Journal of Plant Interactions

ISSN: 1742-9145 (Print) 1742-9153 (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

Mycorrhizal association and morphology in orchids

Kullaiyan Sathiyadash , Thangavelu Muthukumar , Eswaranpillai Uma & Radha Raman Pandey

To cite this article: Kullaiyan Sathiyadash , Thangavelu Muthukumar , Eswaranpillai Uma & Radha Raman Pandey (2012) Mycorrhizal association and morphology in orchids, Journal of Plant Interactions, 7:3, 238-247, DOI: 10.1080/17429145.2012.699105

To link to this article: https://doi.org/10.1080/17429145.2012.699105

Published online: 04 Jul 2012.

Journal of Plant Interactions
Vol. 7, No. 3, September 2012, 238–247

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Mycorrhizal association and morphology in orchids

Kullaiyan Sathiyadash[a]*, Thangavelu Muthukumar[a], Eswaranpillai Uma[a] and Radha Raman Pandey[b]

[a]*Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore 641 046, India;* [b]*Department of Life Sciences, Manipur University, Canchipur, Imphal, India*

(*Received 30 January 2012; final version received 29 May 2012*)

We investigated the mycorrhizal associations in 31 adult wild or cultivated green orchids (22 epiphytic, 8 terrestrial, and 1 species with both epiphytic and lithophytic life-forms) from different vegetation types of Western Ghats, southern India. All the orchids examined were mycorrhizal with the extent of colonization varying with species and life-forms. Mycorrhizal association has been reported for the first time in 25 orchids. The entry of mycorrhizal fungi into the roots was mostly through root hairs. In certain epiphytic species, the fungal entry was directly through the epidermis. The fungi formed highly coiled hyphal structures (pelotons) within the root cortex, and their size was related to the cell size. The fungal invasion of the cortical cells was through cell-to-cell penetration. The cortical cells contained intact and lysed pelotons, and their ratio varied with species and life-forms. No significant relationship existed between root hair characteristics and the extent of colonization. Chlamydospores and microsclerotia-like structure were frequently found within the cortical and root hair cells. The liberation of fungal reproductive structures was by spiral dehiscence of the root hairs.

Keywords: orchid mycorrhizae; pelotons; microsclerotia; root hairs

Introduction

The Orchidaceae with over 700 genera and 25,000 species is one of the largest plant families on earth encompassing around 10% of flowering plant species (Dressler 1993). They exhibit large variations in their floral features, life-forms, habitat distributions, and trophic patterns (Gardes 2003). Members of this taxon grow in a wide range of habitats and have a substantial variety of life-history strategies ranging from epiphytic to terrestrial and nutritional modes from autotrophic to heterotrophic (Mc Cormick et al. 2004). Orchids have several unique characteristics and much of their diverse presence may be attributed to their relationship with mycorrhizal fungi (Smith and Read 2008). The production of minute seeds with minimal nutrient reserves renders orchids to be dependent upon mycorrhizal fungi for the provision of resources necessary for germination and growth during early stages of plant development (Rasmussen 1995; Arditti and Ghani 2000; Rasmussen and Whigham 2002). This myco-heterotrophic mode of nutrition in orchids has evolved independently several times during plant evolution (Bidartondo 2005).

There is a widespread assumption that the photosynthesis in adult phase will relieve the green orchids from their fungal dependence (Smith and Read 2008). Nevertheless, carbon gains from photosynthesis are likely to be minimal in the majority of green photosynthetic orchids growing in deeply shaded tropical forests (Bidartondo et al. 2004). As terrestrial orchids include some of the most valuable components of plant communities worldwide, it is of prime concern to determine the sustentation of orchids throughout their life cycle under natural conditions (Batty et al. 2002). Fungi are probably involved in the uptake of mineral nutrients, but this has not been conclusively proven, and the physiological interactions between fungi and adult photosynthetic species are yet to be understood (Smith and Read 2008).

Root architecture has a functional significance in terms of nutrient uptake by plants, mycorrhizal dependence as well as on the fitness of plants (Lambers et al. 2006). Changes in root hair numbers and length are considered to be an adaptation to varying availability of the nutrient resources (Bates and Lynch 2001). Plant species with more and/or long root hairs are more efficient in accessing the nutrients from substrates than species with less and/or short root hairs. Baylis (1975) showed that coarse and thick rooted plants with less developed root hairs were strongly dependent on mycorrhizal fungi for their nutrient and therefore were highly mycotrophic. Although the impact of root architecture on the extent of mycotrophy is well resolved for arbuscular mycorrhiza (Fitter 1982), such relationship between root architecture and the extent of mycotrophy is unknown for orchidaceous mycorrhiza.

One of the changes in the host root in response to fungal invasion is an increase in cortical cell size. In the ground orchid *Spathoglottis plicata*, the fungal invasion and peloton formation have resulted in a 3–5% increase in root cortical cell size (Senthilkumar

*Corresponding author. Email: ksd.bio@gmail.com

ISSN 1742-9145 print/ISSN 1742-9153 online

http://dx.doi.org/10.1080/17429145.2012.699105
http://www.tandfonline.com

and Krishnamurthy 1998a). This suggests that fungal peloton size should be closely related to the host cell size. However, information about the relation between host cell and fungal peloton dimensions are lacking. A study by Rasmussen and Whigham (2002) suggests that the host cells in orchidaceous mycorrhiza digest the pelotons within them in a controlled manner. The carbohydrates present in the fungal hyphae are released, which are then absorbed by the host cells (Athipunyakom et al. 2004). As this process is influenced by the host, the ratio of intact to lysing pelotons could differ among plant species and may also differ based on the mycorrhizal dependence.

Indian orchids are found at varying altitudes and climatic conditions. India posses certain orchid-rich areas like the eastern Himalayas and the western and southern Indian hills (Singh 2001). It is estimated that about 1300 orchid species belonging to 140 genera exist in India with Himalayas as their main habitat, whereas other species remain scattered in the Eastern and Western Ghats (Singh 2001; Misra 2007). The north-eastern India has one of the world's most diverse orchid floras (~800 species) followed by Western Ghats (~300 species) and north-western Himalayas (~200 species). The diversity among Indian orchids is so large that they may range from small- to large-flowered, terrestrial to epiphytic, and autotrophic to heterotrophic orchids. In general, terrestrial orchids are more common in north-western India, epiphytic orchids in north-eastern India, and small-flowered orchids in Western Ghats. There are about 199 species (belonging to 67 genera) of orchids distributed in the South Indian state of Tamil Nadu (Henry et al. 1989). However, limited reports are available on the mycorrhizal status of Indian orchids (Vij and Sharma 1988; Senthilkumar and Krishnamurthy 1996, 1998a, 1998b; Senthilkumar et al. 2000; Madhaiyan et al. 2003; Muthukumar and Sathiyadash 2009; Murugan et al. 2010; Muthukumar et al. 2011), and information on the mycobionts associated with Indian orchids are also scarce. Senthilkumar (2003) has reported the association of *Rhizoctonias* with the endangered orchids *Gastrochilus acaulis*, *Polystchya concreta*, and *Nervilia prainiana* of Kolli Hills, India. Similarly, Saha and Rao (2006) have reported eleven fungal isolates, including *Rhizoctonia repens* (=*Epulorhiza repens*), *Rhizoctonia solani, Cochliobolus spicifer*, and *Trichoderma viride*, associated with four epiphytes, two ground and two hybrid orchids. Similarly, association of *Ceratobasidium* with *Dactolorhiza hatagirea* (Aggarwal and Zettler 2010) and *Rhizoctonia solani* with *Zeuxine strateumatica* (Kumar and Kaushik 2004) have also been reported.

Wang and Qiu (2006), in their checklist compilation on the occurrence of mycorrhizae in land plants, listed the occurrence of orchidaceous mycorrhiza in 83 taxa, which is only a small fraction of the thousands of orchids distributed worldwide. Furthermore, Brundrett (2009) also pointed out that orchids are undersampled group, because of their occurrence in specialized habitats. Hence, the objectives of carrying out this study are four-fold. First, we examined the mycorrhizal status of certain orchid species growing in diverse habitats of South India. Second, we assessed the role of root architecture on the extent of mycotrophy. Third, we investigated the possibility of any relation between the host cell and fungal peloton dimensions, and finally, we evaluated the proportion of intact to lysing or lysed pelotons in different orchid species.

Materials and methods

Study sites

Roots of thirty-one orchid species (five individuals per species) were collected between November 2009 and January 2010 from six different areas and vegetation types in the Western Ghats. The characteristics of the study sites are presented in (Table 1).

Sample collection

Root samples of 31 orchid species (22 epiphytic, 1 lithophytic and epiphytic, and 8 terrestrial) were collected from five individuals at two different growth stages (vegetative and reproductive). Care was taken not to damage the roots during collection. Roots were washed and preserved in FAA (formalin–acetic acid–70% alcohol: 5:5:90; v/v) solution until processing.

Determination of root morphology

Ten randomly selected root bits (approximately 1cm long) were mounted in water on microscopic slides for the assessment of root hair characters. The number of root hairs per centimeter root length was counted at 20 × magnification in a dissecting microscope (Zeiss, West Germany). To estimate the length and breadth of the root hair, measurements were made at 100 × using a compound microscope (Olympus BX50, Japan) fitted with an ocular scale.

Estimation of orchid fungal colonization

Fixed roots were cleared in 2.5% KOH (Koske and Gemma 1989), acidified with 5N HCl, and then stained overnight with trypan blue (0.05% in lactoglycerol). The stained roots were examined with compound microscope (200 ×) for the presence of fungal structure. Magnified intersection method of Mc Gonigle et al. (1990) used for estimating arbuscular mycorrhizal (AM) colonization was adapted for estimating root length colonization. In addition, the number of fungal hyphae and pelotons intersections was also recorded. Quantification of root length colonized by fungal hyphae as well as the intact and lysed pelotons, in addition to total root length colonization, was made.

Table 1. Characteristics of the study sites.

	Study site					
	Nilgiri	Velliangiri	Coimbatore	Wayanad	Salem	Kallar
Location	11°28′N & 76° 63′E	10°58′N & 76° 73′E	11°04′N & 76° 93′E	11°48′N & 76° 26′E	12°4′N & 78° 30′E	12°27′N & 75° 16′E
Altitude (m. a. s. l)	2073	520–1840	426–550	2100	290	360
Annual rainfall (mm)	1520–1700	500–7000	500–700	2322	930.7	1400
Vegetation type	Forest	Sholas	Forest	Forest	Forest	Forest

Distribution of fungal colonization and measurement of cell and peloton dimensions

Mycorrhization was also evaluated on stained hand sections of roots. Transverse sections of the fixed root were taken with a gap of 2–3 mm from each other. Thin and complete sections were stained within tryphan blue and were picked randomly for analysis. Pelotons with apparently intact hyphae were assumed to be intact and functional, whereas densely stained pelotons with poorly distinguishable hyphal structures were considered to be lysing.

The proportion of cortical volume occupied by pelotons was scored on each section using an eight-step scale (12.5–100%) of Rasmussen and Whigham (2002) with a slight modification. Cross hairs were attached to a circular cover glass dividing the circumference into eight equal parts from the centre. The whole circumference was taken as 100% with each division of 12.5%. The divisions were further subdivided in four parts of 3.125% each. Five hand sections from different root pieces of a species were mounted, and the sections were scored under a dissection microscope. The length and breadth of the 50 cells and pelotons in the selected sections were recorded.

Statistical analysis

Data on mycorrhizal colonization and root hair morphology were subjected to analysis of variance (ANOVA) to determine the significance of the variation measured. Linear regression analysis was used to assess the relationship between cell size and peloton size. Similarly, Pearson's correlation was used to assess the relationship between root hair characters and the extent of mycorrhization. The percentage root length colonization data was arcsine square-root transformed prior to statistical analysis.

Results

All the orchid species examined in the present study were mycorrhizal to varying extents. The extent of colonization significantly ($P < 0.05$) varied among species ($F_{31,64} = 14.76$) and life-forms ($F_{31,64} = 24.23$). Percentage root length colonization ranged from 34% (*Luisia zeylanica*) to 79% (*Acampae praemorsa*) in epiphytes, 42% (*Eulophia epidendraea*) to 92% (*Habenaria roxburgii*) in terrestrial, and 74% (*Oberonia ensiformis*) in lithophytic orchids (Table 2).

Colonization patterns

Epiphytic and lithophytic species

All epiphytic species occurred in association with accumulated organic debris, mosses, and other plants. Aerial roots were colonized only when they were in contact with the substrate, while the roots which were not in contact were free from colonization. All the species examined had colonization ranging from 34%

Table 2. Root hair morphology, extent of mycorrhizal colonization and cortical volume occupied by colonized cells in South Indian orchids.

Plant species	CS	LF	Root hair characteristics			% Colonization			RILP	Ratio of cortical volume occupied
			RHN	RHL	RHB	RLDP	RLIP	RLTC		
Acampae praemorsa (Roxb.) Blatt. & Mc Cann.	VI	E	9.7±1.2[a]	131.0±9.3	9.1±0.0	24.7±6.2	54.28±5.0	79.15±5.4	2.02±0.15	27.18±10.03
Aerides ringens Fischer	VI	E	9.7±2.2	56.1±15.8	9.1±0.0	31.4±4.5	20.48±1.7	51.86±6.1	0.27±0.03	46.75±8.63
Bulbophyllum termulum Wt.	II	E	3.0±0.6	243.7±4.7	37.7±1.4	21.1±6.8	44.14±6.9	65.24±10.2	0.58±0.01	47.48±5.35
Calanthe triplicata (Willem.) Ames	I	T	15.3±0.3	232.1±66.1	11.1±1.3	30.0±1.1	14.00±0.6	44.00±1.7	1.96±0.18	32.07±1.83
Coelogyne mossiae Rolfe	II	E	3.0±0.6	370.7±3.5	41.0±2.1	30.0±1.1	33.33±0.9	63.33±1.2	4.87±0.39	12.73±1.17
Cymbidium aloifolium Sw.	VI	E	15.3±0.9	236.6±9.5	12.1±1.5	28.0±1.2	49.51±3.5	77.55±4.0	3.38±0.57	31.83±2.30
Cymbidium pendulum Sw.	I	E	10.7±1.20	218.5±5.1	10.6±1.5	17.6±1.3	32.29±2.1	49.91±3.2	1.12±0.06	6.76±1.21
Dendrobium herbaceum Lindl	IV	E	6.7±0.88	461.0±6.7	40.0±1.1	21.5±1.5	18.25±2.1	39.75±2.7	0.47±0.03	15.57±7.01
Epidendrum sp.	IV	E	6.7±0.9	1067.7±33.8	8.3±0.9	13.1±3.6	33.25±1.0	46.35±0.6	0.71±0.01	25.11±1.57
Eulophia epidendraea (Retz.) Fischer	VI	T	6.0±0.6	358.3±33.5	12.7±1.4	15.1±2.5	26.81±8.8	42.27±10.6	0.82±0.01	30.92±4.55
Gastrochilus acaulis Kuntze	II	E	4.0±0.6	542.7±1.4	16.7±0.3	6.3±0.7	30.33±1.4	36.67±2.0	0.57±0.03	7.47±0.91
Habenaria longicorniculata Graham	II	T	9.7±0.9	653.0±8.5	18.0±1.1	17.9±1.0	57.37±5.6	75.27±6.3	0.47±0.00	21.39±6.23
Habenaria rariflora A. Rich.	II	T	2.7±0.3	894.7±2.6	22.3±1.2	47.7±1.4	39.00±0.9	86.67±2.0	0.60±0.02	38.97±7.95
Habenaria roxburgii Nicolson	V	T	13.0±1.5	179.0±36.8	10.6±1.5	10.7±3.1	78.35±5.4	89.05±3.3	0.75±0.01	43.73±16.22
Luisia birchea (A. Rich.) Bl.	II	E	14.0±2.1	151.5±8.0	9.1±0.0	20.7±3.3	44.77±4.8	65.44±1.6	2.39±0.71	40.50±14.40
Luisia pulniana Vatsala	V	E	28.3±8.8	196.9±40.1	16.7±5.5	34.9±11.7	25.12±2.6	60.00±14.3	1.07±0.26	65.89±23.83
Luisia zeylanica Lindl	II	E	16.0±3.1	236.3±37.8	18.2±0.0	11.7±0.9	22.67±1.5	34.33±1.9	1.88±0.92	15.08±1.16
Malaxis versicolor (Lindl.) Santapau & Kopadia	I	T	15.3±0.9	90.9±10.5	9.1±0.0	53.3±2.4	37.67±1.5	91.00±3.8	1.77±0.13	84.47±9.41
Oberonia bruniana Wt.	I	E	7.0±0.6	90.6±5.3	18.2±0.0	28.3±4.0	25.64±1.9	53.98±3.9	2.48±0.61	35.92±6.62
Oberonia ensiformis (Sm. ex Rees.) Lindl	I	E	15.3±0.9	178.2±8.5	18.2±0.0	45.7±2.3	12.33±1.2	58.00±3.0	1.76±0.53	54.17±9.37
	I	L	14.7±1.2	390.4±21.0	9.1±0.0	42.3±1.4	31.67±0.9	74.00±2.3	0.67±0.07	47.46±4.14
Oberonia mucronata (D. Don) Ornemed & Seident	I	E	11.0±0.6	300.0±34.4	12.1±3.0	41.7±0.9	19.33±0.9	61.00±0.6	0.82±0.05	59.26±2.17
Oberonia platycaulon Wt.	I	E	7.3±1.4	203.0±39.4	18.2±0.0	25.7±1.8	51.33±0.9	77.00±1.5	0.96±0.19	37.75±2.01
Oberonia verticillata Wight	I	E	7.0±1.1	181.8±10.5	18.2±0.0	28.0±1.7	16.33±1.2	44.33±2.9	2.93±0.76	20.67±1.33
Polystachya concreta (Jacq.) Garay & H. R. Sweet	II	E	13.3±2.0	190.9±18.9	18.2±0.0	37.8±9.2	35.73±1.2	73.49±7.9	0.85±0.09	15.15±1.62
Rhyncostylis retusa Bl.	II	E	21.0±1.5	372.7±13.9	18.2±0.0	23.4±6.7	31.75±10.8	55.19±8.0	1.51±0.22	57.62±25.51
Robiequetia josaphiana Manilal & Singh	II	E	6.7±0.9	130.3±10.9	18.2±0.0	13.0±2.1	41.67±0.9	54.67±1.2	1.84±0.08	14.51± 2.17
Satyrium nepalense Don.	II	T	10.3±1.2	836.3±197.6	30.3±6.1	21.6±8.4	41.54±5.5	63.14±6.6	2.60±0.87	31.33±6.18
Sirhookeriana lanceolata (Wt.) O. Kuntze	IV	E	17.0±3.1	366.6±66.5	24.2±3.03	26.8±5.0	38.6±2.7	65.39±7.0	3.17±1.69	13.55±0.94
Spathoglottis plicata Bl.	III	T	35.0±1.7	1196.8±84.3	15.1±3.03	52.3±1.2	29.7±0.9	82.00±1.1	3.21±0.35	4.61±0.23
Vanda testaceae (Lindl.) Reich. f.	VI	E	31.0±2.1	533.3±156.2	30.3±8.02	29.9±1.1	23.6±1.2	52.56±1.6	0.84±0.20	40.44±13.41
Vanilla planifolia Andrews	III	E	24.7±3.2	1166.5±40.1	21.2±3.03	32.3±1.4	19.3±0.9	51.67±2.2	8.99±3.01	12.61±1.88

Note: CS, collection site; I, Nilgiri; II, Velliangiri; III, Coimbatore; IV, Wayanad; V, Salem; VI, Kallar; LF, life-form; E, epiphyte; L, lithophyte; T, terrestrial; RHN, root hair number; RHL, root hair length; RHB, root hair breadth; RLDP, root length with degenerating peloton; RLIP, root length with intact peloton; RLTC, root length with total colonization; RILP, ratio of intact and lysed pelotons.
[a]Mean±S.E.

(*Luisia zeylanica*) to 79% (*A. praemorsa*) (Table 2). Colonization zones were normally in the regions adjacent to the substrate (Figure 1a), often in specific regions of cortex. The average percentage of root cortex colonized was $<40\%$. Where colonization occurred, the cortical cells contained pelotons in various stages of development and lysis. Root hairs of some epiphytes contained hyphae and microsclerotia-like structures. Similarly, fungal hyphae were seen on the root surface at the point of contact with the substrate. The percentage of root length with intact pelotons ranged from 12% (*O. ensiformis*) to 78% (*A. praemorsa*) while with degenerating pelotons ranged from 6% (*Gastrochilus acaulis*) to 45% (*O. ensiformis*).

The only lithophytic species *O. ensiformis* examined also occurred as an epiphyte at the same site and had 74% of its root length colonized and the mycorrhizal morphology resembled the other epiphytic species. The percentage of root length with intact and degenerating pelotons was 31% and 42%, respectively. The cortical volume containing colonized cells averaged 30.61 ± 7.87 (mean $\pm$ S.E., $n = 23$) in epiphytic and 46.47% in lithophytic species (Table 2).

Terrestrial species

In terrestrial orchids, the fungal hyphae entered the roots frequently through root hairs (Figure 1b,c) or directly through the root surface (Figure 1b,c). Cortical colonization was commonly observed in young and old roots, which occurred in patches at times occupying up to 80% of the cortex (Table 2). A feature that was apparently connected with the presence of endophyte within roots in most of the terrestrial species was the distortion of the root hairs in the colonized root portions. Usually $<55\%$ of the cortical volume was colonized in the young roots. However, older roots of these plants had extensive colonization with partially digested or degenerating pelotons and their ratio varied with species (Table 2). Intact nuclei were seen in cells containing degenerating pelotons. Microsclerotia-like structures were abundant in the cortical regions and in root hairs. Intercalary or terminal chlamydospores occurred in root hairs in clumps or long chains. These chlamydospores were liberated through spiral dehiscence of the root hairs (Figure 1i). The cortical volume containing colonized cells was 55.08 ± 13.35 (mean $\pm$ S.E., $n = 8$) in terrestrial species. The percentage of intact pelotons in terrestrial species ranged from 14% (*Calanthe triplicate*) to 78% (*Habenaria roxburgii*), whereas degenerating pelotons ranged from 11% (*Habenaria roxburgii*) to 53% (*Malaxis versicolor*) (Table 2).

Ratio of intact to degenerating pelotons

The ratio of intact to degenerating pelotons varied with species (Table 2). Generally, it was lower for epiphytic (1.53) compared to terrestrial forms (2.10). The lithophytic species *O. ensiformis* had the ratio of <1.

Relationship between cell and peloton dimensions

There were great variations in cell and peloton sizes among orchids (Figure 2). In certain species, the cortical cells were tightly packed with the fungal hyphae, while in others the pelotons consisted of loose coils of hyphae. There was significant relationship between cell and peloton's length and breadth.

Root morphology and extent of colonization

Root hair number ($F_{31,64} = 4.93$), length ($F_{31,64} = 34.82$), and breadth ($F_{31,64} = 8.44$) varied significantly ($P < 0.01$) between species (Table 2). Generally, terrestrial species had numerous and long root hairs compared to epiphytic and lithophytic species. The extent of colonization was not related ($P > 0.05$) to root hair number ($r = 0.120$), length ($r = 0.097$), and breadth ($r = -0.156$).

Discussion

All the adult green orchids examined in the present study were mycorrhizal which is in accordance with the fact that adult orchids are mycorrhizal dependent and usually have mycorrhizal roots or tubers (Rasmussen 2002; Rasmussen and Whigham 2002; Bidartondo et al. 2004). Mycorrhizae have previously been reported for *S. plicata* (Hadley and Williamson 1972; Senthilkumar and Krishnamurthy 1996, 1998a, 1998b), *Vanilla planifolia* (Madhaiyan et al. 2003), *Calanthe triplicata* (Kaliamoorthy 2007), *O. ensiformis*, *O. platycaulon*, *Malaxis versicolo*r (Bagyalakshmi et al. 2010), and *Rhyncostylis retusa* (Radhika and Rodrigues 2007). Examined in the present study and to best of our knowledge, the mycorrhizal status are reported for the first time for the other 25 species.

In this study, the extent of fungal colonization and the cortical volume occupied by colonized cells in terrestrial species were higher as compared to epiphytic and lithophytic species. This is in accordance with the observations of Hadley and Williamson (1972) and Goh et al. (1992), where heavy mycorrhization have been reported in temperate and tropical terrestrial orchid species. In terrestrial orchids, new roots are produced in each season and are rapidly mycorrhized by fungi originating from the soil. Examination of terrestrial orchid roots in our study clearly indicated that the fungal entry into the root was primarily through the root hairs. This corroborates the observations of Senthilkumar and Krishnamurthy (1998b) where the fungal entry into root of *S. plicata* was always confined to the part of the root which had root hairs (Currah et al. 1988; Vij and Sharma 1988). The fungus enters the root hairs frequently at their tips, infrequently slightly away from the tip or rarely

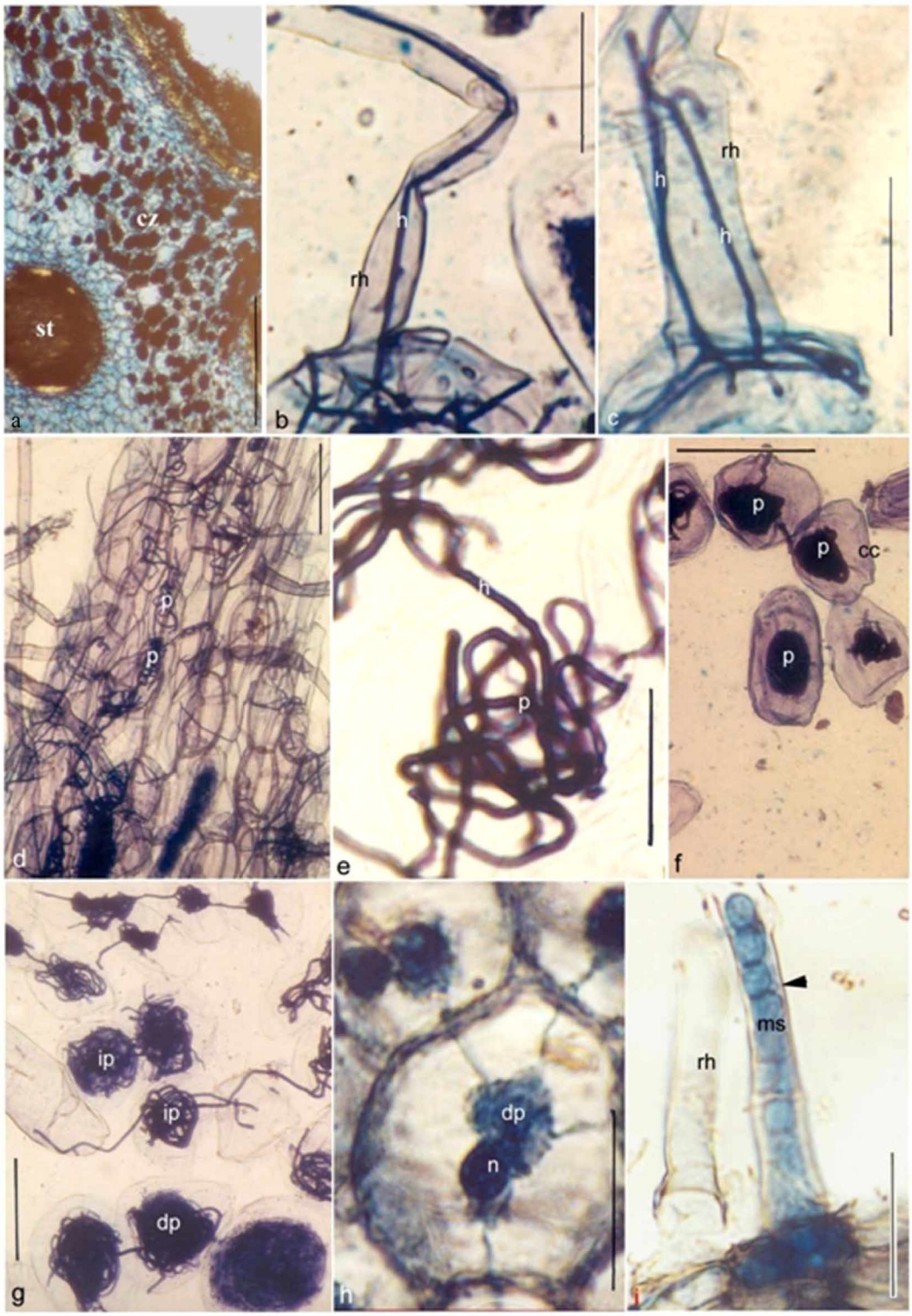

Figure 1. Mycorrhizal association in orchids. a. Transverse section of *Gastrochilus acaulis* epiphytic root showing colonization zone (cz) and stele (st); b and c. Fungal hyphae (h) within root hairs (rh) of the epiphytic *Luisia zeylanica* (b) and terrestrial *Malaxis versicolor* (c); d. Pelotons (p) in root cortical cells of the epiphytic *Coelogyne mossiae*; e–g. Intact pelotons (ip), cortical cells (cc), hyphae (h) and degenerating pelotons (dp) in the terrestrial *Habenaria rarifolia* (e), epiphyte *Vanilla planifolia* (f) and terrestrial *Spathoglottis plicata* (g); h. Degenerating peloton (dp) and intact nucleus (n) in terrestrial *Habenaria rarifolia*; i. Microsclerotia like structures (ms) within root hairs (rh) of epiphytic *C. mossiae*. Scale bars: a. 50 μm, b–i. 100 μm.

at the base. Normally, only single hypha entered each root hair, but occasionally two or more hyphae could be seen within the root hair as observed by Senthilkumar and Krishnamurthy (1998b). After the entry of the fungal hyphae, root hairs usually lost their straight cylindrical nature and became crooked

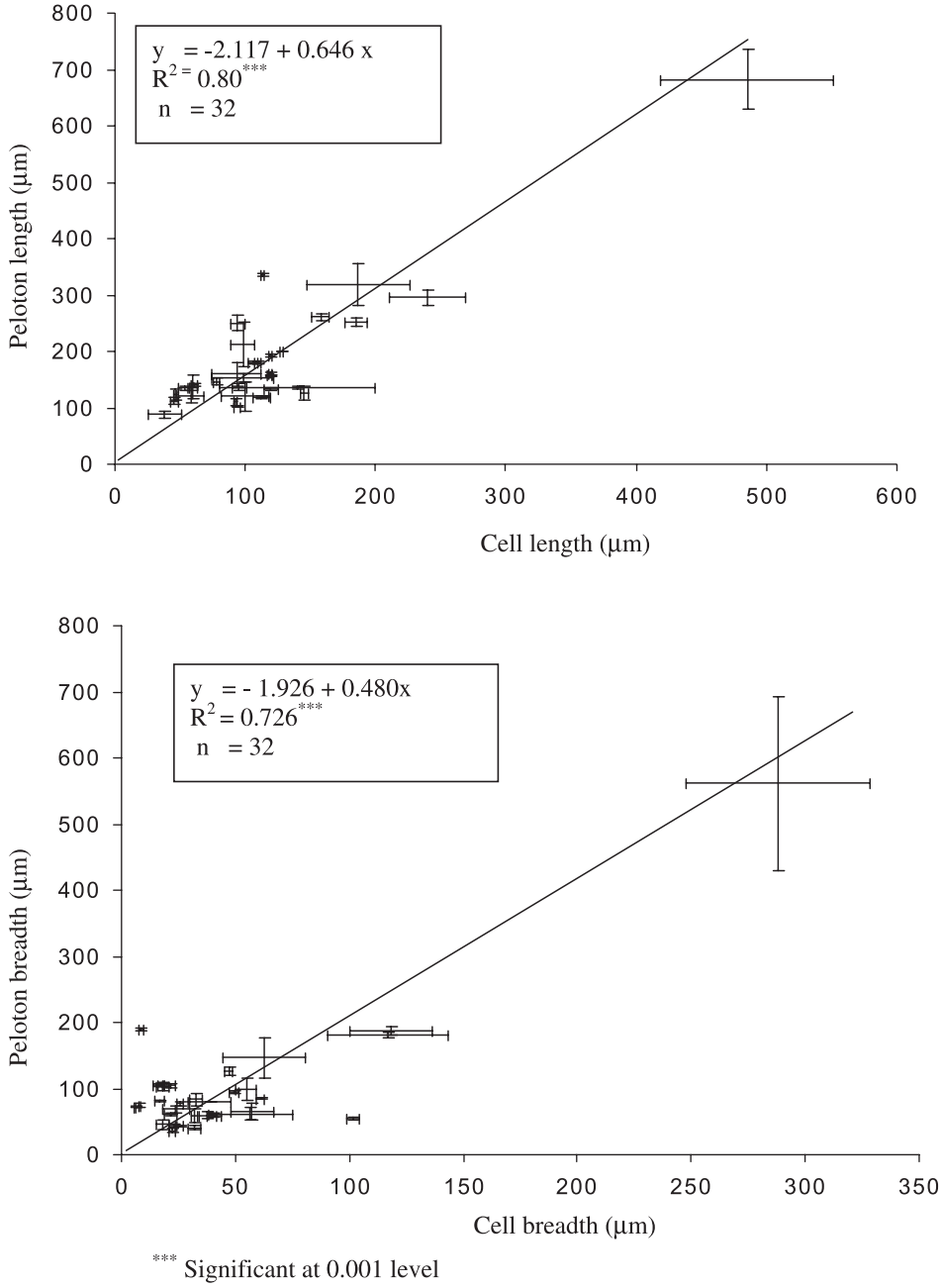

Figure 2. Scatter diagram showing relationship between root cell and peloton dimension in orchids.

and distorted to various degrees mimicking the colonization pattern involving the nodulating bacterium (Senthilkumar and Krishnamurthy 1998b). The distortion of the root hair may be limited to the tip or may extend to different lengths towards the base of the hair depending upon the locus of the hyphal entry. In fact, the distorted appearance of root hairs is indicative of the fungal presence (Peterson and Farquhar 1994). No special vesicular, or appressorial, or swollen structures was evident at the points of hyphal entry as in AM (Smith and Read 2008). Within the root, the fungus spread from cell to cell and extensively colonized the cortex.

The localization of the colonization zone towards the region where the roots were in contact with the substrate in epiphytic and lithophytic species is in line with the observations where mycorrhizal colonization has been reported to be frequent in regions of the root adjacent to the substrate (Bermudes and Benzing 1989; Lesica and Antibus 1990; Richardson et al. 1993). Aerial roots that were free of substrate were devoid of colonization (Goh et al. 1992). In addition, roots of epiphytic orchids had moderate levels of colonization compared to terrestrial species (mean 56.83% vs 72.12%), contrasting the widely held view that epiphytic orchids have very low colonization levels compared with terrestrial species (Rasmussen 2002).

Cell-to-cell penetration of the fungal hyphae neither distorted nor induced any thickening in the cell wall, suggesting a local hydrolysis (Peterson and Currah 1990; Beyrle et al. 1995). Growth and anastomosis of the intracellular hyphae resulted in

the formation of complex pelotons, which increase the interfacial area between the symbionts. The extent of hyphal coiling (peloton compactness) varied among orchid species. In the present study, the peloton dimensions were related to cell dimension indicating that the size of pelotons seems to depend on the host cell size.

The development of pelotons and their subsequent lysis follow a time scale pattern (Rasmussen and Whigham, 2002) and the ratio of intact: lysed pelotons varied with species and habitats. Lysis of the fungus in orchid mycorrhizae is generally regarded as a manifestation of plant defense against invasion, and it has also been assumed as important for the transfer of nutrients from the fungus to the host (Smith and Read 2008). However, there is no clear evidence against this hypothesis, except that the growth response to colonization appears to start before the lysis of the pelotons (Rasmussen 2002). In orchids, traditionally two mycorrhizal types have been recognized based on the mode of peloton lysis. In phytophagy, the fungal tips of the intracellular hyphae lyse and the hyphal contents are released into the host cell, whereas in tolypophagy there is an overall collapse and breakdown of the peloton hyphae as observed in our study (Burgeff 1936). In the present study, all the mycorrhizae in orchids examined were of tolypophagy type characterized by the total collapse and disintegration of pelotons. The average ratio of root length with intact to lysed pelotons was higher in terrestrial (2.10) as compared to epiphytic (1.53) and lithophytic (0.74) species. Hence, it appears that terrestrial orchids are less dependent on fungi for their nutrients compared to epiphytic and lithophytic species. The average root length and the extent of cortical region containing the lysed pelotons are higher and compensates for the intact to lysed pelotons ratio. Rasmussen and Whigham (2002) reported that the older roots of several terrestrial orchids contained more root cortical cells with lysed pelotons than the intact (young) pelotons. In addition, the increased presence of intact pelotons indicates the continued presence of active fungal hyphae at any given time. The continued dependence on fungi can be due to the fact that most adult terrestrial orchids have few unbranched roots that are thick owing to a highly developed cortex (Rasmussen 1995). Such a root character provides the root system of terrestrial orchids with a small surface areas which are not usually considered favorable to water and ion uptake (Rasmussen and Whigham 2002).

Root hair characters (number, length, and breadth) of orchids analyzed in this study were not related to the extent of fungal colonization which is in direct contrast with the AM association, where the root characters determine the extent and dependency of the hosts on the mycorrhizal fungus (Brundrett 2002). This disparity between the two mycorrhizal forms may be due to the variation in fungal types involved (Bidartondo et al. 2004). Although green adult orchids possess mycorrhizal fungi in their roots (Rasmussen 2002), the direct uptake of nutrients by fungi and their transport to roots has yet to be conclusively proved as for AM association (Brundrett 2004).

Orchid roots in the present study had moniliform structures resembling those of *Tulasnella calospora* (*Epulorhiza repens*) in the cortical and root hair cells, which clearly indicates that the roots also serve as venues for the reproduction of the fungus as well as for their exit from their root cortex to the rhizosphere (Curtis 1939). During liberation, the fungus does not exit as hyphae, but as moniliform cells or microsclerotia as observed by Senthilkumar and Krishnamurthy (1998b). Furthermore, root hairs containing fungal reproductive structures undergo a characteristic spiral dehiscence as observed in the present study and as also reported by Senthilkumar and Krishnamurthy (1998b).

Traditionally, identification of the orchid mycobionts has involved culture dependent approaches. However, currently culture-independent molecular approaches are employed to identify the mycobionts in roots using fungal specific primers (Kristiansen et al. 2004; Kennedy et al. 2011; Tondello et al. 2012). Although molecular approaches eliminates the time consuming culture step, cultivation based approaches are still essential to establish the true biological entity of the mycobiont and to ascertain their role in the symbiosis (Steinfort et al. 2010; Valadares et al. 2012). Though our findings clearly indicate the widespread occurrence of mycorrhizae in South Indian orchids, further studies on the characterization of the mycobionts would enable us to understand the role of mycobiont in these adult green orchids.

References

Aggarwal S, Zettler LW. 2010. Reintroduction of an endangered terrestrial orchid *Dactylorhiza hatagirea* (D. Don) Soo, assisted by symbiotic seed germination: first report from the Indian subcontinent. Nature Sci. 8:139–145.

Arditti J, Ghani AKA. 2000. Numerical and physical properties of orchid seeds and their biological implications. New Phytol. 145:367–421.

Athipunyakom P, Manoch L, Piluek C. 2004. Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. Kasetsart J Nat Sci. 38:216–228.

Bagyalakshmi G, Muthukumar T, Sathiyadash K, Muniappan V. 2010. Mycorrhizal and dark septate fungal associations in shola species of Western Ghats, southern India. Mycoscience. 51:44–52.

Bates TR, Lynch JP. 2001. Root hairs confer a competitive advantage under low phosphorus availability. Plant Soil. 236:243–250.

Batty AL, Dixon KW, Brundrett M, Sivasithamparam K. 2002. Orchid conservation and mycorrhizal associations. In: Sivasithamparam K, Dixon KW, Barrett RL, editors. Microorganisms in plant conservation and biodiversity. Dordrecht (the Netherlands): Kluwer. p. 195–226.

Baylis GTS. 1975. The magnoloid mycorrhizal and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB, editors. Endomycorrhizas. New York (NY): Academic press. p. 373–389.

Bermudes D, Benzing DH. 1989. Fungi in neotropical epiphyte roots. Biosystems. 23:65–73.

Beyrle HF, Smith SE, Peterson RL, Franco CMM. 1995. Colonization of *Orchis morio* protocorms by a mycorrhizal fungus: effects of nitrogen nutrition and glyphosate in modifying the responses. Can J Bot. 73: 1128–1140.

Bidartondo MI. 2005. The evolutional ecology of mycoheterotrophy. New Phytol. 167:335–352.

Bidartondo MI, Birghardt B, Gebauer G, Bruns TD, Read DJ. 2004. Changing pattern in the dark; isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc R Soc Lond B. 271:1799–1806.

Brundrett M. 2002. Co-evolution of roots and mycorrhizas of land plants. New Phytol. 167:335–352.

Brundrett M. 2004. Diversity and classification of mycorrhizal association. Biol Rev. 79:437–495.

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil. 320:37–77.

Burgeff H. 1936. Samenkeimung der Orchidéen. [Seed germination of orchids]. Jena (Germany): Gustav Fischer. German.

Currah RS, Hambleton S, Smreciu A. 1988. Mycorrhizae and mycorrhizal fungi of *Calypso bulbosa*. Am J Bot. 75:739–752.

Curtis TJ. 1939. The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. Am J Bot. 26:390–399.

Dressler RL. 1993. Phylogeny and classification of the Orchid family. Portland (OR): Dioscoride.

Fitter AH. 1982. Morphometric analysis of root systems: applications of the technique and influence of soil fertility on root system development in two herbaceous species. Plant Cell Environ. 5:313–322.

Gardes M. 2003. An orchid-fungus marriage-physical promiscuity, conflict and cheating. New Phytol. 154:4–6.

Goh CJ, Sim AA, Lim G. 1992. Mycorrhizal associations in some tropical orchids. Lindleyana. 7:13–17.

Hadley G, Williamson B. 1972. Features of mycorrhizal infection in some Malayan orchids. New Phytol. 71:1111–1118.

Henry AN, Chitra V, Balakrishnan NP. 1989. Flora of Tamil Nadu, India. Coimbatore (India): Botanical Survey of India.

Kaliamoorthy S. 2007. Pattern of mycorrhizal infection in the roots of *Aerides maculosum* Lindl. and *Calanthe triplicata* (Willem.) Ames. Mycorrhiza News. 19:14–18.

Kennedy AH, Taylor Dl, Watson LE 2011. Mycorrhizal specificity in the fully mycoheterotrophic *Hexalectris* Raf. (Orchidaceae: Epidendroideae). Mol Ecol. 20:1303–1316.

Koske RE, Gemma JN. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res. 92:486–488.

Kristiansen KA, Freudenstein JV, Rasmussen FN, Rasmusen HN. 2004. Molecular identification of mycorrhizal fungi in *Neuwiedia veratrifolia* (Orchidaceae). Mol Phylogene Evol. 33:251–258.

Kumar R, Kaushik P. 2004. Isolation of a cellulose producing mycorrhizal fungus *Rhizoctonia solani* from *Zeuxine strateumatica* (Linn.) Schltr. J Orchid Soc India. 18:11–108.

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann Bot. 98: 693–713.

Lesica P, Antibus RK. 1990. The occurrence of mycorrhizae in vascular epiphytes of two Costa Rican rain forests. Biotropica. 22:250–258.

Madhaiyan M, Santhanakrishnan P, Pragatheswari D. 2003. Rapid detection and assessment of orchid mycorrhizal colonization in *Vanilla planifolia* Andr. roots. Mycorrhiza News. 14:10–13.

Mc Cormick MK, Whigham DF, O'Neill J. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol. 163:425–438.

Mc Gonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A method which gives an objective measures of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol. 115:495–501.

Misra S. 2007. Orchids of India – a glimpse. Dehradun (India): Bishen Singh Mahendra Pal Singh.

Murugan T, Sathiyadash K, Muniappan V, Muthukumar T. 2010. The mycorrhizal status of south Indian epiphytic orchids. J orchid Soc India. 24:29–33.

Muthukumar T, Sathiyadash K. 2009. Mycorrhizal morphology of Nun's orchid [*Phaius tankervilliae* (Banks ex L' Herit.) Blume.]. Mycorrhiza News. 21:9–11.

Muthukumar T, Uma E, Karthikeyan A, Sathiyadash K, Sarah Jaison, Priyadharsini P, Ishworani C, Muniappan V. 2011. Morphology, anatomy and mycorrhizae in subterranean parts of *Zeuxine gracilis* (Orchidaceae). Ann Biol. 33:127–134.

Peterson RL, Currah RS. 1990. Synthesis of mycorrhizae between protocorm of *Goodera repens* (Orchidaceae) and *Ceratabasidium cereale*. Can J Bot. 68:1117–1125.

Peterson RL, Fraquhar ML. 1994. Mycorrhizas-integrated development between roots and fungi. Mycologia. 86:311–326.

Radhika KP, Rodrigues BF. 2007. Orchid mycorrhizal colonization in *Rhyncostylis retusa* (L.) Blume. Mycorrhiza News. 19:22–23.

Rasmussen HN. 1995. Terrestrial orchids. From seed to mycotrophic plant. Cambridge (UK): Cambridge University Press.

Rasmussen HN. 2002. Recent developments in the study of orchid mycorrhiza. Plant Soil. 244:149–163.

Rasmussen HN, Whigham DF. 2002. Pheonology of roots and mycorrhiza in five orchid species differing in phototropic strategy. New Phytol. 154:797–807.

Richardson KA, Currah RS, Hambleton SM. 1993. Basidiomycetous endophytes from the roots of neotropical epiphytic Orchidaceae. Lindleyana. 8:127–137.

Saha D, Rao AN. 2006. Studies on endophytic mycorrhiza of some selected orchids of Arunachal Pradesh – 1. Isolation and identification. Bull Arunachal For Res. 22:9–16.

Senthilkumar S. 2003. Mycorrhizal fungi of endangered orchid species in Kolli, a part of eastern ghats, South India. Lankesteriana. 7:15–156.

Senthilkumar S, Britto SJ, Krishnamurthy KV, Hariharan C. 2000. Biochemical analysis of mycorrhizal roots of *Aerides maculosum*. Phytomorphol. 50:273–279.

Senthilkumar S, Krishnamurthy KV. 1996. Certain peculiar features of mycorrhizal association in the ground orchid *Spathoglottis plicata* Blume. Mycorrhiza News. 8:9–11.

Senthilkumar S, Krishnamurthy KV. 1998a. A cytochemical study on the mycorrhizae of *Spathoglottis plicata*. Biol Planta. 41:111–119.

Senthilkumar S, Krishnamurthy KV. 1998b. The role of root hair in the mycorrhizal association of the ground orchid *Spathoglottis plicata* Blume. Mycorrhiza News. 10:15–17.

Singh DK. 2001. Orchid diversity in India: an overview. In: Sood A, Pathk P, Sehgal RN, Shekhar N, Sharma M, editors. Orchids: science and commerce, Dehradun (India): Bishen Singh Mahendra Pal Singh. p. 33–65.

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis. 2nd ed. San Diego (CA): Academic press.

Steinfort U, Verdiigo G, Besoain X, Cisternas MA. 2010. Mycorrhizal association and symbiotic germination of the terrestrial orchid *Bipinnulus fimbriata* (Poepp.) Johnst (orchidaceae). Flora. 205:811–817.

Tondello A, Vendramin E, Vallani M, Baldan B, Squartini A. 2012. Fungi associated with the southern Eurasian orchid *Spiranthes spiralis* (L.) Chevall. Fungal Biol. 116:543–549.

Valadares RB, Pereira MC, Otero JT, Cardoso ES. 2012. Narrow fungal mycorrhizal diversity in a population of the orchid *Coppensia doniana*. Biotropica. 44:114–122.

Vij SP, Sharma M. 1988. Mycorrhizal association in North Indian orchidaceae: a morphological study bibliotheca. Mycologia. 91:467–503.

Wang B, Qiu YL. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza. 16:299–363.