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ARBUSCULAR MICORRHIZAL FUNGI ASSOCIATION IN TWO COFFEE FARMS WITH DIFFERENT CULTIVATION AT LAM DONG

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have an important role in agriculture because of the benefits on plant and ecosystem. However, mycorrhizal association is affected by many factors such as vegetation and farming conditions. In this study, AMF system on soil and roots of coffee were investigated from two coffee farms with different cultivation method in Lam Dong Province, one was not applied fertilizer in 4 years and the other was conventional. The density, the type of mycorrhizal spore and fungal infection rate on coffee roots are different between two coffee farms. Based on morphology, there are 119 types of AMF spore in both coffee farms and most of them belongs to genera *Acaulospora, Gigaspora, Entrophospora* and *Glomus*. Spore types RE7, W6 and W1 belonged to *Acaulospora* and Y5 belonged to *Entrophospora* appeared in both farms. Besides, spore types B7, RE10, Yc, RE1 and Y1 were recorded in high density (1-4 spores /g soil). All of them were the potential strains for developing the VAM fertilizer specialized to coffee plantation.

Keywords: AMF, coffee, farming, mycorrhiza, spore.

1. INTRODUCTION

Mycorrhizas is a symbiotic association between fungi, roots, and soil. Among mycorrhizal types, arbuscular mycorrhizae (AM) is the most common. In this symbiotic relationship, the fungal extra- radical hyphae help plants absorb water and nutrients specially minerals such as Phosphorus, Potassium, Nitrogen etc. [1]. According to Andrade et al. [2], the AM association increases resistance of plants in drought condition, high metal concentrations, salinity and temperature stresses and protects the host against pathogens such as bacteria, nematodes [3, 4]. In addition, AM fungi (AMF) also improve the soil structure through interaction with soil biota, fungal hyphae action, the excretion of glycoproteins and other extracellular compounds [5].

Coffee is an important crop in the world and has a great influence on the Tay Nguyen Highland, Viet Nam. However, the quantity and quality of coffee are also unstable because of

applying chemical fertilizers in long time. One of the solutions to improve the situation is using soil microorganisms, including using AM fungi.

The presence of AM on the roots of coffee, was first observed by Janse in 1897 [2]. Until recently, studies have shown that the effect of a symbiotic association between fungi and the roots of coffee, when compared AMF inoculated coffee group to non-inoculated group on *Coffea arabica* [6]. Until now, most research on the isolation, identification, and interaction between AMF with the roots of coffee has been studied primarily in South America and Africa [7-9]. Although Southeast Asia and Vietnam are a coffee-growing area, researches for AMF on coffee have not not been paid attention yet. The initial study of AMF for Vietnam coffee will contribute farming solutions and create products to help improve the quality of the coffee.

2. MATERIALS AND METHODS

2.1. Soil sampling and treatment

Soil samples were taken from two farms at Lam Dong Province. The first farm (G1) is at Mimosa, Ward 10, Da Lat City, Lam Dong Province. The second (G2) is at Hiep An Commune, Duc Trong District, Lam Dong Province.

The samples were collected in rain season, August 2015. Each farm was divided into 3 plots; 5 soil samples were collected in each plot at 4 corners and center of plot; each soil samples were collected at canopy cover of coffee, included both soil and roots; finally, all 5 soil samples were mixed into one. Each soil samples were measured pH and moisture. After that, soil samples were homogenized manually, and root fragments were separated. Soil characteristics such as organic matter, soil texture, total Nitrogen, total Phosphorous and available Phosphorous were analyzed. The analyzed standards were followed TCVN 8941:2011 (organics matter (OM)), TCVN 6647:2000 (soil texture), TCVN 6498:1999 (total Nitrogen), TCVN 8940:2011 (total Phosphorus) and TCVN 8661:2011 (available Phosphorus).

2.2. Collecting spore

AM fungal spores were extracted from 50 g soil by the wet sieving [10] with sucrose density gradient centrifugation technique [11]. After receiving spores, the number and types of spore were counted directly on the filter paper grid of 0.5 cm. AMF spores were classified by color and morphology. Species diversity of AMF spores were calculating using Shannon-Wiener diversity index [12] and Simpson's reciprocal index [13].

2.3. Staining AMF spore

Spores were stained by PVLG and Melzer's reagent. Stained spores were observed under stereo microscope and identify to genus following as description of Morton and Benny [14] and the standards in INVAM [15].

2.4. Staining and quantifying mycorrhizal root

Fragmental roots (2-4 cm long segments) were soaked in H_2O_2, for 2-3 minutes to remove phenolic compounds; washed again with 10% KOH overnight and boiled for 30-60 minutes in boiling water. After that, all fragmental roots were soaked in 5 % lactic acid solution for 3-5 minutes; stained in Trypan blue for 10-15 minutes, washed again with lacto-glycerol solution and immersed in 50 % glycerol. The roots were observed under the microscope in 20 % glycerol

mounting solution. The grid line intersect method [16, 17] was using to calculate the fungal infection rate.

2.5. Data analysis

All data is recorded, stored and processed by MS Excel 2013 software (Microsoft, WA, United States). Independent means were compared using an independent t – test.

3. RESULTS AND DISCUSSION

3.1. Characteristics and mycorrhizal systems at two coffee farms

Both coffee farms G1 and G2 were planted *Coffea arabica* (Katimor) and used rain water for irrigating. G 1 farm was covered by pine forest, planted 25-year-old coffee trees, intercropped with persimmon (*Diospyros kaki)*, and have not been manured fertilizers (both inorganic and organic) for 4 years. G 2 was purely planted 4-year-old coffee trees and applied inorganic fertilizer every year. In G 2, before coffee was planted, banana tree (*Musa* spp.*)* had been cultivated for 6 years and daylily flower (*Hemerocallis citrina*) had planted for 2 years. The soil in both farms are rich soil with similar soil texture.

Table 1. Soil characteristics in two coffee farms (mean (SD)).

	G 1	**G 2**	**t-value**	**df**	**p-value**
pH	6.35^{a} (0.16)	6.47^{a} (0.19)	0.8466	4	0.2224
Moisture (%)	54^{a} (5.57)	33^{b} (7.71)	3.7866	4	0.0097
Organics matter (%)	10.27^{a} (1.35)	5.30^{b} (0.61)	4.6391	4	0.0048
Total P (%)	0.04^{a} (0.01)	0.05^{a} (0.03)	0.6310	3	0.2863
Available P (mg/100g)	36.93^{a} (4.85)	20.30^{b} (3.29)	4.9154	4	0.0039
Total N (%)	0.26^{a} (0.01)	0.12^{b} (0.02)	12.9653	3	0.0005

Two independent means were compared by t-test, one tail with $\alpha = 0.05$.

Different superscript letters (a, b) within rows show significantly different

The soil characteristic of G 1 farm is better than G 2 farm with higher moisture, organic matter and available Phosphorous (Table 1). It could be caused by both the high cultivation density of G 2 farm and the fallow condition of G 1 farm. The extraordinary height of organics matter of G 1 farm could be caused by its fallow.

The density and diversity of spores and fungal infection rates of G 2 were significantly higher than G 1 (Table 2) with $t = 1.4414$, $df = 28$, $\alpha = 0.1$. The results are consistent with previous studies of Hendrix et al. [18] and Oehl et al. [19] that AFM diversity is not only affected by soil type but also related to the previous vegetation.

Table 2. AMF association of two farms.

	G 1	G 2	t-value	df	p-value
Type of spore	48	71	1.9803	28	0.0288
Density of spore (spore/g soil)	15	23	1.4942	28	0.0731
Rate of fungal infection (%)	12.85	17.95	1.4414	28	0.0803
Shannon – Wiener index	H' = 2.845	H' = 2.925			
Simpson's reciprocal index (1/D)	1/D = 11.716	1/D = 12.189			

Two independent means of rate of fungal infection were compared by t-test, one tail with $\alpha = 0.1$.

3.2. Identify some typical spores in the soil of two coffee gardens

Based on the colors and morphology, there are 48 types of spore in G1, 71 types in G2. There are several types of spores appeared in both farms such as RE7, W6, W1, Y5. There are many types of spores only appear in G2, mainly belong to *Glomus*.

Some spore types appear with high density in each farm (1-4 spores / g soil): B7, RE10 in farm G1; Yc, RE1 and Y1 in farm G2. Based on some of the characteristics of color, size, number of spore's wall, wall surface and spore stalk to identify preliminary some typical spores in the soil of two farms (Table 3).

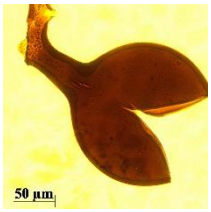
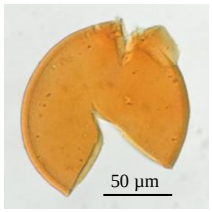
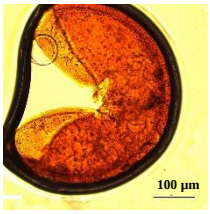
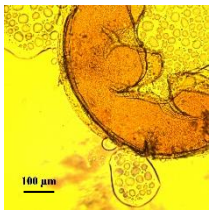
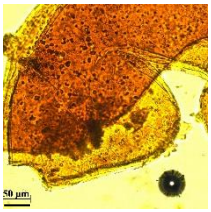
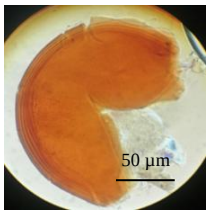
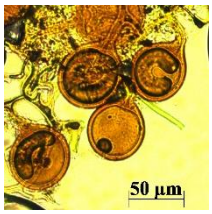
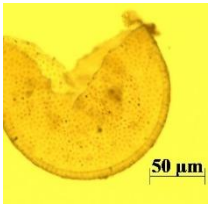
Spore types RE7, RE19, W6 and W1 (Table 3): Spores are non-stalk, usually orange to brown, young spores can be white or yellow and mature spores are red or brown. Spore size is in the range of 60-380 µm, globose or subglobose, sometimes oval. There are 2-3 spore wall layers, wall surface is often spines or polygonal projections with or without a reticulum. They should be belonged to genus *Acaulospora*.

Spore types Y1 (Table 3): Spores usually has large size, about 300 µm, creamy white or pale yellow, globose or subglobose. There are 3 spore wall layers, staining dark red-brown to a very dark red-purple in Melzer's reagent, the outer layer is often smooth surface. Spores has bulbous stalk. It should be belonged to genus *Gigaspora*.

Spore type RE1 and Yc (Table 3): Spores are usually yellowish brown or dark red-brown, globose, subglobose, ellipsoidal or irregular in shape. The size of spore is about 85-157 µm. There are 2 spore wall layers with transparent outer layer and smooth. Spores have a stalk with funnel shape. They should be belonged to genus *Glomus*.

Spore type B7 and Yc (Table 3): Spores are without stalk, usually yellow-orange to dark brown, the average size of about 120 µm, globose or subglobose. Wall spores usually have 4 or more layers, the outer surface is often smooth and shiny. They should be belonged to genus *Entrophospora*.

Table 3. Some typical spores in two coffee gardens staining in Melzer's reagent.

Photo	Genus	Color Shape,	Photo	Genus	Color Shape
RE1 	*Glomus*	red globose, stalked	B7 	*Entrophospora*	light brown globose, shiny
RE7 	*Acaulospora*	Dark-red to red globose	Y1 	*Gigaspora*	Yellow, globose, stalked
RE10 	*Acaulospora*	red-brown, globose	Y5 	*Entrophospora*	Yellow, globose, smooth
W6 	*Acaulospora*	White, globose, glossy	Yc 	*Glomus*	Yellow, globose, stalked
W1 	*Acaulospora*	White, globose, rough			

4. CONCLUSION

AMF association depends on not only soil nutrients but also the vegetation and farming conditions. The soil of G1 has more nutrients than G 2, however, density, diversity and AM infection rate are lower than G 2. *Acaulospora*, *Gigaspora* and *Entrophospora* are the genus appearing mostly in two gardens.

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