In vitro multiplication and restoration of selected rare, endangered and threatened plants of India

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Target 3 of *Global Strategy for Plant Conservation* (GSPC) emphasizes the need for developing appropriate *ex situ* conservation models with protocols for practical conservation of endangered plant species. In India, at least 1500 out of the 17,000 higher plant species are threatened to various levels. As part of the GSPC, a range of rare, endangered and threatened (RET) species was multiplied through embryo and tissue cultures and reintroduced/translocated into selected forest segments of the southern Western Ghats in peninsular India. The establishment rates of terrestrial and epiphytic orchids (*Ipsea malabarica*, *Paphiopedilum druryi*, and *Vanda coerulea*) studied over 12–24 month period varied from 54–85%. However, the establishment rate of the epiphytic *Dendrobium heterocarpum* was poor (15%). Both the rattan palms (*Calamus travancorica*, *C. nagabettai*) multiplied through both embryo and tissue cultures showed high rates (75–89%) of establishment. Micropropagated plants of the tree species (*Blepharistemma membranifolia*, *Calophyllum apetalum*) and a woody shrub (*Decalepis arayalpatra*) were also established at high rates (84–91%) of success. The results suggest: a) both embryo and tissue cultures may be employed for species recovery. However, for purpose of retaining genetic diversity, embryo cultures may be desirable; b) there is no difference in establishment rates between embryo and tissue-derived plants of orchids and rattans; c) Micropropagated plants reintroduced during pre-monsoon/monsoon showers (May–June; October) were best established.

Introduction

Botanic garden communities across the world are concerned about the cascading loss of ecosystems, habitats and species from the face of the Earth. Apart from the man-made landscape changes and injudicious exploitation of resources, the Earth is warming up now more than at any point of time in its history. Global warming is already stated to have taken a toll of more than 650 animal species and an unknown number of plant species. Recent estimates are so alarming that 1,00,000 out of an estimated 4,00,000 plant species are doomed to become extinct within the next 50 years. Ways and means have to be found to conserve the endangered taxa. *In situ* conservation of plant species is ideal but impractical due to various reasons. In India hardly 4.5% of the natural forests are under protection. Hence, there is an urgent need for conserving the species of prospective value through *ex situ* methods, including biotechnological interventions. Where conventional methods fail, biotechnological approaches serve as a crisis management tool for conserving species that are critically endangered and are on the verge of extinction. In the present investigation, embryo and tissue cultures were successfully tested for multiplication and recovery of selected RET plant species of India.

Materials and Methods

Nine RET species, comprising medicinal plants (*Decalepis arayalpathra* (Joseph & Chandra.) Venter, *Calophyllum apetalum* Willd., *Blepharistemma membranifolia* (Miq.) Ding Hou.), orchids (*Paphiopedilum druryi* (Bedd.) Stein, *Ipsea malabarica* (Reichb.f.) Hook.f., *Dendrobium heterocarpum* Wall. ex. Lindl., *Vanda coerulea*) and rattans (*Calamus travancoricus* Bedd.Ex.Becc. and Hook.f., *C. nagabettai* Fernandez and Dey) were used for *in vitro* multiplication.

Direct axillary shoot formation in single node cultures of *D. arayalpathra*, *B. membranifolia* and *C. apetalum* was achieved as described earlier (Lakshmi and Seeni 2001, 2003). Green pod cultures of *I. malabarica*, *D. heterocarpum* and *P. druryi* were established (Mitra *et al*., 1976) liquid nutrient medium supplemented with 0.5gl^{-1} casein acid hydrolysate (CH) following the method of Gangaprasad *et al*. (1999). Embryo cultures of *Calamus* species were raised in Murashige & Skoog (MS) agar medium containing 0.1mgl^{-1} thidiazuron (TDZ). After 3–12 weeks after culture initiation, shoots/protocorms differentiated up on

the nodes, embryos or seeds were detached and subcultured in nutrient regimes containing low concentrations of growth regulators. Shoots were rooted in basal or auxin supplemented media through 1-4 transfers and rooted plants were hardened before establishment in the nursery.

Reintroduction of the 2–3 month old nursery plants was made in selected forest segments in pits of $30cm^3$ made in the floor, planting one plant in each before the onset of monsoon (May–June; October). Seedlings of *I. malabarica* were planted in groups of 100 in beds made in 10 different sites in the Silent Valley. Epiphytic orchids were tied directly on to the trunks of selected trees. Establishment and growth of the plants were monitored periodically.

Results

Out of the three medicinal plants tried, *Decalepis arayalpathra* is a shrub and *C. apetalum* and *B. membranifolia* are trees. Nodal explants responded with single axillary shoot formation in *D. arayalpathra* and 3–7 axillary buds in others (Table 1). The axillary shoots showed rapid growth and *in vitro* derived shoots/nodes could be repeatedly subcultured at 6–8 week intervals to scale up multiplication so that 300–600 shoots could be raised in a year starting from a single node. Seeds of *D. heterocarpum*, *P. druryi*, *and I. malabarica* were germinated optimally using casein acid hydrolysate to produce protocorms. During subculture, each protocorm produced 1–2 seedlings or secondary protocorms from which up to 14.8 shoots differentiated as in *I. malabarica*. In *V. coerulea* up to 35 shoots differentiated from the base of each leaf in 12 months. Each zygotic embryo of the rattan species *C. travancoricus and C. nagabettai* cultured in MS medium responded with the proliferation of 5–8 shoots in 2–3 months in presence of $0.1mgl^{-1}$ TDZ and were subcultured to produce 136 and 144 shoots respectively in a year.

Table.1 Culture initiation and multiplication of selected RET plant species.

Species	Status	Explant.	Mean No. of shoots or protocorms/ expalant* (%response)	Medium	No. of shoots raised/ explant/ year	Nursery establishment (%)
Decalepis arayalpathra	Endangered	Node	1.0 (75)	MS+2.0 BA	300	64
Calophyllum apetalum	Vulnerable	Node	3.1 (68)	MS+2.0 BA	300	90
Blepharistemma membranifolia	Threatened	Node	3.7 (84)	½MS+2.0 BAP	600	83
Paphiopedilum druryi	Threatened	seed	1.5 (20)	Mitra et al $+0.5gl^{-1}$ YE (liquid)	1–2	70
Ipsea malabarica	Vulnerable	seed	2.0 (80)	Mitra et al $+0.5gl^{-1}$ CH (liquid)	14.8	65
Vanda coerulea	Endangered	leaf	3.5 (90)	Mitra et al $+1.0\ mgl^{-1}$	35	80
Dendrobium heterocarpum	Vulnerable	seed	1.2 (70)	Mitra et al $+0.5gl^{-1}$ CH	1–2	50
Calamus travancorica	Threatened	Embryo	8.5 (60)	MS + $0.1mgl^{-1}$ TDZ	136	72
Calamus nagabettai	Endangered	Embryo	5.2 (50)	MS + $0.1mgl^{-1}$ TDZ	144	93

* initiation period varied for different species

The orchids propagated through seed culture produced roots in the multiplication medium itself and thus no separate rooting phase was required for these species. However, addition of $1.0\ mgl^{-1}$ NAA induced rooting in foliar meristem derived shoots of *V. coerulea*. Addition of 0.5–2 mgl-1 IBA induced the formation of 2–5 hardy roots in *D. arayalpathra*, *C. apetalum* and *B. membranifolia* which was essential to get satisfactory

rates of establishment of the rooted plants in the nursery. High concentrations of NAA (3.0 mgl^{-1}) were essential to get rooting from zygotic embryo derived shoots of rattans.

Except for *Vanda coerulea*, all the species were reintroduced into native habitats. The planting trials were made during May and June or October in different years when the south-west monsoon/ north-east rains facilitated significant (54–91%) establishment after 12 months of observation (Table 2). However, *D. heterocarpum* showed poor establishment (15%). The medicinal plants showed vigorous growth and attained 0.75–1.0m height in a year. The new growth in *Vanda coerulea* and *D. heterocarpum* was associated with emergence of leaves and green root tips in three months when the roots got firmly attached to the tree trunk. Seedlings of *Paphiopedilum druryi*, endemic to Argasthyamalai, were established in their natural habitat (about 1300m) with the formation of a pair of new leaves. However, *I. malabarica* remained as such during monsoon season (June–September) and later the leaves got dried up. During next monsoon (June), new growth occurred with profuse formation of leaves but the plants did not flower even after two years. The rattan species reintroduced at different localities showed more or less equal rates of establishment (72–90%). They attained a mean height of 30cm and 76 cm in the first and second years respectively.

Table 2. Reintroduction/ translocation trials with micropropagated plants of RET species

Sl. No.	Species	Place of reintroduction	Season/ month	No. of plants reintroduced	% establishment after one year
1	*Decalepis arayalpathra*	Kallar	SW monsoon	100	85
2	*Calophyllum apetalum*	Palode		125	91
3	*Blepharistemma membranifolia*	Alantur, Kannur,Palode, Trivandrum	(June)	170	80
4	*Paphiopedilum druryi*	Agasthyamalai	September	50	54
5	*Ipsea malabarica*	Silent Valley	September	1000	80
6	*Vanda coerulea*	Ponmudi	SW monsoon (July)	20	85
7	*Dendrobium heterocarpum*	Ponmudi		73	15
8	*Calamus travancorica*	Peppara, Kulathuppuzha,	SW monsoon (June)	175	72
9	*Calamus nagabettai*	Aryankavu		280	90

Discussion

Conservation of biodiversity in general and RET species in particular has to be achieved through a combination of strategies and action plans. The present study is probably the first concerted effort to micropropagate nine RET species of Indian origin and assess their establishment in natural localities upon reintroduction/translocation. The results presented in Table 1 and 2 confirm the amenability of the selected species for biotechnology-based multiplication and recovery of the species through reintroduction/translocation. However, the poor establishment of the epiphytic *D. heterocarpum* requires further investigation.

Perusal of literature reveals that in plants where natural spread is limited and in situ conservation is insufficient, micropropagation and ecorestoration may be particularly beneficial. Axenic seedlings of the endangered *Paphiopedilum rothchildianum* from Malaysia were not only used for sale but also for successful reintroduction into the forests of Sabah (Grell et al 1988). A number of embryo/spore culture-derived ferns and orchids have also been successfully reintroduced by the Royal Botanic Gardens, Kew. Already published reports on in vitro multiplication and conservation of *Bletia urbana*, *Epidendrum ilense*, *Ipsea maabarica*, *Vanda coerulea* and *Vanda spathulata* also vouch for the use of tissue culture under certain situations (Rubluo *et al* 1989, Christenson 1989, Gangaprasad *et al* 1999, Seeni & Latha 2000 and William Decruse et al 2003). Since retention of genetic diversity is desirable in any conservation programme, seed and embryo cultures may be preferred over tissue culture in plants. If seeds are not set or scarcely available, tissue culture may be an option as demonstrated in *B. membranifolia*, *C. apetalum* and *D. arayalpatra* where the

reintroduced population(s) will be homogeneous. However, the desirability of conserving more genetic diversity through embryo/seed cultures than nature otherwise prefers would be a subject of discussion.

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