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IN VITRO PROPAGATION OF A RARE MEDICINAL PLANT *ABRUS LAEVIGATUS* E. MAY

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Abstract

An efficient protocol was developed for in vitro propagation of *Abrus laevigatus* E. May. through induction of Plantlets by indirect organogenesis in nodal segment derived callus tissues. Yellowish green, fragile, nodular callus was induced at the cut surface of the nodal segments cultured on MS fortified with 5.0 mg/1 BAP, 0.2 mg/l KIN and 0.1 mg/1 IBA. The callus differentiated into adventitious shoots when it was sub-cultured on to MS supplemented with 3.0 mg/l BAP + 0.5 mg/1 Kin + 0.5 mg/1 NAA. On an average 6.87 ± 0.26 shoots developed. These micro-shoots were rooted in half-strength MS containing 1.0 mg/1 IBA and the rooted plantlets were transferred to soil after acclimatization.

Keywords: *Abrus laevigatus* E. May, In vitro propagation, Callus

Introduction

Abrus laevigatus E. May. commonly known as 'Kunch' in Bengali is a deciduous woody climber of the family Fabaceae. Shiny white colored seeds can easily recognize it. Since last long, this plant species has been in use for its medicinal value [1]. Different plant parts of this species contain various kinds of alkaloids such as glycerrhizin, precol, abrol, abrasine, abrin A and abrin B, which indicate its medicinal value [2,3].

In nature the propagation *of Abrus laevigatus* E. May. through seeds is difficult because of their hard seed coat - a trait which explains its sparse distribution. It is, therefore important to develop a protocol for *in vitro* propagation to save this medicinally important taxon from further depletion of its population, at the same time to meet up the demand of the traditional medicine industry. *In vitro* propagation has proven as a potential technology for mass scale production of medicinal plant species [4-10]. So far, our knowledge goes; no report has been published on *in vitro* propagation of *Abrus laevigatus* E. May. The present investigation reports the *in vitro* propagation technique that can be used as a potential tool for large-scale production of this medicinal plant.

Materials and Methods

Mature seeds of *Abrus laevigatus* E. May. were collected from botanical garden and sown in earthen pots for raising seedlings. Juvenile twigs from one-year-old mature plant were used as source of explants. Juvenile twigs were surface sterilized with $HgCl_2$ solution (0.1% w/v) for four to six min. Thereafter, explants were rinsed with sterile distilled water. Nodal segments of twigs made into pieces (0.5 cm) were cultured on 8% (w/v) agar solidified MS supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kin) at different concentrations and combinations. The pH of the medium was adjusted to 5.8 before autoclaving.

Regenerated shoots were sub cultured at an interval of 14 - 20 days. Once the shoot buds developed, they were further cultured for elongation in the same medium. Elongated shoot buds were rooted on half strength MS fortified with different concentrations of auxins (NAA and IBA) alone. All cultures were incubated at 25 ± 2°C under 16/8 hr photoperiod. After 12 weeks, plantlets with roots were planted successfully in pot soil through gradual acclimatization.

Results and Discussion

Within seven to 15 days of culture, callus formed at the cut surfaces of nodal explants, when grown on MS supplemented with 2, 5 and 8 mg/1 BAP and Kin either alone or in combination with 0.1 - 1.0 mg/1 NAA, IAA and IBA (Table 1). Maximum (80%) callus formation took place on MS fortified with 5.0 mg/1 BAP with 0.5 mg/1 NAA after two successive subcultures. In this combination light yellowish green and nodular callus developed. Callus was also induced in BAP and Kin supplemented medium. However, BAP was found to be more effective than Kin for callus induction (Table 1).

Plate.1 Callus from Stem explant

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cytokinin-enriched medium in species exhibiting strong apical dominance.

The highest numbers of shoots (6.87 ± 0.26) were developed in MS with 2.0 mg/1 BAP + 0.5 mg/1 Kin + 0.5 mg/1 NAA (Table 1).

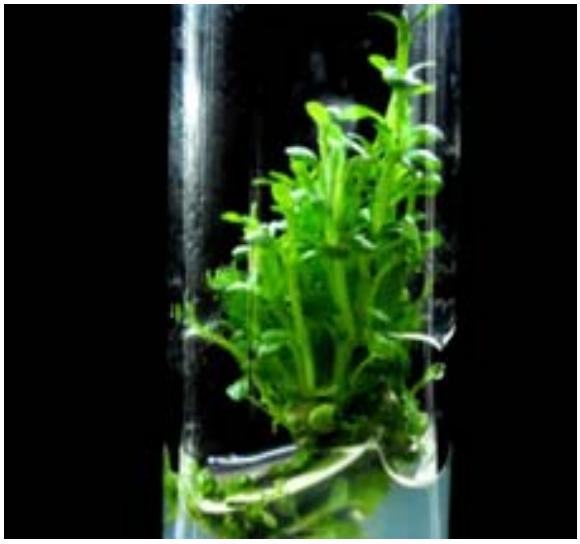

Plate.2 Multiple shoots formation from callus

There were significant differences in regeneration frequencies, number of shoots/culture and length of shoots/culture. As stated by Martin [6] the high morphogenic efficiency of node segments derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis.

Shoot buds developed from callus culture elongated. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days. Shoot regeneration *via* callus phase was the simplest way to induce somaclonal variation and thus can pave the way for improvement of the species [13]. Such indirect organogenesis was reported in many medicinal plant species including *Asparagus cooperi* [14], *Plumbago zeylanica* [15], *Holostema adakodien* [7], *Rauvolfia tetraphylla* [8], *Gloriosa superba* [16], *Phellodendron amurense* [9] etc. When shoot buds started elongation and leaves developed in the nodal zone, quick abscission of leaves took place. It remained a problem for keeping the shoot buds healthy. Similar results were previously reported in other medicinal plants species [17, 18]. Martin [7] considered that necrosis and abscission of leaves and shoots were due to the accumulation of ethylene, they used $AgNO_3$ or $CoCl_2$ for resolving this problem. However, in the present study abscission could not be resisted by the use of either $AgNO_3$ or $CoCl_2$.

Table 1. Effect of different concentrations and combinations of growth regulators on the adventitious shoot regeneration

Growth regulators(mg/l)					Mean No. of shoots*/ callus	Length (cm) of shoots
BAP	Kin	NAA	IAA	IBA		
Hormone free Medium					-	-
5.0	0.2				-	-
5.0	0.6				-	-
5.0	1.0				-	-
5.0	0.2	0.1			2.43 ± 0.35	0.90 ± 0.10
5.0	0.6	0.5			6.87 ± 0.26	1.93 ± 0.17
5.0	1.0	1.0			3.33 ± 0.18	1.28 ± 0.13
5.0	0.2		0.1		1.10 ± 0.10	0.67 ± 0.15
5.0	0.6		0.5		2.00 ± 0.55	0.82 ± 0.31
5.0	1.0		1.0		0.80 ± .1C*	0.18 ± 0.07
5.0	0.2			0.1	.43 ± 1.2C**	0.34 ± 0.05
5.0	0.6			0.5	1.15 ± .10C*	0.65 ± 0.16
5.0	1.0			1.0	0.70±.03C**	0.19 ± 0.06

Rooting experiments were carried out in MS supplemented with 0.1-1.5 mg/1 either NAA or IBA. Medium containing 1.0 mg/1 IBA proved to be the most effective for rooting of micro-shoots than that of any concentration of NAA (Table 2). In this medium the highest percent (70) and number (3.23 ± 0.27) of roots formed at the cut end of micro shoots within two weeks of culture.

The effectiveness of IBA in rooting has been reported in many medicinal plants [7, 19]. shoots with strong and stout root system were acclimatized outside growth chamber for a week. These juvenile plants were transferred to earthen pots, which were placed in natural environment containing mixture of soil and manure (1:1). 75% plants survived in nature. Mean values within columns followed by the same letter are not significantly different at 5% level.

Table 2. Effect of half-strength MS with different concentrations of auxins on root proliferation in *in vitro* grown shoots from callus cultures of *Abrus laevigatus* E. May

Growth regulators (mg/l)		% of shoots producing roots	No. of roots*/ shoot	Average length (cm) of roots*
Control		-	-	-
NAA	IBA			
0.1	-	-	-	-
0.5	-	27	0.98 ± 0.25	0.45 ± 0.11
1.0	-	40	1.43±0.27	0.72 ± 0.l0
1.5	-	-	-	-
-	0.1	-	-	-
-	0.5	50	1.17±0.12	1.13±0.06
-	1.0	70	1.93 + 0.23	1.33±0.10
-	1.5	47	1.67±0.35	0.91 ± 0.l0

*Mean values within columns followed by the same letter are not significantly different at 5% level.

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