Venkata Naveen Kasagana et al /J. Pharm. Sci. & Res. Vol.3(8), 2011,1378-1386

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Conservation Of Medicinal Plants (Past, Present & Future Trends)

Venkata Naveen Kasagana* and Swathi Sree Karumuri.
Department of Pharmaceutics,Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi-626130. Tamilnadu, India.

Abstract: Conservation of threatened species of medicinal plants and their habitats and Support for livelihood security through protection of wild medicinal plants based on sustainable harvesting. This deals about the promotion of sustainable medicinal plant cultivation through the process of building IPR and field gene bank. In-situ conservation of medicinal plants in and around the mountains and national park areas and ex-situ techniques involving cryopreservation and conducting ethno medical survey to explore utilization of medicinal plants. This involves the research on the propagation and cultivation methods of selected indigenous medicinal plants for human and livestock disease. Impact on wild populations of medicinal plants through harvesting and other activities that involves the conservation of medicinal plants on-farm pilot propagation and cultivation trials of medicinal plants on past, present and future scenario. Development and implementation of appropriate management options and guidelines for sustainable harvesting of medicinal plants by applying various conservation techniques. The output will be field gene bank established serving research and conservation. Guidelines for sustainable harvest of medicinal plants and its Cultivation practices can be developed by providing income generating activities such as incentives and also creating market opportunities for both import and export and for formalizing traditional medicine.

INTRODUCTION:

Medicinal plants and traditional medicine play an important role in the health care system of most developing countries. The traditional health care practice is mainly dependent on medicinal plants collected from the wild. In spite of this, the medicinal plant biodiversity is being depleted due to man-made and natural calamities. Moreover, the indigenous knowledge associated with the conservation and use of medicinal plants is also disappearing at an alarming rate. The fact that medicinal plants could be used as sources of revenue for farmers, the Institute of **Biodiversity Conservation (IBC)** has initiated the development of a project on **Conservation and Sustainable Use of Medicinal Plants (CSMPP).** (Singh BM, 2001)

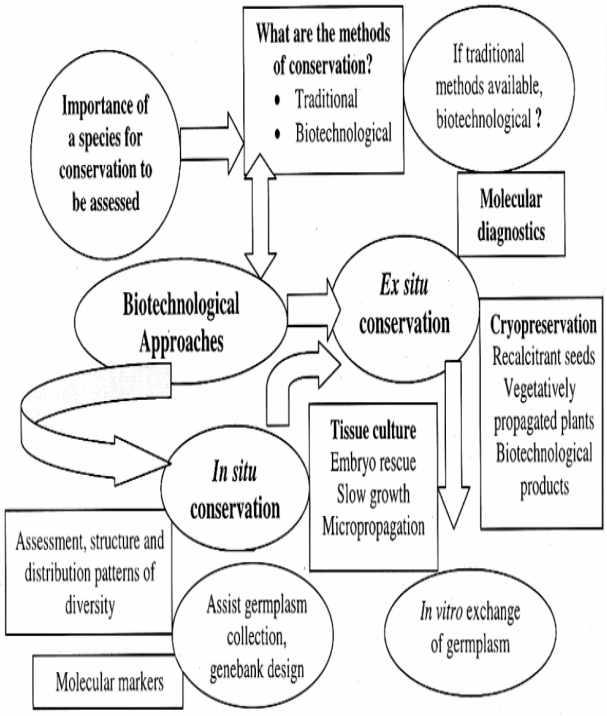
NEED FOR CONSERVATION:

The goal of conservation is to support sustainable development by protecting and using biological resources in ways that do not diminish the world's variety of genes and species or destroy important habitats and ecosystems. In general, it involves activities such as collection, propagation, characterization, evaluation, disease indexing and elimination, storage and distribution. The conservation of plant genetic resources has long been realised as an integral part of biodiversity conservation. There are two methods for the conservation of plant genetic resources, namely **In-Situ & Ex-Situ conservation.**

On the other hand, *ex situ* conservation 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, DNA storage, pollen storage, in vitro conservation, field gene banks and botanical gardens. (Sarma, S., 2003)

***In-situ and ex-situ* Conservation and Sustainable Use of Medicinal Plants**



Field gene banks provide easy access to conserved material for use, they run the risk of destruction by strategies using the tools of biotechnology are increasingly being applied towards conservation of plant genetic resources. These include (a) *in vitro* conservation (b) *in vitro* propagation and re- introduction of plants to their natural habitats, and (c) molecular marker technology. Several *in vitro* techniques have been developed for storage of vegetatively propagated and recalcitrant seed producing species. In general, they fall under two categories: (i) slow growth procedures, where germplasm accessions are kept as sterile plant tissues or plantlets on nutrient gels; and (ii) cryopreservation, where plant material is stored in liquid nitrogen. Slow growth procedures provide short- and medium-term storage options, while cryopreservation enables long- term storage of the plant material.(Chamberlain, D. F., 1982)

Traditional Methods of Conserving Medicinal Plants

The rural people who constitute the bulk of population are heavily dependent on the vegetation around them for fuel wood and for medicine. They are mainly subsistence farmers, and cannot afford alternative fuels, let alone the high prices of modern medicine. As a result vegetation is lost and environmental degradation takes place. Major steps have been taken towards conserving the medicinal plants. They include: discouraging cutting down indigenous trees and encouraging the local people to plant fast-growing exotic and indigenous trees for domestic use, the inauguration of a national tree planting day and the creation of nature reserves. However, despite this intensified drive towards conservation, it is still difficult to prevent local people from destroying the plants around them.The planting of fast-growing exotics is not a complete solution to the problem of environmental degradation, mainly because the locals still need indigenous plants as a source of medicine and for crafts such as carving. Local people do not approve of the planting of medicinal plants because of their belief that indigenous plants lose their curative properties when cultivated.

GERMPLASM TECHNIQUE FOR CONSERVATION:

Germplasm conservation of vegetatively propagated crops, forest species especially those with recalcitrant seeds in live gene banks in fields poses tremendous problems in terms of required land space and labour input during annual or perennial replanting, testing and documentation. The advantage of ***in vitro*** or reduced growth storage include little space necessary in growth rooms for maintaining thousands of genotypes and the absence of diseases and pest attack in culture vessels. Furthermore, *in vitro* storage eliminates the need for long and frustrating quarantine procedures during move ment and exchange of germplasm.

Disadvantages of Germplasm:

- ♣ Some crops do not produce viable seeds
- ♣ Some seeds remain viable for a limited duration only and are recalcitrant to storage
- ♣ Seeds of certain species deteriorate rapidly due to seed borne pathogen
- ♣ Some seeds are very heterozygous not suitable for maintaining true to type genotypes

Although the most economical means of germplasm storage for seed propagated species is in the form of seeds, this is not always feasible because of the following reasons:

- ☞ Some crops do not produce viable seeds.
- ☞ Some seeds remain viable for a limited duration only and are recalcitrant to storage.
- ☞ Seeds of certain species deteriorate rapidly due to seed borne pathogen.
- ☞ Some seeds are very heterozygous not suitable for maintaining true to type genotypes.

Effective approach to circumvent the above problems may be application of **cryopreservation technology**

CRYOPRESERVATION TECHNIQUE FOR CONSERVATION OF PLANTS

- ♣ **"Cryopreservation"** is defined as the viable freezing of biological material and their subsequent storage at ultra low temperatures (-196C)"using liquid nitrogen.
- ♣ The use of liquid nitrogen, either by itself or as a source of nitrogen gas, is based on the following unique combination of features:

• Chemically inert
• Relatively low cost
• Non-toxic
• Non-flammable
• Readily available

Factors determining the Conservation Protocol

• Applicability.
• Range, time, reproductive biology, storage suitability, infrastructure.
• Need Driven and Technology driven
• Security, Efficiency, Accessibility and Sustainability.
• Resource requirement.
• Risks in conservation
• Regeneration capability and time.
• Cost and returns.

New cryopreservation techniques:

✓ **Encapsulation and dehydration, Vitrification, Encapsulation and vitrification, Desiccation, Pregrowth, Pregrowth and desiccation, Droplet freezing**.

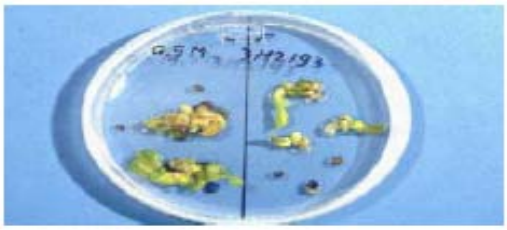
Cryopreservation procedures

✓ Three different procedures have been used for cryopreservation of plant cells: two-step freezing, vitrification and encapsulation-dehydration.
✓ Two-step freezing: This procedure includes an incubation of cells in a mixture of cryoprotectants (total concentration of 1–2M), which causes moderate dehydration of the cells, followed by a slow freezing step (for example, 1°C/min down to app –35°C).
✓ Vitrification: This procedure is based on severe dehydration at non-freezing temperatures by direct exposure to concentrated cryoprotectants (total concentration ranging from 5–8M), followed by rapid freezing.
✓ Encapsulation-dehydration: Cells are encapsulated in alginate beads, cultured on medium with increased sucrose concentration, air-dried using silica gel or the airflow of a flow cabinet and directly transferred to liquid nitrogen.

Fundamental aspects of cryopreservation:

☞ Freezing behaviour of plant tissues ,Ultra structural aspects of freezing adaptation
☞ Physical and biochemical studies of cryopreservation, induced damage, Molecular changes, Molecular mechanisms of freezing tolerance.

Systems to which cryopreservation techniques applied


Undifferentiated plant cells, Embryonic suspension, Callus, Pollen, Seeds, Somatic embryos, Shoot apices, Genotype considerations.

Cryoprotectants

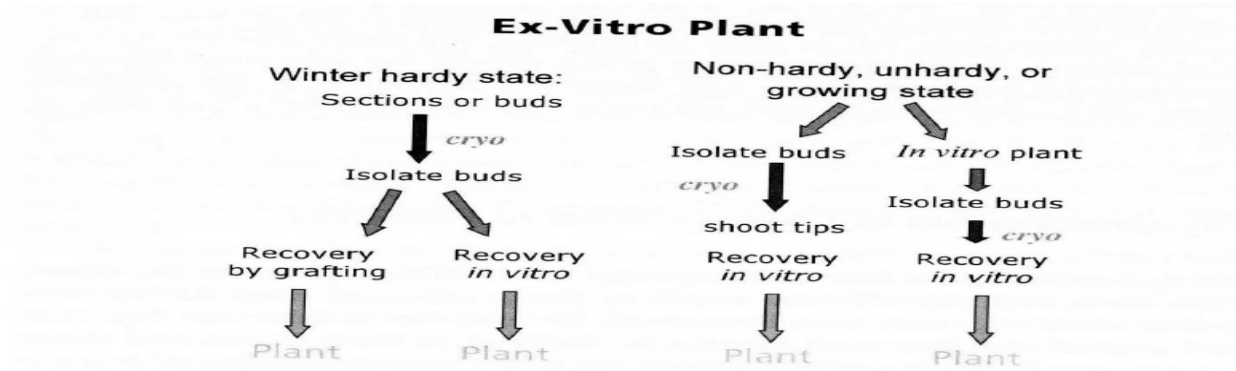
Cryoprotectants protect lowly frozen cells by one or more of the following mechanisms:
•Suppressing high salt concentrations
•Reducing cell shrinkage at a given temperature
•Reducing the fraction of the solution frozen at a given temperature
•Minimizing intracellular ice formation

VITRIFICATION

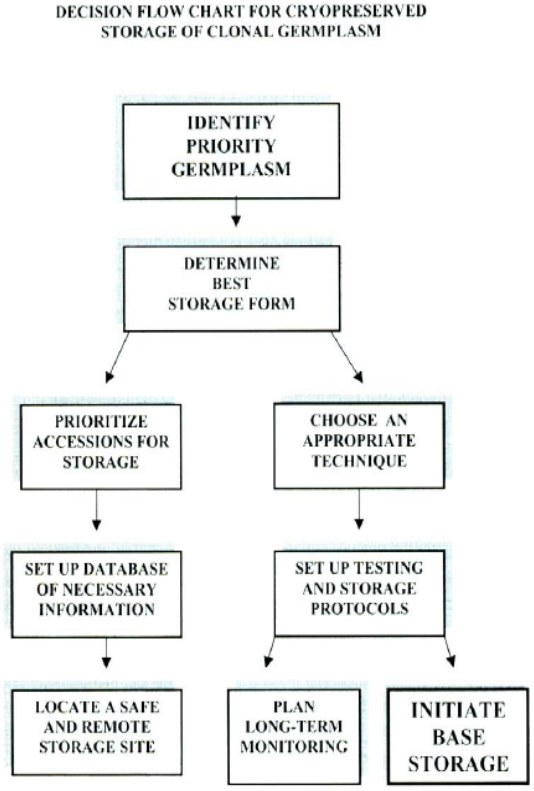
An "arrested liquid" state known as a *glass* is achieved.

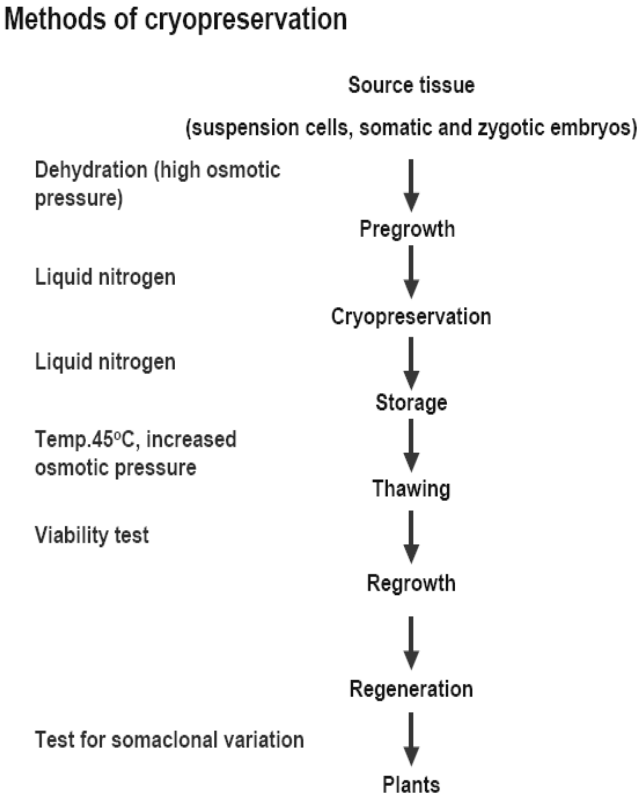
Vitrification is this conversion of a liquid into a glass. Glass is a liquid that is too cold to flow.
A vitrified liquid is essentially a liquid in molecularstasis. Vitrification is solidification due to increasedviscosity rather than to crystallization.
Vitrification techniques were successfully applied to a variety of complex biological materials

Cryopreservation of vegetative buds and shoot tips



Options in using vegetative buds or shoot tips from clonal lines for cryopreservation





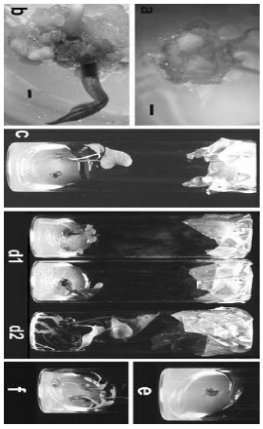
(Gupta A K ,2003)

Cryopreservation in medicinal plants

Cryopresevation provides an opportunity for conservation of endangered medicinal plants.

For example, low temperature storage has been reported to be effective for cell cultures of medicinal and alkaloid producing plants such as *Rauvollfia serpentina, D. lanalta, A. belladonna, Hyoscyamus* spp

Cryopreservation has been used successfully to store a range of tissue types, including meristems, anthers/pollens, embryos, calli and even protoplasts.



TISSUE CULTURE TECHNIQUES USED FOR CONSERVATION

Cell suspensions:

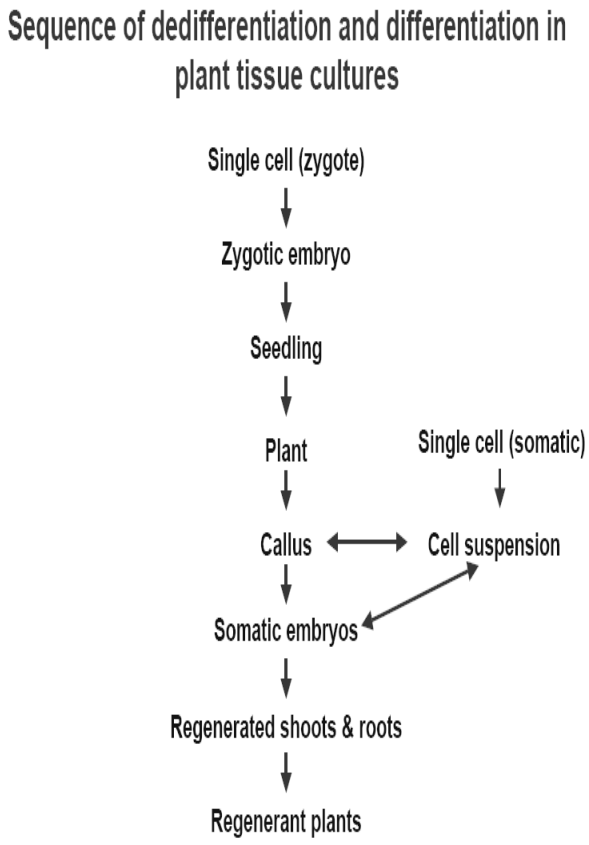
a. Pre-treatment: The cell suspension is inoculated at high density into standard medium containing 6% mannitol and cultured under standard conditions.

b. The suspension is harvested when the cell will be dividing rapidly and chilled on ice.

c. Cryopreservation: A double strength cryoprotectants solution(1M dimethylsulfoxide (DMSO) + 1M glycerol + 2M sucrose)was prepared and one volume of

cryoprotectants solution is added to one volume of cell suspension and the mixture is incubated on ice for 1hr.

d. The mixture is dispensed as 1 ml aliquots and the ampoules are cooled at 1oC min-1 until they reach -35oC.
e. Storage: The ampoules are stored in or over liquid nitrogen.
f. Thawing: The ampoules are dropped into sterile water at about 40oC with a ratio of 4 ampoules to 150 ml water.
g. Regrowth: The cells in suspension are transferred to several layers of 5 cm filter paper on the surface of a 9 cm agar plate containing a growth medium and are incubated under standard conditions. The cells and upper layers of filter paper are then transferred to fresh medium, until after 5 – 6 days, the cells alone are transferred to agar medium.



Zygotic embryos:

a. Pregrowth: The embryos are excised aseptically from the seeds then in an open petri dish, are exposed to a sterile airflow in a laminar flow cabinet for 3h.

b. Cryopreservation: The embryos contained in plastic ampoules are immersed in liquid nitrogen.
c. Thawing: The plastic ampoules are transferred to a water bath at 37 – 38oC then placed on moist sterile filter paper in petri dishes for 10 days.
d. Regrowth: After 10 days in the absence of medium the embryosare added to a standard growth medium to stimulate growth into plants Zygotic embryos

Adventitious buds:

a. Pregrowth: uniformed nodes (5 mm in length) containing an adventitious bud are removed from stem sections of plants.
b. Nodes are placed on agar medium containing 0.7 M sucrose and incubated under standard conditions for two days then transferred to a nylon membrane contained in a dish.
c. Cryopreservation: Nodes are transferred to plastic ampoules then immersed in liquid nitrogen and stored under liquid nitrogen.
d. Thawing: Nodes are thawed in a water bath at 25oC.
e. Regrowth: Because of their size, nodes can be transferred individually to a standard growth medium and the adventitious bud is stimulated to develop as a shoot.

ADVANTAGES OF TISSUE CULTURE

Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- To quickly produce mature plants.

- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: orchids and nepenthes.
- To clean particular plant of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Application of Tissue Culture and Cryopreservation Techniques

- Species which are difficult to regenerate by conventional methods and the only way to save them from extinction is to propagate them by tissue culture.
- Species where population has decreased due to over exploitation and thus initial bulking of the stock can be taken up by tissue culture.
- Species which show lot of variability in terms of the active principles with medicinal properties. Tissue culture of selected clones will help in sustainable harvest and fetching better prices both in the domestic and international market.
- Trees with medicinal properties or elites can be identified based on their potential of yielding higher amount of active principle.

Ex situ conservation of plants: It involves three methods namely, **field gene banks, seed banks and in vitro storage**. Of these, seed banks are the most efficient and effective method of conservation for orthodox seed. It is an effective and compact method of storage. The seeds are placed in packets and stored in medium term storage facilities (maintained at 00C to 50C temp. and 15% to 20% relative humidity) as active collections. Most of the material is also kept in long-term storage facilities (held at colder temperatures, -20 to -180C).Most seed samples are expected to remain viable for 20 to 30 years in medium term storage and for up to 100 years in long term storage depending upon the species, the initial seed quality and specificity of storage environment and general state of infrastructure. (Hajra, 2001)

NATIONAL CRYOBANK & GENETIC CONSERVATION

• National cryobank at NBPGR has responsibility to conserve desiccation sensitive seeds, vegetative tissues, pollen and selected orthodox seed species.

• Infrastructure facilities include 6 large capacity cryotanks capable of accommodating 30,000 to 40,000 samples of varied germplasm.

• Presently about 6,000 accessions are stored in cryobank in the form of seeds, embryo, embryonic axes, shoot apices and pollen.

GENE BANK PRESERVATION

A Gene bank conserves plant genetic wealth. The rich heritage of plants, which feeds and sustains humankind, is conserved through seeds, vegetative propagules, tissue culture, embryos, gametes or cells, DNA etc. Besides orthodox seeds, vegetatively propagated clonal material and recalcitrant species are maintained under *in-vitro*, Cryo and field conditions.

The importance of gene banks has been recognised since long and they have been in existence in various parts of the world for a long time. In India the largest, most modern gene bank was opened in New Delhi only a few years ago. This bank is primarily intended for the storage of crop species and their wild relatives. Despite the subject having been flagged at recent discussions, no concrete action has yet started with respect to the setting up of a gene bank for forest germplasm. Given the rapid rate of destruction of forests around the world, including the Indian sub-continent, there is certain urgency about conserving the genetic material of the Indian forests.

The purpose of the Gene Bank is to

• Undertake and promote long-term conservation of plant genetic resources employing ex-situ conservation for seeds, in-vitro cultures and cryopreservation techniques and assist in in-situ conservation efforts.

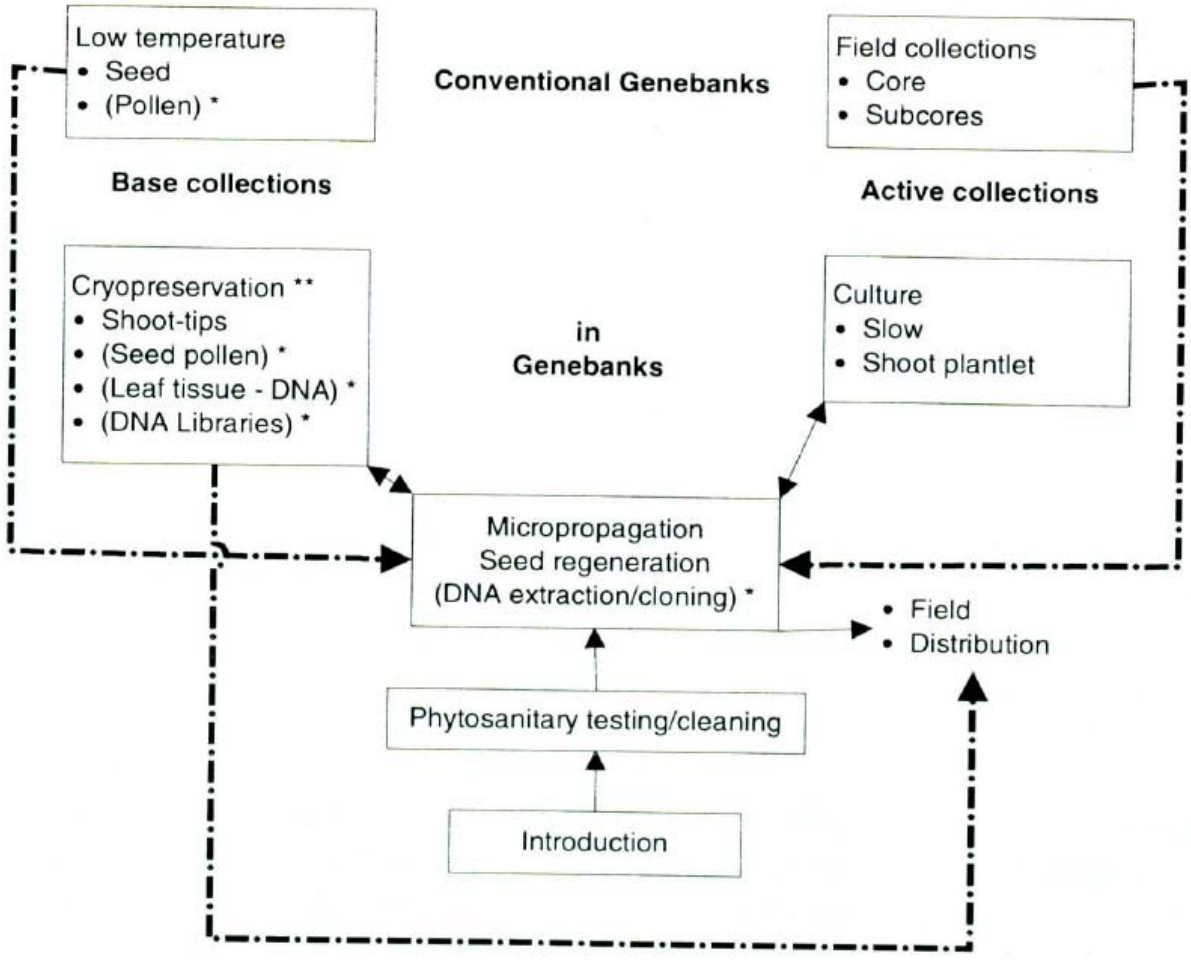
- Act as the repository of collected material, elite material and endangered material, as also a regional repository of duplicate collections as a part of the global system.
- Monitoring and maintenance of the existing collections, facilitating the organisation of regeneration program.
- Ensuring availability of exotic and indigenous germplasm through periodic seed increase for evaluation, utilization and conservation.
- Conducting research related to medium and long-term conservation of germplasm.
- Developing and operating a database and information network system on forest genetic resources
- Support/assist in organisation of post-graduate education and short/medium-term training courses on Forest germplasm activities at national, regional, and international levels.

GENETIC CONSERVATION

To expand the current tissue culture, propagation, and storage of all endangered species of plants is possible. Emphasis will be placed on the "Genetic Safety Net" (GSN) species in efforts to achieve complete genetic safety net coverage for these living critically endangered plants, including capture of the maximum genetic diversity remaining in wild populations.

Conservation scenario in the world

- ☞ Most of the 5,554505 accessions are stored in seed banks –3610428 accessions.
- ☞ 1,400,000 *ex situ* conserved accessions 25% the information concerning to storage types is missing.
- ❖ 90% seed banks (88)
- ❖ 8% FGB (11)
- ❖ 2% *in vitro* storage
- ❖ 1.2% cryopreserved



Nearly USD 250 million are spent annually for *ex situ* conservation 85% of the costs for seed (90% of total accessions) unit cost amounting to USD 44/ accession.

Economics of conservation

Costs of conservation of potatoes in Germany US$

Cryo	22.29
FGB	50.59
In vitro	137.65

In India on an average 19.86 US$ in spent per accession.

Case studies

1. Cassava

☞ *In vitro* conservation techniques are well established in cassava

☞ Routinely used to store over 5000 accessions Cryopreservation protocols are under development

2. Potato:

☞ *In vitro* conservation techniques are routinely used for propagation, disease elimination, slow growth storage and distribution of potato germplasm.

3 .Musa species:

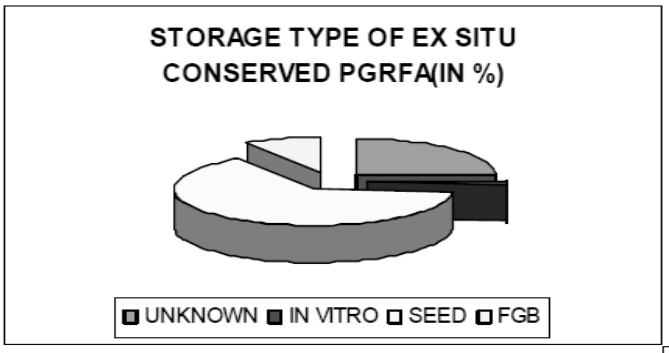
☞ In vitro techniques are well developed and practiced for both conservation and use.

☞ Cryopreservation research is in progress at INIBAP Belgium.

Sweet potato germplasm: distribution of plantlets and tuberlets (1990–95)

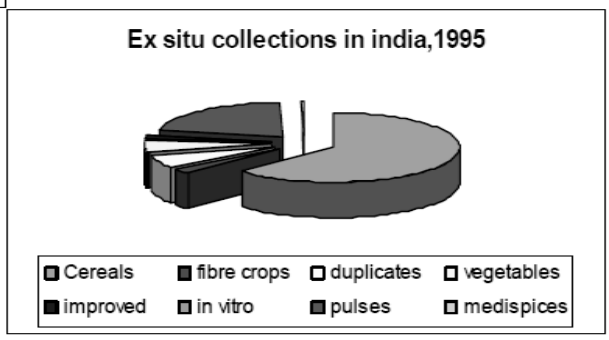
Regions*	Years						
	1990	1991	1992	1993	1994	1995	
ESEAP	38	546	158	493	397	890	Units
	19	284	101	256	309	431	Accessions
LAC	106	546	604	248	219	56	Units
	26	290	275	122	124	113	Accessions
SSA	12	488	238	393	1,012	462	Units
	6	246	119	217	505	237	Accessions
SWA	24	237	96	214	56	0	Units
	12	118	48	78	28	0	Accessions
MENA	12	126	60	106	102	17	Units
	6	64	30	53	51	8	Accessions
OTHERS	52	255	136	0	199	104	Units
	23	43	66	0	97	52	Accessions
TOTAL	244	2,198	1,292	1,454	1,985	1,586	Units
	92	1,045	639	726	1,114	784	Accessions

*Regions: ESEAP (East, Southeast Asia & the Pacific), LAC (Latin America & the Caribbean), SSA (Sub-Saharan Africa), SWA (South & West Asia), MENA (Middle East & North Africa). Data from CIAT



Comparison between world and Indian holdings in germplasm *ex situ*

***In vitro* India-0.6%**
World 1%



CONCLUSION:

The overall conclusion of the presentation is to initiate and support for conservation, management and sustainable utilization of medicinal plants for human and livestock health care and to promote in-situ conservation and sustainable uses of medicinal plants in and around site of global significance. It also promotes the Conservation of threatened species of medicinal plants and their habitats for livelihood security through conservation of wild medicinal plants based on sustainable harvesting and by implementing various conservation techniques.

REFERENCES:

[1]. Singh BM, Sharma KD, Katoch M, Guleria S & Sharma TR, Molecular analysis, Royle - an endangered medicinal herb of northwestern Himalaya, Plant Genetic Resour Newslett , 124 (2001) 57-61.

[2]. Sarma, S., Meghalaya, the land and forest.A remote sensing based study. NEHU, Shillong, 2003.

[3]. Champion, H. G. and Seth, S. K., A Revised Survey of Forest Type of India, Govt Publication, New Delhi, 1968.

[4]. Chamberlain, D. F., Revision of Rhododendron II, Notes from the Royal Botanic Garden, Edinburgh, 1982, vol. 39, no. 2.

[5]. Mao, A. A., Singh, K. P. and Hajra, P. K.,Rhododendrons in Floristic Diversity and Conservation Strategies in India, BSI,Kolkata, 2001, vol. IV, pp. 2167–2195.

[6]. Rao Kameswara, C., Geetha, B. L. And Suresh, Geetha, Report, Compiled from the 1997 IUCN Red List of Threatened Plants,Ministry of Environment and Forests, Govt of India, 2003, pp. 40–41.

[7]. Indian Journal of Traditional Knowledge Vol. 8(1), January 2009, pp. 29-34 Received 30.09.2008; Revised 06.12.2008.

[8]. MoEF, Annual Report (1999-2000) (Ministry of Environment and Forests, Government of India, New Delhi),2000.

[9]. Saxena S, Chandak V, Ghosh SB, Sinha R, Jain N and Gupta A K (2003) Costs of conservation of Agro biodiversity in India. In Detlef Virchow (Ed) Efficient Conservation of Crop genetic diversity: Theoretical approaches and Empirical studies. pp 137 – 174.