In Vitro Cell.Dev.Biol.—Plant (2011) 47:110–122
DOI 10.1007/s11627-011-9344-9

INVITED REVIEW

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

Peringattulli Narayanan Krishnan · S. W. Decruse · R. K. Radha

Received: 24 March 2010 / Accepted: 5 January 2011 / Published online: 1 February 2011

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 biotechnoligical 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

P. N. Krishnan (✉) · S. W. Decruse · R. K. Radha
Plant Biotechnology and Bioinformatics Division,
Tropical Botanic Garden and Research Institute,
Palode, Thiruvananthapuram 695 562, India
e-mail: peringattulli@yahoo.co.uk

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

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

Table 1. (continued)

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

[a] 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 *Garcenia 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
Acorus calamus	90	18	Radha and Seeni, unpublished
Blepharestemma membranifolia	78	24	Laksmi and Seeni 2001a
Calophyllum apetalum	85	12	Lakshmi and Seeni 2003
Celastrus paniculatus	95	8	Laksmi and Seeni 2001b
Decalepis arayalpathra	84	24	Gangaprasad et al. 2005
Heracleum candolleanum	85	18	Radha and Seeni, un published
Mahonia leschenaultii	80	18	Radha and Seeni, 2010c
Morinda umbellata	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
Bacopa monnierii	Multiple shoot clumps	20	1/2 MS+2% sucrose; polypropylene (pp) capped bottles	Satheesh et al. 2003, Sharma et al. 2007
Baliospermum montanum	Shoot tip cuttings	12	1/2 MS+1.33 μmBA+1% agar; 25°C; Culture tubes with pp caps	TBGRI 1997[a]
Curcuma spp. (wild species)	Multiple shoot clumps	9–12.5	MS+24.6 μM 2iP	Tyagi et al. 2004
Decalepis arayalpathra	Shoot tip cuttings	>7	1/2 MS+4.44 μmBA+2% mannitol; 25°C; Culture tubes with pp caps	TBGRI 1997[a]
Garcenia indica	Shoot cultures	11	MS+0.5 μM BA	Malik et al. 2005
Geophila reniformis	Shoot tip cuttings	12	1/2 MS; 25°C; Culture tubes with pp caps	TBGRI 1997[a]
Hemidesmus indicus	Shoot cultures	18–22	1/2 MS+20 g/l sucrose	George et al. 2010
Holostemma annulare	*In vitro* Nodes (Axillary shoot and roots developed during storage)	12	1/2 MS+2% mannitol; 25°C; Culture tubes with pp caps	TBGRI 1997[a]
Piper barberi	Shoot tip cuttings (Roots formed during storage)	28	1/2 MS+1.0% agar; Bottles with pp caps	TBGRI 1997[a]
Piper longum	Shoot tip cuttings (Roots formed during storage)	12	1/2 MS+1% sucrose; 25°C; Bottles with pp caps	TBGRI 1997[a]
Rauvolfia serpentina	*In vitro* nodes with 2 axillary buds	15	MS+4.44 μM BA+0.54 μM NAA; 15°C; Culture bottles with pp caps	Neelam and Chandel 1992
Utlaria salicifolia	Shoot cultures	24	1/2 MS+ 40 g/l sucrose	George et al. 2010
Zingiber sp	*In vitro* rhizome	16–20	MS; 16 h light	Tyagi et al. 2006

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

[a] % Germination/ regeneration in control given in *parenthesis*

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

Acknowledgements The authors acknowledge the Director, TBGRI for providing facilities and encouragement and the Department of Biotechnology, Government of India for providing financial assistance.

References

Ajithkumar D.; Seeni S. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. *Plant Cell Rep* 17: 422–426; 1998.

Anand A. Studies on genetic stability of micropropagated plants and reintroduction in an endemic and endangered taxon: *Syzygium travancoricum* Gamble. *J Plant Biotechnol* 5: 201–207; 2003.

Anand M. P. H.; Harikrishnan K. N.; Martin K. P.; Hariharan M. *In vitro* propagation of *Kaempferia rotunda* Linn. "Indian crocus": a medicinal plant. *Phytomorphology* 47: 281–286; 1997.

Animesh B.; Bari M. A.; Mohashweta R.; Bhadra S. K. *In vitro* regeneration of *Aristolochia tagala* CHAMP. - A rare medicinal plant of chittagong hill tracts. *J Biosci* 15: 63–67; 2007.

Anish N. P.; Dan M.; Bejoy M. Conservation using *in vitro* progenies of the threatened Ginger *Boesenbergia pulcherrima* (Wall.) Kuntze. *Int J Bot* 4: 93–98; 2008.

Anitha S.; Kumari B. D. R. Stimulation of reserpine biosynthesis in the callus of *Rauvolfia tetraphylla* L. by precursor feeding. *Afr J Biotechnol* 5: 659–661; 2006.

Anu A.; Babu K.; Nirmal John C. Z.; Peter K. V. *In vitro* clonal Multiplication of *Acorus calamus* L. *J Plant Biochem Biotechnol* 10: 53–55; 2001.

Asha K. I.; Nair G. M. Direct Organogenesis in *Dioscorea* Species. *Indian J Plant Genet Resour* 18: 250–257; 2005.

Asha K. I.; Nair G. M. Shoot Multiplication in *Dioscorea* Species. *Indian J Plant Genet Resour* 20: 130–136; 2007.

Avik R.; Sabita B. Cryopreservation of *in vitro* grown nodal segments of *Rauvolfia serpentina* by pvs2 vitrification. *Cryoletters* 29: 321–328; 2008.

Baksha R.; Miskat A. A. J.; Rahima K.; John L. M. *In vitro* rapid clonal propagation of *Rauvolfia serpentina* (Linn.) Benth. Bangladesh. *J Sci Ind Res* 42: 37–44; 2007.

Becker H.; Sauerwein M. Manipulation of the biosynthetic capacity of plant cell structures *In:* Chadwood RV. In: Rhodon M. J. C. (ed) Secondary products from plant tissue culture. Clerendan Dvers, Oxford, pp 43–58; 1990.

Bejoy M.; Dan M.; Anish M. P. Factors affecting the *in vitro* multiplication of the endemic ginger, *Curcuma haritha* Mangaly and Sabu. *Asian J Plant Sci* 5: 847–853; 2006.

Benjamin B. D.; Roja G.; Heble M. R. Agrobacterium mediated transformation of *Rauvolfia serpentina*: regeneration and alkaloid synthesis. *Plant Cell Tissue Organ Cult* 35: 253–257; 1993.

Benoy J.; Satheeshkumar K. *In vitro* mass multiplication of *Ophiorrhiza mungo* Linn. *Indian J Exp Biol* 42: 639–642; 2004.

Benoy J.; Satheeshkumar K.; Seeni S. A protocol for high frequency regeneration through nodal explant cultures and *ex vitro* rooting of *Plumbago rosea* L Pakistan. *J Biol Sci* 10: 349–355; 2007.

Biotech Consortium India Limited. Summary report on market survey on tissue cultured plants. Department of Biotechnology, Government of India and Small, Farmers' Agri-Business consortium. http://dbtmicropropagation.nic.in/surveytcp.pdf: pp.1–14; 2005.

Chaudhury R.; Chandel K. P. S. Germination studies and cryopreservation of seeds of black pepper (*Piper nigrum* L.)- a recalcitrant species. *Cryoletters* 15: 145–150; 1994.

Chaturvedi H. C.; Jain M.; Kidwai N. R. Cloning of medicinal plants through tisuue culture-A review. *Indian J Exp Biol* 45: 937–948; 2007.

Chithra M.; Martin K. P.; Sunandakumari C.; Madhusoodanan P. V. Silver nitrate induced rooting and flowering *in vitro* of rare rhoeophytic woody medicinal plant, *Rotula aquatica* Lour. *Indian J Biotechnol* 3: 418–421; 2004.

Chithra M.; Martin K. P.; Sunandakumari C.; Madhusoodanan P. V. Protocol for rapid propagation, and to overcome delayed rhizome formation in field established *in vitro* derived plantlets of *Kaempferia galanga* L. *Sci Hortic* 15: 113–120; 2005.

D'Souza L.; Rajendra K.; Vinitha C.; Alice C. A.; Smitha H.; Icy D. Micropropagation and conservation of medicinal plants. In: Khan I. A.; Khanum A. (eds) Role of Biotechnology in medicinal and aromatic plants. Ukaaz, Hyderabad, pp 57–82; 1998.

Decruse S. W.; Gangaprasad A.; Seeni S.; Sarojini Menon V. Micropropagation and ecorestoration of *Vanda spthulata*, an exquisite orchid. *Plant Cell Tissue Organ Cult* 72: 199–2002; 2003.

Decruse S. W.; Seeni S. Pushpangadan, P. Cryopreservation of alginate coated shoot tips of *in vitro* grown *Holostemma annulare* (Roxb.) K. Schum, an endangered medicinal plant: Influence of preculture and DMSO treatment on survival and regeneration. *Cryoletters* 20: 243–250; 1999.

Decruse S. W.; Seeni S.; Pushpangadan P. Effect of cryopreservation on seed germination of selected rare medicinal plants of peninsular India. *Seed Sci Tech* 27: 501–505; 1999.

Decruse S. W.; Seeni S. Ammonium nitrate in the culture medium influences regeneration potential of cryopreserved shoot tips of *Holostemma annulare*. *Cryoletters* 23: 55–60; 2002.

Decruse S. W.; Seeni S. Seed cryopreservation is a suitable storage procedure for a range of *Piper* species. *Seed Sci Tech* 31: 213–217; 2003.

Decruse S. W.; Seeni S.; Nair G. M. Preparative procedures and culture media effect on the success of cryostorage of *Holostemma annulare* shoot tips. *Plant Cell Tissue Organ Cult* 76: 179–182; 2004.

Delse P. S.; Sailas B.; Molly H. Micropropagation of *Rotula aquatica* Lour.-an important woody mediicnal plant. *Phytomorphology* 52: 137–144; 2002.

Dennis T.; Surabhi S. Multiple shoot induction and callus regeneration in *Sarcostemma brevistigma* Wight and Arnott, a rare medicinal plant. *Plant Biotechnol Rep* 3: 67–74; 2009.

Engelmann F. Importance of cryopreservation for the conservation of plant genetic resources. In: Engelmann F.; Takagi H. (eds) Cryopreservation of tropical plant germplasm. Current research progress and application. IPGRI, Rome, pp 8–20; 2000.

Faisa M.; Ahmad N.; Anis M. An efficient micropropagation system for *Tylophora indica*: an endangered, medicinally important plant. *Plant Biotechnol Rep* 1: 155–161; 2007.

Falk D. A.; Knapp E.; Guerrant E. O. An introduction to restoration genetics. Society for Ecological Restoration, US Environment Protection Agency, Washington DC; 2001.

Falkenhagen H.; Kuzovkina I. N.; Alterman I. E.; Nikolaeva L. A.; Stockigt J. Alkaloid formation in hairy roots and cell suspensions of *Rauwolfia serpentina* Benth. *Nat Prod Res* 3: 107–112; 1993.

Fay M. F. Conservation of rare and endangered plants using *in vitro* methods. *In Vitro Cell Dev Biol Plant* 28: 1–4; 1992.

Fay M. F. In what situations is *in vitro* culture appropriate to plant conservations? *Biodivers Conserv* 3: 176–183; 1994.

Fulzele D. P.; Satdive R. K.; Pol B. B. Growth and production of camptothecin by cell suspension cultures of *Nothapodytes foetida*. *Planta Med* 67: 150–152; 2001.

Fulzele D. P.; Satdive R. K.; Pol B. B. Untransformed root cultures of *Nothapodytes foetida* and production of camptothecin. *Plant Cell Tissue Organ Cult* 69: 285–288; 2002.

Gangaprasad A.; Decruse S. W.; Seeni S.; Nair G. M. Micropropagation and restoration of *Decalepis arayalpathra* (Joseph and Chandra) Venter- an endemic and endangered ethanomedicinal plant of Western Ghats. *Indian J Biotechnol* 4: 265–270; 2005.

Gangaprasad A.; Decruse S. W.; Seeni S.; Sarojini Menon V. Micropropagation and restoration of the endangered malabar daffordil orchid *Ipsea malabarica* (Reich.b.f.) Hook.f. *Lindleyana* 14: 38–46; 1998.

Gangaprasad A.; Lakshmi G. N.; Radhakrishnan K.; Seeni S.; Nair G. M.; Pushpangadan P. Micropropagation of *Utleria salicifolia*,- an endemic endangered ethanomedicinal plant of the Western Ghats. *J Med Aromatic Plant Sci* 25: 19–24; 2003.

George S.; Geetha S. P.; Anu A.; Indira B. *In vitro* conservation studies in *Hemidesmus indicus, Decalepis hamiltonii* and *Utleria salicifoliaIn:*Proceedings of the 22nd Kerala Science Congress, Kerala State Council for Science Technology and Environment, Thiruvnanthapuram, Kerala, India: 258–259; 2010.

Gerald M.; Geetha S. P.; Sudhakar S. R.; Raghu A. V.; Indira B.; Ravindran A. N. An efficient micropropagation system for *Celastrus paniculatus* Willd.: a vulnerable medicinal plant. *J For Res* 11: 461–465; 2006.

Gerald M.; Geetha S. P.; Raghu A. V.; Indira B.; Ravindran P. N. Micropropagation of *Holarrhena pubescens*. *J Trop Med Plants* 6: 111–118; 2005.

Ghosh B.; Mukherjee S.; Jha T. B.; Jha S. Enhanced colchicine production in root cultures of *Gloriosa superba* by direct and indirect precursors of the biosynthetic pathway. *Biotechnol Lett* 24: 231–234; 2002.

Ghosh S.; Ghosh B.; Jha S. Aluminium chloride enhances colchicine production in root cultures of *Gloriosa superba*. *Biotechnol Lett* 28: 497–503; 2006.

Gilani A. H. Role of medicinal plants in modern medicine. *Malays J Sci* 24: 1–5; 2005.

Giridhar P.; Gururaj H. B.; Ravishankar G. A. *In vitro* shoot multiplication through shoot tip cultures of *Decalepis hamiltonii* Wight and Arn., a threatened plant endemic to Southern India. *In Vitro Cell Dev Biol Plant* 41: 77–80; 2005.

Gokhale M.; Bansal Y. K. Direct *in vitro* regeneration of a medicinal tree *Oroxylum indicum* (L.) Vent. through tissue culture. *Afr J Biotechnol* 8: 3777–3781; 2009.

Gopi C.; Vatsala T. M. *In vitro* studies on effects of plant growth regulators on callus and suspension culture biomass yield from *Gymnema sylvestre* R.Br. *Afr J Biotechnol* 5: 1215–1219; 2006.

Grell E.; Schmude H. N. F.; Lamb A.; Bacon A. Re-introducing *Paphiopedilum rothchildianum* to Sabah, North Borneo. *Am Orchid Soc Bull* 57: 1238–1245; 1988.

Hayashi T.; Kasahara K.; Sankawa U. Efficient production of biologically active diterpenoid by leaf organ culture of *Scoparia dulcis*. *Cell Cult Biotechnol* 46: 517–520; 1998.

Hettiarachchi A.; Fernando K. K. S.; Jayasurya A. H. M. *In vitro* propagation of Wadakaha (*Acorus calamus* L). *J Natn Sci Coun SriLanka* 25: 151–157; 1997.

Hilton M. G.; Rhodes M. J. C. Factors affecting the growth and hyocyamine production during batch culture of transformed roots of *Datura stramonium*. *Planta Med* 59: 340–344; 1993.

Indira Iyer R.; Jayaraman G.; Ramesh A. Direct somatic embryogenesis in *Myristica malabarica* Lam., an endemic, threatened medicinal species of Southern India and detection of phytochemicals of potential medicinal value. *Indian J Sci Technol* 2: 11–17; 2009.

Islam R.; Karim M. R. Plant regeneration from excised cotyledon of *Aegle marmelos* Corr. *Pak J Bot* 26: 393–396; 1994.

Jain M.; Rathore A. K.; Khanna P. Influence of kinetin and auxins on the growth and production of diosgenin by *Costus speciosus* (Koen.) Sm. callus derived from rhizome. *Agric Biol Chem* 48: 529; 1984.

Jayakumaran Nair A.; Sudhakaran P. R.; Madhusudana Rao J.; Ramakrishna S. V. Berberine synthesis by callus and cell suspension cultures of *Coscinium fenestratum*. *Plant Cell Tissue Organ Cult* 29: 7010; 1992.

Johnson M.; Alias A. Somoclonal variation studies on *Phyllanthus amarus* Schum and Thonn Iran. *J Biotechnol* 5: 240–245; 2007.

Joy P. P.; Thomas J.; Mathew S.; Skaria B. P. (2001) Scope of medicinal, aromatic and dye yielding crops in Kerala. In: National seminar cum workshop on "Scope of medicinal, aromatic and dye yielding crops in dry land agriculture." Central Research Institute for Dryland Agriculture, Hyderabad, India; 11–13 Dec

Kameswara Rao N. Plant genetic resources: advancing conservation and use through biotechnology. *Afr J Biotechnol* 3: 136–145; 2004.

Karmarkar S. H.; Keshavachandran R.; Nazeem P. A.; Girija D. Hairy root induction in adapathiyan *(Holostemma ada-kodien* K. Schum.). *J Trop Agric* 39: 102–107; 2001.

Karthikeyan K.; Chandran C.; Kulothungan S. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Centella asiatica*. *Indian J Biotehnol* 8: 232–235; 2009.

Karuppusamy S. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. *J Med Plant Res* 3: 1222–1239; 2009.

Khan T.; Krupadanam D.; Anwar S. Y. The role of phytohormone on the production of berberine in the calli cultures of an endangered medicinal plant, turmeric (*Coscinium fenestratum* l.). *Afr J Biotechnol* 7: 3244–3246; 2008.

Kim O. T.; Bang K. H.; Shin Y. S.; Lee M. J.; Jang S. J.; Hyun D. Y.; Kim Y. C.; Senong N. S.; Cha S. W.; Hwang B. Enhanced production of asiaticoside from hairy root cultures of *Centella asitica* (L.) Urban elicited by methyl jasmonate. *Plant Cell Rep* 26: 1914–1949; 2007.

Kirillova K. V.; Smirnova M. G.; Komov V. P. Sequential isolation of superoxide dismutase and ajmaline from tissue cultures of *Rauwolfia serpentina* Benth. *Appl Biochem Microbiol* 37: 160–163; 2001.

Krishnan P. N.; Sudha C. G.; Seeni S. Rapid propagation through shoot tip culture of *Trichopus zeylanicus* Gaertn., a rare ethnomedicinal plant. *Plant Cell Rep* 14: 708–711; 1995.

Krombholz R.; Mersinger R.; Kreis W.; Reinhard E. Production of forskolin by axenic *Coleus forskolhii* roots cultivated in shake flasks and 20 L glass jar bioreactors. *Planta Med* 58: 328–333; 1992.

Laksmi G. N.; Seeni S. Micropropagation and restoration of *Blepharestemma membranifolia* (Miq.) Ding Hon., an endemic and threatened medicinal tree of the Western Ghats. In: Proceedings of the 13th Kerala Science Congress, Kerala State Council for Science Technolgy and Environment, Thiruvananthapuram, Kerala, India: 124–128; 2001a.

Laksmi G. N.; Seeni S. Rapid *in vitro* multiplication and field establishment of *Celastrus paniculatus* sub sp. *paniculatus* Willd. (Celastraceae), a medicinal woody climber. *Indian J Exp Biol* 39: 697-–704; 2001b.

Lakshmi G. N.; Seeni S. Rapid clonal multiplication of *Morinda umbellata* Linn (Rubiaceaqe), a medicinal liana through culture of nodes and shoot tips from mature plant. *Phytomorphology* 52: 77–81; 2002.

Lakshmi G. N.; Seeni S. *In vitro* multiplication of *Calophyllum apetalum* (Clusiaceae), an endemic medicinal tree of the Western Ghats. *Plant Cell Tissue Organ Cult* 75: 169–174; 2003.

Lincy K. A.; Remashree A. B.; Sasikumar B. Indirect and direct somatic embryogenesis from aerial stem explants of ginger (*Zingiber officinale* Rosc.). *Acta Bot Croat* 68: 93–103; 2009.

Loc N.; Doan D.; Tae K.; Moon Y. Micropropagation of zedoary (*Curcuma zedoaria* Roscoe)– a valuable medicinal plant. *Plant Cell Tissue Organ Cult* 81: 119–122; 2005.

McGlaughlin M.; Karoly K.; Kaye T. Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, *Abronia umbellata* subsp. *breviflora* (Nyctaginaceae). *Conserv Genet* 3: 411–420; 2002.

Malabadi R. B.; Mulgund G. S.; Nataraja K. Effect of triacontanol on the micropropagation of *Costus speciosus* (Koen.) SM. using rhizome thin sections. *In Vitro Cell Dev Biol Plant* 41: 129–132; 2005.

Malik S. K.; Chaudhury R.; Kalia R. K. Rapid *in vitro* multiplication and conservation of *Garcinia indica*: A tropical medicinal tree species. *Sci Hortic* 106: 539–553; 2005.

Mandal B. B.; Chandel K. P. S.; Dwivedi S. Cryopreservation of yams (*Dioscorea* spp.) shoot apices by encapsulation-dehydration. *Cryoletters* 17: 165–174; 1996.

Mandal B. B.; Sharma D. S.; Srivastava P. S. Embryogenic Cultures of *Dioscorea bulbifera* L. by encapsulation-dehydration. *Cryoletters* 30: 440–448; 2009.

Martin K. P. Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Rep* 21: 112–117; 2002.

Martin K. P. Clonal propagation, encapsulation and reintroduction of *Ipsea malabarica* (Reichb. F.) J. D. Hook., an endangered orchid. *In Vitro Cell Dev Biol Plant* 39: 322–326; 2003.

Massot B.; Milesi S.; Gontier E.; Bourgaud F.; Guckert A. Optimized culture conditions for the production of furanocoumarins by micropropagated shoots of *Ruta graveolens*. *Plant Cell Tissue Organ Cult* 62: 11–19; 2000.

Maunder M. Plant reintroduction: an overview. *Biodivers Conserv* 1: 51–62; 1992.

Muthukumar B.; Arockiasamy D. I.; Natarajan E. Direct organogenesis in *Datura metel* L., from *in vitro* nodal explants. *Indian J Biotechnol* 3: 449–451; 2004.

Natesh S. Conservation of medicinal and aromatic plants in India- an overview.*In:* Kamaruddin MS. In: Natesh S.; Osman A.; Azizol A. K. (eds) Medicinal and aromatic plants: strategies and technologies for conservation. Forest Research Institute, Kuala Lumpur, pp 1–11; 1999.

Nath T. K.; Chandra S. N.; Tiwari V.; Deo Singh B. Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. *Plant Cell Tissue Organ Cult* 63: 179–185; 2000.

Neelam S.; Chandel K. P. S.; Srivastava V. K. *In vitro* propagation of *Coleus forskohlii* Briq., a threatened medicinal plant. *Plant Cell Rep* 10: 67–70; 1991.

Neelam S.; Vimala D. S.; Ruchira P. *In vitro* propagation of a threatened, anticarcinogenic herb Curculigo orchioides Gaertn. *J Plant Biochem Biotechnol* 16: 63–65; 2007.

Neelam S.; Chandel K. P. S. Low-temperature storage of *Rauvolfia serpentina* Benth. Ex Kunrz.- an endangered, endemic medicinal plant. *Plant Cell Rep* 11: 200–203; 1992.

Nikam T. D.; Savant S. R. Callus culture and micropropagation of *Ceropegia sahyadrica* Ans. and Kulk.: an edible starchy tuberous rare asclepiad. *Indian J Plant Physiol* 12: 178–180; 2007.

Nikam T. D.; Savant S. R. Multiple shoot regeneration and alkaloid cerpegin accumulation in callus culture of *Ceropegia juncea* Roxb. *Physiol Mol Biol Plants* 15: 71–77; 2009a.

Nikam T. D.; Ebrahami M. A.; Patil V. A. Embryogenic callus culture of *Tribulus terrestris* L. a potential source of harmaline, harmine and diosgenin. *Plant Biotechnol Rep* 3: 243–250; 2009b.

Nisha A.; Narasimhan S.; Manjula S.; Nair G. M. Direct Organogenesis in *Geophila reniformis* D.Don, an important medicinal herb. *J Plant Biotechnol* 6: 189–192; 2004.

Nishritha B.; Sanjay S. *In vitro* propagation of a high value medicinal plant: *Asparagus racemosus* Willd. *In Vitro Cell Dev Biol-Plant* 44: 525–432; 2008.

Noman A. S. M.; Islam M. S.; Siddique N. A.; Hossain K. High frequency induction of multiple shoots from nodal explants of *Vitex negundo* using silver nitrate. *Int J Agric Biol* 10: 633–637; 2008.

Pal A.; Roy A. Embryo culture of *Costus speciosus* (Koen.) Sm. to regenerate variable diosgenin yielding clones. *Plant Cell Rep* 10: 565–568; 1991.

Papiya M.; Mandal B. B.; Bhat K. V.; Biswas A. K. Cryopreservation of Asian *Dioscorea bulbifera* L. and *D. alata* L. by vitrification: importance of plant growth regulators. *Cryoletters* 30: 110–111; 2009.

Prakash S.; Elangomathavan R.; Seshadri S.; Kathiravan K.; Ignacimuthu S. Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. *Plant Cell Tissue Organ Cult* 78: 159–165; 2004.

Prathanturarug S.; Soonthornchareonnon N.; Chuakul W.; Phaidee Y.; Saralamp P. Rapid micropropagation of *Curcuma longa* using bud explants pre-cultured in thidiazuron supplemented liquid medium. *Plant Cell Tissue Organ Cult* 80: 347–351; 2005.

Preetha T. S.; Hemanthakumar A. S.; Decruse S. W.; Krishnan P. N.; Seeni S. Effect of synthetic auxins on somatic embryogenesis from leaf derived callus of *Kaempferia galanga* L- an endangered medicinal plant. *Phytomorphology* 58: 117–124; 2008.

Pushpangadan P.; Seeni S.; Jacob T. Conservation of plant genetic resources in India- establishment of gene bank for medicinal and aroatic plants at Tropical Botanic Garden and Reserach Institute. G-15 GEMAB Newsletter Nos 5 and 6: 10-12; 1994.

Radha R. K.; Seeni S. (2010c) *In vitro* propagation and regeneration of *Mahonia leschenaultii* Nutt. a tree species of Western Ghats. *In vitro*Cell Dev Biol-Plant (in press)

Radha R. K.; Shereena S. R.; Divya K.; Krishnan P. N.; Seeni S. (2010a) *In vitro* propagation from seedling explants and anthaquinone production from *in vitro* shoots of *Rubia cordifolia* Linn., a medicinal plant. 97th Indian Science Congress, University of Kerala, Kariavattom, India.3–7 Jan

Radha R. K.; William Decruse S.; Amy M. V.; Krishnan P. N. (2010b) Zygotic embryo cryopreservation of *Celastrus paniculatus*, Golden Jubilee National Symposium on Plant Diversity Utilization and Management, Department of Botany, University of Kerala, Kariavattom, India. 27–29 May

Radha R. K.; William Decruse S.; Krishnan P. N. Cryopreservation of excised embryonic axes of *Nothapodytes nimmoniana* (Graham) Mebberly, a vulnerable tree species of the Western Ghats Indian J Biotechnol. *Indian J Biotechnol* 9:435-437; 2010d.

Radha R. K.; William Decruse S.; Seeni S.; Ganeshan S. (2006) Cryopreservation of embryonic axes of recalcitrant seed species *Myristica malabarica* Lam., a rare medicinal plant of the Western Ghats. National Seminar on Plant Resources of Western Ghats. Indian Institute of Science, Bangalore, Karnataka, India 7–8 December

Raghu A. V.; Geetha S. P.; Gerald M.; Indira B.; Ravindran P. N. *In vitro* clonal propagation through mature nodes of *Tinspora cordifolia* (Willd.) Hook.f. and Thomas.: an important ayurvedic medicinal plant. *In vitro Cell Dev Biol Plant* 42: 504–508; 2006a.

Raghu A. V.; Geetha S. P.; Gerald M.; Indira B.; Ravindran P. N. Direct shoot organogenesis from leaf explants of *Embelia ribes* Burm. f.: a vulnerable medicinal plant. *J For Res* 11: 57–60; 2006b.

Rajasri B.; Sabita B. *In vitro* multiplication of *Coleus forskohlii* Briq.: an approach towards shortening the protocol. *In Vitro Cell Dev Biol Plant* 37: 572–575; 2001.

Rama Rao B.; Vijay Kumar D.; Amrutha R. N.; Jalaja N.; Vaidyanath K. Effect of growth regulators, carbon source and cell aggregate size on berberine production from cell cultures of *Tinospora cordifolia Miers*. *Curr Trends Biotechnol Pharm* 2: 269–276; 2008.

Ramavat K. G.; Dass S.; Mathur N. The chemical diversity of bioactive molecules and therupetic potential of medicinal plants. In: Ramavat K. G. (ed) Herbal drugs: Ethnemedicine to Modern Medicine. Springer, Heidelberg, pp 7–32; 2009.

Ratnam W.; Teik N. L. Practical applications of biotechnology in conservation of medicinal and aromatic plants. In: Kamaruddin M. S.; Natesh S.; Osman A.; Azizol A. K. (eds) Medicinal and aromatic plants: strategies and technologies for conservation. Forest Research Institute, Kuala Lumpur, pp 53–62; 1999.

Roja G.; Heble M. R. The quinoline alkaloids camptothecin and 9-methoxycamptothecin from tissue cultures and mature trees of *Nothapodytes foetida*. *Phytochemistry* 36: 65–66; 1994.

Roja G.; Heble M. R. Indole alkaloids in clonal propagules of *Rauwolfia serpentina* Benthe ex kurz. *Plant Cell Tissue Organ Cult* 44: 111–115; 1996.

Rubluo A.; Chavez V.; Martinez A. *In vitro* seed germination and reintroduction of *Bletia urbana* (Orchidaceae) in its natural habitat. *Lindleyana* 4: 68–73; 1989.

Samarajeewa P. K.; Dassanayake M. D.; Jayawardena S. D. G. Clonal propagation of *Gloriosa superba*. *Indian J Exp Biol* 31: 719–720; 1993.

Sarasan V.; Soniya E. V.; Nair G. M. Regeneration of Indian sarasaparilla, *Hemidesmus indicus* R.Br., through organogenesis and somatic embryogenesis. *Indian J Exp Biol* 32: 284–287; 1994.

Sarker K. P.; Islam A.; Islam R.; Hoque A.; Joarder O. I. *In vitro* propagation of *Rauvolfia serpentina* through tissue culture. *Planta Med* 62: 358–359; 1996.

Sasikumar S.; Raveendar S.; Premkumar A.; Ignacimuthu S.; Agastian P. Micropropagation of *Baliospermum montanum* (Willd)Muell. Arg. a threatened medicinal plant. *Indian J Biotechnol* 8: 223–226; 2009.

Satheesh Kumar K.; Seeni S. *In vitro* multiplication of *Nothopodites foetida* (Wight) Sleumer through seedling explant cultures. *Indian J Exp Biol* 38: 273–277; 2000.

Satheesh Kumar K.; Seeni S. Production of plumbagin (5-hydroxy-2methoxy 1, 4-naphtoquinone) in callus and cell suspension cultures of *Plumbago indica* Linn. *Indian J Biotechnol* 1: 305–308; 2002.

Satheesh Kumar K.; Seeni S. *In vitro* mass multiplication and production of roots in *Plumbago rosea*. *Planta Med* 69: 83–86; 2003.

Satheesh G.; Geetha S. P.; Sudhakar R. S.; Indira B.; Ravindran P. N. *In vitro* medium-term conservation of *Bacopa monnieri* (L.) Pennell—the memory plus plant—under slow-growth conditions. *PGR News Lett* 151: 49–55; 2003.

Sayeed Hassan A. K. M.; Roy K. S. Micropropagation of *Gloriosa superba* L. fhrough high frequency shoot proliferation. *Plant Tissue Cult* 15: 67–74; 2005.

Seeni S.; Latha P. G. *In vitro* multiplication and eco-rehabilitation of the endangered Blue Vanda. *Plant Cell Tissue Organ Cult* 61: 1–8; 2000.

Sen J.; Sharma A. K.; Sahu N. P.; Mahato S. B. Production of forskolin in *in vitro* cultures of *Coleus forskohlii*. *Planta Med* 58: 324–327; 1992.

Shahanaz Beegum A.; Martin K. P.; Zhang C. L.; Nishita I. K.; Ligimol S. A.; Madhusoodanan P. V. Organogenesis from leaf and internode explants of *Ophiorrhiza prostrata,* an anticancer drug (camptothecin) producing plant. *Electron J Biotechnol* 10(1): 7; 2007.

Shalaka D. K.; Sandhya P. Micropropagation and organogenesis in *Adhatoda vasica* for the estimation of vasine. *Pharm Mag* 5: 539–363; 2009.

Sharad T.; Pankaj S.; Kanchan S. *In vitro* propagation of *Pterocarpus marsupium* Roxb.: an endangered medicinal tree. *Indian J Biotechnol* 3: 422–425; 2004.

Sharma N.; Satsangi R.; Pandey R.; Vimala Devi S. *In vitro* clonal propagation and medium term conservation of brahmi (*Bacopa monnieri* (L) wettst). *J Plant Biochem Biotechnol* 16: 139–43; 2007.

Shilpashree H. P.; Ravishankar R. *In vitro* plant regeneration and accumulation of flavanoids in *Hypericum mysorense*. *Int J Integr Biol* 8: 43–49; 2009.

Shin S. Studies on the production of anthraquinone derivatives by tissue culture of *Rubia* species. *Arch Pharm Res* 12: 99–102; 1989.

Singh B.; Sood N. Significance of explant preparation and sizing in *Aloe vera* L.- A highly efficient method for *in vitro* multiple shoot induction. *Sci Hortic* 122: 146–151; 2009.

Sivakumar G.; Krishnamurthy K. V.; Hao J.; Paek K. Y. Colchicine production in *Gloriosa superba* calluses by feeding precursors. *Chem Nat Compd* 40: 499–502; 2004.

Soniya E. V.; Das M. R. *In vitro* micropropagation of *Piper longum*—an important medicinal plant. *Plant Cell Tissue Organ Cult* 70: 325–327; 2002.

Soniya E. V.; Sujitha M. An efficient *in vitro* propagation of *Aristolochia indica*. *Biol Plant* 50: 272–274; 2006.

Sreekumar S.; Seeni S.; Pushpangadan P. Production of 2-hydroxy 4-methoxy benzaldehyde using root cultures of *Hemidesmus indicus* R.Br. *Biotechnol Lett* 20: 631–635; 1998.

Sreekumar S.; Seeni S.; Pushpangadan P. Micropropagation of *Hemidesmus indicus* R.Br. for cultivation and production of 2-hydroxy 4-methoxy benzaldehyde. *Plant Cell Tissue Organ Cult* 62: 211–218; 2000.

Srinath R.; Govinda Raju B. Role of plant biotechnology in production of secondary metabolites. In: Irfan A. K.; Atiya Khan (eds) Role of biotechnology in medicinal and aromatic plants, Vol 2. Ukaaz, Hyderabad, pp 392–403; 1999.

Stanly C.; Keng C. L. Micropropagation of *Curcuma zedoaria* Roscoe and *Zingiber zerumbet* Smith. *Biotechnol* 6: 555–560; 2007.

Sudha C. G.; Krishnan P. N.; Pushpangadan P. *In vitro* propagation of *Holostemma annulare* (Roxb.) Schum, a rare medicinal plant. *In vitro Cell Dev Biol Plant* 33: 57–63; 1998.

Sudha C. G.; Krishnan P. N.; Pushpangadan P.; Seeni S. *In vitro* propagation of *Decalepis arayalpathra*, a critically endangered ethnomedicinal plant for *ex-situ* conservation. *In vitro Cell Dev Biol Plant* 41: 648–654; 2005.

Sudha C. G.; Krishnan P. N.; Seeni S.; Pushpangadan P. Regeneration of plants from *in vitro* root segments of *Holostemma annulare* (Roxb.) K.Schum., a rare medicinal tree. *Curr Sci* 78: 503–506; 2000.

Sudha C. G.; Obul Reddy B.; Ravishankar G. A.; Seeni S. Production of ajmalicine in hairy root cultures of *Rauvolfia micrantha* Hook., a rare and endangered medicinal plant. *Biotechnol Lett* 25: 631–636; 2003.

Sudha C. G.; Seeni S. *In vitro* multiplication and field establishment of *Adhatoda beddomei* C.B. Clarke, a rare medicinal plant. *Plant Cell Rep* 13: 203–207; 1994.

Sudha C. G.; Seeni S. *In vitro* propagation of *Rauvolfia micrantha,* a rare medicinal plant. *Plant Cell Tissue Organ Cult* 44: 243–248; 1996.

Sudha C. G.; Seeni S. Establishment and analysis of fast-growing normal root culture of *Decalepis arayalpathra*, a rare endemic medicinal plant. *Curr Sci* 81: 371–374; 2001.

Suja A. (2005) Medicinal plants of Western Ghats. In: Sahyadri E.News: Issue XII http://www.ces.iisc.ernet.in/biodiversity/sahyadri_enews/newsletter/issue12/Index.htm

Sundravelan R.; Desireddy B.; Ciddi V. Production of camptothecines from callus cultures of *Nothapodytes foetida* (Wight) Sleumer. *Indian J Biotechnol* 3: 452–453; 2004.

Suzuki H.; Matsumoto T.; Mikami Y. Effects of physical factors and surface active agents on the formation of anthraquinones by *Rubia cordifolia* cells in suspension culture. *Agric Biol Chem* 49: 519–520; 1985.

Swapna T. S.; Binitha M.; Manju T. S. *In vitro* multiplication in *Kaempferia galanga* Linn. *Appl Biochem Biotechnol* 118: 233–241; 2004.

Thengane S. R.; Kulkarni D. K.; Shrikhande V. A.; Joshi S. P.; Sonawane K. B.; Krishnamurthy K. V. Influence of medium composition on callus induction and camptothecin(s) accumulation in *Nothapodytes foetida*. *Plant Cell Tissue Organ Cult* 72: 247–251; 2003.

Tyagi R. K.; Anuradha A.; Akkara Y. Conservation of *Zingiber* germplasm through *in vitro* rhizome formation. *Sci Hortic* 108: 210–219; 2006.

Tyagi R. K.; Yusuf A.; Dua P.; Agrawal A. *In vitro* Plant Regeneration and Genotype Conservation of Eight Wild Species of *Curcuma*. *Biol Plant* 48: 129–132; 2004.

Vanisree M.; Hsin-Sheng T. Plant cell cultures- an alternative and efficient source for the production of biologically important secondary metabolites. *Int J Appl Sci Eng* 1: 29–48; 2004.

Verma P. C.; Singh D.; Rahman L.; Gupta M. M.; Banerjee S. *In vitro* studies in *Plumbago zeylanica*: rapid micropropagation and establishment of higher plumbagin yeilding hairy root cultures. *J Plant Physiol* 159: 547–552; 2002.

Vines G. (2004) Herbal harvests with a future: towards a sustainable source for medicinal plants, Plant life International.www.plantlife.org.uk

Wochok Z. S. The role of tissue culture in preserving threatened and endangered plant species. *Biol Conserv* 20: 83–89; 1981.

Yamamoto O.; Yamada Y. Production of reserpine and its optimization in cultured *Rauwolfia serpentina* Benth. cells. *Plant Cell Rep* 5: 50–53; 1986.

Yamuna G.; Babu N. K. Cryopreservation of black pepper somatic embryos. In: Proceedings of the 22nd Kerala Science Congress, Kerala State Council for Science, Technology and Environment. Thiruvananthapuram, Kerala, pp 241–242; 2010.

Yamuna G.; Sumathi V.; Geetha S. P.; Praveen K.; Swapna N.; Nirmal Babu K. Cryopreservation of *in vitro* grown shoots of ginger (*Zingiber officinale* Rosc.). *Cryoletters* 28: 241–252; 2007.

Yogananth N.; Jothi Basu M. TLC method for the determination of plumbagin in hairy root culture of *Plumbago rosea* L. *Glob J Biotechnol Biochem* 4: 66–69; 2009.