

The Diversity of Arbuscular Mycorrhizal Fungi in the Black Cumin Rhizosphere (*Nigella sativa* L.) in Cianjur, West Java, Indonesia

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) is a type of fungus that can form a symbiotic mutualism with most plants. Some AMF can only be symbiotic with a certain plant species. This research aims to determine and obtain the genus AMF from black cumin (*Nigella sativa* L.) accessions from America, Turkey, Hong Kong, Slovenia, India, and Kuwait accessions which had been grown in West Java, Indonesia. Three samples from each accession, four replications each, were collected for examination. The results showed that six genera of AMF were found in the rhizosphere of black cumin: *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, *Dentiscutata*, and *Entrophospora*. The genus *Glomus* was predominantly found in the Indian accession, i.e. 96.42 spores.

Keywords: black cumin, diversity, fungi, exploration, AMF

Introduction

A Mycorrhiza is a symbiotic association and a mutualistic relationship between a vascular host plant and fungi of the Glomeromycota phylum (Peterson et al., 2004). Mycorrhiza can promote nutrient absorption by the plants; the host plants supplied the carbohydrates as a source of nutrients to the mycorrhiza (Gaur and Kaushik, 2011), resulting in a mutualistic association. The association of AMF with host plants has important roles in the growth and development of plants (Lekatompessy and Sukiman, 2015). AMF can increase the absorption of phosphorus (P) (Chairani, 2007) and uptake of copper (Cu), Manganese (Mn), ferum (Fe) (Sreenivasa et al., 1993) through expansion of root absorption area in the presence of external hyphae that grows and develops through the roots of the plants. In addition, mycorrhiza fungi can improve the absorption of

immobile elements on the soil and increase uptake and transport of highly mobile elements such as N (Marschner and Dell, 1994; Liu et al. (2000).

AMF form symbiotic associations with the roots of more than 80% of terrestrial plants (Smith and Read 2008). This broad spread of AMF indicates that the effect of specific host plants on AMF activities is very small (Smith and Read, 2008). Klironomos and John (2000) reported that AMF successfully colonized 96% of the species of grass and horticulture plants. This means that around 4% AMF has a specific host and are not able to colonize well on species of grass and horticulture plants. Therefore, AMF exploration in a plant such as black cumin is of fundamental importance to understand the level of colonization and the number of AMF spores in this species.

Material and Methods

All accessions of black cumin were grown on polybags with Andosol soil in Pacet, Cianjur, West Java, Indonesia. There were six accessions of black cumin from America (N1), Turkey (N2), Hong Kong (N3), Slovenia (N4), India (N5) and Kuwait (N6). Three soil samples were collected from each accession and repeated four times so that there were 72 samples in total. The research consisted of five stages: soil sampling, AMF isolation, spore counting, AMF identification, and AMF colonization calculation. All stages of the research were conducted from October to November 2017.

Soil Sample Collection

Soil samples of 300 g each were collected from the depths of 5 to 20 cm from the soil surface at four different points of the rhizosphere area. The samples were then stored in plastic bags and labelled. Twenty gram of soil sample was used for AMF isolation. Root

samples for AMF colonization analysis were taken from four points of the rhizosphere and placed in plastic containers containing 70% alcohol

AMF Isolation

For AMF isolation, a modified method by Pacioni (1992) and Brundrett et al. (1996) were used and described below:

- The soil sample was placed into the glass and mixed with 1 L of water and stirred to get fine particles
- Sieves of 500 μm , 125 μm , and 63 μm were arranged and placed under running water.
- The soil suspension was poured into a stratified sieve (size 500 μm , 125 μm , and 63 μm). This step was repeated until the soil suspension became clear.
- Soil deposits of 125 μm and 65 μm were removed and transferred into centrifuge tubes and 60% sugar solution was added with a ratio of 3:2, shaken and centrifuged for 3 minutes at 2500 rpm.
- The supernatant was poured into a plastic funnel with millipore paper to filter the sample into the glass bottles. The previously used millipore paper was divided into eight gradients to facilitate observation.
- The filtered spores were then washed, cleaned with water from a spray bottle to prevent spore lysis.
- Spores that had been trapped in the millipore paper were removed using a tweezer into a petri dish
- Spore counting and genus identification were observed under a compound microscope (Olympus SZ261) under 40x magnification

Spore Counting

Spores were counted using a hand counter under the Olympus SZ61 Microscope under 40 x magnifications. The area of the millipore were divided into eight sections; spores were counted from the 1st to the 8th section using a hand counter under a compound microscope; the number of spores from each section were added up to get the total spore count.

AMF Identification

Spores were identified based on their AMF morphology according to the method of INVAM (2018). Each sample consists of 20 g rhizospheric soil.

AMF Colonization Calculation

Observation of AMF colonization was conducted after cleaning and staining the roots. Root coloration was scored using the method of Clapp et al. (1995) described below:

- The roots were washed thoroughly using a sieve

and then immersed in 20% (w/v) KOH solution for 1 day until the roots turned white.

- The solution was removed and the roots were washed under running water, then immersed in a solution of 0.1 M HCl for 3 to 4 minutes without washing
- The roots were soaked in a trypan blue solution (0.25 g in 475 mL lactic acid and 25 mL of aquadest) for 24 hours followed by the root washing
- Soak the roots in a de-staining solution of 25 mL aquadest and 475 mL lactic acid for 24 hours
- The roots were then cut into approximately 10 mm length and lined on a glass slide then covered with a cover slip; each slide has 10 pieces of roots.
- Two samples were prepared from each plant. Each sample was observed under the compound microscope; fragmented root sections characterized by hyphae, arbuscular or vesicles were photographed and recorded.

The percentage of root colonization was calculated using the formula developed by Brundrett et al. (1996):

$$\% \text{ colonization} = \frac{\sum \text{colonized field of view}}{\sum \text{whole field of view}} \times 100 \%$$

Result and Discussion

The Diversity of AMF Genus

Six genera of AMF were found from the rhizosphere of black cumin accessions (Table 1). *Glomus* is the most commonly discovered genus, with more than 96%, followed by *Gigaspora*, *Acaulospora*, *Scutellospora*, *Dentiscutata*, and *Entrophospora* (Table 1).

The presence of *Glomus* in each of black cumin accession indicates that *Glomus* has a very wide dispersal area. This is in accordance with Hartoyo et al. (2011) that *Glomus* has the widest distribution compared to any other AMF genus. The presence of *Glomus* species that are more than other genera is also the cause of frequent encounters of this genus (INVAM 2018). The optimum pH of planting medium, which was neutral to alkaline, might have provided an optimal environment for *Glomus* germination.

The highest number of spores was found in Indian accession (96.42 spores). It is suspected that in general the Indian AMF accession is more active compared to AMF of other accessions. The American and Kuwait accessions were found in six genera of AMF, i.e. *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, *Dentiscutata*, and *Entrophospora*. The number of spores found was still relatively low, i.e. 4.32 spores per g of sample. Growing black cumin in polybags might be the one of the causes of the small AMF population. In addition, the often rainy field conditions affect the increase in the number of

Table 1. Quantity of AMF spores in black cumin rhizosphere

AMF Genera	Accessions*					
	N1	N2	N3	N4	N5	N6
<i>Glomus</i>	79.17	79.58	76.17	82.33	96.42	83.58
<i>Gigaspora</i>	2.42	1.08	1.08	1.42	1.33	1.08
<i>Acaulospora</i>	1.83	0.42	1.67	1.08	1.08	0.83
<i>Scutellospora</i>	0.17	0	0	0	0	0.83
<i>Dentiscutata</i>	0.92	0.75	0.67	0.58	0.58	0.25
<i>Entrophospora</i>	0.17	0	0.08	0.17	0	0.17

*N1 = American accession, N2 = Turkey accession, N3 = Hong Kong accession, N4 = Slovenian accession, N5 = Indian accession, and N6 = Kuwait accession

spores. Even though the average rainfall during the study was classified as low at 21.95 mm, but the high intensity of rain often occurred. According to Delvian (2003) the formation of spores is influenced by the amount of rainfall and soil moisture fluctuations.

Differences in the number of spores among genera could possibly due to differences in exudates released by each accession of black cumin. According to Giovannetti et al. (1993) germination of spores and growth of hyphae are influenced by root exudates.

AMF Colonization

AMF colonization observation showed that all

accessions of black cumin were infected by AMF (Figure 3). This indicates an association between AMF and black cumin, and a successful symbiosis of AMF with black cumin. One of the successes of infection can be seen from AMF colonization characterized by the discovery of hyphae in the root tissue of plants (Figure 2).

The highest colonization value in the accessions of India (N5) and Kuwait (N6) is 8.33% followed by Hong Kong 5.83% accession, 5% US, Turkey, and Slovenia 3.33%. The percentage of infections found in the multiple accessions of black cumin is still low. According to O'Connor et al. (2001) colonization values of <10% is considered low, 10-30% is

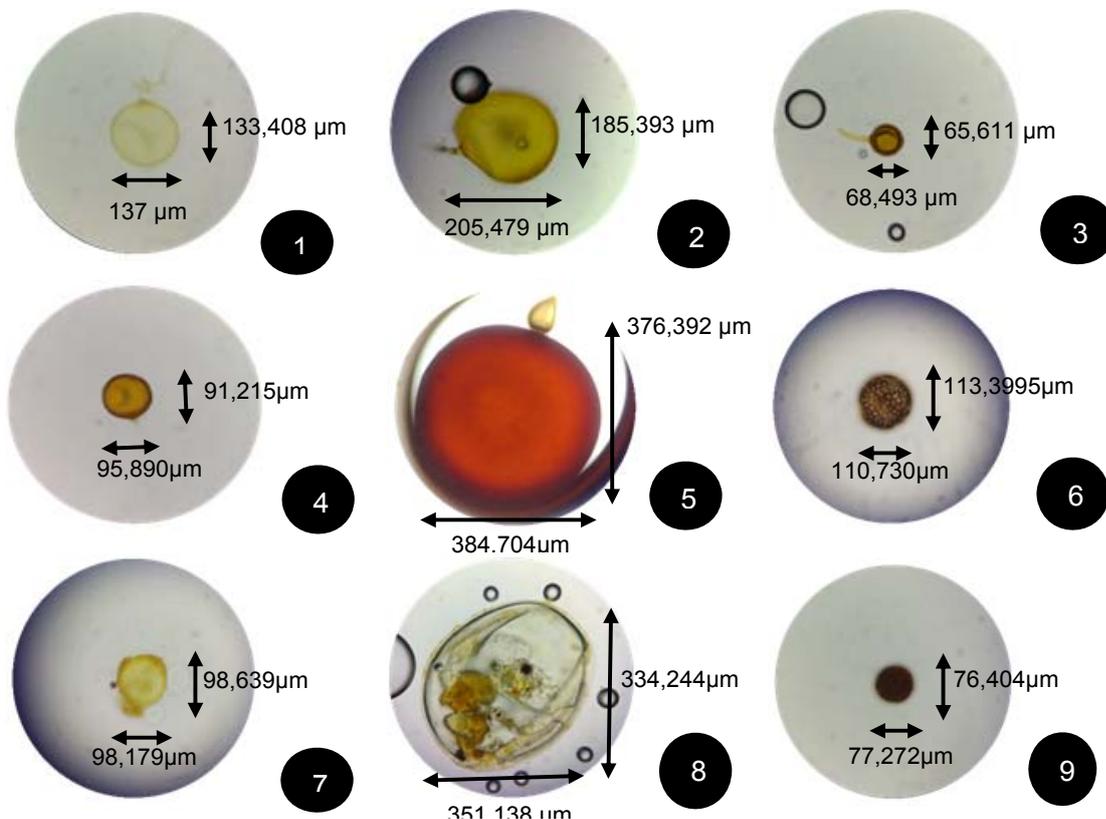


Figure 1. *Glomus* sp 1 (1), *Glomus* sp 2 (2), *Glomus* sp 3 (3), *Glomus* sp 4 (4), *Gigaspora* (5), *Dentiscutata* (6), *Acaulospora* (7), *Scutellospora* (8), *Entrophospora* (9).

moderate, whereas >30% is high. This low value of colonization was possibly related to the fact that the planting medium was less optimal for AMF growth. According to Chalimah et al. (2007) one of the factors that determine spore germination and AMF colonization is the composition and pH of the planting medium. The planting media in this study was soil, manure, and charcoal husk (2: 1: 1 v/v) with a pH of 7.05. According to Goltapeh et al. (2008) the colony growth will be optimal at pH 5.6. The increasing pH of the planting medium from 4.7, 5.6 to 6.4 increased the values of AMF colonization (Nurlaeny et al., 1996; Singh, 2004).

The condition of the planting medium that is suspected to have a high availability of P might have caused the less activity of the AMF. AMF activity will be optimal for planting media with low P availability, whereas in planting media where P availability is high, AMF activity will not affect host plants (Swift,

2004). P availability in the rhizosphere is related to soil pH. Acidic soil media conditions will increase the value of AMF colonization (Singh, 2004). The successful utilization of AMF can be seen in the size of colonization. The higher the AMF colonization in the host plant the higher symbiotic association between AMF and the host plants.

Conclusion

AMF can readily associate and form a symbiotic relationship with various accessions of black cumin grown in Indonesia. Six genera of AMF were found in the rhizosphere of black cumin: *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, *Dentiscutata*, and *Entrophospora* with the genus *Glomus* to be predominant (96.42 spores) in black cumin Indian accession.

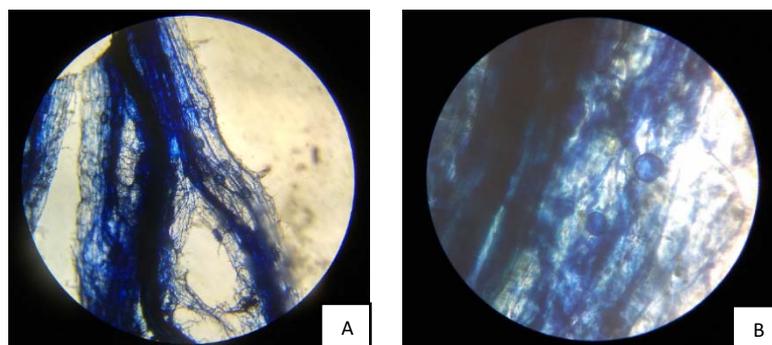


Figure 2. AMF colonization of black cumin roots in the form of vesicular (A), arbuscular (B).

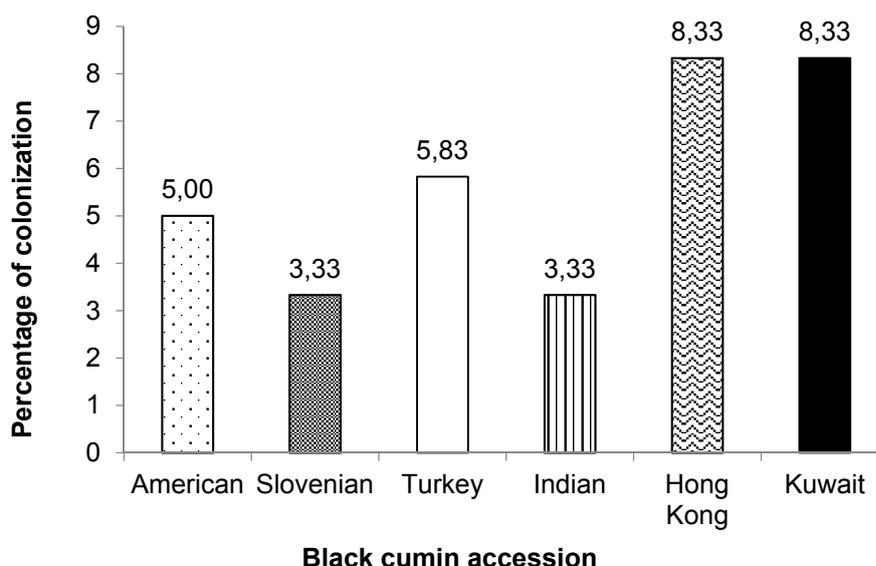


Figure 3. Percentage of AMF colonization in the rhizosphere of six accessions of black cumin

Acknowledgement

The authors thanked Lembaga Pengelola Dana Pendidikan (LPDP) and Penelitian Terapan Unggulan Perguruan Tinggi 2018 (PTUPT) for funding this research, and the manager of IPB Pasir Sarongge Experimental Station for facilitating the study.

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