Controlling *Rhizoctonia* Damping-off of Chinese Mustard by Using Endomycorrhizal *Rhizoctonia* spp. Isolated from Orchid Mycorrhizae

Jr-Hau Jiang and Si-Loi Tam, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; Takeshi Toda, Faculty of Bioresource Science, Akita Prefectural University, Shimo-shinjo, Akita 010-0195, Japan; and Lung-Chung Chen, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

Abstract

Jiang, J.-H., Tam, S.-L., Toda, T., and Chen, L.-C. 2016. Controlling *Rhizoctonia* damping-off of Chinese mustard by using endomycorrhizal *Rhizoctonia* spp. isolated from orchid mycorrhizae. *Plant Dis.* 100:85-91.

Inoculation of hypovirulent *Rhizoctonia* spp. has been recognized as an effective strategy for protecting plants against damping-off caused by pathogenic *Rhizoctonia* spp. In this study, endomycorrhizal *Rhizoctonia* spp. isolated from fungal pelotons in orchid plants were used for controlling *Rhizoctonia* damping-off of Chinese mustard. According to phylogenetic analysis and anastomosis group (AG) determination, the virulence of three isolates of multinucleate *Rhizoctonia solani* in AG-6; eight isolates of binucleate *Rhizoctonia* in AG-A, AG-B, AG-G, AG-P, and AG-R; and two isolates of binucleate *R. repens* were evaluated using test plants. All

isolates, except that in AG-R, caused low disease severity in 10-day-old radish (0.10 to 0.61), cucumber (0.28 to 0.54), and Chinese mustard (0.18 to 0.65). By contrast, pathogenic isolates in AG-4 killed almost all test plants with symptoms of collapsed hypocotyl and wilted leaves (0.88 to 0.96). Of the 13 endomycorrhizal *Rhizoctonia* isolates assessed, AG-P isolates Cno10-3 and CalS1-2 provided 91 and 100% protection, respectively, against *R. solani* AG-4 in 26-day-old Chinese mustard. This study revealed that endomycorrhizal *Rhizoctonia* spp. in orchid have the potential to biologically control damping-off of Chinese mustard.

Orchidaceae is one of the largest and most diverse plant families, including an estimated 20,000 to 35,000 species in the plant kingdom (12). Several researchers have confirmed that the primary mycorrhizal fungi associated with green orchid are Ceratobasidiaceae, Tulasnellaceae, and Sebacinaceae fungi (13,38); these fungi can be recognized as the form genus *Rhizoctonia* (33,40,49). Genus *Rhizoctonia* was first described by De Candolle (1815) and is considered a heterogeneous assemblage of filamentous fungal taxa (16). Although many reports have listed various saprophytic and symbiotic taxa, the organisms belonging to this genus are typically pathogens, which are primarily associated with host roots (39). Pathogenic *Rhizoctonia solani* Kühn often severely damages crops, ornamental plants, and trees (16).

Genus *Rhizoctonia* comprises multinucleate *Rhizoctonia* spp. (MNR), binucleate *Rhizoctonia* spp. (BNR), and uninucleate *Rhizoctonia* spp. (36). Most MNR and BNR are further divided into various anastomosis groups (AGs). At present, the multinucleate *R. solani* (teleomorph = *Thanatephorus cucumeris* (A. B. Frank) Donk) contains 14 AGs (AG-1 to AG-13 and AG-BI) (9,16), whereas BNR (teleomorph = *Ceratobasidium* D. P. Rogers) are divided into at least 16 AGs (AG-A to AG-I, AG-K, AG-L, and AG-O to AG-S) (36). Within the same AG, isolates may exhibit similar characteristics, such as types of disease symptoms caused and host preferences (21). Moreover, several studies have demonstrated that some BNR (e.g., isolates in AG-B_(O), AG-G, and AG-P) are hypovirulent to crops and can provide protection from damping-off (19,39). Alternative methods of control such as using biocontrol organisms have been considerably investigated since soil fumigation was prohibited (35).

Various hypovirulent BNR isolated from soil have been confirmed to act against pathogenic *Rhizoctonia* spp., and certain isolates have yielded 75 to 95% protection (41). In soybean (22,32), bean (18,50),

cucumber (20), cotton (19), tomato (26), and spinach (27), certain isolates of hypovirulent BNR have been shown to act as inducing agents, leading to systemic resistance against pathogenic fungi such as *R. solani*, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, and *F. oxysporum* f. sp. *spinaciae*. Recently, isolates of *Ceratobasidium* from orchid have been used to suppress rice sheath blight disease caused by *R. solani* AG-1 IA (25). Moreover, certain MNR and BNR species are mycorrhizal symbionts of orchid, whose minute seed pass through a nonphotosynthetic phase in which they depend on an external supply of nutrients and require an association with a compatible fungus to obtain sufficient nutrition for germination (3). According to the classification of AG determination, certain isolates in three AGs of MNR (AG-5, AG-6, and AG-12) and four AGs of BNR (AG-A, AG-C, AG-E, and AG-I) have been reported to form orchid mycorrhizae with wild orchid (10,31,40,47). Mosquera-Espinosa et al. (25) indicated that isolates of BNR (teleomorph = *Ceratobasidium*) that originated from an association with terrestrial orchid are more likely to survive in soil environments and provide effective biological control compared with *Rhizoctonia* fungi isolated from epiphytic orchid species adapted to live on tree bark.

No studies have involved using endomycorrhizal *Rhizoctonia* fungi to control vegetable damping-off caused by pathogenic *R. solani* AG-4, a major pathogen on a wide variety of hosts (e.g., radish, cauliflower, cabbage, carrot, and cucumber) (46). The damping-off caused by *R. solani* AG-4 is particularly severe in plug trays if the surviving inoculum touches emerging seedlings of Chinese mustard (*Brassica rapa* var. *chinensis*). Isolates of endomycorrhizal *Rhizoctonia* spp. in our study were obtained from fungal pelotons in the roots or rhizomes of wild orchid. Little is known regarding the pathogenicity, virulence, and mycorrhizal symbiosis of orchid mycorrhizal fungi, particularly endomycorrhizal *Rhizoctonia* spp. Thus, the objectives of this study were to (i) identify isolates of endomycorrhizal *Rhizoctonia* sampled from fungal pelotons in wild orchid in Taiwan according to morphology, AG determination, and phylogenetic analysis of the internal transcribed spacer (ITS) region with 5.8S ribosomal DNA (rDNA-ITS); (ii) analyze the virulence of endomycorrhizal *Rhizoctonia* isolates on radish, cucumber, and Chinese mustard; and (iii) determine which endomycorrhizal *Rhizoctonia* isolates can control vegetable damping-off caused by *R. solani* AG-4.

Materials and Methods

Endomycorrhizal *Rhizoctonia* spp. Sections of the root cortices of wild orchid exhibiting fungal pelotons were excised, surface

Corresponding author: L.-C. Chen; E-mail: lcchen5a08@gmail.com

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is published online.

Accepted for publication 26 April 2015.

sterilized in a 1% sodium hypochlorite solution for 1 min, rinsed in sterile water, and transferred to tap water agar (TWA) and potato dextrose agar (PDA) acidified with 0.2% lactic acid to inhibit the growth of bacteria. After incubation for 48 h at 24°C, hyphal tips of *Rhizoctonia* spp. were selected according to the genus *Rhizoctonia*; branching and constriction were apparent near the distal septum of cells in young vegetative hyphae; and conidia, clamp connections and rhizomorphs, and sclerotia without differentiating into the rind and medulla were absent (40).

Nuclear number and AGs. Thirteen representative isolates of endomycorrhizal *Rhizoctonia* from orchid mycorrhizae were initially identified as MNR or BNR after staining with 0.1% trypan blue in lactophenol (6) or 3% KOH and safranin O (4) and observing the hyphal growth after 4 to 7 days of incubation in PDA at 25°C. To determine the AGs of endomycorrhizal *Rhizoctonia* spp., an endomycorrhizal *Rhizoctonia* isolate of unknown AG and a representative tester strain of known AG were grown on PDA for 2 to 3 days. The mycelial plugs obtained from the advancing margins of pure cultures of each isolate were paired with tester strains in TWA and left to grow at 25°C. The overlapping portions of hyphae were transferred to a slide, stained with 0.1% trypan blue in lactophenol, and evaluated for hyphal fusion (9). Three petri dishes were used for each treatment, and experiments were repeated twice. The anastomosis reactions were grouped into four categories (C0 to C3) according to the description by Carling (8).

Virulence determination. Radish (*Raphanus sativus* L.) and cucumber (*Cucumis sativus* L.) were used as the test crops because Juan-Abgona et al. (20) screened hypovirulent *Rhizoctonia* spp. by using these crops. The seed were disinfected with 70% ethanol for 1 min, soaked in 2% sodium hypochlorite for 5 min, then rinsed three times with sterile distilled water. The seed were pregerminated for 2 days at 25°C, and five seedlings were transferred to 2% TWA. Subsequently, a 9-mm mycelial disc obtained from the margin on a 5-day-old mycelium of an endomycorrhizal *Rhizoctonia* isolate was centered on the TWA and maintained in a growth chamber (16-h photoperiod and daytime illuminance of approximately 10,000 lux) at 25°C. Two pathogenic isolates from the seedlings of cabbage (*B. oleracea* var. *capitata*) and Chinese cabbage (*B. rapa* var. *pekinensis*)

that caused damping-off were identified as *Rhizoctonia solani* AG-4 and used as a positive control. Five replicates of each treatment were maintained for 8 days (9) and the experiment was repeated twice. Experiments to determine virulence on radish, cucumber, and Chinese mustard were performed according to a completely randomized design.

For 10-day-old seedlings of radish and Chinese mustard (9), disease severity was determined using the following 5-point scale: 0 = healthy, exhibiting no lesions on the hypocotyl; 1 = minor discoloration of the hypocotyl; 2 = discoloration plus small necrotic lesions (<1 mm) on the hypocotyl or primary root; 3 = discoloration plus large necrotic lesions (>1 mm) on the hypocotyl or primary root; and 4 = collapsed hypocotyl exhibiting wilted leaves or dead seedlings. For cucumber, a modified rating was required to assess disease severity (20), in which 0 = no lesions on the hypocotyl, 1 = one or two yellow-brown lesions (<0.25 mm), 2 = yellow-brown lesions (<0.5 mm) or water-soaked areas covering <10% of the hypocotyl, 3 = yellow-brown to dark brown lesions (>0.5 mm) that coalesce with other lesions, or water-soaked areas covering >10% of the hypocotyl, and 4 = collapsed hypocotyl exhibiting wilted leaves or dead seedlings.

Evaluation of plant protection treatments. Inoculum was prepared by growing each isolate of endomycorrhizal and pathogenic *Rhizoctonia* on PDA for 3 days and transferring three mycelial discs to 200 g of autoclaved barley grain (1:1 dry barley grain/distilled water [wt/vol]) in 500-ml Erlenmeyer flasks. The cultures were incubated at 25°C for 10 days and shaken every 2 days to ensure a uniform distribution of inoculum. Chinese mustard was planted in eight-cell plug trays, with one 10-day-old seedling in each cell, and one barley grain colonized with an isolate of endomycorrhizal *Rhizoctonia* was placed in each cell. The seedlings were regularly watered and incubated in a growth room at 25°C with a 16-h photoperiod of visible light. Six days after planting, each seedling was challenged with one barley grain that was colonized with the virulent *R. solani* AG-4 isolate Cabl2 or Chcab4; the grains were placed at an equal distance (2 cm) from each seedling, except for the noninoculated control. The number of surviving plants was recorded after another 10 days of culturing and the percentage of plant protection was calculated as follows (41): Protection (%) = [(A - B)/(C - B)] × 100, where A is the percentage of surviving

Table 1. Isolates of endomycorrhizal *Rhizoctonia* from fungal pelotons in wild orchid^z

Isolate/accession number	Orchid habitat, locality	Highest Blast hit ^a	Similarity (%)
ANOF0/KJ495962	<i>Anoectochilus formosanus</i> Terrestrial, Yangmingshan	AF354102, isolate 70Rs <i>T. cucumeris</i> strain AG6-HG-I	97 ...
ANOF4/KJ495966	<i>Anoectochilus formosanus</i> Terrestrial, Hsinchu	AF354102, isolate 70Rs <i>T. cucumeris</i> strain AG6-HG-I	97 ...
ANOFD1/KJ495967	<i>Anoectochilus formosanus</i> Terrestrial, Wufong	AF354104, isolate 75Rs <i>T. cucumeris</i> strain AG6-GV	97 ...
Cno3-2/JX514374	<i>Zeuxine</i> sp. Terrestrial, Wulai, Sansia	AY927362, isolate R78 <i>Ceratobasidium</i> sp. AG-A	98 ...
Sno5-12/JX514386	<i>Cleisostoma paniculatum</i> epiphytic, Wulai, Sansia	JQ859885/isolate WUF-ST-RhT4-3 <i>Ceratobasidium</i> sp. AG-B	93 ...
Eno3-3/JX514377	<i>Goodyera procera</i> Terrestrial, Wulai, Sansia	AY927348, isolate R56 <i>Ceratobasidium</i> sp. AG-G	92 ...
Zeus1-1/KJ573103	<i>Zeuxine strateumatica</i> Terrestrial, Tainan	DQ102402, isolate Str14 <i>Ceratobasidium</i> sp. AG-G	99 ...
ANOF2/KJ495964	<i>Anoectochilus formosanus</i> Terrestrial, Nantou	DQ102402, isolate Str14 <i>Ceratobasidium</i> sp. AG-G	99 ...
Cno10-3/JX514376	<i>Cheirostylis hungyehensis</i> Terrestrial, Wulai, Sansia	AB286938, isolate: C-584 <i>Ceratobasidium</i> sp. AG-P	100 ...
CalS1-2/JX514384	<i>Calanthe sylvatica</i> Terrestrial, Wulai, Sansia	AB286941, isolate: C-578 <i>Ceratobasidium</i> sp. AG-P	95 ...
ANOF3/KJ495965	<i>Anoectochilus formosanus</i> Terrestrial, Wulai, Sansia	AB286942, isolate: X4-3 <i>Ceratobasidium</i> sp. AG-R	99 ...
Sno3-3/JX514385	<i>Liparis nakaharai</i> epiphytic, Wulai, Sansia	GU166403, isolate Ps-KT-0-1 <i>Tulasnella calospora</i>	99 ...
Sno6-1/JX514393	<i>Cymbidium ensifolium</i> Terrestrial, Wulai, Sansia	AJ313437, Sp1a <i>Epulorhiza</i> sp.	99 ...

^z Ribosomal DNA-internal transcribed spacer sequences were obtained from GenBank and the reference sequence with highest blast hit was selected based on a known anastomosis group (AG).

plants after colonization with a hypovirulent isolate and challenge inoculation with a virulent isolate, B is the percentage of surviving plants after inoculation with only a virulent isolate, and C is the percentage of surviving noninoculated plants. Experiments for determining plant protection of Chinese mustard grown in soil were performed according to a completely randomized design and repeated twice. Disease severity in 26-day-old Chinese mustard was assessed at the hypocotyl according to a four-point scale (Supplementary Figure S1), in which 0 = no lesions on the hypocotyl; 1 = minor discoloration of the hypocotyl; 2 = discoloration and large, dark brown necrotic lesions on the hypocotyl, and no wire stem symptoms; and 3 = wire stem on the hypocotyl.

Phylogenetic analysis. DNA was extracted using the DNeasy Plant mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The total DNA was recovered in 50 μ l of distilled deionized water and maintained at -80°C . The sequence of rDNA-ITS was amplified using forward primer ITS1 and reverse primer ITS4 (51). A polymerase chain reaction (PCR) was conducted in a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) by using the following thermo profile: initial denaturation at 94°C for 2 min; followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 60 s, and extension at 72°C for 60 s; and final extension at 72°C for 5 min. The PCR products were purified using the MinElute PCR Purification Kit (Qiagen). Clean samples

were eluted with 50 μ l of sterile water and quantified to conduct sequencing reactions. Cycle sequencing was conducted using BigDye version 3.1 chemistry, and sequencing was performed using an ABI 3100 Genetic Analyzer (Applied Biosystems). Both strands of DNA were sequenced. The sequences obtained in this study are available from GenBank under accession numbers listed in Table 1.

The rDNA-ITS sequences underwent a batch BLAST search (1) on the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Results that were highly similar were downloaded and added to the dataset. A multiple sequence alignment of each ITS sequence was generated using Clustal W for multiple alignment (45), packaged by the BioEdit Sequence Alignment Editor (17). Phylogenetic trees were obtained from the sequences analyzed by using Molecular Evolutionary Genetics Analysis software version 5.0 (44) with a neighbor-joining method involving the Kimura two-parameter distance algorithm. In total, 1,000 bootstrap replicates were performed to estimate the node reliability of the phylogenetic trees. Bootstrap support values $>70\%$ were considered significant. Branches that exhibited low bootstrap support values ($<70\%$) were removed.

Statistical analysis. All analyses were conducted using SAS 9.4 (SAS Institute, Inc., Cary, NC). The ordinal data of a rating scale were calculated by applying a nonparametric methodology (34). The rank

Fig. 1. Endomycorrhizal *Rhizoctonia* isolated from fungal pelotons in cortical cells of roots or rhizomes in wild orchid **A**, *Anoectochilus formosanus*; **B**, *Goodyera procera*; and **C**, *Cymbidium lancifolium*. **D** to **F**, Fungal pelotons (white arrows). **G**, to **I**, Cultural types of endomycorrhizal *Rhizoctonia* isolates. **J** to **L**, Multinucleate and binucleate *Rhizoctonia* isolates are identified by the genus concept of *Rhizoctonia* and the number of nuclei per cell in young vegetative hyphae (black arrows). Scale bar represents 3 cm (**A**), 10 cm (**B** and **C**), 1,000 μ m (**D**), 400 μ m (**E** and **F**) and 20 μ m (**J** to **L**).

assigned to each observation was determined through the PROC RANK and PROC MIXED procedure used to compute test statistics and levels of significance. Estimated relative treatment effects for disease severity were calculated using $\hat{p}_i = 1/N(\bar{R}_i - 1/2)$, in which values are between 0 and 1, where i is the treatment, N is the number of observations, and $\bar{R}$ is the mean rank. In addition, 95% confidence interval (CI) was generated using LD_CI macro (5). A smaller value of $\hat{p}_i$ indicates a lower value of disease severity after a treatment. Values of $\hat{p}_i$ for two treatments differed significantly when the CI for each $\hat{p}_i$ did not overlap. Data on plant protection were analyzed using a one-way analysis of variance. Mean separation was assessed using a protected post-hoc Tukey's test ($P = 0.05$) after significant results from an F test in SAS 9.4 were determined.

Results

Phylogenetic analysis. Isolates of endomycorrhizal *Rhizoctonia* sampled from fungal pelotons in orchid (Fig. 1) located at six localities in Taiwan were identified (Table 1). Selected isolates were further identified as BNR and MNR according to the number of nuclei per cell in the young vegetative hyphae (Fig. 1J to L). One representative isolate from each orchid was further analyzed. In all, 3 isolates of MNR and 10 isolates of BNR were obtained in this study and assigned to multinucleate *R. solani* (teleomorph = *T. cucumeris*),

BNR (teleomorph = *Ceratobasidium*), and binucleate *R. repens* (teleomorph = *Tulasnella calospora*) according to the BLAST results (Table 1).

Phylogenetic analysis was conducted to group the isolates into three large clades (Fig. 2), labeled *Thanatephorus* (MNR), *Ceratobasidium* (BNR), and *Tulasnella* (BNR). The first two clades were categorized into different AGs. Isolates in clade *Thanatephorus* (MNR) formed an AG-6 clade exhibiting 88% bootstrap support and included reference sequences of *R. solani* AG-6 (mycorrhizal association, Japan; AF153780, AF153787, and AF153790) (37) and AG-6 GV (soil, Japan, AF354104) (15). Three isolates of putative AG-6 (ANOF0, ANOF4, and ANOFD1) were paired with a known representative tester strain of *R. solani* AG-6 GV (isolate NKN-2-1, Japan, provided by Ogoshi) and exhibited C2 reactions with the tester.

In clade *Ceratobasidium* (BNR), five subclades were classified according to isolates of AG-A (99% bootstrap support), AG-B (98% bootstrap support), AG-G (85% bootstrap support), AG-P (77% bootstrap support), and AG-R (100% bootstrap support). Isolates of AG-P and AG-U were grouped into the same subclade, because anastomosis was confirmed between certain isolates of AG-P and AG-U (36). Each isolate of a putative AG was paired with a known representative tester strain of AG-A (isolate AH-1), AG-G (isolate AH-9), and AG-P (isolate C-578) (Japan, provided by Ogoshi).

Fig. 2. Phylogeny of endomycorrhizal *Rhizoctonia* isolates from orchid and related fungi based on ribosomal DNA-internal transcribed spacer (ITS) sequences using the neighbor-joining method. One ITS sequence from *Sebacina vermifera* (AF20272) was defined as an outgroup. Numbers at the nodes show bootstrap values (1,000 replicates; only values above 70% are shown) from the corresponding neighbor-joining tree. MNR = multinucleate *Rhizoctonia* spp. and BNR = binucleate *Rhizoctonia* spp.

All isolates obtained from the orchid plants exhibited C2 reactions with their tester isolates. According to the high similarity of rDNA-ITS sequences and the phylogenetic analysis (36,37), isolates ANOF3 and Sno5-12 were recognized as AG-R and AG-B, respectively. ANOF3 was highly homologous with *Rhizoctonia* AG-R isolates in GenBank (accession numbers HQ269823 and AB286942), having 98 and 99% identities (11,36). Sno5-12 was grouped with *Rhizoctonia* AG-B (accession number JQ859885), with 93% identity (14), forming a cluster with AG-B_(O) (accession numbers AB219143 and DQ102430) and supported by a 98% bootstrap value.

The *Tulasnella* (BNR) clade, which exhibited 100% bootstrap support, included reference sequences of *T. calospora* (from orchid, Thailand, GU166403; GU166407) (28) and *Epulorhiza* spp. (from orchid, Singapore, AJ313439; AJ313437) (23). Isolate Sno3-3 from the orchid *Liparis nakaharai* shared a 99% sequence identity with *T. calospora* (GU166403, teleomorphic *R. repens*), and isolate Sno6-1 from the orchid *Cymbidium ensifolium* shared a 98% sequence identity with *Epulorhiza* sp. (AJ313437) (99% bootstrap support).

Virulence determination in vitro. All isolates sampled from orchid, except for isolate ANOF3 (AG-R), were mildly virulent to the 10-day-old radish, cucumber, and Chinese mustard, ranging in relative treatment effects ($\hat{p}_i$) according to disease severity from 0.1 to 0.61 in radish (noninoculated control, $\hat{p}_i = 0.1$), 0.28 to 0.54 in cucumber (noninoculated control, $\hat{p}_i = 0.28$), and 0.18 to 0.65 in Chinese mustard (noninoculated control, $\hat{p}_i = 0.18$); however, isolate ANOF3 was highly virulent to the test plants (particularly to cucumber; $\hat{p}_i = 0.89$) (Table 2). Among the isolates of endomycorrhizal *Rhizoctonia*, both Sno3-1 and Sno6-1 did not cause the test plants to become diseased. Nevertheless, both pathogenic *R. solani* AG-4 isolate Cabl2 and Chcab4 were highly virulent to radish, cucumber, and Chinese mustard, killing almost all test plants that exhibited wilted cotyledons and collapsed hypocotyl ($\hat{p}_i = 0.88$ to 0.96). In this study, mild symptoms were rated as <2 according to a 0-to-4 scale.

Plant protection evaluation. The relative disease severity from hypovirulent isolates and from hypovirulent isolates challenged by virulent isolates on Chinese mustard, as well as the percentage of plant protection provided by the hypovirulent isolate, are shown in

Table 3. Almost all plants died when inoculated with *R. solani* AG-4 isolate Cabl2 ($\hat{p}_i = 0.96$), suggesting that Cabl2 expressed stable virulence to Chinese mustard grown in soil compared with the pathogenic isolate Chcab4 ($\hat{p}_i = 0.85$). Most isolates of endomycorrhizal *Rhizoctonia*, except for two isolates of *Tulasnella* (Sno6-1 and Sno3-3) and one isolate of *Ceratobasidium* (Sno5-12), protected Chinese mustard against *R. solani* AG-4 isolate Cabl2 (Table 3). *R. solani* AG-6 isolates (ANOF0, ANOF4, and ANOFD1) reduced disease severity and afforded moderate plant protection (45.8 to 52.4%). Regarding endomycorrhizal BNR (*Ceratobasidium*) treatments, all isolates except for Sno5-12 significantly reduced the relative treatment effects ($\hat{p}_i = 0.14$ to 0.57), yielding various levels of plant protection (41.1 to 100%). The *Ceratobasidium* AG-P isolates CalS1-2 and Cno10-3 provided the highest protection of 91 and 100% with the lowest relative treatment effect ($\hat{p}_i = 0.14$ and 0.20), respectively, against *R. solani* AG-4 (Cabl2).

Discussion

Identifying the anamorphic form of genus *Rhizoctonia* in species-level taxonomy is difficult because the teleomorph of *Rhizoctonia* is rarely induced under laboratory conditions (37). The diversity of endomycorrhizal *Rhizoctonia* isolates from orchid reflects various AGs. Moreover, isolates of *R. solani* AG-6 form a close relationship with BNR (AG-P, AG-R, AG-S, and AG-U). *R. solani* AG-6 and AG-12 have been reported to exhibit mycorrhizal associations with wild orchid (10,31). Phylogenetic analysis of rDNA-ITS sequences has shown that some BNR are paraphyletic with MNR (15,24,48). In the present study, we found that BNR isolates were clarified as mycorrhizal fungi associating with orchid. Although these AGs contain pathogenic isolates (40), certain isolates have been reported to be hypovirulent or nonpathogenic isolates that protect plant hosts against pathogens (19,20,22,39).

Several researchers have suggested that virulence determination should involve evaluating diseases according to a continuum between highest and lowest virulence (39) and have recommended using suitable hosts for identifying hypovirulent *Rhizoctonia* isolates that could control pathogens (19,20). Juan-Abgona et al. (20) indicated that cucumber cultivars were more susceptible than radish cultivars when tested in vitro against isolates of *Rhizoctonia*, and

Table 2. Relative ranking of disease severities on 10-day-old seedlings of radish, cucumber, and Chinese mustard that were inoculated with individual isolates of endomycorrhizal and pathogenic *Rhizoctonia* spp.^y

Isolate	AG ^z	Radish		Cucumber		Chinese mustard	
		$\widehat{p}_i$	CI for $\widehat{p}_i$	$\widehat{p}_i$	CI for $\widehat{p}_i$	$\widehat{p}_i$	CI for $\widehat{p}_i$
<i>Thanatephorus</i>							
Cabl2	4	0.88	0.86–0.90	0.91	0.90–0.92	0.93	0.92–0.93
Chcab4	4	0.96	0.95–0.96	0.92	0.91–0.92	0.93	0.92–0.93
ANOF0	6	0.40	0.35–0.46	0.38	0.32–0.45	0.43	0.36–0.48
ANOF4	6	0.61	0.54–0.67	0.41	0.34–0.49	0.63	0.58–0.69
ANOFD1	6	0.56	0.49–0.62	0.35	0.29–0.41	0.65	0.61–0.70
<i>Ceratobasidium</i>							
Cno3-2	A	0.55	0.49–0.60	0.51	0.44–0.59	0.34	0.28–0.41
Sno5-12	B	0.47	0.43–0.51	0.32	0.27–0.36	0.38	0.32–0.47
Eno3-3	G	0.51	0.46–0.56	0.40	0.33–0.47	0.61	0.55–0.66
ZeuS1-1	G	0.48	0.44–0.52	0.54	0.47–0.62	0.61	0.53–0.68
ANOF2	G	0.50	0.45–0.54	0.50	0.42–0.57	0.35	0.28–0.44
Cno10-3	P	0.48	0.44–0.52	0.48	0.40–0.56	0.41	0.35–0.47
CalS1-2	P	0.55	0.49–0.60	0.54	0.47–0.62	0.39	0.33–0.46
ANOF3	R	0.77	0.71–0.82	0.89	0.88–0.90	0.82	0.77–0.85
<i>Tulasnella</i>							
Sno3-3	...	0.10	0.09–0.11	0.28	0.27–0.30	0.18	0.16–0.19
Sno6-1	...	0.10	0.09–0.11	0.28	0.27–0.30	0.18	0.16–0.19
Control	...	0.10	0.09–0.11	0.28	0.27–0.30	0.18	0.16–0.19

^y Disease severity was originally rated on a 0-to-4 scale and the single variable ranked using the PROC RANK procedure. A relative disease severity [$\hat{p}_i = 1/N (\bar{R}_i - 1/2)$] was calculated using a mean rank formula ($\bar{R}_i = 1/n_i \sum_{k=1}^n R_{ik}$) and confidence intervals (CI) were generated using the LD_CI macro. Values of $\hat{p}_i$ between two accessions are significantly different from each other if 95% CI for each $\hat{p}_i$ does not overlap.

^z Anastomosis group.

isolates identified as hypovirulent should consistently exhibit a hypovirulent reaction on other crops when tested in vitro and in soil. In this study, endomycorrhizal *Rhizoctonia* isolates caused slight discoloration at the basal site of the hypocotyl and a low disease severity in 10-day-old radish, cucumber, and Chinese mustard under an in vitro conditions. An exception was the ANOF3 isolate (AG-R), which was more virulent to tested plants. The results revealed that almost all of the endomycorrhizal *Rhizoctonia* spp. isolated from wild orchid exerted low virulence on test plants. However, certain AGs of genera *Thanatephorus* and *Ceratobasidium* are pathogenic on a range of hosts. Thus, the virulence of endomycorrhizal *Rhizoctonia* isolates should be evaluated using several plants in further research.

Previous studies have revealed that *R. solani* AG-4 is an economically vital soilborne plant pathogen that affects various hosts (2,46). In the present study, the damping-off of Chinese mustard was significantly reduced when seedlings grown in soil were protected before a challenge inoculation with *R. solani* AG 4. However, the percentages of plant protection caused by isolates of endomycorrhizal *Rhizoctonia* varied. *Rhizoctonia* fungi may exhibit similar characteristics, such as types of disease symptoms caused or host preferences within the same AG (21). The ability of endomycorrhizal *Rhizoctonia* spp. to stimulate the germination and growth of orchid seed has been predicted according to phylogenetic relationships (29). Therefore, we expected that closely related isolates or AGs might be similar in plant-protective ability. According to AG determination, certain isolates of BNR from soil have been used in greenhouse or field trials to ameliorate damping-off

in various crops, such as bean (e.g., AG-G and AG-R) (18,39,50), cucumber (e.g., AG-G, AG-P, and AG-B_(O)) (20,39), and cotton (e.g., AG-G and AG-R) (19). We found that endomycorrhizal *Rhizoctonia* isolates are related to the aforementioned hypovirulent BNR isolates of AGs. Although certain *Rhizoctonia* spp. are plant pathogens, the nutrient requirements of endomycorrhizal *Rhizoctonia* spp. remain unknown (25,48).

In experiments conducted using plug trays, isolates from the terrestrial orchid species *Cheirostylis hungyehensis* (Cno10-3) and *Calanthe sylvatica* (CalS1-2) yielded the most effective plant protection. Both isolates belonged to AG-P and exhibited mild relative treatment effects on the disease severity in 10-day-old test plants and high levels of plant protection with mild relative treatment effects in 26-day-old Chinese mustard grown in soil. Certain isolates of hypovirulent *Rhizoctonia* exhibit abilities to protect seedlings against pathogenic *Rhizoctonia* isolates through competition of nutrients (9) and colonization of infection sites (42), for example. Plant protection is sometimes effective when the control agent is related to the pathogen because their requirements are likely similar (43). The evaluated isolates of AG-6 and AG-P produced abundant mycelium and rapidly colonized on the hypocotyl of the seedlings, as did the pathogen *R. solani* AG 4. Extensive colonization is typically considered a favorable sign when attempting to control pathogens (39). Sneh and Ichielevich-Auster (41) demonstrated that isolates in AG-B_(O) and AG-P induce plant resistance and protected cucumber seedlings. Furthermore, phenylalanine ammonia lyase, peroxidase, the pathogenesis-related (PR)-2 family of proteins, chitinase (PR-3 family), salicylic acid, pectic substances, and suberin have been involved in resistance responses (7,18). In the present study, plants colonized by AG-P isolates accumulated pectic substances and phenolic compounds in the hypocotyl that were not apparent in noninoculated controls. The isolates of AG-P exhibited the strong ability to colonize the base regions of seedlings, producing loose and small infection cushions that may not be effective in penetration and colonization (unpublished). Recently, colonizing *Arabidopsis thaliana* with hypovirulent BNR isolates of AG-A and AG-B_(O) induced genes involved in the systemic acquired resistance, induced systemic resistance, and phytoalexin production pathways (35). Plant protection provided by isolates of AG-P sampled from orchid may involve fungal competition regarding space and nutrition and induce innate immune system responses in plants. The combination of high protection levels and a low tendency for disease severity suggests that isolates of AG-P are the most promising candidates for controlling *Rhizoctonia* damping-off in Chinese mustard. Although certain isolates of AG-P were recognized as plant pathogens, some isolates were considered hypovirulent BNR that could control damping-off (39). Applying AG-P to host plants, as previously reported, requires further investigation. A recent study by Veldre et al. (48) indicated that *Rhizoctonia* fungi isolated from soil and symbiont generally exhibited a low ability to cause plant disease compared with pathogenic *Rhizoctonia* fungi isolated from the host.

The isolates of endomycorrhizal *Rhizoctonia* described in this study were obtained from the fungal pelotons in the cortical cells of orchid roots or rhizomes, suggesting that the fungi had functional mycorrhizal associations with orchid plants. Our findings revealed that hypovirulent isolates of *Rhizoctonia* can be identified in orchid. Moreover, isolates of endomycorrhizal *Rhizoctonia* may be effective in controlling the *Rhizoctonia* damping-off of Chinese mustard grown in soil and yield low levels of virulence in radish, cucumber, and Chinese mustard under in vitro conditions. Such isolates may be applicable for treating vegetable crops. Thus, we can justify that two endomycorrhizal isolates of AG-P are useful for biological control. However, understanding the mode of plant protection provided by isolates of AG-P is necessary for developing efficient procedures and preparations and for improving the efficacy of application practices.

Acknowledgments

We thank Y. I. Lee (Botany Department, National Museum of Natural Science, Taichung, Taiwan) and J. F. Xu (National Chung Hsing University, Taichung, Taiwan) for providing the native orchid plants used in this study.

Table 3. Relative ranking of disease severities on 26-day-old Chinese mustard grown in soil inoculated with individual isolates of endomycorrhizal and pathogenic *Rhizoctonia* spp. and inoculated with putatively protective isolates on 10-day-old plants than challenged with pathogenic *Rhizoctonia* isolates 6 days later^x

Isolate	AG ^y	Inoculating with each isolate		Challenging with pathogenic Cabl2 after inoculating putatively protective isolate		
		$\hat{p}_i$	CI for $\hat{p}_i$	$\hat{p}_i$	CI for $\hat{p}_i$	PP (%) ^z
<i>Thanatephorus</i>						
Cabl2	4	0.96	0.95–0.96	...	...	...
Chcab4	4	0.85	0.77–0.90	...	...	...
ANOF0	6	0.46	0.41–0.51	0.50	0.40–0.61	45.8 cd
ANOF4	6	0.42	0.41–0.43	0.48	0.38–0.57	48.7 cd
ANOFD1	6	0.42	0.41–0.43	0.49	0.40–0.59	52.4 cd
<i>Ceratobasidium</i>						
Cno3-2	A	0.42	0.41–0.43	0.51	0.40–0.62	41.1 de
Sno5-12	B	0.44	0.40–0.47	0.65	0.56–0.72	17.9 ef
Eno3-3	G	0.52	0.45–0.59	0.48	0.38–0.58	54.8 cd
Zeus1-1	G	0.44	0.40–0.47	0.39	0.30–0.49	75.4 bc
ANOF2	G	0.42	0.41–0.43	0.57	0.50–0.64	44.6 de
Cno10-3	P	0.46	0.41–0.51	0.20	0.14–0.29	91.1 ab
CalS1-2	P	0.42	0.41–0.43	0.14	0.11–0.18	100 a
ANOF3	R	0.51	0.44–0.58	0.44	0.35–0.53	60.7 cd
<i>Tulasnella</i>						
Sno3-3	...	0.42	0.41–0.43	0.71	0.65–0.75	4.8 f
Sno6-1	...	0.42	0.41–0.43	0.71	0.65–0.75	8.9 f
Control	...	0.42	0.41–0.43	0.73	0.69–0.77	0 f

^x Disease severity was originally rated on a 0 to 3 scale and the single variable ranked using the PROC RANK procedure. A relative disease severity $\hat{p}_i = 1/N (\bar{R}_i - 1/2)$ was calculated using a mean rank formula ($\bar{R}_i = 1/n_i \sum_{k=1}^{n_i} R_{ik}$) and confidence intervals (CI) generated using the LD_CI macro. Values of $\hat{p}_i$ between two accessions are significantly different from each other if 95% CI for each $\hat{p}_i$ does not overlap.

^y Anastomosis group.

^z Percent plant protection (PP) was calculated using the formula Protection (%) = $[(A - B)/(C - B)] \times 100$, where A = the percentage of surviving plants after colonization with a hypovirulent isolate and challenge inoculation with a virulent isolate, B = the percentage of surviving plants after inoculation with only a virulent isolate, and C = the percentage of surviving noninoculated plants. Values followed by the same letter were not significantly different according to post hoc Tukey's test ($P = 0.05$) by using one-way analysis of variance procedure.

Literature Cited

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. H., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25: 3389-3402.
- Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. *Annu. Rev. Phytopathol.* 20:329-347.
- Arditti, J. 1992. *Fundamentals of Orchid Biology*. John Wiley & Sons, New York.
- Bandoni, R. 1979. Safranin O as a rapid nuclear stain for fungi. *Mycologia* 71: 873-874.
- Brunner, E., Domhof, S., and Langer, F. 2002. *Nonparametric Analysis of Longitudinal Data in Factorial Experiments*. John Wiley & Sons, New York.
- Burpee, L., Sanders, P., Cole, H., and Kim, S. 1978. A staining technique for nuclei of *Rhizoctonia solani* and related fungi. *Mycologia* 70:1281-1283.
- Cardinale, F., Ferraris, L., Valentino, D., and Tamietti, G. 2006. Induction of systemic resistance by a hypovirulent *Rhizoctonia solani* isolate in tomato. *Physiol. Mol. Plant Pathol.* 69:160-171.
- Carling, D. E. 1996. Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. Pages 37-47 in: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. B. Sneh, S. Jabajihare, S. Neate, and G. Dijst, eds. Kluwer Academic Publisher, Dordrecht, The Netherlands.
- Carling, D. E., Baird, R. E., Gitaitis, R. D., Brainard, K. A., and Kuninaga, S. 2002. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology* 92:893-899.
- Carling, D. E., Pope, E. J., Brainard, K. A., and Carter, D. A. 1999. Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. *Phytopathology* 89:942-946.
- Copes, W. E., Rodríguez-Carres, M., Toda, T., Rinehart, T. A., and Cubeta, M. A. 2011. Seasonal prevalence of species of binucleate *Rhizoctonia* fungi in growing medium, leaf litter, and stems of container-grown *Azalea*. *Plant Dis.* 95:705-711.
- Cribb, P. J., Kell, S. P., Dixon, K. W., and Barrett, R. L. 2003. Orchid conservation: A global perspective. Pages 1-24 in: *Orchid conservation*. K. W. Dixon, S. P. Kell, and P. J. Cribbs, eds. Natural History Publications, Kota Kinabalu, Malaysia.
- Dearnaley, J. D. 2007. Further advances in orchid mycorrhizal research. *Mycorrhiza* 17:475-486.
- Fang, X. L., Finnegan, P. M., and Barbeti, M. J. 2013. Wide variation in virulence and genetic diversity of binucleate *Rhizoctonia* isolates associated with root rot of strawberry in Western Australia. *PLoS One* 8:E55877.
- Gonzalez, D., Carling, D. E., Kuninaga, S., Vilgalys, R., and Cubeta, M. A. 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia* 93:1138-1150.
- González García, V., Onco, P., and Rubio Susan, V. 2006. Review. Biology and Systematics of the form genus *Rhizoctonia*. *Span. J. Agric. Res.* 4:55-79.
- Hall, T. A. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41:95-98.
- Jabaji-Hare, S., Chamberland, H., and Charest, P. M. 1999. Cell wall alterations in hypocotyls of bean seedlings protected from *Rhizoctonia* stem canker by a binucleate *Rhizoctonia* isolate. *Mycol. Res.* 103:1035-1043.
- Jabaji-Hare, S., and Neate, S. M. 2005. Nonpathogenic binucleate *Rhizoctonia* spp. and benzothiadiazole protect cotton seedlings against *Rhizoctonia* damping-off and *Alternaria* leaf spot in cotton. *Phytopathology* 95:1030-1036.
- Juan-Abgona, R. V., Katsuno, N., Kageyama, K., and Hyakumachi, M. 1996. Isolation and identification of hypovirulent *Rhizoctonia* spp. from soil. *Plant Pathol.* 45:896-904.
- Keijer, J., Korsman, M. G., Dulleman, A. M., Houterman, P. M., de Bree, J., and Van Silfhout, C. H. 1997. In vitro analysis of host plant specificity in *Rhizoctonia solani*. *Plant Pathol.* 46:659-669.
- Khan, F. U., Nelson, B. D., and Helms, T. C. 2005. Greenhouse evaluation of binucleate *Rhizoctonia* for control of *R. solani* in soybean. *Plant Dis.* 89:373-379.
- Ma, M., Tan, T. K., and Wong, S. M. 2003. Identification and molecular phylogeny of *Epulorhiza* isolates from tropical orchids. *Mycol. Res.* 107:1041-1049.
- Moncalvo, J. M., Nilsson, R. H., Koster, B., Dunham, S. M., Bernauer, T., Matheny, P. B., Porter, T. M., Margaritescu, S., Weiß, M., and Garnica, S. 2006. The cantharelloid clade: Dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* 98:937-948.
- Mosquera-Espinosa, A. T., Bayman, P., Prado, G. A., Gomez-Carabali, A., and Otero, J. T. 2013. The double life of *Ceratobasidium*: Orchid mycorrhizal fungi and their potential for biocontrol of *Rhizoctonia solani* sheath blight of rice. *Mycologia* 105:141-150.
- Muslim, A., Horinouchi, H., and Hyakumachi, M. 2003. Control of *Fusarium* crown and root rot of tomato with hypovirulent binucleate *Rhizoctonia* in soil and rock wool systems. *Plant Dis.* 87:739-747.
- Muslim, A., Horinouchi, H., and Hyakumachi, M. 2003. Suppression of *Fusarium* wilt of spinach with hypovirulent binucleate *Rhizoctonia*. *J. Gen. Plant Pathol.* 69:143-150.
- Nontachaiyapoom, S., Sasirat, S., and Manoch, L. 2010. Isolation and identification of *Rhizoctonia*-like fungi from roots of three orchid genera, *Paphiopedilum*, *Dendrobium*, and *Cymbidium*, collected in Chiang Rai and Chiang Mai provinces of Thailand. *Mycorrhiza* 20:459-471.
- Otero, J. T., Bayman, P., and Ackerman, J. D. 2005. Variation in mycorrhizal performance in the epiphytic orchid *Tolumnia variegata* in vitro: The potential for natural selection. *Evol. Ecol.* 19:29-43.
- Pascual, C. B., Raymundo, A. D., and Hyakumachi, M. 2000. Efficacy of hypovirulent binucleate *Rhizoctonia* sp. to control banded leaf and sheath blight in corn. *J. Gen. Plant Pathol.* 66:95-102.
- Pope, E. J., and Carter, D. A. 2001. Phylogenetic placement and host specificity of mycorrhizal isolates belonging to AG-6 and AG-12 in the *Rhizoctonia solani* species complex. *Mycologia* 93:712-719.
- Poromarto, S. H., Nelson, B. D., and Freeman, T. P. 1998. Association of binucleate *Rhizoctonia* with soybean and mechanism of biocontrol of *Rhizoctonia solani*. *Phytopathology* 88:1056-1067.
- Roberts, P. 1999. *Rhizoctonia-Forming Fungi: A Taxonomic Guide*. Royal Botanic Gardens, Kew, UK.
- Shah, D. A., and Madden, L. V. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. *Phytopathology* 94:33-43.
- Sharon, M., Freeman, S., and Sneh, B. 2011. Assessment of resistance pathways induced in *Arabidopsis thaliana* by hypovirulent *Rhizoctonia* spp. isolates. *Phytopathology* 101:828-838.
- Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S., and Sneh, B. 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience* 49:93-114.
- Sharon, M., Kuninaga, S., Hyakumachi, M., and Sneh, B. 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. *Mycoscience* 47:299-316.
- Smith, S. E., and Read, D. J. 2008. *Mycorrhizal Symbiosis*, 3rd ed. Academic Press, London.
- Sneh, B. 1996. Non pathogenic isolates of *Rhizoctonia* (np-R) spp. and their role in biological control. Pages 473-483 in: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. B. Sneh, S. Jabajihare, S. Neate, and G. Dijst, eds. Kluwer Academic Publisher, Dordrecht, The Netherlands.
- Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* Species. American Phytopathological Society, St. Paul, MN.
- Sneh, B., and Ichielevich-Auster, M. 1998. Induced resistance of cucumber seedlings caused by some non-pathogenic *Rhizoctonia* (np-R) isolates. *Phytoparasitica* 26:27-38.
- Sneh, B., Ichielevich-Auster, M., and Plaut, Z. 1989. Mechanism of seedling protection induced by a hypovirulent isolate of *Rhizoctonia solani*. *Can. J. Bot.* 67:2135-2141.
- Sneh, B., Yamoah, E., and Stewart, A. 2004. Hypovirulent *Rhizoctonia* spp. isolates from New Zealand soils protect radish seedlings against damping-off caused by *R. solani*. *N. Z. Plant Prot.* 57:54-58.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731-2739.
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673-4680.
- Tu, C., Hsieh, T., and Chang, Y. 1996. Vegetable diseases incited by *Rhizoctonia* spp. Pages 369-377 in: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. B. Sneh, S. Jabajihare, S. Neate, and G. Dijst, eds. Kluwer Academic Publisher, Dordrecht, The Netherlands.
- Uetake, Y., Obayashi, K., Ogoshi, A., and Tsutsui, K. 1988. Identification of *Rhizoctonia* species isolated from orchids. *Ann. Phytopathol. Soc. Jpn.* 54: 114.
- Veldre, V., Abarenkov, K., Bahram, M., Martos, F., Seloosse, M. A., Tamm, H., Kõljalg, U., and Tedersoo, L. 2013. Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales, Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecol.* 6:256-268.
- Warcup, J. 1981. The mycorrhizal relationships of Australian orchids. *New Phytol.* 87:371-381.
- Wen, K., Seguin, P., St-Arnaud, M., and Jabaji-Hare, S. 2005. Real-time quantitative RT-PCR of defense-associated gene transcripts of *Rhizoctonia solani*-infected bean seedlings in response to inoculation with a nonpathogenic binucleate *Rhizoctonia* isolate. *Phytopathology* 95:345-353.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Snunsu, and T. J. White, eds. Academic Press, San Diego, CA.