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In-vitro Propagation of *Datura innoxia* from Nodal and Shoot Tip Explants

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Abstract The present paper describes a prime and easy-to-use protocol for large scale production of plantlets through shoot tip culture of *Datura innoxia*, the plant having phytoremediation potential and the method is useful for the ex-situ conservation of other phytoremediational important species. A simple micro-propagation method from nodal and shoot tip explants were reported here for Datura innoxia, at contaminated site nearby Bhopal. This plant is having considerable high phytoremediation potential. There are only few research papers about the plant regeneration from nodal segments of *Datura innoxia.* Therefore, by considering the pharmaceutical and phytoremediation importance of this plant, it is necessary to provide efficient tissue culture protocols for it. The plant regeneration from nodal segments is considered to be one of the most promising ways for multiplying a selected variety true to its type showing the same agronomic characteristics. Here multiple shoots were induced in vitro from the stem nodal and shot tip segments on Murashige and Skoog (MS) medium containing 6-benzylaminopurine (BAP) alone or in combination with naphthalene acetic acid (NAA) and kinetin (KIN). High frequency of micro shoots were obtained from the explants on MS medium supplemented with various concentrations of BAP (0.1 to 1.0mg/l) along with NAA. The nodal explants produced the highest number (3 per shoot) of shoots per culture with a mean length of approximate 4.5cm. The shoot tip explants produced maximum number of (6 to 7) shoots per culture on the same medium, average length of the in vitro shoots being approximate 4 cm. Moreover, the nodal segment, which had a pre-existing meristem, is suitable for clonal propagation because it is easily manipulated, has a high proliferation rate and maintains clonal fidelity. This tissue culture protocol helps to regenerate large number of *Datura innoxia* plantlets having hyper accumulator properties for heavy metals.

Keywords: *Datura innoxia, MS Media, shoot tip culture, nodal explants, in-vitro, phytoremediation, heavy metal contamination, urban regeneration*

1. Introduction

A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil.

Industrial development is an ongoing phenomenon in developing countries like India, but many industries produce harmful wastes and discharge directly in to land. Most of our industries are at the preliminary phase and cannot manage to pay for as invest in waste matter management and pollution control due to little income edge. Enormous number of contaminants and waste materials containing heavy metals are disposed into the environment.

Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem. A wide range of inorganic and organic compounds cause contamination, these include heavy metals, combustible and putrescible substances, hazardous wastes, explosives and petroleum products. Major component of inorganic contaminates are heavy metals they present a different problem than organic contaminants.

There are only few reports about tissue culture of Datura. Therefore, by considering the phytoremediation importance of this plant, it is necessary to provide efficient tissue culture protocols for it.

Datura, is a genus of nine species of *vespertine* flowering plants belonging to the family *Solanaceae*. They are known as Angel's Trumpets, sometimes sharing that name with the closely related genus *Brugmansia*.

There are only few reports about tissue culture of Datura. Therefore, by considering the pharmaceutical importance of this plant, it is necessary to provide efficient tissue culture protocols for it.

2. Materials and Methods

The technique involves the isolation, inoculation and regeneration of plant cells, tissues, organs under controlled conditions in culture vials, containing synthetic

nutrient medium. Both the chemical compositions of the medium and the controlled environmental conditions (light, temperature, humidity, aeration etc.) effectively control the expression of any genotype or phenotype potential in the explants.

Murashige and skoog's (1962) nutrient medium was used throughout the experiment. In addition, media were supplemented with growth regulators, other additional vitamins, organic supplements and carbon sources.

2.1. Plant Material

Bhopal is sitting a top a highly toxic sludge underground. There are many hazardous waste sites in industrial area in Bhopal. Hazardous waste may contain environmental contaminants such as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water

Datura innoxia plants were brought from industrially contaminated site at Bhopal.

2.2. Explants and Surface Sterilization

Small tender twigs were collected from contaminated sites, cut into 0.5-1.0cm nodal segments and used as explants for the induction of multiple shoots. Explants were washed thoroughly under running tap water for 15 min and then were surface-sterilized by antifungal agent, soap solution and HgCl2 solution and washed thrice with sterile distilled water.

2.3. Culture Medium and Conditions for Plant Regeneration

Under a laminar flow, cabinet explants were inoculated aseptically on MS (Murashige and Skoog, 1962) medium supplemented with various concentrations of 6- Benzyl amino purine (BAP) alone or in combinations with naphthalene acetic acid (NAA) and kinetin (KIN) . All media were adjusted to pH 5.8, and 0.8% agar and 30g l^{-1} sucrose were added. About 15ml of the medium were dispensed in each culture bottle and sealed with plastic cover before autoclaving at 121℃ for 15min under pressure of 15 Pis. The media were left to cool as slant in the culture room until use. All cultures were maintained at 16hr light of 1000lux using fluorescent lamps at 25 ± 2 ℃. Results were observed at regular intervals and data were collected from three independent experiments and presented as average.

2.4. Effects of Basal Medium Strength on Multiple Shoots Induction

In evaluations on the abilities of different basal media to support shoot culture establishment, full and half strength of either MS or B5 (Gamborg et al. 1968), salts and their combination (full MS salt and B5 vitamins) were supplemented with 0.5mg/L BAP in combination with 0.5mg/l NAA.

2.5. Shoot Induction Experiment

The effect of season, age of explant and the effect of various cytokinins on initiation of shoots were studied simultaneously. For these studies the nodal explants were inoculated on Murashige and Skoog (1962) basal medium supplemented with cytokinins like BAP and kinetin KIN, in the concentration of (0.5-2.0) mg/l alone or in combination with other cytokinins of each, containing sucrose 30g and gelled with agar 4g/l. In addition auxins like IAA or NAA (0.1-1.0 mg/l) were used for promoting the shoot induction.

2.6. Medium Used in Shoot Initiation

- Medium 1 MS + 0.1 mg/l BAP
- Medium 2 MS + 0.3 mg/l BAP
- Medium 3 MS + 0.5 mg/l BAP
- Medium 4 MS + 1.0 mg/l BAP
- Medium 5 MS + 0.1 mg/l KIN
- Medium 6 MS + 0.3 mg/l KIN
- Medium 7 MS + 0.5 mg/l BAP + 0.5 mg/l KIN
- Medium 8 MS + 1.0 mg/l BAP + 0.5 mg/l KIN

3. Results and Discussion

Number of experiments was carried out for initiation of shoot from nodal explants and apical meristem. The measurement of growth was taken by the percentage of, number of shoots initiated per explants and shoot length.

Table 1. Initiation Trials: Contamination and Survival by *Datura innoxia*

Species	Collection Dates	Total # Attempted	% Contaminated	% Survival
Datura Innoxia	05/07/11	58	55	80%
	02/08/11	20	60	9
	15/08/11	35	40	4
	07/09/11	25	10	54

Table 2. Effect of different concentrations of plant growth hormones on nodal and shoot tip explants of *Datura innoxia*

	Nodal Explants			Shoot Tip Explants		
PGR mg/l	% of Response	No. of Shoots in Culture	Avg. Length of Shoots (cm)	% of Response	No. of Shoots in Culture	Avg. Length of Shoots (cm)
BAP						
0.3	10	12	1-2	20	13	1-2
0.5	90	25	1-4	75	15	1-3
0.7	15	12	1-2	20	10	1-2
Kinetin						
0.3	11	10	1	12	11	1
0.5	65	20	1-3	60	14	1-4
0.7	13	10	1-2	15	9	1-2
NAA						
0.3	5	7	1	10	9	1
0.5	70	25	1-3	60	45	1-3
0.7	10	8	1-2	5	10	2

Graph showing percentage of response of nodal explants of *Datura inoxia* towards different plant growth hormones and their concentrations.

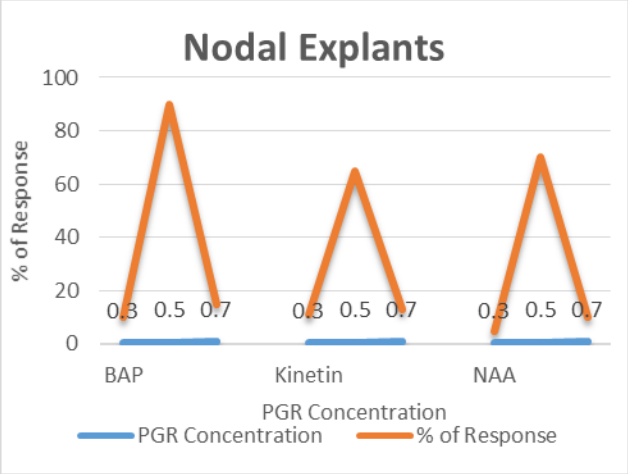


Figure 1. Graph of PGR Vs % of response of nodal explant

Graph showing percentage of response of shoot tip explants of *Datura inoxia* towards different plant growth hormones and their concentrations.

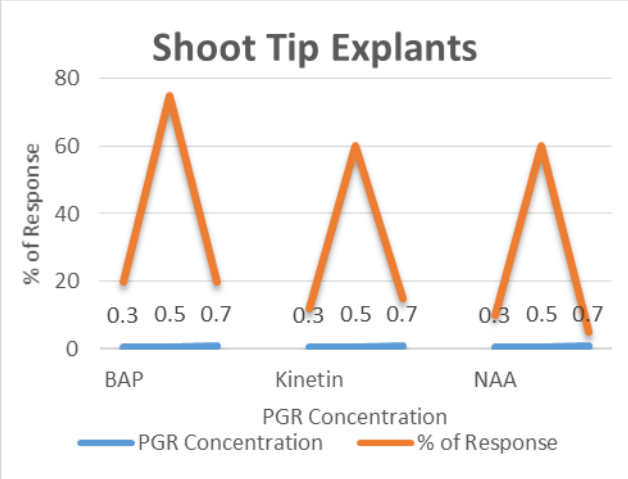


Figure 2. Graph of PGR Vs % of response of shoot tip explant

In the preliminary experiment effect of cytokinins alone or in combination were tested. Maximum (70-80%) number of bud break and initiation of shoot was reported in BAP alone (0.5-1.0mg). About 12-20 shoots were developed. The combination of BAP and Kinetin contained media, shows 70-75% of initiation was found with the formation of only one shoot with lots of callus formation.

In order to optimize a suitable medium for mass multiplication of shoots from a single initiated nodal region, the highest number of shoots was observed in the medium containing higher concentration of BAP (1.0-2.0 mg/l). These media show about 15-20 number of shoots per culture, when sub-cultured in the same fresh medium after 15 days duration. The lengths of shoots elongate 4-5cm and increases with culture duration.

Cytokinins, especially BAP, were reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation. Nodal and shoot tip explants of Datura were inoculated on MS medium supplemented with BAP,NAA and KIN at different concentrations (0.1,0.2,0.3,0.4,0.0.5,0.6 and 0.7mg/l) for the production of multiple shoots.

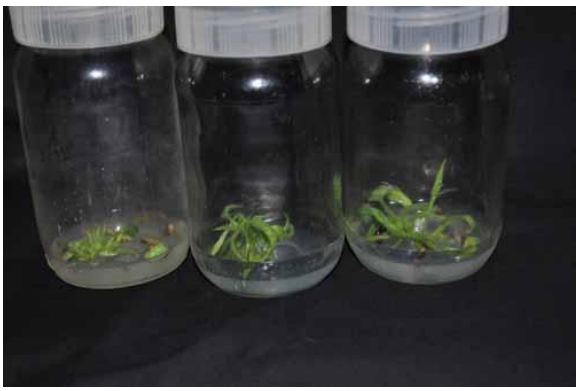
The proliferation efficiency of nodal explants from healthy plants was significantly higher than that of shoot tip explants after five to six weeks. As a supplement of 0.5mg/l BAP resulted in maximum proliferation (95%) was observed in nodal explants. The nodal explants produced the highest number (42.00 ± 4.42) (Table 1) of shoots per culture with a mean length of 9.45 ± 4.13cm. These explants were capable of directly developing multiple shoots on MS medium containing different concentrations of BAP or KIN.

Nodal explants as the best source of multiple shoot induction have also been suggested in case of other medicinal plants also. The results showed that BAP alone or in combination with NAA was more effective for shoot multiplication.

4. Conclusion

Biodiversity prospecting would lead to the discovery of wild plants that could clean polluted environments of the world. This subject is at its infancy with a great hope of commercial hype. The desire to capitalize on this new ideas need to provide strong incentives for conserving nature

Photograph 1. Initiation of shoot from shoot tip explant



Photograph 2. Initiation of shoot from nodal explant

Phytoremediation is the most powerful tool against the industrial pollution because it takes advantage of natural plant processes. It requires less equipment and labor than other methods since plants do most of the work. Trees and plants can make a site more attractive as well. In this concern *Datura innoxia* is very important plant having highest capacity of phytoremediating the soil contamination.

Tissue culture studies on Datura provide a fast culture method in the form of in vitro propagation through soot tip and nodal explants. Locally adapted tissue cultured

plants could be used for seed increase or transplantation onto degraded sites. Plant tissue cultures also offer important technical advantages compared with whole plants. Because in vitro plant cultures are grown and maintained free from microbial contamination.

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