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MULTIPLE SHOOT REGENERATION FROM *IN VITRO* LEAF EXPLANT OF *ATROPA ACUMINATA* ROYLE

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ABSTRACT

A successful regeneration protocol was obtained for conservation of the medicinally important plant species of Kashmir Himalaya viz., Atropa acuminata Royle. During the present study, callus derived from in vitro leaf explant on MS medium adjuvanted with BAP (3mg/l) was subcultured for shoot regeneration. Multiple shoots regenerated on MS medium fortified with different concentrations of BAP alone and in combination with Kn and IAA. The relative concentration of cytokinin (BAP) to Kinetin (Kn) greatly affected the multiple shoot regeneration. Maximum number of shoots regenerated on MS medium supplemented with BAP (3mg/l) + Kn (3mg/l).

Keywords: *Atropa, BAP, callus, explant and shoot regeneration*

1. INTRODUCTION

Atropa acuminata is commonly known as Indian Belladonna. It is a perennial plant and grows about 1.6 m tall. It has simple leaves which are ovate with entire margins. The flowers are solitary, bell-shaped and yellowish white in colour. They are hermaphrodites and are pollinated by insects [1]. Flowering period is from June to July and the seeds ripe from August to October. The black fruits are berries. The rhizome of this plant has been traditionally used as a sedative [2] antidote in cases of mushroom or toadstool poisoning, analgesic, antispasmodic, hallucinogenic, mydriatic, narcotic [3] diuretic, anodyne [4], arthritis related inflammatory disorders, muscle and joint pain, muscle spasms [5] sore throat, ulcerative colitis [6]. In folklore medicines, the plant is used for several inflammatory disorders such as arthritis, asthma, conjunctivitis, encephalitis, pancreatitis, peritonitis, acute infections and neuro inflammatory disorders [7]. *A. acuminata* serve as one of the most important source of medicinally important tropane alkaloids, including atropine, scopolamine and hyoscyamine [8]. The drugs atropine and hyoscyamine extracted from the plant act as stimulants to the sympathetic nervous system and are employed as antidotes to opium [9]. *A. acuminata* contains highly oxygenated oleanane triterpenes such as 2α, 3α, 24-trihydroxyolean-12-ene-28, 30-dioic acid and 2α, 3α, 24, 28-tetrahydroxyolean-12-ene [10]. Monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinine are present as main constituents [11].

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2. MATERIALS AND METHODS:

In vitro leaf explants were cultured on MS basal medium, MS medium supplemented with different concentrations and combinations of plant growth regulators both individually and in combinations. Auxins like 2, 4- D; IAA; NAA; IBA and cytokinins like BAP and Kn were used in concentration range of 0.1-5 mg/L. The pH of the medium was adjusted to 5.8 prior to gelling with agar was dispensed in culture tubes and flasks and sterilized by autoclaving at 121ºC temperature and 15 lbs pressure for 15 minutes. The cultures were incubated under controlled conditions in the culture room under the regime of 16h light period (500-3000 lux) and 8h dark period and temperature of 22±4Cº.

3. RESULTS AND DISCUSSION

3.1. Shoot regeneration

Callus was achieved from *in vitro* leaf explant when inoculated on MS medium enriched with BAP (1mg/l), BAP (2mg/l), BAP (3mg/l), BAP (4mg/l) and BAP (5mg/l) (Fig.1) are effective for inducing fragile and creamish, fragile and creamish, compact and light green, hard and creamish and compact and light green callus in 20%, 40%, 80%, 60% and 60% cultures within 38, 36, 23, 29 and 31 days of inoculation respectively (Table 1). Dai et al. (2003) also obtained callus from *in vitro* leaf of *Populus canescens* and *Populus grandidentada* on MS medium and WPM medium containing four combinations of cytokinins and auxins.

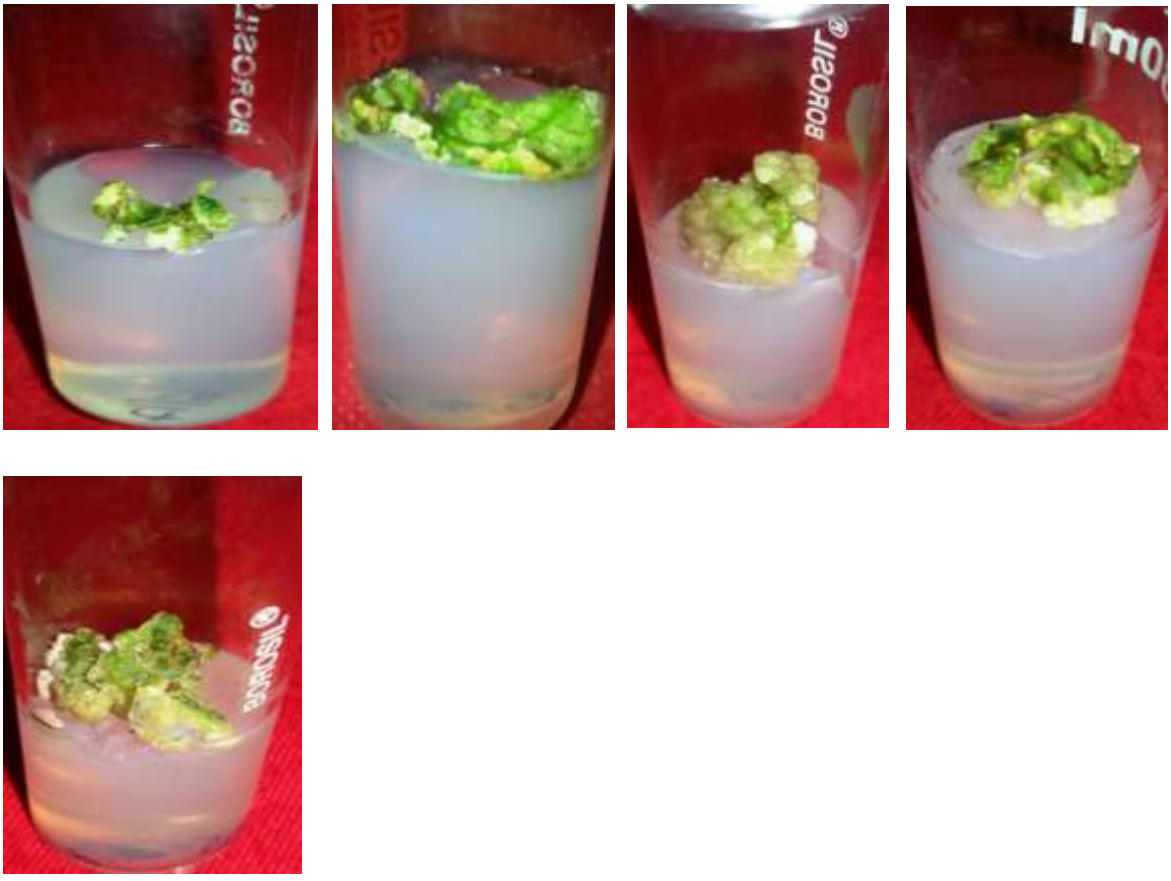

Fig.1: Callus production from *in vitro* leaf explant on MS medium containing

a) BAP (1mg/l) b) BAP (2mg/l) c) BAP (3mg/l) d) BAP (4mg/l) e) BAP (5mg/l)

Table 1: Effect of plant growth regulators on callus production from *in vitro* leaf explant

Treatments	Number of days taken	Texture and color of callus	% culture
MS basal	-	-	-
MS+BAP (1mg/l)	38	Fragile and creamish	20
MS+BAP (2mg/l)	36	Fragile and creamish	40
MS+BAP (3mg/l)	23	Nodular and light green	80
MS+BAP (4mg/l)	29	Hard and creamish	60
MS+BAP (5mg/l)	31	Nodular and light green	60

10 replicates per treatment

3.2. Shoot regeneration

The *in vitro* leaf derived callus when subcultured on MS medium supplemented with BAP (1mg/l), BAP (2mg/l), BAP (3mg/l), BAP (4mg/l) and BAP (5mg/l) regenerate shoots with 3.8±1.39cm, 4.2±1.71cm, 7.0±2.93cm, 3.6±1.20cm and 2.8±0.86 cm mean number of shoots and 1.9±0.12cm, 1.9±0.14cm, 2.3±0.17cm, 2.2±0.25cm and 1.8±0.18cm mean length of shoots in 40%, 60%, 80%, 70% and 60% cultures within 35, 39, 18, 24 and 29 days respectively. Shoots were also regenerated from cytokinin–cytokinin combination on MS medium supplemented with BAP (3mg/l) in combination with Kn (1mg/l), BAP (3mg/l) in combination with Kn (2mg/l) and BAP (3mg/l) in combination with Kn (3mg/l) (Fig.2) with 4.8±1.49cm, 5.6±1.69cm and 15.2±8.86cm mean number of shoots and 1.6±0.14, 2.6±0.15, 5.1±0.20 and 2.3±0.13 mean length of shoots in 50%, 40%, 90% and 60% cultures within 42, 38 and 20 days respectively. (Table 2). Jose et al. (1992) also obtained shoot regeneration from *in vitro* leaf of *Camellia reticulata* on WPM. However, they used WPM medium instead of MS medium.

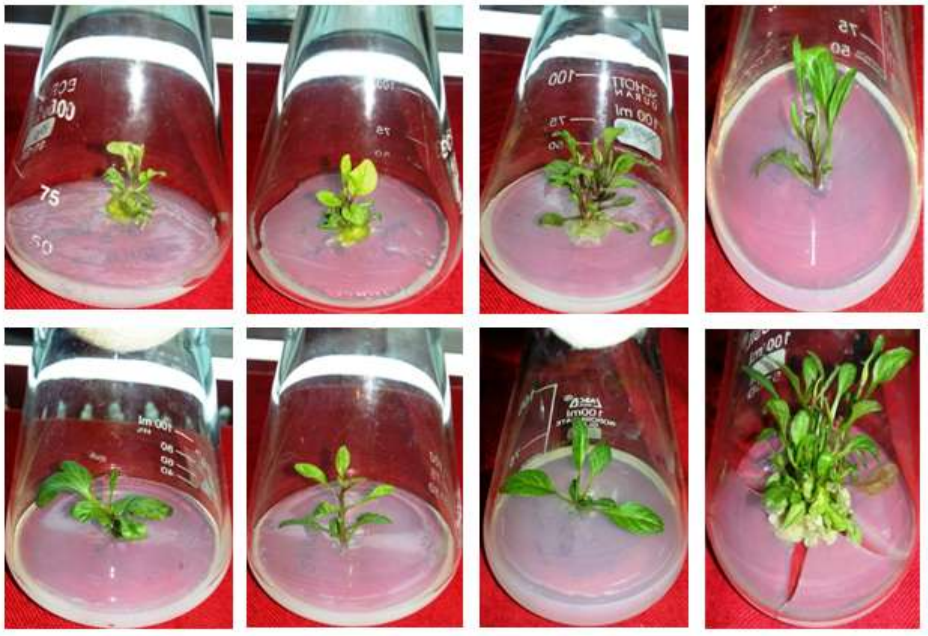

Fig.2: Shoot regeneration from *in vitro* leaf explant on MS medium containing

a) BAP (1mg/l) b) BAP (2mg/l) c) BAP (3mg/l) d) BAP (4mg/l) e) BAP (5mg/l)

f) BAP (3mg/l) + Kn (1mg/l) g) BAP (3mg/l) + Kn (2mg/l) h) BAP (3mg/l) + Kn (3mg/l)

Table 2: Effect of plant growth regulators on shoot regeneration from *in vitro* leaf derived callus

Treatments	Number of days taken for shoot regeneration	Mean number of shoot (±SE)	Mean length of shoots (cm)±SE	% culture response
MS+BAP (1mg/l)	35	3.8±1.39	1.9±0.12	40
MS+BAP (2mg/l)	39	4.2±1.71	1.9±0.14	60
MS+BAP (3mg/l)	18	7.0±2.93	2.3±0.17	80
MS+BAP (4mg/l)	24	3.6±1.20	2.2±0.25	70
MS+BAP (5mg/l)	29	2.8±0.86	1.8±0.18	60
MS+BAP (3mg/l)+Kn(1mg/l)	42	4.8±1.49	1.6±0.14	50
MS+BAP (3mg/l)+ Kn(2mg/l)	38	5.6±1.69	2.6±0.15	40
MS+BAP (3mg/l)+ Kn(3mg/l)	20	15.2±8.86	5.1±0.20	90

10 replicates per treatment

4. CONCLUSION

An efficient and rapid propagation protocol of *A. acuminata* plants was developed using *in vitro* leaf as explant. Among all the plant growth regulators, BAP was proved to be the most effective for callus induction and BAP (3mg/l) in combination with Kn (3mg/l) proves best for shoot regeneration.

REFERENCES

[1.] Nasir, Y. J. (1972) *Atropa acuminata* Royle ex miers in *Hook Flora of Pakistan.* 1:138.

[2.] Rhodes, J. B., Abrams, J. H., Manning, R. T. (1978) Controlled clinical trial of sedative-anticholinergic drugs in patients with the irritable bowel syndrome. *Journal of Clinical Pharmacology, 18: 340–345*.

[3.] Grieve, (1984) *A Modern Herbal. Pengium* ISBN 0-14-046-440-9.

[4.] Chiej, R. (1984) *The Macdonald encyclopedia of medicinal plants.* Macdonald & Co (Publishers) Ltd.

[5.] Chopra, R. N., Nayar. S. L., Chopra. I. C. (1986) *Glossary of Indian medicinal plants* (Including the Supplement) Council of Scientific and Industrial Research, New Delhi.

[6.] Kaul, M. K. (1997) *Medicinal plants of Kashmir and Ladakh.* Indus Publications, New Delhi, India pp. 173.

[7.] Shanafelt, T. D., Barton, D. L., Adjei, A. A., Loprinzi, C. L. (2002) Path physiology and treatment of hot flashes In: Mayo Clinic proceedings. *Mayo Clinic, 77: 1207–1218.*

[8.] Nisar, A., Malik, A. H., Zargar, M. A. (2013) *Atropa acuminata* blunts production of pro-inflammatory mediator's eicosanoids, leukotrienes, cytokines *in vitro* and *in-vivo* models of acute inflammatory responses. *Journal of Ethnopharmacology, 147:584-594.*

[9.] Phillipson, J. D., Handa, S. S. (1975) N-oxides of hyoscyamine and hyoscine in the Solanaceae *Photochemistry, 14: 999-1003.*

[10.] Mehmood, M. A., Anis, I., Khan, P. M., Riaz, M., Makhmoor, T., Choudhary, M. I. (2002) Highly oxygenated triterpenes from the roots of *Atropa acuminata*. *Natural Product Letters, 16: 371–376.*

[11.] Jayakanthi, J., Dhanarajan, M. S., Vijay, T. (2011) Found main constituents of *Atropa acuminata* belongs to monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinine *International Journal of Pharmacy and Pharmaceutical Sciences, 3: 0975-1491.*