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Rapid *In Vitro* Micropropagation of Two Different Cultivars of *Phaseolus calcaratus* RBL-1 and RBH-35

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A successful protocol for rapid proliferation of adventitious shoot from hypocotyl and leaf explants of two cultivars of *Phaseolus calcaratus* cv., RBL-1 and RBH-35 is described. A high frequency of multiple shoot buds was obtained directly from cut ends of the explants on MS medium supplemented with different (1-10 mgl^{-1}) concentrations of auxins viz. 2, 4-dichlorophenoxyacetic acid (2, 4-D), indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) alone and in combination with cytokinin, 6-benzyladenine (BA). Lower concentrations of auxins (0. 1 and 0. 5 mgl^{-1}) alone and in combination with different concentrations of BA were in general found to be the best for multiple shoot differentiation within 3-4 weeks of culture. Rooting of *in vitro* regenerated shoots was induced by NAA (0. 1 mgl^{-1}) alone or a combination of IBA and BA (0. 1+ 0. 1 mgl^{-1}). Regeneration was much higher from hypocotyl than from leaf.

Introduction

Grain legumes (edible legumes or seed legumes) are an important human and animal dietary constituent in the developing countries[1]. The improvement of legumes through tissue culture is essential for their continued exploitation as a source of human nutrition and other products[2]. Early attempts to regenerate legumes from tissue cultures were frustrating and several authors highlighted the recalcitrance of leguminous tissue under *in vitro* conditions[3,4]. Plant regeneration has been reported in several *Phaseolus* species viz., *P. aconitifolius* Jacq. syn *Vigna aconitifolia*[5,6], *Phaseolus vulgaris*[7-9]. Rice bean or red bean (*Phaseolus calcaratus* Roxb. syn. *Vigna umbellata* Thumb. Ohwi and Ohashi) is a tropical food legume of potential value. However, *in vitro* studies of this species have not yet been attempted. The present study is a maiden attempt to assess the regenerative capacity of the explants of *P. calcaratus* Roxb. under different hormonal conditions.

Materials and Methods

Two cultivars of *Phaseolus calcaratus*, RBH-35 and RBL-1 were used as experimental materials. The seeds of these two cultivars obtained from the Directorate of Pulses Research Centre of IARI, Kanpur (U. P.), India were surface sterilized with 0. 1% mercuric chloride (w/v) for 5 min. and then rinsed with sterile distilled water 4 or 5 times. The seeds were germinated on sterile moist filter paper in petriplates or on sterile moist cotton in flasks (100 ml) at 22-25°C in dark. Four-seven day old seedlings were used as the source of the explants.

Hypocotyl (1 cm) and leaf explants (0. 5 cm^2-1 cm^2) were washed with distilled water and surface sterilized with 0. 1% (w/v) mercuric chloride solution for 5 min. followed by a thorough washing

with sterile double distilled water. Explants were aseptically inoculated onto agar (8 $g l^{-1}$) solidified Murashige and Skoog's medium[10] containing 30 $g l^{-1}$ sucrose (Qualigens), which was supplemented with different concentrations of auxins such as IBA, 2, 4-D, and NAA alone and in combination with a cytokinin, BA. The media were adjusted to pH 5.8 prior to autoclaving for 15 min. at 1.1 kg cm^{-2}. Cultures were incubated at 25±2°C with a photoperiodic regime of 16 h light (40-50 $\mu Em^{-2} sec^{-1}$)/8 h dark for callusing and organogenesis. Calli obtained from different explants were periodically subcultured after every 4 weeks. For each treatment a minimum of 16 replicates were kept and each treatment was repeated three times.

Results and Discussion

In the present study, moderate to very high callusing was observed at various hormonal combinations and concentrations in the two cultivars used. The two kinds of explants markedly differed in the capacity to undergo organogenesis and the hypocotyl proved much more suitable than the leaf in both the cultivars. Out of all the auxins tried, lower concentrations (0.1, 0.5 mg l^{-1}) of NAA alone or in conjunction with different BA concentrations induced the best adventitious shoot bud

Table 1. Morphogenetic response of hypocotyl explans of two cultivars of *Phaseolus calcaratus* after 120 days of culture.

Plant Growth Regulator (mg l^{-1})	Cultivar RBH-35%		Cultivar RBL-1	
	Explants Producing shoot	% Plantlet formation	% Explants Producing shoot	% Plantlet formation
0.1 NAA	100	100	100	66.6
0.5 NAA	79.1	79.1	100	50.0
1 NAA	0	0	87.5	66.6
0.1 IBA	62.5	62.5	66.6	66.6
1 IBA	0	0	83.3	83.3
0.1 2, 4-D	0	0	50.0	50.0
0.1 NAA+0.1 BA	70.8	16.6	100	0
0.1 NAA+0.5 BA	75.0	25.0	100	50.0
0.1 NAA+1 BA	50.0	37.5	100	0
0.1 NAA+5 BA	100	16.6	71.4	0
0.1 NAA+10 BA	100	50.0	0	0
0.5 NAA+0.1 BA	100	100	50.0	25.0
0.5 NAA+0.5 BA	100	50.0	0	0
1 NAA+0.1 BA	100	50.0	0	0
1 NAA+0.5 BA	75.0	54.16	0	0
1 NAA+1 BA	66.6	66.6	0	0
0.1 IBA+0.1 BA	100	100	100	100
0.1 IBA+0.5 BA	25.0	23.0	0	0
0.5 IBA+0.1 BA	37.5	37.5	50.0	50.0
0.5 IBA+0.5 BA	33.3	33.3	0	0
1 IBA+0.1 BA	41.6	40.0	0	0
1 IBA+1 BA	100	100	0	0
5 IBA+0.1 BA	100	100	0	0
5 IBA+0.5 BA	79.16	79.16	0	0
5 IBA+1 BA	41.7	41.7	0	0
5 IBA+5 BA	100	66.6	0	0
10 IBA+0.1 BA	100	100	0	0
0.1 IBA+0.5 BA	100	100	0	0

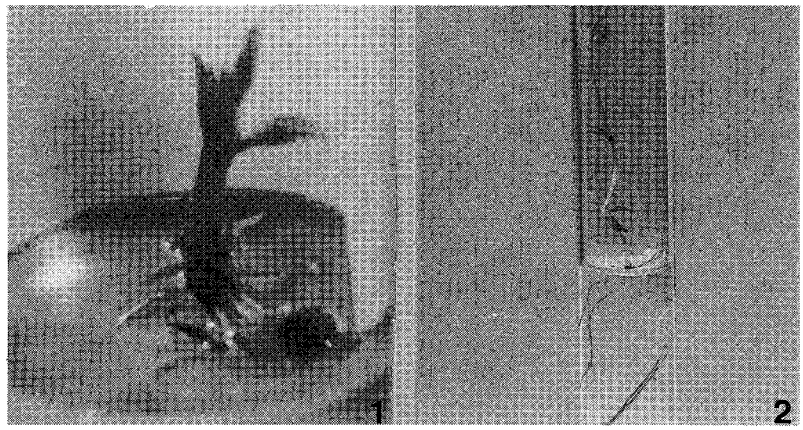

Fig. 1 Shoot buds arising from hypocotyl of cv. RBL-1 on 0.1 mgl^{-1} NAA after 3 weeks of culture.

Fig. 2 Whole plant formation in cv. RBH-35 on 0.1 mgl^{-1} IBA+ 0.1 mgl^{-1} BA after 6 weeks of culture.

development directly from the explant surface in both the cultivars (**Table 1, Fig. 1**). In general this reponse was quite well marked in cultivar RBL-1 where all the explants produced greenish shoots on its surface. With increasing concentrations (1-10 mgl^{-1}) shoot differentiation suffered a decline and proved completely ineffective for shooting in both the cultivars. In the case of IBA only 1 mgl^{-1} was effective for shoot differentiation in cultivar RBL-1 and 0.1 mgl^{-1} in RBH-35. Maximum shooting (100%) was observed with both low as well as high concentrations of IBA in combination with BA in the cultivar RBH-35 while in cultivar RBL-1 only low concentrations of both IBA and BA produced maximum (100%) shooting. 2, 4-D alone at low concentration (0.1 mgl^{-1}) was found effective for direct shoot bud regeneration in cultivar RBL-1, while no shoot differentiation from hypocotyl was observed in cultivar RBH-35. Therefore, 2, 4-D was suppresive for shoot regeneration in this species as reported earlier[11,12].

The shoots directly regenerated roots 7-9 days after the initiation of adventitious shoot formation. In both the cultivars maximum rooting of the regenerated shoots wasobserved with NAA (0.1 mgl^{-1}) when auxin was applied singly. In combined treatment of auxin and cytokinin in both the cultivars, maximum rooting (100%) of the shoots was observed with 0.1/0.1 mgl^{-1} IBA/BA (**Fig. 2**).

Based on the results obtained, it can be concluded that with auxin alone cultivar RBL-1 induces a higher degree of shoot buds than cultivar RBH-35. But the latter showed a higher degree of shooting than the former in auxin and cytokinin-combined treatments. Variation in morphogenic potential of the two cultivars may be attributed to the influence of genotype on differentiation under *in vitro* conditions as reported earlier[13,14].

The results described suggest the potential of hypocotyls to quickly differentiate into adventitious shoots and plantlets by manipulation of NAA, IBA and BA. Since regeneration of plants has been difficult to achieve in *Phaseolus calcaratus* in the past, this material is open for further research in future.

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