

# Mycorrhizal Fungi from *Spathoglottis plicata* and the Use of these Fungi to Germinate Seeds of *S. plicata* *in vitro*

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## ABSTRACT

Healthy roots from mature plants of terrestrial orchid, *Spathoglottis plicata* (Blume.) collected from Chiang Mai, Chanthaburi, Nakhon Ratchasima and Bangkok were used to isolate mycorrhizal fungi. A modification of Masuhara and Katsuya's method was employed to isolate these fungi from the peloton. Identification was based on morphological characteristics. Nuclei were stained with safranin O using Bandoni's method revealing that all isolates were binucleate. Three genera and four species of mycorrhizal fungi were identified: *Epulorhiza repens*, *Rhizoctonia globularis*, *Rhizoctonia* sp. and *Sabacina* sp. (*Epulorhiza* sp. anamorph). *In vitro*, symbiotic seed germination and development on seeds of *S. plicata* were tested using *Epulorhiza repens* and *Rhizoctonia globularis*. It was found that 99.2% of seeds inoculated with *Epulorhiza repens* and 96.3% of seeds inoculated with *Rhizoctonia globularis* germinated within 21 days, whereas only 15% of seeds incubated in the absence of fungi germinated. About 8.1% of seeds inoculated with *Epulorhiza repens* and 1.7% of seeds inoculated with *Rhizoctonia globularis* initiated leaves after 60 days of incubation, whereas in the absence of fungi they did not develop into seedlings and mortality gradually occurred. The seedlings became more mature and ready to be planted after 127 days.

**Key words:** mycorrhizal fungi, *Spathoglottis plicata*, germinate, *in vitro*

## INTRODUCTION

*Spathoglottis* is a terrestrial orchid, widely distributed in Southeast Asia and Pacific region. Many species have bright, beautiful, medium-sized flowers, which make them worthy subjects for cultivation. Several species and particularly the much improved hybrids, have been used in some gardens in the tropic. This extremely variable

species is native to India, Thailand, Malaysia, Indonesia and the Philippines. It generally inhabits the grassy lowlands and is common in the peninsular region of Thailand. Five species are known to occur in Thailand. Out of these, *S. plicata* and *S. lobbi* are widespread (Kamemoto and Sagarik, 1975).

Orchid seeds are extremely small with limited nutrients. Naturally, they need mycorrhizal

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fungi for germination (Harley and Smith, 1983). The fungus infects orchid seeds through rhizoids and form hyphal coils (pelotons) in the host cell. The pelotons are eventually digested by the orchid, thus supplying the necessary energy for germination (Rasmussen, 1995).

Most orchid mycorrhizal fungi isolated and identified are in the genus *Rhizoctonia*. The first isolations were made by Noel Bernard and one of them was identified as *Rhizoctonia repens* Bernard, a species commonly found as a ubiquitous orchid endophyte (Hadley, 1982). The main characteristics of orchid mycorrhizal fungi are right-angle branching, constriction at branch point, septum in the branch hypha near its point of origin, sclerotia formation, chains of monilioid cells and the number of nuclei in the young cells (Rasmussen, 1995). The plant pathogen species, *Rhizoctonia solani* (teleomorph : *Thanatephorus*, Ceratobasidiales) are multinucleate, whereas *Ceratorhiza* (teleomorph : *Ceratobasidium*, Ceratobasidiales) and *Epulorhiza* (*Tulasnella*, Tulasnelles and *Sebacina*, Sebacinales) are binucleate. *Rhizoctonia*-like fungi include the anamorphic genera *Ceratorhiza*, *Epulorhiza*, *Moniliopsis* and *Rhizoctonia* of a variety of teleomorphs stages of *Ceratobasidium*, *Tulasnella*, *Sebacina* and *Thanatephorus* (Moore, 1987). Teleomorphic states are rarely encountered in the field or laboratory (Warcup and Talbot, 1971). Some of these are well known as plant pathogens on a wide variety of crops (Sneh *et al.*, 1991). Very limited studies have been carried out to determine the genetic relationships among orchid *Rhizoctonia*-like endophytes and identified them on the basis of the molecular characteristics (Otero *et al.*, 2002).

Attempts to propagate orchids from seed using symbiotic fungi have been successful in Europe (Rasmussen *et al.*, 1991), Australia (Beyrle and Smith, 1993), United States of America (Zettler *et al.*, 1999), Canada (Utetake and Peterson, 1998) and Japan (Takahashi *et al.*, 2001), Taiwan (Wang *et al.*, 2004), Singapore (Narmatha *et al.*, 2000).

Our objectives were to isolate and identify the naturally occurring mycorrhizal fungi of *Spathoglottis plicata* and to examine the ability of seedlings and mycorrhizal fungi to promote the germination, growth and development of *S. plicata* seeds *in vitro*.

## MATERIALS AND METHODS

### Fungal isolation

Roots of *Spathoglottis plicata* were collected from Chanthaburi, Chiang Mai, Nakhon Ratchasima and Bangkok and roots of *Goodyera procera* from Chiang Mai. Mycorrhizal fungi were isolated using a modification of the Masuhara and Katsuya method (1994). The root segments were sterilized in 5% sodium hypochlorite (NaOCl) for surface sterilization. After shaking for 5 min, they were rinsed three times with sterile water. The roots were then cut into longitudinal sections and observed for the presence of hyphal coils (pelotons) under a stereo-microscope in sterile condition. The epidermal layer was then removed to avoid contamination. The pelotons were transferred from the inner cortex, macerated with a fine needle, scaped in a drop of sterile water and rinsed 3-5 times in sterile water. A single peloton was placed on Masuhara and Katsuya 1/6 NDY medium containing 100 mg/l streptomycin and 50 mg/l tetracycline and incubated at 28°C. After 3 to 7 days of incubation, hyphal tips were transferred onto a potato dextrose agar (PDA) slant. Pure cultures were maintained on slant PDA for identification.

### Fungal identification

Macroscopic features were examined on colony growth pattern, color, sclerotia formation, etc. Fungal growth rate was measured from the colony on PDA. For microscopic examination, fertile hyphae were mounted in sterile water on a microscopic slide, covered with a cover slip, and examined under a light microscope. Hyphal and monilioid cells were measured. The number of

nuclei per cell was stained with safranin O-KOH using Bandoni's method (1979).

### ***In vitro* seed germination with mycorrhizal fungi**

*In vitro* symbiotic seed germination was tested using a modification method of Zettler and McInnis (1993). *Spathoglottis plicata* seeds were collected from Rapee Orchid Garden, Kasetsart University, Bangkok. The orchid flowers were cross-pollinated by hand. Mature capsules were collected one month after pollination before seed dehiscent. They were sterilized with 70% ethyl alcohol and burned in the flame. Mature seeds were taken from the inner part of capsules and put in sterile water. One millilitre of sterile water containing seeds was pipetted onto a 1×4 cm filter paper strip (Whatman No. 1) in a petridish; the filter paper was placed on 20 ml solidified Dixon's oat-medium (2.5 g rolled oats, 7 g agar, 1,000 ml water; Dixon, 1987), pH 7.0, prior to autoclaving. Plates were inoculated with a 1 sq cm agar block containing mycelium and placed in the dark. Uninoculated seeds (the control) on a filter paper were incubated in a petri dish and kept in the dark. Five replicate plates were inoculated with the two mycorrhizal fungi, and five uninoculated plates served as the controls. All plates were incubated in the dark at 28°C for 21 days to determine seed germination. The seedlings were transferred onto fresh oatmeal agar in 5 × 10 cm bottles covered

with a rubber plug and parafilm and placed by the window.

### **Assessment of seed germination and development**

Percentage of seedling was examined 21, 60 and 127 days after incubation. Germination and developmental stages were assessed on a scale of 0-6: 0=no germination, 1=germination (seed coat ruptured by enlargement embryo), 2=presence of rhizoids, 3=presence of leaf primordium, 4=appearance of the first true leaf, 5=elongation of initial leaves, 6=elongation of root (Zettler and McInnis, 1993).

## **RESULTS AND DISCUSSION**

Three genera and four species were identified as follows: *Epulorhiza repens*, *Rhizoctonia globularis*, *Sebacina* sp. and unidentified *Rhizoctonia* (Table 1). Most *Rhizoctonia* did not produce conidia, yet the teleomorphic state of this genera produced spores. Nuclear staining revealed that all strains were binucleate. The number of nuclei present in the young cells was one of the morphological features used to identify the *Rhizoctonia*. Pure cultures were maintained on PDA slant and liquid paraffin for further studies on molecular phylogeny and symbiotic seed germination. Named isolates are

**Table 1** Mycorrhizal fungi isolated from orchid hosts from various locations.

Fungal isolate	Orchid hosts	Location
<i>Epulorhiza repens</i> (KUFC 14-6)	<i>Spathoglottis plicata</i>	QSB, Chiang Mai
<i>Epulorhiza repens</i> (KUFC 2-7)	<i>Spathoglottis plicata</i>	Chanthaburi
<i>Epulorhiza repens</i> (KUFC 15-0)	<i>Spathoglottis plicata</i>	Kasetsart Univ., Bangkok
<i>Rhizoctonia globularis</i> (KUFC 5-21)	<i>Goodyera procera</i>	QSB, Chiang Mai
<i>Rhizoctonia globularis</i> (KUFC 14-9)	<i>Spathoglottis plicata</i>	QSB, Chiang Mai
<i>Rhizoctonia</i> sp. (KUFC 14-3)	<i>Spathoglottis plicata</i>	QSB, Chiang Mai
<i>Sebacina</i> sp. (KUFC 37-5)	<i>Spathoglottis plicata</i>	Nakhon Ratchasima

\* QSB = Queen Sirikit Botanic Garden

described below.

### Description of mycorrhizal fungi

#### *Epulorhiza repens* (Bernard) Moore

Basionym : *Rhizoctonia repens* (Bernard)

Teleomorph : *Tulasnella calospora* (Boud.) Juel

On PDA, colony reaching 9 cm in diam after 10 days (growth rate at 28°C approximately 0.4 mm/hr). Colony colorless, with thin, white to cream, margin submerged, entire glabrous and zonate (Figure 1A). Vegetative mycelium growth mostly within the agar medium. On CMA, mycelium nearly completely submerged, white to cream. Sclerotia minute, composing of loosely arranged clusters of monilioid cells (Figure 1B). Vegetative hypha 3.0-4.7 µm, binucleate, septate, hyaline, constricted at branch points. Monilioid cells, thin walled, hyaline, ellipsoidal to spherical 8.5-15.5 × 7.1-11.5 µm in short, branched or unbranched.

HOST: Root of *Spathoglottis plicata*, Chanthaburi, Queen Sirikit Botanic Garden (Chiang Mai), Kasetsart University

*Rhizoctonia repens* was originally isolated and described by Bernard in 1909 as orchid symbiont. The fungus produced monilioid cells and pelotons formed in the culture of the fungus. The main characteristics of the taxon were the creamy white colour of the mycelium and the presence of monilioid cells in short chains. It was later reported from various orchid species in Germany by Burgeff (1936) and U.S.A. by Curtis (1939) (Currha *et al.*, 1987). Warcup and Talbot (1967), using soil on agar casing method, induced the teleomorph, *Tulasnella calospora* (Boudier) Juel, of isolates identified as *Rhizoctonia repens*.

In this study, *Epulorhiza repens* was identified as *Rhizoctonia repens*, using the cultural and the size, shape and organization of monilioid cells as described by Currha *et al.* (1987).

*E. repens* was a species commonly obtained from orchid endophyte (Hadley, 1982) This

mycorrhizal fungi distribute in Asia. Many publications reported that the mycorrhizal fungi associated with orchid in the tropical area were identified as *Rhizoctonia repens* (*E. repens*). In India, Senthikumar and Krishnamurthy (1998) studied on a cytochemical on the mycorrhizae of *Spathoglottis plicata*. The plants were maintained in pots containing compost rich garden soil and natural inoculum of the mycorrhizal fungus *Epulorhiza repens* (= *Rhizoctonia repens*). *E. repens* was also the most frequently isolated fungus from *Spiranthes sinensis* as reported by Terashita (1982) in Japan.

#### *Rhizoctonia globularis* Saksena & Vaartaja

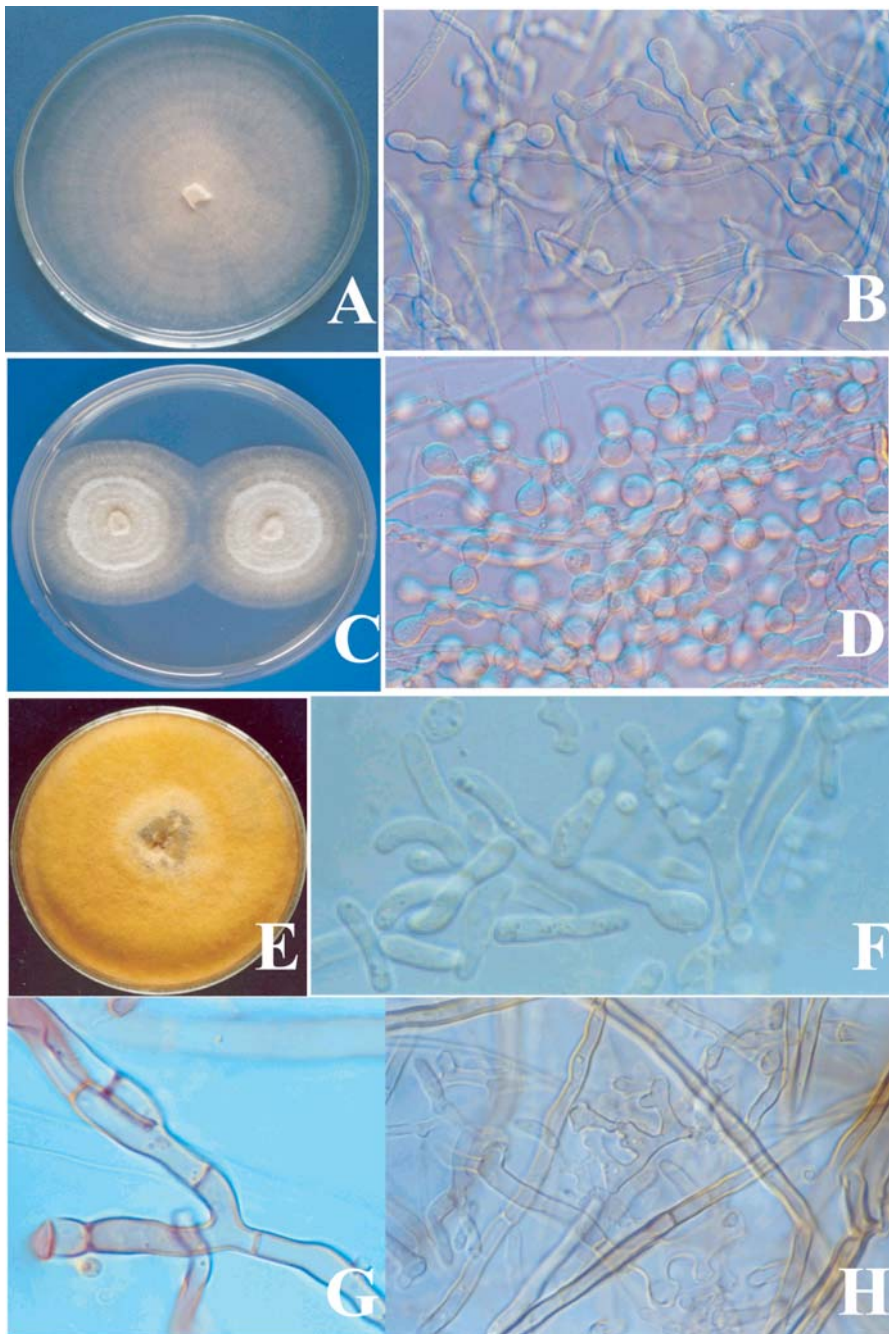
On PDA, the colony was colorless and had uniform surface growth, hyaline, radial mycelium with fine zone 0.25 mm apart (Figure 1C). In the old culture, the surface mycelium was appressed, and the colony became smooth, translucent, and glistening. Aerial mycelium was absent. The vegetative hyphae septate, 2.5-3.5 µm wide, branching often at nearly right angles but more commonly at 45° angle. Monilioid cells, were produced in short, branched, chain of 3-8 cells (Figure 1D). They were hyaline, globose, 8-10.5 µm diam.

*R. globularis* is close to *R. repens* as described by Burgeff and Curtis. The conspicuous radial growth of surface mycelium with fine zones and the smaller monilioid cells with their typical mode of attachment in a chain distinguish *R. globularis* from *R. repens* (Saksena and Vaartaja, 1960).

HOST : Root of *Spathoglottis plicata*, Queen Sirikit Botanic Garden (Chiang Mai). Root of *Goodyera procera*, Queen Sirikit Botanic Garden (Chiang Mai).

In this study, *Rhizoctonia globularis* was isolated from the roots of *Goodyera procera* and *Spathoglottis plicata* from Queen Sirikit Botanic Garden, Chiang Mai.





**Figure 1** *Epulorhiza repens* (KUFC 14-6): colony on PDA, 10 days at 28 °C (A); elipsoid to spherical monilioid cells 250X (B); *Rhizoctonia globularis* (KUFC 5-21): colony on PDA, 10 days at 28 °C showing concentric zonation (C); globose monilioid cells 250X (D); *Rhizoctonia* sp. (KUFC 14-3): colony on PDA, 16 days at 28 °C (E); irregular-shaped spores 1000X (F); hypha branching near right angle 1000X (G); formation of short, stubby, swollen side, branches 400X (H).

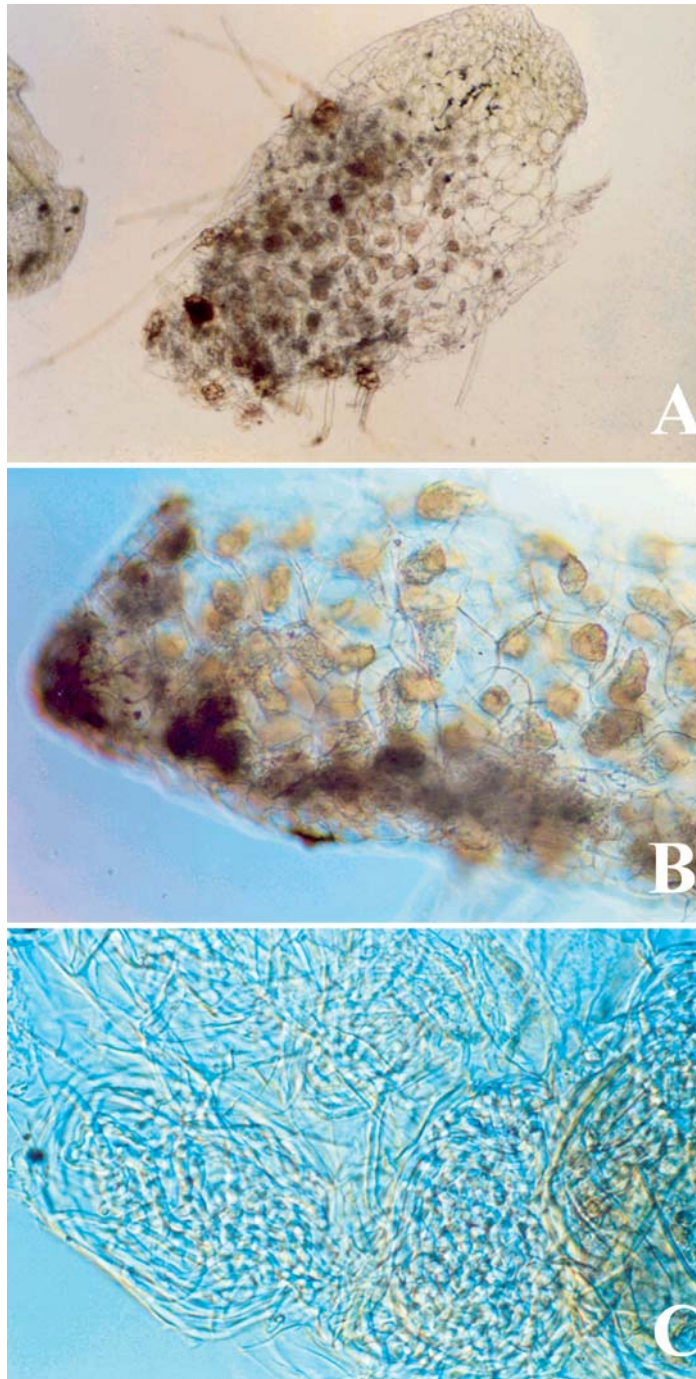


Figure 2 *Spathoglottis plicata* protocorms colonized by *Epulorhiza repens* (KUFC 14-6), 21 days after sowing incubation at 28 °C, forming pelotons in root cortical cells, 100X(A), 400X(B) and 1000X(C).

***Rhizoctonia* sp.**

On PDA, colony reaching 9 cm in diam. after 16 days (growth rate at 28°C approximately 0.02 mm/h). Colony cream, later yellow, with cottony aerial mycelium (Figure 1E). Vegetative hyphae septate, hyaline becoming yellow, brown when mature, branching at nearly right angles similar to the genus of *Rhizoctonia* (Figure 1G,H), and monilioid absent. Abundant cylindric shape spores (Figure 1F).

Host : Root of *Spathoglottis plicata*, Queen Sirikit Botanic Garden, Chiang Mai

***Sebacina* sp.**

Thin culture growth, little or no aerial mycelium. Probasidia fusoid – clavate developing an apical papillate prolongation at an early stage. Hyphae smooth, hyaline 3-5 µm, binucleate, lacking clamp connections. *Sebacina* sp. was isolated from *Spathoglottis plicata* in Khao Yai, Nakhon Ratchasima. The monilioid cells were found and the basidia were produced in the culture but spores were absent. This fungus was also isolated from mycorrhizae of *Platanthera orbiculata* (Currah *et al.*, 1990). The anamorphic state of *Sebacina* sp, was *Rhizoctonia globularis* (Saksena and Vaartaja, 1960). Warcup and Talbot (1967) induced the culture of *R. globularis* to form small fructification in soil-on agar plates, Urrbrae red clay as the soil. This perfect state proved to be a species of *Sebacina* with ovoid, cruciately-septate probasidia. The genus was based on teleomorphic material found in nature on twigs, grass, wood, etc., rather than on cultural or anamorph characteristics. Warcup and Talbot (1967) described the cultural characteristics of the teleomorphic state of *Sebacina vermifera* which isolated from Australian orchids and this fungus was associated with all the members of *Caldeninae* except *Lyperanthus*.

HOST : Root of *Spathoglottis plicata*, Nakhon Ratchasima

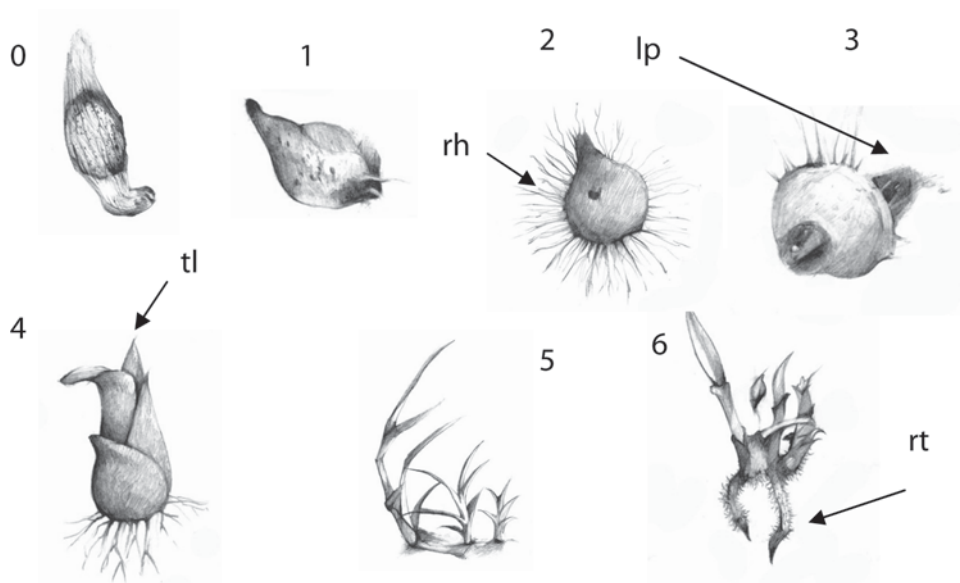
**Germination with fungi**

Seeds of *S. plicata* germinated within 21 days for all treatments. They increased in size, rupturing their testae. The development of fungi infected protocorms that ultimately grew beyond stage 1 was extremely rapid after 21 days after sowing, with the initiation and elongation of rhizoid in stage 2 (Figure 2; Table 2). Most (99.2% and 96.3%) *S. plicata* seeds germinated within 21 days when inoculated with *E. repens* (KUFC 14-6) and *R. globularis* (KUFC 5-21). Seedling development inoculated with *E. repens* (KUFC 14-6) continued growing to 60 days after sowing, with 5.1% of them initiated rhizoid, 30% initiated leaf primordium, 47.5% initiated the first true leaf and 8.1% initiated leaves (Figure 3; Table 2). The remaining of seedling germinated 2.4% but they did not develop into the other stages. For seeds inoculated with *R. globularis* (KUFC 5-21), they initiated rhizoid, leaf primordium, first true leaf and leaves 42.2, 28.6, 16.4 and 1.7 % respectively after 60 days. About 10.8% of them reached stage 1. In contrast, asymbiotic seedlings germinated 15% but did not progress much beyond testa splitting. The seedling development continued up to 127 days after sowing; 42.8 and 12.6% of the seedlings inoculated with *E. repens* (KUFC 14-6) and *R. globularis* (KUFC 5-21) developed root with hair in stage 6 (Figure 3).

The results showed that *E. repens* isolate (KUFC14-6) was more effective than *R. globularis* (KUFC 5-21) in symbiotic germination (figure 4A-D). It might be specific between the fungus and orchid host because *E. repens* isolate (KUFC 14-6) was isolated from *S. plicata* whereas *R. globularis* was isolated from *Goodyera procera*. Narmatha *et al.* (2000) isolated *Rhizoctonia* isolate SP1a from *Spathoglottis plicata* and studied symbiotic seed germination. They reported that *S. plicata* seeds germinated with *Rhizoctonia* isolate SP1a germinated 75% and the *S. plicata* seedlings with leaves and roots were obtained within the 8<sup>th</sup> week of inoculation with the fungal isolate.

**Table 2** Percentages of seed/seedling in developmental stages 21, 60 and 127 days after sowing fungal-inoculated seed and the control (non-inoculated).

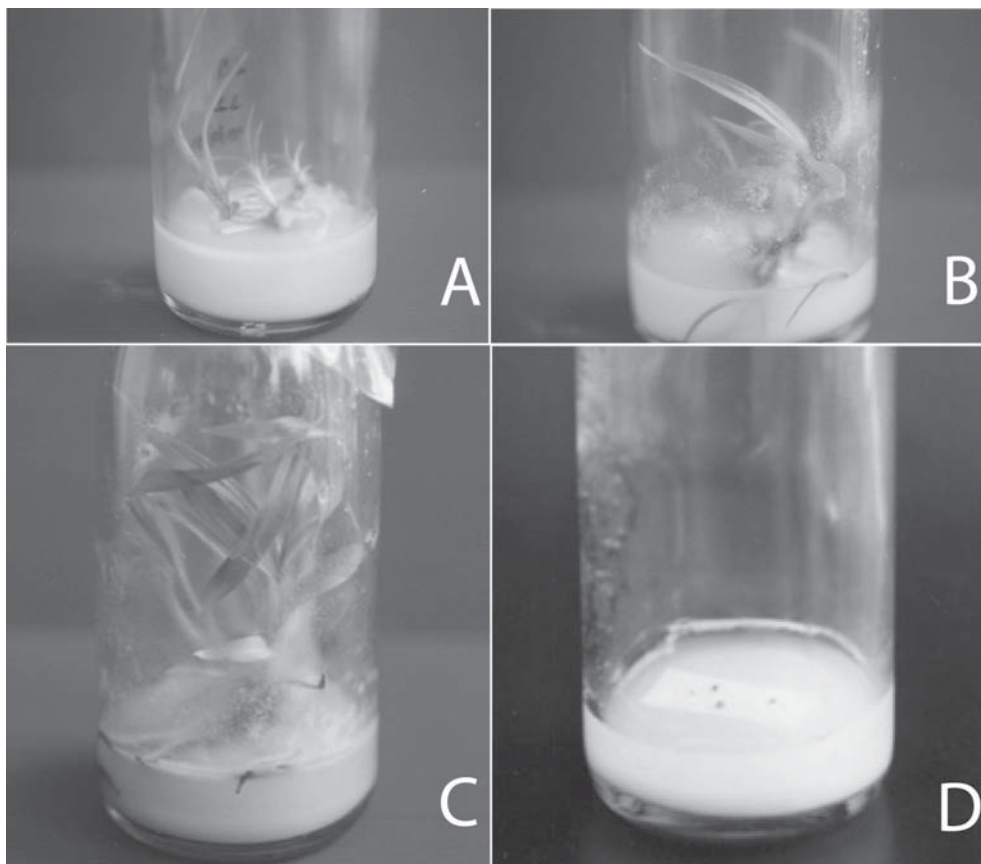
Treatment		Stages of development (%)						
		0	1	2	3	4	5	6
21 days	<i>Epulorhiza repens</i> (KUFC 14-6)	0.8	7.1	92.1	0	0	0	0
	<i>Rhizoctonia globularis</i> (KUFC 5-21)	3.7	28.6	67.7	0	0	0	0
	Control (Asymbiotic)	85	15	0	0	0	0	0
60 days	<i>Epulorhiza repens</i> (KUFC 14-6)	0	2.4	5.1	30.0	47.5	8.1	0
	<i>Rhizoctonia globularis</i> (KUFC 5-21)	0	10.8	42.2	28.6	16.4	1.7	0
	Control (Asymbiotic)	0	0	0	0	0	0	0
127 days	<i>Epulorhiza repens</i> (KUFC 14-6)	0	0	0	14.5	12.7	30.0	42.8
	<i>Rhizoctonia globularis</i> (KUFC 5-21)	0	0	14.0	17.3	31.0	25.1	12.6
	Control (Asymbiotic)	0	0	0	0	0	0	0



*P. Athipunyakom*

**Figure 3** Developmental stages of *Spathoglottis plicata* in symbiotic germination : stage 0 = no germination ; stage 1 = germination (seed coat ruptured by enlarged embryo) ; stage 2 = presence of rhizoids (rz) ; stage 3 = presence of leaf primordium (lp) ; stage 4 = appearance of the first true leaf (tl) ; stage 5 = elongation of initial leaves ; stage 6 = elongation of root.





**Figure 4** *In vitro* symbiotic germination of *Spathoglottis plicata* seedlings after 127 days incubation at 28 °C , seedlings associated with *Epulorhiza repens* (KUFC 2-7) (A), *Rhizoctonia globularis* (KUFC 5-21) (B), *Epulorhiza repens* (KUFC 14-6) (A) and Control (D).

*Rhizoctonia* isolate SP1a was not identified to species. Anderson (1991) reported an isolation of *Epulorhiza repens* from *Spiranthes magnicamporum*, thus, it supported the germination and development of the seeds of this species (Figure 2 and Figure 4 A-D).

### CONCLUSION

Three genera and four species of mycorrhizal fungi were identified: *Epulorhiza repens*, *Rhizoctonia globularis*, *Rhizoctonia* sp. and *Sebacina* sp. Among them, *E. repens*, *Rhizoctonia* sp. and *Sebacina* were associated

with *S. plicata*, whereas *R. globularis* was associated with the two hosts: *S. plicata* and *G. procera*. All isolates were binucleate. *In vitro* seed germination of *S. plicata* indicated that *Epulorhiza repens* (KUFC 14-6) was the most effective mycorrhizal fungi to stimulate growth and development. The seedlings became more mature and ready to be planted after 127 days.

### ACKNOWLEDGEMENTS

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## LITERATURE CITED

- Anderson, A.B. 1991. Symbiotic germination, asymbiotic germination and growth of *Spiranthes magnicamporum* (Orchidaceae). **Lindleyana** 6: 183-186.
- Bandoni, R. 1979. Safranin O as a rapid nuclear stain for fungi. **Mycologia** 71: 873-874.
- Beyrle, H.F. and S.E. Smith. 1993. Excessive carbon prevent greening of leaves in mycorrhizal seedlings of the terrestrial orchid *Orchis morio*. **Lindleyana** 89 (2): 97-99.
- Currah, R.S., L. Sigler and S. Hambleton. 1987. New records and new taxa of fungi from mycorrhizae of terrestrial orchids of Alberta. **Can. J. Bot.** 65: 2473-2482.
- Currah, R.S., A. Smreciu and S. Hambleton. 1990. Mycorrhizae and mycorrhizal fungi of boreal species of *Platanthera* and *Coeloglossum* (Orchidaceae). **Can. J. Bot.** 68: 1171-1181.
- Dixon, K. 1987. Raising terrestrial orchids from seed, pp.47-100. In W.K. Harris (ed.), **Modern Orchid Growing for Pleasure and Profit**. Orchid Club of Southern Australia Inc. Adelaide.
- Hadley, G. 1982. Orchid mycorrhiza, pp. 81-118. In J. Arditti (ed.). **Orchid Biology : Reviews and Prespective, II** Cornell University Press, Ithaca, New York.
- Harley, J.L. and S.E. Smith. 1983. **Mycorrhizal Symbiosis**. London. Academic Press. 483 p.
- Kamemoto, H. and R. Sagarik. 1975. **Beautiful Thai Orchid Species**. The orchid society of Thailand, Aksornsampan Press. 186 p.
- Masuhara, G. and K. Katsuya. 1994. *In situ* and *In vitro* specificity between *Rhizoctonia* spp. and *Spiranthes sinensis* (Persson) Ames. var. *amoena* (M. Bieberstein) Hara (Orchidaceae). **New Phytol.** 127: 711-718.
- Moore, R.T. 1987. The genera of *Rhizoctonia*-like fungi; *Ascorhizoctonia*, *Ceratorhiza* gen. nov., *Moniliopsis* and *Rhizoctonia*. **Mycotaxon** 29: 91-99.
- Narmatha, L.S., J.Y. Wong, T.K. Tan and C.S. Loh. 2000. Symbiotic abilities of mycorrhizae isolated from terrestrially grown and epiphytic orchids, pp. 56. In the **3<sup>rd</sup> International Symposium on Rhizoctonia (ISR 2000)**, 17-20 August 2000. National Chung Hsing University, Taichung, Taiwan (ROC).
- Otero, J.T., J.D. Ackerman and P. Bayman. 2002. Diversity and host specificity of endophyte *Rhizoctonia*-like fungi tropical orchids. **Am. J. Bot.** 89(11): 1852-1858.
- Rasmussen, H.N. 1995. **Terrestrial Orchids from Seed to Mycotrophic Plant**. Cambridge University Press. 444 pp.
- Rasmussen, H.N., B. Johansen and T.F. Andersen. 1991. Symbiotic *in vitro* culture of immature embryos and seeds from *Listera ovata*. **Lindleyana** 6(3):134-439.
- Senthikumar, S., and K.V. Krishnamurthy. 1998. The role of root hairs in the mycorrhizal association of the ground orchid, *Spathoglottis plicata* blume. **Mycoorhiza News** 10(2): 15-17.
- Sneh, B., L. Burpee, and A. Ogoshi. 1991. **Identification of Rhizoctonia species**. St. Paul APS press. 133 p.
- Saksena, H.K. and O. Vaartaja, 1960. Descriptions of new species of *Rhizoctonia*. **Can. J. Bot.** 38: 931-943.
- Takahashi, K., I. Ogiwara and N. Hakoda. 2001. Inoculation of *Habenaria* (Pecteilis) *radiata* with two isolates of orchid mycorrhizal fungi : germination and growth response to separate, stimulateneous, and consecutive inoculation. **Lindleyana** 16(2): 102-108.
- Terashita, T. 1982. Fungi inhabiting wild orchids in Japan (II). Isolation of symbionts from *Spiranthes sinensis* var. *amoena*. **Trans. Mycol. Soc. Japan** 23: 319-328.
- Wang, T.Y., C.F. Lin and Y.T. Lin. 2004. Effect of orchid mycorrhizal fungi on seed germination of *Dendrobium*. pp. 60. In

**Abstract the 8<sup>th</sup> Asia Pacific Orchid Conference in Taiwan, 6-8 March.**

- Warcup, J.H. and P.H.B. Talbot. 1967. Perfect states of Rhizoctonias associated with orchids I. **New Phytol.** 66: 631-641.
- Warcup, J.H. and P.H.B. Talbot. 1971. Perfect states of Rhizoctonias associated with orchids II. **New Phytol.** 70: 35-40.
- Utetake, Y. and R.L. Peterson. 1998. Association between microtubules and symbiotic fungal hyphae in protocorm cells of the orchid species, *Spiranthes sinensis*. **New Phytologist** 140: 715-722.
- Zettler, L.W. and T.M. McInnis, Jr. 1993. Symbiotic seed germination and development of *Spiranthes cernya* and *Goodyera pubescens* (Orchidaceae): Spiranthoideae) **Lindleyana** 8(3): 155-162.
- Zettler, L.W., J.C. Burkhead and J.A. Marshall. 1999. Use of mycorrhizal fungus from *Epidendrum conopseum* to propagate *Encyclia tampensis* from seed *in vitro*. **Lindleyana** 14(2): 102-105.