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Callus induction from various explants of French bean (*Phaseolus vulgaris* L.)

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ABSTRACT: Callus cultures were studied in white seed induced mutant obtained from *Phaseolus vulgaris*L., cv. varunto help establish a suitable protocol for a prospective *in vitro* program. Callus cultures were initiated from the axillary leaves, axillary shoots, node, internode, and root segments.The initiation and growth of callus were evaluated on MS medium with 3% sucrose, 0.4% agar, 1.5 mg.l^{-1}BAP, and three levels of IAA. The highest callus relative growth was obtained on medium with 0.5 mg.l^{-1}IAA and 1.5 mg.l^{-1}BAP.

Key words: French bean, callus, in vitro culture, *Phaseolus vulgaris* L.

Abbreviations: BAP: N^6-Benzylaminopurine; IAA: Indole Acetic Acid; MS: Murashige and Skoog

Introduction

French bean (*Phaseolus vulgaris*L.) is an economically important crop and comprises one of the major grain legumes forhuman consumption in Latin America, Africa and Asia (Delgado-Sanchez et al., 2006; Varisai Mohamed et al., 2006). Despite its importance the production growth rates in French bean arelimited by viral, fungal, bacterial pathogens, insects,lack of drought tolerance and nutritional deficiencies. Therefore, there is considerable scope in development of new bean cultivars withuseful agronomical traits (Aragado et al., 1996).

Plant biotechnology together with conventional breeding methods offer scope in bean improvement since resistance to biotic / abiotic stresses could be increased and theseed quality, plant architecture and reproductive modes could bebeneficially altered through this procedure (Veltcheva et al., 2005). Nevertheless, a reliable and efficient *in vitro* culturesystem that may lead to efficient differentiation, shootdevelopment and whole plant regeneration becomes an essentialprerequisite for improvement of common bean throughgenetic transformation/ mutagenesis protocols (Svetleva et al., 2003; Varisai Mohamed et al., 2006). In addition to geneticimprovement, the*in vitro* culture forms an important tool for therecovery and conservation of germplasm besides proving helpfulin embryo rescue system (Delgado-Sanchez et al., 2006).

Materials and Methods

White seed mutant of*Phaseolus vulgaris*L. cv.varundeveloped through application of 0.05 % EMS was used in the present studies. The presoaked seeds were washed in tap-water containing 0.1% (v/v) Tween 20. The seeds were then washed in double distilled water. The seeds were taken to the laminar airflow and surface sterilized by dipping in 70% (v/v) alcohol for 2 min. Then seeds were washed thoroughly in distilled sterilized water. The explants were disinfected with 0.5% (w/v) mercuric chloride for 5 min. Finally, the seeds were rinsed with sterilized distilled water five times. The surface sterilized seeds were inoculated on 0.4% Agar forseedling formation.The in vitro grownseedlings of *Phaseolus vulgaris* L. were used as a source of explants. Callus cultures were initiated from axillary leaves, axillary shoots, node, internode, and root segments from *in vitro* grown seedlings on MS medium(Murashige and Skoog, 1962). All the cultures were incubated in a growth room under a 16 h photoperiod (cool, white fluorescent light) and the temperature was maintained at 25 ± 2°C with 2 - 7% relative humidity.

Results and Discussion

The explants of *Phaseolus vulgaris* L.showed callus initiation after five days of inoculation and the well-developed callus was obtained after 13 days. A combination of 1.5 mg l^{-1} BAP and 0.5 mg l^{-1} IAA was found to be suitable for high frequencyof callus from stem as explants *Table1*. When nodal explants were cultured on MS medium with 1.5 mg l^{-1} BAP and 0.5 mg l^{-1}IAA, the highest percentageof callus could be observed as compared to leaves, internode and root explants.

It has been demonstrated that adenine, adenosine andadelynic acid have cytokininlikeactivity and when they are added tothe culture medium they help improve growth or toreinforce the response normally attributable to cytokininaction. In this sense, adenine stimulates somaticembryogenesis and caulogenesis, enhances growth ofisolated meristem tips, induces proliferation of axillaryshoots in shoot cultures and promotes adventitious shootformation indirectly from calli or directly from explants(Van Staden et al. 2008).

Fig.1 Callus induction from different explants in French bean (*Phaseolus vulgaris* L.)

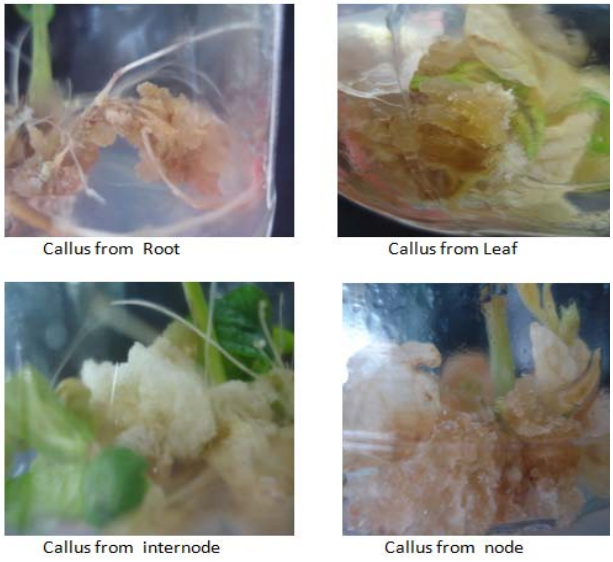

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Table 1 Effect of various concentrations of IAA and BAP on callus inductioninFrench bean (*Phaseolus vulgaris* L.)

Treatments		Leaves	Node	Internode	Root
IAA	BAP				
0	0	-	-	-	-
0.5	1.5 mg	++	+++++	++++	++++
1.0	1.5 mg	++	+++	+++	++
1.5	1.5 mg	++	+++	++	+
2.0	1.5 mg	++	+++	++	+
2.5	1.5 mg	++	+++	++	+

+ indicates the frequency of callus induction in *Phaseolus vulgaris* L.

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