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In vitro multiplication of the woody legumes Leucaena leucocephala and Albizzia procera

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ABSTRACT

HOSSAIN, M., D. HANUS, C. KEVERS & Th. GASPAR (1993). In vitro multiplication of the woody legumes Leucaena leucocephala and Albizzia procera. *Saussurea* 24: 1-6. In English, English and French abstracts.

Desinfected seeds of the leguminous trees *Leucaena leucocephala* and *Albizzia procera* were allowed to germinate *in vitro* to provide seedlings as explant sources for micropropagation. The organogenic capacity of segments of excised cotyledons, hypocotyls and roots, and of cotyledonary nodes, was tested on Murashige and Skoog and Lloyd and McCown media supplied with different combinations of cytokinins and auxins. Both species developed shoots from cotyledonary buds. Such shoots could be used as further sources of nodes and shoot tips for continued axillary proliferation. Adventitious budding and shooting occurred on root and hypocotyl segments of *Albizzia* only. Shoots of *Leucaena* rooted without special treatment at the end of the multiplication cycle. Shoots of *Albizzia* necessitated a specific auxinic rooting medium, after a prior passage on a medium without growth regulator. Plantlets of both species were successfully acclimatized in soil in a greenhouse.

RÉSUMÉ

HOSSAIN, M., D. HANUS, C. KEVERS & Th. GASPAR (1993). Multiplication in vitro de Leucaena leucocephala et de Albizzia procera (Légumineuses ligneuses). *Saussurea* 24: 1-6. En anglais, résumés anglais et français.

Des graines désinfectées des Légumineuses forestières *Leucaena leucocephala* et *Albizzia procera* ont été mises à germer *in vitro*. Les plantules issues de germination ont servi de sources d'explants pour la micropropagation. La capacité organogénétique de fragments de cotylédons, d'hypocotyles et de racines, et de nœuds cotylédonaires, a été testée sur les milieux de Murashige et Skoog et de Lloyd et McCown, en présence de différentes combinaisons de cytokinines et d'auxines. Des pousses feuillées ont été générées à partir des bourgeons cotylédonaires chez les deux espèces: elles ont servi de source de nœuds et d'apex pour poursuivre la multiplication par prolifération axillaire. Des bourgeons adventifs se développant en pousses feuillées sont apparus sur les fragments de racines et d'hypocotyles d'*Albizzia* seulement. Les pousses feuillées de *Leucaena* s'enracinent sans traitement particulier à la fin du cycle de multiplication. Les pousses feuillées d'*Albizzia* nécessitent pour s'enraciner un traitement auxinique après un passage sur milieu sans régulateur de croissance. Les plantules (enracinées) des deux espèces sont ensuite acclimatées en serre sans problème particulier.

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Introduction

Many developing countries including Bangladesh have problems of deforestation and a large demand for timber, fuelwood and construction materials. Large-scale micropropagation can offer solutions to some of these problems. Tree legumes are well known for their utility to mankind. The interest in their *in vitro* propagation is evidenced by the numerous investigations on *Acacia* (SKOLMEN & MAPES, 1976; DUHOUX & DAVIES, 1985; DEWAN & al., 1992), *Sesbania* (KHATTAR & MOHAN RAM, 1983; HOSSAIN & al., 1990; PELLEGRINESCHI & TEPFER, 1993), *Dalbergia* (NATARAJA & SUDHADEVI, 1984), *Leucaena* (DHAWAN & BHOJWANI, 1985), *Albizzia* (TOMAR & GUPTA, 1988; VARGHESE & AMARJEET, 1988; SINHA & MALLICK, 1993), *Cassia* (GHARYAL & MAHESWARI, 1990), *Bauhinia* (KUMAR, 1992; MATHUR & MUKUNTHAKUMAR, 1992) and *Parkinsonia* (MATHUR & MUKUNTHAKUMAR, 1992). Relatively less success however has been achieved, probably because in vitro manipulations of organs of mature plants are less amenable than juvenile tissues (AHUJA, 1991). Before establishing the necessary techniques of rejuvenation of adult materials, an obligatory step when micropropagation will concern selected elite trees, the present investigations dealing with *Leucaena leucocephala* (Lam.) de Wit and *Albizzia procera* (Benth.) will utilize seeds and the resulting seedlings as explant sources.

Materials and methods

Plant material source. — Initial explants were taken from seedlings obtained from seeds cultured in sterile conditions. Seeds were surface sterilized by soaking in concentrated sulphuric acid for ten 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, 0.2 mg/l 2 IP 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/cm^2 pressure for 20 min.

Culture conditions. — 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.

Micropropagation media. — For multiplication and rooting, MS and WPM (LLOYD & MCCOWN, 1980; MCCOWN & LLOYD, 1983) media were used. They were supplemented with different concentrations of auxins (NAA, 2,4-D), cytokinins (BAP, 2IP) and activated charcoal (AC 0.5 g/l). 100 ml aliquots were dispensed into 600 ml Le Parfait glass recipients. The latters were covered by glass lids fixed with a plastic Reynolon (Reynolds Incorp.) film.

Results

Germination

Eighty to ninety percent germination of the seeds was obtained, delivering healthy seedlings as explant sources in about one (*Leucaena*) or two (*Albizzia*) months.

Media*	Shoot number	Shoot length (mm)	Root/Shoot	Root length (mm)
MSO	1.2	17	1.8	71
MS BAP 1 - NAA 0.1	1.0	51	–	–
MS BAP 1 - 2.4 D 1	1.4	50	1.8	14
MS BAP3	1.0	36	1.0	41
MS/3 BAP 0.1	1.2	61	1.4	66
WPM BAP 0.5	1.0	43	–	–
WPM 2IP 1.5	2.1	69	2.9	134
MS 2IP 1	1.4	55	2.0	73

*Additives in mg/l

Table 1. - Characteristics of shoots raised from cotyledonary buds of *Leucaena leucocephala* on various media. Measurements after 8 weeks of culture.

	Hypocotyl segments		Cotyledonary nodes	
Media*	Number of adventitious shoots	Length of adventitious shoots	Number of axillary shoots	Length of axillary shoots
MS BAP1 NAA 0.1	4.3 ± 1.3	25.0 ± 8.2	2.0 ± 0.7	14.5 ± 3.7
MS 2 IP 1	2.6 ± 0.5	40.5 ± 6.8	1.2 ± 0.4	24.2 ± 6.4

*Additives in mg/l

Table 2. - Mean number and length of adventitious and axillary shoots obtained from hypocotyl segments and cotyledonary nodes of *Albizzia procera* after 60 days.

Media*	Shoot number	Shoot length (mm)	Root/Shoot	Root length (mm)
WPM 2IP 1.5	1.0	39	1.6	43
MS 2IP 1	1.0	34	1.2	26
MSO	15.	16	1.8	20
MSO + A.C.	1.5	34	1.0	50

*Additives in mg/l

Table 3. – Characteristics of shoots raised from node explants (shoots raised from cotyledonary buds) of *Leucaena leucocephala* on various media. Measurements after 4 weeks of culture.

Multiplication

A. From seedlings' organs. — All parts of seedlings *i.e.* hypocotyl, root and cotyledon segments and cotyledonary nodes were tested for multiplication on MS and WPM media supplied with cytokinins with or without auxin.

Leucaena leucocephala. After sixty days of culture, root and cotyledon explants suffered from necrosis in all the media used. Non-organogenic calli of different shapes were observed on hypocotyl segments. Table 1 indicates that all the media permitted the production of axillary shoots from cotyledonary buds. The medium WPM with 2IP 1.5 mg/l favoured the highest shoot amount and allowed the highest shoot elongation. Such shoots raised from the cotyledonary buds immediately rooted without necessitating a transfer on a specific rooting medium, except on the media MS (BAP 1- NAA 0.1) and WPM (BAP 0.5). The highest rooting rate and root elongation were also registered on the medium WPM (2IP 1.5 mg/l).

Albizzia procera. Cotyledon segments died rapidly. Adventitious buds developing into shoots appeared on root segments only on the medium MS with BAP 1 mg/l and NAA 0.1 mg/l (data not given). Hypocotyl segments also differentiated adventitious buds at their both ends on several media. Axillary buds from cotyledonary nodes generally developed into shoots. Table 2 shows the comparison of formation of adventitious and axillary shoots from segments of hypocotyl and cotyledonary nodes respectively on MS BAP 1 mg/l + NAA 0.1 mg/l and MS 2IP 1 mg/l after two months of culture. The number of adventitious shoots on hypocotyls was higher on MS BAP 1 mg/l + NAA 0.1 mg/l than on MS 2IP 1 mg/l. However the length of the adventitious shoots obtained in these two media was reverse.

B. Continued from shoots raised on seedlings' organs. — Shoots raised from cotyledonary buds (for the two trees) or from adventitious buds (*Albizzia*) were further used as sources of node or shoot tip explants.

Leucaena leucocephala. Node and shoot tip explants were cultured on the two most performant media of the preceding experiment, *i.e.* on WPM (2IP 1.5) and MS (2IP 1), compared to MSO (without growth regulators) with and without active charcoal. MSO media allowed a higher number of axillary buds developing into shoots than WPM and MS media supplied with 2IP (Table 3) but the latter favoured higher shoot elongation, occurring during simultaneous rooting. Node and shoot tip explants from such shoots, placed on the same media, reacted as similar explants from shoots raised from cotyledonary buds. This thus allowed continued shoot production through axillary proliferation. A mean multiplication rate of five (five node cuttings per shoot) per multiplication cycle was recovered.

Albizzia procera. Axillary proliferation could be continued through explants from either primary adventitious or axillary shoots, still using MS BAP 1- NAA 0.1 or MS 2IP 1.

Rooting and transfer ex vitro

Shoots from *Leucaena leucocephala* rooted automatically without special treatment at the end of the multiplication cycle. Such plantlets were successfully transplanted and acclimatized in soil.

Shoots of *Albizzia procera* were tested for rooting on WPM 1/2 (half strength minerals) and WPM 1/2 supplied with IBA 1 mg/l. They rooted with difficulty on these media when directly originated from the multiplication media containing BAP or 2IP but they did after a one cycle passage on a MSO (no regulators) medium with or without active

charcoal, which did not affect their multiplication rate on the contrary (Table 4). Such rooted shoots could be successfully acclimatized in the greenhouse.

Medium	Multiplication rate (nodes/plant)	Shoot length (mm)	Callus at base (%)
MSO	4.0	12.4 ± 3.3	100
MSO + A.C.	5.0	21.4 ± 5.8	20

Table 4. – Multiplication rate, shoot length and callus obtained from node explants taken from adventitiously produced shoots of *Albizzia procera*, after 35 days.

Discussion

The results indicate that there is a considerable potential for rapid in vitro mass propagation of *Leucaena leucocephala* and of *Albizzia procera* using the above mentioned culture methods, *i.e.* through axillary proliferation of cotyledonary nodes for both trees, and also through adventitious regeneration from root and hypocotyl segments for *Albizzia*. MS medium with BAP and 2IP as cytokinins appears suitable, but WPM, favourably used for woody plants in general (AHUJA, 1991) was also exploited here successfully. The beneficial effect of charcoal, prior to rooting, was noticed as already observed for *Kalmia latifolia* (KEVERS & GASPAR, 1992).

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