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RESEARCH ARTICLE

MICROPROPAGATION STUDIES OF A MEDICINAL PLANT *Aristalochia indica*

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ABSTRACT

The development of *in vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines. *Aristalochia indica* is used in traditional remedy. In the present study, attempts have been made to develop a simple, reliable and reproducible protocol for micropropagation from different explants of *Aristalochia indica*. Shoot tip and nodal segments showed elongation without multiplication when either NAA or KIN was used in MS medium. Shoot multiplication was obtained when cytokinins like BAP was used. BAP alone also induced multiple shoots. The regenerated individual shoots were rooted in MS medium containing 1 mg dm^{-3} indole-3-butyric acid (IBA). Regenerated plants grew well when transferred to soil.

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INTRODUCTION

Plants have been an important source of medicine for thousands of years apart from being source of food, fibre, wood etc. Even today, the World Health Organisation estimates that up to 80 per cent of people still rely mainly on traditional remedies such as usage of herbs for their medicines. Plants are also one of the important sources of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modelled based on plant substances. The most popular analgesic, aspirin was originally derived from species of *Salix* and *Spiraea*.

Some of the most valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plant sources (Katzung, 1995). The development of *in vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines. Moreover, steady supply of raw material can be maintained. Micropropagation of various plant species, including many medicinal plants has been reported (Withers and Anderson, 1986). Plant regeneration via somatic embryogenesis from single cells, that can be induced to produce an embryo and then a complete plant, has been demonstrated in many medicinal plant species. Somatic embryos, which are bipolar structures, arise from individual cells and have no vascular connection with the maternal tissue of the explants (Haccius, 1978). Embryos

may develop directly from somatic cells (direct embryogenesis) or development of recognizable embryogenic structures is preceded by numerous, organized, non-embryogenic mitotic cycles (indirect embryogenesis). Somatic embryogenesis has a great potential for clonal multiplication. Under controlled environmental conditions, somatic embryos germinate readily, similar to their zygotic counterpart.

Most valuable phytochemicals are products of plant secondary metabolism. Their complex structural features are difficult to synthesize. Plant cell cultures are an attractive alternative source to whole plant for the production of high-value secondary metabolites. The accumulation of secondary products in plant cell cultures depends on the composition of the culture medium and on environmental conditions (Gastaldo *et al*., 1994).

Aristalochia indica grows in wild throughout the low hills and plains of India from Nepal to west Bengal and south India. It belongs to the family Aristalochiaceae under the order Piperales.

Vernacular names

Tamil	:	Eesuvaramooli,
Hindi	:	Isvaramul,
English	:	Indian birthwort,
Kanadam	:	Isvaberusa,
Malayalam	:	Garudakkoti,
Sanskrit	:	Garalika,
Telugu	:	Esvaraveru

It is a perennial shrubby glabrous twiner with a long woody root stock. Leaves are simple, alternate, short and petiolelate, entire with some what undulated margins. Flowers greenish white or light purplish in axillary cymes or fascicles with swollen or inflated basal part, contracted middle part and narrowly, funnel shaped distal part, seeds flat and winged. It is known from the literature that parts of this plant such as roots, leaves, fruits are medically important. Roots are bitter acid, astringent, thermogenic, purgative, anodyne, depurative, digestive, anthelmintic, stomachic, cardiotonic, anti-inflammatory, diuretic, sudorifi and tonic. It contains aristolochin ($C_{17}H_{19}O_3N$). They are useful in ulcers, inflammations, leprosy, leucoderma, skin disease, intestinal worms, cardiac debility, abdominal disorders in children and all types of poisonous bites and sting. (Warrier *et al.,*1994). Leaves are used to treat Cholera, bowel complaints. A paste made out of the leaves is good for inflammations. In the present study, attempts have been made to develop a simple, reliable and reproducible protocol for micropropagation from different explants of *Aristalochia indica*.

MATERIALS AND METHODS

The plants are collected from the medicinal plants garden, at Naloor, Madurai district, Tamilnadu, and served as the source of explants. Various explants like shoot tips (1.0-1.5cm), nodal segments (3.0-4.0cm), tender leaves (2nd and 3rd leaves from the apex) and internodal segments (2.0-3.0cm) were used to study the regeneration potential of the plant. Aseptic explants such as shoot tips (0.3-0.5 cm), single nodes (1.0-2.0 cm), leaf discs (1cm2) and internodal segments (1.0-1.5 cm) were dissected out and inoculated aseptically on basal medium consisting of Murashige and Skoog's (1962) salts and vitamins, 3% sucrose, pH-5.7-5.8 and 0.8% agar (Quixcel, India). Basal medium was supplemented with various concentrations of NAA and BA for auxiliary shoot multiplication, 2,4-D /NAA and BA/kinetin for callus induction and adventitious bud regeneration and BA/kinetin alone for direct regeneration. The medium was buffered to pH 5.8. All cultures were maintained in a 12-h photoperiod at 24 ± 1^0 C and a photon flux density of 70 μ mol $m^{-2}s^{-1}$. The calli were transferred either to the same initiation medium or two to three subcultures on the MS basal medium, the calli were placed on MS basal medium containing different concentration and combinations of 2, 4-D, IAA, IBA, BA and KN.

RESULTS AND DISCUSSION

Pharmaceutical companies depend largely upon materials procured from naturally occurring stands that are being rapidly depleted. Plant tissue culture is an alternative method of commercial propagation. The levels and kinds of plant growth regulators included in the culture medium largely determine the success of tissue culture work. Root

differentiation from unorganised callus tissue are and a ratio of ~ 4 favours the development of

Table 1. *In vitro* callusing response of different explants of *Aristalochia indica* under different hormone combinations.

Explants	*Hormones*			Response
	2,4- D (mg/l)	**6-BA (mg/l)**	**NAA (mg/l)**	
Leaf disc	2.6	---	---	Leaf curl formation
	2.2	---	---	Leaf curl formation
	2.0	---	---	Good callusing
	1.8	---	---	No callusing
Nodal region	2.2	---	---	Callus formed at the base of the nodal segment
	2.1	---	---	Callus formed at the base of the nodal segment
	2.0	---	---	Callus formed at the base of the nodal segment
	1.8	---	---	Poor Callus formation
	1.6	---	---	Poor Callus formation
	1.4	---	---	No response
	0.5	---	---	No response
	1.0	---	---	No response
Shoot tip	1.5	---	---	No response
	2.0	---	---	No response
	2.5	---	---	No response

Table 2. *In vitro* plantlet regeneration of shoot tip, nodes, leaf disc derived callus of *Aristalochia indica* as influenced by different hormones

Explants	*Hormones*			Response
	2,4- D (mg/l)	**6-BA (mg/l)**	**NAA (mg/l)**	
Shoot tip	---	2.2	0.2	Slow shoot growth
	---	2.4	0.4	Slow shoot growth
	---	2.6	0.6	Rapid Shoot growth
	---	2.8	0.4	Rapid Shoot growth
	---	3.0	0.1	Rapid Shoot growth
Nodes	---	3.0	1.0	Multiple shoot growth
	---	2.8	0.8	shoot growth
	---	2.6	0.6	Slow shoot growth
	---	2.4	0.4	No response
	---	2.2	0.2	No response
	---	2.0	0.1	No response
	---	2.0	0.1	No response
	---	2.4	0.4	Shoot bud formation
Callus	---	2.6	0.6	Shoot bud formation
	---	2.8	0.8	Rapid proliferation of multiple shoots
	---	3.0	1.0	Rapid proliferation of multiple shoots

closely regulated by the relative concentrations of auxins and cytokinin in the medium (Ammirato, 1983; Rout and Das, 1997). Auxin:cytokinin ratio of ~10 yield rapid growth of undifferentiated shoots. The gibberellins stimulate growth of organs but generally do not favour organ initiation. Percentage of bud break and shoot multiplication was generally higher in MS medium compared to

the multiplication of *Eucommia colmoider* was

Plate 1A: Callus initiation from leaf explants of *Aristalocia indica*

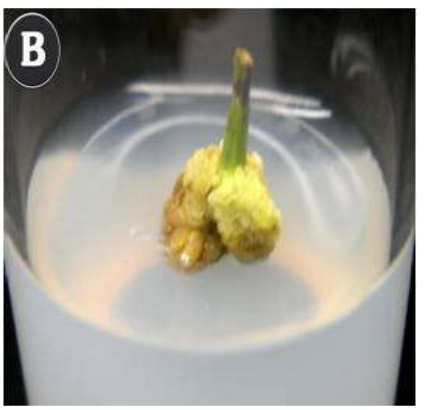

Plate 1B: Callus induction from base of the nodal explant

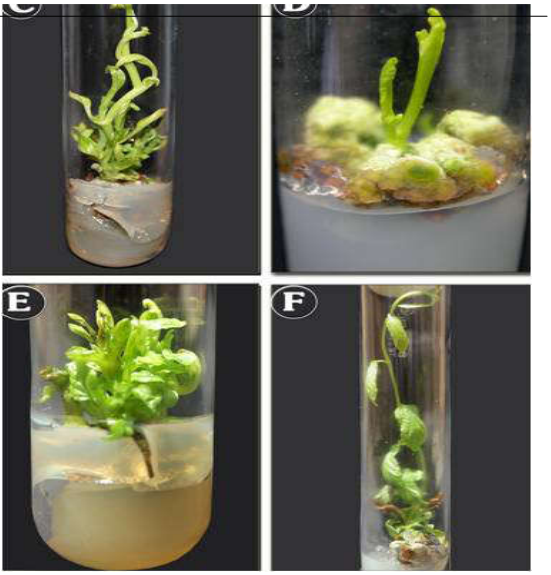

Plate 1 C, D, E & F: Shoot proliferation from shoot tip

Plate 2. Regenerated plant-lets

higher in MS basal medium. Different explants from *Aristalochia indica* like shoot tips, nodal segments and leaf disc were used to initiate cultures. Shoot tips and nodal segments showed elongation without multiplication when either NAA or KIN was used in MS medium. Shoot multiplication was obtained when cytokinins like BAP was used (Plate1 – C to F). BAP (1 - 5 mg dm^{-3}) alone also induced multiple shoots (7 - 8) in *Aristolochia.* Manjula *et al*. (1997) reported shoot multiplication of *Aristolochia* 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.

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 2). Meagre callusing was also noted along with the shoot buds. Similar results have been reported in *Adenophora triphylla* where BAP with NAA induced direct shoot buds from leaf and internode explants. Callus was developed from leaf disc and internodal segments when cultured on MS medium supplemented with 2,4-D (Table 1; Plate1 - A & B). This on further subculture in media containing 4 mg dm^{-3} BAP, developed shoots within 3 weeks (Plate 1 C & D; Table 2). This is in agreement with the results in *Curculigo orchioides* where BA could induce shoot buds from callus (Dhenuka *et*

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 critical step in somatic embryogenesis is the ability to form embryos that will develop into complete plantlets (P late- 1 F). On transfer to MS basal medium these somatic embryos formed complete plantlets with well-developed shoot and root systems. Regenerated plants grew well under both growth chamber and mist chamber conditions (Plate 2).

Summary

Aristalochia indica is used in traditional remedy. In the present study, attempts have been made to develop a simple, reliable and reproducible protocol for micropropagation from different explants of *Aristalochia indica*. Fast growing calli derived from leaf disc explants were found to be regenerable. Multiple shoots were induced from such calli. Complete plantlets regenerated and grew well when transferred to soil.

REFERENCES

Ammirato, P.V. 1983. Somatic embryogenesis and plantlet development in suspension cultures of the medicinal yam, *Dioscorea floribunda*. *Am J Bot*., 65: 89–95.

Chen, L.J., Hu T.W. and Huang, L.C., 1995. A protocol toward multiplication of the medicinal tree, *Eucommia ulmoides* Oliver. *In vitro Cell Dev Biol Plant*., 31:193–8.

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

Gastaldo, P., Carli, S. and Profumo, P. 1994. Somatic embryogenesis from stem explants of *Aesculus hippocastanum. Plant Cell Tiss. Organ Cult*., 39:97 – 99.

Katzung, B.G., 1995. *Basic and Clinical Pharmacology,* ed 6, London: Prentice Hall International (UK) Limited.

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

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tabcco tissue cultures. *Physiol. Plant*., 15:473–497.

Rout, G.R. and Das, P. 1997; *In vitro* organogenesis in ginger (*Zingiber officinale Rosc*.). *J Herbs Spices Medicinal Plants*. 4(4):41–51.

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

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

Warrier, P.K., Nambiar V.P.K. and Raman Kutty., 1994. Indian medicinal plants: A compendium of 500 species. Vol. I Orient Longman, India. Withers LA, Anderson PG. *Plant Tissue*

Culture and its Agricultural Applications. London: Butterworths.

Withers, L.A. and Anderson, P.G. 1986. *Plant Tissue Culture and its Agricultural Applications*. London: Butterworths

Zhang, L., Kai, G., Xu, T., Pi, Y., Zhang, H., Sun, X. and Tang, K., 2003. Efficient regeneration of tetraploid *Isatis indigotica* plants *via* adventitious organogenesis from hypocotyls explants. - *Biol. Plant*., 48: 121-124.