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Arbuscular mycorrhizal fungi associated with tree peony in 3 geographic locations in China

Zhaoyong SHI^{1,2,*}, Yinglong CHEN^{3,4}, Xiaogai HOU¹, Shuangcheng GAO¹, Fayuan WANG¹

¹College of Agriculture, Henan University of Science and Technology, Luoyang, China

²Laboratory for Earth Surface Processes, Ministry of Education, Peking University, Beijing, China

³College of Life Sciences, Hebei University, Baoding, China

⁴School of Earth and Environment and UWA Institute of Agriculture, University of Western Australia, Perth, Australia

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Abstract: The diversity of arbuscular mycorrhizal fungi (AMF) is of great interest because of their potential function in ecosystems. Tree peony is an important traditional ornamental and medicinal plant with economic significance. We examined the mycorrhizal status of the rhizosphere of 14 common cultivars of tree peony (*Paeonia suffruticosa*) in 3 different geographic locations in China. Root samples of all cultivars were colonized by AMF. The mean percentage of root length colonization, vesicles, and arbuscules were 39%, 3.6%, and 6.0%, respectively. AMF species richness varied from 5 to 11, and spore density ranged from 20 to 61 per 50 g of rhizospheric soil. The average AMF species diversity (Shannon–Wiener index) was 1.92, ranging from 1.64 to 2.18. A total of 31 AMF species belonging to 3 genera were identified in the rhizospheric soil. *Glomus* (21) was the dominant genus, followed by *Acaulospora* (7) and *Scutellospora* (3). *G. aggregatum* was the most commonly distributed species, with an occurrence frequency of 71.4 and a relative abundance of 13.6%. This study focused on the comparison of AM fungal diversity associated with tree peony in various original cultivar groups. This knowledge will help in selecting suitable AM inoculums for cultivation in the different original cultivar groups of tree peony.

Key words: Colonization rate, species diversity, spore density, tree peony

1. Introduction

Tree peony (a woody shrub of the Chinese Mudan, genus *Paeonia*, family Paeoniaceae) is native to China and there are 8 species with over 1000 cultivars grown in the temperate regions around the world (Hong and Pan, 1999). China was the first place of domestication and has been the origin center for cultivated tree peony for about 1600 years.

Arbuscular mycorrhizae (AM), the most commonly distributed plant symbionts, play key roles in ecosystems (Smith and Read, 2008). Many studies have shown their potential influence on the growth responses in coexisting plant species, and even on ecosystem processes and plant diversity in natural communities (van der Heijden et al., 1998a, 1998b; Klironomos et al., 2000). Recent studies have demonstrated that arbuscular mycorrhizal fungi (AMF) are important both ecologically and physiologically in floriculture and medical crops (Koltai, 2010; Closa and Goicoechea, 2011; Garmendia and Mangas, 2012; Tüfenki et al., 2012). These studies showed that co-occurring plant species vary considerably in seed germination, plant growth,

flowering, nutrition acquisition, and stress resistance in response to mycorrhizal colonization along a continuum from highly responsive, obligate mycotrophic species to facultative mycotrophic and nonresponsive species.

Tree peony, with its special feature of large, pretty, and fragrant flowers, has become an important source of valuable floriculture. It also has great value for herbal medicine and food ingredients (Fan et al., 2012). Tree peony is being cultivated as a commercial floricultural plant. In addition, the planting area of tree peony in China has been expanded to more than 7000 ha and therefore the prospective development of peony flowers is thought to be of great economic potential. Due to the numerous functions of AMF, such as the benefits of AM symbiosis on the growth and development of plants, including a variety of ornamental horticulture plants (Koltai, 2010), fundamental research on AMF diversity associated with tree peony in different geographic locations and cultivar groups in China is urgently needed.

In recent years, attention has been paid to AMF diversity and function in Chinese tree peony. Studies

* Correspondence: shizy1116@126.com

showed abundant AMF colonization on roots (Guo et al., 2007; Guo and Liu, 2010), growth promotion (Chen et al., 2010), nutrient acquisition improvement (Tong et al., 2010), and enhanced plant resistance to temperature stress (Li et al., 2009). Tree peony in China can be classified into 4 cultivar groups according to place of origin: Zhongyuan cultivar group, Xibei cultivar group, Xinan cultivar group, and Jiangnan cultivar group (Wang et al., 2004). Unfortunately, previous studies were mainly conducted on the Zhongyuan cultivar group and thus the mycorrhizal status of the other cultivar groups of tree peony remains unknown. This report presents results of the AM status of the 14 most common cultivars of tree peony (*Paeonia suffruticosa*) from 3 of the 4 cultivar original groups in China.

2. Materials and methods

2.1. Sample collection

The 14 most common cultivars of tree peony in the Zhongyuan (5 cultivars), Xinan (5), and Xibei (4) groups were examined in the present study. One representative tree peony garden was selected from each tree peony cultivar group. These were the State tree peony garden in Luoyang representing the Zhongyuan cultivar group, the Pengzhou tree peony garden representing the Zinan cultivar group, and the Hanzhong tree peony garden representing the Xibei cultivar group. Detailed descriptions of the cultivars are given in Table 1. Soil samples were collected from the rhizospheres of 4 randomly selected plants for each cultivar, which means there were 4 repeats for each cultivar. Care was taken when collecting individual plants to make sure the objective plant roots were sampled. Samples were taken to the laboratory for determination of root colonization. Soil samples (ca. 500 g) were air-dried for spore extraction. In addition, soil Olsen P was analyzed from a mixed soil sample of each tree peony cultivar group.

2.2. Assessment of AM colonization

Fresh roots (ca. 0.2 g) were washed free of soil and cleaned in 10% (w/v) KOH at 90 °C in a water bath for 20–30 min depending on the degree of lignification of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5–1.0-cm segments and stained with 0.5% (w/v) acid fuchsin (Biermann and Linderman, 1981). From each sample, 30 root fragments (ca. 1 cm long) were mounted on slides in a polyvinyl alcohol solution (Koske and Tessier, 1983) and examined for the presence of AM fungal structures. The percentage of root length colonized by AM fungal structures was estimated according to the method of Trouvelot et al. (1986).

2.3. Extraction and counting of AM fungal spores

Spores or sporocarps were extracted from 20 g of air-dried subsample of each soil sample in triplicate by wet sieving

followed by flotation–centrifugation in 50% sucrose (Dalpé, 1993). The finest sieve used was 25 µm (500 holes per square inch). The spores were collected on grid-patterned (4 × 4 mm) filter paper, washed 3 times with distilled water to spread them evenly over the entire grid, and counted using a dissecting microscope. A sporocarp was counted as 1 spore. The number of spores was expressed as the average of 3 replicates. For observation and identification of spore characteristics, spores were mounted on glass slides in polyvinyl lactoglycerol (PVLG) and PVLG + Melzer's reagent and then identified to species using current taxonomic criteria (Morton and Redecker, 2001) and information published by INVAM on the internet (<http://invam.caf.wvu.edu>).

Spore density, species richness, relative abundance, and frequency of AMF were expressed as follows: spore density (SD) = number of AM fungal spores in 50 g of soil; species richness (SR) = number of AM fungal taxa found in 50 g of soil; relative abundance (RA) = (number of spores of a species or genus/total spores) × 100%; and frequency (F) = (number of samples in which the species or genus was observed/total samples) × 100%. Species diversity was assessed using the Shannon–Wiener index as follows:

$$\text{Shannon–Weiner index} = -\sum (P_i \ln[P_i]),$$

where P_i = the number of individuals in a particular species divided by the total number of individuals in all species.

2.4. Statistical analysis

The data on the percentage of root length colonized by arbuscular mycorrhiza were normalized by arcsine transformation prior to statistical analysis, and spore density counts and estimates of species richness were subjected to square root transformation. Other data were analyzed without transformation. The root colonization, spore density, and AM fungal diversity index results are shown as arithmetic mean values with standard errors. The parameter of each cultivar was denoted by employing the corresponding mean of all cultivars in the same cultivar groups. The data were subjected to one-way analysis of variance and means were compared by least significant difference at the 5% level. The statistical tests were applied using SPSS 13.0.

3. Results

3.1. Colonization status of AMF

All 14 cultivars were colonized by AMF but the degree of colonization varied among plant species and locations (Figure 1). Typical AM fungal structures, i.e. arbuscules and vesicles, were observed in the roots of all cultivars. The percentage of root length colonization varied from 20% to 58% with an average of 39%. The colonization of arbuscules varied from 0.6% to 7.2% with a mean of 3.6%, and the colonization of vesicles ranged from 2.4% to 10.1%

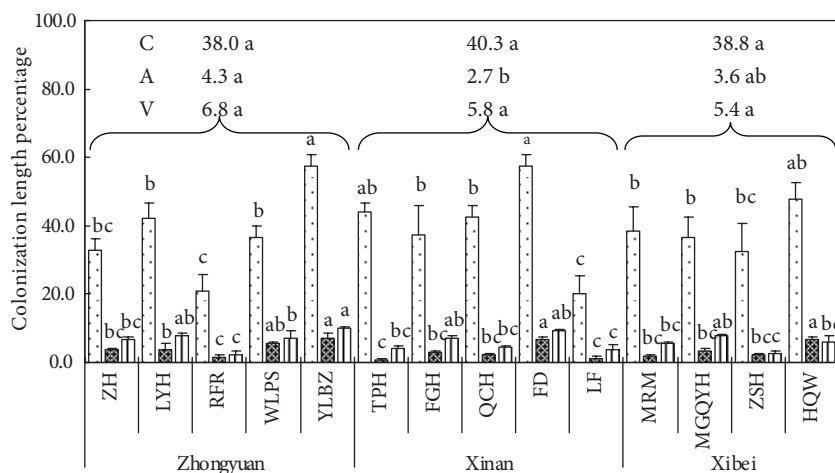


Figure 1. Arbuscular mycorrhizal (AM) fungal colonization in roots of different cultivars and cultivar groups (above columns) of tree peony. The total colonization length percentage (C, dot-filled), arbuscular (A, net-filled) and vesicular (V, vertical line-filled) percentage of roots of tree peony are presented with standard errors. Bars with different small letters are significantly different in each parameter at the level of 0.05.

with an average of 6.0%. As far as different tree peony cultivars were concerned, the highest colonization rates were observed in cultivars of YLBZ in the Zhongyuan cultivar group, FD in the Xinan cultivar group, and HQW in the Xibei cultivar group.

No significant difference was observed in the total colonization length and vesicular percentage of roots of tree peony (Figure 1). The arbuscular percentage was significantly higher in the Zhongyuan cultivar group than in the Xibei cultivar group. The colonization and typical structures of AM (arbuscules and vesicles) were observed in all samples (Figure 1). The occurrence frequency of arbuscules was lower than that of vesicles in all cultivars except for HQW in the Xibei cultivar group.

3.2. Genera, species, relative abundance, and frequency of AMF

A total of 31 taxa representing 3 genera of AMF were isolated and identified (Tables 1 and 2) from the rhizosphere of the 14 cultivars. These species belonged to the genera *Acaulospora* (7), *Glomus* (21), and *Scutellospora* (3). Among the 31 taxa, *Glomus aggregatum* was the most abundant (13.6%) and the most frequent (71.4%) species when the 3 tree peony cultivar groups were considered together (Table 1). For each cultivar group, the most frequent species were *G. cerebriforme* (26.8%) in the Zhongyuan cultivar group, *G. aggregatum* (33.9%) in the Xian cultivar group, and *G. convolutum* and *G. clarum* (28.6%) in the Xibei cultivar group. As for AM fungal species abundance, the most abundant species was *G. cerebriforme* in the Zhongyuan cultivar group, *G. aggregatum* and *G. clarum* in the Xian cultivar group, and

G. convolutum in the Xibei cultivar groups. When all the different cultivar groups are considered, 19, 16, and 21 AMF were observed in the Zhongyuan, Xinan, and Xibei cultivar groups, respectively.

3.3. Spore density and species richness of spores of AMF

The rhizosphere spore densities of the 14 cultivars ranged from 21 to 61 per 50 g of soil, with an average of 40. Significant variations were observed among the different cultivars in the different tree peony cultivar groups (Figure 2). The spore density of AMF in the rhizosphere of MRM in the Xibei cultivar group was the highest (58) and that of ZH in the Zhongyuan cultivar group was the lowest (25).

The species richness of AMF ranged from 5 to 11 with an average of 7.7 (Figure 2). The highest species richness was observed in the cultivar MGQYH in the Xibei cultivar group, with a mean value of 10.3, while the lowest appeared in the cultivar YLBZ in the Zhongyuan cultivar group, with a mean value of 5.8. SD and SR were also significantly different among the 3 cultivar groups (Figure 2). The highest SD and SR values were in the Xibei cultivar group, with mean values of 52.6 and 9.4, respectively.

3.4. Species diversity of AMF

The AM fungal diversity differed among cultivars and geographic locations (Figure 3). The highest species diversity (2.2) was in the cultivar ZSH in the Xibei cultivar group, and the lowest (1.6) was in the cultivar YLBZ in the Zhongyuan cultivar group (Figure 3). Significant differences in species diversity were observed among the 3 cultivar groups (Figure 3). The diversity of AMF was highest in the Xibei cultivar group.

Table 1. Genera and species of AMF in the rhizosphere of different cultivars and groups of tree peony.

Groups	Locations	Available P of soil (mg/kg)	Cultivars	AMF species
Zhongyuan cultivar group	State peony garden of Luoyang, Henan Province 34°42'27.97"N, 112°23'34.53"E	18.53	ZH (Zhi Hong)	<i>Acaulospora elegans</i> , <i>Glomus aggregatum</i> , <i>G. caledonium</i> , <i>G. clarum</i> , <i>G. convolutum</i> , <i>G. mosseae</i> , <i>G. sinuosum</i> , <i>G. vesiculiferum</i> , <i>Scutellospora nigra</i>
			LYH (Luo Yang Hong)	<i>A. elegans</i> , <i>G. aggregatum</i> , <i>G. australe</i> , <i>G. caledonium</i> , <i>G. clarum</i> , <i>G. convolutum</i> , <i>G. hoi</i> , <i>G. tortuosum</i>
			RFR (Rou Fu Rong)	<i>A. denticulate</i> , <i>A. elegans</i> , <i>G. aggregatum</i> , <i>G. claroideum</i> , <i>G. clarum</i> , <i>G. convolutum</i> , <i>G. etunicatum</i> , <i>G. hoi</i> , <i>G. mosseae</i> , <i>G. tortuosum</i> , <i>Scut. nigra</i>
			WLPs (Wu Long Peng Sheng)	<i>A. bireticulata</i> , <i>A. denticulate</i> , <i>G. aggregatum</i> , <i>G. caledonium</i> , <i>G. claroideum</i> , <i>G. clarum</i> , <i>G. convolutum</i> , <i>G. etunicatum</i> , <i>G. hoi</i> , <i>G. tortuosum</i> , <i>G. trimurales</i> , <i>G. vesiculiferum</i> , <i>Scut. nigra</i>
Xinan cultivar group	Pengzhou peony garden, Sichuan Province 30°58'41.29"N, 103°57'17.74"E	19.82	YLBZ (Ying Luo Bao Zhu)	<i>A. excavata</i> , <i>G. australe</i> , <i>G. clarum</i> , <i>G. convolutum</i> , <i>G. etunicatum</i> , <i>G. hoi</i> , <i>G. microcarpum</i> , <i>G. mosseae</i> , <i>G. trimurales</i>
			TPH (Tai Ping Hong)	<i>A. elegans</i> , <i>A. rehmsii</i> , <i>G. caledonium</i> , <i>G. claroideum</i> , <i>G. etunicatum</i> , <i>G. hoi</i>
			FGH (Fu Gui Hong)	<i>A. denticulate</i> , <i>A. elegans</i> , <i>G. aggregatum</i> , <i>G. caledonium</i> , <i>G. claroideum</i> , <i>G. coronatum</i> , <i>G. hoi</i> , <i>G. trimurales</i> , <i>Scut. nigra</i>
			QCH (Qian Ceng Hong)	<i>A. bireticulata</i> , <i>A. rehmsii</i> , <i>G. aggregatum</i> , <i>G. claroideum</i> , <i>G. clarum</i> , <i>G. etunicatum</i> , <i>G. globiferum</i> , <i>G. trimurales</i>
Xibei cultivar group	Hanzhong peony garden, Shaanxi Province 34°10'42.44"N, 106°58'25.52"E	16.88	FD (Feng Dan)	<i>A. rehmsii</i> , <i>A. spinosa</i> , <i>G. australe</i> , <i>G. claroideum</i> , <i>G. clarum</i> , <i>G. coronatum</i> , <i>G. etunicatum</i> , <i>G. trimurales</i> , <i>Scut. nigra</i>
			LF (Lu Fen)	<i>A. rehmsii</i> , <i>G. aggregatum</i> , <i>G. claroideum</i> , <i>G. etunicatum</i> , <i>G. globiferum</i> , <i>G. hoi</i> , <i>G. trimurales</i> , <i>Scut. nigra</i>
			MRM (Mei Ren Mian)	<i>A. bireticulata</i> , <i>A. rehmsii</i> , <i>G. aggregatum</i> , <i>G. australe</i> , <i>G. claroideum</i> , <i>G. constrictum</i> , <i>G. etunicatum</i> , <i>G. hoi</i> , <i>G. mosseae</i>
			MGQYH (Mei Gui Qian Ye Hong)	<i>A. bireticulata</i> , <i>A. foveata</i> , <i>A. rehmsii</i> , <i>G. aggregatum</i> , <i>G. claroideum</i> , <i>G. constrictum</i> , <i>G. coronatum</i> , <i>G. fragile</i> , <i>G. fulvum</i> , <i>G. hoi</i> , <i>G. trimurales</i>
Xibei cultivar group	Hanzhong peony garden, Shaanxi Province 34°10'42.44"N, 106°58'25.52"E	16.88	ZSH (Zhu Sha Hong)	<i>A. foveata</i> , <i>A. rehmsii</i> , <i>G. aggregatum</i> , <i>G. caledonium</i> , <i>G. claroideum</i> , <i>G. constrictum</i> , <i>G. hoi</i> , <i>G. microcarpum</i> , <i>G. tenue</i> , <i>Scut. cernadensis</i> , <i>Scut. minuta</i>
			HQW (Hong Qiang Wei)	<i>A. elegans</i> , <i>A. rehmsii</i> , <i>G. aggregatum</i> , <i>G. caledonium</i> , <i>G. cerebriforme</i> , <i>G. claroideum</i> , <i>G. fragile</i> , <i>G. fulvum</i> , <i>G. mosseae</i>

Table 2. Frequency and relative abundance of AM fungal species in the rhizosphere of different cultivars and groups of tree peony.

AMF species	Frequency (%)				Relative abundance (%)			
	Zhongyuan	Xinan	Xibei	Total	Zhongyuan	Xinan	Xibei	Total
<i>Acaulospora bireticulata</i>	5.4	7.1	12.5	25.0	0.3	0.5	1.6	2.3
<i>A. denticulata</i>	8.9	5.4	0	14.3	1.2	0.5	0	1.7
<i>A. elegans</i>	17.9	8.9	3.6	30.4	1.4	0.6	0.1	2.2
<i>A. excavata</i>	7.1	0	0	7.1	0.5	0	0	0.5
<i>A. foveata</i>	0	0	14.3	14.3	0	0	0.8	0.8
<i>A. rehmsii</i>	0	28.6	28.6	57.1	0	6.0	4.0	10.0
<i>A. spinosa</i>	0	7.1	0	7.1	0	0.9	0	0.9
<i>Glomus aggregatum</i>	23.2	19.6	28.6	71.4	2.7	3.9	7.0	13.6
<i>G. australe</i>	12.5	7.1	7.1	26.8	1.3	1.2	0.6	3.1
<i>G. caledonium</i>	10.7	14.3	14.3	39.3	0.9	2.2	1.8	5.0
<i>G. cerebriforme</i>	0	0	7.1	7.1	0	0	1.2	1.2
<i>G. claroideum</i>	8.9	33.9	26.8	69.6	1.1	6.0	4.5	11.6
<i>G. clarum</i>	26.8	10.7	0	37.5	5.2	1.4	0	6.6
<i>G. constrictum</i>	0	0	19.6	19.6	0	0	2.9	2.9
<i>G. convolutum</i>	25.0	0	0	25.0	2.2	0	0	2.2
<i>G. coronatum</i>	0	8.9	5.4	14.3	0	0.8	0.2	0.9
<i>G. etunicatum</i>	17.9	28.6	7.1	53.6	2.1	4.5	1.3	7.8
<i>G. fragile</i>	0	0	14.3	14.3	0	0	1.7	1.7
<i>G. fulvum</i>	0	0	14.3	14.3	0	0	0.99	1.0
<i>G. globiferum</i>	0	14.3	0	14.3	0	2.0	0	2.0
<i>G. hoi</i>	23.2	21.4	19.6	64.3	2.2	3.4	3.3	8.9
<i>G. microcarpum</i>	3.6	0	7.1	10.7	0.5	0	1.0	1.6
<i>G. mosseae</i>	8.9	0	14.3	23.2	1.0	0	2.5	3.5
<i>G. sinuosum</i>	7.1	0	0	7.1	0.5	0	0	0.5
<i>G. tenue</i>	0	0	7.1	7.1	0	0	0.8	0.8
<i>G. tortuosum</i>	12.5	0	0	12.5	1.0	0	0	1.0
<i>G. trimurales</i>	5.4	26.8	7.1	39.3	0.3	2.3	0.9	3.6
<i>G. vesiculiferum</i>	7.1	0	0	7.1	0.4	0	0	0.4
<i>Scutellospora cerradensis</i>	0	0	5.4	5.4	0	0	0.4	0.4
<i>Scut. minuta</i>	0	0	3.6	3.6	0	0	0.1	0.1
<i>Scut. nigra</i>	10.7	16.1	0	26.8	0.4	0.9	0	1.3

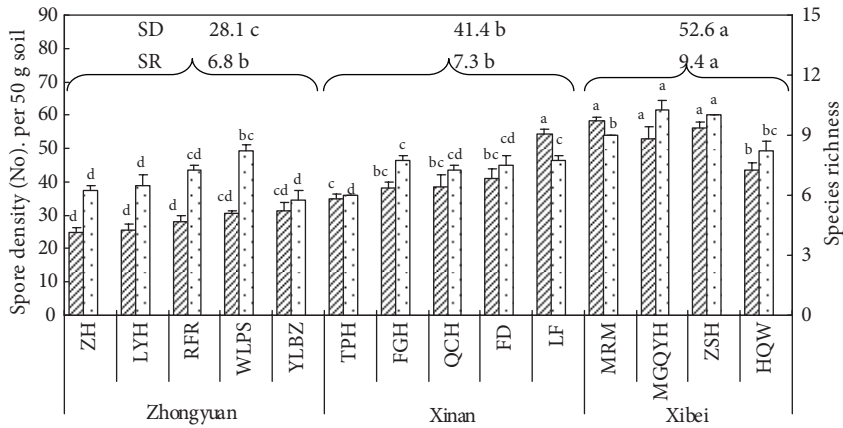


Figure 2. Spore density (SD) and species richness (SR) of arbuscular mycorrhizal fungi in the rhizosphere of different cultivars and cultivar groups (above columns) of tree peony. Spore density (acclititous line-filled) and species richness (dot-filled) of AMF are presented with standard errors. Bars with different small letters are significantly different in each parameter at the level of 0.05.

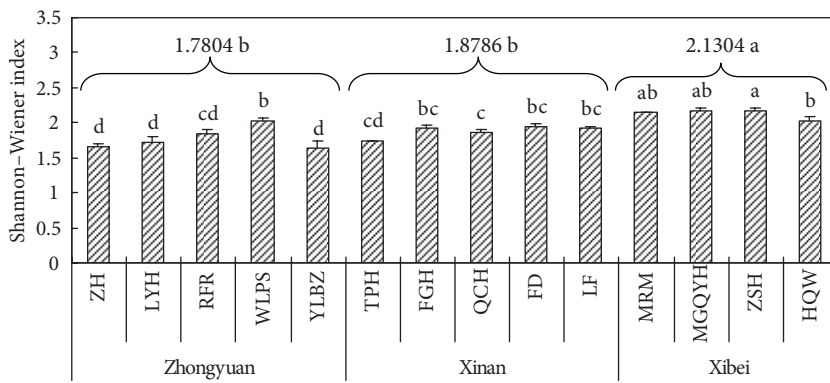


Figure 3. Diversity (Shannon–Wiener with standard errors) of arbuscular mycorrhizal fungi in the rhizosphere of different cultivars and cultivar groups (above columns) of tree peony. Bars with different small letters are significantly different at the level of 0.05.

4. Discussion

This study explored the colonization of roots and the diversity of AMF associated with tree peonies based on 3 different cultivar groups in China. The colonization status of the roots of tree peonies in different cultivars and among different cultivar groups indicated that the AMF are ubiquitous in the most common tree peony in 3 cultivar groups in China and reflects the fact that the majority of plants are associated with AMF (Smith and Read, 2008). The dominance of AMF in tree peony was also in agreement with the observations documented for other horticultural plants in previous studies (Guo et al., 2007; Guo and Liu, 2010; Bechem and Alexander, 2012; Yamato et al., 2012). The occurrence frequency of arbuscules in all cultivars except for HQW in the Xibei cultivar group was lower than that of vesicles, which is in

accord with our studies in other plant species or ecosystems (Shi et al., 2006a, 2006b, 2007). One possible reason is the characteristic of arbuscules being short-lived, which is related to the absorption and storage of soil nutrients (Shi et al., 2007).

In the matter of the AM species, this result showed that *Glomus* was the dominant genus in the rhizosphere of tree peony, which is consistent with other investigations conducted in the Zhongyuan cultivar group (Guo et al., 2007; Guo and Liu, 2010). The difference in AM fungal species and genera among cultivars may be led by ecological factors because many ecological factors affect the growth, development, and distribution of AM fungi (Smith and Read, 2008), but it could be also genotypic variability in mycorrhization, which requires further investigations. Our results showed that different tree peony cultivars are

associated with different AM fungal communities. This may be related to the choice between host plants and AMF during their evolution because many researchers have confirmed that they could influence each other (Smith and Read, 2008). This finding will benefit our selection of AM fungal species when we consider introducing inoculum to tree peony.

The variation of spore density and species richness of AMF among different tree peony cultivars and different cultivar groups is possibly related to soil characteristics, especially soil available P concentrations, which has been supported by many studies (Smith and Read, 2008; Ong et al., 2012; Aytok et al., 2013). In this study, our result also showed that the highest SD and SR of AMF were observed in the Xibei cultivar group with the lowest soil available P concentration. Certainly, other edaphic factors and ecological factors may also influence species density (Shi et al., 2007; Smith and Read, 2008).

The AM fungal diversity that we observed in the different cultivar groups was similar to that of a study done by Guo et al. (2007) in the Zhongyuan cultivar group and other ecosystems such as grassland (Gai et al.

2009), and these levels of fungal diversity are lower than that of a forest ecosystem (Shi et al., 2006a). Two possible explanations could be (1) the effect of the diversity of host plants and (2) the influence of long-term fertilization. In our study, the tree peony samples were collected from tree peony gardens with good management measures, which led to low plant diversity and long-term balanced fertilization. Many studies have shown that the diversity of the host plants determined AM fungal diversity (Smith and Read, 2008; Chen et al., 2012). At the same time, Lin et al. (2009) showed that long-term balanced fertilization decreases AM fungal diversity.

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