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Review Article

In Vitro Propagation for Conservation of Rare and Threatened Plants of India – A Review

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Abstract

India has a rich biological diversity due to its varied climatic, altitudinal variations and ecological habitats. There have been increasing rates of threats of depletion to these biological resources due to immense biotic and abiotic stresses. Indiscriminate collection of plants for their medicinal, ornamental, perfumery uses, etc. and habitat loss and degradation are potential causes of threats. Conventionally, there are two methods of conservation: *in situ* and *ex situ* conservation, both are complementary to each other. *In situ* methods allow conservation to occur with ongoing natural evolutionary processes, *ex situ* conservation via *in vitro* propagation also acts as a viable alternative for increase and conservation of populations of existing bioresources in the wild and to meet the commercial requirements. A review highlighting various *in vitro* protocols developed for selected rare and threatened plant species of India has been done to highlight the significance of *ex situ* conservation in cases where regeneration through conventional methods is difficult to undertake and species are left with low population in the wild.
Keywords: *in vitro* propagation, conservation, rare, threatened.

Introduction

India is one of the twelve megadiversity countries of the world with a rich diversity of biotic resources (Bapat *et al*., 2008). Out of 34 hotspots recognised, India has two major hotspots - the Eastern Himalayas and the Western Ghats. India harbours about 47 000 species of plants of which 17 000 are angiosperms (Bapat *et al*., 2008). A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category. On a global basis, the IUCN has estimated that about 12.5% of the world's vascular plants, totalling about 34 000 species are under varying degrees of threat (Phartyal *et al*., 2002). IUCN recognises the following categories: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient and not evaluated. Critically endangered, endangered and vulnerable together form the threatened category. Each of these threatened categories can be deduced on any of the five criteria that reflect extinction risk:

- Declining population (past or projected): this includes species with high harvests, especially in destructive fashion.
- Narrow distribution, fragmentation and decline or fluctuation: several endemic species *prima facie* appear to be natural candidate for qualifying as threatened as per this criterion.
- Small population size and decline: absolute population number low and rate of decline high.
- Very small population or very restricted distribution: absolute population numbers extremely meagre.
- Quantitative analysis of probability of extinction: simulations using deterministic and stochastic population models.

Species with small populations that are not at present endangered or vulnerable but are at risk are called rare. These species are usually localised within restricted geographical areas or habitats or are thinly scattered over a more extensive range (Singh *et al*., 2006).

A species may become threatened and vulnerable with extinction due to any of the following natural or manmade causes (Singh and Chowdhery, 2002):

- Population crash/fragmented smaller populations.

- Loss of specific pollinators
- Loss of reproduction.
- Low seed germination capability
- Loss in genetic variability
- Habitat degradation or destruction (clearing of land habitat of plant and animal species) for human settlement and other commercial purposes, etc.
- Over exploitation: removal of timber, fuel, fodder and other commercially important species in excess of which the ecosystem cannot sustain.
- Competition: ecologically better suited species replacing the weaker ones.
- Pathological causes: outbreak of diseases, epidemics.
- Environmental factors: due to change of environment beyond the tolerance limit of the species.

The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicine (Kala, 2005) resulting into the increasing demand for medicinal plants. Plants that are useful as ornamentals, timber, perfumery trade, etc. are also being exploited from the natural habitat. Intensive grazing activity, habitat destruction and seedling mortality have limited the distribution of *Meconopsis simplicifolia* (Sulaiman and Babu, 1993), a plant species of horticultural value. Indiscriminate collection and severe habitat loss are the two potent factors responsible for the depletion of orchids like *Vanda coerulea* (Seeni and Latha, 2000) and *Renanthera imschootiana* (Seeni and Latha, 1992). Similarly, bamboos which are distinct and fascinating plants associated with unique elements of biodiversity with a wide range of values and with over 1500 documented uses are intrinsically vulnerable to deforestation. The vulnerability of some bamboo species is increased by the simultaneous flowering and subsequent death of the entire populations in cycles of 20-120 years. A recent study revealed that around 40% of bamboos in Asia-Pacific region are potentially threatened due to the small amount of forest cover remaining within their natural ranges (Bystriakova *et al*., 2004). Many bamboos in the American continent may be of conservation concern and the 1997 IUCN Red List of Threatened Plants contained 12 species of woody bamboos such as *Chusquea aperta*, *Guadua calderoniana* from the Americas and *Thamnocalamus tessellatus* from Africa. There are reports of *in vitro* micropropagation of bamboos through somatic embryogenesis (Rao *et al*., 1985; Lin *et al*., 2004) and axillary branching (Das and Pal, 2005) as well as rhizome induction (Kapoor and Rao, 2006) which can provide some respite from this alarming scenario but concrete efforts are required for conservation of this “wonder” agri-horticultural crop.

Methods of Conservation

Conventionally, *in situ* conservation allows evolution to continue within the area of natural occurrence, and *ex situ* conservation provides a better degree of protection to germplasm compared to *in situ* conservation. However, both *ex situ* and *in situ* conservation are complementary and should not be viewed as alternatives (Wang *et al*., 1993). *Ex situ* conservation includes germplasm banks, common garden archives, seed banks, DNA banks and techniques involving tissue culture, cryopreservation; incorporation of disease, pest and stress tolerance traits through genetic transformation and ecological restoration of rare plant species and their populations. *Ex situ* conservation has gained international recognition with its inclusion in Article 9 of the Convention on Biological Diversity (Glowka *et al*., 1994). *In vitro* propagation of rare and threatened plants is generally undertaken to enhance the biomass and conserve the germplasm especially when population numbers are low in the wild. Tissue culture technique has been successfully used when wild grown plants are difficult to propagate through conventional ways. *In vitro* propagation or micropropagation is a viable alternative for species which are difficult to regenerate by conventional methods; where populations have decreased due to over exploitation by destructive harvesting and can effectively be used to meet the growing demand for clonally uniform elite plants. When species have been over collected by hobbyists for medicine, food or fragrance, *in vitro* propagation can provide an alternate source of plants and alleviate pressures on wild populations.

Tissue culture can also be used when wild grown plants are difficult to propagate for *ex situ* preservation in botanical gardens. Such plants can be used as a source of seed for long-term storage and if seed is not produced, the tissue culture lines themselves can be cryopreserved. Propagated plants might also be used for *ex situ* studies on the biology of threatened plant species. Biotechnology offers avenues for maintenance, genetic improvement and efficient use of endangered plant resources and products (Bapat *et al*., 2008). Tissue culture is used for conservation of biological diversity by multiplication of plant species that have extremely small populations, for species with restricted reproductive capabilities and for recovery and reintroduction (Bramwell, 1990). The main areas of research in plant tissue culture viz. micropropagation, anther and microspore culture, somaclonal variations and mutagenesis, protoplast culture and somatic hybridization are some of the effective tools for regeneration and conservation of endangered plants (Bapat *et al*., 2008). Production of phytochemicals from cell cultures is advantageous and *in vitro* studies on secondary metabolites, biotransformation, cryopreservation of valuable cell lines, immobilization and understanding enzymatic pathways will generate new data as well as counter the reduction of production on medicinal plants (Bapat *et al*., 2008).

The *in situ* conservation has greater advantages over *ex situ* conservation as the species remain within the nature's ongoing evolutionary process and thus adapt to the changing natural conditions and compete with other species whereas, in the latter, the process of evolution is disrupted. But at the same time the protected areas are not always safe and vulnerable to loss and destruction (Singh and Chowdhery, 2002). Therefore, concerted efforts of both *in situ* and *ex situ* conservations are needed and should not be viewed as alternatives (Wang *et al*., 1993). *In vitro* culture techniques have been used in many germplasm repositories all over the world to supplement other *ex situ* methods for conservation of plant species particularly those which are either vegetatively propagated, produce recalcitrant seeds or are rare/endangered (Bapat *et al*., 2008).

There are four complementary strategies for biodiversity conservation: *in situ* strategy, *ex situ* strategy, reduction of anthropogenic pressures and rehabilitation of endangered species (Singh *et al*., 2006). Under natural conditions, each organism possesses a range of tolerance to variations in its physical and chemical environment. The organism responds to variations in environmental conditions in terms of their growth, reproduction and distribution. Any of the physical or chemical components of the environment that may inhibit the growth of living organisms, through either its lack or excess, is said to be a limiting condition or limiting factor. The organisms show wide distribution due to wide ranges of tolerance for all the factors and restricted distribution if the tolerance range for one or more than one factor is narrow. If some of the environmental factor shifts beyond the tolerance range of an organism, the organism can come to the resting stage or migrate or it can acclimate or it fails to adapt to the changes (Singh *et al*., 2006) in the environment and biosphere as such.

Global Policies and Networks for Conservation of Biodiversity

The Convention on Biological Diversity, in force since 1992, is the major international conservation convention. The Global Strategy for Conservation of Plants was adopted with the intention to harmonise with existing international initiatives addressing various aspects of plant conservation. This was formulated to address the relative invisibility of plants in international conservation flora and the actual loss of plant species. Target 8 of the Global Strategy for Plant conservation aims to secure 60% of threatened plant species in accessible *ex situ* collections, preferably in the country of origin, and 10% of them are included in recovery and restoration programmes. As part of Botanic Garden Conservation International's (BGCI) contribution to Target 8, a Plant Search Database has been developed to identify all those plants that are in cultivation in botanic gardens (Leadlay, 2005). There are over 150 000 taxa recorded in Plant Search provided by 637 gardens, of which over 11 000 species are recorded as globally threatened (Oldfield, 2007).

Realizing the importance of conservation of the national heritage, the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India was established in 1976 with the national responsibility for the collection, evaluation, conservation and exchange of germplasm of various agri-horticultural crops. National Facility for Plant Tissue Culture Repository was established in 1986 with the financial assistance from the Department of Biotechnology, Government of India, India. This facility has made significant progress in the fields of *in vitro* conservation, cryopreservation and molecular characterization of germplasm of various plant species. More than 1900 accessions are being maintained under slow growth conditions in the *in vitro* repository (Anonymous, 2006-2007).

The development of reliable *in vitro* protocols are of great importance for conservation of rare and threatened plant species by virtue of producing uniform planting material for offsetting the pressure on the natural populations especially for medicinal and ornamental plants. Concerted international and national efforts have been initiated to conserve and to sustainably use the biodiversity. The micropropagation unit at Royal Botanic Garden, Kew is involved in propagation and maintenance of more than 3000 plant taxa, from all over the world, for over 30 years (Sarasan *et al*., 2006). The use of various approaches of biotechnology in conservation of biodiversity and plant genetic resources has been described by various authors (Fay, 1992; Rao, 2004; Bapat *et al*., 2008).

The present review summarises the protocols reported for propagation and conservation of a few important selected rare and threatened plant species and the feasibility of their large-scale propagation.

Critically Endangered Plants

Arnebia euchroma (Royle) Johnston

A. euchroma (Boraginaceae) is distributed in the Pamirs, the Tien Shan, The Himalaya and Western Tibet (Anonymous, 1985). Shikonin possesses anti-bacterial, anti-fungal, anti-inflammatory and wound healing properties. Furthermore, *A. euchroma* exhibits potent anti-HIV activity (Manjkhola *et al*., 2005). Hence, the species is harvested indiscriminately for its medicinal uses.

Manjkhola *et al*., (2005) reported organogenesis and somatic embryogenesis in *A. euchroma* callus cultures from leaf explant on MS (Murashige and Skoog, 1962) medium supplemented with 2.5µM IBA and 2.5µM BAP and 72% plantlets survived under nursery conditions. The use of leaf as explant helped in avoiding the destruction of the mother plants. Somatic embryos were encapsulated for use as synthetic seeds. Jiang *et al.* (2005) obtained shoots via cotyledonous explant on TDZ supplemented LS (Linsmaier and Skoog, 1965) medium.

Decalepis arayalpathra (Joseph & Chandra.) Venter

D. arayalpathra (Periplocaceae) is endemic to southern forest of the Western Ghats, India (Nayar, 1996). Plant exploration studies in this region have revealed its habitat specificity and the occurrence of only small populations in the crevices of the rocks and accordingly, it is enlisted as critically endangered (CAMP-1, 1995). Recent pharmacological investigation of the root extract of the plant has revealed immunomodulatory and anti-cancerous properties (Subramoniam *et al*., 1996). It is estimated that more than 90% of the plant species used by the industry are collected from the wild and more than 70% of the plant drugs involved destructive harvesting and very few plants are in cultivation (Sudha and Seeni, 2001). The natural regeneration as well as conventional propagation of *D. arayalpathra* is beset with several factors like poor fruit set and scanty seed germination.

Sudha and Seeni (2001) established fast growing normal root cultures of *D. arayalpathra* from leaf and internodal explants of *in vitro* raised shoot cultures and also detected a root specific aromatic compound, 2-hydroxy-4-methoxy benzaldehyde using TLC. Cotyledon with shoot tip explant produced a maximum number of multiple shoots (Sudha *et al*., 2005) but the shoots were thin and fragile and showed low percentage of survival (40%). These shoots rooted on medium supplemented with auxins like NAA with formation of callus at the base of the shoots. The rooted plantlets of *D. arayalpathra* were reintroduced to its natural habitat at Kallar Reserve Forest, Thiruvananthapuram, India with 84% survival after two years (Gangaprasad *et al*., 2005).

Endangered Plants

Ceropegia candelabrum L.

C. candelabrum (Asclepiadaceae), known as the 'Glabrous goglet flower' is a perennial herb found at the edges of moist deciduous forests (Beena *et al*., 2003). Root tubers contain the alkaloid ceropegine which is used in Indian Ayurvedic drug preparations (Beena *et al*., 2003).

Beena *et al*., (2003) established a protocol for *in vitro* propagation of *C. candelabrum* through axillary bud multiplication by using BAP (8.87µM) in combination with 2.46µM IBA. Shoots were rooted on ½ strength MS medium supplemented with IBA with a maximum of seven roots per shoot on 0.49µM IBA. Somatic embryogenesis from leaf and internode segment was achieved by Beena and Martin (2003) on ½ or ¼ strength of MS medium containing 0.23µM or 0.45µM 2,4-D. Higher number of somatic embryos was achieved in suspension culture and on transfer to ¼ strength MS solid medium, 50% somatic embryos germinated and developed into plantlets.

Chlorophytum borivilianum Sant. et Fernand.

C.borivilianum (Liliaceae), an endangered species (Purohit *et al*., 1994) is valued for the dried fasciculated roots having aphrodisiac properties and forms an important ingredient of herbal tonics prescribed in the Ayurvedic system (Kirtikar and Basu, 1975). Due to large scale and indiscriminate collection of wild material, *C. borivilianum* is rapidly disappearing from natural habitats (Purohit *et al*., 1994).

Shoot regeneration from shoot bases and immature floral buds along with inflorescence axis of *C. borivilianum* has been achieved *in vitro* (Purohit *et al*., 1994; Sharma and Mohan, 2006). MS basal medium containing 22.2µM BAP produced maximum shoots (11) per explant (Purohit *et al*., 1994). Arora *et al*., (2006) achieved somatic embryogenesis from seedling and leaf explants. Shoots rooted on B5 medium supplemented with 0.57µM IAA and showed 90% survival when transferred to soil.

Decalepis hamiltonii Wight & Arn.

D. hamiltonii (Asclepiadaceae) is endemic to the Deccan Peninsula and Western Ghats of India. The roots provide potent bio-insecticide activity against storage pests at lethal and sub-lethal levels (Indian patent no. 130/del/98) and are also potent antimicrobial agent (George *et al*., 1999). Clonal propagation of *D. hamiltonii* using nodal explant has been reported by Bais *et al*. (2000) and Anitha and Pullaiah (2002). Reddy *et al*. (2001) obtained rooting of microshoots from nodal explants on IBA (4.4µM) exhibiting 100% rooting and showed 90% field survival. Giridhar *et al*. (2004) induced somatic embryogenesis from leaf cultures of *D. hamiltonii* and 70% of the rooted plantlets on IBA on transfer to field survived.

Gloriosa superba L.

G. superba (Liliaceae) is a valuable tropical medicinal plant. Corms are thermogenic, abortifacient, alexteric and antipyretic (Somani *et al*., 1989). Somani *et al*. (1989) obtained shoots from corm explants on MS + 3mg/l Kn. The multiple shoots formed microcormlets at the base of each shoot. Sivakumar and Krishnamurthy (2000) reported as many as 35 shoots on average from shoot tip explant on basal medium consisting of MS salts and B5 vitamins + 9.84µM 2-iP + 2.32µM Kn. Hassan and Roy, (2005) developed a protocol for propagation of *G. superba* using terminal shoot tips and stem nodes. Shoots rooted on ½ strength MS + 1.0mg/l IBA or 0.5mg/l IAA. Ninety per cent plants survived in the field.

Ipsea malabarica (Reichb.f.) J.D. Hook.

I. malabarica (Orchidaceae), commonly known as ‘The Malabar Daffodil Orchid’, is an endemic and endangered orchid of the Western Ghats of India (Martin and Pradeep, 2003). Martin and Pradeep (2003) reported *in vitro* storage of *I. malabarica* at whole plant level. Shoots developed from rhizome explants on ½ strength MS medium supplemented with 3% sugar and 1.5mg/l Kn developed 25 shoots over a period of 14 months. Elimination of Kn and sugar individually from the above medium increased the time of subculture with a reduction in the number of shoots. Exclusion of sugar and growth regulator was optimum for *in vitro* conservation and this medium facilitated storage for 27 months.

Martin (2003) accomplished clonal propagation and encapsulation of *in vitro* formed bulbs using rhizome and its reintroduction to the natural habitat. Half strength MS medium supplemented with 6.97µM Kn induced four shoots per explant within 50 days. Transfer of the isolated shoots increased rate of shoot multiplication to more than ten shoots and subsequent culture developed bulbs. *In vitro* bulbs were encapsulated and 100% conversion to plantlets on growth regulator-free ½ strength MS or 6.97µM Kn supplemented medium was observed. Fifty plantlets were reintroduced into their natural habitat (Martin, 2003).

Picrorhiza kurroa Royle ex Benth.

P. kurroa (Scrophulariaceae), commonly known as 'Kutki', is endemic to alpine Himalayas and grows in the inner ranges from Kashmir to Sikkim (Chandra *et al*., 2006). The extract of runners and roots of this plant has been used since long in several Ayurvedic preparations, prescribed in hepatic disorders (Chandra *et al*., 2006).

Upadhyay *et al*. (1989) developed a micropropagation method through forced axillary branching using terminal and nodal cuttings using BAP. Rooting of microshoots was obtained on MS + 1.0μM NAA in 20 days. Chandra *et al*. (2006) achieved *in vitro* shoot multiplication using nodal segments. 65% survival of plantlets was achieved in the greenhouse and these were transferred to field and 80% survival was noted after three months. Wawrosch *et al*. (2003) investigated the influence of rooting conditions which could help in the establishment of the plants *ex vitro*.

Psoralea corylifolia Linn. (Syn: *Cullen corylifolia* (L.) Medik.)

P. corylifolia (Fabaceae) is an endangered plant (Jain, 1994) and is used as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions (Sahrawat and Chand, 2001). Pharmaceutical companies largely depend upon material procured from naturally occurring stands which are being depleted rapidly, raising concern about possible extinction. *P. corylifolia* is propagated by seeds. However, seed germination percentage is very low (5–7%). No alternative mode of multiplication is available to propagate and conserve genetic stock of this plant (Chand and Sahrawat, 2002).

Saxena *et al*., (1997) reported plantlet regeneration via organogenesis in callus cultures derived from mature leaves and stems, petioles and roots of young seedlings of *P. corylifolia*. The number of shoots obtained was more (5.8-22.4) in case of explants derived from seedlings than in mature plants (3.4-4.8). Rooting was observed on MS medium with 2% sucrose containing either NAA or IBA but with intervening callus. Ninety five to ninety eight per cent rooted plants survived in the greenhouse. *In vitro* plant regeneration of *P. corylifolia* was achieved from hypocotyl segments (Sahrawat and Chand, 2001), root segments (Chand and Sahrawat, 2002), cotyledonary node (Jeyakumar and Jayabalan, 2002). Axillary shoot multiplication from nodal explants of *P. corylifolia* was achieved by Faisal and Anis (2006) using TDZ.

Pterocarpus marsupium Roxb.

P. marsupium (Fabaceae) commonly known as 'Bijasal', is one of the most important multipurpose forest tree legumes of India, valued greatly for its excellent timber and for its pharmaceutical properties. Two important phenolic constituents, marsupsin and pterostilbene, isolated from the heartwood of *P. marsupium* are reported to possess anti-hyperglycemic activity (Manickam *et al*., 1997). Hard fruit coat, low germinability coupled with poor seed viability is responsible for its diminishing population size and inclusion in the list of depleted plant species (Anis *et al*., 2005).

Das and Chatterjee (1993) attempted micropropagation of *P. marsupium* using seedlings and coppiced shoot explants without any response. However, plant regeneration from cotyledonary node of *P. marsupium* has been reported by several authors (Chand and Singh, 2004; Anis *et al*., 2005; Husain *et al*., 2007). Husain *et al*. (2007) employed a two step procedure for rooting by first giving pulse treatment with IBA (200μM) for 4 days followed by subsequent transfer to semisolid half strength MS medium containing IBA (0.2μM) + phenolic acids. The acclimatized plantlets showed 70% survival in the greenhouse.

Renanthera imschootiana Rolfe

R. imschootiana (Orchidaceae), popularly called Red Vanda, is an extremely endangered epiphytic orchid of North-Eastern India, distributed in the hill tracts of the Cachar district, Assam, Manipur, Nagaland, Mizoram, India and Burma. This species is of great horticultural value and as a progenitor of many outstanding hybrids such as *Aranthera* Leong Kok, *Rendopsis hiiaka* and *Renanthopsis* Jan Stokes (Seeni and Latha,1992).

Seeni and Latha (1992) reported leaf base regeneration of the plants on MS + BAP and NAA. Differentiation of up to 10 shoot buds free of callus and protocorm-like bodies occurred in 10-12 weeks which was enhanced on coconut water and banana pulp during subculture. Shoots were rooted on MS + 5μM NAA preferably in conjugation with 1% activated charcoal. Laishram and Devi (1999) also obtained regeneration of plantlets using excised shoot tip, axillary buds and segment of young leaves.

Vanda coerulea Griff. ex Lindl.

V. coerulea (Orchidaceae), popularly known as the 'Blue Vanda of Asia', is a perennial epiphyte growing in the Khasi and Jaintia Hills of Meghalaya in India and in the northern ranges of Thailand and Burma. It has bred for qualities such as flower size, floriferousness, vigour and cold tolerance in modern vandaceous hybrids (Motes, 1988). The species is also important ethnobotanically as the juice from its leaves is used to cure diarrhoea, dysentery and dermal disorders (Nadkarni, 1954).

Seeni and Latha (2000) described rapid multiplication of *V. coerulea* through shoot tip and leaf base culture of both mature plants and axenic seedlings. The morphogenic responses differed among the explant types and sources of explants cultured. The plantlets were transplanted and established at the frequency of 95-100%. These plantlets were then transferred on to host trees and more than 85% established at Ponmudi and 70% at Palode, Kerala, India.

Vij and Aggarwal (2003) reported regeneration of *V. coerulea* using foliar explants. Leaves (<3.0cm) could regenerate shoot buds with 75% frequency in cytokinins (Kn/BAP) supplemented VW (Vacin and Went, 1949). Nearly 50 plantlets were harvested after 24 weeks. Kanika and Vij (2004) obtained up to 80 PLBs in 8 weeks on Mitra medium (Mitra *et al*., 1976) containing BAP, 2,4-D and coconut water. Malabadi *et al*. (2004) demonstrated the potential of TDZ in inducing PLBs with callusing from thin shoot tip sections of *V. coerulea*. The use of TDZ for longer than 8 weeks resulted in formation of fasciated or distorted shoots. Rooting was achieved on 11.42μM IAA supplemented ½ strength VW basal medium.

Vulnerable Plants

Coleus forskohlii Briq.

C. forskohlii (Lamiaceae) grows wild in the sub-tropical Himalayas, distributed from the hills to Nepal ascending up to 2000m, and in Bihar, Deccan Peninsula and Gujarat. Its roots produce a labdane diterpeniod, forskolin, lower blood and intraocular pressure and are an anti-inflammatory (Mukherjee *et al*., 1996).

Sen and Sharma (1991) obtained shoot multiplication from shoot tips of 30-d-old seedlings (150 shoots/ shoot tip in 4 months) in MS medium + 2mg/l BAP. Sharma *et al.* (1991) achieved *in vitro* multiplication using nodal explants (12.33±1.10) on MS medium supplemented with 2.0mg/l Kn + 1.0mg/l IAA in 6 weeks. Almost one hundred per cent *in vitro* plantlets survived in soil. Bhattacharyya and Bhattacharya (2001) could not induce multiple shoots from nodal explants but reported complete plantlets in 35-40d in a one-step procedure by culturing shoot tips in MS medium containing 0.57μM IAA + 0.46μM Kn, reducing the culture period with multiplication rate of 12.5 shoots per explant.

Gymnema sylvestre R.Br.

G. sylvestre (Asclepiadaceae), popularly called as 'Gurmar' is distributed over most parts of India and Africa. Natural stands of *G. sylvestre* are threatened with extinction due to its indiscriminate collection and over exploitation of natural resources for commercial purposes and to meet the requirements of the pharmaceutical industry.

Micropropagation by axillary bud proliferation of *G. sylvestre* has been reported (Reddy *et al*., 1998; Komalavalli and Rao, 2000). Reddy *et al*., (1998) could induce maximum number of shoots (7) from nodal explant of mature plants in combination of 5mg/l BAP and 0.2mg/l NAA with callus at the base of shoots. *In vitro* propagation was markedly influenced by the seedling age, medium type, plant growth regulators, complex extracts and antioxidants (Komalavalli and Rao, 2000). Maximum number of shoots was obtained from nodal explants from 20-day-old seedlings in MS medium supplemented with 1mg/l BAP, 0.5mg/l Kn, 0.1mg/l NAA malt extract and citric acid, each of 100mg/l. Rooting was achieved in ½ strength MS supplemented with 3mg/l IBA. Kumar *et al*. (2002) obtained somatic embryos from hypocotyl, cotyledon and leaf explants. Maximum frequency of embryogenic callus was achieved from hypocotyls.

Holostemma ada-kodien Schult.

H. ada-kodien (Asclepiadaceae), popularly known as Jivanti or Jivani, is indigenous to India (Martin, 2003). It provides the essential raw material for more than 34 ayurvedic preparations and is one of the major ingredients of the drug Jivanti, which is listed in the indigenous system of medicine. Owing to indiscriminate collection of root tubers as raw material for the ayurvedic drug preparations and other anthropogenic reasons, it is listed in the first red list of medicinal plant of South India as a vulnerable species (CAMP-1, 1995).

Sudha *et al*. (2000) described plant regeneration from chlorophyllous root segments derived from *in vitro* rooted plants on MS basal medium supplemented with 0.2mg/l BAP. The protocol described can be considered as an alternative means to enhance the *in vitro* multiplication rate for clonal propagation and is also advantageous as it eliminates the stage of rooting prior to transfer to field. Martin (2003) induced somatic embryogenesis using leaf, internode and root explants on MS medium supplemented with 1.0mg/l 2,4-D. Fifty per cent of the embryos underwent maturation and conversion upon transfer to 1/10 MS basal solid medium. Ninety per cent plantlets survived in the field.

Rauvolfia serpentina Benth. ex Kurz.

R. serpentina (Apocyanaceae) commonly known as 'Sarpagandha', is a valuable source of the alkaloid reserpine. Roots are used as a valuable remedy for high blood pressure, insomnia, anxiety, excitement, schizophrenia, insanity, epilepsy, hypochondria and disorders of the central nervous system (Roy *et al*., 1994).

Roy *et al*. (1994) established a protocol for propagation of *R. serpentina* using shoot tips and lateral buds from field grown plants. Rooting was achieved on ½ strength MS + 1.0mg/l IBA + 1.0mg/l IAA medium. Ninety five per cent of the plantlets survived on transfer to field. Ahmad *et al*. (2002) established plantlet regeneration system from shoot and nodal explants of field grown plants and from calli of leaf and internode explants of *in vitro* grown shoots. Shekhawat and Kataria (2005) obtained 3 to 5 shoots per node by axillary bud proliferation on MS medium + 10µM BAP + 0.5µM IAA. A promising *in vitro* propagation of *R. serpentina* was developed using shoot tips on MS medium supplemented with 4.0mg/l BAP + 0.5mg/l NAA which gave the highest percentage of response with 7 or 8 multiple shoots per culture (Baksha *et al*., 2007). Sharma and Chandel (1992) reported storage of nodal cultures of *R. serpentina* at reduced temperature. After 15 months of storage, the cultures maintained at 15 C were viable, showing 70% survivability in field.

Tylophora indica (Burm f.) Merrill

T. indica (Asclepiadaceae) have long been used for the treatment of asthma, bronchitis, whooping cough, dysentery, rheumatic gouty pains and hydrophobia (Faisal *et al*., 2007). Pharmacological activity is attributed mainly to the presence of alkaloid tylophorine and tylophorenine. Besides, root contains a potential anti-tumour alkaloid tylophorinidine (Mulchandini *et al*., 1971).

Various authors have described protocols for propagation of *T. indica* by using different methods such as somatic embryogenesis through leaf explants (Manjula *et al*., 2000; Jayanthi and Mandal, 2001), axillary bud multiplication through nodal segment (organogenesis) (Faisal *et al*., 2007), indirect shoot regeneration from leaf, stem and petiole via callus (Faisal and Anis, 2003; Faisal *et al*., 2005). Manjula *et al*. (2000) achieved production of up to 30 embryoids with high conversion rate to plantlets and its survival (90%) in soil. Jayanthi and Mandal (2001) also achieved 25 embryos per callus with more than 80% survival rate. The plantlets were found to be true-to-type through RAPD analysis and were transported to Gudalur forests of Western Ghats, India.

Rare Plants

Rotula aquatica Lour.

R. aquatica (Boraginaceae) is distributed in India, Srilanka, Tropical South East Asia and Latin America. The root tuber is astringent, bitter, diuretic, laxative for piles and is also useful in treating coughs, cardiac disorders, dysurea, blood disorders, fever, ulcers and uterine diseases (Sebastian *et al*., 2002).

Sebastian *et al*. (2002) developed a propagation protocol using nodal explants from mature plants. Maximum number of shoots per node was achieved on Woody Plant Medium (Lloyd and McCown, 1981) supplemented with 6.0mg/l BAP. Rooting was obtained on ½ strength WPM medium supplemented with 0.5mg/l IAA, which showed 5.7±0.14 roots. Seventy per cent survival of the plantlets was recorded. Martin (2003) reported axillary bud multiplication and indirect organogenesis. Fifteen shoots were obtained in combination of BAP (1.0mg/l) with IBA (0.5mg/l) in MS medium through axillary bud multiplication. Chithra *et al*. (2005) described somatic embryogenesis and encapsulation of somatic embryos from internode and leaf explants.

Withania somnifera (L.) Dunal.

W. somnifera (Solanaceae) has antibiotic, antiviral, antiamoebic, antiarthritic and anti-inflamatory properties (Kurup, 1956). It is an imperative need and compulsion of the recent times to take steps to preserve this rare medicinal plant (Sivanesan and Murugesan, 2005). Various protocols were reported for *in vitro* propagation using different parts of the plant, mature as well as seedlings of *W. somnifera*. Sen and Sharma (1991) achieved shoot multiplication from shoot tips of aseptically germinated seedlings. However, there was less survival in the soil. Kulkarni *et al*. (2000) described direct regeneration of shoots from nodes, internodes, hypocotyl and embryos. Maximum shoot regeneration (24 shoot) was obtained from nodal segments in 0.2mg/l TDZ. Leaf explants were used by Sivanesan and Murrugesan (2005) for regeneration of plantlets. Protocols through indirect organogenesis of *W. somnifera* were also established (Manickam *et al*., 2000; Rani *et al*., 2003).

Table - 1: *In vitro* propagation of some rare and threatened plant species of India

Botanical Name	Family	Explant used	References
Adhatoda beddomei C.B. Clarke[En]	Acanthaceae	Node	Sudha and Seeni,1994
Celastrus paniculatus Willd. [En]	Celastraceae	Stem	Maruthi *et al*., 2004
Dendrobium moschatum (Buch-Ham) Swartz[En]	Orchidaceae	Stem	Kanjilal *et al*., 1999
Gentiana Kurroo Royle.[CR]	Gentianaceae	Shoot tips, nodes	Sharma *et al*., 1993
Geodorum densiflorum (Lam) Schltr. [En]	Orchidaceae	Rhizome	Sheelavanthmath *et al*., 2000
Kaempferia galanga Linn.[En]	Zingiberaceae	Rhizome	Shirin *et al*., 2000
Meconopsis simplicifolia (D.Don) Walp.[En]	Papaveraceae	Hypocotyl, cotyledon	Sulaiman and Babu, 1993
Nardostachys jatamansi D.C. [CR]	Valerianaceae	Petiole	Mathur, 1993
Nepenthes khasiana Hook. f. [En]	Nepenthaceae	Node	Latha and Seeni, 1994
Pimpinella tirupatiensis Balk. & Subr. [En]	Apiaceae	Hypocotyl	Prakash *et al*., 2001
Pittosporum napaulensis (DC.) Rehder & Wilson [R]	Pittosporaceae	Node	Dhar *et al*., 2000
Rheum emodi Wall. [En]	Polygonaceae	Shoot tips	Lal and Ahuja, 1989
Saussurea obvallata (DC.) Edgew. [En]	Asteraceae	Root, hypocotyl, cotyledon, leaf	Dhar and Joshi, 2005
Sternbergia fischeriana (Herbert) Rupr. [En]	Amaryllidaceae	Bulb scale	Mirici *et al*., 2005
Syzygium travancoricum Gamble[CR]	Myrtaceae	Node	Anand *et al*., 1999

CR, critically endangered; En, endangered; R, rare

Table 1 shows some rare and threatened plants of India which have not been mentioned in the text for which *in vitro* propagation has been done.

Conclusions

The prime importance of *in vitro* propagation of rare, critically endangered, endangered and vulnerable plants would be to generate a large number of planting materials from a single explant without destroying the mother plant and subsequently their restoration in the natural habitat, thus conserving the biodiversity. The significance of an efficient *in vitro* protocol would be to obtain maximum number of plantlets in minimum period of time with proper rooting along with acclimatization in the field. The different regeneration systems which have been developed need to be field tested and the field data is collected so that the complete technology packages could be ready for commercialization and transfer to the user agencies (Anonymous, 2000).

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