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# ORCHIDS - MYCORRHIZAE

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**Abstract**

The present paper includes data on orchid flora available from Andhra Pradesh, their endemics, and also reviews the orchid-mycorrhizal status along with author's data on orchidoid mycorrhiza.

## Introduction

PLANT DIVERSITY is quite amazing in structure, function and economic value. Orchids are not only important in medicine but also add beauty and charm to the landscape. IUCN report (Anonymous, 1996) has recorded 20,000 orchid species in the world. Most of the orchids are distributed between 30° North and South of Equator. High rainfall, relative humidity, temperatures, substratum, latitude and altitude affect distribution of orchid flora. Greater diversity of orchids is found in the tropics. Orchidaceous plants occur as epiphytes, lithophytes, terrestrials and as saprophytes. Charaka Samhita and other ancient books have recorded the medicinal value of orchids and awareness of these plants dates back to Vedic period. Classical work of Hortus malabaricus by Van Rheede (1678-1693) introduced Indian orchids. About 1296 species belonging to 155 genera of orchids are recorded from India (Singh *et al*., 2019) and constitutes 6% of the world orchids. Orchids account for nearly 8% of angiosperm species diversity (Chase *et al*., 2015; Willis, 2017). The predominantly occurring orchids are *Bulbophyllum,* followed by *Dendrobium, Eria, Habenaria, Oberonia* and others. The Eastern Himalayas represent 870 species of orchids belonging to 160 genera and 290 species of orchids are known to occur in Western Himalayas. About 380 species are distributed in peninsular regions. Andaman and Nicobar Islands represent 115 genera.

Orchid genera have become important as commercial product because of their great potential in horticulture. There are more than one lakh registered hybrids all over the world representing *Aerides, Calanthe, Coelogyne, Cymbidium, Dendrobium, Vanda etc*. Orchids are not only known for their ornamental value but many of them are used in traditional medicine as well (Pathak *et al.,* 2010). The leaves of *Vanda tessellata* and roots of *Acampe praemorsa are* used to cure rheumatism (Ahmed *et al*., 2002 and Kumar *et al*., 2000). Whole plants of *Aerides odorata* are known to be used in curing tuberculosis (Dash *et al*., 2008). Leaves and stem of *Cleisostoma williamsonii* have found place in Ayurveda for curing bone fractures, while root extract of *Calanthe triplicata* is used in controlling diarrhoea and tooth cavities. Tubers of *Epipactis latifolia* are used to cure nervous disorders, while tuber extract of *E. nuda* is said to be useful as a blood purifier. The flowers of *Dendrobium hookerianum* yield dye whereas whole plant of *Habenaria clavigera* may find place as an anti AIDS agent.

The orchid flowers with their varied colors, sizes and beauty attract the attention of entrepreneurs, floriculturists, pharmacists, amateur orchid lovers, and others. Nowadays, orchid growing has become a commercial project. It is a multi-million dollar business in Netherlands, California, Thailand, and Singapore. The orchids represent 8-10% of the international market. At present, India represents 1% of orchid market in the world indicating that orchid industry is still at its embryonic stage. The over exploitation and habitat destruction made some species of *Dendrobium, Paphiopedilum, Vanda etc*. rare and led to their entry into the Red Data Book. Nearly 147 species of orchids in the Indian sub continental region are under threat. Protection of natural habitats, encouraging biosphere reserves, multiplication of orchids in botanical gardens, orchidaria and other measures will help in conserving the orchid flora. Setting of seed/gene banks and multiplication through tissue culture (Anuprabha *et al.,* 2017; Arora *et al.,* 2016; Decruse and Gangaprasad, 2018; Kaur *et al.,* 2017; Pathak *et al.,* 2017; Sibin and Gangaprassad, 2016) will ensure conservation. The Botanical Survey of India has brought several threatened species of orchids under cultivation at orchidaria in Howrah, Shillong and Yercaud. Orchids contribute to the rich plant wealth of India as they have medicinal value, besides being important in floriculture. Orchid taxa from Andhra Pradesh are represented by 78 species belonging to 36 genera. These include

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*Acampe, Aerides, Bulbophyllum, Cymbidium, Dendrobium, Eulophia, Habenaria, Liparis, Nervilia, Oberonia, Pholidota,* and *Vanda. Habenaria ramayyana* is an endemic, while *Geodorum densiflorum* and *Vanda tessellata* are common orchids of Andhra Pradesh (Raju *et al*., 2008).

## Orchid Mycorrhizae

Orchidoid mycorrhizae are characteristic of approximately 20,000 species in the family Orchidaceae. Orchids pass through an obligate mycoparasitic stage in the course of normal development. Orchid seeds are dust like, having minute spherical embryo with no endosperm and a thin seed coat. The seed cannot grow until a fungus has infected it. Fungal contact makes the orchid seed to grow into a protocorm. Once the protocorm has grown to a sufficient size, the shoot starts to grow, producing a structure termed the mycorrhizome, this helps in seedling growth. Orchids grow in association with a number of fungi: *Armillaria mellea, Epulorrhiza*, and *Rhizoctonia solani* (Hajong and Kapoor, 2016; Hossain *et al*., 2013) which are well-known plants pathogens. Lab tests have shown that an embryo paired with the non suitable fungus can be quickly killed by the fungus. Thus, it is not surprising that orchids have specialized cells and structures for mycorrhizae. In adult plants, much of the plant body contains antifungal phytoalexins (Hadley, 1982) and this is presumably true of embryos and protocorms as well. In orchid embryos, cells near the suspensor end enlarge and undergo nuclear replication repeatedly, and then these cells are infected by the fungus, which forms a tightly coiled hyphal structure termed as a *peloton*. As with all endocellular mycorrhizae, the fungus does not actually penetrate the cell membrane. The dynamic pelotons remain there for a longer time and then get digested by the plant. The empty cells might be reinfected or the plant may enlarge new cells, so that the band of mycorrhizae moves progressively down the plant as it grows. Few orchids are infected seasonally and adults may be non-mycorrhizal for some part of the year. Others are continuously infected, but the infection is confined to enlarged cells. The roots often become mycorrhizal, and in some species, mycorrhizal root fragments can grow into new plants when they are separated from the parent rhizome. There is an enormous variation in patterns of organ growth and mycorrhiza across orchid genera and the variation across the family is likely to be one of the reasons that is why orchids have been able to live in so many different habitats throughout the world. There are a number of achlorophyllous orchids that remain mycotrophic throughout their lives but this can be seen as retention of juvenile traits.

A number of fungi found in orchid mycorrhizae belong to the anamorphic genus *Rhizoctonia*. They include teleomorphic genera *Sebacina, Thanatephorus*, *Tulasnella*. Non-chlorophyllous orchids often seem to parasitize basidiomycetes, including members of *Armillaria, Coriolus, Favolaschia, Fomes, Hymenochaete, Marasmius, Thelephora*, *Tomentella,* and *Xerotus*. Fungi obtain carbohydrates outside of orchid from a variety of other sources. Many of these fungi are saprophytic, able to break down dead organic matter in the soil. *Armillaria mellea*, *Rhizoctonia solani/ Thanatephorus cucumeris* are better known as plant pathogens while others are ectomycorrhizal in the roots of some trees. Orchid taxa vary in their fungal specificity and the embryos of photosynthetic orchids appear to form mycorrhizal associations with a fewer fungal species than the adults. It is relatively common to find many endophytic fungal species in adult orchids. However, fungal isolates from adults have failed to produce mycorrhizal associations with embryos of the same species. In the non-chlorophyllous orchids, all available evidence suggests that the plants are highly host specific, parasitizing only one or a few fungal species. Often, these plants are parasitized by ectomycorrhizal fungi so that the carbohydrates that the orchid gains comes from the trees surrounding it. Kaushik (1983) reported mycorrhizal association in 53 species of the orchids showing fungal pelotons and hyphae in transections in cortical cells of roots with photomicrographs and drawings; and elongated cells packed with mycorrhizal pelotons and septate hyphae in photomicrographs of longitudinal sections prepared by microtomy of roots of *Calanthe gracilis*.

The fungal partners are generally able to break down complex organic materials; the orchids that grow with them are able to tap unusual substrates for nutrients including the bog peat, highly calcareous soils, and the dust and debris on tree branches. Indeed, minute and highly dispersible orchid seeds and mycotrophic habit are undoubtedly the features that made them successful as epiphytes. About 70% of the orchid species are epiphytic and they comprise perhaps two-thirds of epiphytic vascular plant species.

Vij *et al*. (2002) have reviewed the status of orchidoid mycorrhiza in techniques to investigate them in an in-depth manner. Vij and Sharma (1983, 1988) have studied the mycorrhizal association in North Indian orchidaceae and mycorrhizal endophytes. Further, Vij *et al*. (1985) have elaborately documented mycorrhizal endophytes of *Spiranthes lancea*. Sharma *et al*. (1988) have presented status report on mycorrhizal endophytes in some orchids. Singh and Varma (2000) have presented an account on orchidaceous mycorrhizal fungi. Varma

(1994) has shown beneficial role of mycorrhizal fungi in the early establishment of orchid seedlings raised from tissue culture techniques. Katiyar *et al*. (1986) have reported mycorrhizal association in terrestrial orchids. Kumar and Krishnamurthy (1998) also conducted the cytochemical study on the mycorrhizae of *Spathoglottis plicata.* Kaushik and Pal (2011) described identification features, classification, and cultural characteristics of various species of *Rhizoctonia*. Kaushik and Pal (2012) also assessed the lignin degrading capacity of *Rhizoctonia solani* isolated by them from *Vanda testacea* and carried qualitative and quantitative estimation of degradation caused by mycorrhizal fungus. Rajkumar and Kaushik (2007) isolated a cellulose producing mycorrhizal fungus *Rhizoctonia solani* from *Zeuxine strateumatica*.

Orchids are in the association with mycorrhiza in all the natural habitats. They depend on mycorrhizal fungi either throughout their life cycle or at least until the initial establishments of seedlings (Weber and Webster, 2001). Orchids have been described as mycotrophs (fungus-fedders) by Zettler (1997). The phenomenon of mycotropism in terrestrial orchid species has been documented. Voluminous data has been published on the natural infection that occurs in orchids; however work on tropical species is very scarce (Hadley, 1984).

## Mode of Fungal Entry and Colonization

The transverse sections of infected roots of *Acanthephippium bicolor* showed that the fungal entry is through the distorted portion of root epidermal cells. The fungal hyphae entered the velamen tissues before reaching the exodermal region. Following this, the fungal hyphae were found to enter the cortical region through the passage cells, which were not ligated as in the other cells of the exodermis. This was confirmed cytochemically using toluidine blue O (Gahan, 1984). Unlike in *Acanthephippium bicolor,* the mode of entry was through root hair. The fungal entry through the root hair has been reported by Senthilkumar and Krishnamurthy (1998) in *Spathoglottis plicata* and by Weber and Webster (2001) in *Dactylorhiza maculata* sp. *ericetorum.* The rest of the process that leads fungal hyphae to enter the cortical region was similar to the manner as observed in *Acanthephippium.* The fungal colonization began in the inner cortical cells.

The colonization of the fungus in the host root system was calculated as per the formulae given by Hadley and Willamson (1972). The mean percentage of colonization was recorded from the infected zone of young and old roots. The results are presented in Table 1.

Tightly interwoven coils called pelotons formed are considered to be the most distinctive character of an orchid mycorrhiza (Currah and Zelmer, 1992) and reflect the establishment of a successful, stable symbiosis (Zettler, 1997). Fungal colonization was also noticed at times in a few passage cells of exodermis. The colonization usually began in the inner cortical cells. The fungal hyphae were also observed in the outer cortical region. In the present study, the transverse section of *Paphiopedilum villosum* showed that pelotons occupied the entire cell cavities, particularly in the inner cortex region. Similar observations were made in *Acanthephippium bicolor.* The pelotons were densely stained with toluidine blue O. The fungal hyphae extending from the matured pelotons were found to be finer. The cortical zone having matured pelotons forms the digestion layer (Burgeff, 1959). Unlike the digestion layer, the early stages of fungal colonization appeared to be in loose aggregates of hyphae. Similar observations were made in *Spathoglottis plicata* (Senthilkumar and Krishnamurthy, 1998). Cytochemical study with toluidine blue O (McCully, 1966) showed positive results for high DNA content and the results were similar to Senthilkumar and Krishnamurthy (1999). The staining done using coomassie brilliant blue showed positive staining fungal hyphae, indicating high protein content. The digestion of fungal pelotons in the cortical cells, especially in *Acanthephippium bicolor,* showed the release of lipid droplets. This was positively stained by Nile blue sulphate. However, the digesting pelotons in *Paphiopedilum villosum* did not show the presence of lipids. The degradation of lipids and polyphosphates was observed in the degenerating hyphae of *Platanthera* (Richardson *et al*., 1992) and in *Spathoglottis plicata* (Senthilkumar and Krishnamurthy, 1998). The tropical epiphytic orchids are less dependent on mycotrophy

Table 1. Mycorrhizal infection of cortex tissue in *Acanthephippium bicolor* and *Paphiopedilum villosum.*

| Parameters | *Acanthephippium bicolor* | | *Paphiopedilum villosum* | |
|---|---|---|---|---|
| | Young root | Old root | Young root | Old root |
| Mycorrhizal Colonization (%) | 17 ± 5 | 25 ± 1 | 78 ± 4 | 89 ± 6 |
| Undigested Pelotons (%) | 94 ± 1.0 | 93 ± 0.1 | 92 ± 1 | 96 ± 1 |
| Digested Pelotons (%) | 6 ± 0.5 | 6 ± 0.4 | 9 ± 1.1 | 4.5 ± 1.0 |

due to availability of sunlight throughout the year (Wang *et al*., 2011), the mycorrizal association in *Acanthephippium bicolor* is perhaps an alternative nutritional requirement developed by the plant to enhance its survival. The presence of pelotons in older roots of both *Acanthephippium bicolor* and *Paphiopedium villosum* may be attributed to the fact that the orchids achieve a balance between digestion and re-infection (Fig. 1 A-F).

## Host Influence

Orchid mycorrhiza also have wall associated exocellular acid phosphatase activity which disappears within host cells but in contrast to ericoid mycorrhiza, the role of the latter modulating the fungal enzyme activity has not been investigated. Cytochemical studies have provided evidence that when orchid endomycorrhizal fungi invade host cells, they produce active polyphenoloxidases which accumulate in the host fungus interface (Salome *et al*., 1983). These enzymes are likely to promote degradation or inactivation of fungistatic phenolic (phytoalexins) which are frequent in orchids and accumulate with mycorrhizal infection. A controlled balance between phytoalexin production and its inactivation by the fungus is no doubt a stabilizing factor in maintenance of the orchid symbiosis.

## Post-Infection Growth Stimulus

Stimulation of growth can take place before intracellular lysis of the endophyte occurs. In the absence of external nutrients, infecting hyphae are rapidly lysed and little or no growth stimulus occurs. The hypothesis is posed that digestion of the endophyte is a defense reaction not a pre-requisite for a growth stimulus and that nutrients are transferred across the living fungus-host interface and stimulate growth (Hadley and Williamson, 1971).

## Genomics

The mitochondrial ribosomal subunit (Ls) DNA was used to identify the orchid mycorrhizal fungi found in roots of *Dactylorhiza majalis.* The gene amplified using DNA extracted from single peloton obtained from fresh and silica gel dried roots. Furthermore, sequencing a variety of well-characterized orchid isolates expanded the fungal database of the mitochondrial ribosomal Ls DNA. Polymerase chain reaction product length variants present in *D. majalis* were sequenced and identified using the expanded database. This analysis revealed two different peloton-forming fungi in samples from *D. majalis,* which some times occurred together as a single or two taxa peloton within the same cortex cell. The first taxon belonged to the genus *Tulasnella* and the

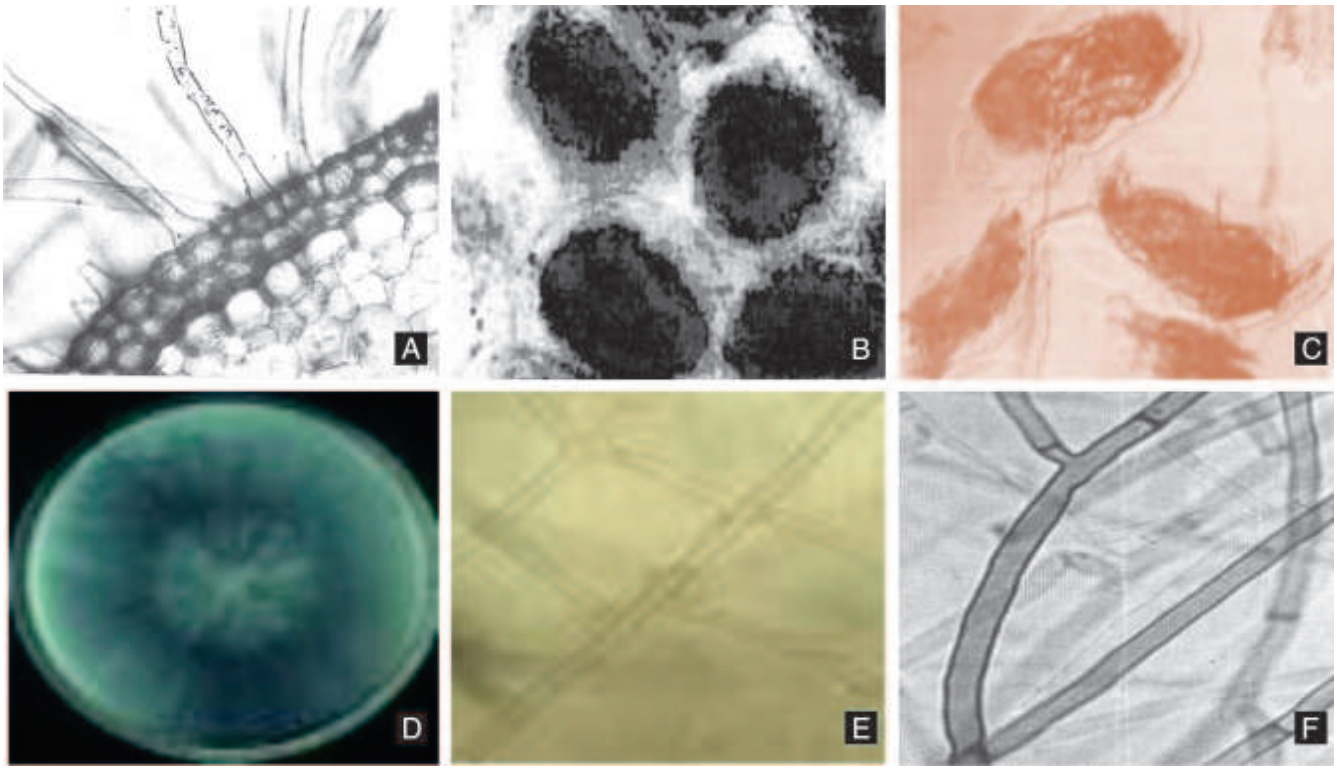


Fig. 1 A-F. Orchid mycorrhiza in *Acanthephippium bicolor*: A-C, Entry of fungus into the root followed by peloton formation in the mid cortex; D, Culture plate of an orchid mycorrhizal fungus; E, Hyphae of *Rhizoctonia*; F, Fungal isolate from root.

second taxon was distantly related to bacteria (Kristian *et al*., 2001). Mycorrhizal associations in the terrestrial and aerial roots of 20 epiphytic orchid species including *Arundina graminifolia* and *Dendrobium crumentum* were examined. The results showed that terrestrial orchids harbour extensive mycorrhizal fungi. The degree of association depends on the distribution and density of fungal flora in the environment. Free-hanging aerial roots are devoid of any fungus. It is thus possible to use aerial root explants for micropropagation (Goh *et al*., 1992). *Rhizoctonia* like fungus with 144 species or variants of terrestrial orchids from the SouthWest region of Western Australia was studied in terms of abundance and location of endophyte in host tissues, seasonality of infection, and fire ecology of the endophyte (Ramsay *et al*., 1996).

## Role of Mycorrhizae in Orchids

The existence of mycorrhiza in orchids was apparently first noted in 1824 by the German naturalist Heinrich Link Bernard (1899) who showed root fungus association in the seedling of *Neottia nidus-avis*, almost all fungi isolated from orchids have been assigned to the form genus *Rhizoctonia (*Arditti, 1982; Burgeff, 1959) and some species bearing clamp connections were also isolated. These fungi included *Corticium catonii* and *Marasmius coniatus var*. *didymoplexis.* Masuhara and Katsuya (1989) investigated the effect of *Rhizoctonia repens* and *R*. *solani* on seed germination and early growth of some terrestrial orchids. Ramakrishna *et al*. (1991) have discussed at length the role of mycorrhiza in orchids.

Orchid endophytes utilize a variety of carbon sources and require all the essential minerals by degrading various substrates, through the production of various enzymes (Hadley and Williamson, 1972; Smith, 1974). Indeed, some orchid endophytes are found to be soil saprophytes and many others may be parasitic (Warcup, 1975) on a variety of hosts (*e.g*. *Armillaria mellea, Ceratobasidium cornigerum*, *and Thanatephorus cucumeris).* Some mycorrhizal fungi may be host specific (Burgeff, 1959).

There are two stages of infection in the life cycle of many orchids; i) primary infection of the germinating seedling and ii) the reinfection of the new roots of the adult. Living epidermal hair cells and basal cells of seeds are the sites of infection (Burgeff, 1959) then hyphal clusters called pelotons are formed by the hyphae which are thinly enveloped by the host cytoplasm and subsequently surrounded by the encasement layer. During later stages, the hyphae start to degenerate, collapse, become disorganized and are digested by the host. All orchids require an external source of nutrients for seed germentation and development. The seeds are microscopic and consist of the bare essentials, a seed coat modified for buoyancy, an embryo of 8 to 100 cells and rarely a small amount of undeveloped endosperm. Therefore, the presence of endophytic fungi is very much essential under natural conditions (Harley, 1989).

The asymbiotic germination of orchid seeds on appropriate culture media has revolutionized the commercial orchid growing and hybridization, as every viable seed can be turned into new seedling. Growth of orchids using asymbiotic method have the disadvantage of creating very artificial environment and effecting seedling morphology to some extent but has the advantage of providing a controllable and reproducible milieu for young plants. Yet, another problem with tissue culture of many orchids is that some genera (*e.g. Phalaenopsis, Vanda)* are monopodials that seldom produce lateral growth. Consequently, growers are reluctant to use shoot meristem culture fearing that the removal of the apical dome will lead to eventual death of the plant. Hence, there is always a strong need for propagation of orchids through seeds for commercial purpose and for conservation of orchid wealth which is now at the verge of extinction.

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