Biotec.Res.J.2015; Vol 1(2):135-138
eISSN 2395-6763
OPEN ACCESS

Biotechnological research

Copyright © 2015 Soni and Sharma
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORIGINAL RESEARCH

An efficient *in vitro* method for mass propagation of *Plumbago zeylanica* L. through nodal explants

Vineet SONI[1*], Ira SHARMA[2]
[1]Department of Botany, Mohanlal Sukhadia University, Udaipur-313001, Rajasthan, India
[2]School of Life Science, Jaipur National University, Jaipur-302025, Rajasthan, India
*Corresponding author email: vineetsonijnu@gmail.com

• *Received: 12 June 2015 • Revised: 1 July 2015 • Accepted: 17 July 2015 • Published: 26 July 2015* •

ABSTRACT
A rapid and efficient protocol for the large-scale propagation of a potential medicinal plant, *Plumbago zeylanica* L., through *in vitro* culture of nodal explants is described. High frequency multiple shoots were induced on Murashige and Skoog's basal medium supplemented with 3.0 mg/l kinetin (KIN) and 0.5 mg/l 6-benzyl amino purine (BAP). The *in vitro* developed shoots were rooted best on half-strength MS medium fortified with 1.0 mg/l indole-3-acetic acid (IAA) and 0.5 mg/l 1-naphtalene acetic acid (NAA). The developed micropropagation protocol can be successfully used for large-scale multiplication and conservation of *P. zeylanica*.

KEY WORDS: *Plumbago zeylanica, In vitro Propagation, Plant Growth regulators*

Introduction

Plumbago zeylanica L., (family: Plumbaginaceae) is an important medicinal plant which has traditionally been used in Indian and Chinese systems of medicine for the treatment of various types of diseases such as dyspepsia, piles, diarrhoea, scabies and ulcers, leprosy and rheumatism (Rout *et al.,* 1999; Olagunju *et al.,* 2006). Leaves and roots of *P. zeylanica* contain a naphtoquinone, called plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone), which has earlier been reported to inhibit intestinal carcinogenesis (Sugie *et al.,* 1998), non-small cell lung cancer (Hsu *et al.,* 2006), breast cancer (Kuo *et al.,* 2006), melanoma (Wang *et al.,* 2008), cerebral ischemia (Son *et al.,* 2010), breast and gastric cancer (Manu *et al.,* 2011) and ovarian cancer cells *in vivo* (Sinha *et al.,* 2013).

Unfortunately, due to the indiscriminate and ruthless collection of the roots, the plant population in the natural habitats has declined drastically in recent years. Conventional propagation methods of the plant are rather difficult and insufficient to meet the growing demand of the pharmaceutical companies. Therefore, the present study was carried out to develop and efficient and reproducible *in vitro* method for mass propagation of *P. zeylanica.*

Materials and Methods

Plant material and surface sterilization

Young tender twigs, collected from three years old *P. zeylanica* plants growing in the plant nursery at the Jaipur National University, Jaipur (India), were thoroughly washed under running tap water to eliminate dust particles for 30 min and then with 5% teepol for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water. Then, the twigs were treated with an antifungal agent (Bavistin) for 1 hour and again rinsed three times with sterile distilled water. These all steps were carried out outside the laminar air flow chamber. Thereafter, under a laminar flow chamber, the twigs were surface sterilized in a 0.1% $HgCl_2$ solution for 4 -

6 min followed by thorough washing with sterile distilled water. Finally, the twigs were dissected into 1–1.5 cm nodal segments.

Culture medium and growth conditions

The sterilized nodal explants were cultured on MS Medium (Murashige and Skoog, 1962) supplemented with various concentrations of auxins and cytokinins, either singly or in combination, for shoot multiplication. The pH of the medium was adjusted 5.8 before autoclaving for 15 min at 121 °C. Cultures after inoculation were incubated at 25 ± 2 °C and 65 - 70% relative humidity with photoperiod of 16/8 h at 3000 lux intensity by florescent tubes.

Acclimatization and field transfer

Well rooted shoots were washed in sterile distilled water and transferred to plastic cups (10 cm × 8 cm) containing sterilized mixture of sand and garden soil (1:1 v/v) covered with plastic cover. The cups were incubated at 25 ±2ºC under cool white fluorescent light (70 µmol $m^{-2}s^{-1}$) with 16 h photoperiod. The fully acclimatized plants were finally transferred to the greenhouse.

Results and Discussion

The morphogenetic responses of nodal explants to cytokinins (BAP and KIN) are represented in the table (Table 1). In the present investigation indicates synergistic effect of BAP and KIN on multiple shoot induction from nodal explants of *P. zeylanica*. When KIN alone was used in the medium at a high concentration (3.0 mg/l) it yielded 2.57 shoots per explants, while at low concentration (0.5 mg/l) it yielded a maximum of 1.67 shoots (Fig. 1 A, B). In the same manner, the BAP used in the medium at lower concentration (0.5 mg/l) yielded less shoots while at the highest concentration (3.0 mg/l) it yielded maximum 2.67 shoots (Fig. 1 C). High frequency development of multiple shoots was obtained on medium containing 3.0 mg/l KIN and 0.5 mg/l BAP (Fig. 1 D). The multiple shoots elongated well on the same medium. Other concentrations of BAP and KIN yielded in reduced number of multiple shoots.

Table 1: Effect of KIN and BAP singly or in combination on shoot multiplication in *P. zeylanica*

	Concentration (mg/l)	Response (%)	No. of shoots per explant
KIN	0.0	0.0	00
	0.5	19±2.14	1.67±0.44
	1.0	42±3.82	1.51±0.36
	3.0	58±1.72	2.57±0.67
	5.0	29±4.67	1.73±0.36
BAP	0.0	00	00
	0.5	41±4.23	1.67±0.76
	1.0	55±2.44	2.31±0.42
	3.0	51±1.77	2.67±0.76
	5.0	33±4.17	1.84±0.29
KIN + BAP	0.5 + 5.0	12±3.41	1.76±0.88
	1.0 + 3.0	31±3.36	2.87±0.49
	3.0 + 1.0	71±5.96	5.77±0.97
	3.0 + 0.5	89±4.39	8.36±0.72
	5.0 + 0.5	69±2.24	3.53±0.93

Values are Mean±SD of 30 explants per treatment of 3 replications.

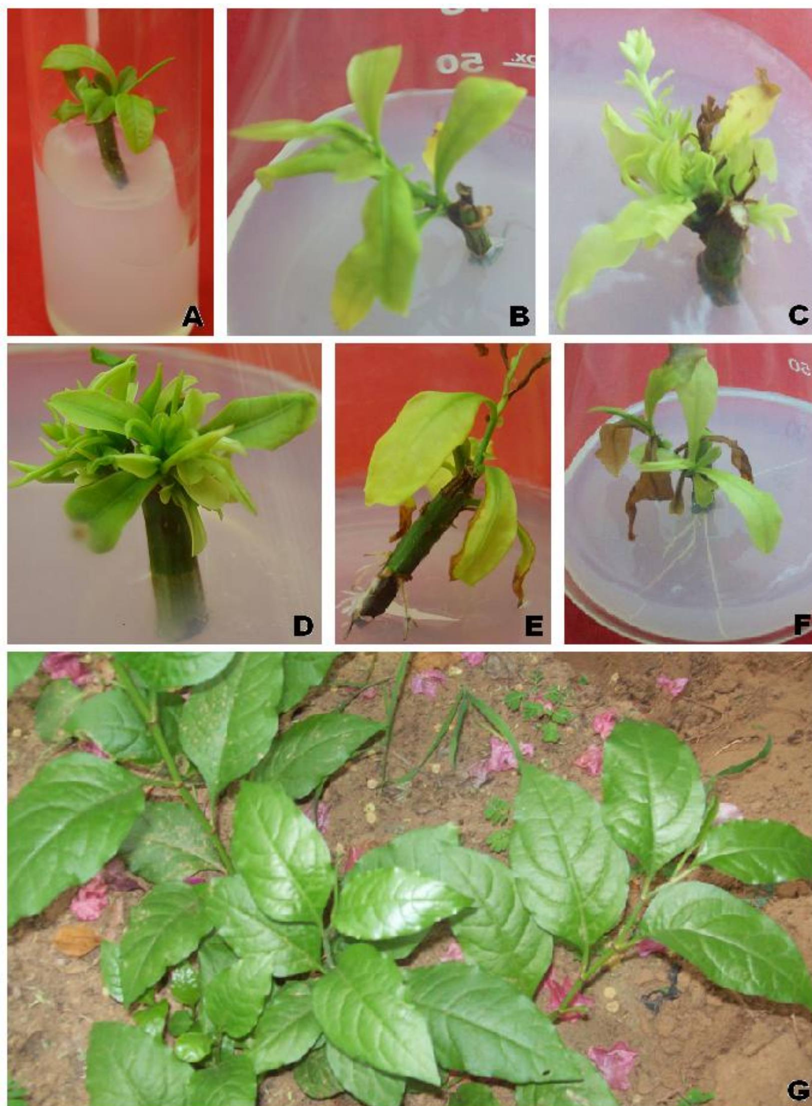

Figure 1: Showing development of multiple shoots *in vitro* (A-D), rhizogenesis (E-F) from nodal explants of *P. zeylanica* and acclimatized plants *in vivo* (G).

Similarly, synergistic effect of BAP and KIN on multiple shoot induction was reported in *Solanum surattense* (Seetharam *et al.,* 2003) and *Gossypium hirsutum* L. cv Narashima (Pathi and Tuteja, 2013).

In order to develop rhizogenesis, the healthy elongated shoots were transferred aseptically to root induction medium. Rhizogenesis was achieved when excised shoots were cultured on ½ MS medium containing 1.0 mg/l IAA (Fig. 1 E). High frequency root induction was observed when NAA was incorporated along with IAA (Fig. 1 F). The percentage of rooting was 85 –95% on ½ MS medium containing 1.0 mg/l IAA and 0.5 mg/l NAA. On the other hand, Lubaina *et al.,* (2011) reported best root induction on MS medium enriched with IBA and NAA. After hardening, the *in vitro* developed plantlets with well-developed shoot and roots were acclimatized successfully and grown in greenhouse (Fig. 1 G). The established plants did not show any variation in morphological or growth characteristics when compared to the mother plant. The developed *in vitro* protocol can be useful for *ex-situ* conservation, commercial propagation and genetic improvement of *P. zeylanica.*

Acknowledgement

The authors are thankful to Prof. P.L.Swarnkar, Prof. C.P.

Malik and Prof. H.N. Verma for their constant guidance, encouragement and support for the completion of the work.

References

Hsu, Y.L., Cho, C.Y., Kuo, P.L., Huang, Y.T.and Lin, C.C. (2006) Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) induces apoptosis and cell cycle arrest in A549 cells through p53 accumulation via c-Jun NH2-terminal kinase-mediated phosphorylation at serine 15 *in vitro* and *in vivo*. J Pharmacol. Exp. Ther., 318(2):484–94.

Kuo, P.L., Hsu, Y.L. and Cho, C.Y. (2006) Plumbagin induces G2-M arrest and autophagy by inhibiting the AKT/mammalian target of rapamycin pathway in breast cancer cells. Mol Cancer Ther, 5:3209-3221.

Lubaina, A.S., Nair, G.M. and Murugan, K. (2011) Shoot multiplication and direct organogenesis of an important medicinal plant *Plumbago zeylanica* L. (Plumbaginaceae). Journal of Research in Biology, 6: 424-428.

Manu, K. A., Shanmugam, M. K., Rajendran, P., Li, F., Ramachandran, L., Hay, H. S., Kannaiyan, R., Swamy, S. N., Vali, S., Kapoor, S., Ramesh, B., Bist, P., Koay, E. S., Lim, L. H., Ahn, K. S., Kumar, A. P. and Sethi G. (2011) Plumbagin inhibits invasion and migration of breast and gastric cancer cells by downregulating the expression of chemokine receptor CXCR4. Mol. Cancer, 10: 107.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tisssue cultures. Physiol. Plant., 15: 473-497.

Olagunju, J.A., Fagbohunka, B.S., Oyedapo, O.O. and Abdul-Azeez, I.A. (2006) Effects of an ethanolic root extract of *Plumbago zeylanica* Linn. on some serum parameters of albino rat. Recent Progress in Medicinal Plants, 11(6): 267-276.

Pathi, K.M. and Tuteja, N. (2013) High-frequency regeneration via multiple shoot induction of an elite recalcitrant cotton (*Gossypium hirsutum* L. cv Narashima) by using embryo apex. Plant Signaling and Behavior, 8(1): 94-99.

Rout, G.R., Saxena, C., Samantaray, S. and Das, P. (1999) Rapid plant regeneration from callus cultures of *Plumbago zeylanica*. Plant Cell Tiss Organ Cult, Vol. 56: 47-51.

Seetharam, Y. N., Jyothisaran, G., Sujeeth, H., Barad, A., Sharanabasappa, G., and Panchal, T. S. (2003) *In vitro* plant regeneration from nodal and leaf explants of *Solanum surattense* Burm. F.: A medicinal plant. Plant Cell Biotech. Mol. Biol., 4: 17-22.

Sinha, S., Pal, K., Elkhanany, A., Dutta, S., Cao, Y., Mondal, G., Iyer, S., Somasundaram, V., Couch, F. J., Shridhar, V., Bhattacharya, R., Mukhopadhyay, D. and Srinivas, P. (2013) Plumbagin inhibits tumorigenesis and angiogenesis of ovarian cancer cells *in vivo*. Int. J. Cancer: 132: 1201-1212.

Son, T.G., Camandola, S., Arumugam, T.V., Cutler, R.G., Telljohann, R.S., Mughal, M.R., Moore, T.A., Luo, W., Yu, Q.S., Johnson, D.A., Johnson, J.A., Greig, N.H. and Mattson, M.P. (2010) Plumbagin, a novel Nrf2/ARE activator, protects against cerebral ischemia. J Neurochem, 112:1316–26.

Sugie, S., Okamoto, K., Rahman, K.M., Tanaka, T., Kawai, K., Yamahara, J. and Mori, H. (1998) Inhibitory effects of plumbagin and juglone on azoxymethane-induced intestinal carcinogenesis in rats. Cancer Lett., 127(1–2):177–83.

Wang, C.C., Chiang, Y.M., Sung, S.C., Hsu, Y.L., Chang, J.K. and Kuo, P.L. (2008) Plumbagin induces cell cycle arrest and apoptosis through reactive oxygen species/c-Jun N-terminal kinase pathways in human melanoma A375. S2 cells. Cancer Lett., 259:82–98.