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Morphogenetic responses of cultured cells of cambial origin of a mature tree – *Dalbergia sissoo* Roxb.

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ABSTRACT

Regeneration of plantlets was achieved from cell suspension derived calli of cambial origin from mature 'elite' trees of Dalbergia sissoo. Callus proliferation occurred on the cambial tissue pieces cultured on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and benzylaminopurine (0.1 mg/l). Suspension cultures were obtained by transferring and agitating callus lumps in liquid medium composed as above. Aggregates of about 30 cells were plated on semi solid medium, which developed into calli. Shoot bud differentiation was observed in the calli transferred to medium devoid of auxin but containing 0.5-2.0 mg/l benzylaminopurine. The isolated microshoots were rooted on modified MS medium containing low organic salts and auxins.

ABBREVIATIONS

BAP - 6-benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; IAA - indole-3-acetic acid; IPA - indole-3-propionic acid; KN - kinetin; MS - Murashige and Skoog (1962); NAA - 1-naphthaleneacetic acid; IBA - indole-3-butyric acid.

INTRODUCTION

In vitro propagation of mature trees has progressed significantly during the last decade (von Arnold, 1988). Organogenesis has been difficult to achieve in woody legume cultures (Datta and Datta, 1985); however, limited success has been reported in recent years (Gresshoff and Mohapatra, 1982; Mohan Ram et al., 1982; Datta and Datta, 1985; Kapoor and Gupta, 1986; Lakshmana Rao and De, 1987; Kumar, 1987; Ravishankar Rai and Jagadish Chandra, 1988; Varghese and Kaur, 1988).

Dalbergia sissoo (Leguminosae) is an important timber yielding tree. It ranks among the finest woods in durability, adaptability and working qualities. Besides, its heartwood contains compunds having the potential for leprosy treatment (Chopra et al., 1958). There are reports on the micropropaga-tion of D. sissoo by culturing root segments and axillary buds (Mukhopadhyay and Mohan Ram, 1981; Datta et al., 1982). Adventitious regeneration directly from organs generally gives a limited number of propagules. This number may be increased several folds by an intervening callus phase that regenerates an increased supply of adventitious shoots (Hussey, 1983). Moreover, cell culture offers many advantages for isolation of mutants in higher plants. As the cells are grown in uniform cultural conditions, reproducible selection schemes can be employed. However, isolation of auxotrophic cell lines and recovery of mutants in higher plants has lagged due to the lack of stable haploid cell lines and suitable selection systems. Therefore, the present investigation was aimed at developing a suitable methodology for rapid propagation of mature 'elite' Dalbergia sissoo lines using cell suspension derived calli which can be exploited in future for isolation of mutants.

MATERIALS AND METHODS

Wood blocks with intact bark were cut from young branches of 'elite' Dalbergia sissoo Roxb. trees. The blocks were soaked in 'Teepol' detergent solution and washed thoroughly under running tap water. These were then surface sterilized with 0.5% aqueous mercuric chloride for 8-10 minutes and washed several times with sterile distilled water. After removing the bark, the cambial layer was scraped off with the help of a sterilized scalpel and cultured aseptically on MS medium (Murashige and Skoog, 1962) containing 3% sucrose, 0.8% agar and various concentrations of auxins and cytokinins either individually or in combinations. The cultures were maintained at 25±2°C under 12 h daily illumination with fluorescent cool light at 45 $Em^{-2}s^{-1}$ intensity and 60-70%

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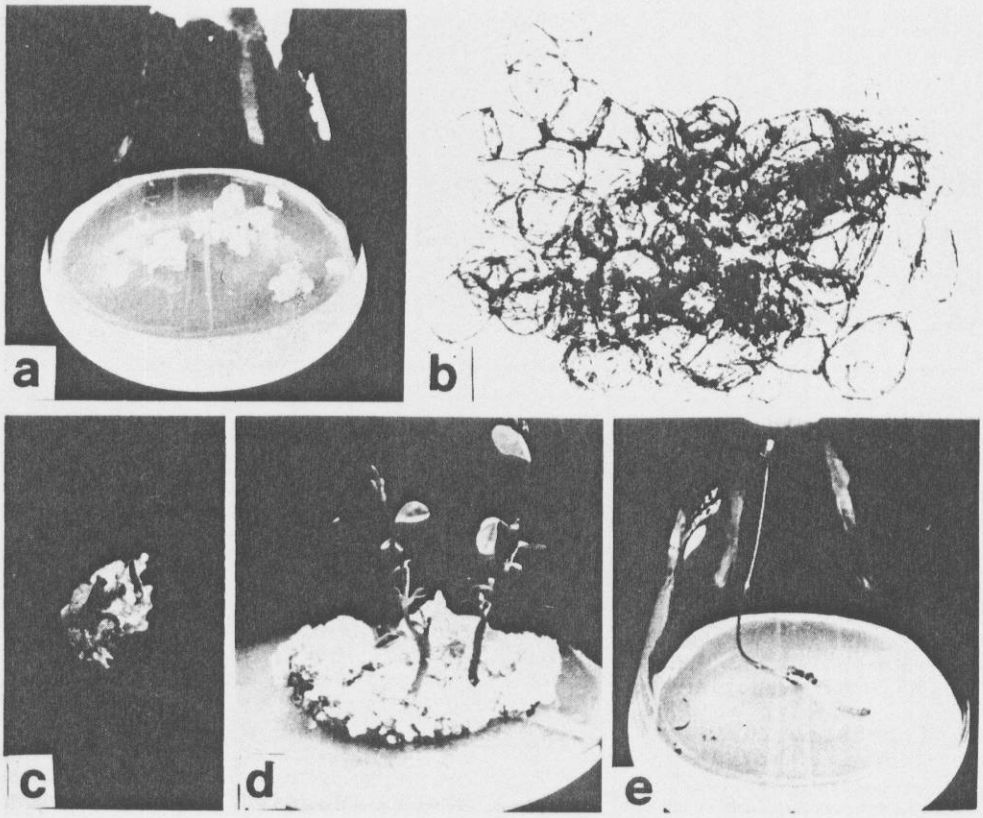

Fig.a Formation of callus lumps on MS+2,4-D (2.0 mg/l)+BAP(0.1 mg/l) from cambial tissues of Dalbergia sissoo

Fig.b Cell aggregates obtained from suspension culture of D. sissoo

Fig.c Differentiation of shoot buds on MS+BAP (1.0 mg/l) in the suspension derived callus of D. sissoo

Fig.d Regeneration of shoots from the callus of D. sissoo

Fig.e Rooted shoots obtained by culturing excised microshoots on MS+IAA+IBA+NAA (1.0 mg/l each)

relative humidity. Suspension cultures were obtained by transferring friable callus lumps (20 d old) to liquid medium of the same composition at 100 rpm with an orbital motion stroke of 2-3 cm. At the first subculture on fresh medium, after 15 d of transfer, large clumps of initial inoculum were removed by passing the suspension through stainless steel mesh of 60 um. Cell aggregates with upto 30 cells were plated on semi-solid medium of the same composition for colony growth. Larger cell colonies were isolated and subcultured on fresh medium to generate calli. Calli (60 d old), were subjected to various growth regulator treatments for organogenesis. For rooting of excised microshoots, half-strength MS medium supplemented with various auxins was used. Experiments were repeated twice.

RESULTS AND DISCUSSION

Callus proliferation occurred on the cambial tissues cultured on MS+2,4-D (2.0 mg/l) + BAP (0.1 mg/l), and the entire explant generated a lump of greenish-white, friable callus within 15 d (Fig.a). Filtered cell suspensions, free of larger clumps, with cell aggregates of ca. 30 cells (Fig.b) when plated on semi-solid medium of same composition formed larger cell colonies after 30 d. However, smaller cell aggregates, i.e. less than 30 cells, failed to grow. On fresh medium, these larger cell colonies grew vigorously and formed calli in 30 d. Organogenesis was observed in the calli subcultured on MS+IAA and/or BAP within 45-60 d. Callus cultures subjected to IAA (0.05-2.0 mg/l) exhibited rhizogenesis after 30 d of subculture. Percentage of culture showing rooting was maximum (45.5±2.8) on MS+IAA (1.0 mg/l) (Table 1). Calli subcultured on MS+BAP (0.05-2.0 mg/l) showed rapid growth and produced 2-6 green nodular structures (i.e. shoot buds) (Fig.c) after 45-60 d, which eventually developed into shoots (Fig.d).

Table 1. Morphogenetic effects of BAP and IAA on the regeneration frequency in callus derived from cell suspension of Dalbergia sissoo[a]

MS+growth regulators (mg/l)		Percentage of cultures showing organogenesis ± S.D.	
BAP	IAA	Shoots	Roots
0	0	-	-
0.05	0	29.1±3.6	-
0.5	0	37.4±4.5	-
1.0	0	60.0±5.8	-
2.0	0	24.6±3.0	-
0	0.05	-	25.0±2.8
0	0.5	-	18.4±2.6
0	1.0	-	45.4±2.8
0	2.0	-	22.2±3.1
0.5	0.5	25.0±3.2	-
1.0	0.5	41.6±6.7	-
1.0	1.0	27.0±3.5	7.6±1.8
0.05	0.5	13.5±1.6	26.4±3.5
0.05	1.0	-	38.1±4.7
2.0	2.0	-	-

[a]Each treatment consisted of 20 replicates
- no response

Table 2. Root formation in excised micro-shoots of D. sissoo cultured on modified MS[a] medium containing different auxins[b]

Growth regulator (mg/l)		Percentage rooting 20 d (± S.D.)	30 d
IAA	0.5	20±2.8	40±2.1
	1.0	30±2.0	50±1.6
	2.0	20±2.0	30±1.6
IBA	0.5	10±0.9	40±1.5
	1.0	30±1.4	60±1.6
	2.0	20±2.0	30±1.6
IPA	0.5	10±1.2	40±0.9
	1.0	40±0.8	70±1.6
	2.0	10±0.8	30±1.2
NAA	0.5	40±0.9	70±1.2
	1.0	60±1.2	80±0.9
	2.0	50±0.4	70±1.2
2,4-D	0.5	-	-
	1.0	-	-
	2.0	-	-
NAA+IBA	1.0+1.0	30±1.4	60±1.2
NAA+IPA	1.0+1.0	30±2.8	60±2.4
NAA+IAA	1.0+1.0	40±1.8	60±1.6
IAA+IBA+IPA	1.0 mg/l each	50±0.8	80±1.2
IAA+IBA+NAA	1.0 mg/l each	60±1.2	90±1.0

[a]MS medium with 1/2 strength inorganic salts
[b]Each treatment consisted of 20 replicates
- no response

Percentage of shoot-forming cultures was maximum (60±5.8) with 1.0 mg/l BAP in the medium (Table 1), and a higher BAP concentration (2.0 mg/l) did not favour regeneration of shoots. Caulogenetic potential using BAP in the present study is in agreement with that of Phillips and Collins (1979) in red clover. The presence of IAA along with BAP always suppressed shoot-formation. A similar antagonistic auxin-cytokinin effect has been observed in callus cultures of Aegel marmelos (Arya et al., 1981). The regenerative callus could be maintained by subculturing on basal MS medium containing 2,4-D (2.0 mg/l) and BAP (0.1 mg/l) for almost 2 years.

Isolated shoots were cultured on half-strength MS medium supplemented with NAA, IAA, IBA, IPA and 2,4-D individually and in combinations over a range of concentrations (0.5-2.0 mg/l) for rooting. Eighty percent of the cultured shoots produced fibrous roots in MS+IAA+IBA+IPA (each at 1.0 mg/l concentration) (Table 2). Combinations of IAA+IBA+NAA (1.0 mg/l each) produced stout tap roots in 90 percent of the cultured shoots (Fig.e). Plantlets were transferred to pots containing a soil:sand (1:1) mixture under glasshouse conditions. Percentage survival in the pots was about 45. Attempts are underway to improve the survival rate of transplants.

Plantlet regeneration from calli of adult trees has been very limited (Jones, 1983). Despite some drawbacks like genetic aberrations (Thomas, 1981) and loss of regeneration potential with the age of the cultures (Murashige, 1974; Halperin, 1986), this approach is considered to be most potential for cloning plant species (Murashige, 1978). The regeneration of plantlets from cambial cultures of mature Dalbergia sissoo trees is similar to the previous report in Ulmus compestris (Jacquiot, 1966). Variant cell lines are usually isolated from protoplasts or more commonly from cell suspension cultures either by plating cell aggregates on semi-solid agar medium (Flick et al., 1981) or by direct selection in cell suspensions. The regeneration protocol described in this experiment offers many advantages for the future isolation of mutants in D. sissoo.

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