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RESEARCH ARTICLE

HIGH FREQUENCY OF MULTIPLE SHOOT INDUCTION IN *Datura metel* L. USING DIFFERENT CONCENTRATIONS OF BAP

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ABSTRACT

Nodal explants were inoculated with basal cut surface down on medium MSB with BAP. The different concentrations BAP ranging from 0.5, 1, 1.5, and 2 mg/lit, were used for obtaining multiple shoots. After 45 days maximum number of multiple shoots were obtained on medium containing 2.0mg/ lit of BAP which was approximately 88 / culture. In the present study 2.0mg/ lit of BAP concentration was found to be the ideal concentration for high frequency of multiple shoots induction. This is the first report of such high frequency of multiple shoot induction. Maximum hyoscyamine 0.0390 mg/g DW and scopolamine 1.660 mg/g DW content was found at 2 mg/lit BAP. Minimum hyoscyamine and scopolamine content was found at 1 mg/lit BAP (0.0164 and 1.154 mg/g DW respectively). We found that difference in content of hyoscyamine and scopolamine was also affected by concentration of BAP i.e. increased concentration of BAP and increased number of multiple shoots showed correlation with increased concentration of hyoscyamine and scopolamine.

INTRODUCTION

The genus *Datura* produces great range of tropane alkaloids, and two of them are hyoscyamine and scopolamine which are important for pharmaceutical industries[1, 2]. World consumption of scopolamine is several folds higher than that of hyoscyamine mainly due to the fact that scopolamine is used as starting material for semi-synthesis of several important drugs[3]. Tropane alkaloids have significant medicinal importance as they are compounds with a variety of pharmacological effects on some human organs such as eyes, nerve system, heart, blood circulation and respiration[4]. Tropane alkaloids inhibit the muscarinergic acetylcholine receptors and show parasympatholytic properties. As such they are used in medicine to treat spasms to sedate patients and for dilation (mydriasis) of pupils. Furthermore tropane alkaloids affect neuronal activities and are known hallucinogens[5]. Hyoscyamine and scopolamine are employed in treatment of gastrointestinal spasm, and particularly in the case of scopolamine as preoperative medicine and in the prevention of motion sickness[6]. The Tropane alkaloids Hyoscyamine[7] and Scopolamine are used in medicine for its anticholinergic activity[8]. Scopolamine is the most valuable tropane alkaloid, preferred for its higher physiological activities and fewer side effects. The world demands for these alkaloids are estimated to be about ten times greater than for hyoscyamine and its recemic form atropine[8].

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According to Nithiya and Arockiasamy[9] indiscriminate collection of plants of *Datura metel* L. has resulted in present sparse distribution of this highly prized medicinal plant species. Since all plant parts are medicinally useful the whole plants are uprooted from the natural population for medicinal use. This plant species is becoming rare in its natural habitat and warrants urgent conservation measures. The two alkaloids hyoscyamine and scopolamine are still extracted from plants as their chemical synthesis is expensive and time consuming. Therefore, their production through biotechnological means is an alternative worth exploring[10]. Obtaining these alkaloids through *in vitro* culture technique remains the focus of considerable research[11]. It is considered a promising direction for industrial-scale production. *In vitro* culture of plant cell or tissue presents a number of advantages over traditional culture[12]. Obtaining hyoscyamine and scopolamine through *in vitro* culture technique is an interesting alternative, since it would guarantee a stable and uniform year-round supply, independent from seasonal variations of field grown plants[7]. In this work we presented high number of multiple shoots production in *Datura metel* L. shoot culture. To our best knowledge it is the first report of such high number of multiple shoots production in this valuable medicinal plant.

MATERIAL AND METHODS

Collection of plant materials: Plant materials for the tissue culture experiments were collected from in and around Pune University area. Seeds were also collected from Pune, Satara,

and Sangli Districts which were grown in Botanical garden of Dept. of Botany, University of Pune.

Sterilization of Explants: For sterilization nodal explants were kept under running water for 30min.with 2-3 drops of Tween-20 followed by a wash of 70% alcohol (v/v) for 10 sec. Then explants were sterilized with 0.1% $HgCl_2$ (w/v) for 3 min. After the treatment of 0.1% $HgCl_2$ the explants were washed three times with sterile distilled water to remove the traces of $HgCl_2$.

Medium used: Surface sterilized explants were inoculated on Murashige and Skoog's medium[13]. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH / HCl before addition of 0.8% agar. Medium was autoclaved at 121ºC at 15 lbs for 20 min. The cultures were incubated at 25 ± 2ºC under photoperiod 16/8 h (light/dark). The light source used was cool white florescent tubes providing an illumination of 2000/lux /m^2 /s.

Inoculation of Nodal Explants: Nodal explants were inoculated with basal cut surface down on medium MSB with BAP. The different concentrations of BAP ranging from 0.5, 1, 1.5, and 2 mg/lit, were used for obtaining multiple shoots. After four weeks multiple shoots was on high number and were subcultured on the new medium of same combinations.

Extraction of alkaloids: The obtained multiple shoots were also checked for the alkaloid content. Alkaloids extractions were carried out using modified method of Berkov [14]. Fresh weight of multiple shoot were taken and dried at 50ºC and take it's dry weight. Powder of dried sample was made and in this sample10 ml of 3%H_2SO_4 was added. Extract were sonicated for 10 min at 33 KHz. Extract was heated at 40-45ºC for 60 min. Extract was filtered using Whatman Filter Paper No. 1 and filtrate was made alkaline using 20 % NH_4OH and pH was adjusted between 9-10. To this filtrate dichloromethane was added in separating funnel and mixed well. Organic phase from separating funnel was collected and kept on water bath at 60ºC until dry residue was obtained. Obtained residue was dissolved in methanol and mixed well using vortex mixer. After mixing it was kept overnight before using for further analysis.

Qualitative methods for Identification of Tropane alkaloids: Thin layer chromatography (TLC) was used for qualitative identification using solvent system Chloroform: Methanol (8:2). The TLC plate was removed from TLC chamber after the solvent had traveled up to 10 cm. and sprayed with Dragendroff locating reagents. Orange color represents presence of alkaloids and background will change to cream color. This was compared with Rf of Std. (Sigma) Hyoscyamine and Scopolamine.

Quantitative method for estimation of tropane alkaloids: Quantification of Hyoscyamine and Scopolamine content was done by spectrophotometric analysis. The cell suspensions grown in different media were used to harvest cells and the biomass was assessed in terms of Fresh weight (FW) and Dry weight (DW). The alkaloid extraction was done as described previously. Std. Hyoscyamine and Scopolamine (Sigma) were used at different concentrations and quantification of experimental samples was carried out using std. graph prepared by using standard of Hyoscyamine and Scopolamine.

RESULT AND DISCUSSION

In order to induce multiple shoots in *D. metel* L. the nodal sectors were cultured on media containing different concentrations of BAP (0.5 -2 mg/lit). After 45 days maximum number of multiple shoots were observed on medium containing 2 mg /lit of BAP which was approximately 88 per culture (Table 1, Figure I, A and B**).** In the present study 2 mg/lit of BAP concentration was found to be the ideal concentration for high frequency of multiple shoots induction (Table 1) (Figure I, A and B). This is the first report of such high frequency of multiple shoot induction. Maximum hyoscyamine 0.0390 mg/g DW and scopolamine 1.660 mg/g DW content was found at 2 mg/lit BAP. Minimum hyoscyamine and scopolamine content was found at 1 mg/lit BAP (0.0164 and 1.154 mg/g DW respectively) (Table 1). In related genera like *Solanum viarum* a combination of 8 mg/ 1 2 ip + 1 mg/lit IAA and 5 mg/lit BAP + 0.5 mg/lit IAA [15] and in *S. trilobatum* 5 mg/lit BAP + 0.5mg/lit IAA[16] were reported to be the most suitable concentration for induction of multiple shoots. This supports our observations. Moreover, the size and physical appearance of shoots formed on each medium did not show any difference except the number of multiple shoots and alkaloid concentration. The number of multiple shoots formed after 30 days differed for all concentrations, suggesting that during this period increasing time period and increasing amount of BAP has effect upon shoot multiplication.

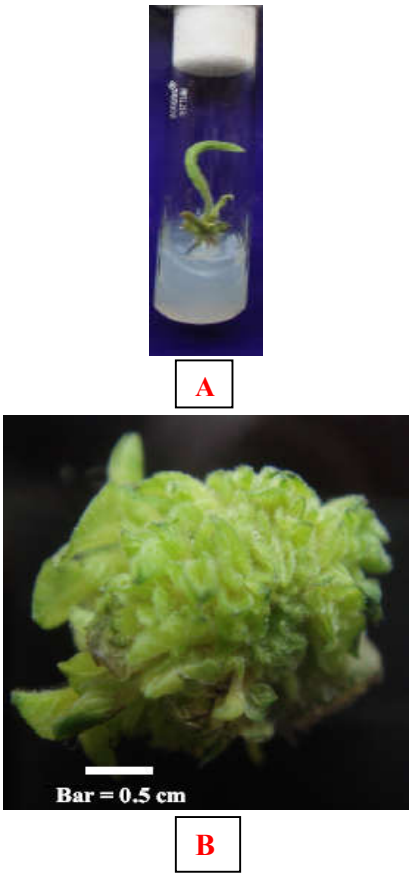

Fig. 1. A – Multiple Shoots induction on medium containing 2.0 mg/ lit BAP. B – Multiple shoots magnified (Bar = 0.5 cm)

Table 1. Multiple Shoot induction and alkaloid concentration in *D. metel* L. on different concentrations of BAP

No	Concentration BAP mg /lit	No of Multiple shoots induced	Hyoscyamine (mg / g DW)	Scopolamine (mg /g DW)
1	0.5	51 ± 0.81	0.0164 ± 0.0003	1.154 ± 0.048
2	1	59.66 ± 1.69	0.0174 ± 0.0002	1.317 ± 0.001
3	1.5	71 ±2.44	0.0293 ± 0.0002	1.546 ± 0.016
4	2	88.33 ± 2.49	0.0390 ± 0.0004	1.660 ± 0.035

*All results are mean of 3 observations ± S.D.

Previous report on *Atropa baetica*[17] indicated that higher number of shoots produced on medium containing 2.0 mg/lit BAP at 24 days. This result showed similarity with our result. In that, concentration of BAP is same in both experiment and it is 2.0 mg/lit BAP, but there is difference in number of multiple shoots. In our experiment we obtained maximum 88.33 shoots (Table 1) (Figure I, B) while Zarate[17] obtained only 3.4 shoots. Zarate[17] also concluded that increasing the concentration of BAP from 0.75-2.0 mg/lit did not result in higher number of shoots. Here we obtained different results as compared with Zarate[17]. Increasing concentration of BAP showed effect on increase in number of multiple shoots in culture (Table 1). Dos Santos[18] presented *in vitro* propagation system for *D. insignis*. They cultured nodal explants on MS supplemented with either BA alone or in combination with 2, 4-D, IAA and best result was found on medium containing 1 mg/lit BAP. It also differed from our results in that we obtained best results on 2 mg/lit BAP concentration (Table 1). We obtained highest hyoscyamine and scopolamine content on 2 mg/lit and it was 0.0390 and 1.660 mg/g DW respectively (Table-1). We found that difference in content of hyoscyamine and scopolamine was also affected by concentration of BAP i.e. increased concentration of BAP and increased number of multiple shoots showed correlation with increased concentration of hyoscyamine and scopolamine. This is the first report of high number of multiple shoots induction and alkaloid production in *Datura metel* L.

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