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Callus induction and shoot regeneration of *Atropa acuminata* Royle-a critically endangered medicinal plant species growing in Kashmir Himalaya

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Abstract

Atropa acuminata Royle, family Solanaceae, is an important medicinal plant species growing in Kashmir Himalaya. During the present study an efficient in vitro protocol has been standardized viz; Callus development and shoot regeneration. Callus development has been achieved from the leaf explant. Callus was obtained when leaf explants were inoculated on MS medium supplemented with BAP and NAA at a concentration of 2.0 mg/l after 62 days of inoculation. Callus developed from leaf explants differentiated into shoots on MS medium supplemented with different concentrations of auxins (IAA, NAA) and cytokinins (BAP). Maximum shoots regenerated on MS medium supplemented with BAP and IAA (3.0 mg/l and 2.0 mg/l) after 19 days.

Keywords: Solanaceae, Explant, Callus, Inoculation, Auxin, Cytokinin.

Introduction

Atropa acuminata Royle is an important medicinal plant belongs to family Solanaceae.[1] It is commonly known as Maitbrand and in Hindi it is known as Sagangur, Angurshefa. *Atropa acuminata* is a perennial plant that grows about 0.9 m tall. It has simple leaves which are ovate with entire margins. The flowers are solitary, bell-shaped and brown in color.

It is endemic to India and is known as Indian Belladonna. It is found in the Western Himalayan ranges, extending from Kashmir at the altitude of 1800-3600m above sea level (asl) to the adjoining hills of the Himachal Pradesh up to 2500m asl. In North West Himalaya it is distributed in Kashmir, Muzzafarabad and Chakrata.[1, 2]

The drugs Atropine and Hyoscyamine extracted from the plant act as stimulants to the sympathetic nervous system and are employed as an antidote to opium.[1, 3] Atropine has a stimulatory effect on the circulatory and respiratory system.[3] It is used to dilate the pupils in eye operations, to relieve intestinal colic and to treat peptic ulcers. The plant can be used to treat the symptoms of Parkinson's disease, reducing tremors and rigidity whilst improving speech and mobility.[4] All parts of the plant are analgesic, antispasmodic, hallucinogenic, mydriatic, narcotic and sedative.[5] It is used against conjunctivitis, fever, encephalitis, muscle and joint pain, acute inflammation, pan- creatitis, peritonitis, scarlet fever.[6,7] Bown, 1995 reported that it is used to treat sunstroke and painful menstruation.[8] Better mann *et al*., 2001 have reported that the aerial parts of this plant have been used in traditional medicine to treat innumerable ailments such

as acute infections, anxiety, asthma, chicken pox.[9] Shanafelt *et al*., 2002 have reported that it is also used against sore throat, ulcerative colitis and whooping cough.[10] Nisar and Akhtar 2013 have reported that the *Atropa acuminata* has been used in folk medicines for several inflammatory disorders such as arthritis, asthma, conjunctivitis, encephalitis, pancreatitis, peritonitis, acute infections and neuroinflammatory disorders.[11]

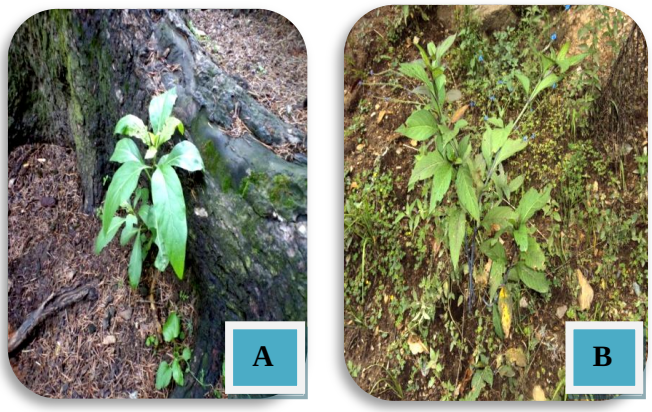

Figure 1: *Atropa acuminata* Royle in natural habitat

Owing to immense medicinal value, the plant is being indiscriminately exploited on a large scale. Unabated as the plant extraction continues to be, far are not the days when this precious legacy will be lost forever. It is indeed a crisis situation for the species which calls for the salvage of whatever is left that the present study for its in vitro propagation and conservation has been taken up.

Materials and Methods

Atropa accuminata was collected from Gulmarg Jammu and Kashmir and were transplanted at the Kashmir university botanical garden (KUBG). The specimen was collected and processed for herbarium preparation and latter deposited at Kashmir University Herbarium (KASH) Voucher Specimen Number 1913 (Ref.No.F1/Herbarium-Specimen vouchers) KU/2013). Leaf explants were collected from plants grown at KUBG. Explants were first thoroughly washed under running tap water in order to remove dirt and dust followed by washing with detergent labolene and surfactant tween-20. The detergent was removed by washing the explants with double distilled water. Then they were treated under laminar air flow hood with chemical sterilant 2% sodium hypochlorite for 8-10 min. This was followed by washing with autoclaved double distilled water and finally inoculation on sterilized nutrient medium.

Medium and culture conditions

Murashige and Skoog's (MS, 1962) medium, gelled with 8% agar was supplemented with different concentrations of auxins and cytokinins both individually and in combination.[12] 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 media was adjusted to 5.8 before autoclaving at 121 °C and 15 lb. The cultures were incubated at 22±4 °C and exposed to a regular photoperiod of 24 hours.

Results

Callus production from leaf explant

Callus was produced when leaf explants were inoculated on MS medium supplemented with BAP + NAA (2 mg/l), BAP (5 mg/l), BAP (2 mg/l) + IAA (5 mg/l) and BAP (2 mg/l) + NAA (5 mg/l) in a period of 62, 29,48 and 43 days of inoculation respectively (Table 1 Fig 2)

Table 1: Effect of different hormones on callus production from leaf explant of *Atropa accuminata*.

MS medium	BAP mg/l	IAA mg/l	IBA mg/l	NAA mg/l	Mean no. of days for callus production	% Response
+	2	-	-	2	62	20
+	5	-	-	-	29	100
+	2	-	-	5	48	40
+	2	5	-	-	43	70
+	-	-	0.5	-	-	0

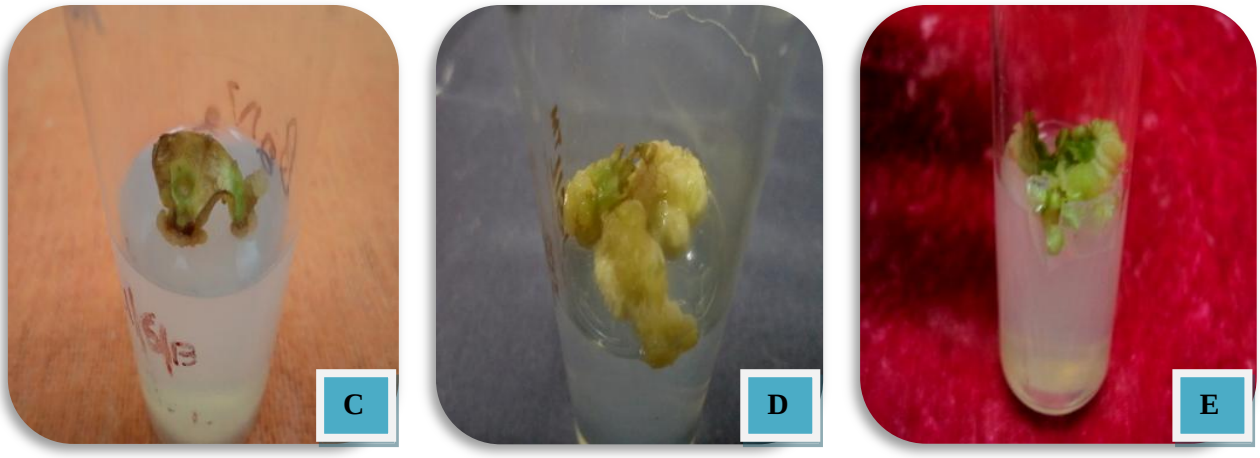

Figure 2: Callus initiation from leaf explant [C MS +BAP (2 mg/l) +NAA (2 mg/l)], [D MS +BAP (2 mg/l) +IAA (5 mg/l)], [E MS +BAP (2 mg/l) + NAA (5 mg/l)].

Callus production from in vitro leaf explant

Callus was also produced when in vitro leaf explants were inoculated on MS medium fortified with BAP (2 mg/l, 3 mg/l, 5 mg/l) in a time period of 17, 34 and 26 days of inoculation. (Table 2 Fig 3)

Table 2: Effect of different hormones on callus production from in vitro leaf explants of *Atropa acuminata*.

MS medium	BAP mg/l	IAA mg/l	Mean no. days for callus production	% Response
+	2	-	17	100
+	5	-	26	40
+	3	-	17	100
+	3	2	25	60

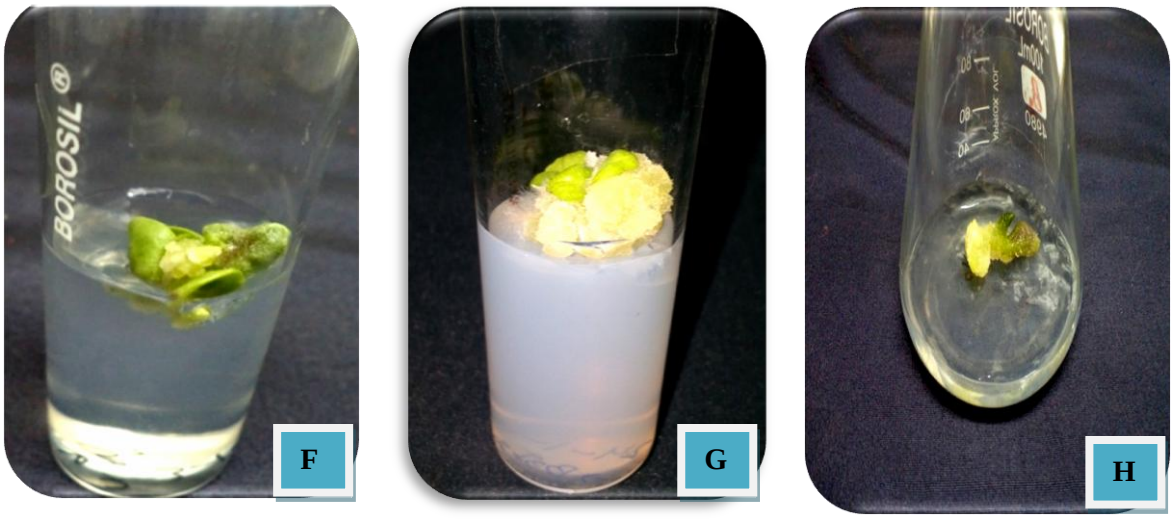

Figure 3: Callus initiation from in vitro leaf explant [F MS + BAP (5mg/l)], [G MS +BAP (3mg/l) +IAA (2mg/l)], [H MS+ BAP (2mg/l)]

Shoot regeneration

For shoot regeneration leaf derived callus was subcultured on MS medium supplemented with different concentrations of cytokinins individually or in combination with auxin. Cytokinins like BAP were used in a concentration of BAP (2 mg/l), BAP (3 mg/l) and BAP (5 mg/l). BAP with IAA also gave good results in a combination of BAP (3 mg/l) + IAA (2 mg/l) and BAP (2 mg/l) + IAA (3 mg/l). MS medium supplemented with BAP (3 mg/l) gives best results after 19 days of subculture (Table 3 Fig 4).

Table 3: Effect of different growth regulators on shoot formation from leaf derived callus of *Atropa accuminata*

MS medium	BAP mg/l	NAA mg/l	IAA mg/l	Average height of shoots	Number of days	% Response
+	2	-	2	4.11±0.27	51	40
+	3	-	2	6.15±0.45	19	100%
+	5	-	-	2.6±0.30	26	60
+	3	-	-	5.6±0.75	19	100%

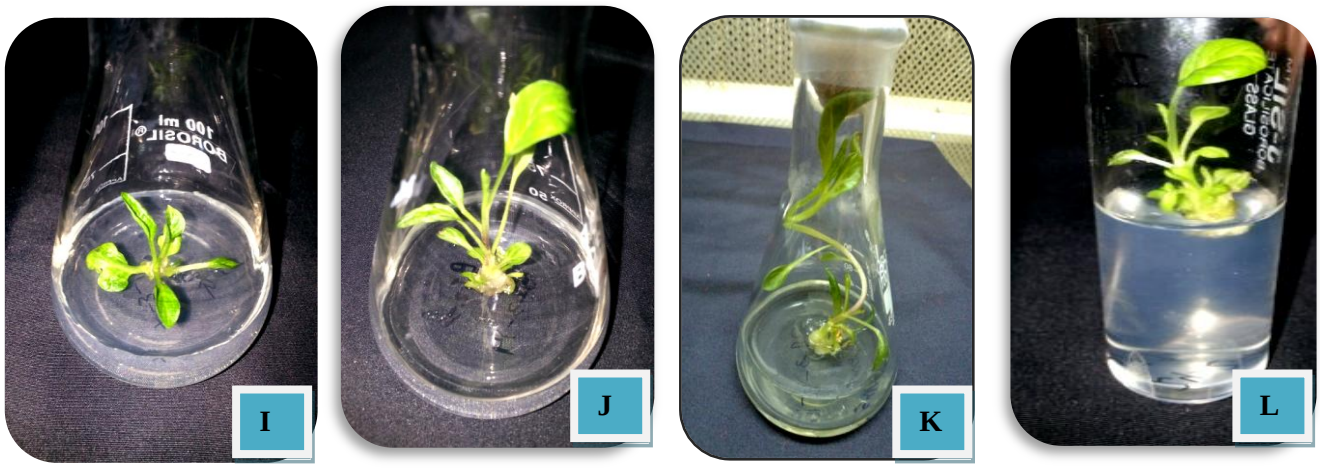

Figure 4: Shoot formation in *Atropa acuminata* on [I MS +BAP (5mg/l)], [J MS+BAP (3mg/l) +IAA (2mg/l)], [K MS+BAP (3mg/l)], [L MS+BAP (2mg/l) +IAA (2mg/l)]

Discussion

During the present study, different hormones, both auxin and cytokinins either individually or in different combinations were tried to produce callus from *Atropa accuminata*. Best results with 100% response were obtained from leaf explant on MS medium supplemented with BAP (5 mg/l) after 62 days. Callus was also produced from in vitro leaf explant with 100% response on MS medium fortified with BAP (2 mg/l, 3 mg/l) after 17 days. The callus produced was hard, green and compact. Callus after subculture produce maximum shoot regeneration on MS medium supplemented with BAP (3 mg/l) alone and in combination of BAP (3 mg/l) +IAA (2 mg/l) after 19 days.

Conclusion

A protocol was developed for callus induction and shoot regeneration of *Atropa accuminata* from leaf explant on MS medium supplemented with different growth regulators. Maximum callus development was achieved in MS medium supplemented with BAP (5 mg/l) and maximum number of shoots developed in MS medium supplemented with BAP (3 mg/l).

Acknowledgement

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Conflict of Interest

We declare that there is no conflict of interests regarding the publication of this paper.

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