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A Protocol for Efficient Plantlet Regeneration from Leaf Derived Callus of Lablab Bean (*Lablab purpureus* var. lignosus (L) prain)

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ABSTRAK

*Satu perbezaan mata tunas pucuk dan gandaan aruhan pucuk daripada kalus daun kacang Lablab (*Lablab purpureus *var. lignosus) telah diperoleh. Aruhan kalus dan penggandaan pucuk pada pelbagai frekuensi diperhatikan menggunakan konsentrasi berbeza dan kombinasi auksin (IAA, 2, 4-D dan NAA) dan sitokinin (BAP). Frekuensi aruhan kalus yang paling tinggi diperhatikan pada medium kultur asas MS yang mengandungi 2, 4-D (3.0 mg/1) dan BAP (0.5 mg/1). Nodular kalus padat hijau berlaku pada NAA (3.0 mg/1) dan BAP (0.5 mg/1). Peratusan pembentukan mata tunas pucuk dan penggandaan yang tertinggi diperoleh daripada kombinasi BAP (2.0 mg/1) dan NAA (0.5 mg/1). Pucuk-pucuk yang dijana semula dipindahkan ke medium kultur asas MS yang mengandungi IBA (1.5 mg/1) untuk aruhan akar. Tumbuh-tumbuhan berakar dipindahkan ke mangkuk plastik dan seterusnya telah berjaya dipindahkan ke ladang.*

ABSTRACT

An efficient shoot bud differentiation and multiple shoot induction from leaf derived callus of Lablab bean (Lablab purpureus *var. lignosus*) *have been obtained. Callus induction and shoot multiplication at various frequencies were observed using different concentrations and combinations of auxins (IAA, 2, 4-D and NAA) and cytokinin (BAP). The highest frequency of callus induction was observed on MS medium containing 2, 4-D (3.0 mg/1) and BAP (0.5 mg/1). The green compact nodular calli occurred on NAA (3.0 mg/1) and BAP (0.5 mg/1). Highest percentage of shoot bud formation and multiplication was obtained from a combination of BAP (2.0 mg/1) and NAA (0.5 mg/1). The regenerated shoots were transferred to MS medium containing IBA (1.5 mg/1) for the induction of roots. Rooted plants were transferred to plastic cups and subsequently these were successfully transferred to fields.*

INTRODUCTION

Legumes are one of the most important groups of crop plants and efforts have been focused to improve the crops, particularly for desirable traits, including their response to in vitro culture manipulation. Since legumes are notoriously recalcitrant to regeneration from tissue culture much effort has been devoted to developing and optimizing efficient in vitro regeneration systems to facilitate a variety of technologies (Geetha *et al.* 1998). The ability to regenerate plants from cultured cells, tissues or organs constitutes the basis of producing transgenic crops. Successful regeneration of legumenous species has been greatly aided by species-specific determination of critical parameters, such as explant source, genotype and media constituents (Parrot *et al.* 1992). Lablab bean (*Lablab purpureus*) var.

Abbreviations

NAA: 1-napthalene acetic acid; IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; 2,4-D: 2,4-dichlorophenoxy acetic acid; BAP: 6-benzyl amino purine.

lignosus (L.) prain is an important high protein grain legume. It belongs to the family of Fabaceae, and grows in tropical and subtropical countries. Recent advances in in vitro culture technologies brought about new techniques for crop improvement. Application of tissue culture techniques to genetic upgrading of economically important plants have been reported (Scowcraft 1977). Only limited success has been reported for in vitro organogenesis and regeneration from *Lablab purpureus* (Sounder *et al.* 1991; Thiruvengadam and Jayabalan 2000a). Shoot and plantlet regeneration from seedling and other explants have been reported in many leguminous pulses like Glycine wigghtii (Pandey and Bansal 1992), *Pisum sataivum* (Ozean *et al.* 1992), *Phaseolus vulgaris* (Zambre *et al.* 1998) and *Macrotyloma uniflorum* (Varisai Mohamed *et al.* 1999). Hence the present investigation was attempted to standardize a protocol for rapid shoot multiplication from leaf explants of Lablab bean.

MATERIALS AND METHODS

Seeds of Lablab bean (*Lablab purpureus* var. lignosus (L) prain) cultivar CO 1 were obtained from Tamil Nadu Agricultural University, Coimbatore, India. The seeds were washed with distilled water 5 times, followed by treatment of 5% sodium hypochloride for 15 min and disinfected with 0.1% $HgCl_2$ for 3 min. The disinfected seeds were rinsed thoroughly with sterile water 6 times and aseptically placed over sterile moist cotton for germination. The young leaves were excised from 7 day old in vitro raised seedlings and cultured in 25 x 150 mm tubes containing 15 mL semisolid MS (Murashige and Skoog 1962) medium with of 3% (w/v) sucrose, 0.8% (w/v) Bacto agar and various concentrations (0.0-5.0 mg/1) of hormones 2,4-D, IAA, IBA and NAA either alone or in combination with (0.5 mg/1) BAP. The pH of the medium was adjusted to 5.8 and autoclaved at 1.06 kg cm^2 for 15 min before inoculation. All cultures were maintained under cool white fluorescent light (80 μ $Em^{-2}s^{-1}$) at 25± 2^0C with a 16 h photoperiod. Shoots obtained in vitro were transferred to MS medium supplemented with different concentrations (0.0 – 2.0 mg/1) of auxins (NAA, IAA and IBA). Histological analysis was done to confirm shoot regeneration from the leaf callus. At least 20-24 explants were cultured in each treatment and all the experiments were repeated three times. The regenerated plants were transferred to plastic cups containing sterile soil, sand and compost in the ratio (1:1:1) in a greenhouse.

RESULTS AND DISCUSSION

Callus initiation was observed within 8 days of culturing the leaf explants of cultivars CO 1 on MS medium supplemented with different concentrations of IAA, 2,4-D and NAA (0.0 – 5.0 mg/1) alone or in combination with BAP (0.5 mg/1). Young leaf explants were more responsive in producing callus than other seedling explants. Similar results were reported in *V. unguiculata* (Kulothungan *et al.* 1995) and *Lablab purpureus* (Thiruvengadam and Jayabalan 2000b). The combination of 2,4-D (3.0 mg/1) and BAP (0.5 mg/1) showed maximum callus induction frequency (70.0 ± 7.00) (Table 1) producing yellowish friable callus. In the present study, leaf explants cultured on MS medium supplemented with (NAA 3.0 mg/1) and BAP (0.5 mg/1) produced greenish compact callus

TABLE 1
The effect of various concentrations of IAA, NAA and 2,4-D in combination with 0.5 mg/1 BAP on callus induction frequency in *L. purpureus*

Auxins (mg/L)	Cytokinins (mg/1)	Callus induction Frequency(% mean ± SD)
IAA	BAP	
0.0	0.0	0.0
1.0	0.5	33.3 ± 3.50^{i}
2.0	0.5	37.0 ± 2.00^{h}
3.0	0.5	48.0 ± 3.61^{e}
4.0	0.5	45.0 ± 5.00^{fg}
5.0	0.5	42.0 ± 4.33^{gh}
2,4-D		
0.0	0.0	0.0
1.0	0.5	49.0 ± 3.00^{de}
2.0	0.5	52.0 ± 2.64^{c}
3.0	0.5	70.0 ± 7.00^{a}
4.0	0.5	57.0 ± 4.58^{bc}
5.0	0.5	46.7 ± 6.11^{ef}
NAA		
0.0	0.0	0.0
1.0	0.5	44.3 ± 4.04^{g}
2.0	0.5	49.6 ± 2.52^{d}
3.0	0.5	63.0 ± 1.73^{b}
4.0	0.5	52.0 ± 2.65^{cd}
5.0	0.5	45.6 ± 351^{f}

Values with the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

(*Fig. 1*) with maximum induction frequency (63.0 ± 1.73). Similar results were reported in *Vitex negundo* (Thiruvengadam and Jayabalan 2001) and *Vigna radiata* (Patel *et al.* 1991). The callus was subcultured on MS medium containing NAA (0.5 mg/1) with different concentrations of BAP (0.5 – 2.5 mg/1) for shoot bud differentiation (Table 2). The highest percentage (81.2 ± 8.5) of shoot initiation from the compact calli occurred on BAP (2.0 mg/1) and NAA (0.5 mg/1). Similar results were reported in *Carrica papaya* (Hossain *et al.* 1993) where adventitious buds were obtained when 2.0 µM BAP and 0.1 µM NAA were used. The buds developed into multiple shoots on medium containing BAP (2.0 mg/1) and NAA (0.5 mg/1) after 5 weeks of culture (*Fig. 2*). Shoots developed (*Fig. 3*) up to approximately 5-10 cm in length and subsequently transferred to rooting media. The highest frequency of root formation (72.0 ± 2.0) (Table 3) was observed on MS medium containing IBA 1.5 mg/1 (*Fig. 4*). Rooted plantlets were successfully transferred to plastic cups. During the initial period of acclimatization, transferred plants were kept under culture room conditions and high relative humidity was maintained by covering the plants with polyethylene bags (creating a humid chamber

TABLE 2
Effect of NAA (0.5 mg/1) in combination with various BAP concentrations on shoot bud regeneration from leaf derived callus of *L. purpureus*

BAP (mg/L)	% of culture showing regeneration (Mean ± SD)	Average number of shoots/culture (Mean ± SE)	Average length (cm) of shoots (Mean ± SE)
0.5	42.5 ± 9.5^{d}	9.7 ±5.0^{cd}	3.6 ± 0.2^{d}
1.0	56.0 ± 6.6^{c}	10.6 ± 3.7^{c}	5.5 ± 2.3^{c}
1.5	65.0 ±12.9^{b}	14.0 ±1.8^{b}	6.4 ± 0.1^{b}
2.0	81.2 ± 8.5^{a}	21.2 ± 1.8^{a}	7.8 ± 0.5^{a}
2.5	40.0 ± 8.1^{de}	7.8 ± 0.5^{d}	3.4 ± 0.3^{de}

Values with the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

TABLE 3
Rooting of shoots of Lablab bean on MS medium supplemented with different concentrations of IAA, NAA, IBA

Growth regulators (mg/1)	shoots forming roots (%)	Average no of roots/ shoots	Root length/ explant
IAA			
0.5	18.3 ± 3.0^{f}	1.0 ± 0.0^{e}	1.0 ± 0.0^{f}
1.0	20.0 ± 2.0^{e}	1.3 ± 0.4^{de}	1.3 ± 0.4^{d}
1.5	38.0 ± 1.6^{cd}	1.6 ± 0.4^{d}	1.5 ± 0.3^{cd}
2.0	22.6 ± 0.9^{de}	0.0 ± 0.0^{ef}	1.1 ± 0.0^{ef}
IBA			
0.5	27.0 ± 1.0^{d}	2.3 ± 0.5^{c}	1.96 ± 0.1^{c}
1.0	48.6 ± 1.5^{c}	3.0 ± 1.0^{b}	2.70 ± 0.6^{bc}
1.5	72.0 ± 2.0^{a}	4.0 ± 0.0^{a}	4.43 ± 0.3^{a}
2.0	54.3 ± 4.0^{b}	3.0 ± 0.8^{bc}	3.00 ± 0.2^{b}
NAA			
0.5	15.3 ± 1.5^{fg}	1.0 ± 0.0^{e}	1.2 ± 0.4^{de}
1.0	20.0 ± 1.6^{ef}	1.0 ± 0.0^{ef}	1.1± 0.2^{e}
1.5	–	–	–
2.0	–	–	–

Values with the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

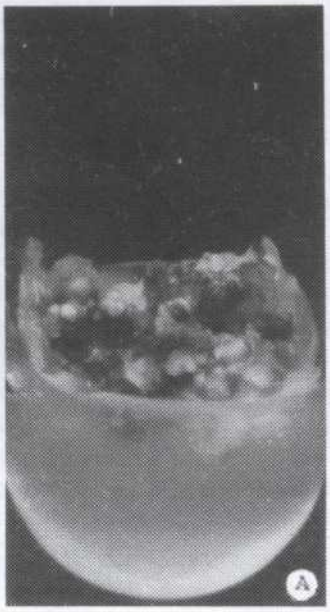

Fig. 1: A greenish compact nodular callus from leaf explant

Fig. 2: Adventitious shoots from leaf derived callus

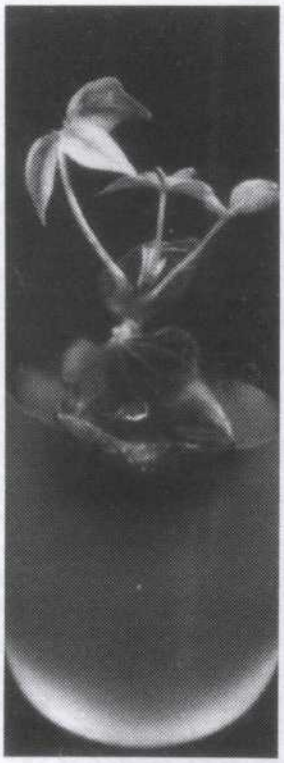

Fig. 3: A single isolated shoot

Fig. 4: Root induction of from regenated shoots

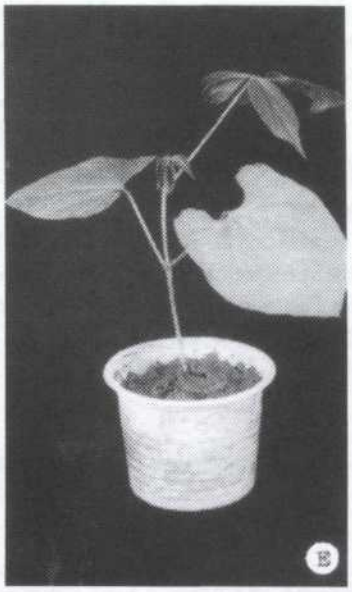

Fig. 5: A hardened plant (after 3 weeks of transfer to plastic cups)

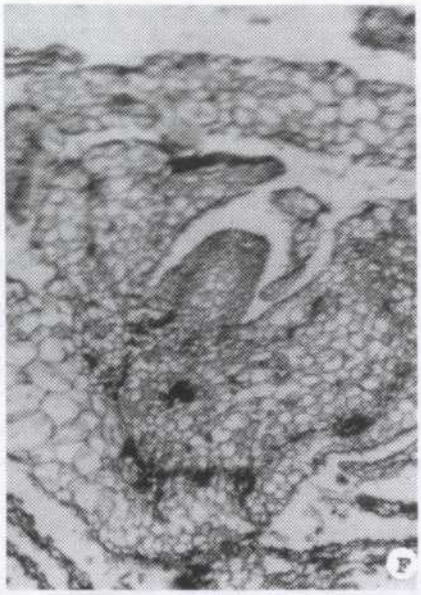

Fig. 6: Histological section showing the development of shoot primordia from the leaf callus

effect). Finally, these plants were successfully transferred to the field (*Fig. 5*). The regeneration of shoot primordia from the leaf callus were histologically analysed confirming indirect regeneration (*Fig. 6*).

The study describes plant regeneration *via* callus culture with high frequency of plant recovery. This plant regeneration protocol may prove to be useful for genetic transformation studies in Lablab bean, for developing, disease-resistant, salt-resistant and herbicide-resistant plants.

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