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In vitro Multiplication of *Atropa acuminata* Royle - An Important Medicinal Plant

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ABSTRACT

A rapid protocol for regenerating shoots from nodal explants of *Atropa acuminata* obtained from *invitro* raised seedlings was worked out. Best results were recorded when nodal segments were cultured on MS medium supplemented with BAP (4.4μM) + IBA (4.9 μM) which resulted in initiation as well as elongation of multiple indirect adventitious shoots. Isolated shoots were rooted on MS basal medium without supplementing any growth regulator.

Key words : *Atropa acuminata* , nodal segments , multiple shoots, Plantlets.

Abbreviations : MS -Murashige and Skoog ; IBA - Indole - 3 - butyric acid ; BAP- 6 -benzyl amino purine .

INTRODUCTION

Atropa acuminata Royle, commonly known as Meit brand, belongs to family Solanaceae. It is a perennial indigenous herb of Kashmir, distributed over temperate parts of western Himalaya extending from Kashmir to Shimla and adjoining areas of Himachal Pradesh. Its chief habitat in J&K State is the Forests of Sindh, Jhelum, Lidder and Chenab Valleys (Chopra *et al.*, 1956).It grows at an altitude of 2000 to 3500m above sea level. The principal alkaloid in the leaves and roots of the plant is hyoscyamine used in many pharmaceutical preparations. It is used as sedative, antispasmodic, narcotic, anodyne for rheumatism, neuralgia, lumbago and local inflammations (Kaul, 1997). Collection of medicinal plants on mass scale from natural habitats is leading to a depletion of plant resources. In India, the drug is still collected from natural sources by uprooting the plant, raising a concern about possible extinction of the species (Gupta, 1988). Realizing the threat of extinction there is a need to develop conservation strategies and quick propagation protocols. For the conservation of valuable genotypes, micro propagation has been widely used for mass multiplication and commercial utilization of a number of medicinal plants (Zarate *et al.*, 1997; Toth *et al.*, 2000; Ahuja *et al.*, 2002; Dias *et al.*, 2002; Kamili *et al.*, 2003, 2004). The present communication describes a protocol for successful micro propagation of *A. acuminata* using nodal segments obtained from *invitro* raised seedlings.

MATERIAL AND METHODS

Certified seeds of *A. acuminata* were procured from Regional Research Institute of Unani Medicine (RRIUM), University of Kashmir and washed with detergent cedepol (0.5% v/v) and 2 - 4 drops of tween-20 (surfactant) under running tap water followed by final rinsing with double distilled water. These seeds were soaked 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 20 - 25 minutes followed by their three times rinsing with autoclaved double distilled water to remove all traces of sterilant. The sterilized seeds were then inoculated on MS basal medium (1962) fortified with 3% sucrose. pH of the medium was adjusted at 5.5 – 5.6 by using NaOH (0.1 N) or HCl (0.1N) before gelling the medium with 0.8% Difco-bactoagar. The medium was finally dispensed into culture vials, which were plugged and autoclaved for 20 min. at 15 lb pressure and 121°C temperature. These cultures were maintained at 25± 3°C with 55 - 65% RH and exposed to 16hour photoperiod provided by cool flourescent tubes (3000 lux).

RESULTS

Seeds of *A. acuminata* when cultured on MS basal medium resulted in full – fledged seedling formation after 6 weeks of their culture (Fig 1). Nodal explants were excised aseptically from these seedlings and cultured on different phytohormonal regimes and combinations the effect of which is depicted in Table 1. Semi compact callus of different degrees was formed at the basal cut ends of each explant. Indirect adventitious shoot regeneration as well as their elongation was evaluated on MS medium using various BAP concentrations (1.1, 2.2, 3.3 and 4.4µM) within 6 – 8 weeks of culture. The average shoot number 2.8 ± 0.74 per explant was observed on BAP (4.4µM) with 60%response.

The combined interaction of BAP (1.1, 2.2, 3.3, 4.4 µM) +IBA (1.2, 2.4, 3.6, and 4.9µM) resulted in compact callus formation at the basal ends which was followed by multiple shoot regeneration and elongation on the same medium. However, average shoot number 6.1 ± 0.83 per explant was observed on BAP (4.4 µM) + IBA (4.9 µM) with 90%response (Fig 2).

Direct root initiation and elongation was observed after subculturing of isolated, elongated shoots onto MS basal medium within 4 weeks of their culture period. The rooting response of shoots was 100% (Fig 3).

Table1: Effect of BAP and BAP + IBA on shoot regeneration from nodal explants of *A. acuminata*.

BAP(μM)	IBA(μM)	Response	% Response	Other morph. genetic responses	Average no. of shoots/ explant*
MS Basal	Control	-	-	-	-
1.1	0	Indirect adventitous shoot regeneration	20	+	1±0.00
2.2	0	1-2 indirect adventitious shoot regeneration.	20	+	1.5±0.50
3.3	0	Multiple indirect adventitious shoot regeneration & elongation	30	+	2.0±0.81
4.4	0	-do-	60	+	2.8±0.74
1.1	1.2	1-2 indirect adventitious shoot regeneration & elongation	30	++	1.5±0.5
2.2	2.4	-do-	30	++	2.8±0.6
3.3	3.6	Multiple indirect adventitious shoot regeneration and elongation	80	+++	4.2±0.74
4.4	4.9	-do-	90	+++	6.1±0.83

*Mean ± S.D ; 10 replicates / treatment
- low callus; ++ moderate callus;+++ profuse callus

Fig. 1

Fig. 2

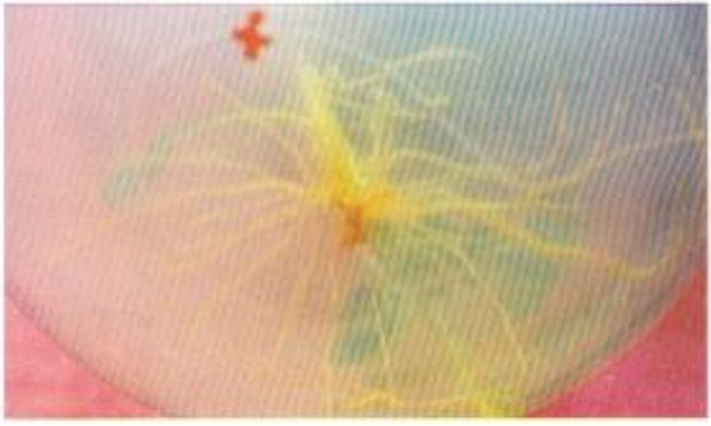

Fig. 3

Fig. 1 - 3: Morphogenetic response of nodal explants of *Atropa acuminata* to various phytohormonal regimes.

1. *invitro* seed germination and seedling formation on MS basal medium (after 6 weeks)
2. Multiple indirect adventitious shoot regeneration and elongation on MS + BAP(4.4μM) + IBA(4.9μM) (after 8 weeks)
3. Root initiation and elongation on MS basal medium (after 4 weeks)

DISCUSSION

In the present study, maximum multiple indirect adventitious shoot regeneration and elongation was observed by culturing nodal segments of *A. acuminata* on MS medium augmented with BAP (4.4 μM). These results are very much in conformity with the observations of Ahuja *et al.* (2002) who achieved similar results in nodal bud culture of *A. acuminata*, using BAP (4.4 μM) fortified MS basal medium. Such results are again supported by the observations in nodal bud culture of *A. baetica* by Zarate *et al.* (1997). Callus regeneration was observed at the basal cut ends of each explant which is in agreement with Toth *et al.* (2000) in *A. belladonna*.

Combined effect of BAP (4.4 μM) + IBA (4.9 μM) on nodal segments also promoted multiple indirect adventitious shoot regeneration and elongation. Such findings are strongly supported by those of Ahuja *et al.* (2002) who also reported multiple indirect adventitious shoot regeneration rate in nodal bud culture of *A. acuminata* under the influence of same phytohormonal combination and concentration.

Rooting of elongated shoots was initiated on MS basal medium only after one week of culturing however long, thin multiple roots were achieved after 4 weeks of culture period. Similar results were recorded by Zarate *et al.* (1997) and Ahuja *et al.* (2002) on *A. baetica* and *A. acuminata* respectively.

The detailed review of the earlier study reveals that there is only scanty published data on organogenesis of the plant species. However, there are few published reports regarding suspension and protoplast culture of *A.. belladona* (Bhandary *et al.*, 1969; Thomas *et al.*, 1970, 1972 ; Bajaj, 1978; Kamada *et al.*,1986 ; Bajaj *et al.*, 1991).

The results of the paper reveal that the protocol developed for micropropagation of *A. acuminata* has the potential to be reproduced and utilized for large scale multiplication vis-à-vis conservation of this medicinal herb — an indigenous threatened medicinal plant.

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REFERENCES

Ahuja, A., Sambyal, M. and Koul, S. 2002. *Invitro* propagation and conservation of *Atropa acuminata Royl ex Lind.* an Indigenous Threatened Medicinal Plant. *J. Plant Biochem and Biotech.* **11** : 121 – 124.

Bajaj, Y. P. S. 1978. Effect of super – low temperature on excised anthers and pollen embryos of *Atropa, Nicotiana* and *Petunia*. *Phytomorphology* .171 – 176.

Bajaj, Y. P. S. and Simola, L. K. 1991. Aropa belladonna L. : *Invitro* culture, Regeneration of plants, Cryopreservation, and the production of tropane alkaloids. In : *Biotechnology in Agriculture and Forestry* **15**. *Medicinal and Aromatic plants III (ed)*. Springer New Verlag Berlin Heidelberg York.

Bhandary, S. B., Collin, H. A., Thomas, E. and Street, H. E. 1969. Root, Callus and Cell Suspension Cultures, from *Atropa belladonna* L. and *Atropa belladonna, cultivar lutea Doll. Ann. Bot.* **33** : 647 – 656.

Chopra, I. C., Sobti, S. N. and Handa, K. L.1956. *Cultivation of Medicinal plants J&K* (Indian Council of Agricultural Research, New Delhi).

Dias, M. C., Almeida, R. and Romano, A. 2002. Rapid clonal multiplication of *Lavandula virdis* L. through *invitro* axillary shoot proliferation. *Plant Cell, Tissue and Organ, Culture*. **68** : 99 – 102.

Gupta, R. 1988. Genetic resources of medicinal plants. *Indian J. Plant Genet. Resources* **1** : 98 -102.

Kamada, H., Okamura, N., Satake, M., Harada, H. and Shimomura, K. 1986. Alkaloid production by hairy root cultures in *Atropa belladonna*. *Plant Cell Reports* **5** : 239 – 242.

Kamili, A. N., Bashir, S., Parray, M. R., Qadri, I. H. and Shah, A. M. 2003. *Invitro* plant regeneration in *Cichorium intybus L.* through nodal culture. *J. Res. Dev.* **3** :15 - 22.

Kamili, A. N., Bashir, S. and Shah, A. M. 2004. Regeneration of plantlets from petiole derived callus of *Artemisia annua* L. *In: Bioresources: Concerns and conservation* (A. N. Kamili and A. R. Yousuf. eds.) pp. 287 – 293. C.O.R.D., Kashmir University.

Kaul, M. K.1997. Medicinal plants of Kashmir and Ladakh. Indus publishing Company New Delhi.

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

Thomas, E. and Street, H. E. 1970. Organogenesis in cell suspension cultures of *Atropa belladonna* L. and *Atropa belladonna cultivar lutea* Doll. *Ann. Bot.* **34** : 657 – 669.

Thomas, E. and Street, H. E. 1972. Factors influencing morphogenesis in excised roots and suspension cultures of *Atropa belladonna*. *Ann. Bot.* **36** : 239 – 247.

Toth, E. T., Onisei, T., Amariei, D. and Lazurca, D. 2000. Variability in tissue culture regenerated plants of *Atropa belladonna*. International Symposium on plant Biotechnology and its contribution to plant Development, Multiplication and Improvement. *ISHS Acta Horticulturae* **289.**

Zarate, R., Cantos, M. and Troncoso, A. 1997. Induction and development of adventitious shoots of *Atropa baetica* as a means of propagation. *Euphytica*. **94** : 361 – 366.