The arbuscular mycorrhizal symbiotic status of *Populus euphratica*, a drought resistant tree species from arid lands

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ABSTRACT

In this study, we isolated arbuscular mycorrhizal fungi (AMF) from rhizosphere of *Populus euphratica*, naturally occurred in Tarim River Basin. We found morphologically similar few AMF spores from rhizosphere of *P. euphratica* under drought stress. The average AMF spore densities were 53/50 and 32/50 g in air dried soil from rhizosphere of mature and seedling of *P. euphratica*, respectively. The AMF spores were simple and *Glomus mosseae* in nature, identified by traditional morphological methods and molecular techniques. The *G. mosseae* fungal spores formed mainly in three different types: alone in the soil, inside of the roots or sporangium. The hypha was single and funnel-shaped at the site of attachment. By optical microscopic observation, the vesicular structures demonstrated a formation by AMF inside the roots of *P. euphratica* tree. The molecular analysis data showed that a mycorrhizal symbiont was established by *G. mosseae* associated with *P. euphratica* root. Moreover, the AMF colonization rate and the infection intensity were not significant high. Specifically, the average colonization frequency (F%) and mycorrhizal infection intensity (M%) were 8.45%, 2.9% for the mature, and 8.62%, 0.3% for the young seedlings of *P. euphratica*, respectively. Copyright © 2013 John Wiley & Sons, Ltd.

KEY WORDS   arbuscular mycorrhizae; nested-PCR; colonization; Populus euphratica

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in natural ecosystems and form intimate symbiotic associations with the fine roots of majority terrestrial plant. It plays a very important role in promoting the absorption of nutrients (especially phosphorus), alleviating drought stress, improving soil structure and regrowth of Bermudagrass, and enhancing the resistance of plant to disease and pests (Koide and Mosse, 2004; Wu *et al.*, 2011). In natural ecosystems, AMF are typically found as mixed communities with multiple species colonizing any given plant root. One species of AM fungus is able to colonize the roots of many host plants, and at the same time, any species of host plant itself could be colonized by different AM fungal species (Gollotte *et al.*, 2004). The most diverse and highly productive terrestrial ecosystems support high abundance and diversity of AMF (Öpik *et al.*, 2006). Because the functionality of the symbiosis is highly variable and dependent upon the identity of the AMF and host species involved (Klironomos, 2003), the composition of AMF species colonizing a given plant has important implications for its fitness. Further, despite the absence of strict host specificity in mycorrhizal symbiosis, a preferential association between some AMF and four *Medicago* species was observed (Pivato *et al.*, 2007), and potato roots were preferentially colonized by one AM fungal species, *Glomus intraradices* (Cesaro *et al.*, 2008).

The AMF has been studied in a wide range of natural and agro-ecosystems. Their presence, diversity and abundance are a function of climatic conditions (Öpik *et al.*, 2006), plant community composition and diversity (Burrows and Pfleger, 2002), and soil disturbance (Jansa *et al.*, 2006). For a number of reasons, the diversity of the original community will be lost to a degree. In recent years, many studies have been carried out on the identification, genetic diversity and community structure of AMF using molecular biology technologies (Mummey and Rillig, 2008; Rodríguez-Echeverría *et al.*, 2009). However, the diversity of indigenous AM fungal species existing in arid land remains largely under-explored. AMF had been reported to play an important role in the reestablishment of the vegetation in disturbed arid ecosystems (Caravaca *et al.*, 2003). It had been demonstrated that establishment of *Retama sphaerocarpa*

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L. seedlings on a degraded semiarid Mediterranean area was promoted by mycorrhizal inoculation (Del Mar Alguacil *et al.*, 2004), and that indigenous AMF inoculation was more effective than allochthonous AMF (Requena *et al.*, 2001). Further, there is some evidence that the indigenous community of AMF played more effective role in promoting the growth of plants in its native soil than introduced alien isolates (Oliveira *et al.*, 2005). *Populus euphratica* is the oldest and the most primitive deciduous tree of desert riparian forest and is widely distributed in arid desert regions of 30–50ºN, e.g. from central to western Asia, North Africa and southern Europe. However, up to more than half of the natural *P. euphratica* trees have disappeared due to water scarcity and the impact of man-made destructions in China since 1950s. There have been many studies reported on *P. euphratica* focusing on its physiological and ecological characteristics (Chen *et al.*, 2003), but less on *P. euphratica* rhizosphere AMF. Our recent works indicated that *P. euphratica* rhizosphere AMF played an important role in improving rhizospheric environment and therefore beneficial to arid riparian community construction (Yang *et al.*, 2008). In this study, we will continue to focus on the indigenous AMF species, especially when breeding or restoration of the *P. euphratica*. Therefore, the objective of the present paper is to address three questions in this regard: (i) Does AM formation occur in the natural *P. euphratica* roots despite the drought stress? (ii) What AM fungal species are associated with natural *P. euphratica* tree? and (iii) Are levels of AM fungal colonization similar in different *P. euphratica* trees?

MATERIALS AND METHODS

Outline of the study sites

The Tarim River basin, with an area of $1\,020\,000\,km^2$, covers the entire southern Xinjiang in Western China. Our study sites are located between Daxihaizi Reservoir and Taitema Lake in the lower reaches of the Tarim River (39°38′–41°45 N, 85°42'–89°17′ E). The area is a flat land with an annual precipitation between 17.4 and 42.0 mm, and average evaporation (potential) at 2500 and 3000 mm. The alluvial plain of the Tarim River is built up by thick quaternary deposits. The deposits consist of fine sand in the upper layer where as clay silt in the deeper layer. According to the United States Department of Agriculture soil classification system, the soil of the lower Tarim River is a member of the Aridisol order. It has a temperate continental climate with extremely arid conditions. The natural vegetation in this region is very different from the surrounding areas, and plant growth cannot be maintained by relying on natural precipitation only. The major plant species growing along riparian zone in this region fed by groundwater and mainly belong to the following families: *Salicaceae*, *Tamaricaceae*, *Leguminosae*, *Apocynaceae* and *Gramineae*. The species include trees like *P. euphratica*, shrubs of *Tamarix* spp., *Lycium ruthenicum*, and herbaceous plants consisting of *Phragmites communis* and so on (Chen *et al.*, 2003). However, in the past 50 years, local intensive economic and social development has vastly increased the consumption of water from the Tarim River, which has caused the stream flow in the lower reaches (at 321 km) ceased completely. Consequently, the groundwater level dropped fast, to a depth of 8–12 m, as a result of the lack of recharging through surface flow. The soil has been seriously desertified, and plant life has seriously degenerated in the region. In order to restore the ecosystem of the lower Tarim River, a series of environmental measures have been taken, including ecological water conveyance project. Nine observation sites were established for monitoring groundwater levels between the Daxihaizi Reservoir and the Taitema Lake (Ye *et al.*, 2010).

Sample collection and processing

The field sampling was conducted in May of 2009. Five mature *P. euphratica* trees with a stem diameter of 25–30 cm and height of 18 m were selected for sampling in the lower reaches of Tarim River at Yinsu section where the groundwater depth fluctuated around 6 m based on the monitoring data in recent years. When groundwater depth was deeper than 4.42 m, the growth of *P. euphratica* would suffer from drought stress and restraint (Fu *et al.*, 2006). The roots of the trees and shrubs in the arid riparian zone deeply buried, and fine roots are less distributed in the shallow soil layer partly because of the dry soil moisture; fine roots of the mature *P. euphratica* tree are relatively abundant in the 80–100 cm soil layer (sandy loam). Therefore, we dug out some soil profiles at the places by 1 m away from *P. euphratica* tree truck and no other plants growing nearby. About 3 kg of rhizospheric soils with the fine roots in 80–100 cm depth soil layer was sampled from multi-points of five mature *P. euphratica* trees rhizosphere. After being rinsed with distilled water, parts of the fresh fine roots were excised into 1.0–2.0 cm pieces and placed into a small glass bottle containing the FAA solution (formaldehyde 5 ml, glacial acetic acid 5 ml and 70% ethanol 90 ml), then stored at 4 °C for the determination of colonization of AMF. The remaining root samples together with the soil samples were naturally dried in the cool condition. These root samples were stored at 4 °C, whereas the soil samples were kept in a cool place, for later use. In addition, the fine roots and soil from five young *P. euphratica* seedlings, height of about 50–70 cm, stem diameter of about 1 cm, adjacent to the river channel, were collected at 15–30 cm depth in consideration of the fact that the mature *P. euphratica* trees had a little of fine roots, and it is hard to get AMF spores from the deep soil samples.

Soil analysis

Soil samples were subsequently analysed in the laboratory for pH (1:5 water soil ratio), soil organic matter ($K_2Cr_2O_7$–H_2SO_4 oxidation method of Walkley–Black), total nitrogen (Kjeldahl), total phosphorus (Colorimetry), total phosphorus (Flame emission spectroscopy), available nitrogen (Diffuse), available phosphorus (Bray-P) and available potassium (NH_4–acetate). Total soluble salts were measured by weighing method (Laboratory of soil physics, institute of soil science, Chinese academy of sciences, 1978).

Morphological observation and counting of AMF spores

About 50.0 g of air dried soil randomly taken from the original collected sample was used to isolate spore or sporocarps using the triple wet sieving method (Liu and Chen, 2007). Those spores or sporocarps were passed through sieve (53 μm pore size) and were placed onto a piece of fine grid filter paper (4 × 4 mm). After being rinsed three times with distilled water, they were spread out in the grid and then were counted under an optical microscope (amplification 30×). Each sporocarp was counted as one spore. Three replicates of the same procedure were conducted for each soil sample. AMF spore density was defined as AMF spore number in 50.0 g air dried soil. Furthermore, intact healthy AMF spores were isolated from the soil sample of rhizosphere. Each spore type was mounted sequentially in polyvinyllactic acid (PVA), and PVA mixed 1:1(V/V) with Meltzer's reagent (Morton, 1988) for identification. Taxonomic identification of spores up to species level was based on spore size, spore colour, wall layers and hyphal attachments by using the identification manual of Schenck and Perez (1990) and the description provided by the International collection of vesicular and AMF (http://invam.caf.wvu.edu). AMF species were identified using Olympus BX51, and their typical taxonomic characters were imaged with Olympus BX51 digital camera.

Observation of the AM structure within P. euphratica and evaluation of AMF colonization

The fixed root fragments were chosen for determination of AMF colonization rate on the basis of the method of Biermann and Linderman (1981). The roots were rinsed with clean water, excised into 0.5–1.0 cm pieces, and then they were put into the little plastic boxes containing 10% (W/V) solution of KOH. After that, the boxes were bathed in 90 °C water for 60 min. Treatment for root transparent was performed as follows: after cooling, the roots were rinsed again with clean water for four to five times, then soaked in a 2% HCl solution for 15–20 min, and finally were stained with 0.05% acid fuchsin and then placed thirty root segments onto a slide and observed with a digital microscope; the colonization frequency (F%) and the mycorrhizal infection intensity (M%) were calculated on the basis of the

abundance grading standards by using 'mycocalc' software. Meanwhile, the fungal structures were observed.

NUCLEOTIDE SEQUENCE ANALYSIS OF 25S RDNA D1/D2 VARIABLE REGION

DNA extraction from single AMF spore

Because the AMF spores isolated from the different *P. euphratica* rhizosphere soil samples were found similar according to the morphological characters, the AMF spores isolated from one of mature and young *P. euphratica* trees rhizosphere soil (code as C3, C3-2, respectively) were selected for nest-polymerase chain reaction (PCR) analysis following the method described by Dong and Zhao (2006) with slight modifications. Briefly, a single AMF spore was picked up using the pipette. It was rinsed with sterile H_2O five times and then put into a sterile 1.5 ml Eppendorf tube, adding 40 μl buffer solution (10 mmol l^{-1} Tris-HCl, 1 mmol l^{-1} EDTA, pH 8.0). When it was fully broken, 10 μl of 20% Chelex-100 was added in order to reduce the impact of metal ions on DNA. The spore lysis mixture was boiled for 10 min then transferred onto an ice bath for 1–2 min, centrifuged at 15000 rpm for 5 min, the supernatant was finally transferred to a new Eppendorf tube, placed in −20 °C for later use.

Nested-PCR amplification

The target region for PCR was the partial sequences of large subunit RNA genes. The first PCR amplification was performed in 20.0 μl of a final volume containing 2.0 μl of 10× PCR buffer solution, 1.6 μl of 2.5 mmol l^{-1} deoxyribonucleotide triphosphates 2.0 μl of 25.0 mmol l^{-1} $MgCl_2$, 2.0 μl of 1.0 μmol l^{-1} primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') (White *et al*., 1990), 2.0 μl of 1.0 μg mol l^{-1} primer NDL22 (5'-TGGTCCGTGTTTCAAGACG-3') (Van Tuinen *et al*., 1998), 0.2 μl of 5 U μl^{-1} Taq DNA polymerase, 2.0 μl of DNA template (single AMF spore DNA) and 8.2 μl of ddH$_2$O. The cycling parameters were 95 °C, 3 min; 93 °C, l min, 55 °C, 1 min, 72 °C, l min for thirty cycles and 72 °C, 5 min for the final extension. The second PCR amplification, a nested-PCR, was conducted using 1:100 dilution of the first PCR product as the template, with primer LR1 (5'-GCATATCAATAAGCGGAGGA-3') (Van Tuinen *et al*., 1998) and primer NDL22 at the same cycling conditions as the first PCR except that the annealing temperature was reduced from 55 °C to 53 °C. The 5.0 μl of the PCR products were examined by electrophoresis on a 1.0% agarose gel.

Nucleotide sequencing of the target DNA fragment and phylogenetic analysis

The PCR products were purified and ligated to pGM-T (TIANGEN company) vector. The ligated vector was transformed into *Escherichia coli* DH5α competent cells. The

positive recombinant clones were screened, and the cloned plasmid DNA was sequenced by Shanghai Bio-Engineering Technology Services. The nucleotide sequences of the clones were basic local alignment search tool (BLAST) against GenBank database. The homologous sequences were retrieved from the GenBank, which were aligned with the clone sequences by use of DNAMAN (Version5.1, Lynnon Biosoft, av St-Louis, Pointe-Claire, Canada) software. A neighbour-joining phylogenetic tree from the clone sequences and the homologous sequences was constructed by DNAMAN (Version5.1, Lynnon Biosoft , av St-Louis, Pointe-Claire, Canada) software, too.

Design AMF species-specific primer and detection of AMF colonization

On the basis of DNA sequencing and phylogenetic analysis of AMF 25S rDNA D1/D2 region, the species-specific primers of AMF, which were used for detecting AMF infection to *P. euphratica* roots, were designed by Primer Primer 5.0 (PREMIER Biosoft International 3786 Corina Way Palo Alto CA 94303-4504 USA) software. The extraction of AMF DNA from the root samples was the same as the method described previously for the extraction of single AMF spore DNA. Two rounds PCR were applied. The PCR products (5.0 μl) were examined by electrophoresis on a 1.0% agarose gel. The PCR products were sequenced and analysed by BLAST searching tool.

RESULTS

Characteristics of P. euphratica rhizosphere soil

Overall, the *P. euphratica* rhizosphere soil in the lower reaches of Tarim River was alkaline, with low content of salt and relatively poor nutrient (Table I). It was extremely deficient in organic matters ($10.0\,g\,kg^{-1}$ below), lack of available nitrogen ($30.0\,mg\,kg^{-1}$ below) and phosphorus ($3.0\,mg\,kg^{-1}$ below). The content of available potassium was in between the state of deficient ($100.0\,mg\,kg^{-1}$ or less) and medium concentrated ($100.0–150.0\,mg\,kg^{-1}$). Except for the pH value, all the salt content, the organic matter and the available potassium in rhizosphere soil under the mature *P. euphratica* trees were relatively higher than those under the young *P. euphratica* plants. Higher content of the organic matter in the rhizosphere soil of the mature plants might be associated with their life time. Root inputs contribute significantly to soil C pools because plants partition up to 40% of assimilated C below ground (Merckx *et al.*, 1986). The high content of the total salt in the rhizosphere soil was related to the sampling location and soil depth when they were collected, because the mature *P. euphratica* were growing on the bank of the river, whereas the young plants were growing adjacent to the river channel, which was covered by water during the water conveyance. The salt in the soil was dissolved and drained by river flow. In contrast, the soil on the bank of the river, where the mature *P. euphratica* trees were grown, without such a process and accumulated relatively high salt contents.

Morphological features of AMF spore isolated from P. euphratica rhizosphere

A small number of AMF spores were isolated from *P. euphratica* rhizosphere soil. The average AMF spore densities were 53/50 g and 32/50 g in air dried soil from rhizosphere of mature and seedling of *P. euphratica*, respectively. They had similar morphological features and then were morphologically identified as *Glomus mosseae* (T. H. Nicolson, Gerd. & Trappe). The morphological characteristics of the fungal spores were as follows: spore is single in the soil, or produced in the root of the plant or formed in sporangium; its shape was round, or nearly round with a diameter of 150–220 μm; its colour was light yellow to brown; its spore wall has three layers, L1 and L2 were colourless and transparent, L3 was light yellow to brown. Single hyphae linked to the spore with a funnel-shaped attachment.

Sequencing and phylogenetic analysis of AMF 25S rDNA D1/D2 variable region

The single AMF spore DNA was diluted and then directly used as DNA template for nested-PCR amplification. After second-round Nested-PCR using primers LR1-NDL22 to amplify the D1-D2 variable region of 25S rDNA, a discrete band for AMF spore with size of approximately 750 bp was specifically amplified (Figure 1). The nucleotide sequences of AMF 25S rDNA D1/D2 variable region were obtained from two clones (C3 and C3-2). The sizes of the fragments were 786 bp and 787 bp for clone C3 and clone C3-2, respectively. The nucleotide sequences of C3 and C3-2 have been deposited in GenBank under accession number GU966530 and GU966531. The nucleotide sequences of

Table I. Soil properties in the rhizosphere of *Populus euphratica* (n=5).

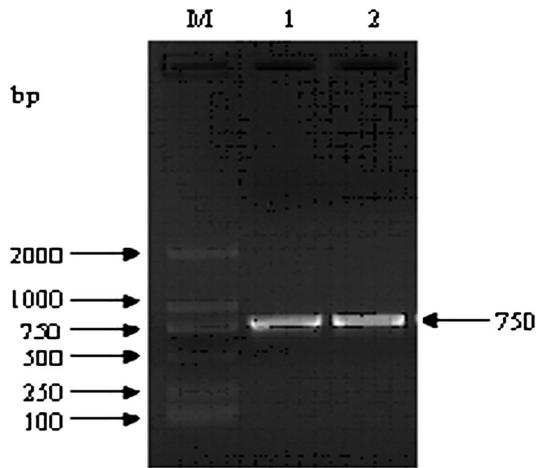| Samples	pH (1:5)	Total salt ($g\,kg^{-1}$)	Organic matter ($g\,kg^{-1}$)	Available nitrogen ($mg\,kg^{-1}$)	Available phosphorus ($mg\,kg^{-1}$)	Available potassium ($mg\,kg^{-1}$)
Mature *P*. *euphratica*	8.29±0.17	3.19±0.26	3.86±0.64	17.48±2.89	0.52±0.01	127.33±25.11
Infancy *P*. *euphratica*	8.50±0.17	0.36±0.09	1.37±0.07	15.16±3.70	0.51±0.01	66.5±4.85

Figure 1. Amplification production of the 25S rDNA D1/D2 variable region.

the two clones were compared with the DNA sequences in GenBank database using BLAST searching tool. Ten highly homologous sequences to C3 and C3-2 clones were retrieved from GenBank. They were aligned with C3 and C3-2 by DNAMAN software and then a phylogenetic tree based on these 12 sequences was constructed with neighbour-joining method at bootstrap using DNAMAN software (Version5.1). It can be referred from the phylogenetic tree (Figure 2) that clone C3 and C3-2 and *G. mosseae* EU346866 and AF396793 were grouped together as cluster. Combined the data from morphological characteristics and 25S rDNA D1/D2 sequence

analysis, we classified that the AMF isolated from the *P. euphratica* rhizosphere in this study was belonging to *G. mosseae*.

AM status and molecular detection of AMF from *P. euphratica* roots

The slides observation under Olympus BX51 showed that AMF formed vesicular structures inside the roots (Figure 3), indicating that the roots of *P. euphratica* were colonized by AMF. Although the AMF were identified by spore morphology and nucleotide sequence analysis as *G. mosseae*, the determination could not be made whether this kind of AMF colonized the roots of *P. euphratica*. After the second-round PCR, the 370 bp target fragment was successfully produced and detected by agarose gel electrophoresis (Figure 4). This DNA fragment was then sequenced and compared with the DNA sequences in GenBank database by BLAST analysis. The result showed that it had highest DNA sequence similarity with the sequences of AMF *G. mosseae* then confirming that *G. Mosseae* can infect and colonize *P. euphratica* root cells.

Colonization of arbuscular mycorrhizal fungi to *P. euphratica* roots

It was observed that either mature *P. euphratica* trees or young *P. euphratica* seedlings was colonized by AMF, but the average colonization frequency (F%) and mycorrhizal infection intensity (M%) were low. These were 8.45%,
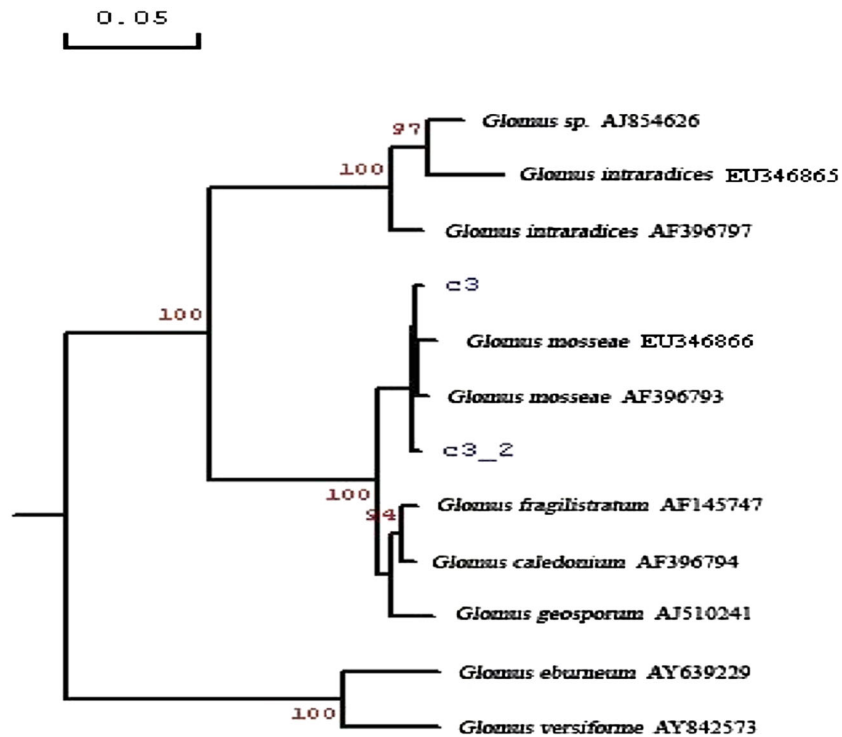

Figure 2. Neighbour-joining tree showing the phylogenetic positions of arbuscular mycorrhizal fungi from *Populus euphratica* rhizosphere soil.
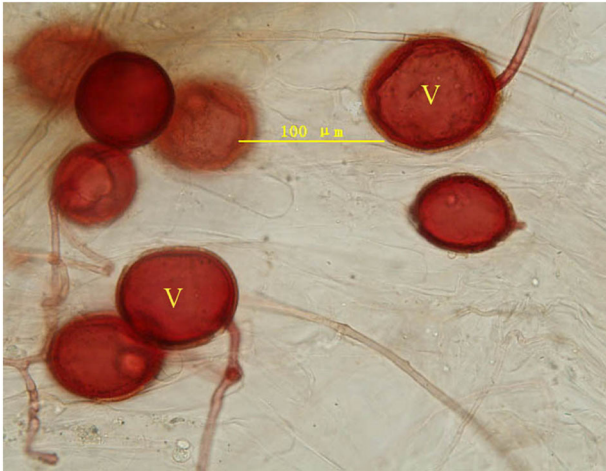
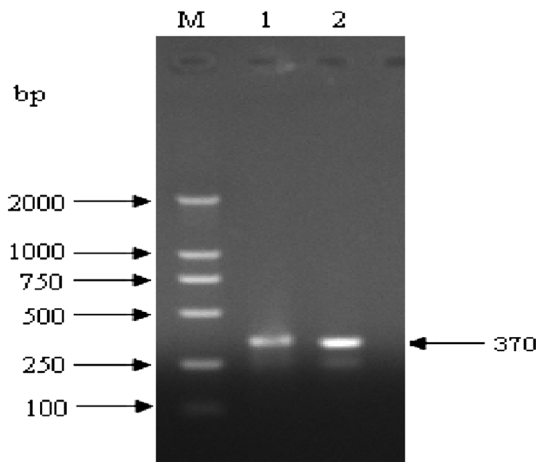Figure 3. Arbuscular mycorrhizal fungi vesicles structure in the roots of *Populus euphratica* (100×).

Figure 4. The second-round polymerase chain reaction (nested-polymerase chain reaction) products on agarose gel electrophoresis. M. DL2000 Markers; 1&2. DDAM-1, 2.

2.9% for the mature *P. euphratica* trees and 8.62%, 0.3% for the young *P. euphratica* seedlings.

DISCUSSION

Arbuscular mycorrhizal fungi spore densities are known to vary greatly in different ecosystems. This study showed that, the AMF spore density from *P. euphratica* rhizosphere soil was lower than the other trees in different region (Cesaro *et al*., 2008). AMF diversity in soil is usually high. The study of Wang *et al*. (2010) showed that *Glomus constrictu*, *Glomus macrocarpum*, *Glomus deserticola*, *Glomus diaphanum* and *Glomus eburneum* were dominant genera in the soil and rhizosphere of *P. euphratica trees in* the middle reaches of Tarim River where the *P. euphratica* did not suffer from severe drought stress. Moreover, the type of

AMF spores in *P. euphratica* rhizosphere soil in the lower reaches of Tarim River under a long-term drought stress was sparsity, and only *G. mosseae* was detected. This provides strong support for the conclusions of other workers that AMF belonging to *Glomus* tend to be dominant in arid ecosystems (Pande and Tarafdar, 2004). Compared with other trees in other region (Cai *et al*., 2008; Li *et al*., 2010), AMF diversity from *P. euphratica* rhizosphere soil was low, too. The possible reason for this phenomenon might be desertification. Desertification reduces the diversity and inoculum potential of AMF (Jasper *et al*., 1991). Moreover, by adaptation, different genera and species of AMF have developed their own host species and ecological environment because appropriate environmental conditions fostered the growth and development of AMF (Haugen and Smith, 1992). In the lower reaches of Tarim River, because of drought and poor nutrients in the soil, less species habituated in the 'green corridor', and their community structure was simple, especially in Yinsu section, no other plants except *Tamarix* were grown near *P. euphratica forest* (the distance between *P. euphratica* tree and the nearest *Tamarix* tree is about 30 m); thus, it was speculated that poor plant species and the severe arid environment resulted in the low level of AMF genera in *P. euphratica* rhizosphere soil under drought stress. In addition, morphology-based identification method bears inconsistency, contingency and limitations; and molecular techniques, on the other hand, excessively depend on PCR, DNA sequencing and PCR amplification using the specific primers (Liu and Feng, 2007). These combined effects made the conclusion in this study that AMF diversity in the rhizosphere of *P. euphratica* was simple and might not be strong enough to be detected. In this sense, it needs to be confirmed in further studies.

It showed that four strains of AMF were isolated from rhizosphere soil of *Phellodendron amurense*, but only two AMF strains successfully colonized *P. amurense* roots (Cai *et al*., 2008). It is also reported that a lot of AMF spores of *Scutellospora calospora* existed in the soil in the Loess Plateau where artificial *Caragana* forest were grown, but this fungus did not colonize the roots of *Caragana* (Liu *et al*., 2009). This proved that not all AMF can infect and colonize the roots of plants. In some extreme conditions, by winning the competition within the community of AMF, one species of AMF would become a predominant species for this environment (Saito *et al*., 2004). This study showed that *P. euphratica* rhizosphere soil was alkaline, which favoured the growth of *G. mosseae*. It was therefore speculated that the selectivity of *G. mosseae* to the specific environment and the good adaptability to its host plants made it become a dominant species in *P. euphratica* rhizosphere soil and then colonized *P. euphratica* roots in the lower reaches of Tarim River. Perhaps, other AMF species might also survived in the rhizosphere soil or even also colonized *P. euphratica* roots; however, they were not

detected because of the limitations of the methods we used or the other factors. Therefore, more works are needed to answer this question.

Generally, under certain conditions, the more specialities can establish between the host and the symbiotic subject, the more the symbiont would be adapted to a specific environment and can use resources more efficiently. For the symbiont, the competition between species is also more moderate (Fox and Morrow, 1981). For *P. euphratica*, the relatively less strain of AMF was found in the rhizosphere soil, the dominant species was *G. mosseae*, which means that the mycorrhizal of *P. euphratica* tree formed by *G. mosseae* colonization were well adapted to the dry environment in the lower reaches of Tarim River, but it enhanced the adaptation of *P. euphratica* to drought stress environment. At high temperatures (32.1–38.0 °C), pepper growth with the *G. intraradices* isolate and the *Glomus* isolate mixture was enhanced relative to non-AM controls (Martin and Stutz, 2004). Under drought condition, mycorrhizal symbiosis had a beneficial effect on *Rosmarinus officinalis* plant water status, enhancing water uptake by improving root hydraulic conductivity and increasing photosynthetic activity (Variations in water status, gas exchange and growth in *R. officinalis* plants infected with *G. deserticola*). Two native Algerian mycorrhizal fungi (*G. mosseae* and *G. intraradices*) were tested for their effect on the growth of micropropagated olive tree (*Olea europaea* L.) (Meddad-Hamza1 *et al.*, 2010). Therefore, from the perspective of resource utilization, the symbiotic structure established by AMF associated with *P. euphratica* roots may be one of the important means for enhancing the resistance of *P. euphratica* forest to the environmental stresses. The information obtained from this study will help to understand diversity of AMF in the extreme arid land. Additionally, it will be very useful to reveal the drought tolerance mechanism of *P. euphratica*, which will also provide the scientific basis for designing the strategies of restoration and conservation of the endangered *P. euphratica* community in such arid desert region.

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