RESEARCH PAPER | Biotechnology | Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

Reduction of Biotic Stress in *Leptadenia Reticulata* (Retz.) Wight & Arn. Using Synthetic Seeds Prepared Via Immobilizing Arbuscular Mycorrhiza Fungi and To Carry Out its Conservation

KEYWORDS: Leptadenia reticulata (Retz.) Wight and Arn, Micropropagation, Arbuscular mycorrhiza fungi

*** Poonam Dhawan**
Department of Biotechnology, Poddar international college. * corresponding author

Shilpi Damor
Department of Biotechnology, Poddar international college

ABSTRACT *The present study reveals the usefulness of Leptadenia reticulata (Retz.) Wight and Arn. for several medicinal purposes and the presence of various phytochemical indicates the potential of this plant as a source of potent drugs. Huge demand and multipurpose uses of this plant in pharmaceutical industries, population bloom, urbanization, over-exploitation and recurring drought and famine make this plant species endangered 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. Our work here is on development of a novel method to conserve this endangered medicinal plant by the means of Micropropagation and raising the in vitro grown plant along with artificial seeds prepared by immobilizing AMF. The development of such a protocol will help us to achieve a large number of individual plants even in the areas where they sustain rarely due to poor survival condition.*

Introduction

Leptadenia reticulata (Retz.) Wight and Arn. plant species are found in arid regions of Rajasthan (Kiran Bala *et al.,* 1989; Pande *et al.,* 1999) which are characterized by poor sandy soils, low organic matter, uncertain and erratic rainfall, high wind velocity and generally experience water deficit during the growth period. Arbuscular mycorrhizal fungi are a major component of rhizosphere microflora in natural ecosystem and play significant role in the re establishment of nutrient cycling in native ecosystem (Peterson *et al.,* 1985).). The population of AMF varies greatly and their distribution is affected by various biotic and abiotic factors (Mohammad *et al.,* 2003). Koske (1981) also reported that distribution of AM fungi is related to soil and environmental conditions. Hayman (1983) reported better establishment of vegetation in arid areas by using AMF as these fungi may often enhance plant absorption of phosphorus and other elements, improve water uptake and its transport to plants, and enable the plants to withstand high temperatures (Yaseen *et al.,* 2011). Turnau and Haselwandter (2002) also considered AMF as a tool for re-establishment of endangered plant species. In the present investigation, *in vitro raised Leptadenia reticulata (Retz.) Wight & Arn.* with and without artificial mycorrhiza seeds were grown in the normal soil. The objective of this study is to determine the response of these seeds to plant survivality and growth.

Material and methods

The work was carried out at Department of Biotechnology, Poddar International College, Jaipur. The soil sampling of both the sites (site where the plant grows i.e. Jodhpur as well as the site where plant does not grow i.e. Jaipur, Fig: A) was done from Durgapura Agricultural Research Centre, Jaipur. The fungus was isolated from the soil in which *Leptadenia reticulata (Retz.)Wight & Arn.* grows. This was done by spreading the soil on PDA plates and incubating the plates at 26° ± 2°C for growth. The isolated colony was identified by biochemical testing from S.P. Institute of Biotechnology, Jaipur. The fungi from the plate were spread onto a glass slide with the help of inoculation loop and stained with trypan blue and observed under microscope. The liquid culture of the isolated AMF was prepared by procuring the fungi from the culture plate and diluting it in 10 ml of water. Further the culture was kept for incubation at 25° ± 2° C for 48 hrs. Sodium alginate (3%) was mixed with liquid culture of AMF and the solution was singly dipped into a calcium chloride solution for a few seconds. The so-formed beads were kept on PDA plates for germination. The plates were kept at an incubation temperature of 28°± 2°C for 5 – 7 days. The growth was observed. The *in vitro* raised plant *Leptadenia reticulata* (Retz.) Wight & Arn was grown in Jaipur soil with the prepared artificial mycorrhizal seeds. Another *in vitro* raised plant *Leptadenia reticulata* (Retz.) Wight & Arn was also grown in the same soil but without the inoculation of prepared artificial seeds. The growth of both the plants was checked alternatively after every 15 days. The length of the plant was recorded. Also, the roots of the *in vitro* raised plant along with the artificial seeds were sampled and stained to confirm the infection of AMF .To determine the colonization, root samples were collected from the *in vitro* grown plants and were washed under running tap water and staining of roots was done by the method of Phillips and Hayman (1970). The roots were cut into pieces of 1.0 cm length and placed in 10 % KOH solution, which was kept at boiling point for about 10 minutes. The root samples were isolated and rinsed with distilled water until the brown colour disappeared. Post clearing bleaching was done with alkaline hydrogen peroxide (0.5% NH_4OH and 0.5% H_2O_2 v/v in distilled water). Roots were rinsed again with distilled water ,treated with 1% HCL and stained with 0.05% w/v trypan blue in lactic acid – glycerol. Assessment of colonization was done microscopically. A segment of root was counted as infected when hyphae, vesicles or arbuscules were observed.

Result and Discussion

Arbuscular mycorrhizal fungi are a major component of rhizosphere microflora in natural ecosystem and play significant role in the re establishment of nutrient cycling in native ecosystem (Peterson *et al.,* 1985). Further, Panwar and Vyas (2002) indicated the significance of AMF in re-establishment and conservation of endangered plants in arid areas.

During the present study it is evident that the AMF were affected by soil pH, organic carbon and phosphorus content (Table-1). A significant positive correlation with pH and organic carbon was recorded during present investigation. The Jodhpur soil had pH of 8.3 which is slightly higher than Jaipur soil (8.1).This difference may be seen to support the fact that Jodhpur soil is alkaline in nature and thus is favourable for growth of AMF. Blaszkowski (1993) while investigating plant communities in Poland observed a significant positive correlation between AMF and soil pH. The major difference noted out was among the initial organic carbon content of both the soils. The organic matter content found in Jaipur soil (0.15 %) out to be was considerably lower than Jodhpur soil (0.19%) which possesses high AMF population. This conclusion was

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

supported in accordance to the finding. A positive correlation with organic carbon content in soil coincides with the findings of Mohammad *et al.*,(2003), who reported the same result while investigating under semi-arid environment of Jordan. Organic matter content in the soil increases the water-holding capacity of the soil (Brady and Weil, 1996;Panwar and tarafdar, 2005; Carrenho*et al.*, 2007) and, therefore, may facilitate a more favourable soil moisture condition for the AMF population. The phosphorus content (5.3 kg/hec) of the soil came out to be high as compared to Jodhpur soil (3.1 Kg/hec) which again suggested a lower population of AMF in Jaipur soil. The rhizosphere soils collected from field and successive pot cultures in Jodhpur have higher AMF spore densities compared to other sites (Panwar and Tarfdar, 2005). This may be because of poor soil fertility (in terms of available phosphorus) which results in higher AMF populations (Norani, 1996; Panwar and Tarafdar, 2005; Carrenho*et al.*, 2007). In general, soils remained alkaline in reaction, became low in organic matter content and available phosphorus status.

The isolated fungus (Fig: B) was confirmed by S.P.Institute of Biotechnology, Jaipur. Mycelium and spore was mounted in lacto phenol for identification. The identification was based on spore and mycelium colour, size, surface ornamentation and wall structure with reference to the descriptions and pictures provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and originally published species descriptions (Fig: C-D).

The investigated sample is *Scutellospora reticulata* **which is** Arbuscular Mycorrhizal Fungi

AMF was thus immobilized for the production of synthetic seed (*Fig: E*). Alginate was used as a matrix for the immobilization of fungus culture. Alginate beads have been widely used for entrapment of BCAs like PGPR and fungi (Trivedi & Pandey, 2008), including AMF (Declerck *et al.*, 1996; Vega *et al.*, 2003). Vega *et al.,* 2003 also worked on growth of micropropagated bananas colonized by root organ culture produced arbuscular mycorrhiza fungi entrapped in calcium alginate beads. Some findings was also reported on immobilization of Pellets (2 mm) of the fungus *T. Viride* and their activity of β-glucosidase was investigated using cellbiose and salicin as substrates (Matteau and Saddler1982). Royer *et al.*, (1983) also reported on hyphal outgrowth from beads that contained viable mycelial fragments. Immobilized fungi were first investigated for a role in biotransformations such as that of cortexolone to hydrocortisone by *Curvularialunata* (Sonomoto *et al.*,1983) and of glucose to itaconic acid by *Aspergillus terreusn* (Horitsu *et al.,* 1983). Immobilized fungi are increasingly studied for application in processes which have traditionally only used free mycelia. The artificial seeds germinated on PDA media after 4-5 days (Fig: F).

The *in vitro* raised plant was grown on Jaipur soil (Fig: G) inoculated with artificial seeds as well as soil without the seeds. The growth in both the cases was recorded alternatively after 15 days. The growth of 6 cm was observed after 30 days (Fig: H) which was measured upto 15 cm after 90 days (Table-2) (Fig: I) in soil inoculated with artificial seeds while the plantlets raised on normal soil were unable to sustain (Fig: J). It led us to conclude that artificial seeds can be used to raise *Leptadenia reticulata (Retz.)Wight and Arn*.in arid as well as semi arid regions with the help of artificial seeds. Positive effect of AMF beads on rice plant has ample of evidences (Selicia& Bagyaraj, 1994; Xiao *et al.*, 2010). Similar results were reported in cowpea (*Vignaunguiculata)* varieties where AM inoculated plants out performed than non-inoculated plants (control) in terms of growth, productivity parameters and nutrient uptake (Yaseen, *et al.*, 2011). Finally in order to make a confirmatory check the roots of the *in vitro* raised plant was stained and observed at Department of Zoology, Jaipur. Cleared and stained roots showed the presence of vesicles, arbuscules and hyphae which confirmed the infection of *in vitro* raised plant with AMF. The fungi identified by (Fig: K-L).

Table-1: Comparison of physicochemical characteristics of both site soils

S. No	Region	pH	Electrical conductivity (Ds/m)	Organic carbon (%)	Olsen P (Kg/ Hec)
1.	Jaipur	8.1	0.9	0.15	5.3
2.	Jodhpur	8.3	0.10	0.19	3.1

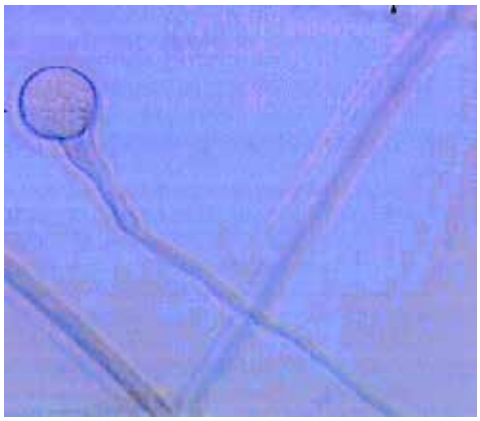
Table-2: Growth of the plant recorded.

S. no.	No. Of days	Length of the plant.	
		Soil inoculated with artificial seeds	Normal soil
1.	30	6 cm	2 cm
2.	60	10 cm	3 cm
3.	90	15 cm	Not survived.

Fig A: soil of both the sites for sampling.

Fig B: Isolated fungi on PDA plate.

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X



(C)

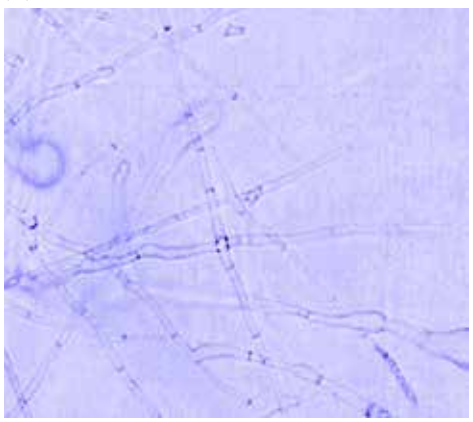


Fig C-D: Arbuscular fungi as identified after staining.

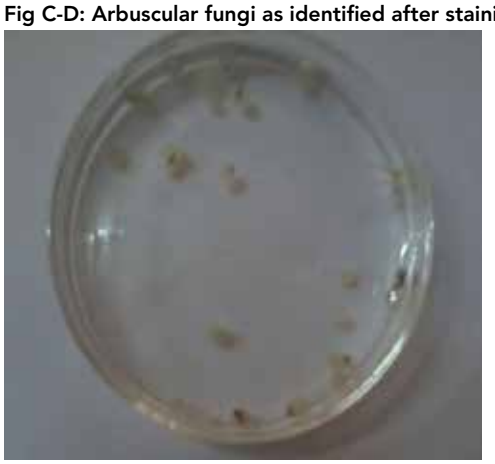


Fig E: Artificial seeds prepared by immobilizing AMF.

B

Fig F: Germinated synthetic seeds on PDA plate.

Fig G: *In vitro* raised plant grown on normal soil along with synthetic seeds.

Fig H: Growth of plant after 30 days (6 cm).

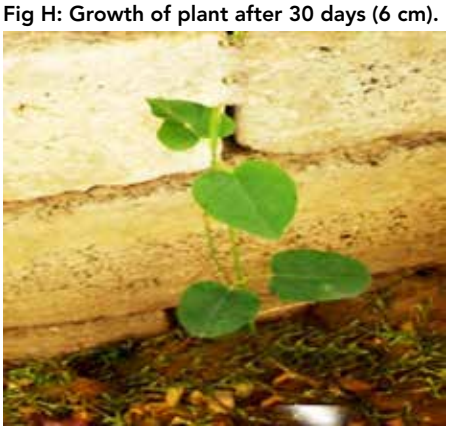


Fig I: growth of plant after 90 days (15 cm).

I

Fig J: *In vitro* plant grown on normal soil along without synthetic seeds.

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

(K)

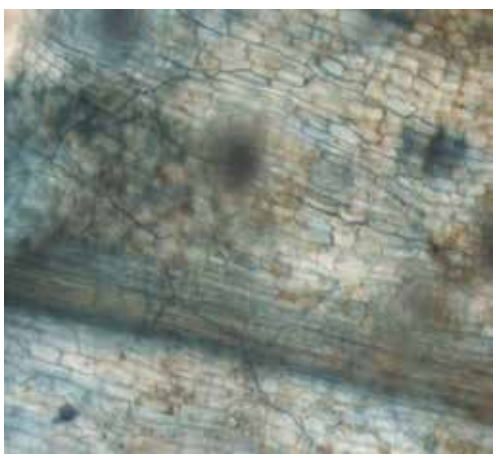


Fig K-L: Fungi infected roots of *in vitro* raised plant.

REFERENCE

1. Blaszkowski, J.1993. The occurrence of arbuscular fungi and mycorrhizae Glomales in plant communities of maritime dunes and shores in Poland. | 2. Brady, N.C., and Weil, R.R. 1996. The Nature and Properties of Soils, 11th ed. Prentice Hall Incorporation, New Jersey, USA 739pp. | 3. Declerck, S., Strullu, D. G. and Plenchette, C. 1996. In vitro mass - production of the arbuscular mycorrhizal fungus, Glomus versiforme, associated with Ri T - DNA transformed carrot roots. Mycological Research 100, 1237 – 1242. | 4. Hayman, D.S.1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Canadian journal of Botany. 61: 944–963. | 5. Horitsu, H.; Takahashi, Y.; Tsuda, J.; Kawai, K.; and Kawano, Y. 1983. Production of itaconic acid by Aspergillus terreus immobilized in polyacrylamide gels. Eur. J. Appl. Microbiol. Biotechnol. 18: 358-360. | 6. Kiran Bala, Rao, A.V. and Tarafdar, J.C., 1989. Occurrence of VAM associations in different plant species of the Indian desert. Arid Soil Research and Rehabilitation 3, 391–396. | 7. Koske, R.E. 1981.A preliminary study of interaction between species of Vesicular arbuscular fungi in a sand dune, 76: 411-418. | 8. Matteau, P. P., and Saddler, J. N. 1982. Glucose production using immobilized mycelial-associated β-glucosidase of Trichoderama E58. Biotechnol. Lett. 4:513–518. | 9. Mohammad, M.J., Hamad, S.R., and Malkawi, H.I.2003. Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. Journal of Arid Environments 53, 409–417. | 10. Norani, A. 1996. An assessment and enumeration of vesicular arbuscular mycorrhizal propagules in som forest sites of Jengka. Journal of Tropical Forest Science .9: 137–146 | 11. Pande, M., Tarafdar, J.C. and Gupta, G.N. 1999. Vesicular arbuscular mycorrhizal infection in arid zone trees of agroforestry systems. Journal of Indian Society of Soil Science. 47:54–57. | 12. Panwar, J. and Tarafdar, J.C.2005. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi . Journal of Arid Environments, 65; 337–350. | 13. Panwar, J. and Vyas, A. 2002. AM fungi: a biological approach towards conservation of endangered plants in Thar Desert, India. Current Science. 82:576–578. | 14. Peterson, R.I., Ashford, A.E., and Allaway, W.G.1985. Vesicular arbuscular mycorrhizal associations of vascular plants on Heron Island, a Great Barrier Reef coral cay. Australian Journal of Botany 33: 669–676. | 15. Royer, G., Livernoche, D., Desrocher, M., Jurasek, L., Rouleau, D., and Mayer, R. C. 1983. Decolorization of kraft mill effluent: kinetics of a continuous process using immobilized Coriolus versicolor. Biotechnol. Lett. 5:321–326. | 16. Selicia, J and Bagyaraj, D., J. 1994. Selection of efficient vesicular - arbuscular mycorrhizal fungi for wetland rice - A preliminary screen. Mycorrhiza 4, 265 - 268. | 17. Sonomoto, K.; Hoq, M.M.; Tanaka, A.; and Fukui, S.1983.Betahydroxylation of cortexolone (Reichstein compound S) to hydrocortisone by Curvularia lunata entrapped in photo-cross-linked resin gels. Appl. Environ. Microbiol. 45: 436-443. | 18. Trivedi, P. and Pandey, A. 2008. Recovery of plant growth - promoting rhizobacteria from sodium alginate beads after 3 years following storage at 4 degrees C. Journal of Industrial Microbiology & Biotechnology 35, 205 – 209. | 19. Turnau, K. and Haselwandter, K.2002. Arbuscular mycorrhizal fungi, an essential component of soil microflora in ecosystem restoration. In: Gianinazzi, S., Schu¨epp, H., Barea, J.M., Haselwandter, K. (Eds.), Mycorrhizal technology in Agriculture. Birkha¨user Verlag, Switzerland, pp. 137–149. | 20. Vega, M. C., Rodriguez - Romero, A. S., Hermoso, C. M. and Declerck, S. 2003. Growth of micropropagated bananas colonised by root - organ culture produced arbuscular mycorrhizal fungi entrapped in Ca - alginate beads. Plant and Soil 254, 329 - 335. | 21. Xiao, T.J., Yang, Q.S., Ran, W., Guo - Hua, X. And Shen, Q. 2010. Effect of inoculation with arbuscular mycorrhizal fungus on nitrogen and phosphorus utilization in upland rice - mungbean intercropping system. Agricultural Sciences in China 9, 528 - 535. | 22. Yaseen, T., Burni, T., and Hussain, F. 2011. Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of cowpea (Vigna unguiculata) varieties. African Journal of Biotechnology. Vol. 10(43), pp. 8593-8598. |