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SCAN ME

Research Paper

EFFECTIVE PROTOCOL FOR CONSERVATION OF *Plumbago rosea* - A PLANT OF NOVEL AND POTENT ANTICANCER COMPOUND PLUMBAGIN

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Abstract

Plumbago rosea L. is valuable medicinal herb and it is widely used in Ayurveda for the treatment of leprosy, diarrhea and dyspepsia etc. *P. rosea* is highly responsible to micropropagation, which is mainly for rapid regeneration of plantlets. The plants were inoculated on MS medium supplemented with different concentrations and combinations of growth hormones. Shoot initiation was observed on MS medium with BAP, Kinetin and IAA. Numerous shoot buds are produced on the combination of 3.5 mg/L BAP, 0.5 mg/L and 1.0 mg/L and the shoot buds were short length. The elongated shoot buds rooted on the MS medium supplemented on combination of 2mg/L and 1 mg/L NAA. The rooted shoots were successfully transferred to the polyhouse and 80% plants were survived.

Key words: *Plumbago rosea,* micropropagation, shoot initiation, root initiation.

INTRODUCTION

It is estimated that about 80,000 plant species are utilized in some form or other by the different systems of Indian medicine [1]. Micropropagation also called microcloning or clonal propagation is the vegetative propagation of plants by employing tissue culture techniques, using cells, tissues, organs, etc. It involves the regeneration of plants from isolated meristematic or somatic cells or tissues. It provides a rapid and reliable means for the mass production of genetically similar and disease free plants through the culture of shoot apices, axillary buds and meristems. It is often used for the propagation of exclusively vegetative species whose multiplication rate is very low, and

also for mass multiplication of superior varieties of hybrids as an alternative to seed propagation [2].

Plumbago rosea is widely used in Ayurveda, Siddha, Unani and Homeopathy and also in uncodified ethnic preparations of the rural folks. Tuberous roots of the plants are reported to have many ethnobotanical uses such as treating oedema, piles, intestinal worms, skin disease, common ward, rheumatism, secondary syphilis, leprosy etc [3,4,5,6]. The roots form an ingredient of more than thirty ayurvedic preparations including herbal extract mixes, massage oil etc [7].

Traditionally root extract of *Plumbago rosea* is used for an abortifacient and anti-fertility medicine. In Myanmar it is also used for leprosy and syphilis. In Ayurvedic preparations the roots are used only after purification to reduce toxicity [8]. The root is also acrid, vesicant, digestive, stimulant, powerful abortifacient and also an oral contraceptive [4]. An ointment made from crushed root mixed with vegetable oil is used as a rubefacient to treat rheumatism and headache. Milky juice of the leaves is applicable on the skin for the treatment of scabies, ringworm and hemorrhoids. In Java, the root is used in the veterinary medicine for expelling worms in horses. It is also useful in the early stages of leucoderma and baldness of head [9].

Members of Plumbaginaceae have also been widely investigated for anti-protozoal, antimalarial, activities [10,11,12]. Besides, different parts of plants have been traditionally used in folk medicines in India, China, and other Asian countries for the treatment of rheumatoid arthritis, dysmenorrhea and cancer. A plant specific active compound present prominently in roots and in traces in other part forming a potential biomarker is plumbagin, a naphthoquinone responsible for many of the activities of the root drugs [13]. Due to these enormous usages of the study species *P. rosea* has to conserve and need to attention on mass propagation for sustainable utilization. In light of this fact the present study focused to develop protocol for mass multiplication of *P. rosea.*

MATERIALS AND METHODS

In vitro regeneration

The application of tissue culture technology for the establishment of protocol for *in vitro* regeneration of study species was done by following the methods as described under:

Sources of plant materials

Healthy plantlets of the study species, *Plumbago rosea* was collected from the individuals raised through clonal propagation and maintained under greenhouse condition.

Explant selection and sterilization

Healthy and immature node segment of the study species was collected from study plant grown under greenhouse condition and were used as explants. These segments were sterilized properly by following standard procedures.

Culture medium

MS (Murashige and Skoog, 1962) basal medium containing 3% sucrose solidified with 0.8% agar (tissue culture grade, Himedia, India) was used.

In vitro studies

Direct Organogenesis

Fresh nodal shoot explants from wild and *in vitro* regenerated (first generation) plantlets derived from nodal segment were cultured on MS medium supplemented with different concentrations and combinations of 0.2 -3.5 mg/L BAP, 0.5 – 3.0 mg/L Kinetin, and 1.0 mg/L IAA (contained in culture bottles). It was repeatedly subcultured once in every 60 days by transferring onto the fresh medium of the same composition up to third and fourth generation. Thus a single explant of nodal segment could conceivably provide new true to type plantlets for further research/ usage.

Rooting of *in vitro* multiplied shoots

The elongated shoots were transferred to MS medium supplemented with different auxins like IBA and NAA at different concentrations for root induction. The rooted plantlets were removed from culture tubes and transferred for acclimatization through hardening.

Hardening

The well-developed healthy plantlets were removed from the culture bottle. Rooted plants were washed to remove agar then dipped in Mancozeb 1% per litre for 2 minutes and were thoroughly washed in running tap water to remove the adhering nutrient medium completely without causing damage to roots. Then the plantlets were soaked in 1% (w/v) fungicide, methyl-3 benzimidizole carbamate (Bavistin) solution for 10-15 minutes and it was transferred to small polytray with coir medium. 19:19:19 N:P:K combination of liquid fertilizer spray the plant at every weeks.

RESULTS

Nodal shoots of *P. rosea* were inoculated on Murashige and Skoog (MS) medium supplemented with different concentration and combinations of Plant growth hormones. The different type of response of shoot induction was observed on MS medium supplemented with 0.2- 3.5 mg/L BAP, 0.5- 3.0 mg/L Kinetin and 1.0 mg/L IAA. All media were solidified with agar and pH was adjusted to 5.8 and autoclaving for 20 minutes at 121° C under 1.2 kg/cm^2 pressure.

The cultured nodal shoots gave various responses to different concentration and combination of plant growth hormones within 30- 40 days. The multiple shoots developed from all media and each media contains 1.0mg/L IAA for inoculation. The best response of shoot induction showed on MS media with 3.5 mg/L BAP, 0.5 mg/L Kinetin and 1.0 mg/L IAA. The result showed the numerous shoots are formed and average length of shoot were short (1cm) with broad leaves.

The combined effect of plant growth hormones of BAP and Kinetin on shoot induction of *P. rosea* showed different response. Ten different combinations of BAP, Kinetin and IAA were added separately media for shoot induction. The number shoots

produced by explants were counted after one month of incubation. Shoot induction of each explant was detected in the entire bottle with medium. The Table 1 showed the different response of shoot initiation with multiplication ratio of shoot and average length of shoots. Each combinations following that is 0.2, 0.4, 0.6, 0.8, 1.0, and 1.5 mg/L BAP and 3.0, 2.5, 2.0, 1.5, 1.0, and 0.5 mg/L Kinetin growth hormones were tested. This study showed that the ratio of shoot initiation was less and 7- 9 days taken for shoot initiation. In another combination of BAP were 2.0, 2.5, 3.0, and 3.5 mg/L Kinetin was 0.5 mg/L and IAA 1 mg/L. This study showed the ratio of shoots was increased but the length was decreased (1cm) and it taken 10- 13 days for initiation of shoots.

Initiated shoots were subcultured after one month. The explant undergoes subsequent subculture in media with frequent growth hormones. Individual shoots from multiple shoots developed from explants separated and transferred to the media.

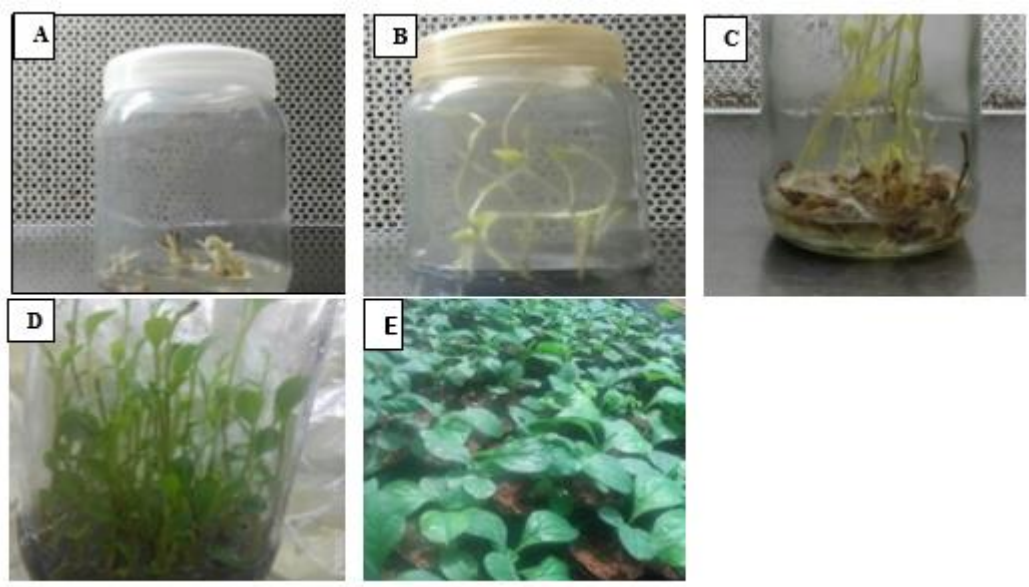
Root formation was induced in the elongated shoots by culturing on the MS medium supplemented with IBA and NAA. The shoots were separated from shoot induction medium and then transferred to the rooting medium. The different concentration and combination of IBA and NAA added separately to the MS media. Shoot buds of *P. rosea* tested for root induction at four combination of IBA and NAA (Table- 2) . The best response was observed with 1.0 mg/L IBA and 2.0 mg/L NAA. It gave numerous roots from each shootlet. In one combination of 2 mg/L IBA and 2mg/L NAA showed the proliferation of callus in the media.

In this study maximum root induction was observed on MS supplemented with combination of 1 mg/L IBA and 2mg/L NAA. The 1 mg/L IBA and NAA combination showed less number of roots and 2mg/L IBA and NAA combination showed the callus roots developed. The ratio of developing roots and days to rooting remarkably varied with different concentrations and combination of IBA and NAA.

Rooted plants were removed from the culture bottles carefully. The rooted plantlets washed to water and dipped in Mancozeb 1% (2 minutes) for removing agar. The rooted plantlets were transplanted to coir medium containing pot tray and it kept in the polytunnel for one months.

Hardening

Rooted plants washed to remove agar then dipped in Mancozeb 1% per L upto 2 minutes. After this process, it transplanted into pot tray with coir medium, this planted pot tray kept in polyhouse for one month to get more roots. 19:19:19 NPK combination of liquid fertilizer spray to the plants at every weeks.



A. Initiation of shoot from node B. Initiation of multiple shoot from explant, C. Shoot elongation and well developed shoots, D. Root initiation with shoots E. Hardened and acclimatized plantlets.

Table 1. Shoot initiation from nodal explant of *Plumbago rosea.*

Growth hormones			No. of days taken for shoot initiation	Multiplication ratio	Shoot length (cm)
BAP (Mg/L)	Kn (Mg/L)	IAA (Mg/L)			
0.2	3.0	1.0	7	1:2	4.0 ± 0.58^{a}
0.4	2.5	1.0	7	1:2	3.8 ± 0.49^{ab}
0.6	2.0	1.0	7	1:2.5	4.0 ± 0.37^{a}
0.8	1.5	1.0	8	1:3	3.0 ± 0.24^{c}
1.0	1.0	1.0	9	1:3	3.5 ± 0.36^{b}
1.5	0.5	1.0	7	1:3.5	3.0 ± 0.45^{c}
2.0	0.5	1.0	10	1:4	1.7 ± 0.67^{d}
2.5	0.5	1.0	11	1:4	1.5 ± 0.85^{e}
3.0	0.5	1.0	13	1:5	1.0 ± 0.37^{f}
3.5	0.5	1.0	13	1:6	1.0 ± 0.26^{f}

Values are expressed as mean±SD (n=6).

Values within the same column not sharing common superscript letters (a,b) differ significantly at $p<0.05$ by DMRT.

Table 2. Root initiation from shoots developed from nodal explant of *Plumbago rosea.*

S.No.	Growth hormones		No.of plants inoculated	No.of roots formed
	IBA Mg/L	NAA Mg/L		
1.	1.00	1.00	20	5±0.25^{c}
2.	1.00	2.00	20	15±0.72^{a}
3.	2.00	1.00	20	12±0.59^{b}
4.	2.00	2.00	20	Callus root

Values are expressed as mean±SD (n=6).

Values within the same column not sharing common superscript letters (a,b) differ significantly at $p<0.05$ by DMRT.

DISCUSSION

Tissue culture point of view several studies have been performed to propagate the plant through *in vitro* technique. The main aim of micropropagation studies is to standardize the *in- vitro* condition and massive production or propagation of the important medicinal plants. Culture of node explant of the study species shows differential response to different combination of plant growth hormones. Shoot induction has developed in a number of medicinally important plants employing micropropagation technique. It differently dependent on appropriate level of plant growth hormones [14, 15, 16, 17, 18]. Shoot rapidly emerged on MS medium with 0.2- 1 mg/L BAP and 1 mg/L IAA. The comparable finding also explained by [19]. The maximum number of shoots are induced in 2- 3.5 mg/L BAP and 0.5 mg/L Kinetin. The comparatively similar shoot induction reported in *P. rosea* [14].

This study demonstrated the essential plant growth regulators for *in vitro* multiplication, as the shoots cultured on basal medium did not multiply and become dead. BAP at a concentration of 0.8- 1.5 mg/L just gave an average of 1:4 shoots. Decreasing the concentration of BAP, decreased in shoot multiplication rate was observed. One of an improved protocol for the micropropagation of *P. zeylanica* L. from nodal explants was developed [20]. The best response of shoot induction was observed on MS basal medium supplemented with 1.0 mg L-1 thidiazuron (TDZ) and 1.0 mg/l Kinetin (KN). Maximum number of shoots when cultured on MS liquid medium supplemented with 1.0 mg/L-1 BAP, 0.5 mg/L-1 IBA and 2.0 mg/L adenine sulfate [21]. The highest multiplication rate of the explants using MS medium supplemented with

Naphthalene Acetic Acid (NAA) (0.01 - 0.05 mg/l) and Benzyl Amino Purine (BAP) (2.0 - 4.5 mg/l) was obtained [22]. The excellent shoot formation in the combination of 1mg/l BAP+ 1mg/l NAA and maximum shoot length was recorded [23].

The highest percentage of callus developed when stem explants cultured on MS medium supplemented with 2.0 mg/L BAP and 1.5 mg/L IAA. The greatest percentage of shoot induction (100%) with shoots induced from callus was cultured on MS medium supplemented with 0.75 mg/L BAP, 1.0 mg/L IAA, NAA. The ratio of rooting in shoots significantly showing variation with different combination and concentration of IBA and NAA [21]. Earlier findings also supported with result obtained in the study species [7, 22, 23].

CONCLUSION

This study revealed that the efficient shoot induction and mass multiplication of *Plumbago rosea* is possible by micropropagation technique. The plant has many diverse medicinal applications including treatment of cancer. Thus there is a current need to conserve this plant for mass production and propagation of good quality plant material. From this study it can be concluded that micropropagation technique will be help for the mass multiplication of *P. rosea* and can be able to overcome drastic diminishing of plant distribution.

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REFERENCE

[1] Lingaraj Patro, Medicinal Plants of India: With special reference to Odisha, International Journal of Advance Research and Innovative Ideas in Education, Vol-2 Issue-5 , 2016, 121- 135.

[2] Bhaskaran. K. K and Ajith Kumar Ramesh.A. R, Introductory Biotechnology molecular Biology, Manjusha Publications Calicut, Xpress offset printer, 2016, 295- 298

[3] Agarval S, Ghosh B, Drug Plants of India (Root Drugs), Kalyani Publishers, New Delhi, 1985, 204–205.

[4] CSIR, The Wealth of India. A dictionary of Indian raw materials and industrial products. Council of Scientific Industrial Research, New Delhi, India, 1969, 162–164.

[5] Mooss N S, Single Drug Remedies, Vaidyasarathy press (P) (Ltd), Kottayam, 1976, 106–110.

[6] Pillai NGK, Menon TV, Pillai GB, Rajasekharan S, Nair CRR, Effect of plumbagin in charmakeela (common wart) a case report. J Res Ayar Sidha, 2, 1981, 12–26.

[7] Chetia S, Handique P J, High frequency in vitro shoot multiplication of *Plumbago indica*, a rare medicinal plant, Curr Sci, 78, 2000, 1–8.

[8] Kirtikar K R, Basu B D, Indian Medicinal Plants Vol.11, M/S Bishen Sing Mahendrapal, New Delhi, India, 1975, 1469–1470.

[9] Okeyo JM, Medicinal plants, GH Schmelzer, A Gurib-Fakim (Eds.) PROTA, 2008, 473.

[10] Dorni AIC, Vidyalakshmi K, Vasanthi R, Rajamanickam G, Dubey G, HPTLC method for the quantification of plumbagin in three *Plumbago* species, Res J Phytochem, 1, 2007, 46–51.

[11] Parekh J, Jadeja D, Chanda S, Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity,Turk. J. Biol, 29, 2005, 203–210.

[12] Saraswathy A, Chandran R, Manohar VM, Wound Healing activity of the chloroform extract of *Plumbago rosea* Linn. and plumbagin, Nat Prod Sci, 12, 2006, 50–54.

[13] Binoy Jose. P Dhanya B P, Krishnan P N, Satheesh kumar, *Plumbago rosea* L. – A Review on Tissue culture and Pharmacological Research, Int. J. Pharm. Sci. Rev. Res., 2014, 246- 256.

[14] Kalpana N Reddy, A.Manoj Kumar, N.B. Shanmugam, Simple and rapid regeneration of *Plumbago rosea* using BAP for Direct and Indirect plant regeneration from leaf and nodal explants, Medicinal and aromatic plant science and biotechnology, 2009, 85-89.

[15] Selvakumar. V, Anbudurai. P. R., Balakumar. T, *In vitro* propagation of the medicinal plant *Plumbago zeylanica* L. Through nodal explants, *In vitro* cellular and developmental biology, 2001, 280- 284.

[16] Yaadwinder Sidhu, *In vitro* micropropagation of medicinal plants by tissue culture, The Plymouth Student Scientist, 2010, 432- 449.

[17] Lakshmanan , G. Bupesh, A.Vignesh , A. Sathiyaseelan and K. Murugesan, Micropropagation and anticancer activity of methanolic extract of Plumbago auriculata Lam. G., International Journal of Advanced Biotechnology and Research ,0976-2612, Vol-7, 2016, 2001-2011.

[18] David Raja. H., mariya Jenifer. A, Steffi. P. F, Thamilmaraiselvi. B., Srinivasan. P and. Tamilvanan. R, Micropropagation of *Plumbago zeylanica*- an important medicininal plant, World Journal Of Pharmacy and Pharmaceutical Sciences , 2018, 2278- 4357.

[19] Bhadra. S. K., Akhter. T, Hossain. M. M, *In vitro* Micropropagation of *Plumbago indica* L. through induction of direct and indirect organogenesis, Plant Tissue Culture and Biotechnology 19(2), 2010, 169- 175.

[20] Antony Ceasar. S., Ayyanar. M. and Ignacimuthu. S., An improved micropropagation protocol for *Plumbago zeylanica* L. An Important Medicinal Plant. Asian Journal of Biological Sciences, 6, 2013, 214-220.

[21] Iyyakkannu Sivanesan and Byoung Ryong Jeong, Micropropagation of *Plumbago rosea* L., African Journal of Biotechnology , vol 8(16), 2009, 3761- 3768.

[22] Idayat T. Gbadamosi and Egunyomi. A, Micropropagation of *Plumbago zeylanica* L. (Plumbaginaceae) in Ibadan, Southwestern, Nigeria, Journal of Medicinal Plants Research, Vol. 4(4) , 2010, 293-297.

[23] Satheesh Kumar. K, Bhavanandan. K. V, Micropropagation of *Plumbago rosea* Linn. Plant cell, Tissue and Organ culture, 1988,275-278.