Research Paper

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Conservation and in Vitro Mass Multiplication of Endangered Medicinal Plant, Leptadenia Reticulata (Retz.) Wight & Arn. Through Nodal Explant

Biotechnology

KEYWORDS : Leptadenia reticulate (Retz.) Wight & Arn. Medicinal plant, nodal segments, Ex-vitro, Asclepiadaceae

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ABSTRACT

An efficient protocol has been developed for plant regeneration from nodal explants of Leptadenia Reticulata (Retz.) Wight and Arn. The population of Leptadenia reticulata (Retz.) Wight and Arn are not sustaining as they cannot reproduce fast enough in the wild to keep up with the demand. Unsustainable extraction, multiple uses and hence the huge demand for this plant by the traditional medicine and industries make this plant endangered. There is a need for development of alternative methods for quick and efficient propagation, for conservation and utilisation of germplasm of Leptadenia reticulata (Retz.) Wight & Arn. A range of various auxins and cytokinins with different concentrations and effect of various media have been investigated for multiple shoot induction using nodes, internodes and shoot tips as explants. The best results were obtained by nodal segment cultured on MS medium fortified with NAA (1.00mg/l) with BAP (4.0mg/l) .The regenerated shoots rooted best on full strength MS medium containing IBA (2.0mg/l). Regenerated plantlets with well-developed shoots and roots were hardened, successfully transferred to soil and maintained in green house. The present in vitro procedure can be used in conservation and mass propagation of this endangered medicinal plant.

INTRODUCTION

The world health organisation has estimated more than 80 %of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs (**Vines, 2004**). Promotion of domestic cultivation of medicinal plants was suggested as a viable long term alternative (**Canter *et al*., 2005**).The application of biotechnology to solve inherent problems of medicinal plants has been a subject of research and reviews (**Dubey *et al*., 2002**).

***Leptadenia reticulata (Retz.)* Wight. & Arn.** (Family-Asclepidaceae) commonly known as jeewanti is an Indian medicinal plant used enormously due to its great value in general debility, involuntary seminal discharge, as a stimulate tonic (**Dandiya & Chopra, 1970**) as a bactericidal, anti fabrifuge, wound healer and in mouth ulcer (**Vaidya, 1965**). Roots are used in many ayurvedic and herbal formulations (**Anon, 1978**) as a cure for ear, nose, and skin infection.(**Kirtikar & Basu, 1998**). It is also used for increasing milk-yielding capacity in cattle (**Anjaria & Gupta, 1967, and Anjaria *et al*., 1975**) and to increase the egg laying capacity of hen in poultry industry. Flowers are good for eye sight. The flowers and tender leaves are used as vegetable (**Shortt, 1887**) and to make bread (**Gammie & Alexander, 1992**). According to ayurveda, is a tonic given for weak debility and such similar conditions. Commonly given to those suffering from weakness and lack of energy to give general strength to their body. A cooling, mucilaginous, demulcent with light strengthening and tonic properties of this plant is traditionally used in the treatment of seminal discharges and snake bite (**Bhatt, *et al*., 2006**). Huge demand and multipurpose uses of these plants in pharmaceutical industries, population bloom, urbanization, over-exploitation and recurring drought and famine in the region make these plants species endangered. Along with it the low viability of seeds and poor capacity of germination rate restricts the propagation of Leptadenia reticulata through seeds. Plant tissue culture as a technology for ex-situ multiplication is fast and uses small amount of shoots and may succeed when other methods fail **(Edson *et. al*., 1997).**The present study is aimed to develop an efficient in–vitro method for conservation and mass-propagation of Leptadenia reticulata through the nodal segments culture followed by successful regeneration micro-propagated plant in the field condition.

MATERIALS AND METHODS

The plant material was collected from Jodhpur University and also planted in earthen pots at the Department of Botany, Poddar International College, Mansarovar, Jaipur. Healthy and young nodal segments were washed under running tap water for 30 min and with 0.1% teepol for 5 minutes. Surface sterilization was done after rinsing with sterile distilled water, in 0.1% HgCl2 for 1 minute and again rinsed thoroughly with sterile distilled water. MS medium used, had the concentration of 3%sucrose and 0.8% agar. The surface sterilized explants were cultured on MS media fortified with various concentrations of plant growth regulators (NAA/BAP) at varied concentration (1.0mg/l – 4.0 mg/l)) for their proliferation and multiplication. The in vitro developed shoots from nodal segments were excised and transferred to MS medium with various auxins (IAA/NAA/IBA) at varied concentration (0.5-4.0 mg/l) for their root induction. The pH of the medium was adjusted to 5.8 prior autoclaving at 121°C for 15 min. All cultures were maintained at 25±2°C with 16 hrs photoperiod under fluorescent light. Each and every experiment were performed with 20 replicates and repeated twice.

After successful root development, *in vitro* developed plantlets were taken out from the culture vessels. The plantlets were then transferred to small plastic pots having a mixture of sterile vermiculite and soil in the ratio of 1:3. After two weeks, the pots were uncovered and brought from partial light to complete sun light gradually and after three weeks for their acclimatization to natural conditions.

RESULTS AND DISCUSSION

During the present investigation, the explants of *Leptadenia reticulata (Retz.)* Wight. & Arn started growing in MS medium supplemented with NAA in combination with BAP within two weeks. The highest percentage and maximum number of shoot induction from nodal segment was observed on MS medium fortified with NAA (1.00mg/l) with BAP (4.0mg/l) (Fig - E) (Table-1). NAA and BAP combinations were rewarding in many fruit tree species (Zimmerman and Swartz, 1994). For the shoot regeneration, cytokinin is effective when used in combination with an auxin (Nike *et al.,* 1999). The combination of NAA and BAP are also well known to induce multiple shootlets in different plant species *(*Tokuhara and Mii, 1993, Melissa *et al*., 1994, Budhiani, 2001; Decruse *et al*., 2003; Daneshvar *et al., 2013*). In constrast to this multiple shoot proliferation has also been reported on combination of kn and IAA in *Peltophroum pterocarpum* from nodal segment (Uddein *et al*., 2005). Some contrast findings have also been reported in aloe vera (Velcheva *et al*., 2005; Hashemabadi and Kaviani , 2008) .The *in-vitro* raised shootlets were transferred to MS media with different concentration of auxins (IBA/IAA/NAA) for rooting. We observed the highest percentage of rootlets per shootlets on MS medium fortified with IBA (2.0 mg/l) (Fig: F) (Table-2). The potential of IBA in root induction has been reported in many species (Epstein *et al*., 1993; Farzin *et al*., 2007; Asghar *et al*., 2011; Rafique *et al.,*2012). In contrast rooting was also observed on IAA in combination with BAP in different plants (Marta *et al.,* 2009; Taware *et al*., 2010; Kalidaas *et al*.,2010; Paul *et al*., 2012). After 30 days, *in vitro* raised plantlets were hardened in polycups (Fig: G) con-

taining mixture of vermiculite soil covered with polypropylene bags and irrigated with diluted liquid MS medium. The plants were kept in a culture room for 15 Days. 55% of plants were successfully established in polycups .After 15 days the polycups hardened plants (Fig: H) were transferred to pots and kept in green house. After one month, the plants were transferred to the fields. On later stages, only 40-50% of the plants survived in field condition for a long time.

CONCLUSION

Thus the present study reveals the usefulness of *Leptadenia reticulata* (Retz.) Wight & Arn. for several medicinal purposes and the presence of various phytochemical indicates the potential of this plant as a source of potent drugs. Hence, there is a need for developing alternative method for quick and efficient method for conservation and utilisation of germplasm of *Leptadenia reticulata* (Retz.) Wight & Arn. The present study has established reliable and repeatable protocol for large scale multiplication of *Leptadenia reticulata (Retz.) Wight & Arn* .through nodal segments.

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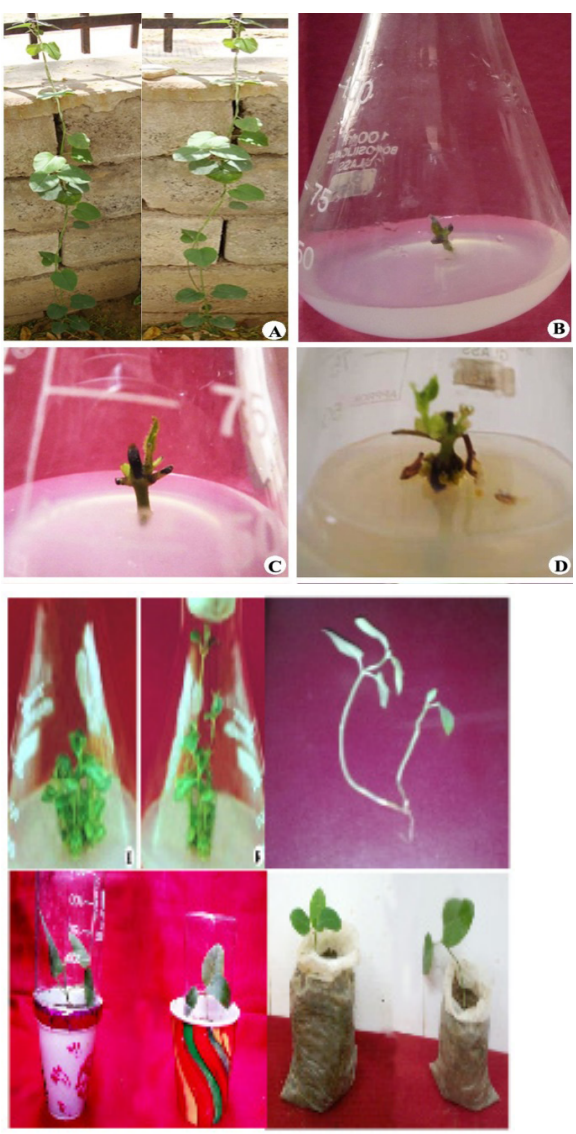

Fig: *In vitro* **mass multiplication of** *Leptadenia reticulata* **(Retz.) Wight & Arn.**
A: *In vivo* **growing plant of** *Leptadenia reticulata* **(Retz.) Wight & Arn.**
B: Inoculation of explants on NAA (1.0mg/l) BAP (4.0mg/l).
C: Initiation of shoot on NAA (1.0mg/l) BAP (4.0mg/l).
D: Initiation of multiple shoot on NAA (1.0mg/l) BAP (4.0mg/l).
E: Multiplication of *in vitro* **raised shoots on NAA (1.0mg/l) BAP (4.0mg/l).**
F: In vitro rooting on IBA (2.0 mg/l).
G: Hardening of the in vitro raised plants.
H: Acclimatization of the plants.

TABLE-1: Effect of NAA and BAP on shoot induction from nodal segments of *Leptadenia reticulata (Retz.) Wight and Arn* on MS medium.

MEDIUM : MS (Full strength) + Sucrose (3.0%) + NAA (1.0mg/l)+ BAP (4.0 mg/l) INOCULUM : Nodal segments INCUBATION : At 25±2°c under 16 hour's photoperiod for 3-4 weeks		
MS media + growth regulators(mg/l)		Number of shoots per node (Mean± S.E.)
NAA	IBA	
1.0	4.0	7.12 ± 0.84
2.0	3.0	4.02 ± 2.84
3.0	2.0	3.14 ± 1.11
4.0	1.0	1.10 ± 0.89

TABLE-2: Effect of IBA on root induction from *in vitro* raised microshoots of *Leptadenia reticulata (Retz.) Wight and Arn* on MS medium after four weeks of culture.

MEDIUM : MS (Full strength) + Sucrose (3.0%) + IBA (2.0mg/l) INOCULUM : Invitroregenerated shoots INCUBATION : At 25±2°c under 16 hour's photoperiod for 3-4 weeks			
MS media + growth regulators(mg/l)		Number of roots per shoot (Mean± S.E.)	Rooting response (%)
IBA	0.5	1.11 ± 0.1	20%
	1.0	2.86 ± 0.14	40%
	2.0	4.20 ± 0.2	70%
	3.0	2.13 ± 0.17	35%
	4.0	1.23 ± 0.26	25%

REFERENCE

1. Anjaria, J., V., Gupta, I., (1967) Studies on lactogenic property of Leptadenia reticulate (Jivanti) and leptaden tablets in goats, sheep, cows and buffaloes. Indian Vet. J., 44, 967974. | 2. Anjaria, J., V., Varia. M., R., Janakiraman, K., Gulati, O., D., 1975, Studies on Leptadenia reticulate: Lactogenic effects on rats. ndian J. Exp. Biol., 13, 448-449. | 3. Anon., 1978, Ayurvedic Formulary of India, Govt of India Press, Faridabad, Part-I, 1st edition, p. 30. | 4. Asghar, S., Ahmad, T., Ahmad, I., Hafiz and Yaseen, M. 2011. In vitro propagation of orchid (Dendrobium nobile) var. Emma white. African Journal of Biotechnology Vol. 10(16), pp. 3097-3103. | 5. Bhatt, T., Jain, V., Jayathirtha, M. G., Banerjee, G.,Mishra, S., H.2002, In vitro regeneration of roots of Phyla nodiflora and Leptadenia reticulate, and comparison of roots from cultured and natural plants for secondary metabolites. Indian J. Exp. Biol., 40, 13821386. | 6. Budhiani E. Micropropagation of Aloe vera through shoot multi¬plication. Indonesia: 2001. | 7. Canter, P., H., Thomas, H., Edzard, Ernest .2005. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology, Trends Biotechnol 23 (4), 180-185. | 8. Dandiya, P., C., Chopra, Y., M., 1970, CNS-active drugs from plants indigenous to India. Indian J. Pharm., 2, 6790. | 9. Danehvar, M.,H., Moallemi,N., and Zadeh, N.,A.2013. the effect of different media on shoot proliferation from shoot tip of aloe vera L.JUNDISHAHPUR J NAT PHARMA PRODUCT 8(2) ;93-7. | 10. Decruse, S.W., Gangaprasad, A., Seeni, S. and Menon, V.S. 2003. Micropropagation and ecorestoration of V. spathulata, an exquisite orchid. Plant Cell. Tiss. Org. Cult. 72: 199-202. | 11. Dubey, T., Guerra, D., J., 2002 use of biotechnology for growing medicinal plants. Rec prog med plants, 5, 47-61. | 12. Epstein, E., Sagee, O. and Zahir, A.1993. Uptake and metabolism of Indole-3 acetic acid and Indole-3 butyric acid by Petunia cell suspension culture, Plant. Growth Regul., 13:31-40. | 13. Farzin, M., Parabia., Bharat., Gami., I. L., Kothari, J. S. S., Mohan. and M. H., Parabia. 2007. Effect of plant growth regulators on in vitro morphogenesis of Leptadenia reticulata (Retz.) W. &A. from nodal explants. CURRENT SCIENCE, 1290 VOL. 92, NO. 9, 1290-1293. | 14. Gammie, Alexander, G.,(1902) A note on plants used for food during famines and seasons of scarcity in the Bombay Presidency. India Bot. Surv., 2, 171196. | 15. Hashemabadi D, Kaviani B. Rapid micro-propagation of Aloe vera L. via shoot multiplication. African J Biotech. 2008;7(12):1892-902. | 16. Kalidass, C., Daniel, A., and Mohan,V. R., 2010. Rapid Micropropagation of Plumbago zeylanica L. An Important Medicinal Plant. Journal of American Science.6(10):1027-1031. | 17. Kirtikar, K., R., Basu, B.,D., 1998, Indian Medicinal Plants, Bishen Singh Mahendra Pal Singh, Dehradun, , 2nd edn, vol.7, pp. 22462247. | 18. Marta, V. M., Andrés, M., and Gatica, Arias. 2009. Effect of BAP and IAA on shoot regeneration in cotyledonary explants of Costa rican melon genotypes Agronomía Costarricense 33(1): 125-131. | 19. Naik SK, Pattnaik S, Chand PK (1999). In vitro propagation of pomegranate (Punica granatum L. cv. Ganesh) through axillary shoots proliferation from nodal segments of mature tree. Scientia Horticulturae, 79: 175-183. | 20. Paul, J., Revathy, I. and Johnson, M.2012. In vitro propagation of Solidago virgaurea L. through nodal culture. Research in Plant Biology, 2(4): 08-15. | 21. Rafique, R., Fatima, B., Mustaq, S., Iqbal, M.S., Rasheed, M., Ali, M. and Hasan, S.Z.U. 2012. "Effect of indole-3-butyric acid (IBA) on in vitro root induction in dendrobium orchid (Dendrobium sabin H.)". Afri. J.Biotech. 11(20):4673-4675. | 22. Shortt, J., 1887 List of wild plants and vegetables used as food by people in famine times. Indian For., 188788, 3, 232238. | 23. Taware, A.S., Mukadam, D.S., Chavan, A.M., and Tware, S.D .2010. Comparative studies of in vitro and in vivo grown plants and callus of Stevia rebaudiana (Bertoni). International Journal of Integative Biology 9(1): 10-15. | 24. Tokuhara, K. and Mii, M. 1993. Micropropagation of Phalaenopsis and Doritaenopsis by culturing shoot tips of flower stalk buds. Plant Cell Rep. 13:7-11. | 25. Uddin, M.S., Nasirujjaman, K.,Zaman, S.and Reza, M.A. 2005. Regeneration of multiple shoots from different explants viz., shoot tip, nodal segment and cotyledonary node of in vitro grown seedling of Peltophorum pterocarpum (DC.) Baker ex.K.Heyne. Biotech4(1) :31-38. | 26. Vaidya, B. G., Nighantu Aadarsha, 1965 Shri Swami Atmanand Saraswati Ayurved Sahakari Pharmacy Ltd, Surat, , vol. 2, p. 1106. | 27. Velcheva M, Faltin Z, Vardi A, Eshdat Y, Perl A. Regeneration of Aloe arborescens via somatic organogenesis from young inflo¬rescences. Plant Cell Tissue Organ Cult. 2005;83(3):293-301. | 28. Vines, G., 2004, Herbal harvests with a future: towards sustainable sources for medicinal plants, Plant Life International. | 29. Zimmerman RH, Swartz HJ (1994). In vitro culture of temperate fruits. In: Vasil IK, Thorpe TA (eds). Plant Cell Tissue Culture. Kluwer Academic Publishers, Dordrecht, pp. 457-474. |