



RESEARCH ARTICLE

EFFECTIVE IN VITRO PROPAGATION OF MEDICINALLY VALUABLE PLANT- ARISTOLOCHIA
BRACTEOLATA, LAM

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ARTICLE INFO

Article History:

Received 04th February, 2016
Received in revised form
26th March, 2016
Accepted 22nd April, 2016
Published online 10th May, 2016

Key words:

Shoot formation,
Herbal drugs,
Micropropagation.

ABSTRACT

Innumerable plants were used in Indian Systems of medicine like Siddha, Ayurvedha and Unani. With increasing demand for herbal drugs, the natural populations of *Aristolochia bracteolata* are threatened with overexploitation. The present study was aimed to develop an innovative micropropagation protocol in *Aristolochia bracteolata*. Nodal and shoot tip explants were used as a starting material for micropropagation. Explants were surface sterilized and aseptically cultured on MS medium supplemented with different plant growth regulators. Best results of axillary bud formation were observed on MS medium containing 2.0 mg/l BAP. The multiple shoot formation from nodal segments were highest in MS medium supplemented with 1mg/l BAP + 2.0 mg/l kinetin. For rooting various concentrations of IBA were used. The rooted plantlets were finally hardened.

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Citation: Lavanya, A., Ambikapathy, V. and Panneerselvam, A. 2016. "Effective in vitro propagation of medicinally valuable plant-Aristolochia Bracteolata, Lam", *International Journal of Current Research*, 8, (05), 30242-30244.

INTRODUCTION

Aristolochia bracteolata. Lam is a member of Aristolochiaceae family commonly known as worm killer. It is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers, succulent herb. In India and the tropics it grows naturally in wet soil, shallow water and marshes. The decoction of leaves of *Aristolochia bracteolata* is used medicinally for treating skin diseases and rheumatism (Anjaria *et al.*, 2002). It was earlier used as a bitter, medicine to treat painful joints and as antihelmenthic. The plant is valued as a nerve and cardio-tonic, broncho-vasodilatory, neuroprotective and hepatoprotective properties. Besides the antioxidant properties, it has been used for the treatment of round worm infection (Nadkarni and Nadkarni, 1954). It is also used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi *et al.*, 2003). *A.bracteolata* possess aristolochic acid which includes antibacterial, anticancer, antiimplantation, antiseptic, antipyretic, anti-inflammatory, antineoplastic,

immunostimulant, antivenom property (URL: <http://www.ars-grin.gov/cgi-bin/duke/chem-activities.pl>). use to their medicinal importance, many scientific studies have been carried out on the phytochemical pharmacological values of *A. bracteolata*. Greater demand for plants especially for food and medicine is one of the causes of their rapid depletion from primary habitats (Boro *et al.*, 1998). Increasing human and life stock populations leads to the harvest of medicinal plants on a mass scale from their natural habitats leading to the depletion of plant resources. In the past two decade plant tissue culture technology has been successfully used in commercial production with there is an increasing demand for their valuable medicinal herb in pharmaceutical markets. The present investigation has been focused to produce or to micropropagate a large number of plantlets and high quality plants.

MATERIALS AND METHODS

Collection of plant material

Twigs of *Aristolochia bracteolata* plant were collected from healthy plants growing in herbal garden, A.V.V.M Sri

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Pushpam College (Autonomous), Poondi was taken for further *in vitro* propagation.



Systematic position:

Class : Dicotyledons
Order : Piperales
Family : Aristolochiaceae
Genus : *Aristolochia*
Species : *bracteolata* Lam
Tamil name : Aaduthinnapalai
Common name: worm killer

Surface sterilization of plants

Young shoots of *Aristolochia bracteolata* were harvested and washed with running tap water to remove superficial dust particle adhering to the surface. The nodal and shoot tip explants were cut into small pieces (about 1.5 cm long) and them treated with tween-20 (2-3 drops/100 ml water) treatment for 15 min which was again followed by thorough rinsing with distilled water (2-3 times) then sterilized with 0.1% mercuric chloride for 3, 4 & 5 minutes under Laminar air flow chamber. After rinsing 3-4 times with sterile distilled water, nodes and shoot tip were cut into smaller segments (0.5 to 1.0 cm) and used as explants.

Establishment of *in vitro* culture

The explants were placed vertically (nodal and shoot tip segments) on solid basal Murashige and Skoog (1962) medium supplemented with 3% sucrose, 0.8 of (w/v) agar and different concentration and combinations of plant growth hormones like BAP (1-3.0 mg/l) Kinetin (2-3 mg/l). IAA or NAA (0.5-1 mg/l). The pH of the medium was adjusted 5.6 to 5.8 with 0.1NaOH before autoclaving at 121°C for 20 minutes. The culture was incubated at a constant temperature of 25±2°C with 16 hours photoperiod (2000 lux) and 8 hours darkness. The observation carried out visually and made weekly and the data were recorded. Nodal segments from the proliferated shoots were sub cultured again for further multiple shoot formation after 2 weeks. The multiple shoots were cut and individuals shoots were placed in MS medium containing IBA (0.5 mg/l) for root induction.

RESULTS

Treatment of nodal and shoot tip explants of *A.bracteolata* with 0.1% HgCl₂ for 3 minutes showed the maximum result in establishment of 100% contamination free viable culture. Auxiliary shoot formation was observed in all the tried media combination. The maximum number of shoot induction were observed in the MS Media supplemented with 2.0 mg/l of BAP which has been the most efficient supplementation as shown in Table : 1. On this medium an average of 5.00 ± 0.45 shoots with mean shoot length were obtained. On kinetin medium, the induction of shoot declined and also made similar observations on medium containing cytokinin with auxins. On BAP supplemented medium, results were very high to those recorded on media supplemented with other plant hormones. On increasing the concentration of BAP to 3 mg/l, a decline in the rate of multiplication of shoot was observed. The shoots were shorter in length. In overall observations of the present study when compared to shoot tip, nodal explants showed maximum response of multiple shoot formation.

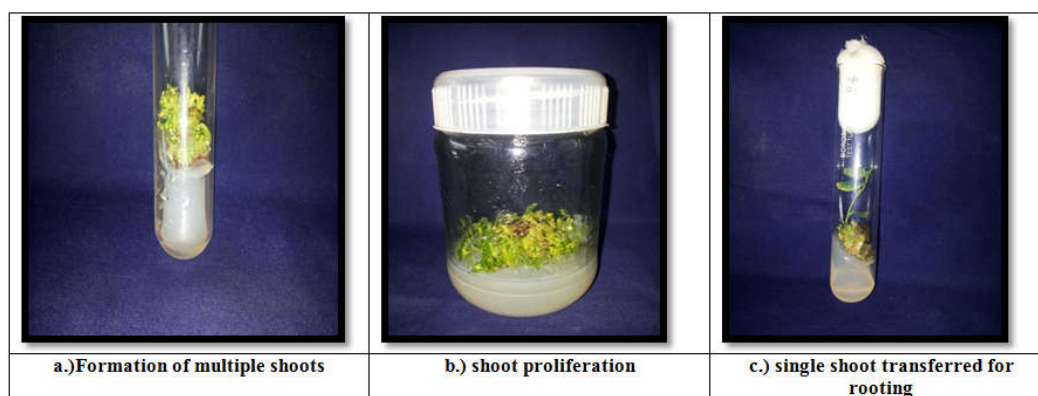


Table 1. Effect of plant growth hormone on shoot multiplication

S.No	MS+ PGR(mg/ml)			Observation after 4 weeks	
	BAP	Kinetin	IAA	Mean shoot number	Mean shoot length(cm)
Control	0.0	0.0	0.0	0.00±0.00	0.00±0.00
1	1.0	0.0	0.0	2.00±0.32	3.00±0.25
2	1.5	1.0	0.0	2.50±0.60	3.56±0.29
3	2.0	0.0	0.0	5.00±0.45	4.20±0.56
4	0.0	1.5	0.0	3.00±0.25	3.42±0.30
5	1.0	0.0	0.5	2.05±0.16	2.50± 0.25
6	3.0	0.0	0.0	2.00±0.35	2.25±0.28

Sub culturing

Periodic sub culturing of shoots were done for long term maintenance of shoot culture. For this MS media supplemented with BAP 2.0 mg/l was tried. The shoots developed were healthy and without any callusing.

Rooting

Rooting was done by using the auxin, IBA. Root induction was observed within 15-20 days. Maximum rooting was recorded in medium containing 1.0 mg/l IBA.

Hardening

In vitro rooted shoots were maintained in rooting medium for over 5-6 weeks. Thereafter they were shifted to pots containing soil: sand: farmyard manure (1:1:1) and maintained in greenhouse for one month and with watering. The healthy plants were then subsequently transferred to field conditions.

DISCUSSION

Effectiveness of HgCl₂ in mitigating infection on *A. bracteolata* explants has also been reported earlier (Shrivastava *et al.*, 1999). Surface disinfected nodal explants showed best results of axillary shoot induction on MS medium supplemented with 1.0 mg/l BAP (Chandra *et al.*, 2002). In the present study 0.1% HgCl₂ proved to be efficient in controlling contamination on nodal and shoot tip explants. In the present investigation, 2.0 mg/l BAP proved to be most efficient supplementation. The observation was high on 1.0 mg/l BAP by Caesar *et al.*, 2009; Vijayakumar *et al.*, 2010; Tanveer *et al.*, 2010. Once the healthy shoots were generated, various combinations of auxins were tested for rooting. The role of auxin is well established for enhancing rooting (Tiwari *et al.*, 2000). The protocol which is made by us is very cost effective and does not need any intermediary hardening technique.

Conclusion

The present investigation produced an efficient protocol for large scale multiplication of *Aristolochia bracteolata* using nodal and shoot tip segments. Thus the study can be used to make this plant available throughout the year for traditional healers, pharmaceutical usages and commercial cultivation and also for the production of secondary metabolites.

Acknowledgement

The authors are thankful to the Secretary and Correspondent, Principal, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur for providing laboratory facilities and Department of Science and Technology for granting financial assistance to INSPIRE FELLOW for research work.

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