Vol-1, Issue-2 PP. 104-109 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

Micro-propagation of *Datura inoxia* as a Heavy Metal Accumulator Plant

Mrs. Ashwini A. Waoo
Research Scholar, M.V.M.
Bhopal, M.P.,
India

Swati Khare
Professor, IEHE
Bhopal, M.P ,
India

Sujata Ganguly
Professor, M.V.M.
Bhopal, M.P.,
India

ABSTRACT

This paper gives a standardize protocol for micro propagation of *Datura inoxia* a best plant proven for phytoremediation. Thus focus was given for callus culture. For callus culture of *Datura inoxia* stem explants were cultured on M. S. basal medium (1962) containing various concentrations and different combinations of growth hormones like KN, NAA and BAP (from 0.1mg/l to 0.5 mg/l. The overall morphological responses of the stem explants indicated that the interaction of these factors leads to shoot-bud proliferation or callus formation. The better response of callus formation was obtained from the stem explants using 0.25-0.5mg/l concentrations of growth hormones.

The present study was carried out to analyze the effects of different growth regulators on formation of callus of *Datura inoxia* in different explants. For this *Datura inoxia* was cultured in M.S. medium supplemented with various concentrations of KN, NAA and BAP. Culture was incubated on (25 ± 2) oC temperatures and photoperiod of 16 hours while callus was observed on different concentration of Auxin or Cytokinin individually or in combinations. Among experimental conditions, the suitable medium for callus induction from stem explant was NAA (0.5mg/L) in alone and BAP (0.25mg/L) + Kinetin (O.25mg/L) in combinations. There are only limited research works related to tissue culture of *Datura inoxia*. Taken into account, the phytoremediation capacity of this plant, there is needed to provide efficient tissue culture protocols or micro propagation method for it.

General Terms

Biodiversity, Environmental Biotechnology, Phytoremediation, Tissue-culture.

Keywords

Auxin, Callus, Cytokinin, *Datura inoxia*, Growth hormones, Micro propagation.

1. INTRODUCTION

Biodiversity is the foundation of research in the area of biotechnology. In spite of this, there are few established techniques for exploiting its potential. The importance of biodiversity is now gaining importance for the cleanup of the metal contaminated ecosystems. Phytoremediation is a thrust research area acquiring commercial significance in the field of environmental biotechnology.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

Phytorermediation is the use of vegetation for in situ treatment of polluted soils, and water. It may be suitable for sites with shallow contamination of organic pollutants, or metal pollutants that are amenable to one of five applications: Phytotransformation, Phytostabilization, Rhizosphere Phytoextraction, Bioremediation, or Rhizofiltration. According to phytoremediation mechanism, plants are exploited as biological reactors that utilize the light energy of sun to remove water and pollutants from the soil or immobilize them. Phytoremediation is the most significant one in study of sub-lethal levels of bioaccumulated contaminants within the tissues / components of organisms, indicating the net amount of pollutants integrated over a period of time [10].

Datura is one of the genuses of Family Solanaceae. Nine species of vespertine flowering plants, belongs to genus Datura. It is also sometimes called as Angel's Trumpets, which means related to genus Brugmansia. *Datura inoxia* commonly called as angel's trumpet, it is a shrubby, short-lived, and perennial plant. This was a native plant of Mexico and Central America, It is about 2-3' tall and sprawls to as much as 3-6' wide. Leaves have a downy texture. Ovate, dark green Single or double, upward-facing trumpets have a sweetly fragrance. Spherical fruits were covered with stiff spines; hence this plant is commonly called as downy, thorn apple. All parts are extremely toxic. During one growing season, devil's trumpet shows 0.5 maximum heights of approximate [1]. *Datura inoxia* is an annual plant that reaches a height of 0.6 to 1.5 meters. Stems and leaves are covered with soft grayish hairs. It has elliptic entire-edged leaves with pinnate venation. All parts of the plant have a foul odor when crushed. The flowers are white, trumpet-shaped, 12–19 cm long. Flowers is seen in early summer until late fall and fruit is a spiny capsule, of 5 cm in diameter. Upon ripping it splits open, dispersing the seeds. The seeds can reside for years in the soil. As per literature, phytochemical studies revealed that Datura is rich in alkaloids, saponins, flavonoids, phenols, essential oils and cardiacs glycoside [2]. It also has insecticidal properties [3].

Present paper concerned with the callus culture of Datura. Callus is undifferentiated, proliferative, unorganized mass of cells. Unorganized callus tissues derived from different explants can be subjected to regeneration via callogenesis and rhizogenesis induced by exogenous plant growth regulators. Callus cultures also enable the production of large no plantlets from some tissues of limiting plant material [4]. There are very few references about the micro-propagation of *Datura inoxia*. Thus this protocol provides a method for callus induction of *Datura inoxia*. According to literature, all living cells are totipotent. In cultures, isolated plant cells or tissues may be induced to form an actively growing undifferentiated, proliferative mass of cells called callus which can be multiplied for an indefinite period by routine sub-culturing.

2. MATERIALS AND METHODS

Datura inoxia explants were collected from the industrially contaminated area in Bhopal city. Stem explants were used for induction of callus cultures. For this M.S. media with various concentrations of PGRs were prepared according to the explants type. M.S. basal medium supplied with 30 g/l sucrose and plant growth regulators was prepared in 500 ml conical flasks. Callus formation depends generally on the age, health and physiological state of the mother plant. The plant which is specifically healthy and vigorous growing shows better response [6]. Thus fresh and healthy explant material should be used for callus culture

2.1 Materials

During experimental protocol following materials were mainly used for callus culture of *Datura inoxia*

1. Nodal explant of *Datura inoxia*.
2. 0.1 % $HgCl_2$
3. Culture Bottles

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

4. Sterilized double-distilled water.
5. Pair of forceps.
6. Scissors or scalpel blades.
7. Plant tissue culture media: Murashige and Skoog (M.S.) medium
8. Plant Growth Regulators

2.2 Sterilization of Explant Material

A piece of stem of Datura plant (approximately 3 cm in length) is taken as an explant. These explants were kept under running tap water to 25 to 30 minutes. They were washed with soap solution for 5 minutes. They were surface sterilized in 0.1 % $HgCl_2$ for 30 seconds and washed 5-6 times with autoclaved distilled water.

2.3 Callus Induction Medium

The callus induction medium consisted of M.S. salts, vitamins, 0.2 -3.0 mg/L of KN, NAA and BAP and 30 g/l sucrose, medium was solidified with 0.8% (w/v) Agar –Agar. For sterilization, medium was autoclaved for 20 min. All cultures were performed in culture bottles. All chemicals used were provided by Hi-media. Explants inoculated with their axes in contact with the callus induction medium positioned upwards on 25-30 ml of solid agar M.S. Medium.

2.4 Inoculation of Explants Material and Callus Induction

In laminar airflow cabinet, an autoclaved scalpel was used to cut out stem segments of uniform size. Explants were inoculated in culture bottles with M.S. basal media supplemented with phyto-hormones for callus induction. Commonly used plant growth regulators (PGR) tissue culture research is the auxins and cytokinins. The concentration of PGR in the culture medium was very critical in controlling the growth and callus formation. Generally formation of callus required the increased concentration of Auxin and a low concentration of Cytokinin added into in the medium. It could stimulate cell proliferation. In this process threshold values of endogenous and exogenous hormones were necessary.

M.S. media [5] with sucrose (3%) and agar (0.8%) with different amount of plant growth regulators was used for induction of callus and shoot regeneration. Different combinations of Auxin, KN, NAA, BAP and IAA were used for callus induction experiments. The pH of media was adjusted to 5.8 by the addition of 0.1% HCl and 0.1% NaOH solution. Media were autoclaved for 15 to 20 minutes at 15 psi and 121^0C. Surface sterilized explants were inoculated into the culture medium in culture room. After aseptic inoculation, the culture vials were incubated at 25 ± 2^0C and were exposed to 16 hours photoperiod [9]. The callus tissues were subjected to serial sub culturing at appropriate time. During this callus obtained was shifted to fresh nutrient media according to requirement of the culture.

2.5 Shoot Regeneration from Callus

About two weeks after transformation to regeneration media, the embryo-derived calli initiate shoot regeneration in most of the treatments [7] [8]. At first, green spots appeared on the surface of callus and these spots were then converted to shoot primordia and subsequently converted to shoots. There were different numbers of regenerated shoots per callus from one treatment to another.

3. RESULT

Under *in vitro* conditions, tissue culture offers the possibility to grow millions of cells. Tissue culture research also revealed the physiological information about the behavior of the plant cells towards various stress conditions. Thus plant tissue culture has opened new avenues in plant improvement. Callus cultures were developed from explants of *Datura inoxia* in M.S. media. Stem explants were cultured on M.S. medium supplemented with various phytohormones with different concentration of KN, NAA and BAP. These phytohormones were used individually or in combinations of each

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

other [9]. Figure 1 and 2 shows the 15 and 30 days old callus. Figure 3 shows the induction of shoot primordial after the supplementation of different combinations of growth hormones.

Fig 1: 15-days Old Callus of *Datura inoxia*

Fig 2: 30-days Old Callus of *Datura inoxia*

Fig 3: Induction of Shoot Primordial from Callus of *Datura inoxia*

The effect of phytohormones from 0.1mg/L to 0.5 mg/L was studied alone and in combinations. The growth of callus induction was recorded till 3 weeks from the beginning and % response after (1, 2, and 3) weeks with all growth regulators in alone and in combinations were recorded. It has been found that when phytohormones were used in combinations good amount of friable callus was obtained in the following media combinations. Table 1 shows average performance of callus induction in response to different concentrations of plant growth regulators after 3 weeks and Table 2 shows the effect of M.S. medium and different concentrations of plant growth regulators.

Table 1. Percentage of Callus Formation after 3 Weeks

Media with Growth Regulators	% of Callus Induction
M.S. + NAA (0.1mg/L) + Kinetin (0.1mg/L)	0%
M.S. + NAA (0.2 mg/L) + Kinetin (0.2 mg/L)	10%
M.S. + BAP (0.25 mg/L) + kinetin (0.25mg/L) + NAA (0.3mg/L)	40%
M.S. + BAP (0.25 mg/L) + kinetin (0.25mg/L) + NAA (0.4mg/L)	60%
M.S. + BAP (0.25 mg/L) + kinetin (0.25mg/L) + NAA (0.5mg/L)	90%

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

Table 2. Effect of M.S. Medium and Different Concentrations of Plant Growth Regulators

Medium	Medium Composition	Callus Induction Days	% of Callus Formation	Morphological Characteristics
M.S.-1	M.S. + NAA (0.1mg/L) + Kinetin (0.1mg/L)	20	-	-
M.S.-2	M.S. + NAA (0.2 mg/L) + Kinetin (0.2 mg/L)	15	10%	Yellowish green, friable
M.S.-3	M.S. + BAP (0.25 mg/L) + kinetin (0.25mg/L) + NAA (0.3mg/L)	10	40%	Yellowish green, friable
M.S.-4	M.S. + BAP (0.25 mg/L) + kinetin (0.25mg/L) + NAA (0.4mg/L)	10	60%	Yellowish green, friable
M.S.-5	M.S. + BAP (0.25 mg/L) + kinetin (0.25mg/L) + NAA (0.5mg/L)	20	90%	Yellowish green, friable

4. CONCLUSION

Datura is a plant with phytoremediational importance; it is a hyper accumulating plant and can survive in higher concentrations of heavy metals. This work provide a useful protocol for callus culture of *Datura inoxia* and its micro propagation which may be used to generate heavy metal tolerating *Datura inoxia* plants in more numbers. It is an excellent example of removal of heavy metal pollution from soil through phytoremediation.

Phytoremediation exploits natural plant mechanisms against the industrial pollution. Tissue culture studies on Datura provide a rapid culture protocol in the form of *in vitro* propagation by callus culture. These types of tissue cultured plants could be used for phytoremediation of industrially contaminated soil. Most experiments used to establish phytoremediation techniques were done with hydroponic culture or plants grown on normal soil. In today's scenario, future efforts must be directed toward research to improve the performance of plants in remediation technologies, in the same manner various results obtained through *in vitro* plant cell and tissue cultures must be pollutant specific.

5. ACKNOWLEDGEMENTS

I would like to thanks to my supervisor and co-supervisor for their valuable guidance and also thankful to Dr. Shagufta Khan, Director, Growtips Biotech Training Institute, Bhopal for her precious support in my research. I would like to thanks to Dr. Aabha Gargava, Principal, Government M.V.M. Bhopal to provide me facilities in M.V.M. college.

6. REFERENCES

[1] Preissel, Hans-Georg, "Brugmansia and Datura: Angel's Trumpets and Thorn Apples" Buffalo, New York: Firefly Books. pp. 117–119. ISBN 1-55209-598-3.

[2] Ayuba VO, Ojobe TO, Ayuba SA (2011), "Phytochemical and proximate composition of *Datura inoxia* leaf, seed, stem, pod and root", Journal of Medicinal Plants Research 5(14): 2952-2955.

[3] Gilman AG (1990), "The Pharmacological Basis of Therapeutics", 8th ed., Pergamon Press, NY. Cited at WWW.erowid.org/plants/Datura faq

[4] Mahakant Jhaand Ramesh Kumar Pandey, "*In vitro* Micropropagation of Datura Metel L. Through Callus Induction From Leaf And Anther Culture", The Bioscan, International quality Journal, 7(1), (2012), 77-80.

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

[5] Murashige T, Skoog F, "A revised medium for rapid growth and bio-assays with tobacco tissue cultures", Physiol. Plant 15, (1962), 473-497.

[6] Amiri, S. and Kazemitabar, S. K., "Enhancement of callus induction and regeneration efficiency from embryo cultures of Datura stramonium by adjusting carbon sources and concentrations", African J. Biotechnology, 10(50), (2011), 10101-10107.

[7] Herouart, D., Gontier, E., Sangwan, R. S., Sangwan, B. S. and Norreel, "Analysis of the potential use of androgenic *Datura inoxia* for the development of cell cultures producing high amounts of tropane alkaloids", J. Exp. Bot. 42(8), (1991), 1073-6.

[8] Engvild, K. C., "Shoot Differentiation in Callus Cultures of *Datura inoxia*", Physiologia Plantarum. 28(1), (1973) 155-9.

[9] A. Waoo, S. Khare, and S. Ganguly, "Comparative Tissue Culture Studies on Lantana Camara and *Datura inoxia* at Heavy Metal Contaminated Site and Phytoremediation Approach at Industrially Contaminated Sites", International Journal of Advances in Biology (IJAB) Vol 1. No .1, August 2014