PROPAGATING ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH COFFEE PLANT BY USING THE HERBACEOUS HOST

DANG HOANG QUYEN
Ho Chi Minh City Open University, Vietnam - dhquyendl@gmail.com

VU THUY DUONG, PHAM NGUYEN DUC HOANG
Ho Chi Minh City Open University, Vietnam

(Received: February 06, 2018; Revised: March 22, 2018; Accepted: April 16, 2018)

ABSTRACT

Coffee (*Coffea* spp.) is one of important industrial crops. Additionally, arbuscular mycorrhizal fungi (AMF) provide many benefits for plants such as increasing nutrient uptake, enhancing tolerance in drought and stress condition, etc. Therefore, preservation and propagation of AMF spores collected from coffee's rhizosphere are necessary for coffee cultivation. The AMF preservation on coffee plant is not feasible because coffee is a long-term plants, which led to study on symbiotic ability of AMF on several short-term host plants (maize (*Zea mays*), plantain (*Plantago* spp.), rice (*Oryza sativa*), beggarticks (*Bidens pilosa*), and bahia grass (*Pensacola bahia*)) to maintain AM association. Investigation of symbiosis ability with four types of AMF spores showed that maize had the highest rate of fungal infection. The total number of AMF spore per 50g soil after 3 months of inoculation on maize reached 352 spores, which was 4.1 times higher than that of the origin while the lowest figure recorded in bahia grass is with only 2.3 times.

Keywords: AMF; Coffee; Maize; Propagation; Symbiosis.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are the most symbiotic species on earth. AMF can associate with 80% of plant species (Strack et al., 2003). The first AMF detection of AMF was recorded in 1842 (Nageli, 1842). Until now, the role of AMF has recorded in many studies. The main AMF benefits has been reported as the increase in nutrient uptake, resistance to stress and drought condition (Auge´, 2001, 2004), tolerance to pathogens (Vaast et al., 1998; Elsen et al., 2003).

On the other hand, coffee (*Coffea* spp.) is an industrial plant which has worldwide economic importance. Coffee creates a significant source of income for developing countries. The genus *Coffea* belongs to the Rubiaceae family, which includes about 100 species (Davis et al., 2006), only two species are commercially cultivated, *Coffea arabica* and *Coffea canephora*. Vietnam is the second largest coffee producer in the world, especially *Coffea canephora*. Coffee is effects directly or indirectly to millions of jobs in Tay Nguyen area and other provinces such as Dong Nai, Binh Phuoc, Son La, and Quang Tri. However, quality and productivity of coffee haven't still stabilized. In 1987, Janse observed the presence of AMF in the coffee plant. After that, there are many studies about the diversity, distribution, the role of AMF in coffee plant, but mostly in Africa and Latin America. In Vietnam, the preservation of native AMF from coffee plants is very important. This AMF source will be used for serving the coffee industry. Host plants that have been successfully used to propagate AMF include alfalfa, corn, soybean, white

clover, onion, sudan grass, bahia grass, narrow leaf plantain, marigold (Corkidi et al., 2008). However these host plants can affect sporulation of AMF communities (Bever et al., 1996). For example, *Gigaspora sinuosum* and *G. clavisporum* do not produce spores when using the trap-plant *Sorghum vulgare* (Guadarrama et al., 2007). Therefore, our research suggests the surveys of different host herbaceous plants to choose the best one for propagating AMF.

2. Materials and Methods

2.1. Preparing AMF spore

AMF spore: AMF spores were extracted from the soil collected from rhizosphere under the coffee canopy by the wet sieving (Gerdemann & Nicolson, 1963) and sucrose density gradient centrifugation technique (Daniels & Skipper, 1982). This mixture is including B7, O3, O5, RE7, RE10, W6, Y3, Y5 (Table 1).

Table 1

AMF spore phylotypes were used in this study

Code	Color, shape	Photo	Code	Color, shape	Photo
B7	Brown, globose		**RE7**	Dark red, globose	
O3	Orange, globose		**W6**	White, globose	
O5	Orange – red, oval		**Y3**	Yellow, globose	
RE10	Red – brown, globose		**Y5**	Yellow, globose	

2.2. Preparing potting soil

Soil and sand (1:1 v/v) were mixed and autoclaved before the experiment. Each pot (19 cm diameter and 14 cm in height) was sterilized by ethanol 70^o. Adding AMF spores into mixed soil and sand. Then put all of them into the pot.

2.3. Treating seed

Seeds of coffee (*C. arabica* and *C. canephora*), maize (*Zea mays*), plantain (*Plantago* spp.), rice (*Oryza sativa*), beggarticks (*Bidens pilosa*), and bahia grass (*Pensacola bahia*) were sterilized with 3% NaOCl in 2 mins and rinsed with sterilized water before sowing.

2.4. Inoculation

Coffee seeds were sown into sterilized sand to prepared clam-leaf seedlings. Five seedlings planted in each potting soil containing 8 AMF spore phylotypes. After 3 months, extracted and counted AMF spore from 50g soil and compared with original quantity.

AMF phylotypes associated with coffee plants were mixed and added to potting soil, sowing five seeds of each herbaceous plant in a pot. After 3 months, extracted and counted AMF spore from 50g soil and compared with original quantity to choose the best host for propagating AMF. Collecting roots to evaluate the rate of fungal infection.

2.5. Rate of fungal infection

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 about 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 the lacto-glycerol solution and immersed in 50% glycerol. The roots were observed under the microscope in 20% glycerol mounting solution. The gridline intersection method was used to calculate the fungal infection rate.

2.6. 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 LSD (α=0.05).

3. Results and Discussion

3.1. AMF phylotypes associated with coffee plants

After 3 months inoculating mixture of AMF spores into potting soil for associating with coffee plants, only the quantity of four in eight phylotypes (O5, W6, Y3, Y5) increased compared with that of the origin (Table 2). Other phylotypes decreased or died (B7, O3, RE10, RE7). Between *C. arabica* and *C. canephora*, the association of diferrent AMF phylotypes to host plant is different. There was significant difference between two coffee species about Y5 phylotype. The root of *C. canephora* associated with Y5 spores stronger than *C. arabica.* Therefore, a significant difference was observed between the average of Y5 spores number of *C. canephora* (244 spores/50g soil) and *C. arabica* (71 spores/50g soil).

Table 2

Number of AMF spores per 50g soil after 3 months of inoculation on coffee plants (Different letters within rows show significantly difference)

Spore code	Origin	*C. arabica*	*C. canephora*
B7	$75^{a} \pm 9$	$0^{b} \pm 0$	$0^{b} \pm 0$
O3	$38^{a} \pm 8$	$0^{c} \pm 0$	$1^{b} \pm 0$
O5	$8^{b} \pm 1$	$45^{a} \pm 23$	$59^{a} \pm 10$
RE10	$20^{a} \pm 1$	$0^{c} \pm 0$	$4^{b} \pm 2$
RE7	$17^{a} \pm 2$	$1^{b} \pm 1$	$4^{b} \pm 2$
W6	$25^{b} \pm 8$	$94^{a} \pm 38$	$135^{a} \pm 74$
Y3	$2^{b} \pm 0$	$26^{a} \pm 15$	$31^{a} \pm 9$
Y5	$33^{b} \pm 9$	$71^{b} \pm 38$	$244^{a} \pm 102$
Total of spore	**219**	**236**	**478**

The rate of four phylotypes O5, W6, Y3, Y5 was 68/219. After three months, the spore quantities were recorded on *C. arabica* (235 spores) and *C. canephora* (478 spores) that were higher than 3.5 and 7 times respectively in comparison with that in the previous soil. Those results indicated that four phylotypes can associate with coffee plants and produce new spores in greenhouse condition.

3.2. AMF associated with herbaceous host plants

Using four phylotypes of spore (O5, W6, Y3, Y5) with the total number of 85 spores/50 g soil for inoculating with five herbaceous plants. After 3 months, the results showed that maize plant was the best host (table 3). Total of spore after 3 months inoculating on maize plant reached 352 spores. Maize plant has a high ability in association with 3 phylotypes of spore (W6, Y3, Y5). Only with O5 phylotype, the number of spores was insignificantly different from the origin.

Table 3

Number of AMF spore per 50g soil after 3 months of inoculation on herbaceous plants (Different letters within rows show significantly difference)

AMF phylotype	Origin	*Plantago* spp.	*Z. mays*	*O. sativa*	*B. pilosa*	*P. bahia*
O5	$16^{b} \pm 9$	$17^{b} \pm 8$	$16^{b} \pm 8$	$38^{a} \pm 18$	$15^{bc} \pm 5$	$3^{c} \pm 1$
W6	$40^{c} \pm 1$	$112^{b} \pm 42$	$206^{a} \pm 58$	$128^{b} \pm 100$	$109^{bc} \pm 34$	$101^{bc} \pm 21$
Y3	$2^{b} \pm 0$	$15^{a} \pm 13$	$14^{a} \pm 6$	$10^{ab} \pm 2$	$17^{a} \pm 7$	$16^{a} \pm 2$
Y5	$27^{b} \pm 8$	$109^{a} \pm 35$	$116^{a} \pm 62$	$91^{a} \pm 30$	$85^{a} \pm 53$	$75^{ab} \pm 14$
Total of spore	**85**	**253**	**352**	**268**	**226**	**195**

Rice plant also associated effectively with 3 phylotypes of spore (O5, W6, Y5). However, total of spore after 3 months inoculating reached 268 spores. Three phylotypes of spore (W6, Y3, Y5) could associate effectively with plantain plant and total of spore after 3 months inoculating reached 253 spores. On beggarticks plant, the association with AMF showed to be lower than that in maize, rice and plantain. It could associate effectively with Y3 and Y5 spore phylotype. The lowest association is bahia grass plant, only one spore phylotype (Y3) has significant difference from the origin and total average of spore was 195 spores, higher than that of the origin only 2.3 times. Plantain, maize, and rice are often selected to propagating AMF, the association with AMF is higher than beggarticks and bahia grass. Although beggarticks and bahia grass appear frequently in the coffee farm, their AMF association and propagation were not effective. Some previous studies showed that *Zea mays* was the best host plant. When using *Mimosa invisa*, *Sorghum bicolor* and *Zea mays* on AMF propagation, resulted spore population and root colonisation were recorded at the highest rate on *Z. mays* (3690 spores/100 cm^3 and 65% root length colonised) (Chaiyasen et al., 2016). In the research of Selvakumar and associates (2016) also showed the same results. Mycorrhizal inoculation by trap culture in maize resulted in longer shoots and roots than sudangrass plants and after second plant cycle, maize plants had the higher percentage of mycorrhizal response in terms of colonization and arbuscules than sudangrass (Selvakumar et al., 2016). Therefore, maize plant was selected as the best host plant for propagating AMF spore collected from the coffee rhizosphere.

3.3. Rate of AMF infection on herbaceous plants

The root of different plants which collected after 3 months inoculated with the mixture of AMF spore was treated and stained with Trypan blue (2.5). The rate of fungal infection was showed in Fig. 1. The results showed that the highest rate of fungal infection (67%) was observed in maize meanwhile the lowest one was reported in beggarticks (15%). These certificated above results, the associated ability of maize root with AMF higher than others, the root colonization in maize was also higher. Root colonization was mainly influenced by host plant and soil texture, the first factor being responsible the highest differences. The highest root colonization percentages observed in maize could be due to higher compatibility between the presence of AMF in the inoculum and the plant (Carrenho et al., 2002).

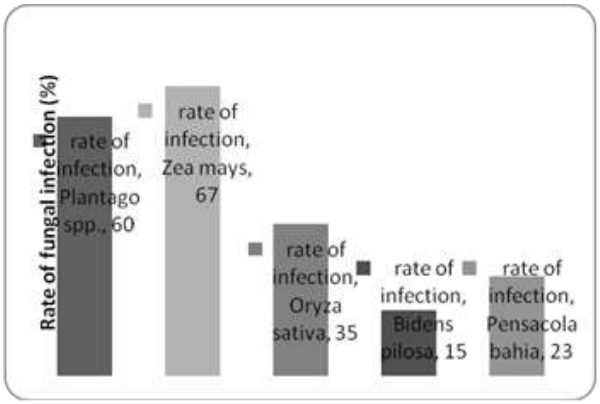

Figure 1. Rate of fungal infection on herbaceous plants

4. Conclusion

In green house condition, there were only 4 AMF spore phylotypes having the increase of AMF quantity (O5, W6, Y3, Y5) after 3 months inoculation on the coffee plants. When using these AMF phylotypes for the experiment on herbaceous host plants, both AMF quantity and rate of fungal infection showed that the best inoculation on *Zea mays*. Therefore, *Zea mays* was selected as the host plant for propagating and preserving AMF■

Acknowledgements

The research was supported by Biotechnology Center of Hochiminh City.

References

Auge´, R. M. (2004). Arbuscular mycorrhizae and soil plant water relations. *Canadian Journal of Soil Science, 84*, 373-381.

Auge´, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11, 3-42.

Bever, J. D., Morton, J. B., Antobovics, J., Schultz, P. (1996). Host dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *J Ecol*, *84*, 71–82.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). Working with Mycorrhizas in Forestry and Agriculture. *AClAR Monograph*, *32*, 183.

Carrenho, R., Sandra, F. B. T., Vera, L. R. B. and Eraldo, S. S. (2007). The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta bot. bras., 21*(3), 723-730.

Chaiyasen, A., Leardwiriyakool, C., David, D. D. and Saisamorn, L. (2016). Influence of host plants and soil diluents on arbuscular mycorrhizal fungus propagation for on farm inoculum production using leaf litter compost and agrowastes. *Biological Agriculture & Horticulture* http://dx.doi.org/10.1080/ 01448765.2016.1187670

Corkidi, L., Evans, M., Bohn, J. (2008). An introduction to propagation of arbuscular mycorrhizal fungi in pot cultures for inoculation of native plant nursery stock. *Native Plants Journal, 9*(1), 29-38.

Daniels, B. A, Skipper, H. D. (1982). Methods for recovery and quantitative estimation of propagules from soil, in: Schenck NC (ed) Methods and principles of mycological research. *The American Phytological Society, St. Paul*, MN 29-35.

Davis, A. P., Govaerts, R., Bridson, D. M., Stoffelen, P. (2006). An annotated checklist of the genus *Coffea* L. (Rubiaceae). *Botanical Journal of the Linnean Society, 152*, 465–512.

Elsen, A., Baimey, H., Swennen, R. and De Waele, D. (2003). Relative mycorrhizal dependency and mycorrhiza–nematode interaction in *Banana cultivars* (*Musa* spp.) differing in nematode susceptibility. *Plant and Soil, 256*, 303-313.

Gerdemann, J. W., Nicolson, T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transaction of the British Mycological Society*, *46*, 235-244.

Guadarrama, C. P., Camargo, R. S. L., Hernández, C. L., Castillo, A. S. (2007). Los hongos micorrizógenos arbusculares de la región de Nizanda, Oaxaca, México. *Bol Soc Bot Méx, 81*, 131-137

Johnson, N. C., Tilman, D. and Wedin, D. (1992). Plant and soil controls on mycorrhizal fungal communities. *Ecology*, *73*, 2034-2042.

Nageli C. (1842). Pilze im Innern von Zellen. *Linnaea*, *16*, 278–285.

Selvakumar, G., Kiyoon, K., Denver, W., Mak, C., Yeongyeong, K., Bongnam, C., and Tongmin, S. (2016). Trap Culture Technique for Propagation of Arbuscular Mycorrhizal Fungi using Different Host Plants. *Korean J. Soil Sci. Fert.*, *49*(5), 608-613.

Strack, D., Fester, T., Hause, B., Schliemann, W., and Walter, M. H. (2003). Arbuscular mycorrhiza: Biological, chemical, and molecular aspects. *J. Chem. Ecol*., *29*, 1955–1979.

Vaast, P., Caswell, C. E. P. và Zasoski, R. J. (1998). Influences of a root-lesion nematode, Pratylenchus cof- feae, and two arbuscular mycorrhizal fungi, *Acaulospora mellea* and *Glomus clarum* on coffee (*Coffea arabica* L.). *Biology and Fertility of Soils*, *26*, 130-135.