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MYCORRHIZAL ASSOCIATIONS IN EIGHT SELECTED TERRESTRIAL ORCHIDS FROM WESTERN GHATS OF GOA

M J Bukhari and D Velip

Department of Botany, Government College of Arts, Science and Commerce, Quepem- 403 705, Goa, India

Abstract

The present study was carried out to assess the mycorrhizal associations in the roots of eight terrestrial orchid species [*Crepidium versicolor* (Lindl.) Sushil K.Singh, Agrawala & Jalal, *Eulophia picta* (R.Br.) Ormerod, *Habenaria crinifera* Lindl., *H. diphylla* (Nimmo) Dalzell, *H. marginata* Colebr., *Nervilia concolor* (Blume) Schltr., *Pecteilis gigantea* (Sm.) Raf., and *Peristylus plantagineus* (Lindl.) Lindl.] from the Western Ghats of Goa. The present communication revealed the occurrence of mycorrhizal associations in the roots of eight selected terrestrial orchid species from Western Ghats of Goa. The colonization was characterized by the presence of pelotons in the root cortical cells.

Introduction

MYCORRHIZAL FUNGI form a mutualistic association with the roots of terrestrial orchids and are considered to be essential for orchid seed germination. These have a significant effect on the vegetative and reproductive growth of the orchid plants. Orchid mycorrhiza is the symbiotic association between fungi and epiphytic and terrestrial species of orchids (Dearnaley, 2007). The association is characterized by the entry of fungal hypahe in the parenchymatous cells of germinating seeds through trichomes and suspensor cells (Smith and Read, 2008) and also through root velamen and cortical cells at adult stage (Clements, 1988), resulting in the formation of intracellular coiled fungal hyphae called pelotons. These coiled fungal hyphal structures, the pelotons serve as a site of nutrient transfer between the symbionts (Dearnaley *et al*., 2016; Rasmussen, 1995; Selosse, 2014) essential for the perpetuation of orchids in their natural habitats. This symbiotic association has a significant effect on the vegetative and reproductive growth of plant (Pandey *et al*., 2013; Perotto *et al*., 2014).

The importance of this mutualistic association with mycorrhizal fungi for germination of orchid seeds and for successful seedling development in terrestrial orchids has been well documented in the earlier reports (Batty *et al*., 2001; Bhatti *et al*., 2017; Rasmussen, 2002). Literature studies revealed the occurrence of mycorrhizal colonization in many orchids (Peterson and Farquhar, 1994; Senthilkumar and Krishnamurthy, 1998; Vij and Sharma, 1983, 1988; Vij *et al*., 1995, 2002; Zettler, 1997). Studies have also revealed how orchids gain mineral nutrients and sometime even organic compounds from interact with orchid mycorrhizal fungi (Li *et al*., 2021). The distribution of orchids in Goa is concentrated mainly in the Western Ghats region, which accounts for 86% of the total orchid diversity of the state (Jalal, 2019). The present communication revealed the occurrence of mycorrhizal associations in the roots of eight selected terrestrial orchids species from Western Ghats of Goa.

Material and Methods

Study Area

The present study was carried out at various localities from Goa *i.e.,* Surla, Gaoundongri, Bhupar-Gaoundongri, Dabhal, Dharbandora, Netravali, Cuncolim, Cotigao, and Pali.

Plant Identification

Plants collected during the present study were identified using flora of Goa (Rao, 1986) and Abraham and Vatsala (1981).

Sampling

During the present study, eight terrestrial orchid species belonging to six genera *i.e*., *Crepidium, Eulophia, Habenaria, Nervilia, Pecteilis,* and *Peristylus* in flowering stage were collected from different localities in Goa (Table 1). Pseudobulb/rhizome of each of the species was carefully removed, stored in polyethylene bags and brought to the laboratory for further processing. The roots were freed from the adhering soil debris by washing under running tap water and preserved in 70% alcohol for microscopic observations.

Mycorrhizal Studies

For mycorrhizal studies, thin free hand sections of roots were taken and stained with 0.05% trypan blue (Phillips

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Table. 1. Data regarding flowering period, locality and habitat of eight terrestrial orchids in Western Ghats of Goa.

Species	Flowering period	Locality	Habitat
Crepidium versicolor (Lindl.) Sushil K.Singh, Agrawala & Jalal	July-August	Bhupar-Gaoundongri	It is mainly found in evergreen forest floor under shade, moist places; it occurs at about 448 m elevation
Eulophia picta (R.Br.) Ormerod	June-September	Netravali	It is found in moist deciduous forest, grows in well-drained soil; it occurs at 102 m elevation
Habenaria crinifera Lindl.	August-September	Surla, Gaoundongri	Grows on well-drained soil with humidity of 70-80%; it occurs at 111 m elevation
H. diphylla (Nimmo) Dalzell	August-September	Cotigao	Grows on well-drained humus rich soil; it occurs at 98 m elevation
H. marginata Colebr.	September-November	Pali, Dabhal	The plant species found in open grasslands and the forest areas; it occurs at 72 m elevation
Nervilia concolor (Blume) Schltr.	May-June	Netravali	It is found in moist deciduous forest, grows in well-drained soil; it occurs at 102 m elevation
Pecteilis gigantea (Sm.) Raf.	September-October	Dabhal, Dharbandora, Cuncolim	It was found in open grassland, lateritic areas, sometimes found in evergreen forest patches ; it occurs at 33 m elevation
Peristylus plantagineus (Lindl.) Lindl.	July-September	Gaoundongri, Netravali	It was found in between the gaps of semi-evergreen forest patches, with humus rich clay soils; it occurs at 130 m elevation

and Hayman, 1970) and were observed under microscope for studies on hyphae and peloton formation. Photographs of the transverse sections were taken using a digital camera of Samsung attached to the microscope.

Results and Discussion

All the selected eight terrestrial orchid species were found growing on varied habitats. Table 1 indicates the data regarding flowering period, locality and habitat of eight terrestrial orchids in Western Ghats of Goa. *Crepidium versicolor* was reported from higher elevation of evergreen forest floors. *Eulophia picta* was collected from moist deciduous forest of Netravali. *Habenaria crinifera* were found growing in well-drained humus-rich soil with humidity 70-80%, at 98 m elevation. *H. diphylla* was found growing on well-drained humus rich soil; it occurs at 98 m elevation. *H. marginata* was found growing in open grasslands. *Nervilia concolor* was collected from moist deciduous forest of Netravali. *Pecteilis gigantea* was found growing in open grassland, lateritic areas, sometimes found in evergreen forest patches as well while, *Peristylus plantagineus* was found between the gaps of semi-evergreen forest patches, with humus rich clay soils at 130 m elevation.

The results on the cross section of roots of all the eight species revealed the presence of pelotons and hyphae. Hyphal colonization was reported in the root hair, velamen tissues, and cortical region of the roots. In all the studied species, the hyphal entry was through the root hair. Our results are in agreement with Senthilkumar and Krishnamurthy (1998) and Sathiyadash *et al.* (2012) where the fungal entry was through the root hair. Pelotons and digested pelotons were concentrated mainly in the cortical region (Fig. 1a-p). In the present study, the extent of hyphal coiling which is known to increase the inter-facial area between the symbionts varied amongst all the presently studied orchid species. Earlier studies reveal that peloton dimensions were related to cell dimension indicating that the size of pelotons seems to depend on the size of the host cell (Sathiyadash *et al.*, 2012). In *Crepidium versicolor* and *Eulophia picta*, major part of cortical region in the cross section showed the presence of pelotons while in the other species, a smaller portion of cortical region was occupied by the pelotons. The pelotons appeared as loosely arranged fungal mycelial network of spherical and hexagonal balls in the parenchymatous cells of the cortical region. The present study revealed that the mycorrhizal fungus which resides in the cortical cells helps in absorbing minerals and other nutrients from the surrounding medium which is then utilized by the host by a process of digestion of the fungal cells within the host tissue. Similar observations were earlier made in other terrestrial species (Vij *et al.,* 1995, 2002; Zettler, 1997). One of the important feature of the orchid mycorrhizae is the lysis of the pelotons (Peterson and Currah, 1990).

Mycorrhizal fungi are well known for the translocation of sugars and phosphates to the orchid plant (Richardson *et al.,* 1992). This type of fungal symbiotic

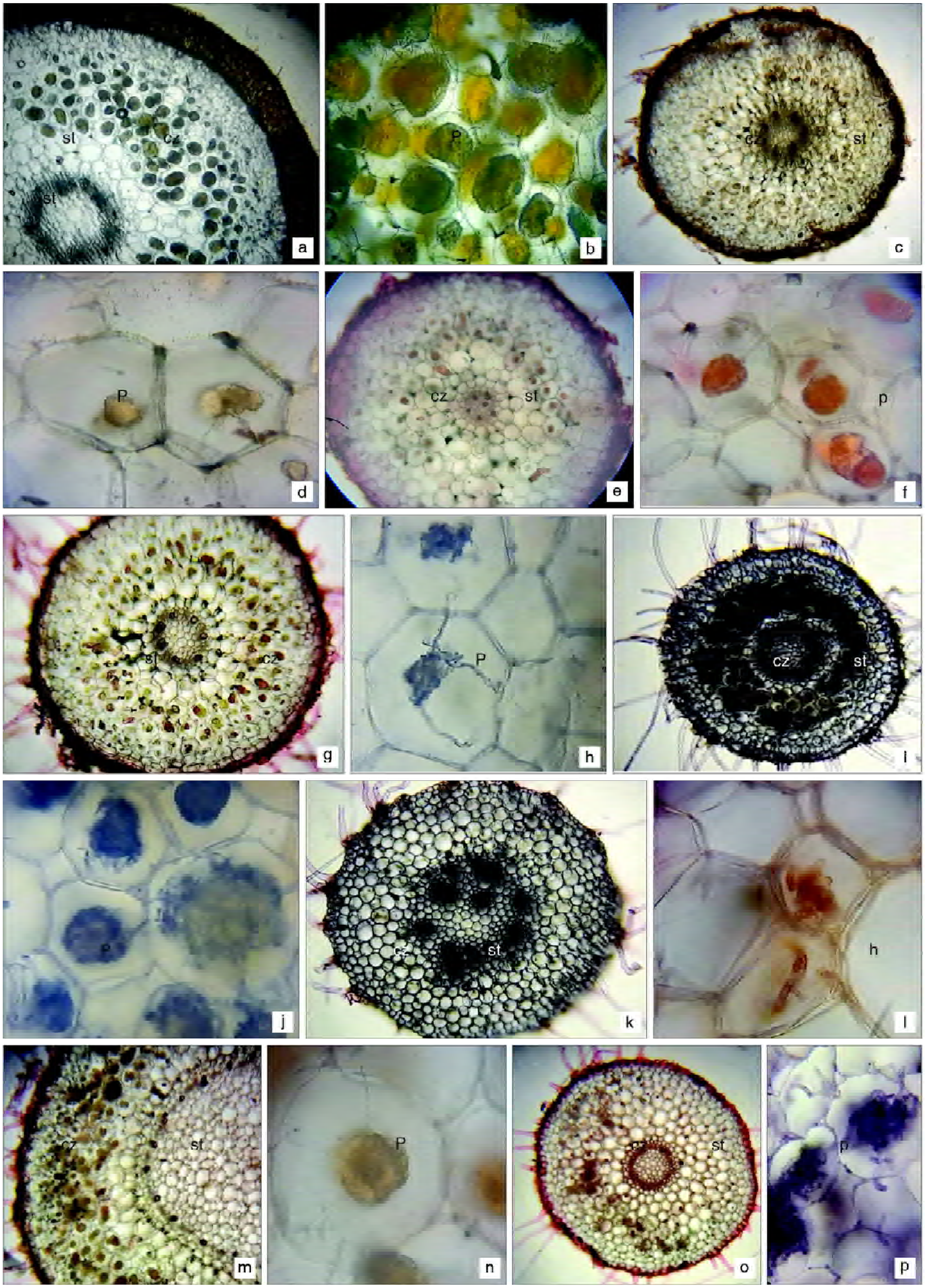

Fig. 1a-p. Mycorrhizal associations in terrestrial orchids from Western Ghats of Goa: a-b, *Eulophia picta*; c-d, *Habenaria crinifera*; e-f, *Habenaria diphylla*; g-h, *Habenaria marginata*; i-j, *Crepidium versicolor*; k-l, *Nervilia concolor*; m-n, *Pecteilis gigantea*; o-p, *Peristylus plantagineus* (cz, colonization zone; s-t, stele; p, pelotons; h, hyphae).

association has been reported earlier in terrestrial orchids (Jyothsna and Purushothama, 2013; Senthilkumar and Krishnamurthy, 1998; Vij and Sharma, 1983, 1988; Vij *et al.,* 1995, 2002; Zettler, 1997). Studies by Yoder *et al.* (2000) has revealed the higher water content in the infected seedlings of both terrestrial and epiphytic orchids than non infected control and their implications in enhanced uptake. Germination is considerably enhanced with mycorrhizal fungi that facilitate the absorption of free water by their orchid seed host. Studies on successful germination of seeds with the aid of mycorrhizal fungi in terrestrial orchids has been studied earlier in some orchids (Bhatti *et al.*, 2017; Rasmussen, 2002). Orchid mycorrhizal fungi have also been studied from other parts of the country. Earlier, Kaushik (1983) gave an illustrated account of intracellular mycorrhizal pelotons and fungal hyphae of 53 species of orchid roots with the help of transections and longitudinal sections. Kumar and Kaushik (2004) showed entrance of the fungus through root hair and observed pelotons in the cortical cells of roots of *Zeuxine strateumatica* and cultured and isolated the enzyme cellulase producing *Rhizoctania solani*. Kaushik and Pal (2011) described pathogenicity and symbiotic nature of the genus *Rhizoctonia.* Pal and Kaushik (2011) studied the antagonistic potential of *Trichoderma viride* to control *Rhizoctonia solani* both isolates from *Rhynchostylis retusa* and *Aerides multiflora respectively.* Subsequently Kaushik and Pal (2012) studied lignin degrading capacity of *Rhizoctania solani* isolated from *Vanda testacea* qualitatively and quantitatively.

During the present study, *Rhizoctonia*-like fungi were noted in the cross section of roots of all the studied species. Earlier studies have reported orchid association with a wide range of fungal species (Waud *et al.*, 2017) and mostly with fungal taxa belonging to *Rhizoctonia*-like species (Dearnaley *et al.*, 2012; Jyothsna and Purushothama, 2013; Weiß *et al.*, 2016). These mycorrhizal fungi play a significant role in rehabilitating threatened orchid species in their natural habitats (Dearnaley, 2007) as they are believed to ensure orchid survival in habitats which either are vulnerable to stressful conditions or these are with limited resources. Some of the selected species in the present study are of ethno-medicinal importance and call for conservation measures. The root paste of *Eulophia picta* is used for curing insect bites (Dash *et al.*, 2008) and is an endangered orchid (Sheelavantmath *et al.*, 2000). The fresh and dried rhizome of *Crepidium versicolor* is used by the tribal and local community of Goa for de-worming. Attempts have already been made towards conservation of some of the commercially important and rare, endangered, and threatened (RET) orchids by propagating them using tissue culture techniques (Anuprabha and Pathak, 2020; Anuprabha *et al.,* 2017; Arora *et al.,* 2016; Bhowmik and Rahman, 2023; Dhillon and Pathak, 2023; Hossain *et al.*, 2012; Kaur *et al.,* 2017; Kirti *et al.,* 2023; Kumari and Pathak, 2021; Mutum *et al.*, 2022; Pathak *et al.,* 2022, 2023; Sunita *et al.,* 2021; Thakur and Pathak, 2021; Tripura *et al.,* 2022; Vasundhra *et al.,* 2021).

To conclude, the present study provides evidence on occurrence of orchid mycorrhizal fungi in all the selected terrestrial species of Western Ghats of Goa. Knowledge of the mycorrhizal fungi is important for programmes aimed at the conservation and management of orchid species. Thus, isolation and identification of mycorrhizal fungi are important to understand the orchid-fungus relationship, which can lead to the development of efficient conservation strategies of the endangered and ethno-medicinally important terrestrial orchids occurring in Western Ghats of Goa.

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