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In-vitro Culture of *Latana camera* from Nodal and Shoot-tip Explants in Phytoremediation Studies

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Abstract —This paper describes a prime and easy-to-use protocol for large-scale production of plant lets through shoot tip culture of Lantana camera, the plant having phytoremediation potential and the method is useful for the ex-situ conservation of other species important for phytoremediation. A simple micro-propagation method from nodal and shoot tip explants were reported here for *Lantana camera*, at contaminated site nearby Bhopal. This plant is having higher phytoremediation potential. There are few research papers about the plant regeneration from nodal segments of *Lantana camera*. Thus it is necessary to work on in-vitro studies of *Lantana camera.*

The plant regeneration from nodal segments were considered to be one of the most promising way for multiplying a selected variety. Here multiple shoots were induced in vitro from the stem nodal and shoot tip explants on Murashige and Skoog (MS) medium containing 6-benzylaminopurine (BAP), alone or with Naphthalene Acetic Acid (NAA) and kinetin (KN). Micro shoots were obtained in high frequency on MS medium having various concentrations of BAP (0.1 to 0.7 mg/l) along with NAA and KN from the explants. The shoot tip explants produced the highest number (3 per shoot) of shoots per culture with a average length of approximate 4.5 cm. The shoot tip explants produced maximum number of (1 to 2) shoots per culture on the same medium, average length of the in vitro shoots being approximate 4 cm. This tissue culture protocol helps to regenerate large number of Lantana camera plantlets having hyper accumulator properties for heavy metals.

Keyword — Latana camera, MS Media, Shoot tip culture, Nodal explants, In-vitro, Phytoremediation.

1. INTRODUCTION

Global industrialization has an effect on environment and the global ecosystem. This corruption of the ecosystem has a negative effect on human health and on all living organisms. Growing industrialization and environmental pollution from technology have started to affect human health

A wide range of pollutants emit from industrial waste and contaminates the soil causing soil pollution. Soil pollution is a worldwide environmental problem. At many hazardous waste sites in industrial area requiring cleanup, the contaminated soil contain a mixture of contaminant types. Numerous industrially contaminated sites exist in the world and new techniques for remediation are urgently needed.

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.

Phytoremediation is a technique in which plants were used to remove pollutants from the environment. It is an echo-friendly method to remediate polluted soil.

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

Lantana is a native of tropical Americas and West Africa. Lantana is commonly known as cariaquillo, filigrana, mille fleurs, sauge, red sage, yellow sage, prickly sage, and lakana. Lantana is an aromatic shrub with quadrangular stems, with prickles. This shrub may be suberect, scrambling, or occasionally clambering (ascending into shrubs or low trees, clinging to points of contact by means of prickles, branches, and leaves) (Howard 1989, Liogier 1995).

There are only few reports available for tissue culture of Lantana. But it has very high potential for phytoremediation. Thus this in vitro study focused on culture of *Lantana camera*, so that a standardize protocol can be prepared.

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 through out the experiment. In addition, media were supplemented with growth regulators, other additional vitamins, organic supplements and carbon sources.

2.1 Plant Material

Lantana camera plants were brought from industrially contaminated sites at Bhopal. Bhopal is sitting on a top of highly toxic sludge underground. There are many hazardous waste sites at 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.

Collection of fresh and healthy twigs from industrially contaminated sites was done and nodal as well as shoot tip explants were obtained from that twigs.

2.2 Explants and Surface Sterilization

Twigs of *Lantana camera* cut into 0.5-1.0 cm from the nodal segments and used as explants for the induction of multiple shoots. Similarly shoot tip region was also excised from the twig. Firstly explants were washed thoroughly under running tap water for 15-20 min. Then surface sterilization was done by antifungal agent (Bavistine), soap solution and different concentrations of $HgCl_2$ solution and washed thrice with sterile distilled water in the laminar air flow chamber.

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 kinetin (KIN) and auxin like naphthalene acetic acid (NAA).

The pH of all media was adjusted to 5.8, and agar concentration was 0.8% and 30 g sucrose was added. About 15 ml of the medium were dispensed in each culture bottle and sealed with plastic cover before autoclaving at 121ºC for 20 min with pressure of 15 psi. All cultures were maintained at 16 hr light of 1000 lux at 28 ± 2 ºC. Periodically results were observed for initiation of shoots from nodal and shoot tip explants.

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 MS media salts and their combination (full MS salt) were supplemented with 0.5 mg/L (BAP,NAA, and KN).

2.5 Shoot Induction Experiment

For these studies the nodal explants and shoot tip explants were inoculated on Murashige and Skoog (1962) basal medium supplemented with cytokinins like BAP and kinetin KN, in the concentration of (0.1-0.7) mg/l alone or in combination with other cytokinins of each, containing sucrose 30 g and gelled with agar 4 g/l. In addition auxins like IAA or NAA (0.1-0.7 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 + 0.3mg/l BAP
- Medium 3 MS + 0.5 mg/l BAP
- Medium 4 MS + 0.7 mg/l BAP
- Medium 5 MS + 0.1 mg/l KN
- Medium 6 MS + 0.3 mg/l KN
- Medium 7 MS + 0.5 mg/l KN
- Medium 8 MS + 0.7 mg/l KN
- Medium 9 MS + 0.1 mg/l NAA
- Medium 10 MS + 0.3 mg/l NAA
- Medium 11 MS + 0.5 mg/l NAA
- Medium 12 MS + 0.7 mg/l NAA

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.

3 RESULTS AND OBSERVATIONS:

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 (0.3-0.5 mg/l).

Table 1: Initiation trials of Lantana camera for Contamination and Survival

Species Used	Explant Collection Dates	Total Number of Attempted	Percentage of Contaminated	Percentage of Survival
Lantana camera	02-06-2011	15	60	20
	01-07-2011	25	70	40
	12-08-2011	30	50	30
	04-09-2011	20	20	70

Table 2: Effect of different concentrations of plant growth hormones on nodal explants of *Lantana camera*

Nodal Explants			
PGR mg/l	% of Response	No. of Shoots in Culture	Average Length of Shoots (cm)
BAP			
0.3	5	6	1-2
0.5	60	12	1-4
0.7	10	5	1-2
Kinetin			
0.3	8	4	1-2
0.5	55	6	1-3
0.7	20	5	1-2
NAA			
0.3	5	6	2
0.5	60	25	1-3
0.7	5	8	1-2

Table 3: Effect of different concentrations of plant growth hormones on shoot tip explants of *Lantana camera*

Shoot Tip Explants			
PGR mg/l	% of Response	No. of Shoots in Culture	Average Length of Shoots (cm)
BAP			
0.3	4	8	1
0.5	75	12	1-3
0.7	20	6	1-2
Kinetin			
0.3	10	9	1
0.5	60	12	1-2
0.7	9	6	1
NAA			
0.3	12	8	1
0.5	60	45	1-3
0.7	10	10	2

Cytokinins, especially BAP, release lateral buds from dormancy and initiate shoot formation. Nodal and shoot tip explants of Lantana were inoculated on MS medium supplemented with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mg/l) of cytokinins BAP, KN and auxins like NAA for the production of multiple shoots. The percentage of survival of shoot tip explant is greater than the nodal explant in case of *Lantana camera*. As a supplement of 0.5mg/l BAP resulted in maximum proliferation (75%) was observed in nodal explants. The nodal explants produced the highest number (12) (Table 1) of shoots per culture with a average length of 1.3 cm. These explants were capable initiating multiple shoots on MS medium containing different concentrations of BAP, KIN, and NAA.

Following graph (figure 1) shows percentage of response of nodal explants of Lantana camera towards different plant growth hormones and their concentrations.

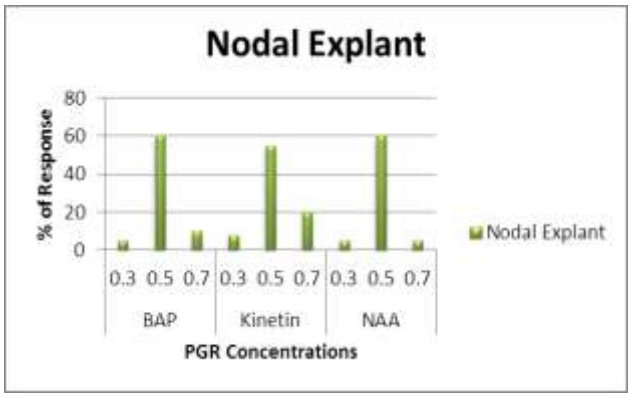

Fig. 1. Graph showing effect of various concentrations of plant growth regulators on % of initiation response of nodal explants

This graph (figure 2) shows percentage of response of shoot tip explants of Lantana camera towards different plant growth hormones and their concentrations.

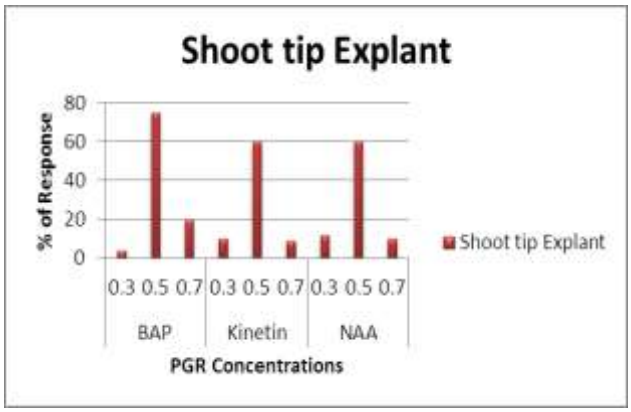

Fig. 2. Graph showing effect of various concentrations of plant growth regulators on % of initiation response of shoot tip explants

Fig. 3. Photograph of shoot initiation form nodal and Shoot tip explants

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. Plants can help clean up many kinds of pollution including metals, pesticides, explosives, and oil. The plants also help to prevent soil erosion by wind, rain, and groundwater and reduce the possibility of carrying pollution away from one site to another site.

Phytoremediation is the most powerful tool against the industrial pollution because it takes advantage of natural plant process. In phytoremediation technology plants act as bioreactors and clean up the pollutants from the soil. In this concern *Lantana camera* has highest capacity of phytoremediating the soil contamination.

Tissue culture studies on Lantana provide a fast culture method in the form of in vitro propagation through soot tip and nodal explants. Plant tissue cultures has many important technical advantages in case of phytoremediation. Because in vitro plant cultures give fast growing genetically similar and pollutant tolerable plantlets in large amount in very little time period. Thus they can be easily used for removing soil pollution.

REFERENCE

[1] Alagumanian S, Saravana Perumal V, Balachandar R, Rameshkannan KM. Rao V (2004) Plant regeneration from leaf & stem explants of Solanum trilobatum L. Sci. Corresp. 86: 1478-1480.

[2] Pauline M. Doran Application of Plant Tissue Cultures in Phytoremediation Research: Incentives and Limitations School of Biological Sciences and Department of Chemical Engineering, Monash University, VIC 3800, Australia; telephone: 61-3-9905-1373.

[3] Zilkah S, Gressel J. 1977. Cell cultures vs. whole plants for measuring phytotoxicity. III. Correlations between phytotoxicities in cell suspension cultures, calli and seedlings. Plant Cell Physiol 18:815–820.

[4] P. Ananthi1, B. D. Ranjitha Kumari1 and A. Ramachandran In vitro propagation of Rorippa indica L. from nodal and shoot tip explants International Journal for Biotechnology and Molecular Biology Research Vol. 2(3), pp. 51-55, March 2011.

[5] Barwal UB, Kerns HR and Wildholm JM (1996). Plant regeneration from callus culture of several soybean genotypes via embryogenesis and organogenesis. Planta, 167: 473-481.

[6] Pauline M. Doran "Application of Plant Tissue Cultures in Phytoremediation Research: Incentives and Limitations", School of Biological Sciences and Department of Chemical Engineering, Monash University, VIC 3800, Australia; telephone: 61-3-9905-1373.

[7] Howard, R.A. 1989. Flora of the Lesser Antilles. Vol. 6. Arnold Arboretum, Harvard University, Jamaica Plain, MA. 658 p.

[8] Liogier, H.A. 1995. Descriptive flora of Puerto Rico and adjacent islands. Vol.4. Editorial de la Universidad de Puerto Rico, San Juan, PR. 617 p.

[9] Priyanka Srivastava, Vikash Sisodia, Rakhi Chaturvedi Effect of culture conditions on synthesis of triterpenoids in suspension cultures of Lantana camara ,L Bioprocess Biosyst Eng (2011) 34:75–80 DOI 10.1007/s00449-010-0448-0

[10] Bhojwani SS, Razdan MK (1996) Plant tissue culture: theory and practice. Elsevier, Amsterdam.

[11] National Risk Management Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268 Introduction to Phytoremediation

[12] Vanessa Ribeiro Affonso,a Humberto Ribeiro Bizzo,b Sharon Santos de Lima,a Maria Apparecida Esquibela and Alice Sato, Solid Phase Microextraction (SPME) Analysis of Volatile Compounds Produced by In Vitro Shoots of Lantana camara L. under the Influence of Auxins and Cytokinins, J. Braz. Chem. Soc., Vol. 18, No. 8, 1504-1508, 2007. Printed in Brazil - ©2007 Sociedade Brasileira de Química.

[13] M. Ghosh – S. P. Singh Comparative uptake and phytoextraction study of Soil induced chromium by accumulator and high Biomass weed species Applied ecology and environmental research 3(2): 67-79. http://www.ecology.kee.hu, ISSN 1589 1623 , 2005, Penkala Bt., Budapest, Hungary

[14] Purnima raizada & Akhilesh S. Raghubanshi, Seed germination behaviour of Lantana camara in response to smoke, Tropical Ecology 51(2S): 347-352, 2010 ISSN 0564-3295 © International Society for Tropical Ecology ,www.tropecol.com

[15] Phytoremediation: Transformation and Control of Contaminants (Environmental Science & Technology: A Wiley Interscience Series of Texts and Monographs); McCutcheon, S.C., Schnoor, J.L., Eds.; John Wiley & Sons, Inc.:

[16] United States Protection Agency Reports (2000): Introduction to Phytoremediation. – EPA 600/R-99/107.

[17] Chaudhry, T.M., Hayes, W.J., Khan, A.G. and Khoo, C.S. (1998): Phytoremediation - focusing on accumulator plants that remediate metalcontaminated soils. – Austraaslian Journal of Ecotoxicology. 4; 37-51.

[18] Mueller, B., Rock, S., Gowswami, Dib, Ensley, D. (1999): Phytoremediation Decision Tree. – Prepared by - Interstate Technology and Regulatory Cooperation Work Group