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PROPAGATION AND CONSERVATION OF SELECTED ORCHIDS OF MANIPUR

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Abstract

Being a part of biodiversity hotspots, the NorthEast India is home to various orchid species. The present study aims to conserve the populations of endangered orchid species of Manipur, using modern biotechnological techniques for the propagation of the species in a time efficient manner, both qualitatively and quantitatively. Literature survey on orchids of Manipur updated a total of 474 species, in the state. During field survey at different sites of the Manipur, we collected and conserved 81 orchid species including four slipper orchids [*Paphiopedilum fairrieanum* (Lindl.)*, P. hirsutissimum* (Lindl. ex Hook.f.)*, P. insigne* (Lindl.) Pfitz., and *P. spicerianum* (Rchb.f) Pfitz.]. Attempts were made to mass multiply some selected orchids using *in vitro* asymbiotic seed germination technique; seedling were obtained in *Dendrobium transparens* and *Vanda coerulea*. Furthermore, to encourage socio-economic upliftment of farmers through entrepreneurship programme, proposals have been put forth through interaction/outreach programmes.

Introduction

ORCHIDS ARE known for their exceptional diversity with about 30,000 species belonging to about 900 genera (De and Medhi, 2015). They are known for their beautiful floral display with varied vibrant colours and exotic morphological features including complex pollination mechanism. They have low fruit set but the *pods* (capsules) once set have enormous seeds accounting up to thousands to millions of dust-like particles (Arditti and Ghani, 2000). In nature, their germination and embryo development require suitable mycorrhizal fungus association (Arditti, 1970; Pal *et al*., 2019). Decrease in number of pollinating agents and habitat destructions due to environmental and anthropogenic factors have eventually led to a steep decline in the natural orchid populations. Orchids are enlisted under Appendix I and II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (http://www.bsienvis.nic.in). There are also certain legislative measures endorsed by the IUCN (International Union for Conservation of Nature) as well as Government of India for the conservation and protection of orchids (https://legislative.gov.in/sites/default/files/A1972-53_0.pdf; De and Singh, 2015).

NorthEast India serves as a home to many native orchid species; orchids signify cultural relevance as well, in its states. Some of the orchids are state flowers in the region *e.g. Dendrobium nobile* (Sikkim), *Paphiopedilum insigne* (Meghalaya), *Renanthera imschootiana* (Mizoram), and *Rhynchostylis retusa* (Assam and Arunachal Pradesh) (http://www.wiienvis.nic.in/KidsCentre/state_symbols_india_8411.aspx). Women use orchid flowers to adorn their hair during festivals and occasions like Bihu dance in Assam. Located at the border of Indo-Myanmar region, the state Manipur is one of the biodiversity hotspots, in the world. In Manipur, flowers of *Dendrobium* are used in the celebration of New Year called *Meitei Cheiraoba*, celebrated during March-April. In Manipuri language, *Meiteilon,* orchids are referred to as *Urei* (which means those that grow on other trees or branches).

Many of the orchids are edible and some possess medicinal properties. Due to their ever increasing horticultural and medicinal values, these are overexploited. The rapid decrease in populations of several species of these plants has ensued Orchidaceae as a prominent subject for conservation. Reconstructing and re-establishment of the disturbed natural habitats, protection of specimen rich areas such as in biosphere reserve, sacred groves, and botanical gardens are considered as an effective mode of conservation (De and Singh, 2015; Murugesan *et al.,* 2017). Alternative conservation strategy *i.e.* application of modern biotechnology techniques such as *in vitro* propagation (Anuprabha and Pathak, 2020; Bhowmik and Rahman, 2020; Kumari and Pathak, 2021; Lalduhsanga *et al.,* 2021; Sembi *et al*., 2020; Sunita *et al*., 2021; Thakur and Pathak, 2021; Vasundhra *et al*., 2021) would help in mass multiplication of the horticulturally and medicinally important orchids on commercial scale, without disrupting their natural genetic populations.

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Material and Methods

Seed Collection

For tissue culture experments, seed explants were obtained from undehisced mature capsules of *Dendrobium chrysotoxum* Lindl.*, D. polyanthum* Wall. ex Lindl.*, D. transparens* Wall. ex Lindl.*, Paphiopedilum hirsutissimum* (Lindl. ex Hook. f.)*, P. spicerianum* (Rchb.f) Pfitz.*, Rhynchostylis retusa,* (L.) Blume, and *Vanda coerulea* (Griff. ex Lindl.), during the field surveys in different parts of Manipur. The collected seeds were stored in -70°C until cultured (Thornhill and Koopowitz, 1992). Collected samples of seeds were checked for their viability rate using 0.2% Trypan blue dye for 3 min, incubated at room temperature and checked through microscope (Strober, 1997). The viable seeds remained unstained while the non-viable seeds stained blue in colour, when observed under microscope.

For net-house hardening and conservation purpose, site visits and field surveys were under taken at Punshilok Langol (Imphal West district), Ningthiching (Churachandpur district), Kotlen (Kangpokpi district), Bungpakhunou (Kamjong district), Kongba Maru (Imphal East district), Mapao village (Kangpokpi district), and Mao village (Senapati district).

Sterilization

Undehisced mature seed capsules were surface sterilized with Tween 20 surfactant followed by rinsing under running tap water. These were treated with 0.1% $HgCl_2$ for 2 min and rinsed with sterile distilled water to wash off the remnants, followed by sterilization with 70% alcohol (30 sec) and subsequent by flame sterilization (2-3 sec). The sterilized seed capsules were then longitudinally dissected with sterile blade and spread evenly on the culture medium, in a laminar air flow.

Medium Preparation

MS medium (half strength) was used for *Dendrobium chrysotoxum, D. polyanthum*, *D. transparens*, *Rhynchostylis retusa*, and *Vanda coerulea*. Sucrose (3%) was used as carbohydrate source, 0.5 gl^{-1} of activated charcoal, 0.8% agar and additional growth supplements such as banana powder were also added. After addition of necessary media composition, pH was adjusted to 5.75±0.05, and nutrient medium was autoclaved at 121°C under 15 psi for 15-20 min. Terrestrial orchid medium (Hi-Media) was used for *Paphiopedilum hirsutissimum* and *P. spicerianum*, instead of MS medium.

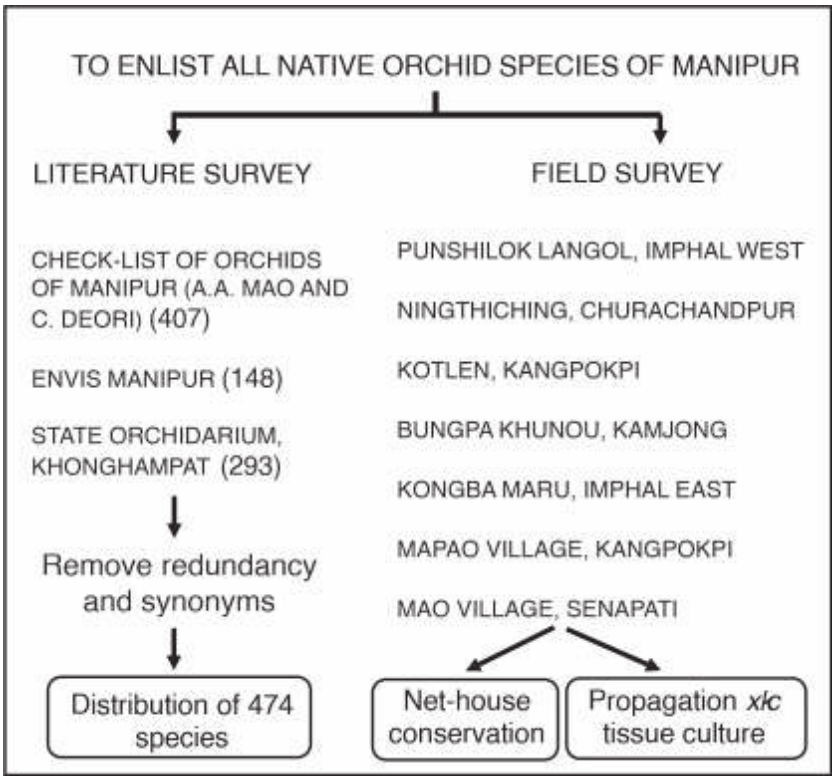

Fig. 1. Flowchart adopted to enlist native orchid species of Manipur through literature and field survey. Literature survey resulted into 474 species in Manipur. Field survey resulted into net-house conservation of 81 orchid species of Manipur and *in vitro* asymbiotic seed germination of seven native species of Manipur.

After inoculation of seeds on the respective culture media, these were incubated in 16/8 hrs (light/dark) photoperiod at a temperature of 24±2°C. Terrestrial orchids (*Paphiopedilum hirsutissimum* and *P. spicerianum*) were incubated in 24 hrs dark condition at 25±2°C for three wks (Chen *et al*., 2015; Yam and Arditti, 2017), after which these were incubated in 16/8 hrs (light/dark) photoperiod condition.

Subculturing and Acclimatization

Sub-culturing was done periodically. The plantlets that have developed 2-3 leaves were cultured for rhizogenesis on rooting medium [NAA (0-2 mgl^{-1}) alone /or BAP (3 mgl^{-1}) with NAA (1 mgl^{-1})]. Mature seedlings that developed velamen on their roots were further sub-cultured into media with gradual decrease in sucrose and additional nutrient concentration for acclimatization. The plantlets that thrive were then transferred to pots with potting mixture containing coarse brick chunks, coconut husk, pieces of bark, and charcoal (Lee and Yeung, 2018).

Results and Discussion

To enlist all native orchid species of Manipur, two modes of survey (literature and field) were conducted (Fig. 1). For literature survey, three sources, *i.e.*, a book entitled *Check-List of Orchids of Manipur* by Mao and Deori (2018) (enlists 407 species), website of Envis Manipur (enlists 148 species) (http://manenvis.nic.in/Database/Orchids_2932.aspx) and the State Orchidarium at Khonghampat (enlists 293 species) resulted into a total of 474 species, after removing the redundant species and the synonyms. Site visits and field surveys at different locations of the state were carried out at Punshilok Langol (Imphal West district), Ningthiching (Churachandpur district), Kotlen (Kangpokpi district), Bungpa Khunou (Kamjong district), Kongba Maru (Imphal East district), Mapao village (Kangpokpi district), and Mao village (Senapati district).

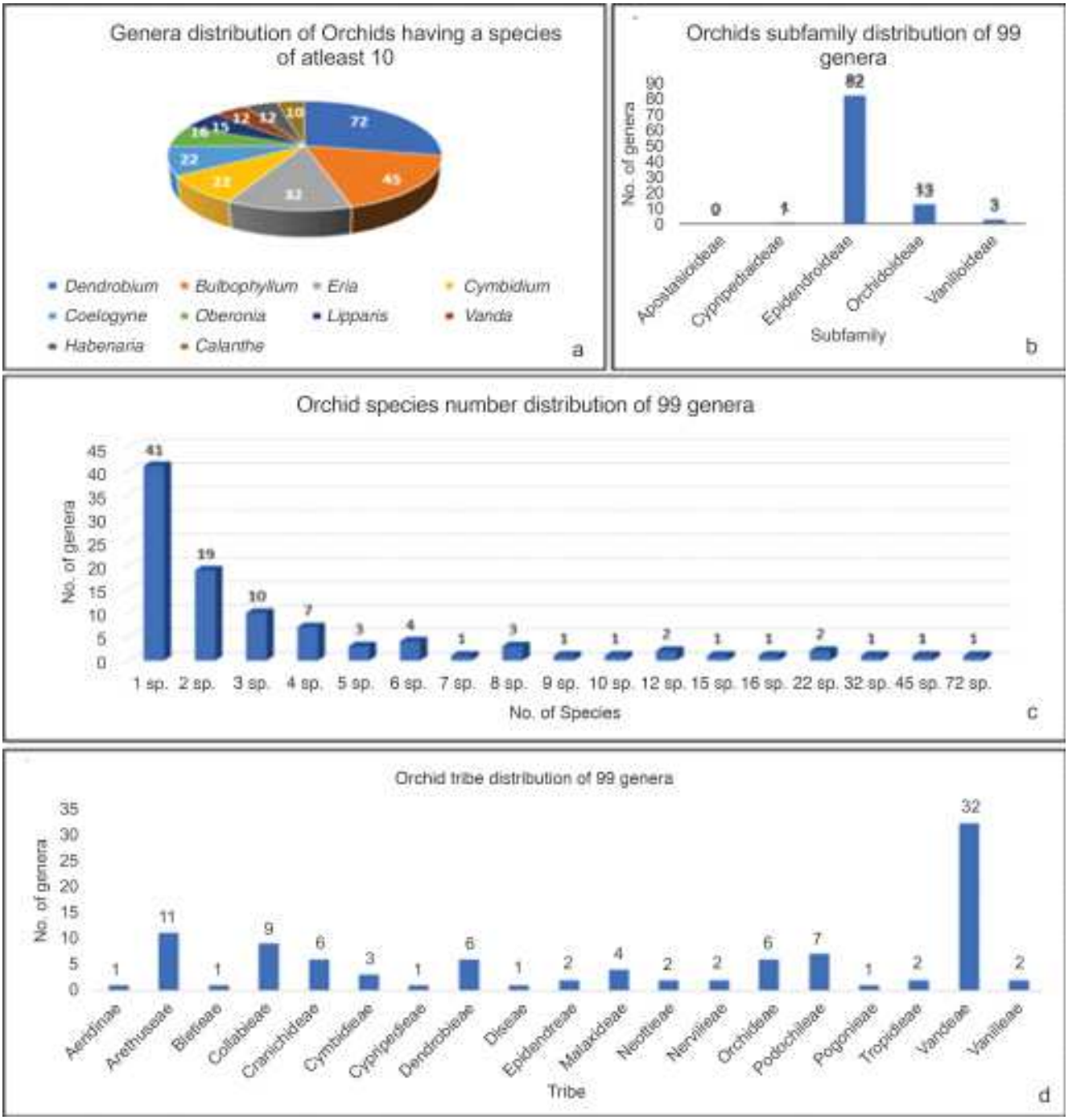

Fig. 2. a-d. Distribution of 474 species: a, 99 different genera with at least 10 species; b, 5 different subfamilies; c, Number of orchid species distributed in different genera; d, Different tribes.

Fig. 3. a-d. Lady's slipper orchids: a, *Paphiopedilum fairrieanum*; b, *P. hirsutissimum*; c, *P. spicerianum*; d, *P. insigne*.

As many as 81 species were collected and maintained at the net-house, IBSD, Takyelpat, Imphal. Attempts were also made for mass propagation of selected seven species (5 epiphytic, 2 terrestrial) using tissue culture technique with a view to conserve these species.

Distribution of 474 species, over 99 different genera indicated *Dendrobium* genera having the highest number of species in Manipur accounting upto 72, followed by *Bulbophyllum* (45) and so on (Fig. 2a). The distribution of these 474 species over the five subfamilies of Orchidaceae showed that Epidendroideae had the highest number of species (with 82 genera) followed by Orchidoideae (13 genera), Vaniloideae (3), and Cypripedoideae (1). There is no representation of the subfamily Apostasioideae which is considered as the most primitive (Zhang *et al*., 2021) (Fig. 2b). There are 41 genera having a single representative species, 19 genera with only two species found in Manipur and so on (Fig. 2c). Distribution of the 99 genera over different tribes indicated Vandeae tribe is most prevalent in the state Manipur (Fig. 2d).

The Cypripedoideae subfamily having a single representative genus found in Manipur is the genus *Paphiopedilum*. With its unique slipper shaped pouch-like modified labellum and long shelf-life blooms, the Cypripedoideae subfamily has been one of the major obsessions for floriculturists and botanists. The present collection also includes four species of *Paphiopedilum* in Manipur namely *Paphiopedilum fairrieanum*, *P. hirsutissimum*, *P. insigne,* and *P. spicerianum* (Figs. 3a-d). There are reports for nine species of *Paphiopedilum,* in India (Chowdhery, 2015).

In vitro asymbiotic seed germination was successfully carried out in five epiphytic (*Dendrobium chrysotoxum, D. polyanthum, Dendrobium transparens, Rhynchostylis retusa,* and *Vanda coerulea*) and two terrestrial (*Paphiopedilum hirsutissimum* and *P. spicerianum*) species (Fig. 4a-g). It was observed that MS (half strength) medium was proved as the most suitable during germination in all the epiphytic orchid species. Addition of supplementary nutrients such as banana powder enhanced the proliferation of protocorms. BAP (3 mgl^{-1}) when used together with NAA (1 mgl^{-1}) in the medium, enhanced growth and multiple shoot formation in protocorms. NAA (1.4 mgl^{-1}) showed effective result for root induction. Its higher concentration (2 mgl^{-1}) accelerated root initiation. The aerial part of the roots developed velamen on maturation. *Dendrobium transparens* and *Vanda coerulea* seedlings with well developed leaves and roots were transferred into the pots with potting mixture, for acclimatization (Fig. 5). The germinating entities failed to develop further despite repeated subculturing in both the terrestrial species (*Paphiopedilum hirsutissimum* and *P. spicerianum*).

Further, awareness and interaction programmes with farmers and locals of Manipur were also undertaken at National Bioresource Park, Haraorou (Fig. 6a-f) and Mao, Senapati district (Manipur) (Fig. 6g-l). Demonstration of orchid farming, hands-on training for orchid cultivation and spread of awareness on orchids as a source of income were organized.

Fig. 4. a-g. *In vitro* asymbiotic seed germination in seven endangered orchids of Manipur: a, *Vanda coerulea*; b, *Dendrobium transparens*; c, *D. polyanthum*; d, *D. chrysotoxum;* e, *Rhynchostylis retusa*; f, *Paphiopedilum spicerianum*; g, *P. hirsutissimum.* (Stereomicroscopic pictures taken after different days of inoculation on nutrient media).

Scientific approaches for *in vitro* propagation for mass production of orchids may not only serve as a mode of conservation but also aid in commercializing the available bioresources in the field of food, pharmaceutical, and floriculture industry. Biotechnological interventions will prove useful in increasing the efficiency of metabolite production in *in vitro* cultured plants. Addition of elicitors in the growth medium such as chitosan has been indicated to enhance the production of phytochemicals in *Vanda coerulea* (Nag and Kumaria, 2018). According to Yeow *et al.* (2020), action of light emitting diodes (LEDs) in PLB cultures shows increase in yield of secondary metabolites in *Dendrobium* hybrids. Such new and improved scientific techniques could be applied to avail economical products for the under privileged section of the society. Thus, conservation, species improvement, and innovative approaches can be

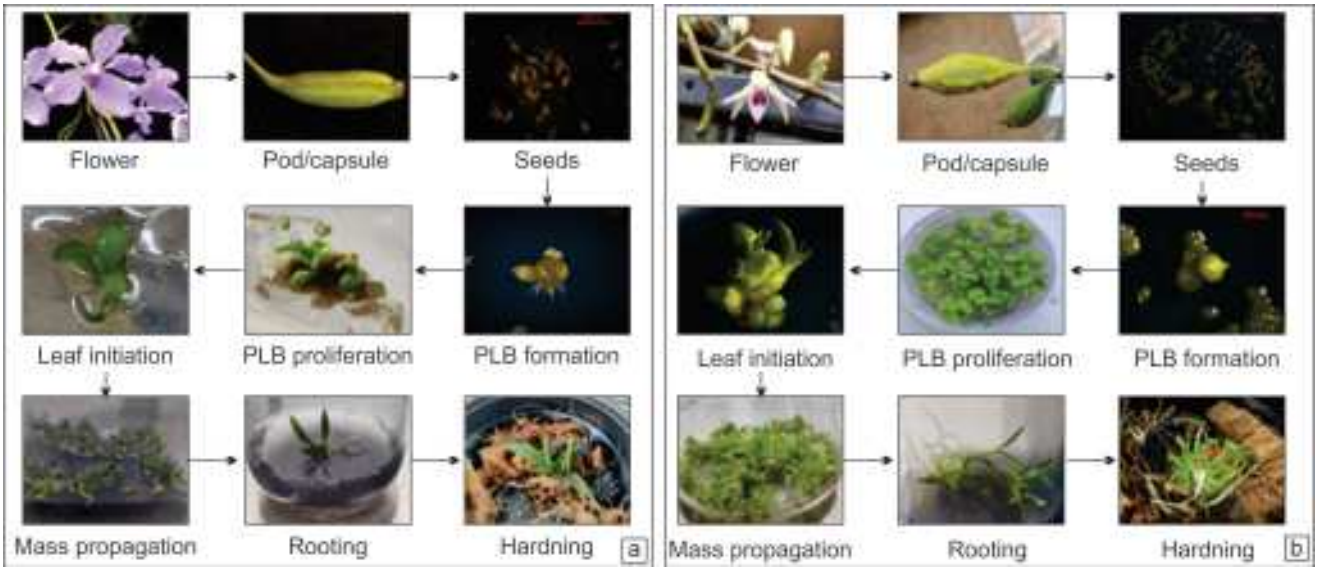

Fig. 5. a-b. Mass propagation through *in vitro* asymbiotic seed germination technique on MS (half strength) medium: a, *Vanda coerulea*, b, *Dendrobium transparens*.

Fig. 6. a-l. The local farmers and the officials of IBSD during various interaction programmes: a-f, One-day interaction programme on orchid cultivation on 19th October, 2020 at National Bioresources Park, Haraorou (Manipur); g-l, Awareness programme on orchid cultivation on 10th April 2021 at Mao, Senapati district (Manipur).

achieved through preservation and protection of the existing natural orchid populations.

The present study aimed for a sustainable maintenance and conservation of the wild orchids of Manipur. As the present data suggests that Manipur as a rich natural orchid germplasm region, it needs to be conserved before unprecedented endangerment of available orchids in this region due to overexploitation. Creating awareness amongst the public regarding their extensive uses in the field of medicine and horticulture would encourage them to cultivate orchids as an additional source of income resulting thereby in promoting orchid farming as an entrepreneurship approach. Demonstration using hands-on training and awareness programmes amongst farmers, locals and potential entrepreneurs would encourage entrepreneurs for income generation through orchid cultivation as a catapult for the upliftment of socio-economic condition of the people of the state. Cultivation of orchids in net-house, conservation in their natural habitats as well as establishment of orchidaria would not only considerably aid in maintaining the natural populations of orchid species but also would attract

visitors thereby promoting ecotourism, in the state.

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