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Full Length Research Paper

The resource investigation and community structure characteristics of mycorrhizal fungi associated with Chinese fir

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Chinese fir is one of the most important commercial timber species in south China. Mycorrhizal fungi has been applied in many trees and showed important effect on enhancing the productive forces and the soil activities, but there is little report about the mycorrhizal fungi on Chinese fir. So this paper investigated the mycorrhizal fungi resources of Chinese fir in Hunan province and analyzed the relation of community with the environment factors. The results showed that, abuscular mycorrhizal fungi (AMF) existed universally in the roots of Chinese fir. 75 specimen of AMF spores were isolated by wet-sieved method from this collection of the rhizosphere soils. They were identified by morphologic method to 4 genera and 9 species of AMF, which 5 species belong to *Glomus*, 1 of *Gigaspora,* 2 of *Acaulospora* and 1 of *Scutellospora*. In the isolated AMF, the importance value of *Glomus mosseae*, *Glomus intraradices* and *Acaulospora laevis* were more than 50% and the spore number of them was significantly higher than other species. These 3 species of AMF were considered as the dominate species in rhizosphere soil of Chinese fir in Hunan province. This research also indicated that, mycorrhiza forming was associated with the environment factors. The AMF spores density has the extremely remarkable correlational dependence with vegetation quantity in Chinese Fir forest (r^2=0.943, $p < 0.05$). Mycorrhiza distribution also associated with seasons and forest age; the most active season of mycorrhiza was between spring and summer and the natural AMF infection rate was increased along with the increasing of forest age. The results will not only provide the theory basis for exploitation and using of mycorrhizal resource, but also enhance the survival rate of seedling and prevent the soil degradation and conserve of soil against erosion.

Key words: Mycorrhizal fungi, vesicle-abuscular, Chinese fir, mycorrhizal inocula.

INTRODUCTION

Chinese fir, also named *Cunninghamia Lanceolata* (Lamb) Hook, is the most important plant and vastly distributed in south China. It is belong to *Cunninghamia* of *Taxodiaceae.* As a kind of important timber plantation, the current demand of Chinese fir increases continuously. To meet the needs of reforestation, young plantations of Chinese fir are established and the trend is rising. But there existed many problems such as the soil decline, the stand productivity decrease and so on. How to keep the rapid-growth, high-yield and sustainable utilization of Chinese fir, enhance the survival rate of seedling afforestation and resolve the series problems which is caused by the successive rotations of Chinese fir plantation, that have became the research focus.

Mycorrhiza is a universal and important symbiosis phenomenon in nature and arbuseular mycorrhiza (AM) is the most widespread mycorrhiza type and it is developing from the terrestrial plant roots and zygomycete fungus

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Abbreviations: VAM, Vesicle arbuseular mycorrhizal; **PVL,** polyvinyl alcohol-Lactic acid phenol; **PVLG,** polyvinyl alcohol-lactic acid-glycerol; **AM,** arbuseular mycorrhiza; **AMF,** abuscular mycorrhizal fungi; **F,** isolation frequency; **RA,** relative abundance; **IV,** importance value.

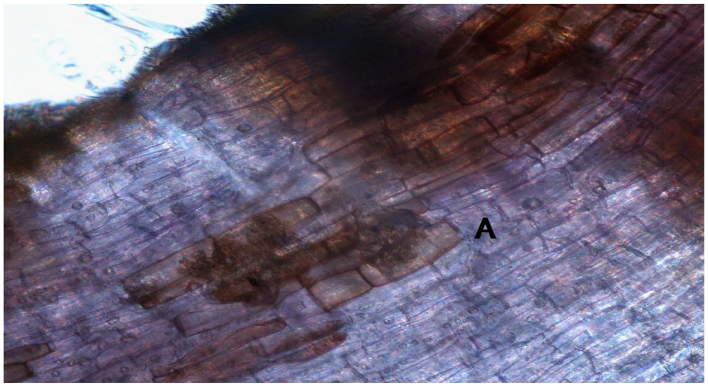

Figure 1. Arbuscular structure in root of Chinese fir. (A, Arbuscular structure, ×40).

(Attia and Awad., 2003; Li and Feng, 2001). In nature, there are about 90% vascular plants that can form AM (Hoeksema and Kummel 2003; Liu et al., 2006). Mycorrhizal fungi show important effect in many aspects, such as improving the soil physics and chemistry character, increasing the soil organic matter content and enhancing the soil activities and the produc-tive forces. It also can enhance plants' ability to absorb water and mineral nutrition, improve resistance to drought (Augé, 2001), salinization and diseases, promote the growth of plant and also can improve the tolerability of plants to the toxic pollution, conserve of soil against erosion. Therefore, as a kind of environment friendly biological preparation, it is necessary to exploit and utilize AM fungal inocula for reconstructing of forest mycorrhiza and restoring green vegetation (Guo and Bi, 1989). Regarding improvement of the ecological status, arbuseular mycorr-hiza can provide a high effective, practical and feasible new biological technology for afforestation. It also will solve the problems of low afforestation quality and low productive forces in our country and as well, help to improve the state of the ecology. Arbuseular mycorrhiza would also help to keep the economy stable and sustain social development (Liu and Chen, 2007, Liu et al, 2009).

However, little report had been obtained so far about the mycorrhizal fungi associated with Chinese fir (Xie and Huang, 1985; Chung et al., 1991). For exploitation of bio-fertilizer of Chinese fir, this paper mainly investigated the mycorrhizal fungi resources and the dominant species associated with Chinese fir forest in Hunan. Then, the relationship of arbuseular mycorrhiza fungi community structure characteristics with vegetation quantity, seasons and forestry age had been studied.

MATERIALS AND METHODS

Samples *collection*

We investigated the arbuseular mycorrhiza fungi and the undergrowth vegetation species of Chinese fir in National Forest Farms of Hunan Province in 2010, which were Da Yunshan, Huang Fengqiao, Dai Shan, Jin Dong and Shan Muhe. 75 representative soil samples of Chinese fir had been selected randomly. It included the different vegetation quantity, forestry age and seasons. We removed the surface sundries of 5 cm depth in four direction of each plot. Then we collected 500 g soil samples in the depth of 10 to 20 cm and collected the roots at the same time. The root samples were rinsed, cleaned with water and preserved in FAA stationary liquid for morphological observation. The soil samples were conserved in 4℃ after natural withering and the spores separation should be completed in 3 month (Zhang et al., 2003).

Observation of the mycorrhiza morphological *characteristics* associated with Chinese *fir*

The root samples were treated and stained by the method of Phillips and Heyman (1970), then the infection intensity was observed and the infection rate was calculated depend on microscope.

Identification of AMF spores associated with Chinese *fir*

Screening spores by wet screened method (Gerdemann and Nicolson, 1963). Water, lactophenol, cotton bule, Melzer's reagent, PVLG (polyvinyl alcohol-lactic acid-glycerol) and PVL (polyvinyl alcohol-Lactic acid phenol) were taken for slice production, respectively. The spores were transferred to glass slide by dissec-ting needle and observed their colour, shape and size. Break the spores for observing the modality character of sporoderm and dis-junctor. Then species identified according to literature and the describe or picture from the Internet (http://invam. caf. wvu. edu).The vesicle arbuseular mycorrhizal (VAM) fungi species were identified following the VAM fungi identify manual (Schenck and Perez, 1990) and new classify units that is publish in "Mycologia" and "Mycotaxon". For the species which had fewer spores, we used expanding propagation by single spore isolation and then were identified.

The community structure characteristics indexes of *AMF and* data analysis

Spore density is the spores' number of AMF in each 100 g soil sample. 1 to 5 spores were 1 grade, 6 to 10 spores were 2 grade, 11 to 15 spores were 3 grades; five spores each were in a grade. Isola-tion frequency (F) = the occurrence number of genus or species/the total soil samples number ×100%. Relative abundance (RA) = the spores' number of a genus or specie/the total spores of AMF×100%. We described the dominant species in same sample place with importance value (IV), IV= (F+RA). If IV≥50% means it is the dominant genus or species, 10%<IV<50% is the common genus or species, IV≤10% is the rare genus or species (Zhao et al., 2001). All data were analyzed by SPSS statistical software.

RESULTS

Morphological characteristics of mycorrhiza associated with Chinese *fir*

The dyeing results of the root showed that, the mycorrhiza mainly existed in the root hair zone, with many hyphae and vesicles of mycorrhizal fungi widely distributed in it (Figures 1 and 2), but in the root cap, mycorrhiza was not found.

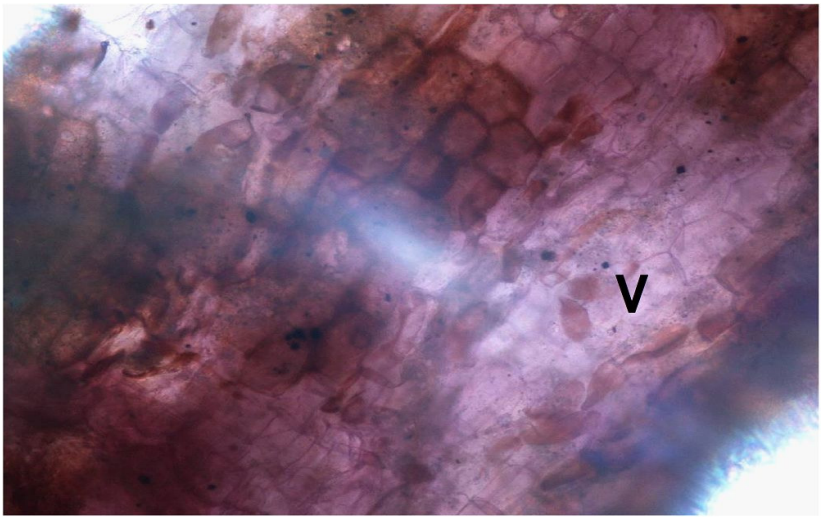

Figure 2. Vesicle in root of Chinese fir (V, Vesicle, ×40).

Table 1. The isolation frequency of AM fungal species isolated in five sample places.

AM fungal species	Isolation frequency (%)				
	DYS	HFQ	DS	JD	SMH
G. mosseae	60.0	73.3	53.3	46.7	33.3
G. constrictum	40.0	46.7	33.3	40.0	20.0
G. macrocarpum	0	0	26.7	0	20.0
G. etunicatum	26.7	33.3	53.3	33.3	26.7
G. intraradices	40.0	60.0	46.7	53.3	33.3
Gigaspora gigantea	20.0	26.7	13.3	0	0
A. laevis	46.7	53.3	33.3	46.7	33.3
Acanlospora sp.	6.7	20.0	13.3	6.7	0
Scutellospora nigra	0	13.3	0.0	0	6.7

According to the distribution and morphological structure observation, it was found that the mycorrhiza form of Chinese fir were same, there were 1 to 22 vesicles/cm^2 in cortex tissue in the root hair zone, the diameter of vesicles between 15 and 80 μm and the shape was rotundity or ellipse. The diameter of hypha is between 2 and 15 μm.

The genus and species *composition* of AMF associated with Chinese *fir*

75 specimens of AMF spores samples had been isolated from 15 Chinese fir forests in Hunan province and the spores collection and identification results showed that, 9 species of AM fungi in 4 genuses had been isolated (Table 1), 5 of *Glomus*, 1 of *Gigaspora,* 2 of *Acaulospora* and 1 of *Scutellospora*.

In Da Yunshan, there were 3 genus and 7 species of AMF, 4 genus and 8 species in Huang Fengqiao, 3 genus and 8 species in Dai Shan, 2 genus and 6 species in Jin Dong and 3 genus and 7 species in Shan Muhe. In these AMF, 5 species belong to *Glomus*, 2 of *Acaulospora*, 1 of *Gigaspora* and 1 of *Scutellospora*.

From Table 2, it showed that the average spore density, isolation frequency, relative abundance and importance value of *Glomus* were respectively 70.53 spores /100g dry soil, 36.0, 12.08 and 48.08%, the IV of *Glomus* was nearly to 50% and it was considered as the common genus. In these isolated AMF, the importance value of *Glomus mosseae*, *Glomus intraradices* and *Acaulospora laevis* were more than 50% and the spore density of them were significantly higher than other species. So these 3 species of AMF were considered as the dominate species in rhizosphere soil of Chinese fir in Hunan province.

The relation of AMF spores density with vegetation quantity

We investigated the vegetation quantity and collected 15 soil samples in the 5 sample places. From Figure 3, we

Table 2. Spore density, isolation frequency, relative abundance and importance value of AM fungi in Chinese fir.

AM fungal Species	Spore density (/100g dry soil)	Isolation frequency (%)	Relative abundance (%)	Importance value (%)
G. mosseae	17.4 ± 2.4	53.3	17.4	70.7
G.constrictum	9.8 ± 2.8	36.0	9.8	45.8
G. macrocarpum	4.2 ± 1.7	9.3	4.2	13.5
G. etunicatum	13.8 ± 2.3	34.7	13.8	48.5
G. intraradices	25.3 ± 2.5	46.7	15.2	61.9
G. gigantea	5.6 ± 2.4	12.0	5.6	17.6
A. laevis	21.6 ± 3.3	42.7	21.6	64.3
Acanlospora sp.	4.8 ± 1.7	9.3	4.8	14.1
S. nigra	2.2 ± 1.5	4.0	2.2	6.2

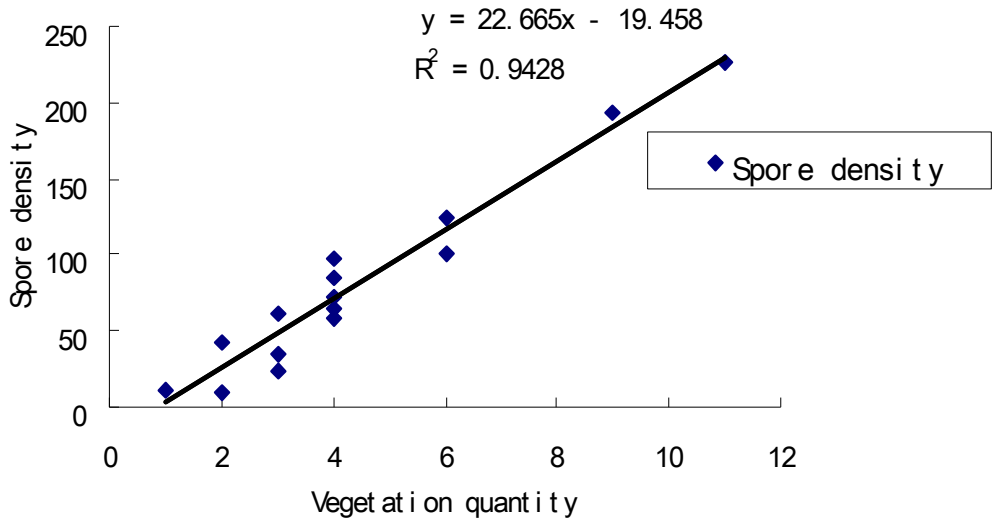

Figure 3. The relationship of plant quantities and spore density of AM fungi.

Table 3. The relation of mycorrhiza distribution and forestry age.

Parameter	1 to 2 year seedling	5 to 10 years plants	15 to 20 years plants
Mycorrhiza	0	12-18	21-32
The plant infection rate	0	24-36%	42-64%
The cell infection rate	0	3-14%	1-24%

can see that the spores density of AMF has the extremely remarkable correlational dependence with vegetation quantity in Chinese fir (r^2=0.943, $p < 0.05$). The vegetation quantity was abundant, but the spores' density was more abundant. From Table 4, it can be seen that in the 4 soil sample, the vegetation quantity was 4 and the spores' density was 17 grade/100 g soil samples. The vegetation quantity of the 5 soil sample was 9, while the spores' density was highest at 38 grade/100 g soil sample. In these 5 sample places, the average spores' density of HFQ sample was 34 grade/100 g soils sample and was remarkably higher than other sample places and the average vegetation quantity was also more than other sample places.

The relation of mycorrhiza distribution with seasons

From the investigation, it was found that mycorrhiza existed universally in Chinese fir roots. The results (Figure 4) showed that in a year, the most active season of mycorrhiza was between spring and summer. At the top

Table 4. The plant quantities and spore density of AM fungi.

Place	Vegetation quantity	Spore density
DYS	3	62
	4	97
	6	124
HFQ	4	85
	9	193
	11	226
DS	1	11
	2	43
	4	58
JD	3	23
	4	72
	6	101
SMH	2	10
	3	34
	4	65

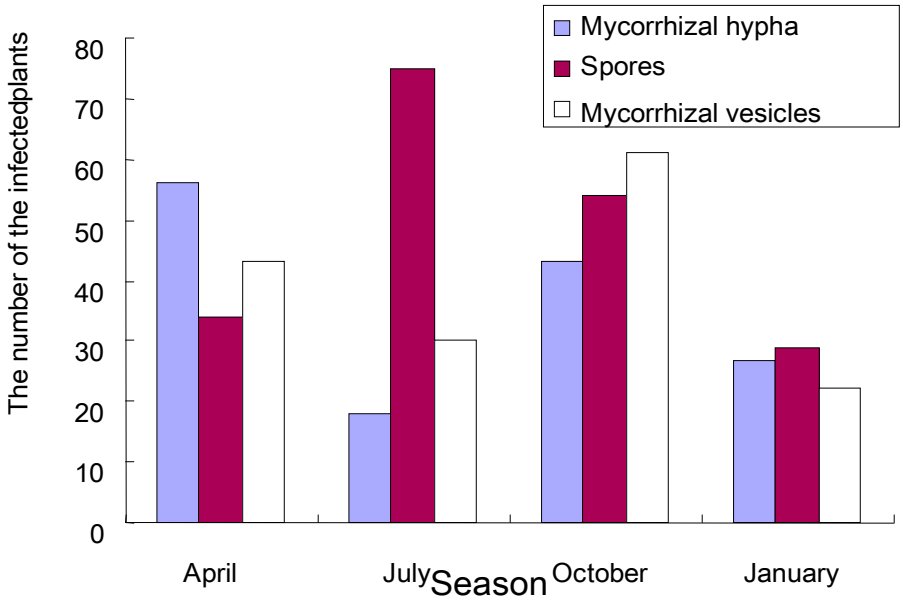

Figure 4. The relation of mycorrhiza distribution with seasons.

soil (0 to 25 cm), in mature age of Chinese fir, a mass of radicle appeared and the root hair was developed well. It was easy to observe the mycorrhizal hypha, but in summer, mycorrhizal hypha decreased. After July, it entered the high temperature and at dry season, the soil moisture reduced, while the radicle and root hair often withered. The hypha was hardly observed, but by the wet screened method, it was easy to catch the spores. In summer, the spores had been observed in all 75 soil samples, in which the spore's number reached the highest than other seasons. From September to October, it was easy to observe the vesicles, while in winter, mycorrhizal fungus activity came to an end.

The relation of mycorrhiza distribution and forestry age

From Table 3, the investigation results of the different age of Chinese fir showed that, in 1 to 2 years, plants of Chinese fir and the natural infection rate of the plant was 0; however, the study could not find any mycorrhizal structure in the 50 seedlings. In 5 to 10 years, 12 to 18 plants of Chinese fir had mycorrhizal structure, and their

plant infection rate was 24 to 36%, while their cell infection rate was 3 to 14%; but for 15 to 20 years old Chinese fir plants, the plant infection rate was 42 to 64%, while the cell infection rate was 1 to 24%. These results indicated that the mycorrhizal distribution was related to forestry age.

DISCUSSION

In this study, an investigation on the mycorrhizal fungi resources of Chinese fir in Hunan province and analyzed the relation of mycorrhiza distribution with vegetation quantity, seasons and forestry age. From this study, it was found that the dominate species in rhizosphere soil of Chinese fir in Hunan province and the distribution of mycorrhiza was also related with the condition of local growth, loose and good soil permeability and better mycorrhiza growth. As such, the infection rate of plant roots and cells were high in Hardens and the soil permeability of mycorrhiza grew worse. The results of this paper will not only provide a scientific basis for exploitation of the environmentally friendly biological preparation of mycorrhizal inocula in Chinese fir forest, but will also improve the ecological environment and keep the economy and as well, boost social sustainable development.

Mycorrhizology is a new branch of mycology. Due to the widest symbiosis formed between fungi and plants in various terrestrial ecosystems and the importance of maintaining ecosystem stabilization and productivity, many developed and developing countries have paid more attention to mycorrhizology.

This research work only identified the mycorrhiza fungi from the morphological characteristics. In future study, the mycorrhiza fungi combined with the molecular biology methods would be identified. As a consequence, it would be more accurate and effective.

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