -15 days. Premature somatic embryo transferred to another medium supplemented with 2, 4-D : ABA (1.0 : 1.5 mg/l) for maturity. Mature embryos were encapsulated into 2% sodium alginate complexed with calcium chloride (30-100 mM) and stored in an airtight container at 4°C. The data were statistically analyzed by using completely randomized design (C.R.D.).

Bud break was obtained within 6-7 days after incubation of the explants. Similar results were obtained in the bud culture of *Hemidesmus indicus* (L.) by Pattnaik and Debata[4]. Optimum callus induction was obtained in 2, 4-D : BAP (3.0 : 0.5 and 2.0 : 0.5 mg/l) while moderate callus induction was observed in 2, 4-D : BAP (1.0 : 0.5 mg/l) and 2,4-D (3.0 mg/l) alone within a week (Table 1). Similar findings have also been reported during the callus induction of *D. sissoo* by Datta and Datta[5]. Combination of BAP : NAA (0.8 : 1.0 mg/l)was found to be most effective for callus growth followed by BAP : KIN (1.0 : 0.5 mg/l) (Table 2). In present study, healthy callus growth was obtained when BAP used in lower concentration because at higher concentrations BAP causes the browning of callus margins which leads to cessation of callus growth[6]. For somatic embryogenesis MS medium was modified by

**Table 1.** Responses of various hormonal combinations on callus induction (%) from axillary buds of Rosewood (*Dalbergia latifolia* Roxb.).

| Hormones | Concentrations (mg/l) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2, 4-D | 1.0 | 2.0 | 3.0 | 4.0 | 1.0 | 2.0 | 3.0 | 0.2 | - | - | - |
| BAP | - | - | - | - | 0.5 | 0.5 | 0.5 | 0.5 | 0.2 | 1.0 | 5.0 |
| NAA | - | - | - | - | - | - | - | - | 5.0 | 0.2 | 0.2 |
| Callus induction (%) | 0.0 | 10.0 | 60.0 | 10.0 | 50.0 | 80.0 | 90.0 | 50.0 | 0.0 | 60.0 | 40.0 |

CD (5%) - 9.4

**Table 2.** Effect of various hormonal combinations on callus volume of Rosewood (*Dalbergia latifolia* Roxb.).

| Hormones | Concentrations (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BAP | 1.0 | 2.0 | 1.0 | 2.5 | 3.0 | 1.0 | 0.5 | 2.0 |
| Kin | - | - | 0.5 | 1.0 | 0.5 | - | - | - |
| NAA | - | - | - | - | - | - | 1.0 | 1.0 |
| $GA_3$ | - | - | - | - | - | 0.5 | 0.0 | 0.5 |
| Increase in callus volume (%) | 62.2 | 66.0 | 59.5 | 44.0 | 10.0 | 53.3 | 82.1 | 45.4 |

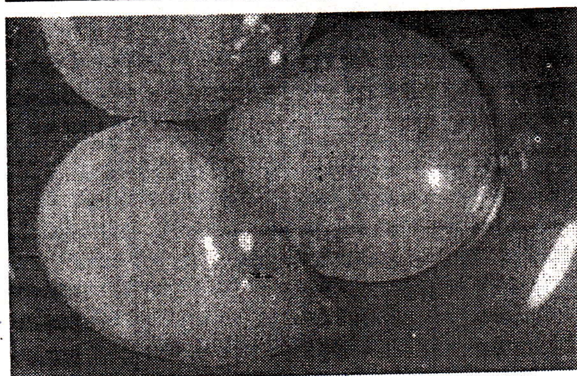

**Fig.1.** Showing encapsulated embryos of *Dalbergia latifolia.*

increasing the concentration of salts, changing sources of nitrogen supply such as casein hydrolysate, glutamine, asparagine, arginine etc. and increasing the concentration of potassium, all these modifications favour somatic embryogenesis[7]. Premature somatic embryos were obtained with the modified MS medium supplemented with 2, 4-D (2.0 mg/l) in one or two sub-culturing. The somatic embryos were then transferred to the modified MS medium with of 2, 4-D : ABA (1.0 : 1.5 mg/l) for maturity at 30°C ± 1°C. Further 3-4 sub-culturing at 14-15 days intervals gives mature somatic embryos. Matured somatic embryos, thus obtained were then encapsulated into 2% sodium alginate complexed with calcium chloride. These encapsulated embryos or synthetic seeds retained their viability when stored in an airtight container at 4°C for 1-2 months[8,9]. Abscisic acid has been found to be critical for embryo maturation[10]. Durzan and Gupta[11] reported that ABA inhibits the cleavage of polyembryony in Douglas-fir which allows for the development and maturation of individual somatic embryos.

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