Effect of various BAP concentrations on plantlet regeneration of *Hyoscyamus niger* L. through nodal segments

Rumisa R. Qadri, Azra N. Kamili and A.M. Shah
Plant Tissue Culture Laboratory, Centre of Research for Development,
University of Kashmir, Srinagar-190006

ABSTRACT

To trigger the morphogenetic response, nodal segments obtained from *invitro* raised seedlings of *Hyoscyamus niger* were cultured on MS (x1/2) basal medium (1962) supplemented with different concentrations of BAP. Maximum elongated multiple shoots were obtained on medium enriched with 5 µM of BAP. Auxin supplemented MS (x1/2) medium showed root initiation and its elongation.

Keywords: Hyoscyamus niger, nodal segments, multiple shoots, plantlets

Abbreviations: MS(×½)- Murashige and Skoog (Half-salt strength); BAP- 6 Benzyl-amino purine; NAA- Napthalene acetic acid

INTRODUCTION

The genus *Hyoscyamus* commonly known as Henbane and locally known as Bazir bhang belongs to family solanaceae. The original range of distribution of *H.niger* embraced Europe, Western and Central Asia, North Africa. Nowadays, it has an almost cosmopolitan distribution because it spread as a weed, was occasionally cultivated and in many places where it was introduced even became naturalized. In India, it occurs in the Western Himalayas from Kashmir to Garhwal at an altitude of about 1,500 m to 3000m. In Kashmir valley, its distribution is rare in certain areas such as Karnah, Gurez and Lolab whereas in Gulmarg and Sonamarg it is found occasionally (Kaul, 1997). *H. niger* is classified as threatened non-endemic plant of Kashmir (Dar *et al*., 2002). Threat status of Rare (R) has been assigned to this herb in Red list of threatened flowering plants of the Kashmir Himalaya by Dar and Naqshi, 2001. However, IUCN has not listed this plant under any threat category.

Henbane is generally a biennial, less frequently an annual herb, reaching a height of more than 50 cm. The dried leaves and flowering tops collected soon after flowering of the plant constitute the drug (Jain, 1996). Principal alkaloids are hyoscyamine, hyoscine, scopolamine and traces of atropine. It is useful for relieving certain painful spasmodic conditions of

muscles, and in hysteria, coughs etc. Seeds of the plant also have medicinal properties; they are usually used as paste and applied locally on pains. It also dilates the pupil of the eye (Kaul, 1997).

Medicinal plants have a tremendous scope in health-care systems under Ayurvedic and Unani systems of medicine being locally available and having least side effects in comparison to modern synthetic drugs. But, the collection of medicinal plants on mass scale from their natural habitats is leading to a depletion of plant resources. Realizing the threat of extinction there is a need to develop conservation strategies and quick propagation protocols. Most of the conventional plant propagation methods are time consuming and labour intensive. With the advent of tissue culture technology it is now possible to produce sufficient planting material in a variety of species in limited time and space irrespective of the climatic conditions. So an attempt was made to develop a protocol for plantlet regeneration from *invitro* raised nodal segments of *H.niger* using different concentrations of BAP.

MATERIAL AND METHODS

Certified seeds of *Hyoscyamus niger* were collected from Regional Research Institute of Unani Medicines (RRIUM), University of Kashmir and thoroughly washed with detergent cedepol (0.5% v/v) and 2-4 drops of tween 20 (surfactant) under running tap water followed by final rinsing with double distilled water. Surface sterilization of seeds was achieved by using 20% sodium hypochlorite for 15 minutes and finally rinsed 3-4 times with autoclaved double distilled water to remove all traces of sterilant. Sterilized seeds were then inoculated on MS (x1/2) basal medium (Murashige and Skoog, 1962) supplemented with 3% sucrose. Procedure for sterilization of medium, pH adjustment and incubation etc remains the same as reported in our earlier communication (Qadri *et al.*, 2005). Normal seed germination and seedling formation was recorded on 8 weeks of culture period. From these seedlings nodal segments were excised for culture purposes.

RESULTS AND DISCUSSION

The main objective of this study was to see the possibility of developing an efficient and reproducible protocol for micropropagation of *H. niger* through nodal segments. Scanning of literature revealed that there is scanty published report on organogenesis of this plant species. Hence, some of the members of the family solanaceae have been taken into consideration for discussion purpose.

Cytokinin BAP was used at various concentrations in the medium. Various responses scored after using different concentrations of BAP on nodal segments of *H. niger* is summarized in Table 1. Different concentrations of BAP (1.1, 2.5, 4.5, 5, 7.5 and 10μM) stimulated direct multiple shoot initiation, proliferation and elongation within 6-8 weeks of culture period. Nodal segments when cultured on medium containing BAP (2.5μM) produced 8-9 shoots per explant with 70% response (Fig.1). On increasing BAP concentration to (5μM) shoot number increased to a maximum of 15-16 shoots per explant with 90% response (Fig. 2). However, higher concentration of BAP (10μM) decreased the shoot number and only 3-4 shoots per explant were observed with 60% response (Fig. 3). Optimum concentration for maximum shoot multiplication was found to be BAP 5μM.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 belladona* and *A. baetica* respectively. Similar results were registered by Grewal *et al.*(1979) in *Hyoscyamus muticus* and Sen and Sharma (1991), Rani and Grover (1999) and Ray and Jha (2000) in *Withania somnifera*. Thus it is firm to believe that low BAP concentration trigger more shoot proliferation in members of family solanaceae and *Hyoscyamus niger* also being a member of the same family showed maximum shoot regeneration at BAP 5μM which becomes the optimum concentration for the shoot multiplication from the nodal segments of the plant.

Table 2 summarizes the results of rooting trials of the isolated elongated shoots of the plant. For rooting of these isolated shoots MS (x1/2) basal medium or medium supplemented with different concentrations of NAA (2.5, 4, and 5μM) were used. On MS (x1/2) basal medium very thin, long thread like multiple roots were regenerated with 80% response (Fig. 4). These results are quite similar to the earlier reports of Zarate *et al.*, 1997 and Ahuja *et al.*, 2002 in case of *Atropa baetica* and *Atropa acuminata* Direct multiple root regeneration was also observed on different concentrations of NAA. Best rooting was achieved on 4μM NAA. Roots were very thick and long and number of roots per explant was 5-6 with 100% response (Fig. 5) whereas roots recorded on 2.5μM NAA were not so thick but the number of roots per explant was as high as 14 -15 per shoot with 90% response (Fig. 6). However, on 5μM NAA roots produced were similar to that obtained on MS (x1/2) basal medium with 100% response. Similar results were recorded by Eapen *et al.* 1978, Kamat *et al.* 1978 and Lorz *et al.*,1979 in *Atropa belladonna*, *Solanum melongena* and *Hyoscyamus albus* respectively who also observed NAA best for rooting. The results of the paper reveal that the protocol developed for micropropagation of *Hyoscyamus niger* has the potential to be reproduced and utilized after refinement for large scale multiplication and conservation of this medicinal herb.

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Fig. 1-6 Plantlet regeneration in *H. niger*
Fig. 1. Direct shoot multiplication on MS (x ½) + BAP(2.5µM)
Fig. 2. Direct shoot multiplication on MS (x ½) + BAP(5µM)
Fig. 3. Direct shoot multiplication on MS (x ½) + BAP(10µM)
Fig. 4. Direct multiple root regeneration on MS (x ½)
Fig. 5. Direct multiple root regeneration on MS (x ½) + NAA (4µM)
Fig. 6. Direct multiple root regeneration on MS (x ½) + NAA (2.5µM)

Table 1. Effect of different concentrations of BAP on multiple shoot regeneration from nodal segments of *Hyoscyamus niger* L.

MS(x1/2) + BAP (µM	Response	Percentage Response	Shoot number Mean ± SD *	Average shoot length (cm), Mean ± SD
1.1	No response	-	-	-
2.5	Direct shoot multiplication	70	9 ± 1.1	2.2±0.2
4.5	Direct shoot multiplication	70	12 ± 1.4	3.2±0.2
5.0	Direct shoot multiplication	90	16 ± 1.5	4 ± 0.7
7.5	Direct shoot multiplication	60	6 ± 0.6	3.6±0.2
10	Direct shoot multiplication	60	4 ± 0.6	3.7±0.2

*Mean ± SD : 10 replicates/ treatment.
Data scored after 8 weeks of culture period.

Table 2. Effect of different concentrations of NAA on root regeneration of *H. niger* L.

Growth media and phytohormones (µM)	Response	Percentage Response	Number of roots/ shoots
MS(x1/2)	Direct root regeneration; roots very thin and long.	80	Numerous
MS(x1/2) + NAA (2.5)	Direct root regeneration; roots thick and long	90	14 15
MS(x1/2) + NAA (4)	Direct root regeneration; roots very thick & long	100	5-6
MS(x1/2) +NAA (5)	Direct root regeneration ; roots thin and long	100	Numerous

Data scored after 8 weeks of culture period.

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