Caspian J. Environ. Sci. 2016, Vol. 14 No.4 pp. 363~371

CJES
Caspian Journal of Environmental Sciences

[Research]

Diversity of arbuscular mycorrhizal fungal spores associated with *Sorbus torminalis* (L.) Crantz

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(Received: May. 2.2016 Accepted: Oct. 19.2016)

ABSTRACT

This study aimed to investigate the diversity and types of arbuscular mycorrhizal fungi (AMF) associated with the wild service tree, *Sorbus torminalis* (L.) Crantz in spring and autumn followed by identifying similarities among the different study sites. Three different sites were selected including Kheiroud, Lalis, and Tarkin, in the Hyrcanian forests, north of Iran. Five rhizosphere soil samples were collected from each site, and the spores were extracted. Based on their morphological features, five species of AMF belonging to two families, i.e., Claroideoglomeraceae (two species) and Glomeraceae (three species) were identified. The species richness of the studied sites was identical with only the Kheiroud site presenting an additional species. *Glomus badium* was the most common AMF species in Kheiroud and Lalis in spring and autumn. However, the most common species in Tarkin was *Septoglomus constrictum*. The Shannon–Wiener indices of diversity and evenness and Simpson's index of dominance (Ds) showed no significant differences among the studied sites in the two seasons. In sum, it is recommended that the colonized wild service tree seedlings be produced with the more relevant species identified in this study than with commercial sources.

Key words: Arbuscular mycorrhizae, Claroideoglomeraceae, *Glomus badium*, Glomeraceae, *Sorbus torminalis*.

INTRODUCTION

The Hyrcanian forests of Iran are temperate, deciduous forests, which have 80 distinct tree species. These forests are located near the south of the Caspian Sea and have a sub-Mediterranean climate (Marvie-Mohadjer 2005). The forests comprise broad-leaved trees with one of the most valuable of these species being the wild service tree. The wild service tree, *Sorbus torminalis* (L.) Crantz is distributed from east to west in the Hyrcanian forests (Sabeti 2002), at elevations ranging from 170 m to 2700 m above sea level (Pourmajidian 2000). This tree is known for its high-quality wood and ecological, ornamental, and medicinal benefits. There is also high demand for its veneer in some European countries (Nicolescu *et al.* 2009). Furthermore, the wild service tree is important for animal and bird feeding (Paganová 2008).

Microorganisms are important components of soil and play a key role in plant nutrition (Smith & Read 2008). AMFs are the most widely available microorganisms in soil, comprising up to 10% of soil microbial biomass (Fitter *et al.* 2011). AMFs, which are in association with more than 80% of terrestrial plants (Parniske 2008), belong to the Glomeromycota family (Schüssler *et al.* 2001). These fungi improve water uptake and the phosphate (P) and nitrogen (N) nutrients of their host plants, increase plant tolerance to cultural and environmental stress (Smith & Read 2008), and provide pathogen protection (Sikes *et al.* 2009). These fungi also have the ability to increase plant tolerance to hard edaphic conditions (Doubková *et al.* 2012) and also improve the growth of their host plants (Wu *et al.* 2011). Different types of AMFs could increase plant growth differently. Their diversity could be affected by soil pH (Dumbrell *et al.* 2010), anthropogenic nitrogen deposition (Egerton-Warburton & Allen 2000), vegetation coverage (Liu *et al.* 2011), and plant diversity (van der Heijden *et al.* 1998). In soil elements, N and P are believed to be important for mycorrhizal fungi growth (Treseder 2004).

Given the aforementioned value of the wild service tree and the effects of different parameters on AMF biodiversity, AMF is considered an important symbiont microorganism for afforestation and also wood and fruit production.

The isolation, identification, and employing of these fungi in the plantations and afforestation sites will be beneficial in improving the establishment of these plants. In this regard, it is important to know which types of AMFs have symbiotic relationships with plant species like the wild service tree. In this study, we hypothesized that the diversity of AMFs associated with *Sorbus torminalis* is different in each studied site. The aims of this study included: to compare the AMF species diversity in the wild service tree rhizosphere, to identify the AMFs that have a symbiotic association with the wild service tree, and finally to determine whether there are a similarities in the AMF spore communities between different sites or not.

MATERIALS AND METHODS

Study sites

The three sites in which the wild service trees were selected for this study, are located in Mazandaran Province (north of Iran). The climate of this region is sub-Mediterranean (Marvie-Mohadjer 2005). Kheiroud (36°40'N, 51°43'E) has an average annual precipitation of 1458 mm. The most common species in Kheiroud are *Fagus orientalis* and *Carpinus betulus*, which are found alongside *Quercus castaneifolia*, *Acer velutinum*, *Tilia platyphyllos*, *Ulmus glabra*, and *Sorbus torminalis.* In this study, the site aspect was south, and the slopes ranged from 30% to 70%. The sampling site elevation was 1000m above sea level and the mean annual temperature was 16°C. Lalis (36°32'N, 51°23'E) has an average annual precipitation of 731mm, and the most common species found here are *Fagus orientalis* and *Carpinus betulus*, together with other species like *Quercus castaneifolia*, *Acer cappadocicum*, *Cerasus avium*, and *Sorbus torminalis*. The aspect for this study was north to northwest, and the slopes ranged from 0% to 60%. The sampling site elevation was 1700 m above sea level and the mean annual temperature was 10°C. Tarkin (36°28'N, 51°51'E) has an average annual precipitation of 1085 mm. The most common species found in Tarkin include *Fagus orientalis*, *Carpinus betulus*, *Tilia platyphyllos*, *Taxus baccata*, and *Sorbus torminalis*. In this study, the aspect was north, and the slopes ranged from 30% to 80%.

The sampling site elevation was 1500m above sea level and the mean annual temperature was 10°C.

The soil characteristics of the studied sites are presented in Table 1.

Soil sampling and analysis

Five wild service trees from each site were randomly selected for measurement during spring and autumn. Five soil samples of about 1 kg were taken from the rhizosphere of selected trees to a depth of 15 cm after the litter and organic horizon removal (Moradi *et al.* 2015). Four soil subsamples from each tree were taken and pooled to get one sample from each tree. The soil samples were then taken to the lab, air-dried, and passed through a 2 mm sieve before being sealed in plastic bags and stored at 4°C for further analysis.

Table 1. Soil chemical properties in studied sites.

Site	Soil texture	pH	SOM (%)	TN (%)	P (ppm)	K (ppm)
Kheiroud	clay and sandy-clay	7.93	8.18	0.22	8.64	311.73
Tarkin	sandy loam	7.62	14.15	0.43	9.46	342.05
Lalis	clay	7.05	9.52	0.25	14.66	532.05

SOM, soil organic matter; TN, total nitrogen; P, phosphorus; K, potassium.

Identification of AMF species

Spores were extracted using the method of Gerdemann & Nicolson (1963) and centrifuged. The spores were then mounted on slides with polyvinyl alcohol + lactic acid + glycerol (PVLG). The PVLG was mixed 1:2 (v/v) with Melzer's reagent and separated by size, color, and shape using a stereomicroscope (Olympus CH2, Japan). Morphological identification was done according to INVAM and Schenck & Ṕerez (1990).

Ecological indices

Spore density (SD), relative abundance (RA), the Shannon–Wiener indices of diversity and evenness, and also Simpson's index of dominance were employed to calculate descriptions of the structures of the AMF communities.

The results were analyzed using SPSS (version 17) for Windows. Ecological factors were assessed using analysis of variance (ANOVA), and Tukey's test was applied to compare the means of the ecological factors at a probability level of 95% ($p<0.05$). Spore diversity was defined by the number of spores in one gram (g) of soil.

$$RA = \frac{(spore\ number)\ of\ species}{the\ total\ number\ of\ spore} \times 100$$

where RA is the relative abundance (%).

Shannon–Wiener index of diversity (H′):

$$H = -\sum P_i LnP_i$$

where Pi is the relative abundance of each identified species per sampling site. Evenness (E):

$$E = \frac{H}{H_{\max}}$$

Simpson's index of dominance (Ds):

$$D = \sum \left[n_i (n_i - 1) / N(N-1) \right]$$

where ni is the number of spores of a species, and N is the total number of identified spore samples.

RESULTS AND DISCUSSION

Species richness and spore density

Five species belonging to two families of AMFs were detected based on the morphological features of 15 rhizosphere soil samples at the three different sites (Tables 2, 3).

Two of these species belong to the family Claroideoglomeraceae with the balance belonging to Glomeraceae.

The identified species at the sites belonging to the Glomeraceae family included *Glomus badium* Oehl, Redecker and Sieverd, *Septoglomus constrictum* Sieverd, Silva and Oehl, and *Glomus macrocarpum* Tul and Tul, while the species belonging to the Claroideoglomeraceae family were *Claroideoglomus etunicatum* Walker and Schüßler and *Claroideoglomus claroideum* Walker and Schüßler.

Table 2. Ecological factors for AMF communities in studied sites for spring (The values are mean ± standard error).

Ecological parameters	Sites												
	Kheiroud					Lalis				Tarkin			
	G. badium	*C. etunicatum*	*S. constrictum*	*G. macrocarpum*	*C. claroideum*	*G. badium*	*C. etunicatum*	*S. constrictum*	*G. macrocarpum*	*G. badium*	*C. etunicatum*	*S. constrictum*	*G. macrocarpum*
RA %	49.3 ± 6.1A	20.7 ± 3.2A	13.0 ± 3.7A	13.1 ± 2.5A	3.3 ± 2.4	36.5 ± 2.6A	22.7 ± 2.3A	20.5 ± 5.6AB	20.3 ± 4.5A	17.2 ±3.3 B	27.5 ± 5.3A	33.8 ± 5.4B	21.4 ± 1.8A
H′	1.2 ± 0.05A					1.3 ± 0.01A				1.3 ± 0.02A			
E	0.8 ± 0.05A					0.9 ± 0.01A				0.9 ± 0.02A			
Ds	0.6 ± 0.03A					0.7 ± 0.005A				0.7 ± 0.01A			

RA, relative abundance; H′, Shannon -Wiener index of diversity; E, Evenness; Ds, Simpson's index of dominance.

Table 3. Ecological factors for AMF communities in studied sites for autumn (The values are mean ± standard error).

Ecological parameters	Sites												
	Kheiroud					Lalis				Tarkin			
	G. badium	*C. etunicatum*	*S. constrictum*	*G. macrocarpum*	*C. claroideum*	*G. badium*	*C. etunicatum*	*S. constrictum*	*G. macrocarpum*	*G. badium*	*C. etunicatum*	*S. constrictum*	*G. macrocarpum*
RA	54.1 ± 4.0A	14.6 ± 2.1A	16.0 ± 3.0A	11.0 ± 2.7A	3.2 ± 0.6	36.8 ± 3.1B	30.0 ± 3.7B	20.3 ± 2.3A	12.8 ± 3.6A	14.1 ± 3.2C	20.8 ± 2.7AB	38.2 ± 5.5B	26.8 ± 2.0B
H′	1.18 ± 0.04A					1.3 ± 0.03A				1.3 ± 0.03A			
E	0.75 ± 0.06A					0.89 ± 0.02A				0.91 ± 0.02A			
Ds	0.6 ± 0.03A					0.7 ± 0.005A				0.7 ± 0.01A			

RA, relative abundance; H′, Shannon -Wiener index of diversity; E, Evenness; Ds, Simpson's index of dominance.

Based on the relative abundance of the species, *G. badium* was the most common AMF species in Kheiroud and Lalis in both spring and autumn. The most common species in Tarkin was *S. constrictum* (Tables 2, 3). With five species, Kheiroud had the highest species richness. The other two sites each had four species of AMF in the wild service tree rhizosphere.

Spore density for spring ranged from 31 to 136 spores per g of soil.

However in autumn, this range was 44 to 154 spores per g of soil with no significant difference (p>0.05) & (Fig. 1).

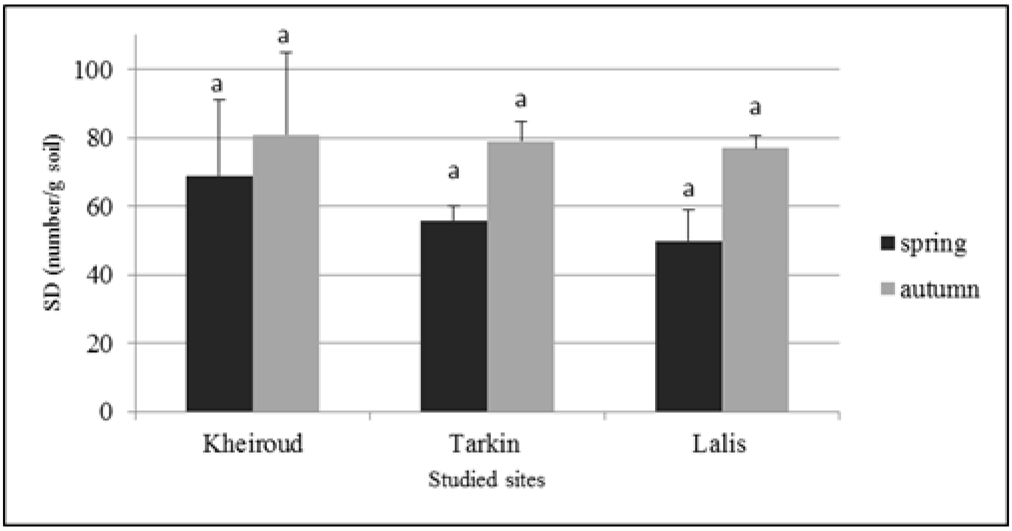

Fig. 1. Spore density in g soil for studied sites in two seasons (data are means of five replications per site. The bars represent standard error).

The AMF spore communities associated with the wild service tree were similar at the three different sites in the northern forests of Iran. Factors such as natural disturbance (Dandan & Zhiwei 2007), niche (Dumbrell *et al.* 2010), changes in soil conditions (Yang *et al.* 2011), nitrogen (Egerton-Warburton & Allen 2000), host plant (Kivlin *et al.* 2011), and soil fertility (Santos *et al.* 2006) significantly affect AMF diversity. In order to assess the maximum diversity of AMF, we chose three different sites in the Hyrcanian forests.

Despite differences between the sites, such as elevation, aspect, slope, organic matter and precipitation, the biodiversity of AMFs were almost similar, and just one additional species was found in the Kheiroud site.

According to our findings, there were no differences in spore richness during the two studied seasons.

This is contrary to the findings of Pagano *et al.* (2013) whose study showed higher species richness in dry seasons.

Ecological factors

Shannon–Wiener indices of diversity, evenness and Simpson's index of dominance are presented in Tables 2 & 3. Further analyses showed no significant differences between these ecological parameters at the studied sites for the two seasons (p>0.05).

There were no significant differences in ecological factors at the sites according to the Shannon–Wiener indices of diversity and evenness and Simpson's index of dominance. Indeed, similarity of spore abundance was evident among the studied sites (Franke-Snyder *et al.* 2001).

In spite of the ecological similarities, there were significant differences in the relative abundance of the AMF species between the sites. This indicates the importance of spore relative abundance in explaining the differences in the plant communities (Lovelock *et al.* 2003; Mangan *et al.* 2004). The relative abundance differences could be explained by the environmental differences between the sites although these environmental differences may have an effect on relative abundance with no biodiversity differences.

Our results showed the same AMF species richness in both seasons, differ from the earlier findings of Pagano *et al.* (2013). However, despite species richness in both seasons, the relative abundance of the species changed between seasons, which is in agreement with findings of Pringle & Bever (2002).

The high number of spores per gram of soil was consistent with the findings of Johnson & Wedin (1997) in the Costa Rican dry forests.

Mosse (1973) and Zhao *et al*. (2002) stated that AMF may not be specific to their host plants, and one species of AMF can form mutualistic associations with many plant species. The results of the present study do not support this research as the wild service trees at the different studied sites had the same AMF species. This suggests that some plant species may have mutualistic relationships with specific AMFs.

The northern forests of Iran are well known for their high biodiversity (Sagheb-Talebi *et al.* 2014). In the present research, we studied the association of AMF with one tree species. Since higher diversity at above-ground results in higher AMF diversity (van der Heijden *et al.* 1998; Chaurasia *et al.* 2005), a lack of AMF biodiversity could point to a reverse influence. Interestingly, the abundance of *G. badium* and *S. constrictum*, the most common symbionts of wild service tree in the northern forests of Iran, was high. This could be due to their adaptation to these sites (Pagano *et al.* 2013). In order to take full advantage of this association, AMF species composition and distribution are paramount (Mangan *et al.* 2004). Indeed, previous research has revealed that some plants favor specific AMF species (Pagano *et al.* 2011), and AMFs are strongly affected by the host plant species (Garbeva *et al.* 2004). In our study, we showed that the wild service tree hosted a specific AMF species in the studied sites, concurred with the findings of Uibopuu *et al.* (2009) who found that plants have selective desire with AMF. These findings significantly endorse the development of inoculum sources for wild service tree nurseries and provide some support for the use of natural AMF sources instead of commercial ones.

CONCLUSION

This study set out to determine AMF species diversity in relation to the wild service tree and the associated differences between sites and seasons. With respect to the detection of same-spore richness at the sites, it revealed a specific consideration for AMF in the wild service tree.

In this study, we demonstrated that AMF diversity was same for the studied sites and seasons. The association of AMF with the wild service tree as a rare, commercial, and valuable tree species could be considered as a significant approach to increase the numbers and protection of these trees. In sum, it is suggested that wild service tree seedlings colonized with the species identified in this research be produced. Microorganisms like AMFs could be one of the most effective solutions to help foresters improve production of this valuable tree species. By using isolated species from natural fields, we may have a better chance for afforestation of the wild service tree. Using the natural AMF of wild service fields as inocula over commercial sources of inocula to produce colonized root seedlings at specific sites may lead to more success in increasing the numbers of this valuable tree.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Iranian Forests, Range, and Watershed Management Organization. This study was supported by the University of Tehran and the Research Institute of Forests and Rangelands of Iran.

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تنوع قارچ‌های میکوریز اربوسکولار در ارتباط با بارانک (Sorbus torminalis (L.) Crantz)

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(تاریخ دریافت: ۹۵/۲۰/۱۳ تاریخ پذیرش: ۹۵/۰۷/۲۸)

چکیده

هدف این مطالعه، بررسی تنوع قارچ‌های میکوریز اربوسکولار همزیست با درختان بارانک در فصل بهار و پاییز می‌باشد. همچنین در این مطالعه تلاش شد تا شباهت‌های موجود در مناطق مورد مطالعه مشخص شود. برای این منظور سه منطقه خیرود، لالیس و تارکین در جنگل‌های هیرکانی شمال ایران انتخاب شدند. از هر منطقه پنج نمونه خاک از ریزوسفر درختان بارانک برداشت و اسپور قارچ‌های میکوریز از این نمونه‌ها استخراج شد. بر پایه خصوصیات مورفولوژیک، پنج گونه قارچ میکوریز متعلق به دو خانواده Claroideoglomeraceae (دو گونه) و Glomeraceae (سه گونه) شناسایی شد. غنای گونه‌ای در مناطق مورد مطالعه یکسان بود و تنها منطقه خیرود یک گونه بیشتر از دو منطقه دیگر داشت. *Glomus badium* فراوان ترین گونه قارچ میکوریز اربوسکولار در مناطق خیرود و تارکین، در بهار و تابستان بود، در حالی که فراوان‌ترین گونه در منطقه تارکین Septoglomus *constrictum* بود. شاخص تنوع شانون وینر، یکنواختی و شاخص غالبیت سیمپسون در مناطق مورد مطالعه و فصل‌های مورد بررسی تفاوت معنی‌داری را نشان نداد. بنابراین با توجه به وجود و شناسایی قارچ‌های میکوریز همزیست با بارانک توصیه می شود که برای تولید نهال‌های میکوریزی از گونه‌های قارچ‌های شناسایی شده در ریزوسفر بارانک به جای استفاده از گونه‌های تجاری استفاده شود.

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