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Micropropagation of *Atropa acuminata* Royle from *in vitro* petiole explant

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ABSTRACT

During the present study, *in vitro* petiole explants of *Atropa acuminata* were subjected to *in vitro* studies so as to develop efficient micropropagation protocols for its regeneration. *In vitro* petiole explant produced maximum amount of callus on MS medium supplemented with BAP (3mg/l) in 80% cultures within 20 days. Shoot regeneration was obtained after sub-culturing the callus on MS medium supplemented with BAP with mean shoot number of 8.6±4.22cm and a mean shoot length of 2.3±0.20 cm in 100% cultures within 13 days. Root regeneration was obtained on MS medium augmented with IBA (0.5mg/l) with a mean number of roots 6.0±2.50cm and mean root length of 1.3±0.11cm with 60% response within 32 days.

Keywords: *Atropa acuminata, micropropagation, explant, callus, shoot regeneration, root regeneration*

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 (Nasir, 1972). 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 (Rhodes *et al*., 1978) antidote in cases of mushroom or toadstool poisoning, analgesic, antispasmodic, hallucinogenic, mydriatic, narcotic (Grieve, 1984) diuretic, anodyne (Chiej, 1984), arthritis related inflammatory disorders, muscle and joint pain, muscle spasms (Chopra,1986) sore throat, ulcerative colitis (Kaul, 1997). 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 (Shanafelt *et al*., 2002). *A. acuminata* serve as one of the most important source of medicinally important tropane alkaloids, including atropine, scopolamine and hyoscyamine (Nisar *et al*., 2013). The drugs atropine and hyoscyamine extracted from the plant act as stimulants to the sympathetic nervous system and are employed as antidotes to opium (Phillipson and Handa, 1975). *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 (Mehmood *et al*., 2002). Monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinine are present as main constituents (Jayakanthi *et al*., 2011).

Materials and Methods

Petiole from *in vitro* raised plants was used for the experimental purpose. *In vitro* petiole 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±4 Cº.

Results and Discussion

The present study focuses on micropropagation of *Atropa acuminata* from *in vitro* petiole explant. Callus was induced when *in vitro* petiole explants were inoculated on MS medium supplemented with BAP (3mg/l), BAP (3mg/l) + IAA (2mg/l), BAP (5mg/l) + IAA (1mg/l) and BAP (5mg/l) + IAA (2mg/l) (Fig.) in 80%, 50%, 30% and 40% cultures within 20, 29, 32 and 30 days. Best response was observed on MS medium enriched with BAP at a concentration of (3mg/L) in terms of percent culture response and minimum number of days taken (Fig.1 and table 1). Similar results were also obtained by Amin *et al*., (2017) from *in vitro* petiole explant on MS medium augmented with BAP (1mg/l) and Kn (1mg/l).

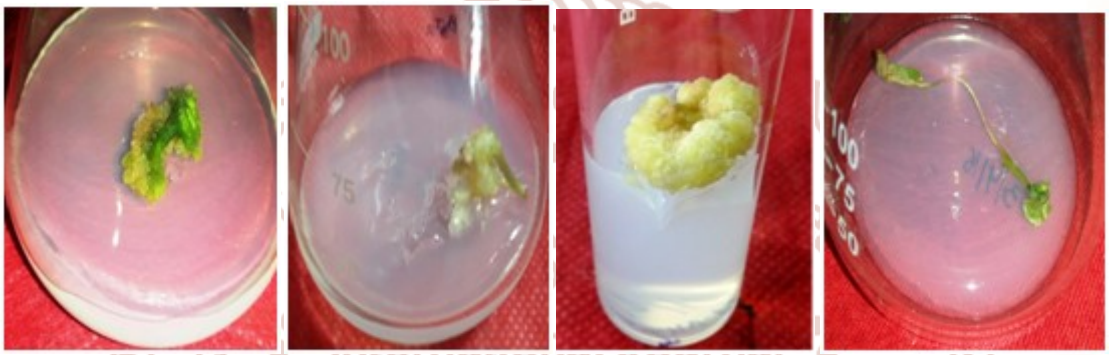

Fig.1: callus production from *in vitro* petiole explant on MS medium containing

a) BAP (3mg/l)
b) BAP (3mg/l) + IAA (2mg/l)
c) BAP (5mg/l) + IAA (1mg/l)
d) BAP (5mg/l) + IAA (2mg/l)

Table 1: Effect of plant growth regulators on callus induction from *in vitro* petiole explant

Treatments	Number of days taken for callus production	Texture and color of callus	% culture response
MS basal	-	-	-
MS + BAP (3mg/l)	20	Hard and light brown	80
MS + BAP (3mg/l) + IAA (2mg/l)	29	Hard and creamish	50
MS + BAP (5mg/l) + IAA (1mg/l)	32	Compact and creamish	30
MS + BAP (5mg/l) + IAA (2mg/l)	30	Fragile and light green	40

10 replicates per treatment

Shoot regeneration

In vitro petiole callus when subcultured on MS medium supplemented with BAP (3mg/l), BAP (4mg/l), BAP (5mg/l), BAP (3mg/l) + IAA (1mg/l), BAP (3mg/l) + IAA (2mg/l), BAP (3mg/l) + IAA (3mg/l), BAP (3mg/l) + IAA (4mg/l), BAP (3mg/l) + Kn (1mg/l), BAP (3mg/l) + Kn (2mg/l), BAP (3mg/l) + Kn (3mg/l), BAP (3mg/l) + Kn (4mg/l) and BAP (3mg/l) + Kn (5mg/l) (Fig.) regenerate shoots with 8.6±4.22cm, 5.6±2.69cm, 4.8±2.35cm, 3.0±0.70cm, 7.2±3.45cm and 5.0±2.07cm mean number of shoots and 2.3±0.20cm, 3.9±0.20cm, 2.7±0.15cm, 4.6±0.29cm, 1.4±0.16cm and 1.5±0.18cm mean length of shoots in 100%, 80%, 60%, 20%, 70%, 40%, 30%, 20%, 50%, 70%, 40% and 30% cultures within 13, 18, 24, 29, 16, 20, 42, 35, 26, 17, 32 and 25 days respectively. Maximum number of shoots was obtained with a mean shoot number of 8.6±4.22cm and mean shoot length of 2.3±0.20cm when BAP at a concentration of (3mg/L) was added to the medium (Fig. 2 and Table. 2). This is the first report of shoot regeneration from *in vitro* petiole explant.

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

a) BAP (3mg/l) b) BAP (4mg/l) c) BAP (5mg/l) d) BAP (3mg/l) + IAA (1mg/l) e) BAP (3mg/l) + IAA (2mg/l) f) BAP (3mg/l) + IAA (3mg/l) g) BAP (3mg/l) + IAA (4mg/l) h) BAP (3mg/l) + Kn (1mg/l) i)

BAP (3mg/l) + Kn (2mg/l) j) BAP (3mg/l) + Kn (3mg/l) k) BAP (3mg/l) + Kn (4mg/l) l) BAP (3mg/l) + Kn (5mg/l)

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

Treatments	Mean number of days taken for shoot regeneration	Mean number of shoots (cm)±SE	Mean length of shoots (cm)±SE	% culture response
MS + BAP (3mg/l)	13	8.6±4.22	2.3±0.20	100
MS + BAP (4mg/l)	18	5.6±2.69	3.9±0.20	80
MS + BAP (5mg/l)	24	4.8±2.35	2.7±0.15	60
MS + BAP (3mg/l) + IAA (1mg/l)	29	3.0±0.70	4.6±0.29	20
MS + BAP (3mg/l) + IAA (2mg/l)	16	5.2±3.45	1.4±0.16	70
MS + BAP (3mg/l) + IAA (3mg/l)	20	5.0±2.07	1.5±0.18	40
MS + BAP (3mg/l) + IAA (4mg/l)	42	3.4±1.02	1.1±0.14	30
MS + BAP (3mg/l) + Kn (1mg/l)	35	1.8±0.58	0.7±0.22	20
MS + BAP (3mg/l) + Kn (2mg/l)	26	2.2±0.73	0.9±0.26	50
MS + BAP (3mg/l) + Kn (3mg/l)	17	7.8±3.69	4.9±0.19	80
MS + BAP (3mg/l) + Kn (4mg/l)	32	1.8±0.58	3.3±0.94	40
MS + BAP (3mg/l) + Kn (5mg/l)	25	3.4±1.02	0.8±0.09	30

10 replicates per treatment

Rooting of regenerated shoots from *in vitro* petiole explant

Roots were regenerated from the shoots of *in vitro* petiole explant inoculated on full strength MS medium. Roots were also regenerated on MS medium supplemented with IBA (0.2mg/l) and IBA (0.5mg/l) (Fig. 25) with 2.2±0.37cm, 3.6±1.20cm and 6.0±2.50 cm mean number of roots and 1.3±0.11cm, 2.6±0.20cm and 1.3±0.11cm mean length of roots in 40%, 30% and 60% cultures within 38, 48 and 32 days of inoculation respectively (Fig. 3 and Table 3).

Fig.3: Rooting of regenerated shoots from *in vitro* petiole explant
a) MS basal b) IBA (0.2mg/l) c) IBA (0.5mg/l)

Table 26: Effect of plant growth regulators on rooting of regenerated shoots from *in vitro* petiole explant

Treatments	Number of days taken for root regeneration	Mean number of roots (cm)±SE	Mean length of roots (cm)±SE	% culture response
MS basal	38	2.2±0.37	1.3±0.11	40
MS+IBA (0.2mg/l)	48	3.6±1.20	2.6±0.20	30
MS+IBA (0.5mg/l)	32	6.0±2.50	1.3±0.11	60

10 replicates per treatment

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