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A conspectus of orchid studies in India

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Abstract: Orchidaceae represents the largest family of flowering plants in India. The country has come a long way from an era of exploration phase, identification and preparation of inventories of orchids, yet it appears to be an unfinished endeavour. Besides documenting the ethnobotanical knowledge of various orchid species, *in vitro* tissue regeneration, micropropagation, and cell culture protocols have also been standardised for many orchid species. However, in contrast to the available diversity, ecological and molecular studies of orchids have not been conducted extensively in the country. Although above (pollinators) and below ground (fungal endophytes) mutualists have been recorded for few orchid taxa, the underlying functional mechanisms, which facilitate the processes, are unexplored. The present review offers insights into the major works that have been carried out on orchids in India, and also highlights several areas of potential research that are neglected or understudied. Most importantly, there is a need to broaden the objectives while training orchidologists. It appears that unification of sub-disciplines is a priority in orchid biology in general, and conservation in particular.

Keywords: Conservation, Ecology, Mycorrhizae, Pollination, Taxonomy, Tissue culture.

Introduction

With more than 28,000 species, Orchidaceae is one of the largest families in the plant kingdom and are known to occupy a variety of niches with habitats ranging from terrestrial to epiphytic and even underground (Christenhusz & Byng, 2016). An estimated one out of every ten flowering plant is an orchid (Gentry & Dodson, 1987). In contrast to its diversity, only a small proportion of orchids have been studied so far (Tandon & Bhardhwaj, 2012). Due to the enormous variability in structural as well as functional traits, orchids are now considered as model system to study complexity and variability (Edens-Meier & Bernhardt, 2014).

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With more than 9% of the angiosperm flora, orchids form the largest group of plants in India (Adit *et al.*, 2020). Ethnobotanical uses of orchids have been well documented in the country (Rajendran *et al.,* 1997; Chowlu *et al.,* 2013; Panda & Mandal, 2013). While floristic and propagation studies on orchids have been popular, research on functional and molecular biology is insufficiently pursued. Change in climate regimes, habitat destruction, fragmentation, and aggressive collection from the wild have made visible adverse impacts on the native orchid flora (Jalal, 2012; Medhi *et al.*, 2012). Integrative understanding of taxonomy and ecology of orchids, using conventional, molecular and *in vitro* techniques will aid in conservation of orchids and develop site-specific strategies for their conservation.

Here, we (i) attempt to compile and highlight the aspects of orchidology in India, and (ii) reiterate core areas of biological studies that can play a crucial role in the development of conservation practices.

Systematic biology

The first record on Indian orchids come from Ayurvedic texts. The medicinal value of some orchids including *Eulophia dabia* (D.Don) Hochr., *Flickingeria nodosa* (Dalzell) Seidenf. and *Malaxis* *rheedei* Blume find their mention in the classical Indian medicinal treatise *Charaka Samhita* from around 100 AD (Caraka *et al*., 2017). Indian orchids were formally introduced to the western world by the Dutch Governor of Malabar (van Rheede, 1692, 1693), in which 17 orchid taxa were described in detail. Based on Rheede's descriptions, Linnaeus (1753) assigned binominals to many plants including orchids and treated them in his genus *Epidendrum* L. Two genera of ornamental plants, namely *Cymbidium* Sw. and *Rhynchostylis* Blume, were also established solely based on Rheede's illustrations. Linnaeus (1753) included 12 orchid species from the Indian sub-continent. Later, Roxburgh (1832) recorded 57 species of orchids belonging to eight genera and proposed many new taxa, which were later re-assigned to other genera based on various floral traits.

During the pre-independence period, significant work on orchids in India was carried out by the following: Griffith (1851) provided an extensive account on the general morphology, methods of pollination and affinities of each of 147 species in 49 genera. However, the most significant contributions about Indian orchids was made by Hooker (1888, 1890), in which 1600 species from British India were described. Hooker (1895) also provided extensive descriptions along with illustrations of 100 orchids. After the establishment of the Botanical Survey of India (BSI) in 1890 at the Royal Botanic Garden, Calcutta (now Acharya Jagadish Chandra Bose Indian Botanic Garden), the Director, Sir George King, launched large scale studies on the Indian flora including orchids. These studies resulted in compilation of several regional floras (Duthie, 1903; Prain, 1903; Cooke, 1906; Haines, 1924; Gamble, 1928), where information on Orchidaceae has been detailed extensively. King and Pantling (1898), inspired by Hooker's work, published an illustrated account on the orchids of Sikkim Himalaya. Collett (1902) described 38 species in 18 genera from Shimla and its neighbouring regions. Duthie (1906) listed 45 genera and 173 species including a few illustrations. Gammie (1905a,b, 1906a,b, 1907a,b,c, 1908a,b, 1909, 1910, 1911, 1912) published a series of papers on orchids of the Bombay Presidency, which included 61 species in 30 genera. Cooke (1906) described 31 genera and 75 species of orchids from the same region. Subsequently, Blatter and McCann (1931a,b,c, 1932) revised the orchid flora of the Bombay Presidency and documented 132 species in 37 genera and described several new species. Brühl (1926) described 461 species belonging to 92 genera from Sikkim and surrounding areas.

By the post-independence period, field-orchidology had become well-established in India. The era represented a phase where new reports of orchids from various regions started coming in. This is primarily due to the variety of ecological habitats and climatic conditions prevalent in the Western Himalayas, Eastern Himalayas and Western Ghats, where orchids are considered to be endemic and represent an important component of the vegetation. Ghose (1953), Pradhan (1976, 1979), Bose and Bhattacharjee (1980) provided preliminary information on the taxonomy of orchids in India. In addition, several inventories were published by various workers (Jain & Mehrotra, 1984; Karthikeyan *et al*., 1989; Kumar & Manilal, 1994; Misra, 2007; Schuiteman *et al*., 2021). Many new species have been reported across varying habitats. Recently published work by Singh *et al*. (2019) enumerated 1256 species in 155 genera, and Misra (2019) listed 1484 species from India. Several revisionary studies on different genera were undertaken by various workers: *Coelogyne* Lindl. (Das & Jain, 1980), *Calanthe* R.Br. and *Cymbidium* (Rathore, 1983), *Oberonia* Lindl. (Ansari & Balakrishnan, 1990), *Bulbophyllum* Thouars (Augustine *et al*., 2001), *Vanda* Gaud. ex Pfitzer (Limansenla *et al*., 2002), *Eria* Schltr. (Agrawala, 2009), Goodyerinae (Bhattacharjee, 2010), *Habenaria* Willd. (Chowdhury, 2011), *Cleisostoma* Blume (Phukan, 2011), *Dendrobium* Sw. (Sabapathy, 2013), Apostasioidae (Chowdhery, 2014), Cymbidieae (Kumar, 2015), *Habenariinae* (Kush, 2015) and Lady's Slipper Orchids (Chowdhery, 2015). Checklists and field guides for different regions exist for Western Himalayas (Raizada *et al*., 1981; Seidenfaden & Arora, 1982; Deva & Naithani 1986; Pangtey *et al*., 1991; Jalal *et al*., 2008; Chowdhery & Agrawala, 2013; Vij *et al*., 2013; Jalal & Jayanthi, 2015; Jalal *et al*., 2019), Northeast India (Tuyama, 1966, 1971; Hegde, 1984; Ghatak & Devi, 1986; Kataki, 1986; Kataki & Hynniewta, 1986; Rao, 1986, 2007, 2010; Singh *et al*., 1990; Shukla *et al*., 1998; Chowdhery, 1998, 2009; Hynniewta *et al*., 2000; Barua, 2001; Chauhan, 2001; Kumar, 2001; Kumar & Kumar, 2005; Pradhan, 2005; Lucksom, 2007; Deb & Imchen, 2008; Sharma *et al*., 2013b; Verma *et al*., 2013; Rao & Singh, 2015; Swami 2016, 2017; Bhattacharjee *et al*., 2018; Gogoi, 2018; Mao & Deori, 2018; Rahamtulla *et al*., 2020), Peninsular India (Santapau & Kapadia, 1966; Abraham & Vatsala, 1981; Joseph, 1983; Kumar & Sasidharan, 1986; Rao, 1998; Kumar & Manilal, 2004; Misra, 2004, 2008, 2014; Kumar *et al*., 2007; Misra *et al*., 2008; Raju *et al*., 2008; Pande *et al*., 2010; Jalal & Jayanthi, 2012; Narayanan *et al*., 2013; Barbhuiya & Salunkhe, 2016; Jalal, 2018, 2019; Prasad *et al*., 2019; Venkaiah *et al*., 2020), and Andaman and Nicobar Islands (Shiva *et al*., 2003; Gupta *et al*., 2004; Pandey & Diwakar, 2008; Karthigeyan *et al*., 2014). Around 10 new orchid names are introduced almost every year from the wild in India. Numerous new distributional records to the existing flora (national and regional) have been added. Although several new orchid species have been described from the subcontinent in recent years, many of them have turned out to be synonyms of previously existing species. To avoid such problems, putative new species need to be checked against the larger group globally, besides cross-checking for ecotypes and morphotypes (morphologically and genetically).

In vitro studies

Rao (1967) was the first to publish on the histogenesis and organogenesis of a few orchid seedlings in India. Subsequent tissue culture studies until the 1980s were rather preliminary and appeared infrequently (*e.g.,* Chennaveeraiah & Patil, 1973; Bapat & Narayanaswami, 1977; Mathews & Rao, 1980).

In vitro research on orchids in India have largely focused on horticulturally important genera (Mitra *et al*., 1976; Sharon & Vasundhara, 1990; Katiyar *et al*., 1991; Kaushik & Kishore, 1991, 1995; Seeni & Latha, 1992, 2000; Piyathi & Murthy, 1995; George & Ravishankar, 1997; Nayak *et al*., 1997, 1998; Kulkarni & Surwase, 1998; Sharma 1998; Sheelavantmath *et al*., 2000; Talukdar, 2001; Saiprasad & Polisetty, 2003; Bejoy *et al*., 2004; Piria *et al*., 2005; Pant *et al*., 2008; Chugh *et al*., 2009; Shadang *et al*., 2009; Guha & Rao, 2010, 2012; Vyas *et al*., 2009; Chauhan *et al*., 2010, 2015; Pant & Swar, 2011; Mahendran & Bai, 2012; Sibin *et al*., 2014; Sibin & Gangaprasad, 2016; Arora *et al*., 2016; Lekshmi & Decruse, 2018; Gurudeva, 2019; Madhavi & Shankar, 2019).

Vij and coworkers established protocols for orchid tissue culture in India and carried out extensive research on genera of horticultural and folk values *viz*., *Aerides* Lour.*, Bulbophyllum, Cattleya* Lindl.*, Coelogyne, Cymbidium, Dactylorhiza* Neck. ex Nevski*, Dendrobium, Eulophia* R.Br. *Goodyera* R.Br.*, Luisia* Gaud.*, Oncidium* Sw.*, Paplionanthe* Schltr.*, Phaius* Lour.*, Rhynchostylis, Satyrium* Sw.*, Vanda* (Vij *et al*., 1984, 1986, 1987, 1989, 1994, 1995, 1997, 2000; Vij & Pathak, 1988, 1989, 1990; Sharma *et al*., 1991; Vij & Kaur, 1992; Vij, 1993; Kaur & Vij, 1995, 2000; Vij & Aggarwal, 2003; Aggarwal *et al*., 2008; Pathak *et al*., 2011, 2017; Anuprabha & Pathak, 2012, 2019; Vasundhara *et al*., 2019). The National Research Centre (NRC) in Sikkim, which was established in 1996, has developed hybrid varieties *in vitro* of several orchid genera of ornamental value (*eg*., between *Cattleya, Cymbidium, Dendrobium, Phaius*) to encourage local farmers to enter the horticultural trade (Nagaraju & Parthasarathy, 1994; Nagaraju & Upadhayaya, 2001; Nagaraju *et al*., 2002, 2003, 2004). Standardized *in vitro* protocols to raise orchid species have also been established for many taxa from Northeast India, *viz*., *Agrostophyllum* Blume, *Cleisostoma*, *Coelogyne*, *Cymbidium*, *Dendrobium*, *Eulophia*, *Paphiopedilum* Pfitzer, *Renanthera* Lour., *Vanda* (Devi *et al*., 1990, 1997, 1998, 2006; Kumaria *et al*., 1990; Sharma & Tandon, 1990; Hazarika &

Sarma, 1995; Datta *et al.*, 1999; Devi & Deka, 2000; Kumaria & Tandon, 2000; Sinha *et al.*, 2001; Jamir *et al.*, 2002; Roy & Bannerjee, 2003; Sinha & Roy, 2004; Temjensangba & Deb, 2005; Das *et al.*, 2007; Deb & Temjensangba, 2007; Shadang *et al.*, 2007; Ng & Saleh, 2011; Dohling *et al.*, 2012; Paul *et al.*, 2012; Mao & Ranyaphi, 2013; Borah *et al.*, 2015).

Cytology and molecular biology

Diversification and speciation in plants is often correlated with chromosome and genome evolution (Alix *et al.*, 2017). Advancement in microscopy and molecular techniques has led to exhaustive cytogenetic research on orchids in India. Initial efforts focused on karyotyping epiphytic orchids to establish variations in this regard (Mehra & Pal, 1961; Mehra & Bawa, 1970; Mehra & Vij, 1972; Kashyap & Mehra, 1983; Mehra & Kashyap, 1983a,b, 1986). The origin of some orchids in nature was attributed to aneuploidy rather than true polyploidy (Sharma & Chatterji, 1961); however, this hypothesis has been tested on only very few taxa. Latha (2002) developed a staining method for rapid and convenient chromosome preparation from root tips of Orchidaceae members based on lactopropionic orcein. Besides, karyomorphological studies on several species of *Cymbidium* have been done in recent years (Sharma *et al.*, 2010, 2012b; Rao *et al.*, 2013).

The previous two decades have seen a rise in molecular and genomic studies on orchids around the world. In India, researchers have begun to employ these methods, and molecular biological tools and procedures are being used to understand the basis of orchid diversity and variation. The research has broadly focused on two topics: (i) barcoding as a tool for molecular taxonomy, and (ii) assessment of diversity through molecular markers. The Department of Botany at the University of Delhi has pioneered DNA barcoding of orchids in India, focusing on several medicinal and threatened species. Research has ranged from checking the applicability of the recommended locus/loci for congeneric species (Parveen *et al.*, 2012; Singh *et al.*, 2012; Khasim & Ramadu, 2018) to diverse orchid taxa (Parveen *et al.*, 2017; Kishor & Sharma, 2018). Several genetic barcoding markers such as ITS, *mat*K, *rbc*L, and *rpo*B and *rpo*C1, amplified fragment length polymorphism (AFLPs), double-digested random amplified DNA (ddRAD), inter simple sequence repeat (ISSRs), random amplification of polymorphic DNA (RAPDs, single primer amplification reactions (SPAR), simple sequence repeats (SSRs) in various taxon-specific combinations aided in assessing genetic diversity in nature (Sreedhar *et al.*, 2007; Sharma *et al.*, 2011, 2012a, 2013a; Chattopadhyay *et al.*, 2012; Bhattacharyya *et al.*, 2013, 2017; Manners *et al.*, 2013; Thakur & Kaur, 2013; Bhattacharyya & Kumaria, 2015; Ramesh *et al.*, 2016; Roy *et al.*, 2017; Rao, 2020). Isolated studies have also been carried out on chloroplast genome sequencing and annotation (Biswal *et al.*, 2017; Konhar *et al.*, 2019) and transcription modulation in selected taxa (Nag & Kumaria, 2018).

Functional biology and ecology

It is widely accepted that above (pollination) and below (fungal endophytes) ground mutualisms have shaped the diversity and occurrence of orchids, and there is a co-evolutionary relationship between the mutualistic partners (Waterman *et al.*, 2011; Selosse, 2014). Distribution patterns of orchids in various ecological regions are more random than regular. The gaps need to be validated through functional and evolutionary ecological studies, which at present have been inadequate in India. Studies on the reproductive ecology of the Indian orchids are scant, with only few isolated species having been superficially studied (Chaturvedi, 2009a,b; Chaturvedi & Chaturvedi, 2010, 2011; Attri & Kant, 2011; Buragohain *et al.*, 2015; Buragohain & Chaturvedi, 2020; Dangat & Gurav, 2020).

Orchid mycorrhizal studies in India have largely focused on molecular characterization of endophytic fungi (Senthilkumar & Krishnamurthy, 1998; Senthilkumar, 2003; Saha & Rao, 2006; Aggarwal *et al.*, 2012; Behera *et al.*, 2013;

Mahendran *et al.*, 2013; Sathiyadash *et al.*, 2013, 2014; Rajulu *et al.*, 2016; Srivastava *et al.*, 2018), besides a single study involving nitrogen metabolizing enzymes (Hajong *et al.*, 2013). Future research should focus on community structure, population biology and other related ecological parameters to assess their implication on orchid fecundity and survival.

It is imperative to gather information on functional ecology of species *in situ* to boost conservation efforts. The declining populations of pollinators due to climate change and loss of habitat have amplified the threat to the survival of orchids, that often depend on specialized pollinators for reproductive success (Abrol, 2012). These aspects are important as they help in understanding the distribution pattern of orchids in various ecosystems, and elucidate the role of mutualistic partners in the reproductive success, and structure and diversity in natural plant communities.

Indian Orchidology: prospects and conservation

Restrictions imposed on the commercial collection of orchids for medicinal and horticultural purposes have resulted in their illegal gathering from natural habitats. Although work on micropropagation using various explants in India is substantial, most of these studies have limited utility. The plants raised by tissue culture in most cases have not been reintroduced to nature because of logistics and requiring a multidisciplinary approach. Folk medicine is one of the major contributors to loss of viable orchid populations through overharvesting. Although an abundance of literature on ethnobotany is available on Indian orchids, preparations from orchid taxa need to be scientifically evaluated for effectiveness. Capacity building through skill enhancement in orchid biology, micropropagation, and nursery cultivation is imperative to boost conservation efforts. Most importantly, critical knowledge of reproductive strategies of most threatened orchids is insufficient. Information on threats, habitat ecology, and population dynamics is also unavailable for the majority of the species. Accordingly, the development of conservation policies is difficult. If pertinent measures are not taken for the protection of orchids, many of them may soon become extinct in nature. For example, *Spathoglottis arunachalensis* Tsering & Prasad, a recently described orchid (Tsering & Prasad, 2020) is not seen any more in the wild, as anthropogenic activities have disturbed its habitat. Many such examples are reminders that *ex situ* and *in situ* conservation measures are a necessity to preserve these plants. The National Orchidarium housed in Shillong (Meghalaya), the Sessa Orchid Sanctuary in Tipi (Arunachal Pradesh), and the Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Trivandrum (Kerala) have become the largest repositories of live orchids in India, and need to be developed as a focal point for the accumulation of a live gene bank of Indian orchids. In lieu of sustainable research, it is imperative that collection and accession of live specimens be promoted instead of herbarium or fixed specimens which are destructive in nature, at least in cases of threatened species. Botanical gardens, orchidaria and sanctuaries can provide accession numbers for such live collections to researchers. This will not only promote research on numerous fundamental aspects, but also can help in the maintenance of a gene pool, to act as a bank for micropropagation, and to serve as reliable repositories for re-introducing species that have disappeared from wild.

Despite having large number of herbarium specimens in various regional institutions/ herbaria, very few have been/ are being digitized in India. Besides, physical access to these specimens is also marred by red tape. To maintain academic and research growth, institutions with rich herbaria should make provisions for loaning/ exchange of specimens, both within and outside the country. It is crucial to understand that conservation of biodiversity can be facilitated through access of genetic resources and collaboration in research.

Conclusions

Research on orchids in general has taken major leaps in the last few years in India. Orchids are being used as model systems to study flowering pattern, developmental biology, and morphogenesis worldwide. Indian orchidologists have described several new species and the first reports of their presence in many regions through continued explorations. *Ex situ* conservation of orchids through *in vitro* methods as well as threat assessment and *in situ* conservation of threatened species is being carried out. Although molecular techniques are being used for resolving species complexes and constructing evolutionary relationships, the research is still in a nascent stage. Conservation genetics is another thrust area, which has been missing in current orchidological studies in the country. It is important to understand the geneflow and interconnectivity between populations to design strategies for conservation and species recovery. Science in India is traditionally an individualistic endeavour and thus hampered by isolated and unequal growth in knowledge; in spite of sincere efforts by many, larger objectives remain distant. This uncoordinated approach is also evident in conservation. Despite advances in orchid studies in India, research is lagging in the conversion of knowledge into applied conservation. It is imperative to design novel approaches of *in situ* regeneration and restoration of habitats, which could support sizeable populations.

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