IN VITRO SHOOT MULTIPLICATION RESPONSE IN SHOOT TIPS OF *ATROPA ACUMINATA* ROYLE ON THREE DIFFERENT NUTRIENT MEDIA - A COMPARATIVE STUDY

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ABSTRACT

In vitro raised shoot tips of Atropa acuminata were cultured on three different nutrient media viz; Murashige and Skoog's medium, Gamborg's medium, and White's medium to score better shoot multiplication response for effective micropropagation. These media were supplemented with same concentrations of BAP ranging from 2.5µM to 20µM.Multiple shoot regeneration was observed on both MS and Gamborg's medium while no response was recorded when explants were cultured on White's medium. Hence low salt medium was not found to be favourable for the growth of the explants.MS was found to be a superior medium for shoot multiplication.

Keywords: *Atropa acuminata*, shoot tips, multiple shoots, nutrient media.

Abbreviations: MS- Murashige and Skoog; B5 – Gamborg' medium; BAP- 6 benzyl-amino purine.

INTRODUCTION

Medicinal plants are now recognized throughout the world as an important component of natural resources of the respective countries. For all practical purposes, medicinal plants are no different from the other economically important species, whether occurring in the wild or cultivated. They are subject to the same risks and need the same degree of protection as other plant resources. There has been a rapid decline in the biodiversity of the world, more particularly during the past two decades or so. Biodiversity losses have been alarming in the developing countries in the tropics and occur due to habitat destruction, over harvesting, pollution, inappropriate and often accidental introduction of exotic plants. Habitat destruction is often related to development projects like land conversion, construction of dams, etc. It is also lost due to sudden natural calamities like floods, cyclones, earthquakes, etc. Conservation of Biodiversity is one of the paramount concerns the world over.

Herbal medicine has been virtually rediscovered in recent years. The renewed interest in herbal medicine is likely to continue due to increasing population and better affordability. Fresh market demands have not only brought in newer opportunities for the herbal drug industry, but are also posing threats to the phytoresources, especially in the developing economics. There is therefore an urgent need to conserve the genetic diversity of medicinal plant resources. With so much of herbal raw materials and finished products being consumed across the world in recent years, there is a greater need today than even before in ensuring that they are safe, efficacious and non – toxic.

In vitro culture techniques have been employed on a number of instances for example, to obtain virus free plant material (Drew *et al*., 1992) to regenerate recalcitrant

woody species or species which do not set seeds (Malamug *et al*., 1991; Vieitez *et al*., 1994), to establish rapid and effective micropropagation protocols (Green and Rhodes, 1982; Morte *et al*., 1992). Recently, particular attention has been paid to *in vitro* techniques as a means to preserve endangered species (Bramwell, 1990; Fay, 1992).

The genus *Atropa* commonly known as Deadly Nightshade and locally known as Meit brand belongs to family solanaceae. In India, it grows wild in fir forests of western Himalaya from Kashmir to its adjoining areas in Shimla at an altitude of 2,100 to 3,500 m above sea level. It has been successfully cultivated in Kashmir as a perennial crop and as a rabi or winter crop at a few places in the plains. In Kashmir Valley, it is occasionally found in Karnah, Gurez, Lolab, Gulmarg and Kishtwar whereas in Sonamarg and Kargil its distribution is rare (Kaul, 1997). The temperate climate is ideally suited for its perennial behaviour. The principal alkaloid in the leaves and the roots of the plant is hyoscyamine and atropine which are used in many pharmaceutical preparations. Roots and leaves are commercial sources of drug which is considered to be anti asthmatic, anti spasmodic, anodyne, diuretic, febrifuge, narcotic and nervine sedative. Roots are employed chiefly for external applications on rheumatism, neuralgia and inflammations etc. In ethno-medicinal uses in the Himalaya root paste is applied on boils and leaves are used in sedation (Kaul, 1997). 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). It is classified as threatened non – endemic plant of Kashmir (Dar *et al*., 2002) and a threat status of Endangered (E) has been assigned to this herb by Kaul, (1997) and Dar *et al*. (2002). However, threat status of critically endangered in north west Himalaya has been attributed to this herb by Ved *et al*. (1998). Realizing the threat of extinction there is a need to develop quick propagation protocols for conservation strategies.

MATERIAL AND METHODS

In vitro grown shoot tips of *A. acuminata* were used as explants. These shoot tips were cut into 1cm segment in previously autoclaved petridishes under laminar air flow cabinet and were aseptically inoculated on three different nutrient media viz; MS medium (1962), B5 medium (1968) and White's medium (1963). These media were enriched with same concentrations of BAP ranging from 2.5µM to 20µM. The pH of the medium was adjusted to 5.5 before gelling the medium with 0.8% Difco- bactoagar. This was followed by dispensing of the medium into culture vials capped with non- absorbent cotton plugs. The medium was sterilized by autoclaving at 15 lb pressure and temperature 121º C, for 15 – 20 minutes before inoculations were performed. These cultures were then maintained at 25 ± 3º C with 55 – 65 % relative humidity and exposed to 16 hour photoperiod provided by cool fluorescent tubes (3000 lux).

RESULTS

Effect of different nutrient media supplemented with same concentrations of BAP (2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20 µM) on induction of shoot number, their length, percent response and degree of callus formation from shoot tips of *A. acuminata* is summarized in Table 1. A comparative response of shoot multiplication induction from the shoot tips on two different nutrient media is depicted in Fig 1. Data was recorded after a time interval of 16 weeks (Subculture after 8 weeks interval)

Shoot multiplication

(i) Response on MS medium (High salt)

Indirect multiple adventitious shoot formation was observed on all concentrations of BAP with MS medium. Callus initiation was noticed after 2nd – 3rd week of culture period from basal end of the explant. Addition of BAP (2.5µM) induced 16± 0.81 shoots per explant with 80% response. On increasing BAP concentration to 15µM shoot number increased to a maximum of 34 ± 0.47 shoots per explant with 90% response (Fig.2). However, a further increase in concentration of BAP (20µM) decreased the shoot number and only 10± 0.67 shoots per explant were observed with only 70% response (Fig.4). Hence optimum concentration for maximum shoot multiplication and high percent response was found to be BAP (15µM) using MS medium – a high salt medium.

(ii) Response on B5 medium (Medium salt)

Multiple adventitous shoot formation was again observed on all concentrations of BAP with B5 medium. Callus induction was noticed from basal end of the explant through which multiple shoot induction was registered. BAP (2.5µM) favoured 10 ± 0.34 shoot formation per explant with 80% response. On increasing BAP concentration to 15µM shoot number increased to a maximum of 31 ± 0.86 per explant with 90% response (Fig.3). However, still higher concentration of BAP (20µM) decreased the shoot number and only 8 ± 0.37 shoots per explant were observed with 60% response (Fig.5). Here again optimum concentration for maximum shoot multiplication was found to be BAP (15µM) in B5 medium – a moderate salt medium.

(iii) Response on White's medium (Low salt)

Shoot tips when cultured on White's medium with various BAP concentrations as used in other media showed no response. Shoot tips lost their green colour and became whitish in appearance. No callus formation and shoot induction were observed even after 8 weeks interval. No subculture could be carried out because the explants necrosed and the cultures had to be terminated.

Table 1: Effect of MS and B 5 media augmented with various concentrations of BAP on indirect multiple shoot regeneration from shoot tips of *Atropa acuminata* Royle. (Data depicted is mean of three repeated experiments)

BAP (µM)	Percent Response		Shoot number Mean ± SD*		Average Shoot length (cm) Mean ± SD*		Degree of callus formation	
	MS	B 5	MS	B 5	MS	B 5	MS	B 5
2.5	80	80	16±0.81	10±0.34	6.2±0.25	5.3±0.70	+	+
5	90	90	21±0.81	12±0.41	7.5±0.37	5.5±0.52	+	+
7.5	90	90	25±0.66	17±0.65	8.3±0.22	6±0.37	+	+
10	90	90	27±0.49	21±0.81	9±0.26	7.4±0.28	++	++
12.5	90	90	29±0.75	24±0.82	12±0.32	9.5±0.61	+++	+++
15	90	90	34±0.47	31±0.86	15±0.45	12±0.35	+++	+++
17.5	70	70	19±0.69	14±0.69	8.5±0.61	7.5±0.38	+++	+++
20	70	60	10±0.67	8±0.37	6±0.37	5.0±0.18	+++	+++

*Mean ± SD: 10 replicates/ treatment.

[a] Data scored after 16 weeks of culture period.

(Subculture after 8 weeks interval)

(+) low callus; (++) moderate callus; (+++) high callus

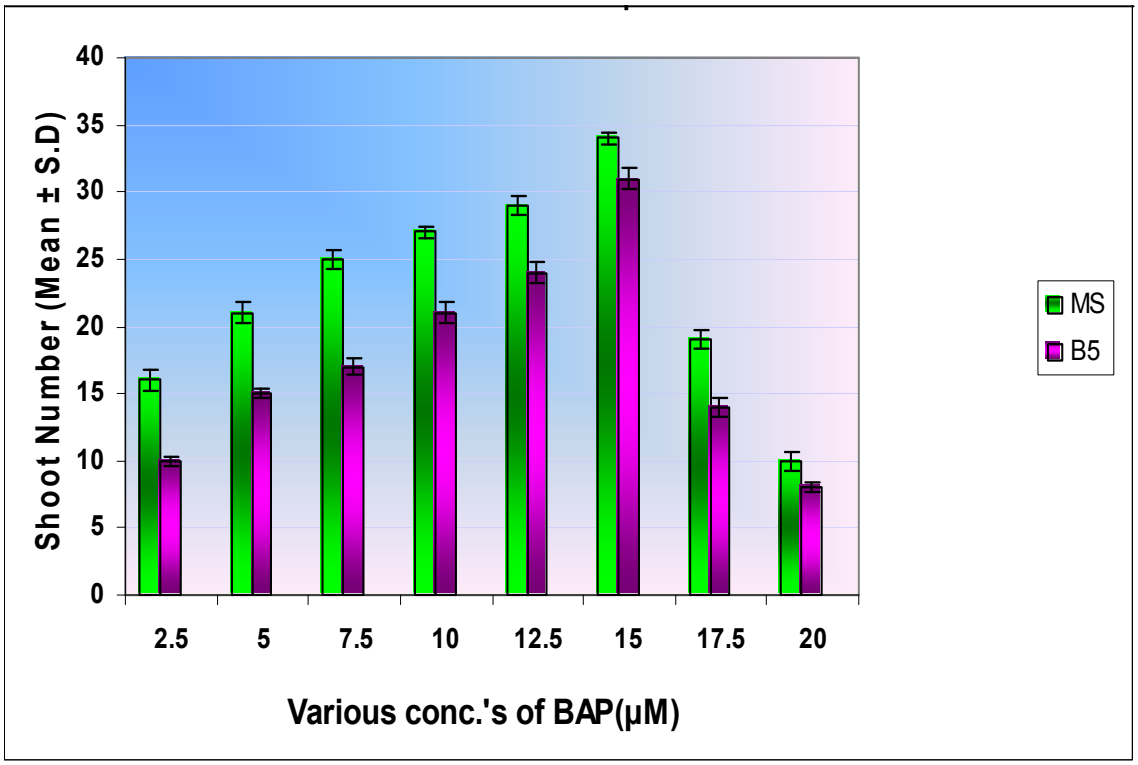

Fig. 1. Effect of MS and B5 media on shoot tips of *A. acuminata*

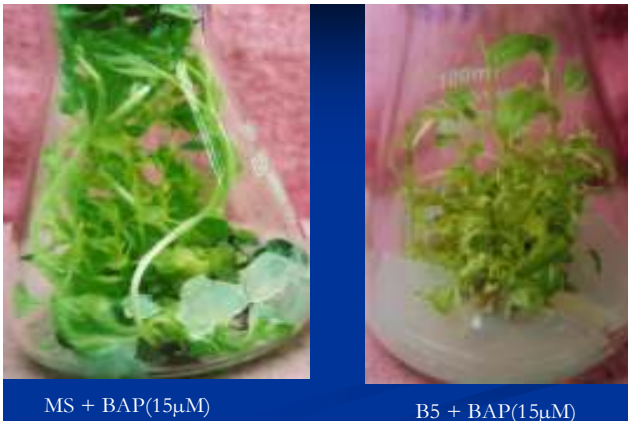

Fig 2

Fig 3

Fig 4.

Fig. 5

Fig. 2-5. Shoot multiplication in *Atropa acuminata*
Fig. 2. Indirect multiple adventitious shoot formation on MS+BAP (15µM)
Fig. 3. Indirect multiple adventitious shoot formation on B5+BAP (15µM)
Fig. 4. Indirect multiple adventitious shoot formation on MS+BAP (20µM)
Fig. 5. Indirect multiple adventitious shoot formation on B5+BAP ((20µM)

DISCUSSION

The main objective of this study was to explore the possibility of raising an effective shoot number for micropropagation protocol. In the present study, three different nutrient media viz. MS, B5 and White's media supplemented with similar concentrations of BAP ranging from 2.5 to 20µM were used. The regenerants were recovered indirectly through callus only on MS and B5 media. Multiple shoots were recorded on all concentrations of BAP in both MS and B5 media, however maximum indirect multiple adventitious shoots (34 and 31 respectively) were observed on both MS and B5 media supplemented with 15µM of BAP. Shoot number was found to be decreasing with still higher concentrations of BAP. Such results are quite similar to the earlier reports of Benjamin *et al*. (1987) and Zarate *et al*. (1997) who also reported decreased shoot number with increased concentrations of BAP in *Atropa belladonna* and *A. baetica* respectively Similar results were again registered by Sen and Sharma (1991), Rani and Groover (1999) and Ray and Jha (2000) in *Withania somnifera*- a member of solanaceae.The two media differ slightly in favouring induction of shoot number and shoot length whereas percentage response and degree of callus formation was nearly similar. Creamish white semi compact callus formation was noticed at the basal cut ends of the explant which is in agreement with Toth *et al.* (2000) in *A. belladonna.*

In present study shoot number had no relation with the degree of callus formation because on 12.5µM and 20µM of BAP, degree of callus formation was same but the shoot number was different. Among the three different nutrient media, MS medium was found most suitable for the overall growth and multiple shoot number followed by B5 medium. Ray and Jha (2000) also reported the similar results in case of *Withania somnifera*. No response was noticed on low salt White's medium in our study. Shoot tips lost their green colour and became whitish in appearance. No callus formation and shoot induction were observed. Hence low salt medium was not at all found to be favourable for desired results. Optimum concentration for obtaining maximum shoot number and length was found to be 15µM used with MS medium (a high salt medium).The data reveals that the protocol for achieving maximum shoot number developed for micro propagation of *Atropa acuminata* has the potential to be reproduced and utilized after refinement for large scale multiplication and conservation of this medicinal herb.

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