ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 5 (3): 287-291, 2006
ISSN 1682-296X
© 2006 Asian Network for Scientific Information

Callus Induction and Organogenesis from Explants of *Aconitum heterophyllum*- Medicinal Plant

Neelofar Jabeen, A.S. Shawl, [1]G.H. Dar,
Arif Jan and Phalisteen Sultan
Division of Biotechnology, Regional Research Laboratory (CSIR),
Sanatnagar Srinagar-190005
[1]Depatment of Botany, Center of Plant Taxonomy,
University of Kashmir-190006

Abstract: A protocol has been developed for *in vitro* shoot proliferation from callus cultures of *Aconitum heterophyllum* Wall. Callus initiation occurs from nodal segments on MS media fortified with NAA (0.5 mg L^{-1}) and BAP (0.25 mg L^{-1}). Callus was transferred on Msmediasupplemented with BAP (0.25 mg L^{-1}) for shoot proliferation. The best response for shoot proliferation was obtained on MS media+ NAA (0.25 mg L^{-1}) + BAP (0.5 mg L^{-1}). The well-developed micro shoots were transferred to root induction media containing MS basalmedia + IAA (1.0 mg L^{-1}). The rooted plantlets were finally transferred to green house for hardening and field transfer.

Key words: *Aconitum heterophyllum, in vitro* propagation, nodal segments, explants

INTRODUCTION

Aconitum heterophyllum Wall. commonly known as 'Atis' or 'Patis' belongs to the family Ranunculaceae and is reputed for its medicinal and pharmaceutical values since long. It is a perennial herb, distributed over temperate parts of western Himalaya, extending from Kashmir to Kumaon. Its chief habitat is in the alpine and sub-alpine areas at elevations of 3000-3500 m, like Gulmarg, Kilanmarg, Sonamarg (Uniyal *et al*., 2002). The tuberous roots of genus *Aconitum* contain alkaloids: benzoylmesaconine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetisinone and benzolylheteratisine (Zhaohong *et al*., 2005; Pelltier *et al*., 1968) plant contain Alkaloids heteratisine, heterophyllisine, heterophylline, hetrophyllidine, atidine, Isoatisine hetidine, hetsinone and benzoylheteratisine (Zhaohong *et al*., 2005). The roots, which have been used mostly as poison than as drug, are now reported to possess significant antipyretic and analgesic properties and a high therapeutic index The *Aconitum* alkaloids mesaconitine and 3 acetylaconitine have been shown to possess anti inflammatory activity (Ameri *et al*., 1998). The plant is used for the treatment of diseases of the nervous system, digestivesystem, rheumatism and fever. *Aconitum* has biological and pharmacological activities such as anti-fungal, anti-bacterial, insecticidal and Brime shrimp cytotoxic activities (Anwar *et al*., 2003). The plant possesses potent immunostimulant property (Atal *et al*., 1986).The root extract exhibits anti-viral activity against Spinach MosaicVirus (SMV) (Patwardhan *et al*., 1990). It is also used for curing hysteria, throat infection, dyspepsia, abdominal pain and diabetes. In the indigenous system of medicine this plant is considered as valuable febrifuge, nervine tonic, especially in combating debility after malaria and in hemoplegia. In view of the continued popularity of *Aconitum heterophyllum* in indigenous as well as modern system of medicine coupled with unscientific and indiscriminate extraction from wild sources has reduced this high value plant species towards rarity and is already in red list (Dar *et al*., 2001). Organized cultivation of *Aconitum* is therefore necessary to ensure the quality and continuous supply of drug. Moreover, the tubers derived from seeds result in large genetic variability, and are frequently infected with fungal diseases, mainly V*erticelium* sp. (Pirone *et al*., 1978). Seedlings are plagued by their variation in quality and quantity, natural uniformity is not maintained throughout. In the past many programmes have been initiated for the

Corresponding Author: Neelofar Jabeen, Division of Biotechnology, Regional Research Laboratory (CSIR), Sanatnagar Srinagar -190005, India

in situ propagation of this endangered medicinal plant. However they are limited by several factors. The potential for regeneration of medicinal plants out of their natural habitat is poor. The germination of seeds and establishment of seedlings is also poor (Khan and Khanum,1998). The number of propagules produced by the natural methods is limited and insufficient for large-scale planting in the wild or under field conditions. Using *in vitro* and associated biotechnological interventions, the removal of such otherwise problems could be possible.

Tissue culture techniques have been used for endangered plants to generate large numbers of propagules, which can be reintroduced in their native habitat. It can enable the mass propagation of this herb from a minimum of plant material so that large quantities of biomass required for extraction of active constituents can be made available throughout the year, without causing further endangerment of the species. This study on *Aconitum heterophyllum* was taken up with a view to develop techniques for its *in vitro* multiplication.

MATERIALS AND METHODS

Fresh viable seeds of *Aconitum heterophyllum* were procured from the Gene bank of Regional Research Laboratory (CSIR), Sanatnagar, Srinagar, Kashmir in the year 2004-2005. Voucher specimen was deposited in the repository of RRL Srinagar, (Voucher No.RRL/AC/Srinagar-2004). Seeds were washed with detergent and 2-4 drops of Tween-20 (Himedia) under running tap water, followed by final rinsing with double-distilled water. These seeds were soaked in double-distilled water for 2-3 days at 4°C in a refrigerator. Surface sterilization of soaked seeds was achieved by using 0.1% (w/v) mercuric chloride for 3-4 min, followed by three times rinsing with autoclaved double distilled water to remove all traces of sterilant. The sterilized seeds were then kept in the dark at 20±2°C on moist absorbent cotton in the petri plates. The germinated Seedlings were inoculated on MS (Murashige and Skoog, 1962) basal media fortified with 3% sucrose. pH of the media was adjusted at 5.8 by using 0.1N NaOH and 0.1N Hcl before gelling the medium with 0.8% agar-agar type (Himedia). The media was finally dispensed into culture tubes, which were plugged and autoclaved for 20 min. at 15 lb pressure and 121°C temperature. These cultures were maintained at 25±2°C with 55-65% relative humidity and exposed to 16 hours photoperiod provided by cool fluorescent tubes (3000 lux).

RESULTS AND DISCUSSION

Seeds of *Aconitum heterophyllum,* cultured on moist absorbent cotton, resulted in full-fledged seedling formation after 4 weeks of their culture (Fig. 1). Nodal explants were excised aseptically from these seedlings and cultured on different phytohormonal regimes, the effect of which is depicted in Table 1. Compact callus was formed on MS medium supplemented with various concentrations of 2,4-D (0.1- 0.4 mg L^{-1}), with 40% response. With NAA (0.1 mg L^{-1}), single shoot, followed by root formation was obtained from the nodal bud. However, comparatively higher concentrations of NAA (0.6 mg L^{-1}) resulted in non-differentiating callus formation with 70% response.

Various BAP concentrations (0.10-0.50 mg L^{-1}) stimulated direct axillary shoot-initiation, proliferation and elongation. The maximum number of shoots survived and grow best on BAP (0.25 mg L^{-1}) with 80% response (Fig. 2). However, higher concentration of BAP (0.5 and 1.0 mg L^{-1}) reduced the number of axillary shoots per explant. The combined interaction of BAP (0.1 mg L^{-1}) + 2, 4-D (0.1 mg L^{-1}) also resulted in shoot proliferation but with lower percentage of shoot formation. Profuse callus was formed on MS medium supplemented

Fig. 1: Seed germination

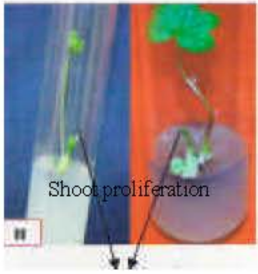

Fig. 2: Shoot proliferation

Table I: Morphogenetic response of nodal explants of *Aconitum heterophyllum* to various phytohormonal regimes

2,4-D (mg L^{-1})	BAP (mg L^{-1})	NAA (mg L^{-1})	Response	% response
MS basal	(Control)	--	--	--
0.1	0.00	0.00	Green callus formed	40
0	0.00	0.10	Single shoot formed from nodal bud. Short adventitious roots were also formed at basal end of shoot.	60
0	0.00	0.60	Friable Callus formation	70
0	0.10	0.00	Shoot proliferation	60
0	0.25	0.00	-do-	80
0	0.50	0.00	-do-	40
0.1	0.10	0.00	-do-	60
0.5	0.25	0.00	White callus formed	80
1	1.00	0.00	Callus	70
0	0.50	0.25	Shoots formed from nodal bud	80
0	1.00	1.00	White nodular callus formation; shoot formation, multiple shoot formation, and multiple shoot regeneration via callus redifferentation.	90

*Data recorded after 4 weeks of culture

Table 2: Rooting response in *Aconitum heterophyllum* after inoculation on MS media supplemented with different concentrations of auxins (observation recorded after 35-45 days).

Media combinations Supplemented with auxin (mg L^{-1})	Mean No. of roots	Rooting in day	Mean length of roots (cm)
Control	0.0	0	0.0
MS	1.8	25-27	2.9
MS+ IAA (0.2 mg L^{-1})	1.3	30-37	1.7
MS+IAA (0.5 mg L^{-1})	1.5	38-40	1.0
MS+IBA (1.0 mg L^{-1})	1.0	40-43	2.0
MS+IBA+IAA (0.5+1.0 mg L^{-1})	2.9	45-48	1.3
MS+IAA (1.0 mg L^{-1})	1.8	26-27	3.2

*The data is based on 5 replicate cultures, while the experiment was repeated thrice mg L^{-1} = milligram per liter

Fig. 4: In direct shoot proliferation

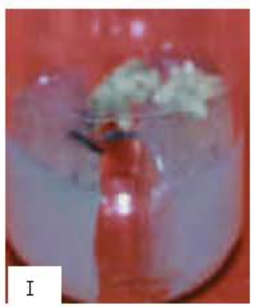

Fig. 3: I: Friable callus; II: Green callus

Fig. 5: Rooting

Fig. 6: Plant in field

by BAP (0.25 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}) with 80% response (Fig. 3). The combined interaction of BAP (0.5 and 1.0 mg L^{-1}) + NAA (0.25 and 1.0 mg L^{-1}) resulted in compact callus formation at the basal ends, which was followed by multiple shoot regeneration and elongation on the same media with 90% response (Fig. 4). Direct root initiation and elongation was observed after sub- culturing of isolated shoots into MS basal media within 4 weeks of their culture period (Table 2). The rooting response of shoots was also initiated on MS basal media fortified with IAA (1.0 mg L^{-1}) after 4 weeks of culturing (Fig. 5). Complete plantlets (6-10 cm) were recovered after 6-8 weeks on rooting media. The healthy plantlets were deflasked and transferred into pots containing sand, soil and vermiculite mixture (1:1:1) (Fig. 6).

In the present study, the maximum multiple direct adventitious shoot regeneration and elongation was observed by culturing nodal segments of *A. heterophyllum* on MS media augmented with BAP (0.25 mg L^{-1}).The results are very much in conformity with other previous studies (Giri *et al*., 1993). Our results are also supported by the observations in liquid culture of *A. napellus* (Watad *et al*., 1995). Callus regeneration was observed at the basal cut ends of each explant, which is in agreement with the anther culture of *A. carmichacli* (Hatano *et al*., 1987).

Combined effect of BAP (1.0 mg L^{-1}) + NAA (1.0 mg L^{-1}) on nodal segments promoted multiple indirect adventitious shoot regeneration and elongation. However, the combined interaction of BAP (1.0 mg L^{-1}) + 2, 4-D (1.0 mg L^{-1}) on nodal segments showed low percentage of response. Such findings are strongly supported by those of Giri *et al*. (1993) who reported the callus formation on MS medium containing 2, 4-D, Kinetin, Coconut water and maintained on 1.0 mg L^{-1} level of NAA. Rooting of the elongated shoots was initiated on MS basal medium fortified by IAA (1.0 mg L^{-1}) 4 weeks after culturing. However, root formation results recorded by Giri and Watad in *A. heterophyllum* and A. *napellus* was obtained best on IBA (1.0 mg L^{-1}) and NAA (1.0 mg L^{-1}), respectively.

The detailed review of the earlier studies reveal that there is only scanty published data on organogenesis of this plant species. However, there are few published reports regarding liquid culture of *A. napellus, A. balfourii* and A. *carmichalli*. (Watad *et al*., 1995; Hatano *et al*., 1987; Pandey *et al*., 2004 and Hatano *et al*., 1988).

Results of the study reveal that the protocol developed for them micro-propagation of *A. heterophyllum* has the potential to be reproduced and utilized for large-scale multiplication viz a viz conservation of this medicinal herb, an indigenous endangered medicinal plant. Friable callus formation was obtained on NAA (0.6 mg L^{-1}) (Fig. 3), but callus maintenance was difficult due to excessive leaching of apparent phenolic compounds. The problem was more acute on hormonal combinations of BAP (0.5 mg L^{-1}) and with 2, 4-D (0.25 mg L^{-1}).

Inclusion of anti-phenolic substances, such as ascorbic acid (10 mg L^{-1}) polyvinylpyrolodine (pvp) 0.5% and activated charcoal (2%) could not overcome this problem. Hence callus induced on 2, 4-D supplemented medium was transferred to NAA (1.0 mg L^{-1}) containing medium. The cultures were sub-cultured after one week's time, so as to prevent the leaching of exudates. The study indicates that *A. heterophyllum* populations in the North-western Himalaya are genetically diverse. At present, rate of its propagation is far less as compared to its exploitation. These species, or at least a significant proportion of its genetic diversity may be lost in near future, if appropriate measures are not taken for its conservation.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. G.N. Qazi Director, Regional Research Laboratory (CSIR). Jammu for research facilities.

REFERENCES

Ameri, A., 1998. The effects of *Aconitum* alkaloids on the central nervous system. Prog. Neurobiol., 56: 211-235.

Anwar, S., B. Ahmad, M. Sultan, W. Gul and N. Islam, 2003. Biological and pharmacological properties of *Aconitum chasmanthum*. J. Biol. Sci., 3: 989-993.

Atal, C.K., M.L. Sharma, A. Koul and A. Khajuria, 1986. Immuno modulating agents of plant origin. I: Preliminary Screening. J. Ethnol Pharmacol., 18: 133-141.

Christopher, J., 1996. Theses plants might spell Death to your cattle Indian Farmings (Review), pp: 43-49.

Dar, G.H., R.C. Bhagat and M.A. Khan, 2001. Biodiversity of Kashmir Himalya, Valley Book House, Srinagar, Jammu and Kashmir, pp: 120-176.

Giri, A., S.A. Paramir and A.P.V. Kumar,1993. Somatic embryogenesis and plant regeneration from callus cultures of *Aconitum heterophyllum* Wall. Plant Cell Tiss. Org., 32: 213-218.

Hatano, K., Y. Shoyama and I. Nishioka, 1987. Somatic embryogenesis and plant regeration from anther *Aconitum carmichaeli*, Debx. Plant Cell Rep., 6: 446-448.

Hatano, K., K. Kamura, Y. Shayama and I. Nishioka, 1988. Clonal multiplicationof *Aconitum carmichaeli* by tip tissue culture and alkaloid contents of clonally propagated plants. Planta Med., 54: 152-155.

Khan, A.I. and A. Khanum,1998. Role of Biotechnology in Medicinal and Aromatic Plants. Ukaaz Publication Shalivahana Nagar, Andra Pradesh, India.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay swith tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Pandey, H., S.K. Nandi, A. Kumar, U.T. Palni, B. Chandra and L.M.S. Palni, 2004. *In vitro* propagation of *Aconitumbal balfourii* Stapf. an important aconite of the *Himalayan alpines*. J. Hortic. Sci. Biotech., 79: 34-41.

Patwardhan, B., D. Kalbag, P.S. Patki and P. Nagsam, 1990. Search of immunomolatory agents: A review. Indian Drugs, 28: 56-63.

Pelltier, S.W., R. Aneja and K.W. Gopinath, 1968. The alkaloids of *Aconitum heterophyllum* Wall: Isolation and characterization. Phytochemistry, 7: 625-635.

Pirone, P.P., 1978. Diseases and Pests of Ornamental Plants. John Wiley and Sons, NewYork.

Uniyal, B.P., P.M. Singh and D.K. Singh, (Eds.), 2002. Flora of Jammu and Kashmir 1 Botanical Survey of India, Kolkata, India, pp: 365-375.

Watad, A.A., M. Kochba, A. Nissima and V. Gaba, 1995. Improvement of *Aconitum napellus* micropropagation by liquid culture on floating membrane rafts. Plant Cell Rep., 14: 345-348.

Zhaohong, W., J. Wen, J. Xing and Y. He, 2005. Quantitative determination of alkaloids in four species of *Aconitum* by HPLC. J. Pharma. Biomed. Anal., pp: 8-12.