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IN-VITRO REGENERATION AND CALLUS FORMATION FROM DIFFERENT PARTS OF SEEDLING OF *MUCUNA PRURIENS* BAK – A VALUABLE MEDICINAL PLANT

SHALINI PATEL*, ANITA MEHTA[1] AND R. K. PANDEY[2]
[1] Dept. of Biotechnology, Ranchi Women's College, Ranchi - 834 001
[2] Dept. of Botany, Ranchi University, Ranchi - 834 008
* Email : shalini_rch@rediffmail.com

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*** Corresponding author**

ABSTRACT
Callus proliferation was studied on cotyledon, leaf and stem explant of *Mucuna pruriens* Bak cultured on Murashige and Skoog's medium (MS) supplemented with 2,4-D, IBA, NAA and BAP alone or in combination. Light brown callus formation was followed by formation of milky white callus on the surface of young excised shoots and leaf tissues of *Mucuna pruriens*. Sometimes green callus was also observed. Development of root and stem with leaves were investigated from excised stem, leaf and cotyledon tissues.

INTRODUCTION

Mucuna pruriens Bak (Fabaceae) commonly known as Kivach, Alkusi, Cowhage, Kaunch, Velvet bean is an economically important medicinal plant found in bushes and hedges and dry deciduous, low forests throughout the plains of India (Sastry & Kavathekar 1990; Singh *et al*., 1996). It is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4-dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter (Daxenbichler *et al*.,1971) used in the treatment of Parkinson's disease (British pharmacopoeia, 1973). That is why it's demand in international market has increased many fold. Large scale formation of callus may be used for L-DOPA production and white and green callus being embryogenic in nature can be exploited for rapid micropropagation.

The great demand of L-DOPA is largely met by the pharmaceutical industries through extract of *Mucuna* from wild population. But commercial exploitation for production is hampered due to it's limited availability. *Mucuna* is annual herbaceous plant which grows only from seeds and is not propagated by cuttings. Micropropagation can provide the opportunity to obtain a rapid and large scale multiplication of the plant. Attempts for production of L-DOPA from callus culture (Brain 1979) and cell suspension (Huizing *et al*., 1985; Wichers *et al*., 1989; Chattopadhyay *et al*., 1994,1995) have been made. Regarding rapid micropropagation none of the protocols reported are suitable due to the low regeneration frequencies. This paper describes an efficient method for high frequency of root and stem (with leaves) formation from in-vitro grown cotyledon, stem and leaf tissues. Present investigation also deals with formation of callus with it's different morphology.

MATERIALS AND METHODS

Culture material

The seeds of *Mucuna pruriens* obtained from wild plants as well as from Dept. of NBPGR, Namkom, Plandu, Ranchi were washed with running tap water and rinsed in cetrimide teepol (5 times dilute) for 2 minutes. Seeds were surface sterilized in 70% ethanol for 1 minute and immersed in 0.1% $Hgcl_2$ for 2 minutes, then rinsed with autoclaved distilled water (5 washes, each for 5 minutes). Seeds were inoculated in test tube (10 X 1.2 cm) containing MS basal media (Murashige and Skoog's , 1962) . Explants obtained from in-vitro plantlets were used as culture materials. Callus having different morphology was also used as culture material.

Culture medium

Solid MS medium containing 3% sucrose with varying concentration of 2,4-D , NAA, IBA, IAA (0.5 – 2.5 mgl^{1}) was used for callus formation and root and shoot regeneration. Combination of auxins (IBA) and cytokinins

(BAP) was also used for plant regeneration. Coconut water (cw - 20% v/v) was also used in MS basal medium for regeneration system. The pH of the media was adjusted to 5.8 before gelling with agar (0.8% w/v) and autoclaved for 15 to 20 minutes at 15 psi at 120°C . The seeds and in-vitro grown leaf, stem and cotyledons were inoculated onto the culture medium (15ml) in culture tubes and incubated in culture room.

Culture conditions

Cultures were incubated at 20 + 5°C under cool fluorescent light (1500 - 2000 lux) with a 16 hours/8 hours light/dark cycle. Each treatment consisted of minimum 10 explants and all experiments were repeated 7 times.

Callusing from different explant

Explants were excised from 10-12 days old in-vitro grown seedlings and transferred to MS medium supplemented with 2,4-D (0.5 – 2.5 mgl^{-1}) and NAA (0.5 – 2.5 mgl^{-1}) for callusing. Combination of IBA (2.5 mgl^{-1}) and BAP (2.5 mgl^{-1}) was supplemented for regeneration system.

Root and shoot formation

Excised leaf, stem, cotyledons were cultured on MS solid medium supplemented with different concentration of NAA (0.5 – 5.0 mgl^{-1}). Coconut water (20% v/v) was also used for plantlet regeneration.

RESULTS AND DISCUSSION

Seeds germinated rapidly under in-vitro condition and formed plantlets in MS medium without any growth regulator. Explants taken from 10-12 days old seedlings resulted in either callus tissue or organogenesis. When NAA (05 – 2.5 mgl^{-1}) and IBA (0.5 – 2.5 mgl^{-1}) were used separately in the media, profused growth of roots was observed from cotyledons, stem but little less from leaf explants. In few cultures roots were measured about 7-10 cm length and 10-12 roots per explants was observed. Roots always showed primary, secondary and tertiary structure (Fig-1). Development of roots was significant part of the work. IBA (0.5,1.0 , 1.5, 2.5 mgl^{-1}) and NAA (0.5,1.0 , 1.5, 2.5 mgl^{-1}) when used separately, was found to be suitable for root formation from cotyledons, leaf and stem . Complete plant regeneration with 2-3 branches from stem tissues was observed in media supplemented with 2.5 mgl^{-1} NAA (Fig-3). When excised juvenile shoot about 2-3 cm in length was cultured horizontally in the medium containing 1.5 mgl^{-1} NAA , trifoliate leaf with long petiole was observed within 2-3 weeks (Fig-4). This investigation differs from the experiments done on *Mucuna pruriens* (Faisal *et al*., 2005) in the sense that they used only nodal segments as explants of 15 days old seedling for different morphogenic response. The nodal segments showed root and shoot induction when they were cultured in MS medium supplemented with various cytokinins and auxins. Media containing coconut water (20% v/v) showed similar result. Coconut water contains a number of cell division factors and free amino acids (Shantz and Steward , 1952).Addition of 20% v/v of coconut water on MS media showed development of shoot and callus on stem . When coconut water along with 1.5 mg/l IBA supplemented with MS media development of root, shoot and callus was observed. Shoot growth and multiple shoot production induced by medium containing coconut water has been reported in a number of tree species of Fabaceae (Nadgir *et al*., 1984; Chandra, 1989).Effect of cytokinins and auxins on micropropagation *Clitoria ternatea* L. which also belongs to the family Fabaceae was observed by Rout (2004). He found that there was no sign of growth when nodal explants were cultured on media without cytokins and auxins. Among growth regulators auxins and cytokinins are widely used. The exogenous growth

Table 2: Effect of different concentration of growth regulators on morphogenic and multiplication response in in-vitro grown *Mucuna pruriens* seedling.

Growth regulator (mgl^{-1})	Leaf Nature of response		Stem Nature of response		Cotyledon Nature of response		Hypocotyl + Cotyledon Nature of response	
	Callus	Organo-genesis	Callus	Organo-genesis	Callus	Organo-genesis	Callus	Organo-genesis
2,4-D								
0.5	+ + +	—	+	—				
1.5	+ + +	—	+ +^{gn}	—	+ +	—		
2.5	+ +	RT	+ + +^{gn}	—	+ + +	—		
5.0	+ + +	—	—	—	+ +	—		
NAA								
0.5	+ + +	—	+ +	—	+	—		
1.5	+ + +	—	+ + +	SH + RT				
2.5	+ +	—	+ + +	SH + RT			—	SH + RT
5.0	+	—	—	—				
IBA + BAP								
2.5 + 2.8	—	—	+ + +	—				
2.5 + 2.5	—	—	+ + +^{w}	—				SH + RT
IBA								
2.5								RT

— = No response, + = Good, ++ = Very Good, +++ = Excellent,.SH = Shooting, RT = Rooting,.W = White Callus,.gn = Green callus

Table 1: Effect of auxin on rooting from in-vitro raised cotyledon, stem, leaf of *Mucuna pruriens* in MS media after four weeks of culture.

Auxins	Cotyledons			Leaf			Stem		
In mgl^{-1}	% rooting	No. of roots per explant	Av. Root Length (cm)	% rooting	No. of roots per explant	Av. Root Length (cm)	% rooting	No. of roots per explant	Av. Root Length (cm)
IBA 0.5	72	4-5	7.5	—	—	—	75	4-5	6.5
IBA 1.0	85	18-20	12.5	30	2-3	0.5	82	15-16	9.5
IBA 1.5	80	10-12	11.0	42	1-2	1.5	85	11-12	10.5
IBA 2.5	60	2-3	2.5	65	4-5	3.5	65	3-4	1.5
NAA 0.5	62	4-5	5.5	—	—	—	60	3-4	4.5
NAA 1.0	80	8-10	9.0	—	—	—	78	7-8	8.5
NAA 1.5	73	6-7	6.5	30	2-3	0.5	69	4-5	5.5
NAA 2.5	60	2-3	1.5	55	4-5	2.5	55	4-5	1.5

Figure 1 : 20 days old cotyledon tissue culture in MS + 2.5 mgl^{-1} NAA showing roots

regulators requirement (type, concentration, auxin to cytokinin ratio) for callus formation depends upon the genotype and endogenous hormone contents of the tissue (Pierik, 1987). The results on impact of various consentrations of auxin and growth regulators have been presented in Table 1 and 2.

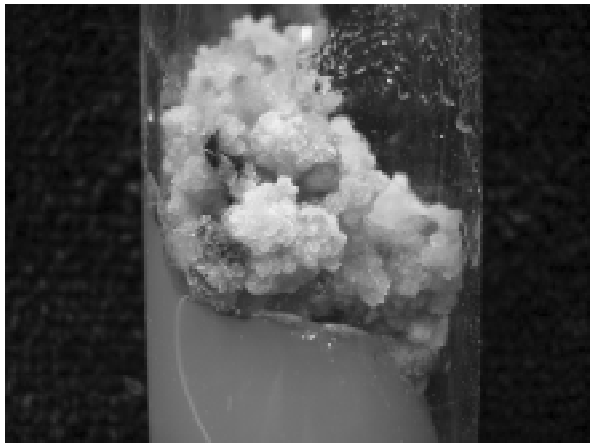

Figure 2: 25 days old stem culture in MS + 2.5 mgl^{-1} 2,4-D showing nodulated calli

Figure 3 : 20 days old culture in MS + 2.5 mgl^{-1} NAA showing branched shoot and roots

Figure 4 : 20 days old juvenile shoot culture in MS + 1.5 mgl^{-1} showing trifoliate leaf

Figure 5: 14 days old 2,4-d grown stem calli culture in MS + 2.5 mgl^{-1} BAP showing green callus.

Type of callus was greatly affected by the type and age of explants and growth regulators used. Initially there was formation of light brown fragile callus on the surface of the explant as well as on the cut region and leaf margin. In 1.5 – 2.5 mgl^{-1} 2,4-D there is profused growth of nodulated calli (Fig-2). If kept in the same media and not subcultured, brown callus was always followed by formation of milky white callus. It was observed during this experiment only. Combination of IBA (2.5 mgl^{-1}) and BAP (2.5 mgl^{-1}) also caused brown callus followed by white callus. When stem and leaf derived 2,4-D grown calli were transferred in media containing 2.5 mgl^{-1} BAP green nodulated calli were noticed within 2 weeks (fig-5). Thus the callus showed differential response according to the growth regulators used. For embryogenic callus leaf was the best explant source (Table 2). The result of the present study is significant, since production of white callus and green callus are being reported in these species for the first time.

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