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Host-related variability in arbuscular mycorrhizal fungal structures in roots of *Hedera rhombea*, *Rubus parvifolius*, and *Rosa multiflora* under controlled conditions

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Abstract The arbuscular mycorrhizal (AM) morphology of three host plant species inoculated with single and mixed fungal culture and the distribution of AM fungal species in roots of the hosts treated with a mixed culture of AM fungi were determined. The aim was to investigate the effect of host plants and AM fungi on AM morphology of coexisting plant species. Noncolonized rooted cuttings of *Hedera rhombea* (Miq) Bean, *Rubus parvifolius* L., and *Rosa multiflora* Thunb. were inoculated with five fungal species as single and mixed culture inocula. The fungal species used were *Gigaspora rosea* and *Scutellospora erythropa*, previously isolated from *H. rhombea*; *Acaulospora longula* and *Glomus etunicatum* from *R. parvifolius*; and *Glomus claroideum* from both plant species. A few hyphal and arbusculate coils were seen in the mixed culture-inoculated roots of *R. parvifolius*; all fungal treatments produced this *Paris*-type AM in *H. rhombea* and *Arum*-type AM in *R. parvifolius*, and *R. multiflora* indicates that AM morphology is strongly controlled by the identity of the host plants used in this study. AM fungal rDNA was extracted separately from roots of each replicate plant species inoculated with the mixed fungal culture, amplified, cloned, sequenced, and analyzed to determine the AM fungal species and their respective proportions in roots of each plant species. *Glomus etunicatum* and *G. claroideum* of the family Glomaceae generally occurred more frequently in *R. parvifolius* and *R. multiflora*, which form *Arum*-types, whereas *S. erythropa*, of the family Gigasporaceae, was the most frequently detected species in *H. rhombea*, which produced *Paris*-type AM. Although the genotype of the plant species used appears to determine the AM morphologies formed, there was preferential association between the hosts and AM fungal inoculants.

Keywords *Arum*-/*Paris*-type mycorrhiza · Host plant/AM fungal identity · Molecular detection · Preferential association · LSU rDNA

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Introduction

Approximately 150 species of arbuscular mycorrhizal (AM) fungi, recently classified in the new phylum Glomeromycota Walker and Schüßler (Schüßler et al. 2001), are known to form symbiotic associations with up to 80% of land plants (Smith and Read 1997). The fungi generally show little or no specificity to the plant species they colonize (Smith and Read 1997); however, there is evidence of preferential selection of the fungi by the host plant species (Bever et al. 1996; Eom et al. 2000; Bidartondo et al. 2002; Vandenkoornhuyse et al. 2002, 2003; Johnson et al. 2003; Gollotte et al. 2004; Landis et al. 2004; Lovelock and Ewel 2005) that is not yet well understood.

It has also been shown that more than one fungal species can colonize roots of an individual plant in a natural ecosystem (van Tuinen et al. 1998; Helgason et al. 1999). However, a majority of the plant species examined during surveys of natural ecosystems in Japan (Yamato and

Iwasaki 2002; Ahulu et al. 2005) formed exclusively only one of the two described morphological types of AM, *Arum*- or *Paris*-type. The AM fungi colonizing each plant species were not characterized in these studies. The *Arum*-type AM is defined on the basis of the presence of extensive intercellular hyphae, which penetrate the root cortical cells to form arbuscules. The *Paris*-type AM is characterized by the absence of intercellular hyphae, which are replaced by extensive intracellular hyphal coils, on some of which arbuscules are formed as intercalary structures, and are referred to as arbusculate coils. Sometimes, the two morphological structures are represented in the same root system, and this has been termed the intermediate type (Smith and Smith 1997). The arbuscules in the *Arum*-type morphology were previously thought to be the only sites for nutrient exchange between the host plant and fungus; however, recent investigations using carrot, petunia, plantain, and potato (Karandashov et al. 2004) and *Allium porrum* and *Asphodelus fistulosus* (Van Aarle et al. 2005) indicate that both hyphal and arbusculate coils in *Paris*-type AM may be involved in nutrient exchange, though arbuscules seemed more effective. In a survey of literature, Smith and Smith (1997) noted that, with few exceptions, plant species belonging to the same family formed the same AM morphology, suggesting that the host plant genotype is important in determining AM morphology.

Brundrett and Kendrick (1990a,b) had earlier proposed that the *Arum*-type is formed in roots having continuous longitudinal air spaces in the plant root cortices, which probably increase the ease of growth and spread of intercellular hyphae, leading to rapid sequential penetration of cortical cells and growth of arbuscules. They also suggested that the occurrence of limited or discontinuous intercellular spaces in roots, or differences between outer and inner cortices, might account for intermediate structures between the *Arum*- and *Paris*-types; however, in spite of the presence of clear intercellular spaces in its root cortex, *Voyria obconica* Prog. still formed *Paris*-type AM (Imhof and Weber 1997, 2000), indicating that AM morphology is not determined by anatomy of the root cortex alone.

Although the host plant identity was previously thought to be solely responsible, recent studies using different AM fungal species to inoculate tomato (Cavagnaro et al. 2001; Kubota et al. 2005) and cucumber (Kubota et al. 2005) have shown that the fungal identity also can determine AM morphology. However, Kubota et al. (2005) found that the noncrop plant species *Clethra barbinervis* maintained *Paris*-type AM when grown under the same conditions as cucumber and tomato. In a survey of *Arum*- and *Paris*-type distribution in a sand dune ecosystem on Niigata University campus (Ahulu et al. 2005), we noted that *Hedera rhombea* (Miq) Bean, an evergreen climber, consistently formed *Paris*-type AM, whereas two deciduous shrubs, *Rubus parvifolius* L. and *Rosa multiflora* Thunb., produced *Arum*-type mycorrhiza. Following evidence of preferential selection of AM fungi by host plant species, we were prompted to do a subsequent study to molecularly determine AM fungal species in field-collected roots of *H. rhombea* and *R. parvifolius* (Ahulu et al. 2006). The results showed that a majority of AM fungal species detected could colonize roots of both plant species, but there were preferences. The most dominant fungus found in *H. rhombea* roots was *Scutellospora erythropa*, a member of the family Gigasporaceae and also isolated as spores in trap cultures of the same plant species, while the AM fungal genotype closest to *Glomus microaggregatum* (Koske, Gemma, and Olexia) was most frequent in roots of *R. parvifolius*.

Considering the ecological importance of AM fungi, it is of great interest to investigate the factors that control the formation of the different morphological structures, as this may lead to understanding the unique function of each structure in the ecosystem. Our aim in the present study was to determine the effect of host plant and fungi on AM morphology by inoculating *H. rhombea*, *R. parvifolius*, and other sand dune plant species that naturally form either *Arum*, *Paris*, or intermediate morphologies with different AM fungal species both as single and mixed culture inocula. The distribution of AM fungal species in the roots of the hosts treated with a mixed culture of AM fungi were also molecularly determined to investigate preferential selection between the host plants and the AM fungal species.

Materials and methods

Plant and fungal material

Up to 1-year-old stems and flushes of new stems of *R. parvifolius*, *R. multiflora*, *H. rhombea*, *Cocculus trilobus* DC., *Ilex crenata* Thunb., and *Ligustrum japonicum* Thunb. were collected from the mixed Pine forest on Niigata University campus. The stems were surface sterilized with 5% sodium hypochlorite and cut into 12-cm pieces. The lower bases of the cut pieces were dipped lightly in a root-promoting hormone, rootone auxin powder (active ingredient 0.2% 1-naphthalenacetamide), and planted in pasteurized sandy soil contained in free draining propagation trays. Stems were sprayed twice daily with tap water for 8–12 weeks to moisturize the soil and to prevent drying of the stem-cuttings until root development.

Inoculum pots of five different AM fungal species (*Glomus claroideum*, *Glomus etunicatum*, *Acaulospora longula*, *Gigaspora rosea*, and *S. erythropa*) were established as pure cultures and the five together as a mixed culture. For pure cultures, 100 spores of each AM fungal

species were surface sterilized with 1% Chloramine T and used to inoculate 3-week-old *Trifolium resupinatum* L. (white clover) seedlings and grown in 50-cm^3 pots of pasteurized sandy soil. Twenty spores of each of the five AM fungal species were used to inoculate the white clover seedlings for the mixed culture inoculum. Sandy soil collected from the source of the fungal species was passed through a 2-mm sieve, pasteurized, and used as growth medium. Three replicates were kept for the single and mixed culture inoculum pots and watered daily with 2–3 mL tap water for 6 weeks.

Roots of the three plant species successfully propagated through stem cuttings (*H. rhombea*, *R. parvifolius*, and *R. multiflora*) were trimmed to 3 cm and planted in pasteurized sand in a 450-cm^3-capacity bag made with 0.2-μm mesh. The mesh permits the passage of fungal hyphae but not of roots. Three bags, each one containing one each of the test plant species, were then placed in a 2-L self-draining plastic pot. Empty spaces surrounding the three bags were filled with pasteurized sand and the prepared inoculum, colonized with white clover seedling with intact potting soil, excavated carefully (to avoid disrupting AM fungal hyphal growth in the soil), and placed in the center of each of the 2-L pots such that the inoculum touched all three bags. The pots were kept in the greenhouse (14 h of daylight and temperature at 25°C) and watered daily with 60 mL tap water per pot.

Harvesting and staining of root samples

Root samples of the three plant species in replicate pots were harvested after 24 weeks of growth. The root samples were first washed gently in running water and then twice with an ultrasound washer at 100 V for 20 s to get rid of fine soil particles on root surfaces. About 0.5-g root samples were blotted dry, cut up into 10-mm pieces, and cleared by heating at 90°C in 10% potassium hydroxide for 1 h. Highly pigmented root samples were further decolorized in alkaline hydrogen peroxide between 20–30 min, depending on the intensity of pigmentation. Roots were washed and acidified in 2% hydrochloric acid for 20 min and stained in 0.05% trypan blue for 10 min in boiling water, washed again, and preserved in lacto glycerol solution.

Determination of extent of colonization and quantification of AM morphological structures

The frequency of mycorrhiza in the root system (*F*%) and abundance of arbuscules in the mycorrhizal parts of the root fragments (*a*%) of each plant species collected after 24 weeks of growth from the different AM fungal treatments was determined with the method of Trouvelot et al. (1986). The abundance of other AM structures, hyphal coils, intercellular hyphae, etc., were quantified as was done for arbuscule abundance but by replacing arbuscules with the respective AM structures. Results were analyzed statistically by ANOVA after arcsine transformation using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). Typical AM morphological structures in roots from each fungal treatment were photographed with an Olympus CAMEDIA digital camera x-1 (Olympus, Tokyo, Japan).

Molecular detection and identification of AM fungal species in plant roots

AM fungal DNA extraction from roots

Replicate root samples of each plant species treated with the mixed fungal inoculum and collected 24 weeks after inoculation were dried separately at 50°C for 48 h. rDNA was extracted from the predried root samples as outlined by Ahulu et al. (2006). The DNA of problematic roots likely to contain large amounts of polyphenolic compounds and polysaccharides was extracted with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) for the first PCR. The partial large subunit (LSU) rDNA of the AM fungi was amplified and cloned as described by Gollotte et al. (2004). Fifteen clones selected randomly from libraries constructed for each replicate sample of the test plant species were purified using Nucleospin® Plasmid (Macherey-Nagel, Düren, Germany) according to the manufacturers' recommendations and sequenced on both strands by MWG-Biotech, Ebersberg, Germany, for analysis. The sequences have been deposited in the European Molecular Biology Laboratory database under accession numbers AM039980–AM039994 and AM086173–AM086204. The frequency of each AM fungal species used in the mixed culture inoculation was determined for each plant species and analyzed statistically by ANOVA followed by Bonferroni posttests using GraphPad Prism version 4.00 for Windows, GraphPad Software (http://www.graphpad.com).

Results

AM morphology and intensity of mycorrhizal colonization

Twenty-four weeks after inoculation, the mixed fungal culture of *G. claroideum* (Schenck and Smith), *G. etunicatum* (Becker and Gerdemann), *A. longula* (Spain and Schenck), *G. rosea* (Nicolson and Schenck), and *S. erythropa* (Koske and Walker) Walker and Sanders produced the highest frequency of colonization in the root system, 66% *H. rhombea*, 70% *R. muliflora*, and 75% *R. parvifolius*. *Gigaspora rosea* produced the least total root system colonization in the three plant species, ranging from 10 to 15% (Table 1).

Table 1 Frequency of AM in the root system of *Hedera rhombea*, *Rubus parvifolius*, and *Rosa multiflora* and abundance of intercellular hyphae, hyphal coils, arbuscules, and vesicles in the root system

Fungal species	*Hedera rhombea*	*Rubus parvifolius*	*Rosa multiflora*
Frequency of mycorrhiza in the root system			
G. rosea	12.0 (2.0) aX	10.0 (2.0) aX	15.0 (1.0) aX
G. claroideum	33.0 (4.4) bX	46.3 (4.0) dY	56.3 (4.7) cY
G. etunicatum	43.7 (4.7) cX	63.0 (1.7) eY	58.3 (2.5) cY
S. erythropa	49.7 (1.5) cZ	33.3 (2.1) cX	43.0 (1.0) bY
A. longula	51.7 (2.1) cZ	22.3 (2.5) bX	42.7 (2.1) bY
Mixed culture	66.0 (3.6) dX	75.0 (3.0) fY	70.0 (1.5) dXY
Two-way ANOVA	df	*F*-value	*P*
Plant species	2	25.40	0.001
Fungal species	5	338.00	0.001
Interaction	10	27.45	0.001
Abundance of intercellular hyphae in mycorrhizal parts of the root fragment			
G. rosea	–	51.7 (3.5) dX	67.0 (3.0) cY
G. claroideum	–	41.0 (1.0) abX	50.3 (4.5) aX
G. etunicatum	–	46.0 (2.0) cX	51.0 (1.7) aY
S. erythropa	–	63.3 (6.4) eX	56.7 (4.0) bX
A. longula	–	62.0 (4.0) eX	60.3 (4.5) bX
Mixed fungal culture	–	38.3 (2.5) aX	55.0 (3.0) aY
Two-way ANOVA	df	*F*-value	*P*
Plant species	1	27.25	0.001
Fungal species	5	24.28	0.001
Interaction	5	9.77	0.001
Abundance of hyphal coils in mycorrhizal parts of the root fragments			
G. rosea	100.0 (0.0) b	–	–
G. claroideum	100.0 (0.0) b	–	–
G. etunicatum	100.0 (0.0) b	–	–
S. erythropa	100.0 (0.0) b	–	–
A. longula	75.3 (1.8) a	–	–
Mixed culture	76.7 (1.1) aY	0.002X[a]	–
One-way ANOVA			
Fungal species	$P<0.001$		
Abundance of arbuscules in mycorrhizal parts of the root fragments			
G. rosea	–	48.3 (3.5) bY	32.0 (2.7) bX
G. claroideum	–	59.0 (5.6) cY	39.3 (2.5) bX
G. etunicatum	–	54.0 (4.0) cY	34.0 (2.0) bX
S. erythropa	–	61.7 (6.0) cY	44.0 (3.6) cX
A. longula	–	12.3 (2.1) aX	10.0 (2.0) aX
Mixed fungal culture	–	62.3 (4.9) cY	33.3 (5.1) bX
Two-way ANOVA	df	*F*-value	*P*
Plant species	1	89.75	0.001
Fungal species	5	178.8	0.001
Interaction	5	7.290	0.003
Abundance of vesicles in mycorrhizal parts of the root fragments			
G. rosea	–	–	–
G. claroideum	–	–	10.3 (1.6) a
G. etunicatum	–	–	14.7 (1.6) a
S. erythropa	–	–	–
A. longula	23.6 (1.6) aX	27.7 (1.8) X	30.0 (2.0) bX
Mixed fungal culture	24.0 (1.3) aY	–	11.7 (1.1) aX
Plant species	$P<0.001$		
Fungal species	$P<0.001$		

Fungal inocula—*Gigaspora rosea*, *Glomus claroideum*, *Glomus etunicatum*, *Scutellospora erythropa*, *Acaulospora longula*, and mixed fungal culture comprising all the five listed species. Means (SD in brackets) followed by the same letters horizontally (X, Y, and Z) and vertically (a, b, c, d, and e) are not significantly different, as determined by Bonferroni's multiple comparison test ($P<0.001$)

df degrees of freedom

[a] The abundance of hyphal and arbusculate coils in mycorrhizal parts of the root fragment

Root colonization in *H. rhombea* was largely in the form of extensive cell-to-cell growth of hyphal coils characteristic of *Paris*-type morphology (Table 1 and Fig. 1a–f). No arbuscules, arbusculate coils, or intercellular hyphae were seen in roots of *H. rhombea*; however, 24% of the total colonization by the mixed inoculum and pure culture *A. longula* was attributed to vesicles (Table 1, Fig. 1a,d).

The mixed culture-inoculated *R. parvifolius* had a few hyphal and arbusculate coils in the root cortex, but the usual cell-to-cell intracellular hyphae associated with *Paris*-type AM were not present (Table 1 and Fig. 2b). All single-species AM fungal treatments produced *Arum*-type in *R. parvifolius* and *R. multiflora* (Figs. 2 and 3). Colonization by mixed and single culture AM fungi ranged from 10–75% and 15–70% in *R. parvifolius* and *R. multiflora*, respectively (Table 1). The mixed inoculum formed the highest colonization in *R. parvifolius* and *R. multiflora* (Table 1). As single culture inoculum, *G. etunicatum* formed the highest colonization in both *R. parvifolius* and *R. multiflora* (Table 1). Frequency of colonization by *G. etunicatum* and *G. claroideum* as single cultures was significantly different in *R. parvifolius*, but not in *R. multiflora* ($P<0.05$) (Table 1).

Details of the abundance of intercellular hyphae, arbuscules, and vesicles in the mycorrhizal root fragments are presented in Table 1. We are confident that intercellular hyphae as referred to in this study were AM fungal hyphae growing longitudinally within the intercellular spaces of the root cortical cells, as opposed to the intracellular type shown by Dickson (2004). The lowest arbuscular colonization was observed in both the *R. multiflora* and *R. parvifolius* plants inoculated with *A. longula* (Table 1, Figs. 2e and 3d,e). Vesicles were generally few and, as expected, confined to roots colonized by *G. claroideum*, *G. etunicatum*, *A. longula*, and the mixed inoculum (Table 1, Figs. 1a,d; 2e; and 3a,c,d). In the roots of *R. multiflora*, the highest proportion of vesicles was formed by *A. longula*. Except in roots of *R. parvifolius* inoculated with *A. longula*, no vesicles were formed when the mixed inoculum, *G. claroideum*, and *G. etunicatum* colonized *R. parvifolius*.

AM fungal species isolated and identified in plant roots inoculated with mixed AM fungal culture

Partial LSU rDNA of AM fungi from roots of *H. rhombea*, *R. parvifolius*, and *R. multiflora* were successfully ampli-

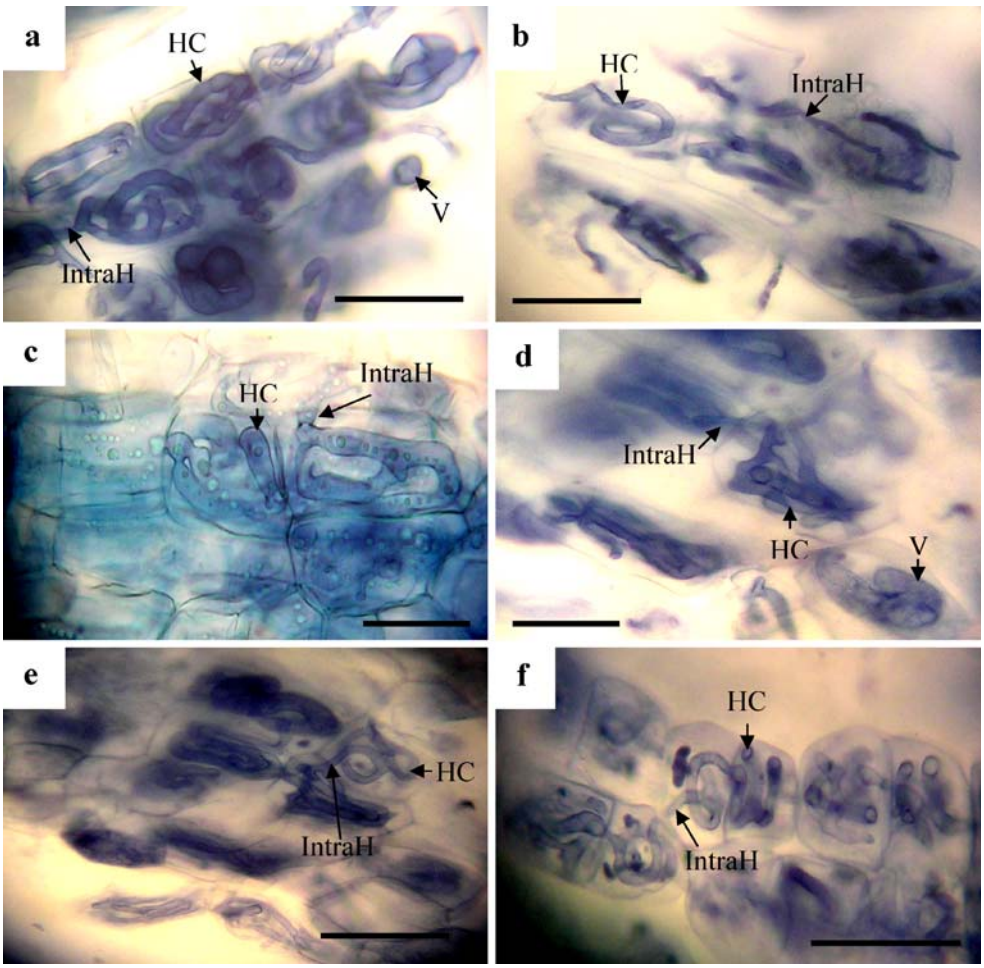

Fig. 1 Typical colonization by **a** mixed fungal culture, **b** *Glomus claroideum*, **c** *Glomus etunicatum*, **d** *Acaulospora longula*, **e** *Gigaspora rosea*, and **f** *Scutellospora erythropa* in association with *Hedera rhombea*. *HC* hyphal coils, *IntraH* intracellular hyphae, *V* vesicles. *Bars*, 100 μm

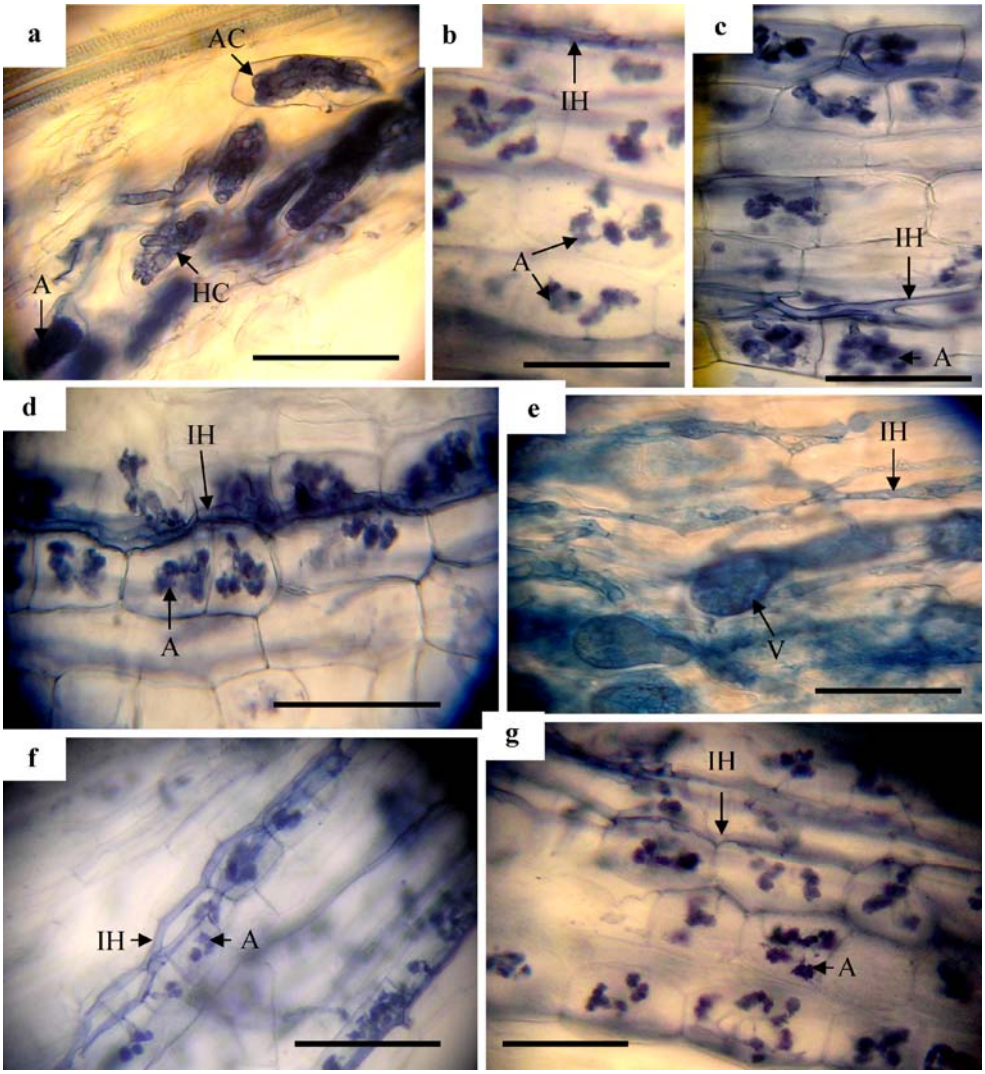

Fig. 2 Typical colonization by mixed fungal culture (**a** and **b**), *Glomus claroideum* (**c**), *Glomus etunicatum* (**d**), *Acaulospora longula* (**e**), *Gigaspora rosea* (**f**), and *Scutellospora erythropa* (**g**) in association with *Rubus parvifolius*. *A* arbuscule, *IH* intercellular hyphae, *HC* hyphal coils, *AC* arbusculate coils, *V* vesicles. *Bars*, 100 μm

fied with the nested PCR approach using the primer pairs LR1/FLR2 (van Tuinen et al. 1998; Trouvelot et al. 1999) and FLR3/FLR4 (Gollotte et al. 2004), respectively. Analysis of 15 representative AM fungal DNA sequences from replicates of each plant species showed some variability, but it was possible to identify clusters of closely related sequences. Except for *G. rosea*, which was not found in the roots of any of the host plant species, each of the remaining four fungal species could colonize at least two plant species. The frequency of DNA sequences of each AM fungal species in roots of plant species treated with mixed culture ranged from a mean of 11.3–60.0% and differed significantly among AM fungi colonizing each plant species ($P<0.0001$) (Table 2). Species of Glomaceae, *G. etunicatum* and *G. claroideum*, generally occurred more frequently in *Arum*-types *R. parvifolius* and *R. multiflora*, whereas *S. erythropa* of the family Gigasporaceae was the most frequently detected species in the *Paris*-type forming *H. rhombea* (Table 2).

Discussion

The actual basis for the formation of *Arum*- or *Paris*-type AM is still unknown. In an earlier study to determine the AM fungal communities colonizing roots of *R. parvifolius* and *H. rhombea* (Ahulu et al. 2006), the two plants were found to accommodate the same fungal taxa in their root systems, but there were preferences. In this study, we found *Arum*-type structures in *R. parvifolius* and *R. multiflora* and *Paris*-type in *H. rhombea* when the three plants were inoculated with the same AM fungal species under pure conditions. This extends two previous studies in which different plant and fungal species were used (Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983; Bedini et al. 2000). On the other hand, recent investigations using tomato (Cavagnaro et al. 2001) and tomato and cucumber (Kubota et al. 2005) have shown that different AM fungi can form either morphology in roots of the same plant species, and sometimes even in the same root system (Dickson 2004).

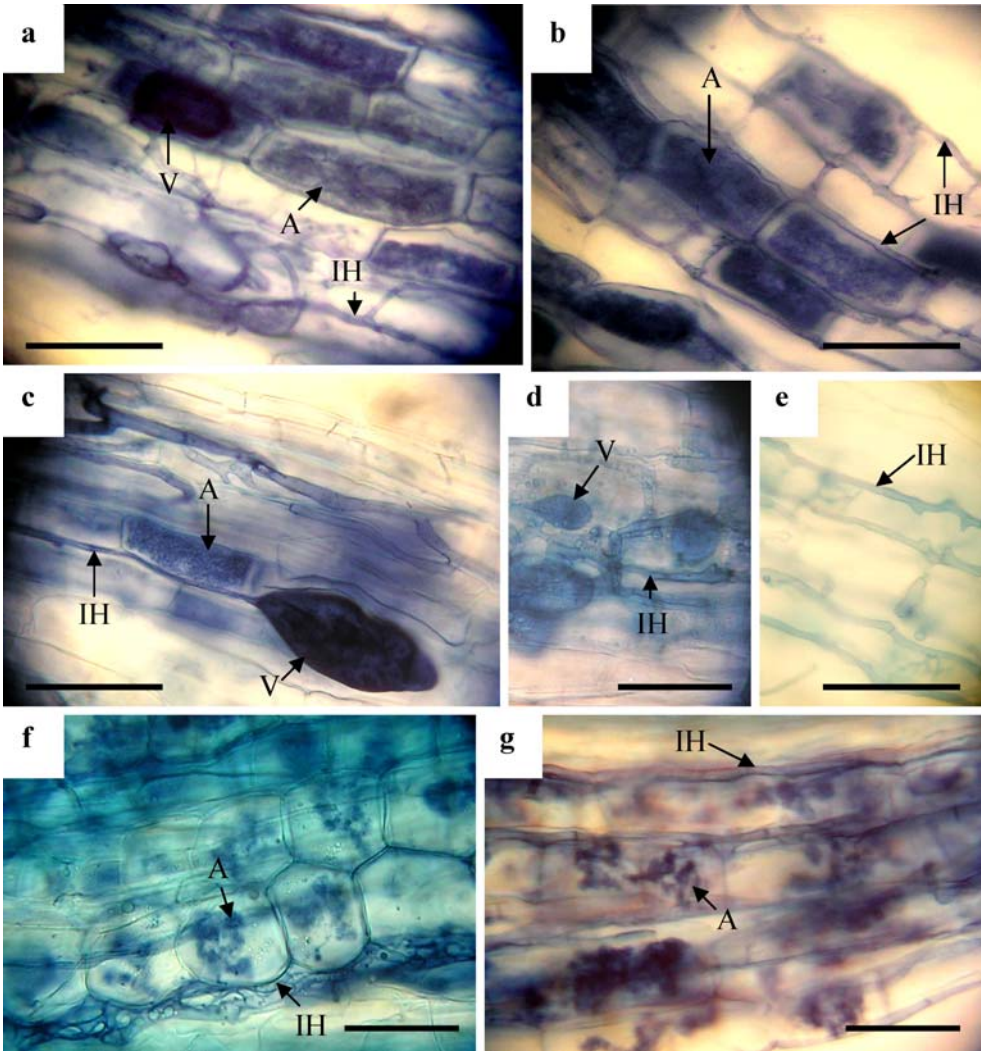

Fig. 3 Typical colonization by mixed fungal culture (**a**), *Glomus claroideum* (**b**), *Glomus etunicatum* (**c**), *Acaulospora longula* (**d** and **e**), *Gigaspora rosea* (**f**), and *Scutellospora erythropa* (**g**) in association with *Rosa multiflora*. *A* arbuscule, *IH* intercellular hyphae, *V* vesicles. *Bars*, 100 μm

In this study, when the same fungal species used as pure culture were used as mixed inoculum, *R. multiflora* and *H. rhombea* formed exclusively *Arum*- and *Paris*-type AM, respectively. Although *R. parvifolius* was predominantly *Arum*-type, a few hyphal and arbusculate coils characteristic of *Paris*-type AM were also found within the same root system. This is in accordance with data from Bonfante-Fasolo and Fontana (1985). Cavagnaro et al. (2001) mentioned that the presence of both intermediate types and *Arum*- or *Paris*-types in some plant families might be accounted for by the presence of different AM fungi. Overlaps of AM fungi colonizing the plants inoculated with mixed culture in this study, particularly between the AM morphologically different plant species, such as, for example, *S. erythropa* in roots of both *Paris*-type *H. rhombea* and *Arum*-type *R. multiflora*, agrees with findings in natural ecosystems that coexisting plant species are indiscriminately colonized by the indigenous AM fungi (Stukenbrock and Rosendahl 2005) to form exclusively one AM morphological type in each plant species (Ahulu et al. 2006).

It is of interest to note that, in spite of the indiscriminate pattern of colonization of the plants inoculated with mixed fungal culture, a majority of sequences from roots of *R. parvifolius* and *R. multiflora* identified with sequences of *G. claroideum* and *G. etunicatum*, whereas a majority of sequences from *H. rhombea* clustered with sequences of *S. erythropa*. Similarly, in a field study (Ahulu et al. 2006), *S. erythropa* was the most dominant fungus found in roots of *H. rhombea*, while a genotype closest to *G. microaggregatum* was most frequently seen in *R. parvifolius*. These findings suggest that, although AM fungal interactions with hosts are generally nonspecific (Smith and Read 1997), preferential associations of some AM fungal species to host plants can exist (Bever et al. 1996; Eom et al. 2000; Bidartondo et al. 2002; Vandenkoornhuyse et al. 2002, 2003; Johnson et al. 2003; Gollotte et al. 2004; Landis et al. 2004; Lovelock and Ewel 2005). This bias of association between the fungal species and the *Arum*-type host plants on one hand and the *Paris*-type plant on the other may be temporary because fungal species not found in plants

Table 2 Mean frequency (%) of DNA sequences attributed to each AM fungal species in roots of *Hedera rhombea*, *Rubus parvifolius*, and *Rosa multiflora* 24 weeks after inoculation with a mixed culture of *Glomus claroideum*, *Glomus etunicatum*, *Gigaspora rosea*, *Scutellospora erythropa*, and *Acaulospora longula*

AM fungal species	Plant species		
	Paris-type *Hedera rhombea*	*Arum*-type *Rubus parvifolius*	*Arum*-type *Rosa multiflora*
Glomus claroideum	11.3 (2.7) a	42.0 (7.3) b	42.0 (2.7) c
Glomus etunicatum	–	38.0 (2.7) b	33.3 (4.7) b
Gigaspora rosea	–	–	–
Scutellospora erythropa	60.0 (8.7) c	–	24.7 (2.7) a
Acaulospora longula	31.3 (6.0) b	18.0 (2.7) a	–
Two-way ANOVA	df	*F*-value	*P*
Plant species	2	0.09677	0.9080
Fungal species	4	46.61	0.0001
Interaction	8	46.59	0.0001

Means (SD in brackets) followed by the same letters vertically are not significantly different, as determined by Bonferroni's multiple ($P<0.0001$)
df degrees of freedom

inoculated with mixed culture could highly colonize the same plant species as single culture inocula. A good example can be seen between *G. etunicatum* and *H. rhombea*. Also, the use of a mix of the five AM fungi in the inoculum may have contributed to enhance the performance of some fungal species and could provide an explanation for the absence of *G. rosea* in plants inoculated with the mixed fungal culture. It should be noted, however, that, when used as single culture inoculum, *G. rosea* formed the lowest total colonization of roots of the three plant species. This supports the findings of the report by Hart and Richard (2002) that species of Gigasporaceae are the slowest colonizers and have extensive hyphal in-soil root colonization. Furthermore, it has been shown that the diversity of AM fungal spores isolated from or present in soils may not necessarily reflect the diversity of AM fungi colonizing roots of individual plant species (Clapp et al. 1995; Kjøller and Rosendahl 2001). Another possible reason for preferential association between host plants and AM fungi might be due to host-specific differences in plant response to AM fungi and in fungal response to plants (Adjoud et al. 1996; Streitwolf-Engel et al. 1997; Van der Heijden et al. 1998).

Our current data show that, under controlled conditions, AM fungal species could indiscriminately colonize host plants that form morphologically different AM, indicating that the formation of either the *Arum*- or *Paris*-type morphology depends on the genotype of host plants used rather than the identity of the colonizing fungi.

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