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SHORT NOTE

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Formation of structures resembling ericoid mycorrhizas by the root endophytic fungus *Heteroconium chaetospira* within roots of *Rhododendron obtusum* var. *kaempferi*

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Abstract A resynthesis study was conducted to clarify the relationship between the root endophyte, *Heteroconium chaetospira* and the ericaceous plant, *Rhododendron obtusum* var. *kaempferi*. The host plant roots were recovered 2 months after inoculation, and the infection process and colonization pattern of the fungus were observed under a microscope. The hyphae of *H. chaetospira* developed structures resembling ericoid mycorrhizas, such as hyphal coils within the host epidermal cells. These structures were morphologically the same as previously reported ericoid mycorrhizal structures. The frequencies of hyphal coils within the epidermal cells of host roots ranged from 13 to 20%. *H. chaetospira* did not promote or reduce host plant growth. This is the first reported study that *H. chaetospira* is able to form structures resembling mycorrhizas within the roots of ericaceous plants.

Keywords Ericoid mycorrhiza · *Heteroconium chaetospira* · Root endophyte

Introduction

The dematiaceous hyphomycete, *Heteroconium chaetospira* (Grove) M.B. Ellis, has been isolated from the wood of deciduous trees (Matsushima 1975), millipede droppings (Ellis 1976), and arable soil (Domsch et al. 1980). The fungus has also been isolated from Chinese cabbage roots grown in wheat field soil. It colonized the inner cortical tissue of the roots of 19 plant species, including Chinese cabbage, without causing any apparent pathogenic symptoms (Narisawa et al. 1998, 2000). This fungus also formed microsclerotia in host root cells (Ohki et al. 2002). These colonizing patterns of *H. chaetospira* in host plant roots were consistent with the dark septate endophytes (DSE) complex (Jumpponen and Trappe 1998).

Recently, *H. chaetospira* has been isolated from the organic layer soil in the subalpine forests in western Canada, where there is an abundance of ericaceous plants (Narisawa et al. 2002). This finding, along with the endophytic nature of *H. chaetospira*, suggested that the fungus may have the potential to form a symbiotic association with ericaceous plants.

The purpose of this study was to determine the potential relationship between *H. chaetospira* and ericaceous plants. For this purpose, we conducted synthesis experiments using *H. chaetospira* isolates with the roots of an ericaceous plant, *Rhododendron obtusum* var. *kaempferi*, in vitro. In addition, host roots containing *H. chaetospira* were examined microscopically.

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Materials and methods

Fungal isolates

Four isolates of *H. chaetospira*; H4007 (MAFF238955) from the roots of Chinese cabbage (*Brassica campestris* L.) (Ibaraki, Japan), OGR3 (MAFF238957) from rice (*Oryza sativa* L) (Tokyo, Japan), BPM3 (available from K. N.) from melon (*Cucumis melo* L.) (Alberta, Canada), and BcaHE2 (available from K.N.) from eggplant (*Solanum melongena* L.) (Alberta, Canada) were used in this study. It was previously confirmed that these isolates colonize host roots through the formation of appressoria on the cell surface and subsequent growth of hyphae within the cells, without any apparent negative effects on host plants such as Chinese cabbage and eggplant (Narisawa et al. 1998, 2000). An isolate of *Oidiodendron maius* Barron (available from F.U. as E97053) was used for comparison. This isolate was obtained from the ericoid mycorrhiza of torch

azalea (*R. obtusum* var. *kaempferi* G. Don) in a stand of *Pinus densiflora* Sieb. et Zucc at Tsukuba, Japan, and was confirmed to form ericoid mycorrhiza (Usuki et al. 2003).

In order to prepare inocula, isolates were grown on CMMY medium [corn meal, infusion form (Difco, Detroit, Mich.), 25 g; malt extract (Difco), 10 g; yeast extract (Difco), 2 g; Bacto agar (Difco), 15 g, per liter] in Petri dishes (90 mm diameter) at room temperature (approximately 23°C) for 3 weeks.

Host plant seedlings

Torch azalea is widely distributed in Japan and was selected as the host. Seeds were collected from a stand of *P. densiflora* at Tsukuba, Japan in 2001, and stored at 4°C in paper bags. The seeds were surface-sterilized in sodium hypochlorite (1% available chlorine) for 5 min, rinsed ten times with sterile distilled water, and germinated axenically on 1.5% water agar in Petri dishes (90 mm diameter).

Fungal inoculation

Pots (polycarbonate,60×60×100 mm) (Asahi Techno Glass, Chiba, Japan) were filled with 150 ml 1:1 mixed peat moss (Hokkaido Peat Moss, Saitama, Japan) and vermiculite (Nittai, Osaka, Japan), and autoclaved for 40 min at 121°C. Five small plugs of mycelium (approximately 0.5 mm diameter) of each isolate cut from the colony on the medium were placed into the soil of each pot. Three 10-day-old axenic seedlings were transplanted into each pot (the controls were uninoculated plants in pots), and incubated in a growth chamber at 20°C under a light:dark cycle (16:8, approximately 180 μmol m^{-2} s^{-2}). There were ten pots for each isolate and control, and the experiment was conducted once.

Two months after inoculation, the plant roots were recovered and washed in running tap water for 3 min to remove superficial debris such as soil particles and fungal hyphae. These washed roots were cut into 1 cm segments, stained with 0.05% lactofuchsin, and observed under an Olympus (Tokyo, Japan) BX50 microscope at ×100 to ×1,000 magnification. If mycorrhizal structures were observed, 30 root segments were selected at random, and at least 100 adjacent epidermal cells in each root segment were observed to estimate the percentage of fungal colonization. The fresh weights of shoots were measured. The mean fresh weight of the three seedlings from each pot was calculated.

Statistical analysis

Differences in the average percentage of cell colonization and plant fresh weight among the treatment groups were tested by the Kruskal-Wallis test, using StatView Statistical Software (Abacus Concept, Berkeley, Calif.). If significant differences were detected, a non-parametric Tukey-type multiple comparison was conducted.

Results

It was observed that fungal hyphae of *H. chaetospira* grew over the root surfaces and occasionally appressorium-like structures were formed at the hyphal tips. From these structures, narrow infection hyphae penetrated the epidermal cells (results not shown) and the penetration hyphae of the three isolates, H4007, BPM3, and BCaHE2, produced hyphal coils where the hyphae accumulated within host epidermal cells (Fig. 1A, B). Hyphae of OGR3 colonized the epidermal cells but did not form hyphal coils.

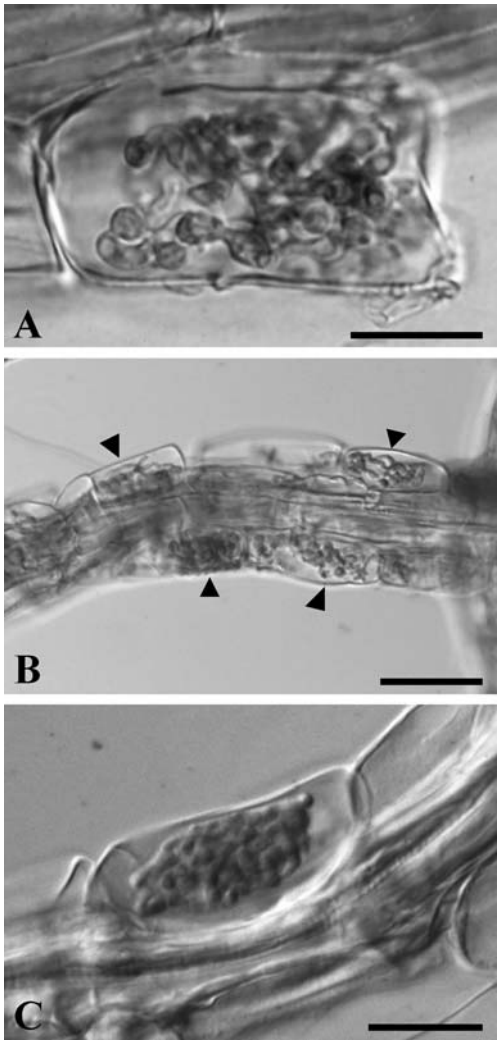

Fig. 1A–C Infection of the root of *Rhododendron obtusum* var. *kaempferi* by *Heteroconium chaetospira* and *Oidiodendron maius*, 2 months after inoculation. **A** Hyphae of *H. chaetospira* (H4007) proliferating in an epidermal cell and forming structures resembling mycorrhiza. **B** Host plant root inoculated with *H. chaetospira* (H4007). *Arrow* Structures resembling mycorrhizas produced in epidermal cells. **C** Hyphae of *Oidiodendron maius* in an epidermal cell forming typical ericoid mycorrhiza. *Bars* **A** 15 μm, **B** 50 μm, **C** 30 μm

The hyphae of *O. maius* grew on the surface of the host roots, penetrated the epidermal cells, and formed hyphal coils typical of ericoid mycorrhizae (Fig. 1C). The hyphal coils of *H. chaetospira* were morphologically identical to those of *O. maius*. *H. chaetospira* was restricted to the epidermal cells and did not advance into the cells of vascular tissues. Hyphae of *H. chaetospira* did not form microsclerotia on or in the epidermal layer.

Roots of the host were extensively colonized by both *H. chaetospira* and *O. maius*. The frequencies of colonization of epidermal cells by the isolates varied (Table 1) . Neither had measurable effects on shoot growth (Table 1).

Discussion

Ericaceous plants have unique, fine "hair roots" that consist of one to three epidermal cell layers surrounding a vascular cylinder. These cells can harbor a diverse flora of fungal endophytes, including ericoid mycorrhizal fungi. Non-mycorrhizal associations are also found in the roots. For example, *Phialocephala fortinii* Wang and Wilcox produced microsclerotia structures on/in the root (Currah et al. 1993; Stoyke and Currah 1993), and some fungal taxa such as sterile mycelia infected and developed loose hyphal coils in the epidermal cells without developing mycorrhizal structures (Perotto at al. 1996). The present results and previous reports suggest that hyphae of *H. chaetospira* are able to develop and form structures resembling ericoid mycorrhizas. The hyphal coils of *H. chaetospira* within host epidermal cells are morphologically identical with typical mycorrhizal structures formed by *O. maius*.

This study has shown for the first time that *H. chaetospira* is able to form structures resembling ericoid mycorrhizas. Other fungal taxa that are known to form ericoid mycorrhizae include *Hymenoscyphus ericae* (Read 1996) Korf and Kernan and its anamorph *Scytalidium vaccinii* Dalpé, Litten and Sigler (Dalpé et al. 1989; Egger and Sigler 1993; Hambleton et al. 1999), *Oidiodendron* species (Dalpé 1986, 1989, 1991; Douglas et al. 1989; Xiao and Berch 1995), *Myxotrichum setosum* (Eidam) Orr and Plunkett, *Gymnascella dankaliensis* (Castellani) Currah, and *Pseudogymnoascus roseus* Raillo (Dalpé 1989). *Acremonium strictum* W. Gams isolated from the roots of *Gaultheria shallon* Pursh and an ectomycorrhizal strain of *Phialophola finlandia* Wang and Wilcox, also formed ericoid mycorrhiza in vitro (Xiao and Berch 1996; Monreal et al. 1999).

H. chaetospira was reported to be a root endophyte colonizing the inner cortical tissue of the roots of 19 different plant species, and is consequently assumed to have a wide host range (Narisawa et al. 2000). *H. chaetospira* has often been isolated from humus-rich soil (Narisawa et al. 2002), and from the mycorrhizal roots of blueberry (*Vaccinium myrtilloides* Michx.) at a subalpine site in western Canada (G. Hill-Rackette, personal communication). Consequently, *H. chaetospira* may occur in the roots of apparently healthy plants as either DSE or ericoid mycorrhizal fungi, depending on the host plant species. The fungus and its association with host roots is circumboreal in distribution; however, it may be particularly abundant in acidic, nutrient-poor, humus soil habitats as well.

In this study, the structures resembling ericoid mycorrhizas of *H. chaetospira* had low average frequencies (13–20%) as compared to previous reports. For example, the frequency of colonization in the root of *G. shallon* by *O. maius* was 90%, and this was the most frequent species isolated from host roots. This was followed by *O. flavum* Szilvinyi (67%), and *S. vaccinii* (50%) (Xiao and Berch 1995). In the field, colonization in the root of *G. shallon* was also high (Xiao and Berch 1996). However, colonization of torch azalea roots in the field was 30% on average (data not shown), which was almost equal to the rate at which mycorrhizal structures formed in vitro. Consequently, the in vitro resynthesis in this study confirmed that *H. chaetospira* could form structures resembling ericoid mycorrhizas in an axenically reared host, and the relatively low rate might depend on the host plant.

One isolate (OGR3 isolated from rice roots) of *H. chaetospira* did not form structures resembling mycorrhizas within host roots. This is not a unique feature with *H. chaetospira*. Other ericoid mycorrhizal fungi (e.g., *O. maius* isolated from Sitka spruce and *Oidiodendron griseum* Robak from wood pulp) have also been shown as non-mycorrhizal isolates (Douglas et al. 1989). This isolate (OGR3) might survive as DSE in roots of ericaceous plants or as a soil saprophyte rather than producing structures resembling mycorrhizas.

Table 1 Ability of four isolates of *Heteroconium chaetospira* and one isolate of *Oidiodendron maius* to form structures resembling mycorrhizas with *Rhododendron obtusum* var. *kaempferi* and their effects on the fresh weight of the host plant, 2 months after inoculation. Values followed by the same letter are not significantly different compared to control (P=0.05)

Fungal species	Structures resembling mycorrhizas	Percentage of cell colonization (%)	Plant fresh weight (mg)
Heteroconium chaetospira(H4007)	+	19.5 a	30.3±5.9 a
H. chaetospira (BPM3)	+	13.2 a	29.3±3.1 a
H. chaetospira (BcaHE2)	+	14.5 a	31.5±5.2 a
H. chaetospira (OGR3)	−	−	27.3±4.4 a
Oidiodendron maius (E97053)	+	31.8 b	23.7±2.8 a
Control	−	−	27.3±1.9 a

Previous work has shown that seedlings of Chinese cabbage inoculated with *H. chaetospira* in a nursery setting had dry weights approximately four times the weight of uninoculated controls (Narisawa et al. 1998), and the relationship between Chinese cabbage and *H. chaetospira* is assumed to be a commensal symbiosis, i.e., at least the host derives benefit from the association. However, in this study, *H. chaetospira* did not affect the growth of ericaceous plants. Similar relations have also been observed between the host plant and *O. maius*. Ericoid mycorrhizal fungi such as *H. ericae* have been known to promote host plant growth if a complex organic form of nitrogen, such as glutathione or bovine serum albumin is provided in vitro (Bajwa et al. 1985; Xiao and Berch 1999). The associations between host plants and fungal endophytes can be changed from mutualistic to pathogenic even within the same host species according to experimental conditions (Jumpponen and Trappe 1998). To confirm the associations between ericaceous plants and *H. chaetospira*, further resynthesis work is necessary under different experimental conditions, focusing mainly on organic nitrogen.

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