ORCHID MYCORRHIZAE: THE FORGOTTEN BIOLOGICAL WORLD

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Orchids are a treasured resource and valuable wealth for botanists, floriculturists, ethonobotanists, ecologists and evolutionary biologists. They are also valued by the product of perfect plant – fungi and plant – animal symbiosis/ mutualisms. The medicinal value and possible orchid flower export for the sub-continent are not yet adequately explored and estimated. In Andhra Pradesh there are 76 orchids reported. More than half are epiphytes while majority are geophytes. Around 18500 species and 1.5 lakh hybrids are reported globally. Fungal stimulus is required for seed germination. Fungal infections lead to endotrophic and intracellular association. Pelotons are formed in the host cells. The digestion of fungus in host cells results in the utilization of nutrients by the host. The fungus gets carbohydrates from the orchid while plant gets nutrients. Mycotrophic way of life has been established in orchids. Fungi like *Armillaria, Rhizoctonia, Corticium, Marasamius, Thanetophora* and *Glomus* have been found to form symbiotic association with orchids. Recently, the growing of orchid seed with the help of culture media has revolutionized the commercial orchid production. In view of problems faced by the orchid growers, there is a need for propagation of orchids through seeds for commercial green house crops and for conservation of orchid wealth which are now at the verge of extinction. All members of the family orchidaceae are thought to form orchid mycorrhizas. The symbionts in roots of an orchid in the field are considered to be ecological symbionts of the plant. A great diversity of fungi can germinate seed in the laboratory than is found in the field. This larger group has been referred to as the physiological symbionts. It is also possible to isolate physiological symbionts from soil adjacent to functional plants in the field but they are not always inside the roots. The fungus recolonizes the plant each year and continues to benefit the host. With more than 18000 species, the orchid family is large and diverse. The fungi associated inside beneficial symbionts are also fungi of no known function. Most of the fungi associated are difficult to identify as they are in vegetative stage or belong to anamorphic fungal genera. 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 mycorrhizae.

Keywords : Diversity, genomics, mycorrhiza, orchid, symbiosis

Orchidoid mycorrhizae, are characteristic of approximately the 20,000 species in the orchidaceae family. Orchids pass through an obligate mycoparasitic stage in the course of normal development. Orchid seeds are dust like, having tiny 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 seedling to grow into a protocorm. Once the protocorm has grown to a sufficient size, the plant shoot starts to grow, producing a structure termed the mycorrhizome, this helps in plant growth. Orchids grow in association with a number of fungi, including species *(Rhizoctonia solani, Epulorrhiza* and *Armillaria mellea)* which are well-known plant pathogens, and lab tests have shown that an embryo paired with the wrong 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, 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 (Saha *et al.* 2006).

These cells are infected by the fungus, which forms a tightly coiled hyphal structure termed a peloton. As with all endocellular mycorrhizae. the fungus does not actually penetrate the cell membrane. The dynamic pelotons remain for longer time, and then get digested by the plant. The empty cells may 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 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.

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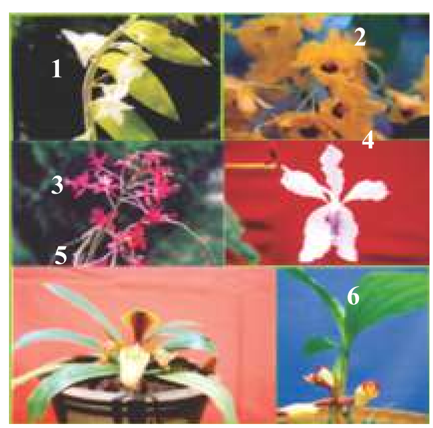
There is enormous variation in patterns of organ growth and mycorrhizal across orchid, genera, and the variation across the family is likely one reason why orchids have been able to live in so many different habitats throughout the world. There are a number of achlorophyllous orchids that remain mycoparasites throughout their lives, but this can be seen as retention of juvenile traits (Senthil Kumar 2003). A number of fungi found in orchid mycorrhizas belong to the anamorphic genus *Rhizoctonia.* They include teleomorphic genera *Thanetophorus, Ypisilonidium*, *Sebacina* and *Tulasnella.* Non-chlorophyllous orchids often seem to parasitize basidiomycetes, including members of *Marasmium, Xerotus, Hymenochaete, Armillaria, Fomes, Favolaschia, Coriolus, Thelephorus* and *Tomentella.* Fungi obtain carbohydrates from outside the orchid, from a variety of sources. Many of these fungi are saprophytic, able to break down dead organic matter in the soil. *Rhizoctonia solani/Thanetophorus cucumeris* and *Armillaria mellea* are better known as plant pathogens. While others are ectomycorrhizal. Orchid taxa vary in their fungal specificity, and the embryos of photosynthetic orchids appear to form mycorrhizas with fewer fungal species than the adults do. It is relatively common to find many endophytic fungal species in adult orchid mycorrhizae. However, fungal isolates from adults have failed to produce mycorrhizae with embriyos 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 parasitize ectomycorrhizal fungi, so that the carbohydrates the orchid gains come from the trees surrounding it (Vij and Sharma 1988).

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, orchid's tiny, highly dispersable seeds and mycotrophic habit are undoubtedly why they are so successful as epiphytes. Around seventy percent of orchid species are epiphytic, and they comprise perhaps two-thirds of epiphytic vascular plant species (Rasmussen 2002).

Plant diversity is quite amazing in structure, function and economic value. Orchids not only form important in medicine but also add beauty and charm to the landscape. IUCN report (Annonymous 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 the orchids being in tropics.

Orchidaceous plants occur as epiphytes, lithotrops, and terrestrials and as saprophytes. Charaka Samhita and other ancient books have recorded the medicinal value of orchids and awareness of orchids dates back to Vedic period. Classical work of *Hortus malabaricus* introduced the Indian orchids. About 1200 species belonging to 177 genera of orchids are recorded from India and constitutes 6% of world orchids (Fig. 1). It also represents 7% of the flowering plants. The predominantly occurring orchids being *Bulbophyllum*, followed by *Dendrobium, Habeneria, Eria, Oberonia* and others .



Figures 1-6 : General view of different orchids
1. *Denobrobium aqueum.* **2**. *Denobrobium fimbriatum.* **3**. *Vanda coerulea.* **4**. *Epidendrum radicans.* **5.** A*canthophippium bicolor.* **6**. *Paphiopedilum villosum.*

The eastern Himalaya represents 870 species of orchids belonging to 160 genera. 290 species of orchids are known to occur in western Himalayas. About 380 species belonging to various genera are distributed in peninsular regions. Andaman and Nicobar represent 115 genera. Orchid genera have become important as commercial product because of their great potential in horticulture. There are 150,00 registered hybrids all over the world representing *Aerides, Calanthae, Coelogyne, Cymbidium, Dendrobium, Vanda* etc. Orchids are not only known for their ornamental value but many of them are used in traditional medicine. The leaves of *Vanda tessellate* were used to cure rheumatism. Similarly roots of *Acampe praemorsa* are used in rheumatism. Whole plants of *Aerides odorata* is known to be used in curing tuberculosis. Leaves and stems of *Cleisosima williamsonii* have found place in Ayurveda for curing bone fractures, while root extracts of *Calanthe triplicate* is used in controlling diarrhea and tooth cavities. Tubers of *Epipactis latifolia* is used to control nervous disorders, while tuber extract of *E. nuda* found useful as blood purifier. The flowers of *Dendrobium hookerianum* yield dye. The whole plant of *Habenaria clavigera* could find place as anti AIDS agent.

The range of flowers, their colours and beauty attracted the attention of aesthesists, business people, floriculturists, pharmacetists, amateur orchid lovers and others. Now orchid growing become a commercial project. It is a multi million dollar business in the Netherlands, California, Thailand, Bangkok 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 in embroyonic state. The over exploitation and habitat destruction made some species of *Dendrobium, Daphiopedihum, Vanda* etc to become rare and entered into red data book. It seems that approximately 147 species of Indian sub-continental orchids are under threat. Protection of natural habitat, encouraging biosphere reserve, multiplication of orchids in botanical gardens, orchidaria and other measure will help to conserve orchid flora. Setting of seed/gene banks and multiplication through tissue culture will ensure conservation. The Botanical Survey of India has brought several species of threatened species of orchids under cultivation at orchidaria in Howrah, Shillong and Yercand.

Orchids from Andhra Pradesh

Orchids form 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 are represented by *Acampe, Aerides, Bulbophyllum, Cymbidium, Dendrobium, Eulophia, Liparis, Nervilia, Oberonia, Pholidola, Vanda* and others. *Habenaria ramayyana* is endemic, while *Vanda tessellate* and *Geoderma densiflorum* are common orchids of AP. (Raju *et al.* 2008).

Mycorrhizal association in orchids

Vij *et al.* (1985) have reviewed the status of orchidoid mycorrhiza at techniques to investigate in an indepth manner. Vij and Sharma (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* (S.W) Baker. Katiyar *et al*. (1988) have presented status report on mycorrhizal endophytes in some orchids. Kullaiyan *et al.* (2012) have presented an account on orchidaceous mycorrhizal fungi. Senthil Kumar (2001) has shown beneficial role of mycorrhizal fungi in the early establishment of orchid seedling raised from tissue culture techniques. Katiyar *et al*. (1988) have reported mycorrhizal association in terrestrial orchids. Senthil Kumar (2001) has conducted for-the first time the cytochemical study on the mycorrhizae of *Spathiglottis plicata.*

Host influence

Orchid mycorrhizae 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 phenolics (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.

Authors observations

Orchids are in the possession of mycorrhiza in all natural situations. They depend on mycorrhizal fungi either throughout their life cycle or at least until the intial establishments of seedlings . Orchids have been described as mycotrophs (fungus-fedders) and the phenomema of mycotropism as the part of terrestrial orchid species has been documented. Voluminous data has been published on the natural infection in orchids, however work on tropical species is very scarce (Hadley and Williams 1972).

Mode of fungal entry and colonization

1.The transverse sections of infected roots of *Acanthophippium* showed that the fungal entry through the distorted portion of root epidermal cells. The fungal hyphae enter velamen tissues before reaching the exodermal region

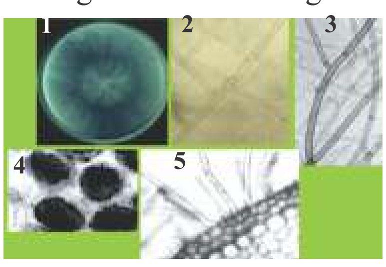


Fig. 2 (1-5) Entry of fungus in the host root system

Orchid Mycorrhiza. 1. Culture plate of an orchid mychorrizal fungus. 2. Hyphae of *Rhizoctina.* 3. Fungal isolate from root (*Acanthophippium*). 4. *Acanthophippium* showing fungal clumps in mid cortex & strach grains in uninfected inner cortex. 5. Profusehair root - serving as entry points of the fungus.

2. Following this the fungal hyphae were found to enter the cortical region through the passage cells, which are not ligated as in the other cells of the exodermis. This was confirmed cytochemically using toluidine blue O. Unlike in *Acanthophippium* the mode of entry was through root hair. The fungal entry through the root hair has been reported by Senthilkumar (2001) in *Spathoglotlis plicata* and in *Dactylorhiza maculate* ssp *ericetorum.* The rest of the process that leads fungal hyphae to enter the cortical region was in the manner as observed in *Acanthophippium.* The fungal colonization begins in the inner cortical cells (Fig. 2).

The colonization of the fungus in the host root system was calculated as per the formulae given by Hadley and Willamson (1971). The results are presented in Table 1. The mean percentage of colonization was recorded from the infected zone of young and old roots.

3.Tightly interwoven coils called peletons formed are considered to be the most distinctive characteristic of an orchid mycorrhiza and reflect the establishment of a successful, stable symbiosis. Fungal colonization was also noticed at times in a few passage cells of exodermis. The colonization usually began in the inner cortical cells.

4.The fungal hyphae were also observed in the outer cortical region. In the present study, the transverse section of *Paphiopedium* showed that pelotons occupied the entire cell cavities, particularly in the inner cortex region. Similar observations were made in *Acanthophippium*. 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 peltons forms the digestion layer (Burgeff 1959). Unlike the digestion layer, the early stages of fungal colonization appeared to be in loose aggregate of hyphae (Fig.3). Similar observations were made in *Spathoglottis plicata* (Senthilkumar and Krishnamurthy 1999). Cytochemical study with toluidine blue O (Mc Cully 1966) showed positive results for high DNA content and the results were similar to Senthil Kumar and Krishnamurthy (1999).

5.The staining using coomassie brilliant blue showed positive staining fungal hyphae, indicating high protein content. The digestion of fungal pelotons in the cortical cells, especially in *Acanthophippium,* showed the release of lipid droplets. This was positively stained by Nile blue sulphate. However, the digesting pelotons in *Paphiopedium* did not show the presence of lipids. The degradation of lipids and polyphosphates was observed in the degenerating hyphae of *Platanthera* and in *Spathogloltis plicata* (Senthilkumar and Krishnamurthy 1999). The tropical epiphytic orchids are less dependent on mycotrophy due to availability of sunlight throughout the year, the mycorrhizal association in *Acanthophippium* is perhaps an alternative nutritional requirement developed by plant to enhance its survival. The presence of peletons in older roots of both *Acanthophippium* and *Paphiopedium* may be attributed to the fact that the orchids achieve a balance between digestion and re-infection (Table 1) .

Table 1 : Mycorrhizal infection in two orchids

Parameters	*Acanthophippium bicolor*		*Paphiopedium villosum*	
	Young root	Old root	Young root	Old root
Mycorrhizal Colonization (%)	17±5	25±1	78±4	89±6
UndigestedPelotons (%)	93±1	92±1	92±1	96±1
Digested Pelotons (%)	6±0.5	6±0.4	9±1.1	4.5±10

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 pelotons obtained from fresh and silica gel dried roots. Furthermore, sequencing a variety of well-charectersized orchid isolates expanded the fungal database of the mitochondial ribosomal Ls DNA. Polyrnerase chain reaction product length variants present in *Dactylorhiza majalis* were sequenced and identified using the expanded database. These analyses revealed two different peloton-forming fungi in samples from *Dactylorhiza majalis,* which some times occurred together as a single two taxa peloton within the same cortex cell. The first taxon belonged to the genus *Tulasnella* and the second taxon was distantly related to bacteria (Kristiansen *et al.* 2001, Alessia Luca *et al.* 2014). Mycorrhizal associations in the terrestrial and aerial roots of 20 tropical orchid species *Arundina graminifolia, Dendrobium crumentum* and hybrids were examined. The results showed that tropical orchids harbor 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 terrestraial orchids from the southwest region of Western Australia were studied in terms of abundance and location of endophyte in host tissues seasonality of infection, and ecology of the endophyte (Ramsay *et al.1*996).

Post infection growth stimulus

Evidence suggests that 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.

Role of mycorrhizae in orchids

1. The hypothesis is posed that the endophyte is a defence reaction not a pre-requisite for a growth stimulus and that nutrients are transferred across the living fungus-host interface and stimulate growth .The existence of mycorrhiza in orchids was apparently first noted in 1824 by the German naturalist Heinrich Link. Bernard (1899) 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, C. octosperum, Marasmius coniatus* var *didymoplexis* and *Gastrodia* resamoides.

Masuhara and Katsuya (1989) investigated the effect of *Rhizoctonia solani* and *R. repens* on seed germination and early growth of some terrestrial orchids. (Martin *et al.* 2004).

2. 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 1971). Indeed some orchid endophytes are found to be soil saprophytes and many others may be parasitic on a variety of hosts *(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; primary infection of the germinating seedling and 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. (Esebastian *et al.* 2014).

3. All orchids require an external source of nutrients for seed germination 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 (Hadley and Williamson 1972).

4. Recently the growing of orchid seed with the help of culture media has revolutionized the commercial orchid growing and hybridization, as every viable seed can be turned into new plant. This has an advantage of providing a controllable and reproducible milieu for young plants. (Fig. 3). Yet another problem with tissue culture of many orchids is that some genera *(Phalaenopsis, Vanda)* are the strong monopodial growers that seldom produce lateral growth. Consequently growers are reluctant to grow tissue culture plants 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 greenhouse crops and for conservation of orchid wealth which are now at the verge of extinction (Singh 1986). Ramakrishnan *et al.* (1991) have discussed at length the role of mycorrhiza in orchids.

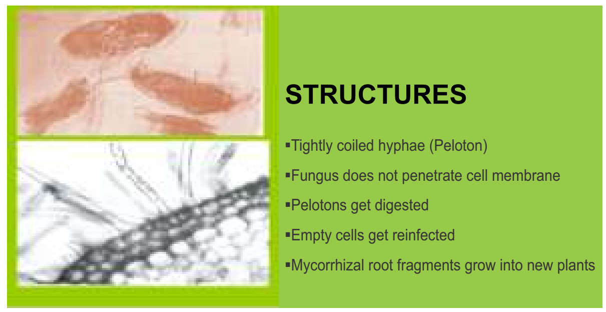


Fig. 3. Digestion of peltons and formation of mycorrhizal root segments.

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