

# Conservation of medicinal plants of Western Ghats, India and its sustainable utilization through *in vitro* technology

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**Abstract** Climate change, alien species, and use of land for intensive farming and development are causing severe threat to the plant genetic diversity worldwide. Hence, conservation of biodiversity is considered fundamental and also provides the livelihoods to millions of people worldwide. Medicinal plants play a key role in the treatment of a number of diseases, and they are only the source of medicine for majority of people in the developing world. The tropical regions of the world supply the bulk of current global demand for “natural medicine,” albeit with increasing threat to populations in the world and its genetic diversity. India is a major center of origin and diversity of crop and medicinal plants. India poses out 20,000 species of higher plants, one third of it being endemic and 500 species are categorized to have medicinal value. The Western Ghats is one of the major repositories of medicinal plants. It harbors around 4,000 species of higher plants of which 450 species are threatened. Currently, the number of species added to the red list category in this region is increasing, and the valuable genetic resources are being lost at a rapid rate. Demand for medicinal plants is increasing, and this leads to unscrupulous collection from the wild and adulteration of supplies. Providing high-quality planting material for sustainable use and thereby saving the genetic diversity of plants in the wild is important. During the last 25 years of intensive research, Tropical Botanic Garden and Research Institute has developed *in vitro* protocol for rapid regeneration and establishment of about 40 medicinally important rare and threatened plants of Western Ghats.

*In situ* conservation alone would not be effective in safeguarding these important species. Thus, utilizing the biotechnological approach to complement *ex situ* conservation program is becoming vital. Propagating biotechnology tools in plant conservation program is a prerequisite to succeed in sustainable use and to complement the existing *ex situ* measures. In addition to propagation, storage of these valuable genetic resources is equally important. *In vitro* slow growth of 35 species and cryopreservation using embryo/meristem/seed in 20 different species of rare medicinal plants of this region is accomplished. Plants developed *in vitro* of ten medicinal plants, which have restricted distribution, were reintroduced in the natural habitat as well.

**Keywords** Conservation · Medicinal plants · Western ghats · *In vitro* conservation · Cryopreservation · Micropropagation

## Introduction

Medicinal plants continue to be an important source of life-saving drugs for humankind, especially in the developing nations. The World Health Organization has estimated that more than 80% of the world population in developing countries depends primarily on herbal medicine for basic health care (Vines 2004). The increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics has renewed the interest in the use of plants and plant-based drugs. Subsequent global inclination toward herbal medicine has advanced the expansion of plant-based pharmaceutical industries. Only a small percentage of medicinal plants traded in India are solely cultivated. The obligatory demand

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for a huge raw material of medicinal plants is met from their wild populations. Over-exploitation and/or destructive harvesting to meet such demand in fact threaten the survival of many rare species (Natesh 1999). Confronted by such unprecedented genetic erosion and disappearance of species and ecosystems, conservation of natural resources assumes paramount urgency.

The objective of conservation of medicinal plant diversity depends on maintaining stability of their ecosystems and utilization of the inherent phytochemicals for developing high-value products (Ratnam and Teik 1999). *In situ* conservation practices through biosphere reserves, sanctuaries, and national parks can preserve the breeding system, but this alone is insufficient for their sustained use. Exploration, collection, characterization, evaluation, domestication/cultivation, and *ex situ* conservation in gene banks eventually support their sustained use supplying quality planting material and certified raw drugs. Modern biotechnological tools, including *in vitro* and cryopreservation techniques, DNA fingerprinting, and bioreactor-mediated bioproduction of phytochemicals, are mostly relevant in this context. Micropropagation and ecorestoration in fact support the *in situ* conservation activities, facilitating population enhancement in species where natural propagation is hindered because of destructive harvesting or reproductive barriers.

### Medicinal Plants of Western Ghats

India, on the tropic of cancer, is a known mega biodiversity center with 8% of the global biodiversity in 2.4% land. The country is 10th among plant-rich countries of the world and 4th among the countries of Asia. Two of the world's 25 hot spots—Eastern Himalayas and Western Ghats—are in India. The sea on the western side, Vindhya and Satpura mountain ranges on the northern side and the semi-arid Deccan plateau on the eastern side protect the Western Ghats. Lying between 8°20'–40' N and 73°77' E, the Western Ghats traverses through the states of Maharashtra, Goa, Karnataka, Tamil Nadu, and Kerala over a 1,600 km distance at an average width of 200 km. This unique land mass of India has one of the most varied tropical and subtropical climates, altitudinal ranges reaching up to 2,800 m and many edaphic conditions. The latter factors confer a variety of micro-climatic/ecological niches that nurture one of the richest endemic flora of peninsular India. With its extremely environmentally heterogeneous biogeographic area, the Western Ghats as a whole is an abode of rich diverse flora. Most of the known types of floristic components comprising nearly 7,000 plant species (Pushpangadan et al. 1994) with 30% endemics are represented here. Medicinal plant species include a variety of life form ranging from

lichens, algae, ferns, herbs, shrubs, climbers, and trees, annuals to perennials. The bulk of these plants with their intraspecific variation represent a chemical and medicinal goldmine as is evident from the strong traditions of natural drug use. Traditionally, natural forests of the Western Ghats region were the source of nearly 500 medicinal plants of which some are used for traditional and folk medicinal practices (Joy et al. 2001). Of the large variety of species available in the Western Ghats, about 50 species hold a very high value in the folk and herbal health forms for the treatment of different forms of ailments (Suja 2005). Modern medicine uses various phytochemicals such as berberine, camptothecin, forskolin, L-Dopa, and reserpine from medicinal plants of Western Ghats; the plants being *Coscinium fenestratum*, *Nothapodytes foetida*, *Coleus forskohlii*, *Mucuna pruriens*, and *Rauvolfia serpentina*. Unfortunately, only a few higher plant species from Western Ghats have been fully documented and prospected. A recent report suggests that only 3–5% of terrestrial plants have been thoroughly investigated (Gilani 2005). Moreover, though the drug discovery from medicinal plants continues to provide new drug leads, use of plant-based medicines is narrow because of several factors. Unavailability of sufficient plant material, selection, and implementation of appropriate high-throughput screening bioassays and the production of bioactive compounds in large quantities are the projected constraints (Ramavat et al. 2009). The necessity of extensive and intensive research on medicinal plants in general is thus evident.

### Micropropagation and Cultivation

Meeting demand of plant raw drugs through cultivation reduces pressure on natural population and is recognized as one of the most effective ways of conservation. In addition, cultivation promises plants or plant parts with both qualitatively and quantitatively uniform chemical constituents required for the preparation of standard formulations (Chaturvedi et al. 2007). The ever-increasing demand for uniform medicinal plants thus warrants their mass cloning through vegetative methods. Plant tissue cultures provide accelerated clonal multiplication of desired clones, where conventional propagation through vegetative method is inadequate, as in *Dioscorea floribunda*, *R. serpentina*, *Aloe vera*, *Picrorhiza kurroa*, and *Zingiber officinale*, as reviewed by Chaturvedi et al (2007). The latter have multiple uses in modern as well as traditional medicine and thus need to be cultivated in large numbers.

The majority of medicinal plants from Western Ghats extensively used in traditional medicine are seldom cultivated. Therefore, natural resources are solely depended upon to meet the demand of such species such as

*Aristolochia tagala*, *C. fenestratum*, *Decalepis arayalpathra*, *Holostemma adakodien*, *Salacia reticulata*, *Trichopus zeylanicus*, *Canarium strictum*, and *Utlaria salicifolia*. Destructive harvesting for stem, roots, or tubers hampers their natural regeneration, leading to depletion in the wild. There has been great interest and progress in *in vitro* propagation of such medicinal plants, and the number of species (Table 1) propagated through tissue cultures increased substantially in the past two decades. Nineteen species are from Tropical Botanic Garden and Research Institute (TBGRI). Different regeneration pathways such as direct, somatic embryos or callus-mediated shoot regeneration were explored in different species to get optimum multiplication rate utilizing different explants. Rooting and field establishment as a part of micropropagation protocol development has been applied successfully without much difficulty. However, in *Asparagus recemosus* in which *in vitro* rooting is difficult, a combination of several PGRs—NAA, Kinetin, adenine sulfate, and phloroglucinol—has been included in one half strength MS medium to get 85% rooting (Nishritha and Sanjay 2008). However, the possibilities of pulse treatment with auxins followed by *in vitro* or *ex vitro* rooting, as proven efficient in other recalcitrant species (D'Souza et al. 1998), has not been well exploited in the medicinal plants of Western Ghats except in a few species such as *Celastrus paniculata* (Gerald et al. 2006). Most of the medicinal plants micropropagated at TBGRI (Table 1) and transferred directly to a mist house without any pretreatment procedures have been shown to exhibit 90% survival and establishment. But, Gangaprasad et al. (2003, 2005) reported only 60% survival and establishment in *D. arayalpathra* and *U. salicifolia* because of their slender elongated stem. In such cases, the propagation protocol itself needs to be modified to get healthy and robust shoots. The micropropagation protocols reported, including those from TBGRI, were based primarily on an experimental scale reaching up to nursery trials. Successful reports of field cultivation trials are available only in a few Western Ghats species such as *A. vera*, *R. serpentina*, and *Z. officinale*. Other trials of medicinal plants such as *D. floribunda*, *Chlorophytum borivillianum*, *P. kurroa*, *Rosmarinus officinalis*, *Solanum khasianum*, and *Saussurea lappa* as reviewed by Chaturvedi et al. (2007) were not as successful. Pilot-scale field cultivation trials of *H. annulare* (unpublished), *Hemidesmus indicus* (Sreekumar et al. 2000) and *P. rosea* (Satheesh Kumar and Seeni 2003) conducted at TBGRI proved early tuberization and increased number/biomass of tubers in tissue-cultured plants. However, large-scale commercial cultivation of any of the Western Ghats medicinal plants utilizing tissue-cultured plants is not well known. This may be because of the higher unit cost of the tissue-cultured medicinal plants, in which the returns are not as cost effective as that of other horticultural crops.

Small-scale cultivation of species like *Kaempferia galanga*, *Holostemma annulare*, *Plectranthus vetiveroides*, *R. serpentina*, *Piper longum*, and *Plumbago rosea* utilizes conventional propagation methods. A market survey on tissue-cultured plants conducted in 2003 (Biotech Consortium India Ltd. 2005) revealed the domestic consumption of 1.55 million tissue-cultured medicinal plants in India, of which a share of 0.42 million is *A. vera*, native to the west coast of India. About 2.0 million tissue-cultured plants of medicinal spices such as ginger, turmeric, and cardamom are also utilized annually. Therefore, the potential of tissue cultures in revolutionizing the commercial agriculture sector enabling mass propagation of elite, high-yielding, and disease-free plants as realized in a few medicinal crops of India is yet to be applied in majority of species from Western Ghats.

### Conservation through Micropropagation and Reintroduction

Reintroduction is the deliberate establishment of individuals of an extint/endangered species into an area and/or habitat where it has become extirpated. The specific aim is to establish a viable self-sustaining population for conservation purposes. The reintroduction of plants is becoming an increasingly utilized strategy in plant conservation and protected area management as proven successful in a variety of species (Maunder 1992). When conventional propagation is insufficient or impaired, micropropagation and reintroduction are recommended (Wochok 1981; Fay 1992; Fay 1994). Such technology has already been successfully demonstrated in a number of plant species such as *Paphiopedilum rothchildianum* (Grell et al. 1988), *Bletia urbana* (Rubluo et al. 1989), *Ipsea malabarica* (Gangaprasad et al. 1998; Martin 2003), *Vanda coerulea* (Seeni and Latha 2000), *Vanda spathulata* (Decruse et al. 2003), and *Syzygium travancoricum* (Anand 2003).

Experimental reintroductions of eight medicinal plants, including the endemic species of Western Ghats such as *Decalepis arayalpathra*, *Mahonia leschenaultii*, *Heracleum candolleianum*, *Calophyllum apetalum*, and *Blepharistemma membranifolia* (Table 2), were attempted at Biotechnology and Bioinformatics Division of TBGRI during 2000–2007. About 100–350 plants were reintroduced into their native habitats, recording 78–95% establishment after 1–2 yr. In addition, a few micropropagated plants were also planted in the natural forest segments of TBGRI campus and are showing luxuriant growth without any special care as reintroduced plants. The utility of a micropropagation system is thus proven effective for the eco-restoration of threatened medicinal plants of Western Ghats. However, the reintroduction carried out at TBGRI is on the experimental scale. Thus, to realize ecological restoration, extended planting of more plants in more than one locality may be affected. Also

**Table 1.** *In vitro* regeneration protocol standardized in medicinal plants of Western Ghats

Species	Regeneration pathway	Explant	Reference
<i>Acorus calamus</i>	Organogenesis	Rhizome buds; Apical shoot	Anu et al. 2001; Hettiarachchi et al. 1997
<i>Adhatoda beddomei</i> <sup>a</sup>	Organogenesis	Nodes	Sudha and Seeni 1994
<i>Aegle marmelos</i> <sup>a</sup>	Organogenesis	Nodes; <i>In vitro</i> nodes	Islam and Karim 1994, Ajithkumar and Seeni 1998
<i>Aloe veera</i>	Organogenesis	Nodes	Singh and Sood 2009
<i>Aristolochia indica</i>	Organogenesis	Node, shoot tip	Soniya and Sujitha 2006
<i>Aristolochia tagala</i>	Organogenesis	Node	Animesh et al. 2007
<i>Asparagus recemosus</i>	Organogenesis	Nodes	Nishritha and Sanjay 2008
<i>Baliospermum montanum</i>	Organogenesis	Nodes	Sasikumar et al. 2009
<i>Blepharostemma membranifolia</i> <sup>a</sup>	Organogenesis	Nodes	Lakshmi and Seeni 2001a
<i>Boesenbergia pulcherrima</i> <sup>a</sup>	Organogenesis	Shoot tips	Anish et al. 2008
<i>Calophyllum apetalum</i> <sup>a</sup>	Organogenesis	Nodes	Lakshmi and Seeni, 2003
<i>Celastrus paniculatus</i> <sup>a</sup>	Organogenesis	Nodes	Lakshmi and Seeni 2001b; Gerald et al. 2006
<i>Centella asiatica</i>	Organogenesis	Node	Nath et al. 2000; Karthikeyan et al. 2009
<i>Ceropegia sahyadrica</i>	Organogenesis	Nodes	Nikam and Savant 2007
<i>Coleus forskohlii</i>	Organogenesis	Stem tip	Neelam et al. 1991; Rajasri and Sabita 2001
<i>Costus speciosus</i>	Organogenesis	Rhizome sections	Malabadi et al. 2005
<i>Curculigo orchiooides</i>	Organogenesis	Shoot bud	Neelam et al. 2007
<i>Curcuma amada</i>	Organogenesis	Rhizome	Prakash et al. 2004
<i>Curcuma haritha</i> <sup>a</sup>	Organogenesis	Rhizome tips	Bejoy et al. 2006
<i>Curcuma longa</i>	Organogenesis	Rhizome buds	Prathantururug et al. 2005
<i>Curcuma zedoaria</i>	Organogenesis	Rhizome bud	Loc et al. 2005, Stanly and Keng 2007
<i>Datura metel</i>	Organogenesis	Nodes	Muthukumar et al. 2004
<i>Decalepis arayalpathra</i> <sup>a</sup>	Organogenesis	Node; Cotyledonary nodes	Gangaprasad et al. 2005; Sudha et al. 2005
<i>Decalepis hamiltonii</i>	Organogenesis	Shoot tips	Giridhar et al. 2005
<i>Dioscorea bulbifera</i>	Organogenesis	Node	Asha and Nair 2007
<i>Dioscorea pentaphylla</i>	Organogenesis	leaf	Asha and Nair 2005
<i>Embelia ribes</i>	Organogenesis	leaf	Raghu et al. 2006b
<i>Geophila reniformis</i>	Organogenesis	leaves	Nisha et al. 2004
<i>Gloriosa superba</i>	Organogenesis	Apical bud	Samarajeewa et al. 1993, Sayeed Hassan and Roy 2005
<i>Hemidesmus indicus</i> <sup>a</sup>	Organogenesis; Somatic embryos	Nodes;	Sreekumar et al. 2000; Sarasan et al. 1994
<i>Holarrhena pubescens</i>	Organogenesis	Nodes, shoot tips	Gerald et al. 2005
<i>Holostemma annulare</i> <sup>a</sup>	Organogenesis	Nodes, <i>In vitro</i> root segments	Sudha et al. 1998, Sudha et al. 2000
<i>Holostemma ada-kodien</i>	Organogenesis	Node	Martin 2002
<i>Hypericum mysorense</i>	Organogenesis	Node	Shilpashree and Ravishankar 2009
<i>Kaempferia galanga</i>	Organogenesis	Rhizome bud	Swapna et al. 2004; Chithra et al. 2005
<i>Kaempferia galangaa</i> <sup>a</sup>	Somatic embryos	<i>In vitro</i> leaf	Preetha et al. 2008
<i>Kaempferia rotunda</i>	Organogenesis	Rhizome bud	Anand et al. 1997
<i>Mahonia leschenaultia</i> <sup>a</sup>	Organogenesis	Nodes	Radha and Seeni 2010c
<i>Morinda umbellata</i> <sup>a</sup>	Organogenesis	node	Lakshmi and Seeni 2002
<i>Myristica malabarica</i>	Somatic embryos (Direct)	Zygotic embryos	Indira Iyer et al. 2009
<i>Nothapodytes foetida</i> <sup>a</sup>	Organogenesis	Axenic seedling nodes	Satheesh Kumar and Seeni 2000
<i>Ophiorrhiza mungo</i> <sup>a</sup>	Organogenesis	Axenic seedling shoots; <i>In vitro</i> nodes	Benoy and Satheeshkumar 2004
<i>Ophiorrhiza prostrata</i>	Organogenesis	Leaf, internode	Shahanaz et al. 2007
<i>Oroxylum indicum</i>	Organogenesis	Apical and axillary buds	Gokhale and Bansal 2009
<i>Piper longum</i>	Organogenesis	Shoot tips	Soniya and Das, 2002

**Table 1.** (continued)

Species	Regeneration pathway	Explant	Reference
<i>Plumbago rosea</i> <sup>a</sup>	Organogenesis	Nodes	Benoy et al. 2007
<i>Pterocarpus marsupium</i>	Organogenesis	Seedling nodes	Sharad et al. 2004
<i>Rauvolfia micrantha</i> <sup>a</sup>	Organogenesis	Nodes; <i>In vitro</i> nodes	Sudha and Seeni 1996
<i>Rauvolfia serpentina</i>	Organogenesis	Shoot tips, nodes	Sarker et al. 1996, Baksha et al. 2007
<i>Rotula aquatica</i>	Organogenesis	Node	Delse et al. 2002; Chithra et al. 2004
<i>Sarcostemma brevistigma</i>	Organogenesis	Nodes	Dennis and Surabhi 2009
<i>Tinospora cordifolia</i>	Organogenesis	Nodes	Raghu et al. 2006a
<i>Trichopus zeylanicus</i> <sup>a</sup>	Organogenesis	Shoot tips of axenic seedlings	Krishnan et al. 1995
<i>Tylophora indica</i>	Organogenesis	Nodes	Faisa et al. 2007
<i>Utlaria salicifolia</i> <sup>a</sup>	Organogenesis	Nodes	Gangaprasad et al. 2003
<i>Vitex negundo</i>	Organogenesis	Nodes	Noman et al. 2008
<i>Zingiber officinalis</i>	Somatic embryos through callus	<i>In vitro</i> Areal stem	Lincy et al. 2009
<i>Zingiber zerumbet</i>	Organogenesis	Rhizome bud	Stanly and Keng 2007

<sup>a</sup> Protocol for micropropagation have been developed at TBGRI

recommended is cloning a sufficient number of propagules collected from source populations to copy maximum genetic diversity (McGlaughlin et al 2002). Ensuring a self-sustained population of endangered species with the full genetic diversity is essential to salvage them from extinction (Falk et al 2001). The exact number of plants that needs to be reintroduced varies with species and heterogeneity of source population. As is done in European countries, a national program on species recovery through biotechnological intervention has been started by the Department of Biotechnology, Government of India, thus giving considerable importance to the necessity of saving endangered taxa through the use of *in vitro* technology.

### Ex situ Conservation Through In Vitro and Cryopreservation

DBT, Government of India in 1992 initiated the establishment of four national gene bank facilities specifically

devoted to the conservation of medicinal and aromatic species of the country. National Gene bank at TBGRI is one among the four to have an objective of conserving the medicinal and aromatic plants of Southern Peninsular India through biotechnological intervention, including *in vitro* and cryopreservation techniques.

**Slow-growth cultures.** Slow-growth procedures allow clonal plant material to be held for 1–15 yr under tissue culture conditions with periodic sub-culturing, depending on species (Kameswara Rao 2004). Medicinal plants of Western Ghats' particularly wild relatives of the established clonally propagated crops like ginger and turmeric (*Zingiber* spp. and *Curcuma* spp.) are conserved as *in vitro* cultures in the *in vitro* repository of National Bureau of Plant Genetic Resources, New Delhi and Indian Institute of Spices Research, Kozhikkode, respectively (Table 3). A few other medicinal plant crops such as *Garcelona indica*, *R. serpentina*, and *Bacopa monnieri* (Table 3) have also been tested for *in vitro* storage. Unpublished results from our laboratory

**Table 2.** Medicinal plants of Western Ghats micropropagated through direct shoot regeneration from axillary or shoot tip meristems with experimental trials conducted for restoration in native habitats

Species	Establishment in the native localities (%)	Observed period (months)	Reference
<i>Acorus calamus</i>	90	18	Radha and Seeni, unpublished
<i>Blepharostemma membranifolia</i>	78	24	Lakshmi and Seeni 2001a
<i>Calophyllum apetalum</i>	85	12	Lakshmi and Seeni 2003
<i>Celastrus paniculatus</i>	95	8	Lakshmi and Seeni 2001b
<i>Decalepis arayalpathra</i>	84	24	Gangaprasad et al. 2005
<i>Heracleum candolleianum</i>	85	18	Radha and Seeni, unpublished
<i>Mahonia leschenaultii</i>	80	18	Radha and Seeni, 2010c
<i>Morinda umbellata</i>	78.7	24	Lakshmi and Seeni 2002

**Table 3.** *In vitro* conservation protocol standardized for the medicinal plants of Western Ghats

Species	Stored material	Optimum period of storage (months)	Culture conditions	Reference
<i>Bacopa monnieri</i>	Multiple shoot clumps	20	1/2 MS+2% sucrose; polypropylene (pp) capped bottles	Satheesh et al. 2003, Sharma et al. 2007
<i>Baliospermum montanum</i>	Shoot tip cuttings	12	1/2 MS+1.33 $\mu$ mBA+1% agar; 25°C; Culture tubes with pp caps	TBGRI 1997 <sup>a</sup>
<i>Curcuma spp.</i> (wild species)	Multiple shoot clumps	9–12.5	MS+24.6 $\mu$ M 2iP	Tyagi et al. 2004
<i>Decalepis arayalpathra</i>	Shoot tip cuttings	>7	1/2 MS+4.44 $\mu$ mBA+2% mannitol; 25°C; Culture tubes with pp caps	TBGRI 1997 <sup>a</sup>
<i>Garcentia indica</i>	Shoot cultures	11	MS+0.5 $\mu$ M BA	Malik et al. 2005
<i>Geophila reniformis</i>	Shoot tip cuttings	12	1/2 MS; 25°C; Culture tubes with pp caps	TBGRI 1997 <sup>a</sup>
<i>Hemidesmus indicus</i>	Shoot cultures	18–22	1/2 MS+20 g/l sucrose	George et al. 2010
<i>Holostemma annulare</i>	<i>In vitro</i> Nodes (Axillary shoot and roots developed during storage)	12	1/2 MS+2% mannitol; 25°C; Culture tubes with pp caps	TBGRI 1997 <sup>a</sup>
<i>Piper barberi</i>	Shoot tip cuttings (Roots formed during storage)	28	1/2 MS+1.0% agar; Bottles with pp caps	TBGRI 1997 <sup>a</sup>
<i>Piper longum</i>	Shoot tip cuttings (Roots formed during storage)	12	1/2 MS+1% sucrose; 25°C; Bottles with pp caps	TBGRI 1997 <sup>a</sup>
<i>Rauvolfia serpentina</i>	<i>In vitro</i> nodes with 2 axillary buds	15	MS+4.44 $\mu$ M BA+0.54 $\mu$ M NAA; 15°C; Culture bottles with pp caps	Neelam and Chandel 1992
<i>Utlaria salicifolia</i>	Shoot cultures	24	1/2 MS+ 40 g/l sucrose	George et al. 2010
<i>Zingiber sp</i>	<i>In vitro</i> rhizome	16–20	MS; 16 h light	Tyagi et al. 2006

<sup>a</sup> Unpublished data from TBGRI. National gene bank for medicinal and aromatic plants- consolidated report for phase I, pp 41–50; 1997

suggest that rooted plants survive longer (up to 28 mo at 25°C) than shoot clumps in culture bottles with polypropylene caps as enclosures. This was proven in a range of wild species of *Piper*, viz., *Piper barberi*, *P. longum*, *Piper galeatum*, and *Piper trichostachyon*. Similarly, with normal incubation temperatures (25°C), *in vitro* shoot tip cuttings of *Baliospermum montanum* and *Geophila reniformis* also survived in culture bottles with polypropylene caps as enclosures for more than 12 mo, with an 80% survival rate (Unpublished). The latter methods are utilized at the *in vitro* bank of TBGRI for routine *in vitro* conservation practices.

**Cryopreservation.** Cryopreservation involves storage of plant material at ultra-low temperatures in liquid nitrogen (–196°C) and is a useful method for germplasm preservation. This is the only available method for long-term germplasm conservation of vegetatively propagated plants and those with recalcitrant seeds. It is also considered safe for the long-term preservation of intermediate seeds. With the advent of new cryogenic procedures such as vitrification and encapsulation–dehydration, the number of plant species, with a proportionate increase in medicinal plants of Western Ghats cryopreserved, has increased significantly in recent years.

Seeds, as the natural regenerating organs of plants, are the preferred choice of material as used in a range of *Piper* spp. (Chaudhury and Chandel 1994; Decruse and Seeni

2003) and *Rauvolfia micrantha* (Decruse et al. 1999). These plants produce intermediate type of seeds. In the latter species, conventional storage is not safe for long-term preservation as moisture content above 7% needs to be maintained to retain viability. However, a number of species/genotypes which are clonally propagated or producing recalcitrant large seeds or otherwise do not set seeds require the intervention of *in vitro* methods for their successful cryopreservation (Table 4). Excised zygotic embryo as utilized in other recalcitrant seed crops (Engelman 2000) has proven useful for the cryopreservation of *Myristica malabarica*, *Nothapodytes nimmoniana*, and *Celastrus paniculatus* (Radha et al. 2006, 2010b, d). In both cases, excised zygotic embryos subjected to simple desiccation under laminar air flow for 90–120 min reduced viability to 68–71%, and 60% of them regenerated into whole plants upon LN storage. TBGRI is utilizing the protocol developed in *M. malabarica*, *N. nimmoniana* for cryobanking. Unpublished results from our laboratory indicate excised zygotic embryos of *Myristica dactyloides* subjected to desiccation for 90–120 min are also suitable for cryopreservation to get 60–65% whole plant regeneration. But the result on *C. fenestratum* is not promising, as cryopreserved embryos recorded only 34% germination against 56% in desiccation control (60 min desiccation). Vitrification and/or encapsulation–dehydration methods are

**Table 4.** Medicinal plants of Western Ghats for which cryopreservation protocols have been standardized

Species	Category	Stored material	Method	% Germination/regeneration after LN storage <sup>a</sup>	Reference
<i>Celastrus paniculatus</i>	Recalcitrant seeds	Zygotic embryos	Desiccation	65 (63)	Radha et al. 2010b
<i>Coscinium fenestratum</i>	Intermediate seeds	Zygotic embryos	Desiccation	34 (56)	TBGRI 2008 <sup>b</sup>
<i>D. bulbifera</i>	Clonal	Shoot tips	Vitrification	58	Papiya et al. 2009
<i>Dioscorea bulbifera</i>	Clonal	Embryogenic callus	Pregrowth + Encapsulation-dehydration	67.8	Mandal et al. 2009
<i>Dioscorea wallichii</i>	Clonal	Shoot tips	Vitrification	37 (71)	Mandal et al. 1996
<i>Holostemma annulare</i>	Clonal/ Orthodox seeds	Shoot tips	Pregrowth + Encapsulation-dehydration	54.2 (60)	Decruse et al. 1999, 2002, 2004
<i>Kaempferia galanga</i>	Clonal	Shoot tips	Pregrowth + Vitrification	50 (64)	TBGRI 2008 <sup>b</sup>
<i>Kaempferia galanga</i>	Clonal	Somatic embryos	Pregrowth + desiccation	42.8 (57.1)	TBGRI 2008 <sup>b</sup>
<i>Myristica dactyloides</i>	Recalcitrant seeds	Zygotic embryos	Desiccation	65 (70)	TBGRI 2008 <sup>b</sup>
<i>Myristica malabarica</i>	Recalcitrant seeds	Zygotic embryos	Desiccation	60 (71)	Radha et al. 2006
<i>Nothapodytes nimmoniana</i>	Intermediate seeds	Zygotic embryos	Desiccation	60 (68)	Radha et al. 2010d
<i>Piper nigrum</i>	Clonal	Somatic embryos	Encapsulation–dehydration; Vitrification	62 (71)	Yamuna and Babu 2010
<i>Rauvolfia serpentina</i>	Intermediate seeds	Nodal segments	Pregrowth + Vitrification	66	Avik and Sabita 2008
<i>Zingiber officinale</i>	Clonal	<i>In vitro</i> Shoot tips	Pregrowth +Vitrification	80	Yamuna et al. 2007

<sup>a</sup> % Germination/ regeneration in control given in parenthesis

<sup>b</sup> Unpublished data from TBGRI. National gene bank for medicinal and aromatic plants—project completion report, pp 41–78; 2008

successful for the cryopreservation of shoot tips, nodal segments, or embryogenic callus of *R. serpentina*, *Z. officinale*, and wild yams of Western Ghats, as proven by different laboratories (Table 4). Several factors such as culture medium, preparative procedures, and  $\text{NH}_4^+$  ions also influence the success of regeneration of shoot tips of *H. annulare* subjected to cryopreservation (Decruse et al. 1999, 2004; Decruse and Seeni 2002). As research carried out by various teams worldwide is progressively improving our understanding of mechanisms involved in *in vitro* and cryopreservation, it is expected that the utilization of cryopreservation in genetic resource conservation will increase steadily in the coming years.

### *In vitro* Production of Secondary Metabolites

Plant cell and tissue culture as a source of secondary metabolites so far has not been commercially successful; the exceptions are *Lithospermum erythrorhizon* and *Panax ginseng* in which cell culture is used for producing shikonin, a natural dye and ginsenosides, an adaptogen, respectively (Srinath and Govinda Raju 1999). A tremendous research and development effort has advanced a number of other *in vitro*-derived secondary products to semi-commercial status,

including vanillin and taxol production in cell cultures (Karuppusamy 2009). In the majority of other medicinal species (Srinath and Govinda 1999; Vanisree and Hsin-Sheng 2004, Karuppusamy 2009), the *in vitro* processes achieved for secondary metabolite production in laboratory conditions have fallen far short of expectations and have never approached commercial status. This may be due to the incomplete understanding of the transport mechanism (nutrients and products across various organelles concerned with biosynthesis and processing), regulation of biosynthesis and enzymology of many biosynthetic steps in organelles (Becker and Sauerwein 1990). The reports available for the production of secondary metabolites from medicinal plants of Western Ghats through *in vitro* methods are also on laboratory scale (Table 5). A research team from TBGRI also explored laboratory scale *in vitro* bioproduction of phytochemicals through root/hairy root/cell suspension culture of *P. rosea* (Plumbagin), *R. micrantha* (ajmaline, ajmalicine), *D. arayalpathra* (2-hydroxy-4-methoxy benzaldehyde), and *H. indicus* (2-hydroxy-4-methoxy benzaldehyde). The team proved increased biomass inheriting the active ingredients (Table 5). Scaling up of plumbagin production in root cultures in a mini-reactor is underway at TBGRI. However, further research is essential for the commercial exploitation for *in vitro* production of any of the medicinal plants from Western Ghats.

**Table 5.** Production of secondary metabolites from medicinal plants of Western Ghats through *in vitro* methods

Species	Pathway	Products	Reference
<i>Adhatoda vasica</i>	Shoot culture	Vasine	Shalaka and Sandhya 2009
<i>Centella asiatica</i>	Hairy roots	Asiaticoside	Kim et al. 2007
<i>Ceropegia juncea</i>	Callus culture	Cerpegin	Nikam and Savant 2009a
<i>Coleus forskohlii</i>	Shoot/ root culture	Forskolin	Sen et al. 1992; Krombholz et al. 1992
<i>Coscinium fenestratum</i>	Callus culture, cell suspension	Berberine	Jayakumaran Nair et al. 1992, Khan et al. 2008
<i>Costus speciosus</i>	<i>In vitro</i> Embryo clones, callus	Diosgenin	Jain et al. 1984, Pal and Roy 1991
<i>Datura stramonium</i>	Hairy roots	Hyocyanine	Hilton and Rhodes 1993
<i>Decalepis arayalpathra</i>	Root cultures	2-hydroxy-4-methoxy benzaldehyde	Sudha et al. 2001
<i>Gloriosa superba</i>	Callus, root cultures	Cochicine	Sivakumar et al. 2004, Ghosh et al. 2002, 2006
<i>Gymnema sylvestre</i>	Callus	Gymnemic acid	Gopi and Vatsala 2006
<i>Hemidesmus indicus</i>	Root culture	2-hydroxy-4-methoxy benzaldehyde	Sreekumar et al. 1998
<i>Holostemma annulare</i>	Hairy roots	unidentified	Karmarkar et al. 2001
<i>Hypericum mysorensense</i>	Shoot cultures	Flavonoides	Shilpashree and Ravishankar 2009
<i>Nothapodytes foetida</i>	Cell suspension, callus, shoot, root cultures	Camptothecin	Roja and Heble 1994, Fulzele et al. 2001, 2002, Thengane et al. 2003, Sundravelan et al. 2004.
<i>Phyllanthus amarus</i>	Callus	Alkaloides	Johnson and Alias 2007
<i>Plumbago indica</i>	Callus and cell suspension cultures	Plumbagin	Satheesh Kumar and Seeni 2002
<i>Plumbago rosea</i>	Hairy roots	Plumbagin	Yogananth and Jothi Basu 2009
<i>Plumbago zeylanica</i>	Hairy roots	Plumbagin	Verma et al. 2002
<i>Rauvolfia micrantha</i>	Hairy roots	Ajmaline, ajmalicine	Sudha et al. 2003
<i>Rauvolfia serpentina</i>	Callus, cell suspensions, hairy roots, shoot cultures	Reserpine, ajmalicine, Super oxide dismutase, ajmaline, Indole alkaloides	Yamamoto and Yamada 1986, Falkenhagen et al. 1993, Benjamin et al. 1993, Kirillova et al. 2001, Roja and Heble 1996
<i>Rauvolfia tetraphylla</i>	Callus	Reserpine	Anitha and Kumari 2006
<i>Rubia cordifolia</i>	Cell suspension culture, callus, <i>in vitro</i> roots	Antraquinones	Suzuki et al. 1985 Shin 1989, Radha et al. 2010a
<i>Ruta graveolens</i>	Shoot cultures	Furfurocoumarins	Massot et al. 2000
<i>Scoparia dulcis</i>	Callus	Scopadulic acid	Hayashi et al. 1998
<i>Tinospora cordifolia</i>	Cell suspension culture	Berberine	Rama Rao et al. 2008
<i>Tribulus terrestris</i>	Embryogenic callus	Harmine, Harmaline, Diosgenin	Nikam et al. 2009b

## Conclusions

The *in vitro* propagation protocols developed for medicinal plants of Western Ghats, including those at TBGRI, are satisfactory in laboratory scale, but their use in commercial scale needs further field cultivation trials. However, the utilization of tissue-cultured plants in a few commercial (medicinal and spice of Western Ghats) crops such as *A. vera*, *Zingiber*, *Curcuma*, and cardamom in revolutionizing the commercial agriculture sector enabling mass propagation of elite, high yielding, and disease-free plants give leads in other species. Conservation through micropropagation and reintroduction successfully demonstrated in a few species such as *D. arayalpathra*, *M. leschenaultii*, *C. apetalum*, *M. umbellata*, *H. candolleianum*, and *B. membranifolia* are on an

experimental scale. Copying the genetic diversity of source population to the restored population is also essential for ecological restoration and saving a species from extinction. The laboratory production of secondary metabolites achieved in medicinal plants of Western Ghats gives leads but necessitates a lot of experimentation at the bioreactor level to elevate, at the very least, into medium level commercial production of phytochemicals. Further researches and insight are thus essential for the effective and sustainable use of medicinal plants from Western Ghats through *in vitro* methods.

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