Vol. 12/2 (2012) 93-102

JOURNAL OF NATURAL REMEDIES

Biotechnological approaches to conserve the wealth of nature: endangered and rare medicinal plant species, a review

Meenakashi Jain, T. Sudhakar Johnson, Prakash Krishnan*

Natural Remedies Pvt Ltd., Veerasandra Industrial Area, Bangalore, Karnataka 560 100

Abstract

The popularization of the health and health related hazards and toxicity associated with the use of synthetic drugs lead to attention and interest in the use of alternatives; plants and plant-based products. However, a large number of medicinal plants remain yet to be investigated for their possible use. Most of the pharmaceutical/nutraceutical industry is highly dependent on wild populations for the supply of raw materials for extraction of medicinally important active compounds. On the other hand, due to a lack of proper cultivation practices, destruction of plant habitats, and the illegal and indiscriminate collection of plants from these habitats, many medicinal plants are severely threatened. Advances in biotechnology offer new methods for conservation of these rare and endangered medicinal plants. The present review is focused on biotechnological tools like *in vitro* culture, micropropagation, mycorrhization, genetic transformation, development of DNA banks and plant part substitution, which can be a potent tool for plant part conservation.

1. Introduction

Prolonged usage of synthetic drugs commonly used in the conventional system of medicine, their associated side effects as well as the uncertainty concerning their safety has paved a way towards an era of alternative system of medicine i.e. herbal medicine.

Plants have been used to overcome the irregularities in the body much before the term “disease” was known to the mankind. A medicinal plant is the one, used in order to relieve, prevent or cure a disease or to alter physiological and pathological process. Some of the oldest known medicinal systems of the world such as Ayurveda of the Indus civilization, Arabian medicine, Chinese and Tibetan traditional medicine of China and Kempo of the Japanese are all based mostly on plants. Medicinal plant therapy is based on the empirical findings of hundreds and thousands of years [1].

The present knowledge concerning the medicinal value of plants is gathered over the centuries through trial and error methods, and is often based on speculation and superstition [2, 3]. The strong historic bond between plants and human

* Corresponding author
Email:prakashkrish07@rediffmail.com

health began to unwind in 1897, when Friedrich Bayer and Co. introduced Aspirin (synthetic analogue of salicylic acid), an active ingredient of willow bark, to the world [4]. Similarly "Taxol" arguably the most celebrated and controversial natural product in recent years has history dates back to 1962 when *Arthur Barclay* collected bark from a single Pacific yew tree, *Taxus brevifolia*. It has since been approved by Food and Drug Administration for use in the treatment of breast cancer and AIDS-related Kaposi's sarcoma [5, 6].

The magnificent rise in the herbal market is credited to the high prices and harmful side effects of synthetic drugs, people rely more on herbal drugs and this trend is growing, not only in developing countries but in developed nations too. The market for herbal drugs has grown at an impressive rate due to a global resurgence in traditional and alternative healthcare systems, and therefore medicinal plants have great economic importance. India has 2.4% of world's area with 8% of global biodiversity. India is home to a great variety of medicinal plants, and is ranked sixth among 12 hotspots of mega diversity countries of the world. The Himalayas is designated as one of the global biodiversity hotspots [7].

World Health Organization (WHO) estimates that about 80% of the population in the developed and the developing nations depend upon traditional medicine, mostly plant drugs for the primary health care needs [8].

Unfortunately due to over exploitation, habitat loss and non-judicious use, many species of medicinal plants have become rare, threatened or endangered. In addition to this, the medicinal plants are highly affected by climate change, such as: increase in carbon dioxide concentration which favors C3 plants over C4 plant, increase in diseases and pest, high rain fall and high salt content in soil etc [9].

Several international, national as well as private bodies namely CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), CBD (Convention on Biological Diversity), UNEP (United Nations Environment Programme, UNEP planned International Union for Conservation of Nature is the world's oldest and largest global environmental organization), Botanic Gardens Conservation International, International Board for Plant Genetic Resources etc. are governed at times to devise feasible and competent strategies toward conserving this near to be threatened medicinal plants diversity [10]. Different approaches towards preserving the botanical diversity are being practiced for medicinal, ornamental, aromatic and other important plants. Substitution of the plant part has been the important method employed by several commercial enterprises, among others, to conserve the diversity. However, there needs to be several integrated and/or independent techniques or methodologies, which when combined together have a promising potential towards the conservation of plant species. In the present review, several approaches practiced worldwide for conserving the biological diversity are discussed.

2.Methods for conservation

United Nations Environment Programme (UNEP) convened a body "Convention on Biological Diversity" (CBD) to frame a protocol substantiating the biological diversity.

In article 8 of The Convention on Biological Diversity (CBD) emphasized on the fundamental requisite of *in situ* conservation of ecosystems and natural habitats [9, 11]. All over the world, the protected areas are the most widely accepted and practically approachable to biodiversity conservation. There are two methods of conservation of medicinal plants

(i) *in situ* conservation

(ii) *ex situ* conservation

In situ conservation focuses on preserving the genetic variation in the location it has been encountered originally i.e. in its natural habitats either in the wild or in traditional farming system. On the other hand *ex situ* involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Approaches to *ex situ* conservation include methods like seed storage, field genebanks and botanical gardens.

Even though the research today is majorly concentrated on developing new methods for *ex situ* conservation of the plant species, article in the CBD (Convention on Biological Diversity) certainly favors *in situ* conservation and regards *ex situ* only as a second best [12].

(i) *In situ conservation*

Conservation of medicinal plant has become a subject of primary importance considering a constant addition of a rare or endangered plant species list. The single most important way to conserve the plant species seems to be by protecting the habitat in which it lives, including the animal and insects which directly or indirectly helps in pollination or dispersal of spores. *In-situ* conservation involves maintenance of genetic variation at its native location although the conservation strategies are still very much in their modulation stage, many still remains unrevealed.

On farm conservation is the widely practiced *in-situ* technique for plant conservation. It is associated with the conservation of plants through the farmers on the farm, the approach is counted within the traditional agriculture system. On-farm is a reasonable and acceptable process, but lags due to the slow growth of the plant. Similar is the home garden technique for conservation which is maintaining the genetic variation of a plant within a limited space to make the maintenance in the native location comparatively cost effective.

Considering the time, cost and uncertainty involved in the above discussed approaches, a more reasonable methodologies for conserving the plants on large scale was introduced, called Genetic reserve conservation. This method is more appropriate for the bulk of wild species, whether closely or distinctly related, because it is easy to maintain a diverse genetic pool in a single reserve and allows continuous evolution of the species. The disadvantage is that the conserved material is not readily available for agricultural exploitation [13,14].

(ii) *Ex-situ conservation*

In-situ conservation, which considered as the method of conserving endangered species in their wild habitats, is promising in protecting indigenous species and maintaining natural communities along with their intricate network of relationships [15]. As habitat degradation and destruction is increasing, *ex-situ* conservation regarded as the process of cultivating and naturalizing endangered species outside of their original habitats, has become a practical alternative [16-18], especially for those overexploited and endangered medicinal plants with slow growth, small abundance and replant diseases [16, 19], e.g. *Paris* species in family Trilliaceae and *Panax* species in family Araliaceae [20]. *Ex-situ* cultivation becomes an immediate action to sustain medicinal plant resources [17, 18].

Understanding the geographical distribution of plant species is essential for their *ex-situ* conservation activities [21, 22]. Although many plant species can be successfully introduced, cultivated and naturalized in a wide range of habitats across countries and continents [23], their growth and distribution in different habitats are based on local indicators [24], e.g. soil properties, climate conditions and environmental features [25]. *Aguilar- Stoen* and *Moe* (2007) found that many medicinal plants thriving in harsh habitats and disturbed areas are of high medicinal efficacy because rocky and dry habitats stimulate their secondary metabolites [26]. Many plants are only found in places where the habitat is congruent with their growth [25], e.g. the propagation and quality of *Banksia serrata* varied among habitats [27]. Variations in growth and metabolites of medicinal plants among niches make *ex-situ* conservation habitat-specific

Genetic variation is maintained away from its original location by collecting the plants in the field gene bank, botanical garden or arboreta, or as a sample of seed, tuber, pollen etc maintained in the artificial conditions.

3. Plant part substitution

Due to the rapid increase of informal trade in medicinal plants/herbs, responsible management of natural medicinal plant resources has become a matter of urgency [28]. Part substitution could possibly fulfill the need of sustainable harvesting by substitution of various parts within the species of the same plant, for instance, substitution of bark or underground parts with leaves of the same plant [29], thereby satisfying the need of commercial enterprises with limited harvesting. *Zschocke* (2000) in his article states that, bark and roots (54%) and bulbs and whole plants (28%) represent the most important ingredients of Zulu herbal medicine sold on local street markets [30].

Cunningham and co workers' revealed that leaf- and fruit harvesting does not damage plants in the same way as debarking [31]. They further suggest few of the possible strategies for conservation [32, 30].

(i) Establish conservation areas and enforce laws against collecting bark.

(ii) Large-scale cultivation.

(iii) Encourage healers/formulators to collect and use alternative plant parts such as leaves and twigs instead of bark and *vice versa*.

Zschocke (2000) further reported the evaluation of differences and similarities between various parts of the same plant with respect to chemical composition and pharmacological properties. The preliminary results of phytochemical and pharmacological comparison of different plant parts of four important South African medicinal plants – *Eucomis autumnalis* (Hyacinthaceae), *Siphonochilus aethiopicus* (Zingiberaceae), *Ocotea bullata* (Lauraceae), and *Warburgia salutaris* (Canellaceae) are described [29].

4. Biotechnological approaches

Realizing the importance and utility of natural plant resources, several medicinal plant species are protected *in-situ*, but failing that *ex-situ* conservation is being resorted to *in-vitro* techniques or biotechnological approaches are becoming increasingly important in the conservation of endangered/threatened plants. This is especially true for the species with reproductive problem and / or extremely reduced population.

Biotechnological approaches are imperative for rapid multiplication and conservation of the critical genotype of medicinal plants. These include

(i) *In vitro* propagation

(ii) Mycorrhization

(iii) Genetic transformation

(iv) Cryopreservation

(v) DNA banks

(i) In vitro propagation

It is *in vitro* regeneration or multiplication (micropropagation) of large number of plants from its part, leaves, seeds, nodes and tubers etc. *In vitro* propagation is used for the production and multiplication of novel plants. Micropropagation has been proved as an important technique for the multiplication of plants in a large scale. Usually, it is carried out either through callus production from explant followed by shoots and roots, or from auxillary explant followed by rooting. *In vitro* regeneration leads to the development of whole plantelet from a single explant under controlled conditions, which after acclimatization can be transferred in the field [9]. *Gottlieb Heberlandt* (1854-1945) cultivated plant tissues in culture *in vitro* and is regarded as father of plant tissue culture [33].

Such a prolific rate of multiplication cannot be expected by any of the *in viv*o methods of clonal propagation. The shoot multiplication usually has a short cycle, results in logarithmic increase in the number of shoots. Tissue culture provides propagules such as minitubers or minicorms for plant multiplication throughout the year irrespective of the season. Using this method stock of germplasm can be maintained for many years. Employing *in vitro* methods more pathogen free plants can be raised and maintained economically. *Gloriosa superba* L, *Rauwolfia serpentina* L. Benth. Ex. Kurz. and *Buchanania lanzan* Spreng are few among the many plants for which *in vitro* propagation has been employed thoroughly [9].

(ii) Mycorrhization

Inoculation of mycorrhizal fungi into the roots of plants is referred as mycorrhization. Mycorrhial fungi are of two kinds: arbuscular Mycorrhizal fungi (AMF) and ectomycorrhizal fungi. These are symbiotic fungi and occur in 90% of the plants [34]. The AMF helps the plant partner by increasing uptake of nutrients in general, and phosphorus in particular [35]. AMF has been extensively studied for the diverse role it plays in the plant growth. [36-38].

If arbuscular mycorrhizal fungi are inoculated to *in vitro*- grown plantlets, they may augment the competence of transplant shock tolerance and growth during the acclimatization phase. *Fortuna et al.* (1992) evaluated transplant shock tolerance by inoculation of *Glomus mosseae* and *Geastrum coronatum* into micropropagated *Prunus cerasifera*. After four weeks growth, 100% survival of plants was recorded. *In vitro* co-culture of plant tissue explants with beneficial microbes induces developmental and metabolic changes, which enhance their tolerance to abiotic and biotic stresses. [39].

(iii) Cryopreservation

Freeze- preservation or cryogenic storage/ cryopreservation, involves transferring biological material at a very low temperature zone (mostly liquid nitrogen is used for cryopreservation). At this low temperature zone all kinds of biological metabolism are suspended thereby eliminating time related biochemical phenomena. The challenge in cryopreservation remains the transition to and from the storage temperature. Cryopreservation works by reducing the amount of free water and increasing the amount of bound water within the stored material. The technique has been proved to be very useful for *Atropa belladonna, Digitalis lanata*, *Hyoscyamus sp*. and *Rauvolfia serpentina*. *Sharma and Sharma* (2003) studied cryopreservation of shoot tips of *Picrorhiza. kurroa* Royle ex Benth (IC 266698), which is also an endangered medicinal plant of India [40]. *Mandal et al.* (2009) cryopreserved embryogenic cultures of *Dioscorea bulbifera* using an encapsulation-dehydration procedure. On sub-culturing they reported 53.3% recovery of growth of embryogenic culture after cryopreservation [41].

(iv) Gene Transformation

Genetic transformation is a powerful tool for enhancing the productivity of novel secondary metabolites of limited yield. Hairy roots, transformed with *Agrobacterium rhizogenes*, have been found to be suitable for the production of secondary metabolites because of their stable and high productivity in hormone-free culture conditions. Genetic transformation facilitates the growth of medicinal plants with multiple durable resistances to pests and diseases. Likewise, transgenes or marker-assisted selection may assist in the development of insect, pest, and drought, salinity resistant plants, which will be needed to fulfill the world's need and save land for the conservation of plant biodiversity in natural habitats. There are more than 120 species belonging to 35 families in which transformation has been carried out successfully by using *Agrobacterium* and other transformations techniques [42].

(v) DNA Banks

DNA banks maintain a library of the "DNA sample" in a way much similar to the "Gene Library". These provide vital information to the conservation scientists. DNA samples may be of three kinds: (i) total genomic DNA, (ii) DNA libraries, (iii) individual cloned DNA fragments.

DNA banks assembled as a means to replace traditional methods of conserving genetic resources. This is important to note as conservation of genome fragments or individual genes are quite a different situation from the conservation of entire genotypes, as living organisms, for their future use. DNA may be a cost effective form for conservation of germplasm depending on the objective of the conservation and the type of use to which it would be applied. For many species that are difficult to conserve by conventional means (either as seeds or vegetatively) or that are highly threatened in the wild, DNA storage may provide the ultimate way to conserve the genetic diversity of these species and their populations in the short term, until effective methods can be developed [43].

Genetic diversity has significant contribution in conservation of plant genetic resources (PGR). There are approaches which are widely applied with their strength and weaknesses. These include *ex situ* and *in situ* conservation. The maintenance of plant populations in their habitats, where they can naturally occur, grow and reproduce is *in situ* conservation. When they grow outside their natural habitat or production areas is referred to as *ex situ* conservation of germplasm. Depending on the biological nature of the species to be conserved, different types of *ex situ* conservation methods are available [44]. The establishment of DNA banks is one of the *ex situ* conservation method which is planned activity. The extraction of genetic material, and storage should be made readily available for molecular applications. DNA resources can be maintained at -20ºC for short- and midterm storage (i.e. up to 2 years), and at -70ºC or in liquid nitrogen for longer periods.

Some important DNA banks includes: (i) The Royal Botanic Garden, Kew, UK, presently the worlds largest and the most comprehensive PGR DNA bank, consisting of over 20,000 DNA specimens representative of all plant families. (ii) The US Missouri Botanical Garden has collection of more than 20,000 plant tissue samples, and provide raw material for the extraction of DNA for its subsequent use in conservation research. (iii) The Australian Plant DNA Bank of Southern Cross University, which was established in June 2002. It contains representative genetic information from the entire Australian flora. (iv) DNA bank of Leslie

Hill Molecular Systematics Laboratory of the National Botanical Institute (NBI) in Kirstenbosch, South Africa, in collaboration with the Royal Botanic Garden, Kew, which preserves genetic material of the South African flora [45].

5. Discussion and Conclusion

Exploitation of a plant wealth for the use in medicine has been a regular practice e.g. aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) [46].

Over-exploitation of the plants for medicinal purpose has dragged certain species to fall in danger of getting threatened or extinct. *Gloriosa superba* L. is a perennial climber from Africa and Southeast Asia, is under threatened category due to its imprudent harvesting from wild as it is extensively used by medicinal industries for its colchicine content [47]. Similarly *Prunus africana* bark harvesting resulted in the species listing in Appendix II of CITES and EU's bark export ban [48]. Considering such serious circumstances, conserving these rare and endangered medicinal plants needs urgent attention.

Efforts are being made at times to conserve the plants in their native land on both small and large scale, conserving the plant species *in situ* necessarily requires protecting the habitat in which it lives, including the animals and the insects. *In situ* conservation is idealistically the most appropriate approach. The only drawback of the methodology is that it is time consuming because of the slow growth and multiplication process. On the other hand *ex situ* methods requires extensive understanding of the geographical as well as physical factors important for the growth of a plant to fulfill the need and to retain the original diversity of a plant. *Ex situ* conservation of the plants provide an advantage of the plant material being easier, to supply plant for propagation, for re-introduction, for agronomic improvement, for research and for education purposes. The notable disadvantage is that the sample of the species conserved *ex situ* may represent a narrower range of genetic variation than that which occurs in the wild.

Researchers across the globe have tried to prevent the illegal and over harvesting of important medicinal plants by introducing a concept of plant part or plant species substitution to satiate the need of the industry. In South Africa, in response to the serious scarcity of *Ocotea bullata* (Burch.), an important medicinal plant, *Cryptocarya species* (Lauraceae) are frequently used as substitute plants [29]. Using the methods to substitute the plant or its part is a promising approach to fulfill the scarcity of the medicinally important compound but the fate of the plant still remains a question.

Due to the unsustainable loophole in different methods, researchers have introduced new biotechnological strategies that seem would open up new vistas in the field of conservation.

M K Rai (2010), cited a list of plants under possible endangered stage and states that In-vitro culturing of endangered plants like Aquilaria malaccensis, Dioscorea deltoidea, Guaicum officinale, Hydrastis canadensis, Nardostachys grandiflora, Panax quinquefolius, Picrorhiza kurroa, Podophyllum hexandrum, Prunus africana, Pterocarpus santalinus, Rauwolfia serpentina, Saussurea costus, Gloriosa superba and Taxus wallichiana will be beneficial because these plants will reach 'critically endangered', or 'possibly endangered' stage [9]. There is a pressing need to deliver mycorrhizal propagules into the roots of the tissue-culture-raised plantlets of endangered medicinal plants during the acclimatization process because the plantlets are devoid of microbes in sterile medium. Consequently, the plants suffer from 'transient transplantation shock'. In order to avoid this bottleneck and for better survival and sustainable plant production, mycorrhization of the micropropagated plantlets is necessary. *Agrobacterium tumifaciens* and *Agrobacterium rhizogenes* are the potent biological tools for transformation of endangered medicinal plants for development of varieties resistant to stress conditions and also for over production of secondary metabolites so that exploitation of these plants will be minimized. Cryopreservation is another technique to preserve the endangered medicinal plants. Moreover, DNA banks would be useful for long-term preservation and sustainable plant productions.

We can summarize here that the plant diversity which is the basis for sustaining the life on the planet, is playing an important role in the bio-economy in the 21st century, predicted to be an era of bio-economy driven by advances of bioscience and biotechnology. Bio-economy may become the fourth economy form after agricultural, industrial, and information and information technology economies, having far-reaching impacts on sustainable development in agriculture, forestry, environmental protection, light industry, food supply and health care and other micro-economy aspects. Thus, a strategic and forward vision for conservation of medicinal plant diversity and sustainable use of plant resources in the 21st century is of far-reaching significance for sustainable development.

6. Acknowledgment

The authors wish to thank Dr Amit Agarwal, Director- Natural Remedies Pvt. Ltd. for providing the necessary facilities required in writing this article. The authors also wish to thank Mr Ashok Kamath, Head- Agronomy Department, Natural Remedies Pvt. Ltd., for his valuable suggestions/advice in compiling this article.

References

1. Gurib- Fakim A. (2006) *Traditions of yesterday and drugs of tomorrow*: *Molecular Aspects of Medicine*, Special Edition, Elsevier Publications, UK.

2. Sen S, Chakraborty R, De B. (2011). *Herbal Medicine*; 1(3–4): 67–75.

3. Hamayun M., Khan AS, Sohn EY, Lee I. (2006). *Lyonia*; 11:101-113.

4. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM, Cornwell T, Pastor I, Fridlender B. (2002). *Trends Biotechnol*.; 20(12):522-31.

5. Cragg GM, Schepartz SA, Suffness M, Grever MR. (1993). *J .Nat. Prod*. 56(10):1657-68.

6. Cragg GM. (1998). *Med Res Rev*;18(5):315-31.

7. Semwal DP, Saradhi PP, Nautiyal BP, Bhatt AB. (2007). *Current Science*; 92(12): 1733-1738.

8. Vines G. (2004). *Herbal harvest with a future: towards sustainable source for medicinal plants, Plantlife International. Plantlife International;* www.plantlife.org.uk.

9. Rai MK. (2010) *Biodicersitas.* 11(3):157-166.

10. Akerele O, Heywood V, Synge H. (1988). *The Conservation of Medicinal Plant*. Cambridge University Press.

11. Tripathi L, Tripathi JK. (2003) *Trop J Pharm Res.* 2: 243-253.

12. Oderson DFP. (2006). *The Government of Barbados: The National report to the Convention of Biological Diversity*.

13. Alterie MA, Merrick LC. (1987). *Economic*

Botany; 41(1):86-96.

14. Nigel Maxted, Brian Ford-Lloyd, John Gregory Hawkes. (1997). *Plant Genetic Conservation: The In Situ Approach*.

15. Meilleur BA, Hodgkin T. (2004). *Biodivers Conserv*.13:663-684.

16. Shinwari ZK, Gilani SS. (2003). *J Ethnopharmacol*; 84:289-298.

17. Rita A, Silvano M. (2006). *Caryologia*; 59:345-349.

18. Barazani O, Perevolotsky A, Hadas R. (2008). *Biol. Conserv*.141:596-600.

19. Chen SL, Wei JH, Huang LF, Guo BL, Xiao PG. (2004).*China J. Chin Mater. Med*. 29:1123-1126.

20. Long CL, Li H, Ouyang ZQ, Yang XY, Li Q, Trangmar B. (2003). *Biodivers. Conserv*. 12:1145-1156.

21. Russell-Smith J, Karunaratne NS, Mahindapala R. (2006). *Biol .Conserv*. 132:22-32.

22. Williams JN, Seo CW, Thorne J, Nelson JK, Erwin S, O'Brien JM, Schwartz MW. (2009). *Divers Distrib*; 15:565-576.

23. Ginnett TF, Demment MW. (1999). *Afr. J. Ecol*. 37:93-106.

24. Ghimire SK, McKey D, Aumeeruddy-Thomas Y. (2004). *Ecol. Soc*. 9:9-24.

25. Pulliam HR. (2000). *Ecol. Lett*. 3:349-361.

26. Aguilar-Stoen M, Moe SR. (2007). *Biodivers Conserv*; 16:1973-1981.

27. Whelan RJ, De Jong NH, Von der Burg S. (1998). *Aust J Ecol*; 23:121-128.

28. Cunningham AB. (1990). *African medicinal plants: setting priorities at the interface between conservation and primary health care*. Report for WWF-Project 3331, Institute of Natural Resources, University of Natal.

29. Zschocke S, van Staden J. (2000). *J Ethnopharmacol*., 71(3):473-8.

30. Mander M. (1997). *The marketing of indigenous medicinal plants in southern Africa: a case study in KwaZulu-Natal. Investigational Report No. 164*, Institute of Natural Resources, University of Natal.

31. Cunningham AB. (1988). *An investigation of the herbal medicine trade in Natal:KwaZulu. Investigational Report No. 29*, Institute of Natural Resources, University of Natal.

32. Cunningham AB. (1991). *Development of a conservation Policy on commercially exploited medicinal plants: a case study from Southern Africa*. In: Akarele, O., Heywood, V., Synge, H. (Eds.), Conservation of Medicinal Plants. Cambridge University Press, pp. 33.

33. Chawla HS. (2002). *Introduction to plant biotechnology*. Oxford and IBH, New Delhi.

34. Williams PG, Roser DJ, Seppelt RD. (1994). *Mycol. Res*. 98: 34-36.

35. Vestberg M, Estaun V. (1994). *Micropropagated plants, an opportunity to positively manage mycorrhizal activities. In: Gianinazzi S, Schuepp H (Eds.) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Birkhauser, Basel.

36. Gianinazzi S, Trouvelot A, Gianinazzi-Pearson V. (1990). *Adv. Hort. Sci*. 4: 25-30.

37. Ponton F, Piche Y, Parent S, Caron M. (1990). Hort Sci 1990; 25: 416-419.

38. Chandra S, Bandopadhyay R, Kumar V, Chandra R.(2010). *Biotechnol Lett*. 32(9):1199-205.

39. Fortuna P, Citernesi S, Morini S, Giovannetti M, Loreti F. (1992). *Agronomie*. 12:825-830.

40. Sharma N, B Sharma B. (2003). *Cryo Lett*. 24 (3): 181-90.

41. Mandal BB, S Dixit-Sharma, Srivastava PS. (2009). *Cryo Let*. 30 (6): 440-448.

42.Birch RG. (1997). *Ann Rev Plant Physiol Plant Mol. Biol*, 48: 297-326.

43. Dulloo E, Nagamura Y, Ryder O. (2006). *DNA storage as a complementary conservation trategy. In: Vicente MC de, Andersson MS (Eds.). DNA banks-providing novel options for gene banks? Topical reviews in agricultural biodiversity*. International Plant Genetic Resources Institute, Rome, Italy.

44. Vicente MC de, Andersson MS, Engels J. (2006). *Introduction and overview. In: Vicente MC de, Andersson MS (Eds.). DNA banks-providing novel options for gene banks? Topical reviews in agricultural biodiversity*. International Plant Genetic Resources Institute, Rome, Italy.

45. Rice N, Henry R, Rossetto M. (2006). *DNA banks: a primary resource for conservation research. In: Vicente MC de, Andersson MS (Eds.). DNA banks-providing novel options for gene banks? Topical reviews in agricultural biodiversity*. International Plant Genetic Resources Institute, Rome, Italy.

46. Vickers A, Zollman C. (1999). *BMJ*. 319:1050 - 1053.

47. Jana S, Shekhawat GS. (2011). *Fitoterapia*. 82(3):293-301.

48. Jimu L. (2011). *Ecol Natural Environ*; 3(4):118-130.