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Mini-Review

A Review of Orchid Mycorrhizae in Korea

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Orchids are evolutionally known to be the most advanced plants in the order Liliales, and comprise approximately 1,000 genera and 35,000 species worldwide. In Korea, more than 110 species of Orchidaceae have been reported to be cultivated or to be collected in the wild. Orchids are mostly dependant on orchid mycorrhizae (OM) throughout or in part of their life cycle. The OM endomycorrhizae belonging to basidiomycetes or rarley ascomycetes are needed for orchid seed germination. Various fungi, including plant pathogenic, antagonistic and symbiotic fungi, were isolated from the roots of orchid native to Korea. The OM fungi collected from the roots of *Cymbidium goeringii* were three species of *Rhizoctonia* namely, *R. repens* (anamorph state of *Tulsanella repens*), *R. endophytica* (*Ceratobasidium cornigerum*), and an unidentified species (possibly an anamorph of *T. calospora*). These symbiotic fungi induced peloton in the cortical cells of orchid roots, and differed biologically and in 18s rDNA sequences from plant pathogenic *Rhizoctonia* species. Also, the mycorrhyzal fungi enhanced the orchid root absorption of nitrogen sources and minerals from the soil. The activity of mycorrhizal fungal hyphae in the roots caused prevention from pathogenic fungi. In nature, the peloton is observed in the cortical cells of *Cymbidium goeriingii* roots, indicating mycorrhizal colonization in the native orchid roots. On the other hand, pathogenic fungi such as *Fusarium* and/or *Rhizoctonia* species are mostly isolated from commercial orchid plants. These suggest that application of symbiotic mycorrhizal fungi should be needed for orchid cultivation in nurseries and at the time of transplanting.

Keywords : mycorrhizae, orchid, *Rhizoctonia*, symbiosis, *Tulsanella*.

Mycorrhizae is the root made from symbiotic interactions between two different organisms, the fungus (myco-) and root (-rrhiza) (Harley, 1969). The mycorrhizal fungus penetrates the root cells and changes the physical structure of the root, resulting in root heteromorphs, and providing physiological benefits for the plants. The symbiotic fungi also benefit from this relation. Mycorrhiza is not artificially made under *in vitro* conditions, but is produced only in nature. In particular, ectomycorrhiza produced from the roots of woody plants was suggested to be an organism independent of or different from the fungus or the root, like lichens (Agerer, 1991). In most cases, the mycobiont fungus obtains the energy source from the plants, provides nutrients to the (phytobiont) plant, and protects the root from environmental stress and plant pathogens. Mycorrhizal fungi increase the solubility of mineral nutrients in the soil, making it easy for the plant to absorb them (Hacskaylo, 1972; Bucking and Heyser, 1994 and 1998; Bucking et al., 1998). Also, they are considered to be 'ecological niche' in the rhizosphere, allocating the carbohydrate nutrients and protecting the plant roots from physical and biological hazards. Phytohormones are involved in these relationships (Alexopoulos and Mims, 1996; Beyeler and Heyser, 1997).

Mycorrhiza is classified into several types according to morphological feautures, role in ecological ranks, or associated plants. It is generally divided into endomycorrhiza and ectomycorrhiza, sometimes including endoectomcyorrhiza mainly found in the roots of azalea (Brubdrett et al., 1996; Susan, 1991). At present, five kinds of mycorrhiza are known: ectomycorrhiza (EM) (mainly formed in the roots of woody plant), arbuscular mycorrhiza (AM), ericoid mycorrhiza (ErM), orchid mycorrhiza (OM), and ecto-endomycorrhiza (Peterson and Farquhar, 1994).

Ectomycorrhizal fungi interact with the roots of woody plants in the surface, and form the mantles in the outer layers of epidermal cells, protecting the roots (Lee et al., 2000ac). Also, they penetrate the cortical cells to the endodermis, sometimes producing internal hyphae and forming Hartig net (Barker et al., 1998). The mantle was reported to be a special parenchyma made from the fungal hyphae, surrounding the external roots (Hacskaylo, 1972; Lee et al., 2000ac). The ectomycorrhizal fungi were, in most cases, reported to be mushroom species.

Arbuscular mycorhiza (AM) is endomycorrhiza, and is most common in agricultural crops. AM fungi penetrate the roots of annual plants (mainly family Graminiaceae), and form vesicles or arbuscules in the root cortical cells (Eom et al., 1994; Koske and Gemma, 1989). Arbuscule is a kind of

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haustorium, a fungal organ that absorbs nutrients in plant root cells, whereas, vesicles are globose bodies produced by the intercalary or terminal swelling of a hypha. Formation of arbuscules and vesicles is stimulated by plant nutrition, especially phosphate. AM fungi were identified to be species of Glomales (Zygomycotonia) inhabiting the soil, and are dispersed by soil insects or small animals.

Ecto-endomycorrhiza has characteristics of ectomycorrhiza, but exhibit an endomycorrhizal character by its intracellular penetration. ErM (Ericaceae, Monotropaceae) is mycorrhiza of the Ericales, which is a kind of ectomycorrhiza. There are two forms of ErM, one with mantel (Hartig net) and the other without mantle (but with invading hyphae in cortex cells), usually forming fine root system. The fungi of ErM eventually degenerate together with the cortex cells.

Characteristics of Orchid Mycorrhiza

Orchids plant. Orchids (Orchidaceae) belong to the order of Liliales, and are often confused with the plants of the lily family (Liliaceae). They are known to be the most evolutionally advanced species in the family Orchidaceae and comprise approximately 1,000 genera and 35,000 species worldwide. More than 110 species of Orchidaceae have been reported in Korea, among which are several commercial species of *Cymbidium* such as *Cymbidium goeringii* known for its beautiful flowers, *Cymbidium kanran* which has fragrant aroma, and *Aerides japonicum* and *Neofinetia falcate* which are used as decorative ornamental plants. The most popular *Cymbidium* for sale in Korea is known to be the hybrids of *Cymbidium sinense* and *C. kanran* or *C. goeringii* produced in England botanical gardens.

Ecologically, orchid plants are divided into two kinds based on their habitation: terrestrial and epiphytic orchids (Arditii, 1992). Terrestrial orchids are well adapted to environmental stresses. They are symbiotic with the fungi in the roots, and survive well under harsh conditions. The epiphytes are less symbiotic with fungi than the terrestrial ones. They originated from subtropical or tropical regions, and are also known to grow on the bark (surface) of woody plants or on the surface of rocks. In some cases, the epiphytes naturally inhabit the twig and trunk of woody plants in heavy rain areas in the tropical regions (Altas and Bartha, 1993). They absorb moisture from the air rather than from the soil.

Non-photosynthetic orchid plants (*Neottia nidusuvis*) were reported to exist symbiotically with fungal endophytes in their roots (Hadley and Williamson, 1972; Richardson et al., 1993). A leafless plant (*Gastrodia elata*) was also reported to be cultivated in wood chips colonized with hyphae of *Armillariella mellea*, and used as an important Korean herbal drug. During the non-photosyntheric portion of the life cycle, and in achlorophyllous orchids, orchids obtain carbon compounds from the fungus. Mature orchid plants may also be provided with mineral nutrients from the mycorrhiza.

The orchid plants differ from other plants by the following features: 1) cellulose and sponge-like structure of cortex holding moisture during the dry season (Altas and Bartha, 1993; Hardley, 1982); 2) perennials or annuals with no leaf or with small shoot for flowering (Sprunger et al., 1991); 3) bilateral symmetry of flowers like the butterfly (Grolier, 1993); 4) very small seeds (more than 4 million seeds in a pod) sized 0.5-1.0 μm and weighing 14 μg (Harley and Smith, 1983); and 5) seeds composed of only 100-150 cells and sized 10-15 μm long (Arditii, 1992; Hadley, 1982). Particularly, orchid seeds are unorganized tissue without any embryo, like the fungal spores, which render them to be symbiotic with the fungi for germination in nature (Hadley, 1982; Zettler and McInnis, 1993).

Orchid mycorrhiza (OM). OM is a kind of endomycorrhiza, formed between plants of Orchidaceae and basidiomycetous, or rarely ascomycetous fungi. The fungi form pelotons, intracellular coils or hyphal aggregates inside the cortical cells (Peterson and Farquhar, 1994). Mycorrhizal fungi are needed for orchid seed germination. Also, orchids show obligate dependence on their mycorrhizal fungi all throughout or in part of their life cycle. Protocorms of orchid cannot obtain nutrients sucessfully and grow without any interactions with mycorrhiza (Harley and Smith, 1983; Uetake et al., 1992). The nurseries of *Spiranthes spiralis* live without photosynthesis, and are supplied with nutrition from the mycorrhizal fungi, producing the flower shoot yearly (Harley and Smith, 1983; Uchida et al., 1986). Thus, mycorrhiza is necessary for seed germination and the successful growth of orchids.

Microflora in orchids roots. Various fungi were isolated from the roots of orchid native to or cultivated in Korea (Riew, 1996). Non-symbiotic isolates were collected together with symbiotic ones from the same roots of *Cymbidium goeriingii*. Some isolates with identical morphology showed different symbiosis.

A group of fungi were isolated from the roots of *Cymbidium goeringii* grown in the fields related to decomposition of organic matter, especially cellulose and hemicellulose. They are species of *Trichoderma*, *Rhizoctonia*, *Gliocladium*, and *Chaetomium*. *Trichoderma* and *Chaetomium* spp. are common species found on litter in the forest (Rifai, 1969; Atlas and Bartha, 1993; Singleton et al., 1992). Six *Trichoderma* species (teleomorph: *Hypocrea; T. aureovirid*; *T. aureoviride, T. hamatum, T. harzianum, T. koningii, T. longibrachiatum*, and *T. viride*) were isolated

and identified based on the manuals of Rifai (1969). Three species of *Gliocladium* (*G. penicillioides, G. roseum*, and *G. virens*), which are similar to *Penicillium* in their morphology and production of some antibiotics of gliotoxin or viridian, were also identified (Domsch et al., 1980). *Trichoderma* and *Gliocladium* species sometimes act as weak pathogen to orchids, and produce certain antibiotics, influencing other flora in the soil (Domsch et al., 1980).

Plant pathogenic fungi mainly including *Fusarium*, *Ascorhizoctonia*, and *Rhizoctonia* were isolated from the roots of orchids grown in greenhouses. *Fusarium* and *Ascorhizoctonia* cause soft or dry root rots in several crops in greenhouses (Nelson et al., 1983; Kommedahl et al., 1988). These fungi may infect orchid plants. Five *Fusarium* species (*F. graminearum, F. oxysporum, F. subglutinans*, and two unknown species) caused soft rot or root rot of orchids (Nelson et al., 1983; Kommedahl et al., 1982; Choi et al., 1997; Peterson, 1991). However, no *Fusarium* species producing red pigments on agar have been isolated from the roots of native and cultivated *Cymbidium goeriingii* orchid in Korea.

Two isolates of *Rhizoctonia repens* were collected from the roots of *Cymbidium goeringii* orchid native to Anmyeon Island and Kochang in Korea showed symbiotic relations with orchids. *R. endophytica* var. *endophytica* isolated from the roots of *Cymbidium sinense* orchid showed a symbiotic relationship with *C. kanran*, but not with *C. goeringii*.

OM fungi. The orchid plants have pelotons in the root cortical cells, which form the mycelial mass of the infecting fungi. In the past, pelotons in the cortical cells were regarded as pathogenic fungi, however, they are now confirmed to be symbiotic fungi. Orchid roots with pelotons are healthier than those without them (Richardson et al., 1993). The pelotons usually persist only for a limited period of time, and become digestive forms in the root cortical cells before degeneration. Various forms of pelotons were observed in the healthy roots of *Cymbidium goeriingii* collected from southweat coast areas of Korea.

The fungus symbiotic to the orchid roots, which was identified as a species of *Rhizoctonia* (a heterogenous group of Fungi Imperfecti), was isolated from the peloton in the cortical cells without any conidial stage throughout the life cycle (Arx, 1974; Currah and Zelmer, 1992; Singleton et al., 1992: Sneh et al., 1991). The symbiotic fungus was reported to be a species of *Tulasnella* in the teleomorph state, in Basidiomycota, differing from *Thanatephorus* of the anastomosis groups (AGs) of *Rhizoctonia solani* known as a root disease pathogen in various economic plants (Sneh et al., 1991). Several studies reported that the fungus of *Rhizoctonia* symbiotic with the orchid plants: 1) form dolipore septa in young vegetative hyphae, differing from the septa of Ascomycotina (Herr, 1979; Moore, 1987; 1996); 2) is dikaryotic in the young cells; 3) sometimes has constricted hyphae, specialized ones called 'monilioid haphae', but usually forms neither clamp connections nor specialized hyphae; 4) has no conidial stage or rhizomorph in the roots or in the soil (Dosmsh et al., 1980; Giman, 1968); and 5) has the perfect (teleomorph) stage, *Tulasnella* (Moore, 1996; Stalpers and Anderson. 1996).

The species of genus *Rhizoctonia* with monilioid mycelia were divided into two groups based on the number of nuclei in the young cells: binucleate and multinucleate (Sneh et al., 1991; Moore, 1987 and 1996). The species of multinucleate *Rhizoctonia* were categorized to be plant pathogenic fungi, including *R. cerealis, R. fumigata, R. oryzae, R. oryzae-sativae*, and *R. solani*. They cause various diseases in plants. However, some isolates of *R. solani* were reported to have symbiotic relationships with the protocorms of orchid (Harvais and Hadley, 1967). This can be attributed to orchids' ability to produce many secondary products that inhibit the fungus.

Nine binucleate *Rhizoctonia* species (teleomorph *Tulsanella*) were known to be symbiotic with the orchid plants or saprobic on the dead woods. Symbiotic fungal species are *R. repens* (Smith, 1966; Currah et al., 1987; Warcup, 1981) [syn. *Epuorhiza repens* (Moore, 1987; Currah and Zelmer, 1992), syn. *T. calospora* (Sneh et al., 1991; Warcup and Tolbot, 1967)], *R. goodyerae-repenstis* (Currah et al., 1989), *R. solani* (Harvais and Hardley, 1967; Smith, 1966), and *R. endophytica* [syn. *R. solani* AG-A (Hardley and Williamson, 1972), syn. *Ceratobasidium cornigerum* (Warcup, 1981; Moore, 1996)]. *T. calospora* and *T. repens* were reported to be identical in several publications, but it is still unclear whether they are the same fungi.

Identification of OM fungi

Morphological characters and simple physiological properties of fungi *in vitro* are useful for fungal identification. However, morphologically and/or physiologically identical fungi may be distinguished from each other by nucleic acid techniques. Species of *Rhizoctonia* were isolated from the roots of *Acianthus, Thelymitra, Pterostylis, Pendunculate, Prasophyllum*, and *Dactylorchis* (Warcup and Talbot, 1967 and 1971). At first, all of *Rhizoctonia* species from orchid plants were believed to be OM because the orchid roots would interact with any species of *Rhizoctonia*, forming OM in nature (Harvais and Hadley, 1967), and all regarded as the same OM. However, they could be discriminated by their teleomorphic states. All *Rhizoctonia* species isolated from orchid plants were identified as species of *Thanatephorus, Ceratobasidium, Ypsilonidium,*

Sebacina, or *Tulasnella* in their telemorphic state, belonging to Basidiomycotina (Harley and Smith, 1983; Sneh et al., 1991 and 1996; Moore, 1987 and 1996; Stalpers and Anderson, 1996).

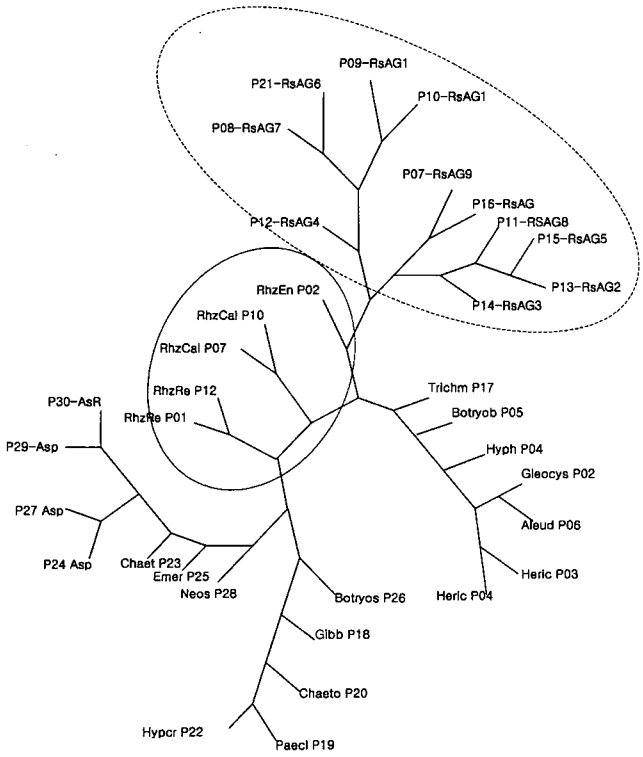

Fig. 1. Dendrogram of 18s rDNA genes of fungal isolates. Orchid mycorrhizal fungi and orchid (Rhzunkn- RhzEnph- isolates) and orchid rhizosphere fungi isolated in the laboratory were compared with the pathogenic *Rhizoctonia solani* AG groups (-RsAG-; *Thanatephorus cucumeris*) and other fungi. Hyph p04; *Hyphodontia alutaria*, Gleocys 02; *Gloeocystidiellum leucoxantha*, Hericium 03; *Hericium ramosum*, Hericium 04; *Hericium ramosum*, Botryobas 05; *Botryobasidium isabellinum*, Aleudiscus 06; *Aleurociscus botryo*, AG groups of Thanatephorus P07-RSAG9: *Thanatephorus cucumeris*, P08-RSAG7: *Thanatephorus cucumeris*, P09-RSAG1: *Thanatephorus cucumeris*, P21-RSAG6; *Thanatephorus cucumeris*, P10-RSAG1; *Thanatephorus cucumeris*, P11-RSAG8; *Thanatephorus cucumeris*, P12-RSAG4; *Thanatephorus cucumeris*. P13-RSAG2; Thanatephorus cucumeris, P14-RSAG3; *Thanatephorus cucumeris*. P15-RSAG5; Thanatephorus cucumeris, P16-RSAG; *Thanatephorus cucumeris*, Trichm P-17; *Tricholoma matsutake*, P30-Asp; *Aspergillus tamari* (Eurotiales), P29-Asp; *Aspergillus flavus* (Eurotiales), P27-Asper; *Aspergillus funigatus* (Eurotiales), P24-Asper; *Aspergillus clavatus* (Eurotiales), P-23 *Chaetosartorya cremea* (Eurotiales), P25; *Emericella nidulans* (Eurotiales), P28; *Neosartorya fischeri* (Eurotiales), Botryo P26; *Botryosphaereia ribis* (Dothideales), P18 Gibbe;r *Gibberella pulicari* (Fusarium), Chaeto P20; *Chaetopsina fulva* (Hypocreales), Hypcro P22p; *Hypocrea lutea* (Hypocreales), Paecomy P19: *Paeclomyces tenuipes* (Hypocreales) (Lee et al., 1999; Lee and You, 2000); *Rhizoctonia repens* [*Tulsanella repens*; RhzPe P-01, RhzPe P-12, RhzPe P-23 (recently isolated from the roots collected from Jeju)], *R. endophytica* (*Ceratobasidium cornigerum*), RhzEn P-02, (weakly pathogenic in orchid), *R. calospora* (*Tulsanella calospora*), RhzCal P-07, RhzCal P-08, and RhzCal P-10.

During the last 8 years, various OM fungi have been isolated from the roots of Korean native orchids (*Cymbidium goeringii*) in the laboratory (Lee et al., 1997ab; 1998abc; 1999abc; 2000b). The OM fungal isolates were compared with the pathogenic fungi, AGs of *R. solani*, by cultural characters [growth rate on potato-dextrose agar (PDA) and hyphal thickness] (Lee and You, 2000), analysis of PCR-RAPD, or the 18s rDNA sequences (Lee and Yoo, 2000). Other fungi isolated from orchid plants have also been examined by using the above methods. Fig. 1 summarizes the result of the 18s rDNA analyses, showing the dendrogram of orchid mycorrhizal fungi isolated from orchid plants and orchid rhizosphere. Three *T. repens* isolates (RhzUnkn P01, RhzUnkn P12, and RhzUnkn P23) were collected from the roots of *Cymbidium goeringii*, while three *T. calospora* isolates (RhzUnkn P07, P08, and P10) were also collected from *Cymbidium goeringii* (but from different locations). Two *Ceratobasidium cornigerum* isolates including RhzEnph P02 were collected from the roots of *Cymbidium sinese* cultivated in a greenhouse. All of the above isolates induced pelotons in the root cells and stimulated root growth of various species of orchid. The hyphal thickness (6-12 μm) observed in the agar medium was similar to that of pelotons in the orchid roots (Lee et al., 2000b; Lee and You, 2000). The fungal isolates were somewhat host-specific. For example, isolate P-02 (teleomorph: *Ceratobasidium cornigerum*) was weakly parasitic on *Cymbidium goeringii* and other species of *Cymbidium*, but symbiotic on a hybrid of *C. kanran*. Interestingly, some isolates influence epiphyte orchids but the mycorrhiza did not affect their growth.

Symbiotic Relations Between Orchids and OM Fungi

Interactions between fungi and plants. Mycorrhizal relations are made by fungal infection and establishment in plant roots. Generally, mycorrhizal fungi are host-specific, but the symbiotic relationships between OM fungi and orchid plants are mostly non-specific (Arditti, 1992). Most endomycorrhizal fungi have biotrophic relations with their host plants, constituting no parasitic nature. On the other hand, OM, a kind of endomycorrhizae involving *Rhizoctonia* species, can be cultured on artificial media. Some OM fungi act rather as parasites. The balance between orchids and OM fungi in a mycorrhizal relation is very delicate, and often too much nutrition has an adverse effect on the seeds or seedlings. Therefore, the degree of mutual dependence in their general relationships is still to be determined.

The benefit of mycorrhizal relations over non-mycor-

rhizal ones to plants can be explained by the former's ability to give ecological natural niches for plants. However, the roles in the mycorrhizal relationships in orchid plants have not been extensively studied and are not clearly understood at present. The symbiotic relations can be detected by the formation of pelotons in orchid root tissues. This can be manifested by the fact that orchid plants with pelotons show healthier life cycle, blooming every year, than those grown from tissue culture (without pelotons) showing no continuous blooming. Except for these aspects, any other features characteristic of mycorrhizal interactions are not understood until now.

Histological aspects. As mentioned above, the most characteristic feature in orchid mycorhyza is the peloton formation in orchid roots. In terrestrial orchids, *Cymbidium goeringii* and *C. kanran* native to Korea, pelotons were formed in cortical cells naturally (Fig. 2A-C) and by artificial inoculation by *R. repense* P-01. This isolate also induced the peloton formation in the root epidermal cells of epiphytic orchids (Fig. 2D-F), and its location is different from that in terrestrial orchids. This is a newly discovered aspect about peloton formation.

Peloton is a mass of entangled hyphae, having various sizes and shapes. They are ellipsoid in shape and sized 5-10 μm in diameter. Under the light microscope, thin individual hypha and/or hyphal mass can be observed (Smreciu and Currah, 1989; Zettler and McInnis, 1993) in the cortical cells of terrestrial orchids. The pelotons in the roots of *Catasetum maculatum* are made from a sterile clamped basidiomycete (Richardson et al., 1993). Even in pure culture, some hyphal tips coil into peloton-like structures as in root cells. With staining, pelotons may be categorized as

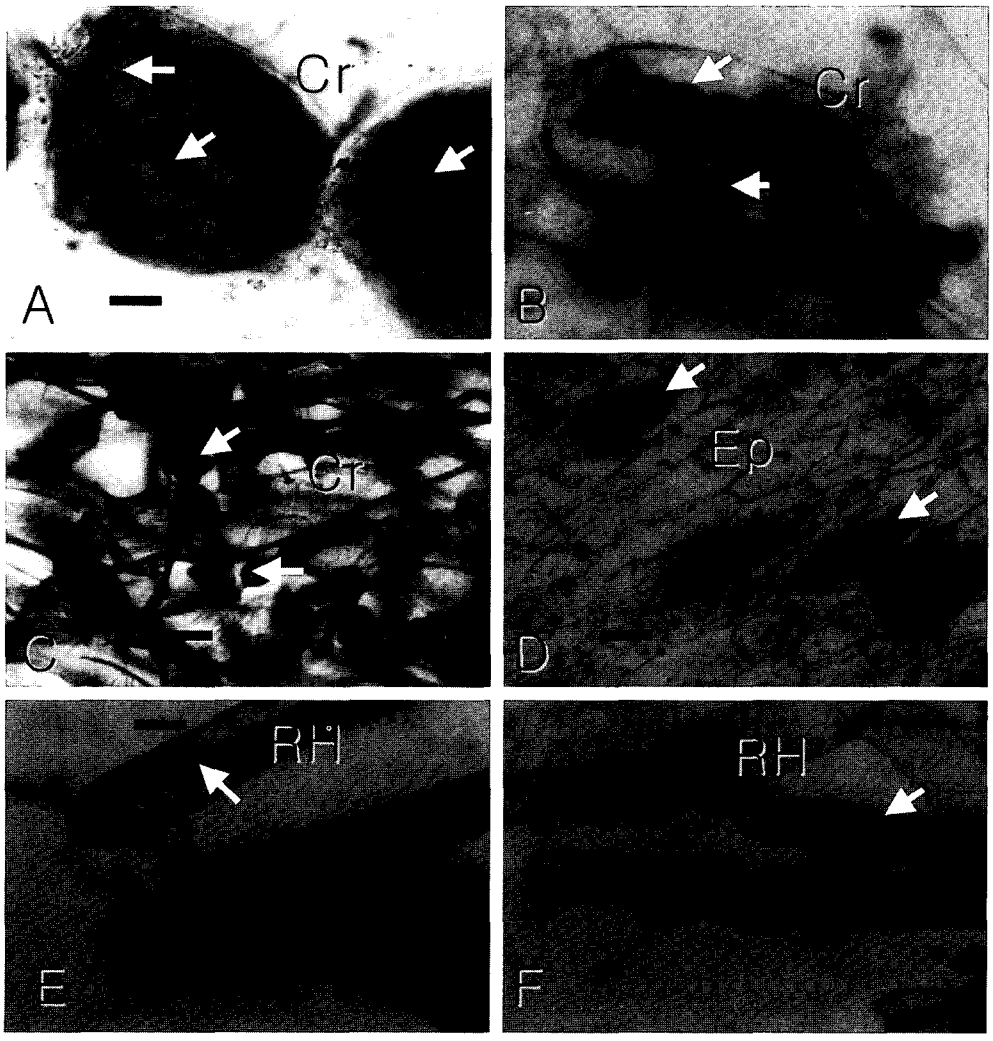

Fig. 2. Light microscopy of pelotons (arrows) formed in orchid root tissues. Pelotons formed in the cortical cell (Cr) of *Cymbidium georingii* (**A**) and of *C. kanran* (**B, C**). Note moniloid-like special pelotons in (B) and (C). Pelotons also formed in epidermal cells (Ep) (**D**) and root hairs (RH) (**E, F**) of epiphytic orchid *Neofinentia falcate*. Bar=10 μm.

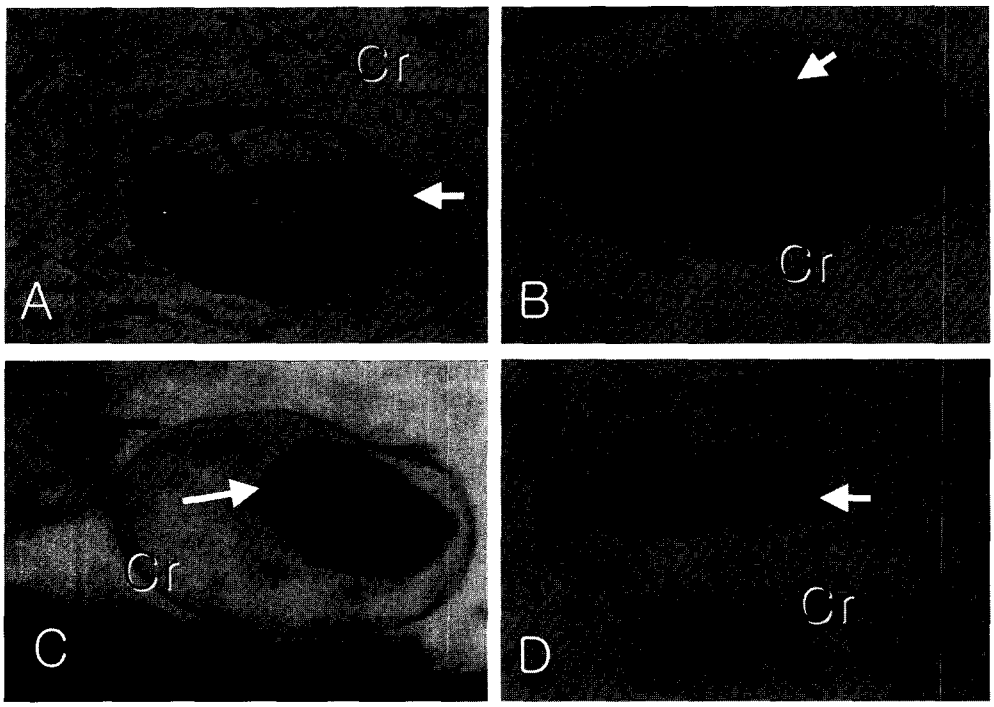

Fig. 3. Light microscopy of the peloton formation (**A**) and digestion process (**B-D**) in the cortical cells (Cr) of *Cymbidium neveo-marpenation*. Arrows indicate various forms of pelotons. Bars=10 μm.

small pointed, pale degenerated, and heavily stained forms. Heavily stained pelotons turn into pale degenerated ones referred to as 'digestive pelotons' (Fig. 3). These three forms of pelotons were readily observed in the roots of orchid collected from the western coast areas of Korea, but not from tissue-cultured orchid roots or those grown in greenhouses. The native and greenhouse-grown orchids (*Cymbidium goeringii*) differed from each other in terms o f blooming. Many commercial orchids have degenerated or dead roots, in which no peloton forms. In the cortical cells of these degenerated roots, non-mycorrhizal fungal hyphae are readily observed. They are believed to be pathogenic fungi because of the formation of haustorium-like structures.

Effect on orchid growth. Mycorrhizal relations in orchid plants are known to have an effect on plant growth. In this study, fungal isolates obtained from the roots of *Cymbidium goeringii* native to the western coast areas or Cheju island in Korea (P-22 and P-23) were applied to young orchid plantlets (Lee et al., 1997abc; Lee et al., 1998abc) or compared with other symbiotic fungi in several orchid species (Lee et al., 2000b). When the collected isolates

Table 1. Growth parameters of *Cymbidium kanran* cv. Haekmyo (a hybrid of Korean and Japanese varieties) inoculated with the orchid-symbiotic fungi[x]

Fungal isolate	Fresh weight (g)	Plant height (cm)	Leaves			Roots			
			Length (cm)	Width (mm)	Number	Number			Length (cm)
						Total	New	Dead	
Control	1.2ab[y]	18.1a	14.3b	5.4b	4.2c	5.4bc	1.5a	1.5x	3.6y
P-01	2.0d	22.3d	17.2c	7.2c	4.1c	6.4bcd	2.8cd	0.0y	5.1x
P-07	1.2a	21.3bcd	17.5c	7.4cd	3.3b	4.6b	0.0e	1.8wx	3.6y
P-08	1.6bc	19.9ab	17.7c	7.1c	3.8bc	5.9bcd	1.7ab	0.3y	3.6y
P-10	1.4ab	20.3bcd	16.9c	6.8c	4.1c	5.4bc	0.8f	0.3y	3.1y
P-12	2.0d	18.2a	13.5b	5.3b	6.1d	7.2d	3.1d	2.3w	5.0x
P-22	2.1d	22.7d	17.1c	7.9d	3.8bc	6.2bcd	2.3bc	0.1y	5.8x
P-23	1.8cd	21.9c	16.8c	7.4cd	3.2b	6.1bcd	2.8cd	0.0y	5.3x

[x]The growth parameters were examined 8 weeks after inoculation.
[y]The same letters in a column denote no significant differences at P=0.05 by Waller-Duncan multiple range test.

Table 2. Growth parameters of *Cymbidium* hybrids 'Gwanum' and 'SaGeo' cultivated for 1 year after inoculation with the symbiotic fungus (*Rhizoctonia repens* P-01)

Orchid cultivar	Orchid mycorrhiza	Fresh root weight (g) /plant (N=4)	No. of bulbs/plant (N=10)		
			Total (T)	Dead (D)	D/T
Gwanum	Control	101.0±5.8*[x]	9.2±1.5	1.7±0.5	0.17±0.05*
	Inoculated	148.0±10.8	11.1±1.9	0.3±0.2	0.03±0.01
SaGeo	Control	96.0±9.4	12.9±2.4	2.8±0.6	0.23±0.06*
	Inoculated	109.0±11.1	10.9±1.9	0.4±0.2	0.04±0.02

[x] Values are averages and standard deviations of given replications. Significant differences (* at P = 0.05) by least significant difference analysis were noted in the number of dead bulbs for both Gwanum and Sageo orchids of *C. kanran* hybrid.

Table 3. Effect of orchid mycorrhizal fungus on the growth of orchids in the greenhouse

Orchid	OMF[x]	Plant height (cm)	No. of shoots	No. of leaves	Leaf width (cm)	Root length (cm)	No. of roots	Fresh weight (g/plant)
Hybrid of	Control	15.5a[z]	2.2a	7.8a	0.7	12.3	5.6a	5.1a
Cymbidium	P-01	18.4b	3.7b	13.1c	0.8	15.7	10.3c	10.7b
kanran[y]	P-02	15.6a	3.4b	11.6bc	0.8	13.6	8.3bc	6.8a
	P-03	15.5a	3.2b	10.3b	0.8	13.8	7.1ab	6.9a
Phalaenopsis	Control	10.5b	1.0	3.8ns	4.6	11.8	13.5b	23.8b
	P-01	12.7c	1.0	3.7	5.0	13.0	13.5b	32.4c
	P-02	8.2a	1.0	3.3	4.7	12.0	10.5a	16.1a
	P-03	10.3b	1.0	3.3	4.6	13.5	13.8b	22.8b

[x] Inoculated with the fungal isolates of *Rhizoctonia repens* (P-01 and P-03) and *R. endophytica* (P-02). Growth of plants was examined 8 months after inoculation.
[y] A hybrid of *Cymbidium kanran* cv. Jeju x *C. kanran* cv. Namguk.
[z] Means followed by the same letter within a column are not different at P = 0.05 by Duncan's multiple range test.

were inoculated to *Cymbidium kanran* cv. HaekMyo (a hybrid of Korean and Japanese varieties), some isolates stimulated plant growth (Table 1). Especially, isolates P-01 and P-22 enhanced the growth of all plants tested, including fresh weight, plant height, and leaf and root growth. Isolate P-01 was identified as *Rhizoctonia repens* (telermorph: *T. repens*). This stimulation of plant growth can be explained by the following: 1) increased absorption of nitrogen sources relative to non-mycorrhizal roots (Lee et al., 1998b); 2) increased absorption of other mineral nutrients such as P, K, and Ca (Lee et al., 1997a and 1998c); and 3) increase of new root formation (Table 1). All plantlets from tissue-cultured plants were dead within 3 months after transplanting into and culturing in soil bed (Lee et al., 1997a, b), suggesting that young plantlets cannot survive without establishment of proper mycorrhizal relations.

Application of OM fungi to orchid bulbs (*Cymbidium* hybrids) increases the production of new roots and decreases the root death probably caused by root rot diseases (Table 2). The same beneficial effects were obtained when three OM fungi were applied to orchid roots of *Cymbidium kanran*, but not that of other orchid *Phalaenopsis* sp. (Table 3). It was observed that *Cymbidium* species native to Korea died or poorly grew during the 2-year cultivation without mycorrhizal association (unpublished data).

When sterilized soil was used for cultivating orchids, the tissue-cultured plantlets died within 3 months after cultivation in the greenhouse. Also, half of roots in the bulb of *Cymbidium* species were dead during the 6-month cultivation, although the fresh weights of non-mycorrhizal orchids were similar to those of mycorrhizal orchids. Moreover, the quality of commercial cymbidium orchids grown in pots without mycorrhizal inoculation was reduced.

Protection against plant pathogens. In orchid plants, the most common fungi are *Trichoderma* and *Fusarium*. The species of *Fusarium* were considered to be very pathogenic on the plantlets of orchids. Also, many other pathogenic fungi or bacteria, which originated from soil materials, were supposed to infest and give harmful effects on orchids. When orchid plantlets planted in Hyponex agar were inoculated with fungi, *Fusarium* sp. killed all plantlets within a week, whereas, *Trichoderma* sp. covering the agar around the plantlets killed them within 5 weeks. Also, *F. subglutinans* and an unidentified *Fusarium* sp. covering the agar plates produced red pigment and caused all plantlets to die within 10 days after inoculation. Several species of *Trichoderma* are used as biological control agents against pathogenic fungi such as *Fusarium* diseases, but may have an adverse effect, probably by toxic antibiotic compounds, especially on young seedlings of orchid plants.

Generally, mycorrhizae are considered to protect plants from soil-borne plant pathogens. However, it is still not established whether OM fungi have such plant-protective activities. As mentioned above, the number of dead roots and bulbs decreased with the inoculation of OM fungi (Tables 1 & 2). The root diseases are probably caused by pathogenic fungi, and in this respect, the OM fungi may prevent the orchid plants from infection by the pathogen. However, further studies are needed to clarify the role of OM in protecting orchid plants from diseases.

Conclusions

Various isolates of orchid mycorrhiza were collected from the roots of *Cymbidium goeringii* orchid native to Korea. Isolates of three *Rhizoctonia* species, *R. repens* (anamorph state of *Tulsanella repens*), *R. endophytica* (*C. cornigerum*), and an unidentified species of *Rhizoctonia* (believed as anamorph of *Tulsanella calospora*, which is the most common OM fungus) were inoculated to orchid roots, and were confirmed to be symbiotic with orchids. These fungal isolates could be differentiated from the plant-pathogenic species of *Rhizoctonia* by the analyses of 18s rDNA sequences and by peloton formation in the cortical cells of orchid root. These fungi enhanced the absorption of nitrogen sources and minerals by the orchid roots. The occupation of mycorrhizal fungal hyphae in the roots was considered to inhibit the infection by pathogenic fungi and bacteria. Thus, OM fungi may be beneficial to orchid cultivation. This kind of benefit to orchid plants may be related to the predominance of mycorrhizal over non-mycorrhizal roots in nature.

Peloton formation was readily observed in the root cortical cells of native *Cymbidium goeriingii* plants, whereas, whereas it was seldom observed in commercial orchids. Instead, many *Fusarium* species were isolated from the roots or bulbs of commercial orchids. Also, the mycoflora of the natural habitat of *Cymbidium goeriingii* was significantly different from that of artificial soil for orchids. The lines of fungus symbiotic with the roots of orchid were isolated from native *Cymbidium goeriingii* roots, but not from the cultivated orchid roots. In most cases, tissue-cultured orchid plants or bulbs were infested with the pathogenic fungus of *Fusarium* or *Rhizoctonia*. Therefore, use of the symbiotic fungi should be considered to produce healthier orchids for commercial purposes.

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