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BRIEF COMMUNICATION

An efficient *in vitro* propagation of *Aristolochia indica*

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Abstract

A rapid and efficient *in vitro* plant regeneration method was developed for *Aristolochia indica*. Multiple shoot formation was induced from shoot tip and nodal explants on Murashige and Skoog (MS) medium with 1 - 6 mg dm^{-3} 2-isopentenyl-adenine (2-iP) or 1 - 4 mg dm^{-3} 6-benzyladenine (BA). Maximum number of shoots were induced with 5 mg dm^{-3} 2-iP alone (about 12 - 14 shoots). Shoot differentiation occurred directly from the leaf bases as well as from the internodes when cultured on 1 - 4 mg dm^{-3} BA and 0.8 - 2 mg dm^{-3} α-naphthaleneacetic acid (NAA) containing medium. Regeneration from the callus occurred when the calli initiated on MS medium containing 0.6 - 4 mg dm^{-3} NAA in combination with 0.8 - 3 mg dm^{-3} BA were transferred to 1 - 6 mg dm^{-3} BA alone containing medium. Elongated shoots were separated and rooted in MS medium containing 1 mg dm^{-3} indole-3-butyric acid. These were then transferred to soil after gradual acclimatization.

Additional key words: auxins, cytokinins, growth regulators, medicinal plant, plant regeneration, tissue culture.

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Aristolochia indica. L., belonging to the family *Aristolochiaceae* is well exploited in the traditional medicines. The plant is a shrubby or herbaceous twiner with woody rootstock. Over-exploitation of the natural population for medicinal use and the lack of systematic efforts at cultivation call the need for *in vitro* culture. Hence, the present study was undertaken in an effort to meet the increasing demands of *A. indica* using *in vitro* propagation techniques.

The plants maintained in the greenhouse of Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram served as the source of explants. Various explants like shoot tips, nodal segments, tender leaves and internodal segments were used to analyse the regeneration potential of the plant. The explants were treated with *Teepol* (a liquid detergent) for 20 min followed by washing under running tap water for half an hour. The explants were then sterilized with 0.1 % mercuric chloride for 6 min and washed several times with sterile distilled water. Leaf pieces, shoot tips, nodal and inter nodal segments were excised and inoculated onto different culture media. The culture media consisted of Murashige and Skoog (1962; MS) basal medium solidified with 0.8 % agar, with 3 % sucrose and 6-benzyladenine (BA), 2-isopentenyl-adenine (2-iP), kinetin (KIN), α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at various concentrations (0.8 to 6 mg dm^{-3}) and combinations. The pH of the media was adjusted to 5.8 using 1 M NaOH or HCl, autoclaved at 121 °C for 20 min and poured into 25 × 150 mm culture tubes. Inoculated tubes were sealed with cotton plugs and incubated under irradiance of 70 μmol m^{-2} s^{-1} for 16-h photoperiod and temperature of 25 ± 2 °C. All experiments were conducted in five replicates.

Shoot tips and nodal segments showed elongation without multiplication when NAA or KIN alone was used in MS medium. Shoot multiplication was obtained when cytokinins like 2-iP or BA were used. Of the various cytokinins used, 5 mg dm^{-3} 2-iP yielded the maximum number of shoots (14) from a single shoot tip or node within 21 d (Table 1). The positive role of 2-iP in multiple shoot formation has been also observed in *Ixora coccinea* (Lakshmanan *et al.* 1997). BA (1 - 5 mg dm^{-3}) alone also induced multiple shoots (7 - 8) in *Aristolochia.* Manjula *et al*. (1997) reported shoot multiplication of *Aritolochia* with 4 mg dm^{-3} BA, but another cytokinin, thidiazuron (1 - 6 mg dm^{-3}) did not promote multiplication but a characteristic swelling was noted at the nodal region.

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Abbreviations: AC - activated charcoal; BA - 6-benzyladenine; 2,4-D - 2,4-dichlorophenoxyacetic acid; IBA - indole-3- butyric acid; 2-iP - 2-isopentenyladenine; KIN - kinetin; MS medium - Murashige and Skoog (1962) medium; NAA - α-naphthaleneacetic acid.

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Development of shoot buds were observed directly from leaf discs as well as from internodal segments when MS medium with 3 mg dm^{-3} BA and 1 mg dm^{-3} NAA were used (Table 1). Meager callusing was also noted along with the shoot buds. Similar results have been reported in *Adenophora triphylla* (Chen *et al*. 2001) where BA with NAA induced direct shoot buds from leaf and internode explants.

Callus was developed from leaf disc and internodal segments when 2,4-D + NAA or IAA alone or in combination with BA and 2-iP were used. 3 mg dm^{-3} BA along with 1 mg dm^{-3} NAA induced morphogenetic

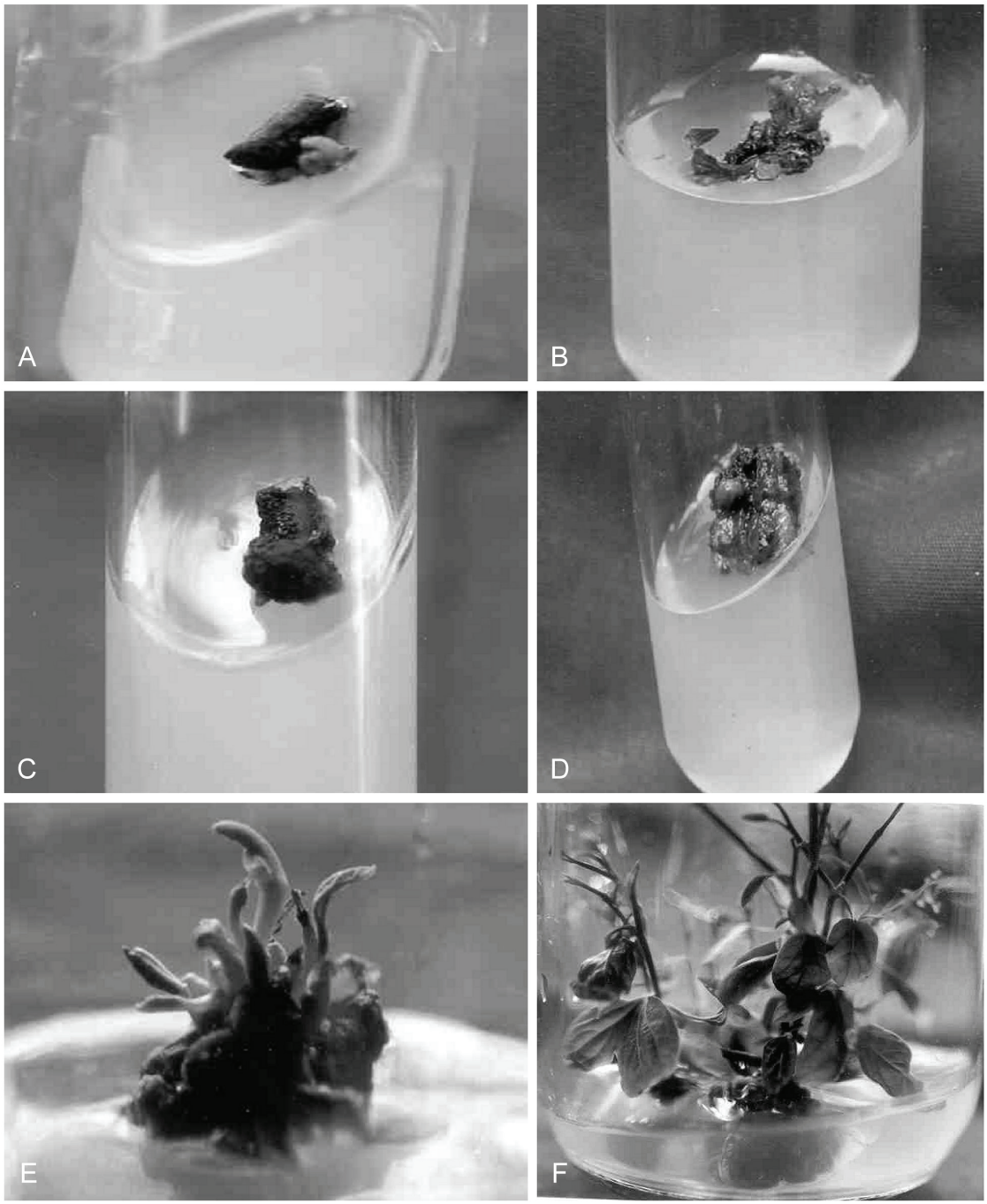

Fig. 1. Morphogenetic responses of *Aristolochia indica* L.: *A, B* - direct regeneration; *C, D, E* - callus induction and regeneration; *F* - multiple shoot formation.

Table 1. Regeneration responses of *Aristolochia indica* cultivated on MS medium with different combinations and concentrations [mg dm^{-3}] of growth regulators for 21 d. Means ± SE, n = 14.

Mode of regeneration	2-iP	BA	NAA	Number of shoots [explant^{-1}]	Survival [%]
Shoot multiplication	1	-	-	2 ± 0.01	90
	2	-	-	4 ± 0.10	88
	3	-	-	5 ± 0.15	92
	4	-	-	6 ± 0.20	90
	5	-	-	14 ± 0.40	91
	6	-	-	5 ± 0.10	90
Direct regeneration	-	3	0.5	-	-
	-	3	1.0	3 ± 0.05	86
	-	3	1.5	3 ± 0.10	85
	-	1	1.0	-	-
	-	2	1.0	-	-
Callus regeneration	-	1	-	-	-
	-	2	-	6 ± 0.01	89
	-	3	-	8 ± 0.15	90
	-	4	-	12 ± 0.30	88
	-	5	-	6 ± 0.10	89

callus. This on further subculture in media containing 4 mg dm^{-3} BA, developed shoots within 3 weeks (Table 1).

This is in agreement with the results in *Curculigo orchioides* where BA could induce shoot buds from callus (Dhenuka *et al.* 1999). Leaching of polyphenols into the culture media was one of the major problems, which influenced the further growth of shoots. Addition of 30 mg dm^{-3} activated charcoal, as well as increased sub-culturing frequently to fresh media reduced the leaching. The regenerated individual shoots were rooted in media containing 1 mg dm^{-3} indole-3-butyric acid (IBA). The promotion of rooting by IBA has been reported in many plant species (Saritha *et al.* 2002, Soniya and Das 2002, Zhang *et al.* 2003). The rooted plantlets were then transferred to greenhouse where they showed about 85 % survival.

The different methods of micro propagation listed can be used for the conservation and exploitation of the plant for various medicinal purposes and for the genetic improvement of the plant as such.

References

Chen, C., Chen, S., Sagarez, A.P., Tsay, H.: Micropropagation of *Adenophora triphylla*.- Bot. Bull. Acad. sin. **42**: 1-7, 2001.

Dhenuka, S., Balakrishna, P., Anad, A.: Indirect organogenesis from leaf explant of the medically important plant *Curculigo orchioides* Gaertin. - J. Plant Biochem. Biotechnol. **8**: 113-115, 1999.

Lakshmanan, P., Lee, C.L., Goh, C.J.: An efficient *in vitro* method for mass propagation of woody ornamental *Ixora coccinia* L. - Plant Cell Rep. **16**: 572-577, 1997.

Manjula, S., Anitha, T., Daniel, B., Nair, G.M.: *In vitro* plantlet regeneration of *Aristolochia indica* through axillary shoot multiplication and organogenesis. - Plant Cell Tissue Organ.Cult. **51**: 145-148, 1997.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-479, 1962.

Saritha, K.V., Prakash, E., Ramamurthy, N., Naidu, C.V.: Micropropagation of *Spilanthes acmella* Murr. - Biol. Plant. **45**: 581-584, 2002.

Soniya, E.V., Das, M.R.: *In vitro* micropropagation of *Piper longum* an important medicinal plant. - Plant Cell Tissue Organ Cult. **70**: 325-327, 2002.

Zhang, L., Kai, G., Xu, T., Pi, Y., Zhang, H., Sun, X., Tang, K. : Efficient regeneration of tetraploid *Isatis indigotica* plants *via* adventitious organogenesis from hypocotyl explants. - Biol. Plant. **48**: 121-124, 2004.