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Short Communication

In vitro Propagation and Conservation of *Atropa acuminata* Royle ex Lindl –An Indigenous Threatened Medicinal Plant

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A micropropagation method has been developed for multiplication and conservation of *Atropa acuminata* by induction of axillary shoot proliferation from shoot tips and nodal explants using Murashige and Skoog (MS) medium supplemented with BAP (1 mg l^{-1}) and IBA (1 mg l^{-1}). Revised tobacco (RT) medium with IAA (1 mg l^{-1}) was found most suitable for shoot elongation. Rooting was highest on full strength RT medium containing IBA (1 mg l^{-1}). *In vitro* raised plantlets were hardened and transferred to soil.

***Key words*:** *Atropa acuminata*, conservation, endangered Indian belladonna, micropropagation.

Indian Belladona (*Aropa acuminata* Royle ex Lindl) belonging to family Solanaceae is distributed over temperate parts of Western Himalaya extending from Kashmir to Shimla and adjoining areas of Himachal Pradesh (1). This is a tall erect, annual herb upto 2 metre in height, woody below, branched above and tillering from the base. The plant is antispasmodic, narcotic, mydriatic, analgesic and antiasthamatic being a source of medicinally important tropane alkaloids - Atropine, Hyoscyamine and Scopolamine(2). The plants are heterogenous due to cross pollination and there is considerable variation in growth and alkaloid content(3). In India, the drug is still collected from natural sources by uprooting its roots. In view of heavy loss of renewable resources the natural populations are becoming scarce and species is declared threatened (4). Realizing the threat of extinction there is a need to develop conservation strategies and quick propagation protocols. As such, *in vitro* techniques appear to be promising alternatives to ensure its regeneration and conservation. A perusal of the literature shows report on production of atropine from hairy root cultures (5) attempts to develop *in vitro* regeneration methods in *A. acuminata* towards micropropagation are obscure. However, *A. belladona* - a related species, has been extensively subjected to tissue culture studies(6). The present communication describes a method for rapid propagation through axillary shoot proliferation in *A. acuminata*.

Plant material of *A. acuminata* was collected from field grown plants in Field Gene Bank at RRL (Br.) Srinagar. Plant material was treated with Tween-20 for 10 min and later washed thoroughly under tap water for 30 min. The shoot tips and nodal segments with axillary buds (about 2.5 cm long) were excised from shoots and used as explants. These explants were treated with bavistin (BASF) 0.1% for 10 min. The explants were surface sterilized with 0.1% (w/v) mercuric chloride for 4 min, and washed with sterile distilled water five times. The shoot tip explants (1.5 cm long) were then trimmed from the lower end prior to culture in test tubes (15 cm x 2.5 cm) containing MS (7) medium with 3% (w/v) sucrose and 0.7% (w/v) Agar. The medium was augmented with 1 mg l^{-1} each of NAA, IBA and 2,4-D alone and in combination with BAP and Kinetin (Kn). (Table 1). For further, development of the multiple shoots, the cultures were transferred to Revised Tobacco medium (8) with IAA 1mg l^{-1}. Primary shoots formed *in vitro* were sectioned into one-node pieces after removing the leaves. The nodal segments containing the dormant axillary buds were cultured on MS medium with IBA+BAP (1 mg l^{-1}) For subsequent multiplication, callus at base was also subcultured on MS medium with IBA + BAP(1 mg l^{-1}) to give multiple shoots. Subcultures were carried out at six week interval. Rooting of elongated shoots (ca. 5-6 cms) was assessed by subculturing on half and full strength Revised tobacco(RT) (8) and B_5 (9) medium containing different levels of IBA. Full strength MS (basal) medium without hormonal supplements served as the control. The pH of the medium was adjusted to 5.8 with 1N NaOH or HCl before it was dispensed into culture vessels and autoclaved at 15 lb/in^2 (121°C) for 20 min. The cultures were incubated at 24 ± 2°C with 16 h (65µm $m^{-2}s^{-1}$)

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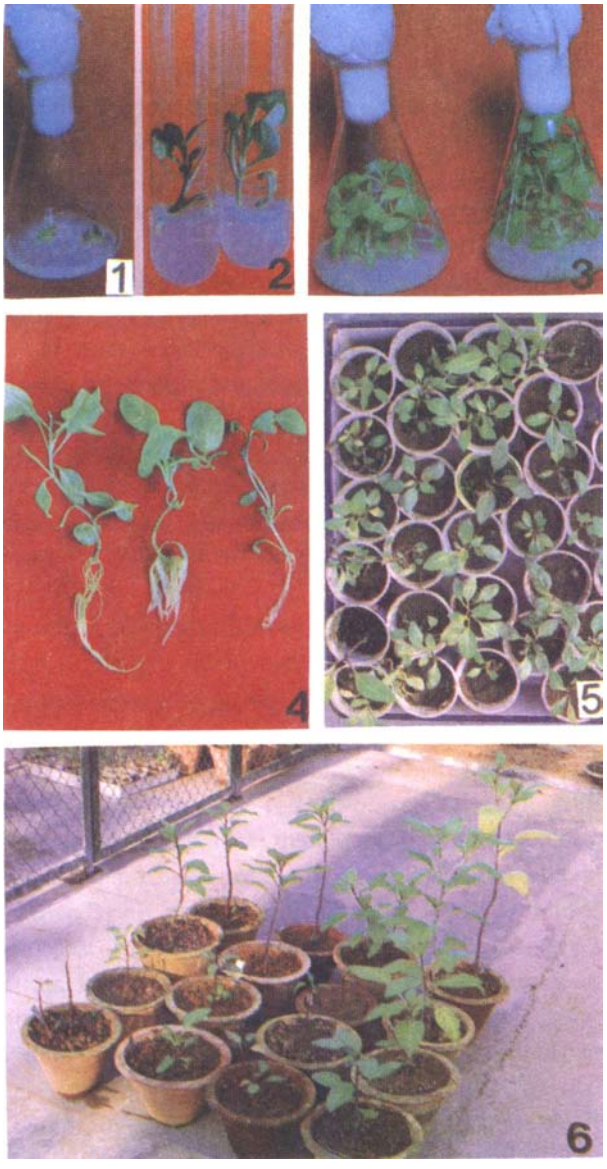
Table 1. Morphogenetic responses of shoot apices and nodal explants of *Atropa acuminata* to various media treatments

BM[a]+PGR (1 mg l^{-1})	Shoot apices			Nodal explants		
	[b]Response %	Shoot number/ culture ± SE	Other morphogenetic response[c]	[b]Response %	Shoot number/ culture ± SE	Other morphogenetic response[c]
BAP	60	1.2±0.6	+	50	2.4±0.6	+
Kn	80	1.3±0.5	+	70	2.8±0.4	+
IAA	80	1.8±0.7	+	80	3.5±0.9	+
IBA	50	1.2±0.6	+	50	3.6±1.6	+
NAA	10	1.3±0.5	+++	10	2.6±1.0	+
2,4-D	10	—	++	20	—	++
BAP+IAA	80	3.1±0.5	++++	80	5.8±0.6	++++
BAP+IBA	80	5.8±0.4	++++	90	4.6±0.8	++++
BAP+NAA	30	3.1±0.6	+++	40	3.5±0.4	+++
BAP+2,4-D	20	—	++	40	—	++
Kn+IAA	70	1.8±0.7	+++	80	3.3±0.3	+
Kn+IBA	60	2.6±0.6	+++	80	2.9±0.4	+
Kn+NAA	40	1.3±0.5	++	50	2.3±0.6	++
Kn+2,4-D	20	—	++	40	—	++

[a]All the treatments contained Murashige and Skoog's (MS) basal salts with 3% (w/w) sucrose.
[b]Data recorded after 3 weeks and represents an avg. of 10 replicates per treatment with two parallel experiments.
[c]+ shoot induction; ++ moderate callusing with nodules; +++ profuse callusing with shoot buds; ++++ multiple shoot differentiation.

photoperiod provided by cool white-flourescent tubes (Philips India). The healthy plantlets with 4-5 nodes and well developed roots were removed from the culture tubes, washed under running tap water to remove the agar and transferred to sterilized garden soil or soilrite in small polypots. The potted plants were maintained inside an intermittent mist chamber under high humid conditions (80-90% RH) for 3 weeks and irrigated with ¼ strength Knop's nutrient solution. Once acclimatized, the plants were transferred to the green house.

Sprouting response of shoot proliferation from nodal and shoot tip explants cultured on MS basal medium supplemented with 1mg l^{-1} of BAP and Kn with IAA, NAA, IBA and 2,4-D as shown in Fig. 1 is summarized in Table 1. Shoot tip explants showed better response than the nodal segments. Combination of BAP and IBA showed substantial increase in the frequency of responding shoot tip explants. The maximum shoot production (5-6 per culture) was obtained on MS medium supplemented with BAP (1 mg l^{-1}) combined with IBA (1mg l^{-1}) after 40 days. Shoot cultures were further multiplied by culturing of nodal segments excised from the primary *in vitro* formed shoots on MS medium containing a combination of BAP 1mg l^{-1} + IBA 1mg l^{-1} (Fig.2). Callusing also appeared at the base of the shoots during repeated subculturing after every six weeks. Percent shoot development as well as number of shoots per node retained the highest values 90% (5 to 6 shoots per node) during the three culture passages. Shoots induced on MS medium with BAP 1mg l^{-1} + IBA 1mg l^{-1} failed to elongate rapidly. Therefore, young micro-shoots were excised and transferred to RT medium supplemented with IAA alone. Maximum shoot growth in terms of shoot length (ca. 20 cms) and number of nodes/shoot was obtained in RT medium supplemented with 1mg l^{-1} IAA (Fig.3). The excised elongated shoots of (6-8 cm) in length showed 80-100 % rooting in the various treatments upon transfer to rooting media (Table 2). The best rooting response in terms of length and number was obtained in full RT medium with IBA 1mg l^{-1} (Fig.4). On replacing IBA with NAA or IAA there was less or no rooting on half strength and full strength MS, RT and B_5 (9) medium. The present results indicate that requirements of growth hormones for the differentiation of shoots or roots or both is very precise and well defined as reported for *A. belladonna* and other medicinal plant species belonging to family Solanaceae (10,11) A success rate of 80% was achieved in transplanting of rooted regenerants with 4-5 nodes to small pots (Fig.5). The plantlets attained a height (30-35 cm) within 60 days of transplantation and grow well in the green house at 18 ± 2°C (Fig. 6). The observations on various morphological characters such as branching pattern, leaf shape, petiole length and area of leaf lamina did not show any significant variation as compared to parent



Figs. 1-6. *In vitro* propagation of *Atropa acuminata*. Shoot bud and shoot proliferation from cultured explants (**1-2**). Well elongated multiple axillary shoot proliferation on RT medium + IAA, 1 mg l^{-1} (**3**). Rooting of shoots in RT basal medium + IBA, 1 mg l^{-1} (**4**). Acclimatization of *in vitro* developed plantlets (**5**). Established hardened plants in green house in soil ready for field transfer (**6**).

plant, indicating that multiplication was true-to-type. Low temperature conservation of shoot cultures is an effective method for long term culture storage (12). Shoot cultures of *A.acuminata* were cold stored at 10°C for 8 months with good results. Stored shoot cultures continue to grow slowly and developed etiolated shoots which quickly turned green and resumed normal growth when retrieved from storage.

Table 2. Effect of various media treatments on *in vitro* rooting in *Atropa acuminata*

Media[a] +IBA (mg l^{-1})	Rooting %	Root number/ shoot (± SE)	Root length (cm ± SE)	Root morphology
½MS[b]+(0.5)	15	4.5+0.7	4.2±0.4	A
½MS+(1.0)	15	5.0+0.3	4.0±0.6	A
MS+(0.5)	20	5.6±1.5	4.8±0.7	A
MS+(1.0)	20	5.6±1.0	5.0±0.4	A
½RT[c]+(0.5)	60	20±1.2	3.0±0.6	A
½RT+(1.0)	60	25±3.0	4.0±1.2	A
RT+(0.5)	60	25±6.0	6.0±0.7	A
RT+(1.0)	100	30±3.0	10.0±0.2	B
½B5[d]+(0.5)	50	25±3.0	4.8±1.8	C
½B5+(1.0)	50	28±4.1	4.1±0.3	C
B5+(0.5)	80	40±6.2	5.5±0.4	C
B5+(1.0)	100	60±7.0	6.2±0.2	C

[a]All treatments contained 3% (w/w) surcrose.
A=Thin fibrous, B = Thin elongated well developed, C = Thick small roots.
[b]MS = Murashige and Skoog basal medium; [c]RT = Revised Murashige and Skoog (Khanna and Staba, 1968) medium, [d]B5 = Gamborg-B5 medium.
Data recorded after 3 weeks and represents an average of 20 replicates per treatment.

In vitro techniques are now successfully applied to a range of threatened and endangered medicinal and aromatic plant species for multiplication and conservation (13-14). The present work describes an efficient multiplication and conservation system for *A. acuminata* using *in vitro* techniques. This system can be adopted for clonal propagation of this medicinally important plant species which otherwise figured as threatened. Further, the plant is heterogeneous due to cross pollination and there is considerable variation in growth and alkaloid content (15). In this context, the presently developed micropropagation system holds promise to maintain clonal uniformity, which is highly desirable from pharmaceutical point of view.

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