1. Conservation of medicinal plants – general introduction

India is floristically rich and is recognized as one of the twelve mega biodiversity centers of the world, ranking 10th among the plant resources rich nations of the world and 4th among the countries of Asia. India is the 7th largest country in the world and Asia’s 2nd largest nation with an area of 3,287,263 sq Km, and is an example of diverse ecosystems. (Swingland, 2001). It is endowed with a rich heritage of medicinal plant wealth. Based on the ethnomedicinal traditional knowledge, utilization and conservation of medicinal and aromatic plants has received considerable attention in recent times, especially in south India. Forests are the primary source of a variety of medicinal plants, while a number of the medicinal plants are also cultivated (FAO, 2003).

Conservation is the process of management of biosphere in order to obtain the greatest benefit for the present generation and maintaining the potential for future. Conservation of plant resources is of global concern because we don’t know what we are losing and what we will need in future.

Conservation methods vary with many biological and environmental factors (Rajasekharan and Ganeshan, 2002). Small isolated populations, endemic and rare species in particular are subjected to genetic drift, inbreeding and their genetic variation is consequently expected to be low compared to that of larger populations which may lead to a decrease in species ability to survive environmental changes and demographic fluctuations, both in short and long term (Bilington, 1991; Gaston and Cunin, 1997a; Karron, 1997). Hence, the maintenance of genetic variation is
essential for long-term protection of a taxon (Hamrick and Godt, 1989; Simberloff, 1988).

1.1. Threats to medicinal plants

There are different primary and secondary factors that pose threat to many medicinal plants. The threats are degradation of habitat due to expanding human activity, forest decline, destructive collection of plant species, invasion of exotic species that compete with native species, increased spread of diseases, industrialization, over exploitation, human socioeconomic change and upheaval, changes in agricultural practices, excessive use of agrochemicals, natural and man-made calamities, genetic erosion etc., In South India, it is estimated that about 70-80 out of the estimated 300 medicinal plants are either endangered or threatened. Hence, there is a necessity to strike a balance between conservation and utilization of these medicinal plants (Rajasekharan and Ganeshan, 2002).

1.2. Need for conservation of medicinal plants

To meet the requirements of expanding regional and international markets healthcare products and needs of growing populations, large quantities of medicinal plants are harvested from forests (Desilva, 1997). In India large number of medicinal plants are extracted from the wild to meet the increasing demand for raw material needed for domestic consumption and for export. As a result, the natural sources are rapidly depleting. Medicinal plants contribute to health, income, agroforestry system, cultural identity and livelihood security. Hence there is a need for conservation, cultivation, maintenance and assessment of germplasm for future use.
Conservation of biological diversity involves protecting, restoration and enhancing the variety of life in an area so that the abundance and distribution of species and communities contributes to sustainable development. The ultimate goal of conservation biology is to maintain the evolutionary potential of species by maintaining natural levels of diversity which is essential for species and populations to respond to long and short term environmental changes in order to overcome stochastic factors failing which would result in extinction.

1.3. Conservation strategies for medicinal plants

The two main strategies are *ex situ* (protection of species outside their natural habitats) and *in situ* (in their natural surroundings) conservation. There is a need for coordinated conservation efforts based on these strategies (Figure 1). More information is required on medicinal plant production, utilization, trade, monitoring the stock of medicinal plants, development of sustainable harvesting practices, preservation of traditional knowledge and intellectual property rights.

World Conservation Union (formerly known as the International Union for Conservation of Nature and Natural Resources) categorized plants Red Data List Categories” (IUCN, 2001) based on the detailed knowledge of the population dynamics and genetics of the species “viz., extinct, extinct in wild, threatened (critically endangered, endangered and vulnerable) and low risk (conservation dependent, near threatened and least concern) and indeterminate where the data is
Conservation biotechnology

Conservation

Propagation

Molecular diversity

( Please refer table 2 for details)

In situ

Ex situ

Reintroduction into nature

Biosphere reserves
Sacred Groves
Sanctuaries
National parks
Protected areas

• Field gene banks, Arboreta such as Pinetum, Bamboosetum, Palmetum and Orchidarium etc.

• Storage of plant parts/tissues viz., buds, seeds, embryos, metistems, callus, ovule, pollen etc.

• Cryo banks and DNA banks

Figure 1: Plant genetic resources - Advancing conservation biotechnology
insufficient. Conservationists focus their attention exclusively on species extinction rather than genetic erosion within individual gene pools, and the latter may be of equal importance in terms of loss of biodiversity (Maxted, 2001).

Hence, it is imperative that viable strategies to conserve the populations and genetic resources of medicinally important species is a must to avoid further loss. Ongoing efforts in India include both *in situ* and *ex situ* conservation measures *viz*, plant tissue culture, introduction of new crop genetic resources, research in habitat restoration, pollution abatement, seed storage and tissue banking etc. (Jackson and Sutherland, 2000).

1.3.1. *In situ conservation*

*In situ* or on site conservation involves maintaining genetic resources in their natural habitats i.e., within the ecosystem to which it is adapted, whether as wild or crop cultivar in farmer’s field as components of the traditional agricultural systems (Damania, 1996) (Figure 1). The key operational steps for establishing *in situ* gene banks for conservation of prioritized medicinal plants include: Threat assessment, establishment of a network of medicinal plant forest reserves, involving local stakeholders, botanical, ecological, trade and ethno-medical surveys, assessing intra-specific variability of prioritized species, designing species recovery programmes, establishment of a medicinal plant seed center etc. Conclusively, no *in situ* conservation project can succeed without the complete cooperation and involvement of local people (Srinivasamurthy and Ghate, 2002).
1.3.2. Ex situ conservation

Ex situ conservation, involves conservation of biodiversity outside the native or natural habitat where the genetic variation is maintained away from its original location (Figure 1) The ex situ genetic conservation fulfills the requirement of present or future economic, social and environmental needs. Conservation also includes propagation and assessment of molecular diversity (Olorode, 2004)

Conservation of medicinal plants include a combination of methods, depending on factors such as geographic sites, biological characteristics of plants, available infrastructure, and network having an access to different geographical areas, human resources and number of accessions in a given collection (Rajasekharan and Ganeshan, 2002).

1.3.2.1. In vitro regeneration

In vitro regeneration include plant/explant growth, maintenance under disease free condition, retention of regenerative potential, genetic stability, and ensuring that there is no damage to the live material. It offers a number of advantages over the in vivo method:

a) great savings in storage space and time

b) possibility of maintaining species for which seed preservation is impossible or unsuitable and

c) disease-free transport and exchange of germplasm, since cultures are maintained under phytosanitary conditions (Natesh, 2000)
In vitro multiplication protocols for fast propagation of a number of red listed medicinal, aromatic and recalcitrant taxa that are difficult to propagate through conventional means would be very useful. Usually, shoot tips or axillary buds are cultured on a nutrient medium containing (i) high levels of cytokinins or (ii) low concentrations of auxin coupled with high-cytokinin content. Somatic embryos, or even axillary buds are encapsulated in hydrosoluble gels to form 'artificial seeds' and have used for rapid propagation of the species. Even more important is the reintroduction of in vitro raised material into their natural habitat and monitoring its performance over several years, to ensure fidelity with respect to active compounds or the marker chemical, vis-a-vis the parents (Natesh, 2000).

The cell culture process itself can result in genetic changes in the regenerated plants. These heritable genetic changes are termed as somaclonal variation. The presence of an undifferentiated callus phase in the regeneration protocol enhances the chances for somaclonal variation among the regenerated plants. These variations can result from simple DNA sequence differences. The cell environment appears to induce a very high frequency of such mutations. Other types of changes that frequently occur in regenerated plants could be due to chromosomal, structural and number changes due to rearrangements in multi-gene families, gene silencing due to changes in DNA methylation, action of jumping genes etc. Hence, it is necessary to avoid the use of auxin and auxin like substances in the meristem multiplication protocols. It is also mandatory to check the fidelity of the plants multiplied from the meristem cultures and plants multiplied from cryo preserved meristems by using RAPD markers.
1.3.2.2. Cryobanks for conservation

Cryopreservation of plant cells and meristems is an important tool for long-term storage of germplasm or experimental material without genetic alteration using a minimum space and maintenance. The development of methods to store apical meristems in liquid nitrogen successfully is needed to aid in the conservation of genetic resources. Cryobanks are basically meant for storage of germplasm. For long-term preservation, cryogenic storage at ultra low temperatures under liquid nitrogen (-150 to -196°C) is the method of choice. Relatively new to plants, cryopreservation has followed advances made in the mammalian systems is achieved either through slow cooling or vitrification. Encapsulation/dehydration is another new technique that offers practical advantages. It is based on the technology originally developed for production of synthetic seeds, i.e., somatic embryos encapsulated in a hydrosoluble gel. Several types of in-vitro raised materials such as meristems/shoot tips, cell suspensions, protoplasts, somatic embryos and pollen embryos of medicinal and aromatic species have been studied from the cryopreservation perspective (Natesh 2000).

1.3.2.3. Low temperature germplasm storage

Preservation by under-cooling has recently been applied to plant tissue cultures. The objective of this approach is to maintain tissues at low temperatures (-10 to -20°C) but in the absence of ice crystallization. The plant tissues are immersed in immiscible oil and the emulsion thus formed can be under cooled to relatively low temperatures thereby circumventing ice formation, one of the most injurious consequences of low temperature storage. Although good recovery has been reported in certain species, this
has only been achieved using a temperature of -10°C and for relatively short storage periods (6-48 hours).

Recently, vitrification, simplified freezing, and encapsulation-dehydration methods have been used for storage of valuable germplasm. These new procedures may replace freeze-induced cell dehydration by removal of all or of a major part of freezable water from cells at room temperature or at 0°C. In the encapsulation-dehydration technique, extraction of water results in progressive osmotic dehydration, additional loss of water is obtained by evaporation and the subsequent increase of sucrose concentration in the beads. In the technique, preculturing encapsulated meristems in medium enriched with sucrose before dehydration induces resistance to dehydration and deep-freezing. The vitrification procedure for cryopreserving meristems involves preculture and/or loading and osmotic dehydration by short exposure of meristems to highly concentrated mixture of cryoprotectants. The encapsulation-dehydration technique is easy to handle and alleviates dehydration process.

1.3.2.4. Seed storage modules

Usually seeds, being natural perennating structures of plants, represent a condition of suspended animation of embryos, and are best suited for storage. By suitably altering their moisture content (5-8%), they can be maintained for relatively long periods at low temperatures (-18 °C or lower). However, in several species, rhizome/bulb or some other vegetative part may be the site of storage of active ingredients, and often, such species do not set seed. If seeds set, they may be sterile or recalcitrant i.e., intolerant of reduction in moisture or temperature, or, otherwise
unsuitable for storage. It is now possible to store materials other than seed, such as pollen or clones obtained from elite genotypes/cell lines with special attributes, *in-vitro* raised tissues/organs, or, genetically transformed material (Natesh 2004).

### 1.4. Constraints for conservation

The IUCN Red Data book lists 34,000 plants with endangered status. The Botanical Garden Conservation International (BGCI) 2000 database indicates that there are about 1846 botanic gardens. In-order to put efforts for *ex-situ* conservation; these botanical gardens have to cultivate several hundreds of endangered, rare and vulnerable plant species, which requires elaborate facilities and extraordinary efforts. Therefore, biologists feel that the *ex situ* conservation should be considered as a complimentary measure of *in situ* conservation for holistic strengthening of conservation.