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In vitro multiplication of the forest tree *Dalbergia sissoo* Roxb.

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ABSTRACT

HOSSAIN, M., D. HANUS, C. KEVERS & Th. GASPAR (1994). In vitro multiplication of the forest tree *Dalbergia sissoo* Roxb. *Saussurea* 25: 69-73. In English, English and French abstracts.

Three weeks old seedlings raised on Murashige and Skoog medium were used as explant sources for the micropropagation of *Dalbergia sissoo* Roxb. Nonorganogenic calli were obtained from hypocotyl segments. Axillary shoots were raised from cotyledonary node segments. Micropropagation could then be efficiently continued from shoot segments and tips on the woody plant medium, full strength with 2IP for multiplication, half strength with IBA for rooting.

RÉSUMÉ

HOSSAIN, M., D. HANUS, C. KEVERS & Th. GASPAR (1994). Multiplication in vitro de *Dalbergia sissoo* Roxb. (ligneux forestier). *Saussurea* 25: 69-73. En anglais, résumés anglais et français.

Des plantules de trois semaines issues de semis in vitro sur le milieu de Murashige et Skoog ont servi de sources d'explants pour la micropropagation de *Dalbergia sissoo* Roxb. Des fragments d'hypocotyles ont généré des cals non-organogéniques. Des pousses feuillées se sont développées à partir des bourgeons axillaires des nœuds cotylédonaire. A partir de ces pousses, la micropropagation peut être continuée efficacement en subcultivant des segments de pousses (avec nœuds) ou des apex sur le milieu de Lloyd et McCown, entier avec 2IP pour la multiplication et dilué de moitié avec AIB pour l'enracinement.

Introduction

In most of the tropical regions including Bangladesh there is an urgent need to protect the existing forest cover from further deterioration and to increase the area for reforestation. Introduction of forest and fastgrowing trees could slow the daily rate of denudation in these areas. The application of tissue culture methods offers new prospects for the multiplication of woody trees. In recent years, numerous studies on in vitro propagation of

Table 1. — Characteristics of axillary shoots obtained from cotyledonary node explants of *Dalbergia sissoo* Roxb. after six weeks of culture on dependence of culture media used.

<i>Media</i>	<i>Shoot length (mm)</i>	<i>Number of nodes</i>	<i>Basal callus (%)</i>
MS 2IP 1.5 mg l ⁻¹	27.4 ± 3.8	4.2 ± 0.4	100
WPM 2IP 1.5 mg l ⁻¹	61.1 ± 10.5	6.2 ± 0.4	100

Table 2. — Elongation and multiplication of axillary shoots from explants of shoot segment with three nodes in WPM medium supplemented with different concentrations of 2IP after six weeks of culture.

<i>2 IP conc. (mg/l)</i>	<i>Shoot length (mm)</i>	<i>Number of nodes</i>
0.5	32.5 ± 8.7	3.0 ± 0.3
1.0	45.0 ± 10.6	3.8 ± 0.4
1.5	53.0 ± 9.1	5.4 ± 0.5
2.0	36.0 ± 7.4	4.2 ± 0.4

Table 3. — Root formation in axillary shoots from single node explant in WPM 1/2 medium with or without IBA after four weeks of culture.

<i>Media</i>	<i>Shoots with roots (%)</i>	<i>Shoot length (mm)</i>	<i>Nb. nodes / shoot</i>	<i>Number of roots/shoot</i>	<i>Mean length of roots (mm)</i>
WPM 1/2	50	19.5 ± 5.2	2.7 ± 0.5	0.5 ± 0.5	23.3 ± 2.9
WPM 1/2 + 1 mg/l IBA	100	19.2 ± 3.8	3.0 ± 0.9	2.3 ± 0.5	19.6 ± 10.3

woody plants have shown that these techniques may be a solution for rapid propagation of selected forest trees (CHALUPA, 1987; McCOWN & McCOWN, 1987; BOULAY, 1987). The objective of the present work was to establish the system of clonal propagation of *Dalbergia sissoo*, an important woody crop for reforestation in Bangladesh. *Dalbergia sissoo* Roxb. is a forest tree yielding high quality of wood. The wood is used in construction and furniture industry and as a source of fuelwood. In this preliminary study, we investigated culture conditions for the production of genetically stable axillary shoots from juvenile plant material, because axillary shoot micropropagation of adult woody material, even in this species, still is difficult (DATTA & DATTA, 1983; DAWRA & al., 1984; SUWAL & al., 1988).

Materials and methods

Initial explants were taken from seedlings obtained from seeds cultured in sterile conditions. Seeds were surface sterilized by soaking in H₂O₂ for twenty minutes. They were washed afterwards six times with sterilized distilled water. The seeds were then placed for germination in MS (MURASHIGE & SKOOG, 1962) medium to which were added 3% sucrose, 0.4 mg/l kinetin, 0.4 mg/l BAP (benzylaminopurine), 0.2 mg/l 2IP (isopentenyladenine) and 0.5 g/l polyvinylpyrrolidone. The medium was adjusted to pH 5.8 and solidified by 7.5 g/l agar and dispensed into glass tubes. The medium was autoclaved at 125° C and at 1.2 Kg/cm² pressure for 20 min.

The cultures were maintained in a growth chamber at 24°C (day) and 20°C (night) with 16 h photoperiod and light intensity of 1000 lux from Sylvania GroLux fluorescent lamps.

For multiplication and rooting MS and WPM (LLOYD & McCOWN, 1980; McCOWN & LLOYD, 1983) media were used. They were supplemented with different concentrations of cytokinin (2IP) and auxin (IBA). 100 ml aliquots were dispensed into 600 ml Le Parfait glass recipients. The latter were covered by glass lids fixed with a plastic Reynolon (Reynolds Incorp) film.

Results and discussion

Healthy seedlings (80% germination occurred) as explant sources for in vitro cultures were obtained after three weeks.

The explants used initially were segments of hypocotyl and root, cotyledonary nodes and excised cotyledons. They were placed on MS or on WPM both with 2 IP 1.5 mg l⁻¹. After thirty days of culture, cotyledon explants turned into callus and root segments did not undergo any change. Nonorganogenic calli of different shapes were raised on hypocotyl segments. Axillary shoots raised from cotyledonary buds on both media. The medium WPM allowed the highest shoot elongation with the highest mean number of nodes (Table 1).

Shoots raised from cotyledonary buds were further used as sources of explants. Shoots tips or segments with a mean of three nodes were cultured on WPM with different concentrations of 2 IP (0.5 — 2.0 mg/l). One axillary shoot raised per explant. Among the four different concentrations, 1.5 mg/l favoured the highest elongation of axillary shoots with the highest number of nodes (Table 2).

Single node explants obtained from culture of shoot segments were used for further multiplication and rooting. After cycles of four weeks on WPM (2IP 1.5 mg/l), a mean multiplication of four nodes/shoot per multiplication cycle was attained. Shoots were also raised from single node explants placed on the rooting media WPM 1/2 (half strength) and WPM 1/2 + IBA (1 mg/l) and they rooted by 50 and 100% respectively (Table 3). Roots were more numerous in the presence of auxin but less longer. Rooted plantlets were successfully transplanted to soil where they continue growing.

In vitro vegetative multiplication of the forest tree *Dalbergia sissoo* thus has been achieved through axillary shoot proliferation using cotyledonary nodes as starting juvenile plant source, and further using explants with multiple nodes and single nodes. Several other authors have also worked on this plant species. SHARMA & CHANDRA (1988) obtained shoot buds by culturing hypocotyl explants and from isolated hypocotyl callus on MS medium containing BAP + IAA/IBA/NAA. The isolated shoots were rooted on MS medium + IBA, NAA or IAA, or IBA + NAA combination. BHATTACHARYA & al. (1990) showed that calli can be obtained from hypocotyl region of seedlings of *Dalbergia sissoo* in MS medium supplemented with auxin namely 2,4-D, NAA and IAA and cytokinin viz BAP used in different concentrations. KUMAR & al. (1991) obtained regeneration of plantlets from cell suspension derived calli of cambial origin from mature elite trees of *Dalbergia sissoo*. Shoot bud differentiation was observed in calli transferred to medium devoid of auxin but containing 0.5-2.0 mg BAP/l. The isolated microshoots were rooted in modified MS medium containing low organic salts and auxins. In our case, the WPM medium of LLOYD & McCOWN (1980), favourably used for woody plants in general (AHUJA, 1991), was exploited successfully when supplemented with 2IP for multiplication and IBA for rooting.

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