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Overview of *In vitro* Strategies to Conserve Rare and Endangered Plant Species in India

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Abstract

India is one of the seventeen mega-diverse countries in the world. The maximum diversity in India has been observed in the Eastern Himalaya, Eastern part of Western Himalaya and the Western Ghats with its endemism. However, due to intrinsic and extrinsic factors, there has been a reduction in the number of species that are threatened, rare and endangered. Therefore, to sustain the populations of these species, immediate action of conservation is required. Biotechnological approaches have been recognized as an efficient method to conserve the rare and endangered plant species.

In the present study, a Google search was carried out along with other scientific databases such as PubMed, SciFinder, Web of Science, and Google Scholar to accomplish a scientific literature search and analysis.

In this review, we have described a few potential *in vitro* methods to protect rare and endangered flowering plants such as tissue culture using different explants, nuclear-DNA assay, cold storage techniques and gene banks. These various strategies are known to have a significant role in the production of a number of plantlets with their quality traits which considerably shows the scope to accomplish the research gap.

Keywords: Conservation; Endangered; Flowering Plants; *In vitro*; India

1. Introduction

The diversity of flowering plants in India is predominantly intensified in 4 biodiversity hotspots, namely Eastern Himalayas, Northeast India and Andaman Islands (Indo-Burma), Nicobar Island (Sundaland) and Western Ghats, out of 34 biodiversity hotspots in the world (Arisdason and Lakshminarasimhan, 2020). In India, about 28% of plants are endemic to the country among which dicotyledonous dominate by 720 genera with 2984 taxa (74%) and monocotyledons are represented by 255 genera with 1061 taxa (26%) (Arisdason and Lakshminarasimhan, 2020). Along with the dominancy, the major flowering families contributing to 7% of the world's documentation are Poaceae, Orchidaceae, Leguminosae, Asteraceae, Rubiaceae, Cyperaceae, Euphorbiaceae and Acanthaceae (Ghosh *et al.* 2017).

These floristically significant areas exhibit an exceptional concentration of endemic species nonetheless experiencing the threats which lead to the higher occurrence of threatened plant species. There are extrinsic factors (Figure 1) such as degradation of habitat, loss of pollinators, overexploitation, introduction of invasive species, global warming, pollution etc. creates the ground for the endangered status of the species however intrinsic factors like poor seed setting and germination, habitat specificity, specialized pollinators, mutualism, mode of dispersal etc. leads to the same (Ravikanth *et al.* 2018).

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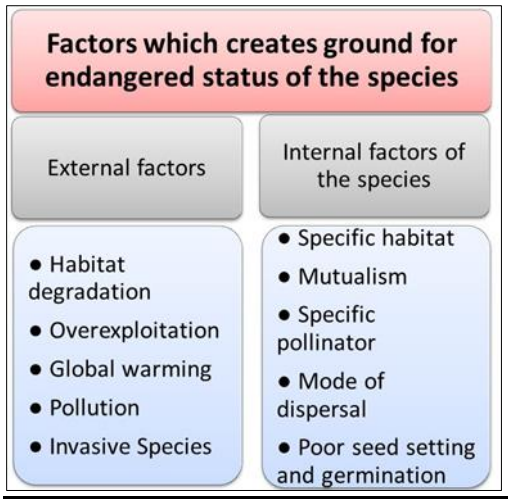

Figure 1 Threats which lead to the occurrence of threatened plant species

Thus, there is a need to develop rapid micropropagation protocols to overcome the constraints of the same to conserve the endangered flora (Nagesh *et al.* 2010). The conservation can be done by using *in-situ* and/or *ex-situ* approaches although *in-situ* strategy may not be sufficient to conserve and/or to preserve such huge bio resources of endangered flora because of intrinsic or extrinsic factors. Thus, the application of *in vitro* methods would be helpful to recover and re-establish endangered plant species (Swamy *et al.* 2018). To accomplish the purpose of conservation of biodiversity, sustainable use of its components and equitable sharing of the benefits arising out of the utilization of biological resources India framed a National Policy and strategy on Biodiversity in 1999, as per the Convention on Biological Diversity (CBD) and built up National Biodiversity Action Plan (NBAP) which was broadly aligned to the global Strategic Plan for Biodiversity 2011-2020. India developed 12 National Biodiversity Targets correlated to the 20 Aichi Biodiversity Targets by the Conference of Parties to the CBD in 2010 at Nagoya, Japan through extensive stakeholder consultations and public outreach (NBAP, 2008).

According to the United Nations Food and Agricultural Organization (FAO), the global population would become near about 9.1 billion in 2050, thus there will be increase in the demand of food production up to 70% which ultimately raise the agricultural land uses and consequently leads to the global challenge of conserving biodiversity. Therefore, the International Treaty on Plant Genetic Resources for Food and Agriculture declared the contribution of farmers towards the diversity of crops, accessing and sharing plant genetic materials (Pathak and Abido, 2014).

2. Methods of Study

To study the various conservation strategies employed in India by *in vitro* methods, a thorough scientific literature search and analysis was carried out. For this, various search strategies have been prepared depending on the scientific database such as PubMed, SciFinder, Web of Science, and Google Scholar. These strategies were further run to collect, screen and analyzed a few relevant records (Figure 2).

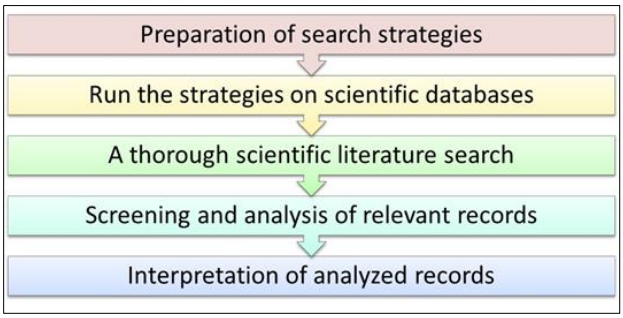

Figure 2 Methods of study

3. Biotechnological approaches

United Nations Environment Programme (UNEP) organized a body "Convention on Biological Diversity" (CBD) to develop a protocol to conserve the biological diversity. There are two methods of conservation of flora viz. *in-situ* and *ex-situ* conservation. *In-situ* conservation deals with the conservation of genetic variation in its natural habitat i.e. at original location itself, while *ex-situ* involves conservation of the species outside the native habitat. Most of the medicinal plant species became endangered because of the overexploitation of the species and are tried to be protected *in-situ* method, but this method is not sufficient and therefore *ex-situ* conservation *in vitro* techniques or biotechnological approaches are becoming increasingly important in the conservation (Jain *et al.* 2012).

Biotechnological approaches are very important in the rapid multiplication and conservation of the critical genotype species especially for the species having problems in germination of seeds, specialized pollinators requirement, mutualism, mode of dispersal, growth form, extremely reduced population etc. (Swamy *et al.* 2018). Conservation of endangered plant species involves preservation and maintenance of species with the use of biotechnological tools such as *in vitro* propagation, molecular conservation strategy, cryopreservation and gene bank (Rai 2010; Jain *et al.* 2012; Manole-Paunescu, 2014; Swamy *et al.* 2018).

The conservation of endangered flora using plant tissue culture techniques is highly accepted biotechnological approaches, which provides the possibilities of rapid multiplication of the species and conserve genetic material (Paunescu, 2009).

3.1. *In vitro* propagation

Plant tissue culture is a rapid and effective *in vitro* technique to regenerate or multiply the plants at large scale (Pathak and Abido 2014). Generally, tissue culture is done through the various explants like shoot tip, nodal segments, leaves, rhizome, roots, seeds etc. *In vitro* culture method is a powerful tool for multiplication, conservation and management of endangered species irrespective of season (Deb *et al.* 2018). *In vitro* protocols developed for endangered species in India highlights the significance of *ex-situ* conservation where reproduction through conventional methods is difficult to carry out and with very low population. Thus, tissue culture technology is used in the conservation of species (Table 1) to make sure the endurance of quick mass propagation of endangered plant species at large scale (Baskaran *et al.* 2011; Sharma and Thokchom 2014).

Due to various intrinsic as well as extrinsic factors like immense flower and seed abortion, poor seed germination, viability of seeds, overexploitation and habitat degradation the plant species such as *Eremostachys superba and Shorea tumbuggaia* have been added under the category of endangered medicinal plant. Therefore, immediate action towards the regeneration and conservation has been taken through the *in vitro* propagation strategies which could be significant advantages over conventional propagation methods (Sunnichan and Shivanna 1998; Shukla and Sharma 2017).

3.1.1. Somatic Embryogenesis

Somatic embryogenesis is the process of formation of embryo like structure from somatic tissue. The somatic embryo may be produced either directly on the explant or indirectly from callus or cell suspension culture. For the first time, Haccius (1978) defined somatic embryogenesis as a non-sexual developmental process, which produces a bipolar embryo from somatic tissue. The first report of plantlet regeneration via *in vitro* somatic embryogenesis was reported in *Daucus carota* (Reinert, 1958; Sharma *et al.*2010).

Somatic embryogenesis (SE) is the most significant biotechnological tool for the rapid propagation of endangered plants. Mature seed embryos callus was used to analyse the extracted proteins from globular, heart/torpedo-shaped, and maturing embryo stages resolved in the 2-DE gels revealed increased protein expression in the developmental stages of the somatic embryos of *Nothapodytes nimmoniana* (Isah, T. 2019). The conservation and recovery of *Nepenthes khasiana* Hook. f through *in vitro* seed germination has been reported by Nongrum *et al.* in 2009. Maximum seed germination was observed on 1/4 MS medium fortified with 2.68 μ*M* NAA found to be produce well developed pitcher plants in 120 days. However, Bahadur *et al.* (2007), performed an experiment using WPM (woody plant medium) + 2% sucrose + 500 mg activated charcoal fortified with various PGR such as NAA - 0.05, 0.1, 0.3 mg/L; IAA - 0.05, 0.1, 0.3 mg/L and BAP - 1.0 mg/L as well as KN - 0.1, 0.3 mg/L and contributed significant results for proliferation of nodal stem and nodal shoot tip explants. Whereas the root initiation was observed in combination of basal medium and different concentration of NAA (0.05, 0.01, 0.5, 1.0, 1.5, 2.0 mg/L).

Table 1 *In vitro* methods conservation strategies of some rare and endangered plants in India

Species	Family	Part of India	Category	Explant	Media and PGR	Ref
Allium stracheyi Baker	Alliaceae	Central Himalaya, India	Medicinal	Seeds	Pre-soaking in 100mg/L GA3 + incubation at 20 ^{0}C + light exposure lowest MGT=5.7 days, with GI=8.11.	Payal *et al.* (2014)
Capsicum frutescens and *Decalepis hamiltonii*	Solanaceae, Apocynaceae	Deccan peninsular India	Culinary spice	Shoot tips from *in vitro* grown seedlings	Triacontanol [CH3 (CH2)28 CH2OH] (TRIA) Axillary shoot proliferation: *C. frutescens*- MS+ 2 µg/L; *D. hamiltonii* - MS + 20 µg/L Rooting: *C. frutescens*- 5 µg/L; *D. hamiltoni*- 10 µg/L	Reddy *et al.* (2002)
Caralluma bhupenderiana Sarkaria	Apocynaceae	South India	Medicinal	Nodal explants	Shooting: MS + 8.87 µ*M* BA Rooting: ½ MS + 2.69 µ*M* NAA	Ugraiah *et al.* (2011)
Caralluma edulis (Edgew.) Benth. & Hook. f.	Asclepiadaceae	Thar Desert of Rajasthan, India	Edible	Nodal segments	Shooting: MS + 2.0 mg/L BAP + additives Shooting: MS + 0.5 mg/L BA + 0.5 mg/L Kn + 0.1 mg/L NAA and additives. Rooting: MS + 1.5 mg/L IBA Ex-vitro rooting: IBA (300 mg/L) for 4 min.	Patel *et al.* (2014)
Ceropegia fantastica Sedgw	Asclepidaceae	Western Ghats	Edible	Nodal segments	Shooting: MS + 1.5 mg/L BAP Rooting: MS (without CaCl2) + 1 mg/L IBA	Chandore *et al.* (2010)
Ceropegia noorjahaniae Ans.	Asclepiadaceae	Western Ghats	Edible and ornamental	Nodal explant	Shooting: MS + 2.0 mg/L BAP Rooting: Half-strength MS medium + 1.0 mg/L IBA Flowering: Half-strength MS medium + 4 mg/L BAP + 5 %, w/v sucrose	Kedage *et al.* (2017)
Ceropegia rollae Hemadri	Apocynaceae	Northern Western Ghats	Edible and medicinal	Nodal explant	Shooting: MS + 3 mg/L BAP. Rooting: MS medium + 2.5 mg/L IBA Flowering: MS medium + 0.6 mg/L IBA	Upadhye *et al.* (2016)
Ceropegia spiralis Wight	Asclepiadaceae	Peninsular India	Medicinal	Young nodal segments	Shooting: MS + 2 mg/L BA + 0.5 mg/L TDZ. Rooting: ½ MS+ 1.0 mg/L IBA	Chavan *et al.* (2011)
Chlorophytum arundinaceum	Liliaceae	Gujarat, India	Medicinal	Cut root portions leaving the stem disc portion	Shoot buds proliferation: ½ MS + 1.0 mg/L BAP, 3.0 mg/L IAA and 3% (w/v) sucrose MS + 3.0 mg/L BAP with 0.1 mg/L NAA Maintainance of regeneration ability: ½ MS + 3.0	Samantaray & Maiti (2011)

					mg/L BAP + 0.1 mg/L NAA + 25 mg/L Adenine Sulphate	
Curculigo orchioides Gaertn	Hypoxidiaceae	Biligiri Rangana Hills of Karnataka	Medicinal	Rhizome	Maximum induction (62%): MS + 0.5-3 mg/L 2,4 D +0.5 mg/L BAP Somatic embryos: 1 mg/L BAP	Nagesh *et al.* (2010)
Dendrobium longicornu	Orchidaceae	Northeast India	Medicinal	Axillary bud segments	Maximum explant response (86.6 %): MS + NAA 30 µ*M* Shoots (4.42) and bud-forming capacity (3.51): MS + 15 µ*M* BAP and 5 µ*M* NAA PLBs (41.48 %): MS + 15 µ*M* BAP and 15 µ*M* 2,4-D	Dohling *et al.* (2012)
Eulophia cullenii (Wight) Bl.	Orchidaceae	Western Ghats	Ornamental	Seeds	Seed germination: effective media: ½ MS, ¼ MS, Knudson-C, and Mitra media (with additives CW/CH/YE) Highest growth index: ¼ MS+CH Rhizome regeneration: 1/2 MS Rhizome multiplication: 22.2 to 44.4 µM BAP	Decruse *et al.* (2013)
Farsetia macrantha Blatt. & Hallb.	Brassicaceae	Thar desert, Barmer district of Rajasthan	Medicinal	Cotyledonary node explant obtained *in vitro* germinated immature seeds	Shoot bud induction: MS + ascorbic acid (50 mg/L) + citric acid (25 mg/L) + l-arginine (25 mg/L) + adenine sulphate (25 mg/L) + 0.5 mg/L BA Maximum number of shoots: MS + 0.5 mg/L BA + 0.25 mg/ L Kin. Rooting: ½ MS + 2.0 mg/L IBA	Choudhary *et al.* (2020)
Homalomena aromatica Schott.	Araceae	Northeast India	Aromatic medicinal herb	Rhizome axillary bud	Shooting: MS + 2.0 mg/L 6-BAP Regenerated shoots rooted on: ½ MS + 0.5 mg/L NAA	Raomai *et al.* (2013)
Hoya wightii ssp. Palniensis	Asclepiadaceae	Pambar Shola of Western Ghats of Tamil Nadu, India	Horticultural importance	Apical bud	Shoot culture: MS + KN (1.0 mg/L) + IBA (0.3 mg/L) Organogenic callus: MS + 1.0 mg/L NAA and 2.0 mg/L 2,4 D Shoot bud induction: MS + 1.0 mg/L BA + 0.5 mg/L IBA + coconut water (15%) Rooting: MS + 0.2 mg/L of IBA	Lakshmi *et al.* (2013)
Ilex khasiana	Aquifoliaceae	Khasi Hills of Meghalaya, India	Holly tree	Nodal explant from seedling	MS + 8.88 mM BAP + 4.64 mM kinetin Callus: MS + 2,4D + 6BAP Rooting: 1/2 MS + 9.84 mM IBA	Dang *et al.* (2011)

				germination Callus: Leaf discs		
Lilium mackliniae	Liliaceae	Ukhrul district of Manipur, India	Ornamental	---	Callus: MS medium + 1-2 mg/L 2,4-D Direct regeneration and somatic embryogenesis: MS + 1.0 mg/L BA Shooting and rooting: MS + 0.5 mg/L BA and 0.5 mg/L NAA	Devi *et al.* (2015)
Musa sp.: Rajeli (AAB), a commercially valuable Indian banana cultivar	Musaceae	Kolhapur	Commercially valuable	Immature male flower primordia from inflorescences	Immature male flower buds cultured on: MS + 4 mg/L 2,4-D, 1 mg/L of IAA and NAA, D-Biotin, 100 mg/L Malt Extract, 100 mg/L L-Glutamine Somatic embryo development: MS+ 0.5 mg/L BAP	Kulkarni & Bapat (2013)
Nepenthes khasiana Hook.f	Nepenthaceae	Meghalaya	---	Seedlings	Axillary shoot: Woody Plant Medium + 2.2 µ*M* BAP Rooting: 2.7 µ*M* NAA	Latha & Seeni (1994)
Nothapodytes nimmoniana (J. Graham)	Icacinaceae	Tamil Nadu, India	Medicinal	Mature seed embryo	Callus: MS + 13.56 µ*M* 2,4-D + 2.22 µ*M* BAP MS + 9.04 2,4-D + 4.44 µ*M* BAP	Isah, T., & Umar, S. (2019)
Nothapodytes nimmoniana Graham	Icacinaceae	Western Ghats of India	Medicinal	Seed embryos	MS + 0.91 µ*M* TDZ Rooting: 1 mg/L IBA	Rajasekharan *et al.* (2008)
Psoralea corylifolia L.	Fabaceae	---	Medicinal	Ten-day-old hypocotyl explants to obtain thin cell layers (tTCL)	MS + 15 µ*M* NAA and 3 µ*M* BA	Baskaran *et al.* (2011)
Rauwolfia tetraphylla L.	Apocynaceae	---	Medicinal	Nodal and shoot tip	Axillary shooting: MS medium + 1.5 mg/L Kn + 0.25 mg/L TDZ; BAP 2.5 mg/L BAP + 0.25 mg/L TDZ. Shooting: MS + 1.5 mg/L BAP + 0.25 mg/L TDZ when transferred to half strength MS Shoots proliferation: MS + 2.0 mg/L BAP + 0.25 mg/L TDZ, when transferred to half and full strength MS Rooting: Axillary shoots + MS + 1.0 mg/L IAA + 1.0 mg/L IBA.	Khan *et al.* (2015)

Renanthera imschootiana and *Vanda coerulea*	Orchidaceae	North-east India and the adjoining areas of Myanmar	Floriculture	Micropropagation: Seeds RAPD: Young leaves from the hybrid seedlings	Seed germination: VW medium + 15% CW Rooting: VW medium + 0.5 mg/L NAA Seedling of hybrid *R. imschootiana* and *V. coerulea*: half-strength MS medium	Kishor, R., & Sharma, G. J. (2009)
Rhododendron dalhousiae var. *rhabdotum, R. elliottii,* and *R. johnstoneanum*	Ericaceae	Northeast India	Ornamental	Nodal explants	39.36 µ*M* Isopentenyladenine + 4.90 IBA	Mao *et al.* (2011)
*Saussurea lappa*Clarke	Asteraceae	Valley of Kashmir and western Himalayas of northern India	Medicinal	Shoot tips of seedlings	Shooting: MS + 0.45 µ*M* TDZ. Multiple shooting: MS + 4.44 µ*M* BAP + 0.45 µ*M* TDZ Rooting: MS + 1.07 µ*M* NAA	Johnson *et al.* (1997)
Saussurea obvallata (DC.) Edgew.	Asteraceae	Himalaya	Medicinal	Epicotyl	Shooting: MS + 1.0 µ*M* Kn + 0.25 µ*M* NAA Rooting: 1/2 MS + 2.5 µ*M* IBA	Joshi, M., & Dhar, U. (2003)
Stemona tuberosa Lour.	Stemonaceae	Eastern Ghats	Wild medicinal plant	Axillary bud	MS + 7.0 mg/L Kn Shoots and tuberous root formation: 7.0 mg/L Kn and 4.0 mg/L TDZ Developed shoots rooted: 1/2 strength MS + 1.0 mg/L IAA	Murthy *et al.* (2013)
Stevia rebaudiana Bertoni	Asteraceae	---	Commercial	Seedling derived node and leaf explants	Shooting: MS+1 µ*M* BA Rooting: 1/2 MS basal	Aggarwal *et al.* (2009)
Swertia chirata Buch. -Ham. Ex. CB Clarke	Gentiaceae	Kashmir to Bhutan and in the Khasi hills in Meghalaya	Medicinal	Immature seeds	-	Parveen, (2010)
Tecomella undulate	Bignoniaceae	North-west and western India	Medicinal	Seeds and cut segments	MS medium + 0.7 µ*M* TDZ + 200 µ*M* IBA	Varshney, A., & Anis, M. (2011)

Trichopus zeylanicus subsp. *travancoricus*	Trichopodaceae	Western Ghats of South India	Medicinal	Branch-petiole explants	Shoot bud: MS + 13.3 µ*M* BA shoot morphogenesis: MS + 4.5 µ*M* 2,4 D Callus: MS + 19.6 µ*M* IBA Root: ½ MS + 2.7 µ*M* NAA	Martin *et al.* (2011)
Vanda coerulea Griff ex. Lindl.	Orchidaceae	Meghalaya	Ornamental	Shoot tips of mature plants, shoot tips and leaf bases of axenic seedlings	Mitra *et al.* + 10% coconut water + 500 mg/L casein hydrolysate + 8.8 µ*M* BAP + 4.1 µ*M* NAA Shooting: shoot tips + agar and agitated liquid media PLB: 35 g/L banana pulp + 30% coconut water + 1.08 µ*M* NAA Rooting: 35 g/L banana pulp + 1.08 µ*M* NAA	Seeni & Latha (2000)
Vigna radiata (L.) Wilczek	Fabaceae	West Bengal	Commercial	Shoot tip	Shooting: B5 medium + 1.074 µ*M* NAA + 22.192 µ*M* BAP. Rooting: Hormone-free B5 medium	Betal, S., & Raychaudhuri, S. S. (1999)
Willisia selaginoides (Bedd.) Warm. ex Willis	Podostemaceae	Idukki district, Kerala	---	Seeds	1/5 MS + 2% sucrose.	Uniyal & Ram (2001)

A process of organogenesis via callus with successful plantlet formation was developed for Anoectochilus elatus. Maximum organogenic callus induction was observed in the internode (77.8 %), followed by node (69.7 %) and leaf explants (64.2 %), on Mitra medium augmented with TDZ (1.0 mg/L) and NAA (0.5 mg/L) and coconut water (10 %). Significant rooting was observed in same medium with AgNO3 (1.0 mg/L). The plants were then transferred to the National Orchidarium, Yercaud, Tamil Nadu, after acclimatization for conservation purpose (Sherif et al. 2016). Rhododendron wattii Cowan acclimatized plants were reintroduced to their natural habitat at Naga Heritage Village, Kisama, Nagaland in 2016 through ex-situ conservation method. Maximum numbers of shoots were obtained from nodal explants on WPM fortified with 39.36 µM 2iP and roots with 2.45 µM IBA and 0.2% (w/v) activated charcoal (Mao et al. 2018).

Ahmad et al. (2018) proposed in vitro regeneration through nodal segments and shoot tips in Decalepsis arayalpathra, medicinal liana of the Western Ghats. The nodal segments were found to be more efficient than shoot tips. MS medium supplemented with 5.0 µM BA + 0.5 µM IAA + 20.0 µM adenine sulphate showed the highest response in nodal segment explant however for rooting half-strength MS medium with 2.5 µM NAA was found to be optimum. The acclimatization of plantlets has been achieved through Soilrite (TM).

Overexploitation of the tubers and poor seed germination of Ceropegia karulensis has found to have very narrowed distribution in the Western Ghats of India. Pandey et al. 2017 developed a protocol for callus induction, somatic embryogenesis and tuberization from various seedling explants. MS media fortified with 2 µM 6-BAP and 1 µM 2,4-D showed highest callus formation however 2 µM BAP and 2 µM NAA with 7% sucrose in MS media showed highest microtuberization. Along with these propagation studies, gas chromatography-mass spectrometry based metabolic profiling was also performed in wild plants and in vitro callus tissues. This proved that the production of various secondary metabolites through the in vitro propagation was unaffected and could be utilized in the conservation strategies. Nayak et al. (2017) proposed the system for high-frequency generation protocol of Blepharispermum subsessile DC. Particle-induced X-ray emission was used to study the accumulation of minerals at various developmental stages of direct organogenesis. It has been found that there is higher accumulation of micronutrients such as Mn, Fe, Ni, Cu, and Zn in the in vitro regenerated roots. The proposed system could be useful to standardize the protocol for generation of this endangered medicinal plant species for its ex-situ conservation. Aponogeton bruggenii Yadav & Govekar has been also successively conserved through the standardization of protocol with multiple shoot induction method using tuber as explant (Jagtap et al. in 2010).

3.2. Molecular conservation strategy

3.2.1. Nuclear DNA Assay

Genetic information is an important tool in the preparation of conservation strategies of endangered plants. Nuclear DNA assay of endangered plant species (Table 2) with multiple DNA markers plays an important role in the implications for genetic enhancement and *ex-situ* conservation (Kumar *et al.* 2014). The molecular adaptation of matK gene in *Nymphaea tetragona* has been studied for the purpose of conservation strategy planning. It has been observed that the adaptive changes at the molecular level associated with varying ecological conditions. Therefore, as the threat to the existing species is anthropogenic, the conservation of *N. tetragona* can be targeted by probable translocation to another site (Dkhar *et al.* 2011).

The various molecular technologies have been used to conserve and maintain the endangered plant species. Random amplified polymorphic DNA (RAPD) has been widely used in the evaluation of genetic relationship among different plant species (Khan *et al.*, 2009). However, in case of use of Inter simple sequence repeat (ISSR) for the assessment of genetic diversity the distribution of nucleotide repeats throughout the genome could be consider and therefore becomes more potent to identify. ISSR markers are more reliable compared to RAPD because of its easy handling and more reproducibility (Wu *et al.*, 2010). *Valeriana jatamansi* from the Himalayas has been studied for its isolated population. In this study it was observed that ISSR markers generated the maximum level polymorphism (89.0%) followed by RAPD (85.8%) and AFLP (67.7%) markers. Therefore, ISSR fingerprinting was more effective than the RAPD assay, in study of *V. Jatamansi* (Kumar *et al.* 2014). *Commiphora wightii* shows a huge population difference therefore crossing the populations may lead to risk of out breeding depression, which can be recognized as interruption of local adaptation (Haque *et al.*2010). *Dysoxylum malabaricum*, an endangered plant in the Western Ghats, faces the pressure due to the overharvesting and developmental activities have resulted in fragmentation and low size of population. Ultimately which leads to the loss of genetic variability hence results in to the inbreeding depression and loss of alleles (Ravikanth *et al.* 2018).

The study of *Nepenthes khasiana* Hook.f, a carnivorous plant from North-East India has been done by using multi-locus analysis. The study reveals that RAPD analysis showed more genetic polymorphism than that of ISSR fingerprinting and more genetic similarity in pooled RAPD-ISSR. As maximum morphological variety in context to the discriminated population the species needs an attention for conservation measures (Bhau *et al.* 2009).

Insufficient number of proper pollinators, fruit development and symbiotic seed germination with mycorrhiza were the reasons responsible for the endangered status of orchid *Anoectochilus elatus* found in the Eastern and the Western Ghats of Tamil Nadu, India. Therefore, an efficient micropropagation protocol has been standardized by Ahamed *et al.* (2017) for eco-restoration programmes. For shooting, MS medium with 1.5 mg/L TDZ and 50 mg/L peptone proved to be significant while for *in vitro* rooting MS medium supplemented with 3 g/L activated charcoal (AC) found to be efficient. The genetic stability of micropropagated plantlets were analyzed through ISSRs molecular markers and showed 2.38% polymorphism and 97.61% monomorphism with true-to-type to mother plant.

Chhajer and Kalia (2016) standardised the protocol for *Tecomella undulata* (Sm.) Seem by using nodal segments from mature tree. Arbitrary (RAPD), semi-arbitrary (ISSR; SCoT), and sequence-based (SSR) markers were used to study the genetic homogeneity. 131 primers (25 each of RAPD, ISSR, SCoT and 56 SSR) were used for the comparison however scorable unambiguous and DNA fragments were produced by 77 (21 RAPD, 20 ISSR, 22 SCoT and 14 SSR) primers. The DNA fragments produced by RAPD, ISSR, SCoT, and SSR primers were 71, 93, 94, and 42 respectively with an average of 3.38, 4.65, 4.27, and 3.0 DNA fragments per primer.

The genetic variations of *Acorus calamus* L., was assessed using RAPD (25 primers) and ISSR markers (17 primers) to design the conservation strategies. The results depicted the polymorphic bands of 33.7% of bands for RAPD markers and 63.7% for ISSR. Homogenous population with low genetic diversity provides monoclonal plantlets therefore the proposed study could be utilized in the designing the conservation strategies of *A. calamus* (Kasture *et al.* 2016). Varghese *et al.* (2016) standardized the clonal strategy of *Hypericum hookerianum* (Hypericaceae), a critically endangered plant of Western Ghats, India by axillary shoot proliferation method. MS medium fortified with 2.325 µ*M* Kinetin and 1/2 MS medium with 2.45 µ*M* IBA showed the maximum shooting and rooting respectively. RAPD analysis and phytochemical analysis was used to confirm the clonal stability of micropropagated plants.1032 amplicons were generated which was monomorphic and true-to-type mother plant which was further confirmed by similarity matrix by Nei's coefficient and phenogram by UPGMA analysis.

Table 2 Molecular conservation strategies of some rare and endangered plants in India

Species	Family	Part of India	Technique	Explant	Ref
Aquilaria malaccensis	Thymelaeaceae	Northeast India	AFLP	Young leaves	Singh *et al.* (2015)
Bacopa monnieri (L.)	Scrophulriaaceae	Central India	RAPD and ISSR analysis	Young leaves	Tripathi *et al.* (2012)
Boswellia serrata	Burseraceae	Central India	RAPD + ISSR Genetic Diversity	Leaves	Vaishnav *et al.* (2019)
Ceropegia evansii McCann	Asclepiadaceae	Western Ghats	RAPD and ISSR analysis	Nodal explant	Chavan *et al.* (2015)
Chlorophytum borivilianum, C. tube rosum and *C attenuatum.*	Asparagaceae	Indian subcontinent	AFLP	Young leaves	Patil *et al.* (2015)
Commiphora wightii	Burseraceae	Thar Desert of Rajasthan, India	RAPD	Fresh young leaves	Haque *et al.* (2010)
Coscinium fenestratum	Menispermaceae	Western Ghats	ISSR	Fresh young leaves	Thriveni *et al.* (2014)
Decalepis arayalpathra	Apocynaceae	Southern Western Ghats	ISSR and RAPD	Leaves	Mishra

Dendrobium nobile Lindl.	Orchidaceae	Northeast India	RAPD	Young leaves	Bhattacharyya *et al.* (2015)
Garcinia indica, G. tinctoria and *G. gummigutta*	Cluciaceae	Western Ghats	Micropropagation and RAPD profiling	Seeds and cut segments	Parthasarathy *et al.* (2013)
Gaultheria fragrantissima Wall.		Eastern Himalayan region	Micropropagation, RAPD and phytochemical	Shoot tips	Bantawa *et al.* (2011)
Lilium mackliniae Sealy	Liliaceae	Manipur, India	Propagation RAPD	Bulb scale	Sahoo *et al* (2018)
Mantisia spathulata Schult. and *M. wengeri* Fischer	Zingiberaceae	Lunglei province of Mizoram	Micropropagation, RAPD, ISSR DAMD, SPAR	Seeds	Sharma *et al.* (2012)
Mantisia wengeri	Zingiberaceae	North East India	SPAR	Flower buds	Sharma *et al.* (2012)
Monoon tirunelveliense	Annonaceae	Kalakkad-Mundanthurai Tiger Reserve in India	DNA extraction: CTAB	Leaves	Viswanathan *et al.* (2015)
Pittosporum eriocarpum Royle	Pittosporaceae	Uttarakhand region of Himalaya	Micropropagation, SCoT, ISSR and RAPD	Nodal explant	Thakur *et al.* (2016)
Renanthera imschootiana and *Vanda coerulea*	Orchidaceae	North-east India and the adjoining areas of Myanmar	Hybridization, Micropropagation and RAPD	Micropropagation: Seeds RAPD: Young leaves from the hybrid seedlings	Kishor, R., & Sharma, G. J. (2009)
Rhodiola imbricata Edgew	Crassulaceae	Trans-Himalayan Leh-Ladakh region of India.	Propagation RAPD HPLC	Seeds	Bhardwaj *et al.* (2018)
*Saraca asoca (*Roxb.) De Wilde	Caesalpiniaceae	Western Ghats region of Karnataka, Maharashtra and Goa	RAPD Genetic Diversity	Fresh leaves	Saini *et al.* (2018)
Shorea tumbuggaia Roxb.	Dipterocarpaceae	Southern Eastern Ghats	Propagation and ISSR	Seeds	Shukla *et al.* (2017)
Tecomella undulata	Bignoniaceae	Rajasthan	RAPD, ISSR, SCoT, and SSR	Leaves	Chhajer, S., & Kalia, R. K. (2016).
Tephrosia calophylla Bedd.	Fabaceae	Southwestern Ghats, India	Microsatellite markers	Leaves	Parine *et al.* (2015)
Valeriana jatamansi	Valerianaceae	Northeast to northwest Indian Himalayas	Nuclear DNA assay RAPD, ISSR and AFLP	Young leaves	Kumar *et al.* (2014).

Vanda coerulea Griff ex Lindl (Blue Vanda)	Orchidaceae	Northeast India	SPAR, RAPD and ISSR	Leaves	Manners *et al.* (2013)

(AFLP: Amplified Fragment Length Polymorphism; DAMD: Directed amplification of minisatellites DNA; ISSR: Inter Simple Sequence Repeat; RAPD: Random Amplified Polymorphic DNA; SCoT: Start Codon Targetted Polymorphism; SPAR: Single primer amplification reaction; SSR: Simple sequence repeats)

3.3. Cryopreservation

In India*, in vitro* conservation and cryopreservation techniques began in the early 1980s. Plant cryopreservation comprises the plant tissue storage, generally seeds or shoot tips are stored in liquid nitrogen (LN) at -196 °C or in the vapour phase of it at -135 °C followed by the re-warming (Kaczmarczyk *et al.*, 2012). Cryostorage plays an important role in the conservation of endangered species (Table 3), especially when species with very few seed formation or quality of seeds (Kaczmarczyk *et al.*, 2012). The cryopreserved samples possibly take few weeks to months or year to produce the micro propagated plants depending upon the species (Sakai & Engelmann, 2007).

Vitrification technique employed in the cryopreservation of shoot tips of *Picrorhiza kurroa*, an endangered medicinal plant, by preculturing of shoot tips at 4ºC for 2 days on MS medium containing 5% DMSO before the dehydration in PVS2 solution for 15 min at 0ºC. After vitrification, the shoot tips are directly immersed in LN2. (Sharma and Sharma, 2003). Vitrification and the encapsulation-dehydration techniques with high frequency of plant regeneration have been applied in the *in vitro* shoot tips of *Dioscorea deltoidea* Wall. Overnight pretreatment of shoot tips on MS medium with 0.3 M sucrose followed by loading with MS using 2 M glycerol and 0.4 M sucrose for 20 min at 25 °C, desiccation for 90 min at 0 °C with PVS2 and quenching in LN. The shoot tips were rewarmed in a water-bath at 40 °C after 1 h of storage in LN which then unloaded with 1.2 M sucrose solution for 20 min and cultured on recovery growth medium. While in encapsulation-dehydration protocol, sucrose-pretreated shoot tips have been encapsulated with 3% calcium alginate, precultured in 0.75 M sucrose (3 days), desiccated (25% moisture) under the laminar air flow, stored in LN (1h) and rewarmed at 40 °C. (Mandal & Dixit-Sharma, 2007).

The rare and endangered species such *as Citrus indica, C. macroptera, C. megaloxycarpa, C. latipes,* etc., has been cryopreserved by techniques of desiccation-freezing, encapsulation and vitrification. (Malik and Chaudhury 2006). The conservation of *Kaempferia galanga* L., an endangered medicinal plant using vitrification technique has been successfully done in 2013 by Preetha *et al.* In this protocol, shoots tips were overnight precultured with MS medium containing 0.4 sucrose, osmoprotected with loading solution (20 min.), desiccated with PVS2 (20 min) at 0 ºC. The cryoprotected shoot tips with rapid freezing in LN followed by rapid thawing produced 50-60% survival and 30-40% regeneration rates.

3.3.1. Synthetic seeds

Synthetic seeds are the encapsulated plant tissues like shoot tips, shoot buds, somatic embryos, cell aggregates, or the tissues which can be cultured as a seed and grown into a whole plant either *in vitro* or in *ex vitro* conditions with the potential of viability after cold storage (Magray *et al.* 2017; Rihan *et al.* 2017). Murashige (1977) was the first researcher who discussed the concept of synthetic seeds, whereas artificial carrot seeds were first produced by Kitto and Janick (1982) (Nandini B. and Giridhar P. 2019).

The advantages of artificial seeds i.e. propagules encapsulation comprises multiplication of recalcitrant and/or vegetatively propagated species. This strategy has been used in the conservation of various plant species, such as orchids in which encapsulated protocorms proved 70% viability even after more than six months of storage at 4^0 C (Paunescu, A. 2009). Synseed technology has been used especially for the plants with poor seed viability and germination, seedless fruit, as well as in the plants in which special dependance of mycorrhizal–fungal symbiosis for germination (Gantait *et al.* 2015). Synseed technology is not only useful for the sterile genotypes but also in the germplasm preservation of endangered and rare plant species (Nandini B. and Giridhar P. 2019).

Among the various *ex situ* conservation methods, synthetic seeds technology thought to be one of the convenient techniques for long-term conservation.

Table 3 Cryopreservation conservation strategies of some rare and endangered plants in India

Species	Family	Part of India	Method	Explant	Ref
Calamus vattayila RENUKA	Arecaceae	Western Ghats of India	Cryopreservation	Mature and immature seeds	Jacob *et al.* (2015)
Dendrobium nobile Lindl.	Orchidaceae	North-east India	Synthetic seed technology: Short-term storage of alginate-encapsulated PLB	Seeds	Mohanty *et al.* (2013).
Holostemma annulare (Roxb.) K. Schum	Apocynaceae	Indian sub-continent	Encapsulation, dehydration and preculture	Shoot tip	Decruse *et al.* (1999)
Ipsea malabarica (Reichb. f.) J.D. Hook	Orchidaceae	Western Ghats of India	Encapsulation	*In vitro* formed bulbs	Martin, K. P. (2003)
Luisia macrantha Blatter & McCann.	Orchidaceae	Western Ghats of India	Dessication and vitrification	Pollinia	Ajeeshkumar, S & Decruse, S. (2013)
Mantisia spathulata and *Mantisia wengeri*	Zingiberaceae	North East India	Cryopreservation of immature seeds	Immature seeds	Bhowmik *et al.* (2011)
Nilgirianthus ciliatus	Acantheceae	Western Ghats of India	Synthetic seeds	Nodal explant	Rameshkumar *et al.* (2017)
Picrorhiza kurroa Royle ex Benth.	Scrophulariaceae	Himalaya	Vitrification	---	Sharma, N., & Sharma, B. (2003)
Picrorhiza kurrooa	Scrophulariaceae	Himalaya	Cryopreservation and synthetic seed production	Seeds	Rawat *et al.* (2012)

3.4. Gene Banks

Gene banks are the bio repositories in which the plant genetic material is preserved for the improvement of the germplasm and conservation purpose.

National Gene Bank has mainly three categories for the conservation of species

- Seed gene bank: Stores the seeds at −18 °C, using 12 long-term storage (LTS) modules
- *In vitro* gene bank: In the form of tissue culture at +4 to +25 °C
- Cryobank: For non-orthodox seed at −160 to −196 °C (in liquid nitrogen) (Akshay *et al.* 2014,).

Government of India has established three gene banks in the country namely, ICAR at the National Bureau of plant genetic Resources (NBPGR), Central Institute of Medicinal and Aromatic plants (CIMAPs) Lucknow and TBFRI in Thiruvananthapuram (Akshay *et al.* 2014, Mandal, 2000). According to the international gene bank databases, National Bureau of Plant Genetic Resources (ICAR-NBPGR) India is one of the largest gene banks among the other gene banks (Mandal, 2000). India's first orchid gene bank has been established at Hengbung, Manipur named as the Centre for Orchid Gene Conservation of the Eastern Himalayan region. In India at the field gene banks of TBGRI, Trivandrum,

various species (600) along with the hybrids (150) of orchids are preserved and maintained. About 90 different orchid genera and commercial orchids are also maintained at NRC for Orchids, Pakyong, Sikkim (Ghosh *et al.* 2017).

Some of the universities and research centres which have been involved in this plant germplasm collection are University of Delhi, Punjab Agricultural University (PAU), North Eastern Hill University (NEHU), National Botanical Research Institute (NBRI), Tropical Botanical Garden and Research Institute (TBGRI), Indian Institute of Horticultural Research (IIHR), Central Tuber Crop Research Institute (CTCRI) and Indian Institute of Spices Research (IISR) (Agrawal *et al.* 2019).

4. Conclusion

In recent decades several plant species in India are known to become endangered and are included under the red-listed plant category. Due to various intrinsic and extrinsic factors, biodiversity seems to be threatened. Thus, the conservation of an endangered species has become an important aspect to prevent the extinction of species. The conservation can be done by using *in situ* and/or *ex situ* methods, although *in-situ* strategies may not be sufficient enough to conserve the endangered species hence the application of *in vitro* methods would be helpful to recover and re-establish endangered plants. In India, various biotechnological approaches have been used to formulate the conservation strategies such as micro-propagation, molecular profiling, cryopreservation and gene bank which act as important tools in the conservation and improvement of mainly endangered species. The use of *in vitro* propagated plantlets for the reintroduction of endangered species is a crucial strategy for conservation. Along with this cryopreservation of vegetative tissues or recalcitrant seeds is also a significant option for long-term storage of germplasm. The levels of genetic diversity and population pattern help in the conservation and management of an endangered species therefore various molecular characterizations can be done to achieve the goal of formulation of a conservation strategy.

These various conservation strategies of endangered plants played a significant role to raise the number of plantlets along with their quality traits which shows the scope for future research to fulfill the existing lacunae on different aspects of the study.

Compliance with ethical standards

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All the authors have no conflict of interest.

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