JOURNAL OF SCIENCE INNOVARE
https://journal.unpak.ac.id/index.php/jsi
Volume 01, Number 02, September 2018, Page 50-53
p-ISSN: 2615-3769 ; e-ISSN: 2615-3750

EXPLORATION AND IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI FROM THE RHIZOSPHERE OF CHILI PLANTS (Capsicum Annuum L) IN BOGOR

Munarti [a)], Annisa Wulan [a)], Agus Utami[a)]

[a)]*Universitas Pakuan, Bogor, Indonesia*
Corresponding Author: munarti@unpak.ac.id

Article history: received 16 Juli 2018; revised 18 Agustus 2018; accepted 01 September 2018

Abstract. Mycorrhiza is ana structural and functional association between specific fungus and the roots of symbiotic relationship between mutualisme functions with a high degree of plant roots. Benefits of mycorrhiza for plant growth and development as its host is to increase the absorption of nutrient elements of soils, as biological barrier against infection of root pathogen, enhancing the resilience of crops to drought and increasing hormone boosters grows. This research aims to identify a arbuskular mycorrhiza fungi isolated from chilli plants rhizosphere in the village of Cilubang Mekar, Gunung Picung, Cikoneng, dan Kampung Baru. The results of this research show that the exploration and characterization of spores that develop on the roots of chilli in the village indicates a growing spores there is the genus *Acaulopora*, *Gigaspora*, and*Glomus*.type of Spore that successfully identified a species of *Glomus* sp 1, *Glomus* sp 2, *Glomus* sp 3.

Keywords: Acaulopora, Gigaspora, Glomus, rhizosphere, mycorrhiza, chilli.

I. INTRODUCTION

Chili (Capsicum annuumL.) is one of the horticultural commodities that has a high economic value. The chili plant contains protein, ascorbic acid (Kumar [1]), β carotene, asparaginase and capsaicin which act as anti-cancer substances. From year to year increasing along with population growth, while chili production in Indonesia is still relatively low. Low chili productivity is caused by many factors such as climate and plant pest organisms (OPT) which can reduce the quality and quantity of chili production.

Some microbes that live in the soil can have symbiosis mutualism with plants that produce a plant resistance to pathogens. Among them are arbuscular mycorrhizal fungi (CMA) which are obligate fungi with spores associated to plant roots. CMA is also antagonistic to plant pathogens such as Fusarium oxysporum fungus and Radhopolus similis nematodes. Mycorrhiza is an organism-originated from the fungus group which illustrates a form of mutualism symbiotic relationship between functions with high-level plant roots (Brundrett [2]).

The benefits of mycorrhiza for plant growth and development as its host increase the absorption of nutrients from the soil, as a biological barrier to infection with root pathogens, increase the resistance of plants to drought and increase growth booster hormones. Arbuscular mycorrhizal fungus isolates from rhizosphere root roots of chili plants so that later can be used as biological control agents of plant pathogens.

The purpose of this study was to determine the diversity and identify arbuscular mycorrhizal fungi isolates from rhizosphere root roots of chili plants.

II. RESEARCH METHODS

This research was carried out for 5 months from November 2017 to March 2018 in the Laboratory of Biological and Biotechnology Resources, Bogor Agricultural University. A sampling was taken in four chili garden locations, namely Cilubang Mekar, Mount Picung, Cikoneng, and Kampung Baru.

Material and tools

The tools used in this study are centrifuges, scales, a set of spore filters (410, 125 and 45 mesh), beakers, Petri dishes, micropipettes, glass preparations, glass covers, stereo microscopes, compound microscopes, needle oceans, cameras, and calculator. The material used was soil samples from chili rhizosphere from five chili garden locations, namely Cilubang Mekar, Cinangneng, Mount Picung, Cikoneng, and Kampung Baru. The staining of the roots required ingredients, namely 10% KOH, 3% H_2O_2, 1% HCl, lactoglycerol, Trypan blue aquades, water and label paper.

The procedure of taking samples

Soil samples were taken from the rhizosphere of chili plants from four chili garden locations, namely Cilubang Mekar, Mount Picung, Cikoneng, and Kampung Baru. Soil sampling was carried out by taking soil in the rhizosphere of about 1000g, the soil taken ranged from 5-15 cm from the soil surface because mycorrhizal spores were commonly found in the topsoil.

Taking root samples is done by holding the base of the plant and taking root samples from the chili plants to taste. The roots used are the roots that are still young because the young roots are part of the roots that

Volume 01, Number 02, September 2018, Page 50-53
p-ISSN: 2615-3769 ; e-ISSN: 2615-3750

produce the highest exudates, so CMA is more likely to infect young plant roots to obtain nutrients from plants. After the soil and root samples are obtained, each sample is then inserted into a plastic bag and given clear information.

Isolation of mycorrhizal fungi

Soil samples taken from the biosphere are weighed as much as 50 grams, then put them into a 1000 ml beaker glass and then pour 1 litter water. The soil aggregate which is broken accidentally by hand makes the spores are free from the soil in 5 minutes. Leave for ± 1 minute until large particles settle. After that, the supernatant liquid was poured into a multilevel filter with a 1 mm sieve hole size, 500 µm, 212 µm, 106 µm (this procedure was repeated 3 times).

After the supernatant liquid has been poured into a multilevel sieve, rinsing with water is carried out to ensure that all the small particles have been carried away by the water. The filter size of 500 µm, 212 µm, and 106 µm was poured into the test tube with the help of a spray bottle and centrifuged at a speed of 5000 rpm for 5 minutes. After that, the centrifugation was poured into a petri dish and then the first stage of spore observation was carried out under a microscopic microscope. The spores found from the first observation were then transferred to a flat preparation with the help of a micropipette.

To see the microscopic characteristics of the spore, an observation was carried out under a compound microscope. Before performing root staining to see the structure of the CMA, the first step is to make the solution. Those are several solutions that need to be made, namely: making 10% KOH, 3% H_2O_2, 1% HCl, lactoglyserol solution and 0.05% trypan blue solution.

After that, the observed roots are colored with the following methods: chili roots are washed cleanly, roots are cut into ± 5cm and then placed them in a 100 ml beaker glass, 10% KOH is added to the beaker glass (until the roots are sinking). Then cover it with aluminum foil, heated at 250ºC for 10 minutes in the microwave. Keep it 10 minutes and removed from the microwave. Put ± 12 hours at room temperature, KOH is removed from the beaker glass. Followed by soaking the roots with 3% H_2O_2 in a glass beaker (until all the roots are sinking), let stand for ± 12 hours at room temperature, after that H_2O_2 is removed. Wash the roots with clean water, after that 1% HCL is added until all the roots sink. Then left it for ± 12 hours at room temperature. Remove the HCL and add Trypan Blue until the root sinks. After that, the beaker glass covered with aluminum foil and heated at 250ºC for 5 minutes at microwave, and then remove the beaker glass from the microwave and later keep it 12 jam in the temperature room. Throw away Trypan Blue from the beaker glass and add laktoglycerol until the roots sink, the beaker glass which is covered with aluminum foil heated at 250ºC for 5 minutes in a microwave, then keep it for ± 12 hours at room temperature. The roots are taken with tweezers, cut them into 3 cm and place them neatly on the preparations. Each preparation consists of 5 pieces of root with a glass cover. Each cutting of the roots which has a structure of the mycorrhizal (vesicular and hyphae) is observed under a microscope.

Identification of mycorrhizal fungi

The making of mycorrhizal spore preparations is intended to assist in the identification process. From the preparation, it is expected that spore morphological information can determine the genus MVA. Identification is done by using the Working with Mycorrhizas in Forestry and Agriculture guidebook (Brundrett [2]). Identification is done using a microscope. The scores obtained were collected based on the morphological characteristics of mycorrhizal spores including spore shape, spore size, and spore color.

III. RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungus (CMA) is a soil microorganism that acts as a microbial decoder, helping plants absorb nutrients from the soil. Microscopic observations on the roots of chili plants with trypan blue staining according to the modification of the method of Phillips [3] showed a rounded structure called vesicles and structures of hyphae mycorrhizal fungi that infect the roots of chili plants. (Figure 1) elongated and branched forms of mycorrhizal hyphae in the roots of chili plants. The structure of vesicles and hyphae shows that there has been infection and colonization between plant roots and mycorrhizal fungi (Sukmawaty [4]). Colonization of CMA in roots is calculated based on present and absent mycorrhizal structures (McGonigle [5]).

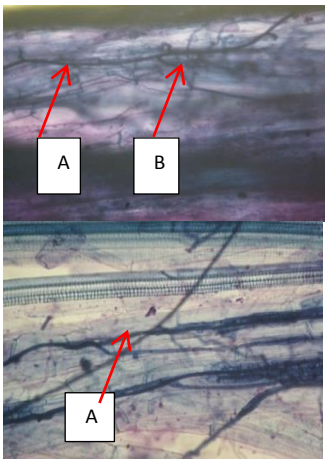

Figure 1 Structure of CMA in root tissue of chili plants, (A) Hyphae and (B) Vesicles.

The results showed that the soil taken from the rhizosphere or around the roots of chili plants in four

Volume 01, Number 02, September 2018, Page 50-53
p-ISSN: 2615-3769 ; e-ISSN: 2615-3750

locations in 50 grams of soil was obtained by 3 genera, namely the mycorrhizal genus which consisted of one species each, namely Gigaspora spp. and Acaulospora spp., while Glomus mycorrhizae consisted of 3 different types based on the morphological characteristics of spores, namely Glomus sp.1, Glomus sp.2, and Glomus sp. 3. Ghasua found arbuscular mycorrhizal fungi Gigaspora spp. and Glomus spp. obtained from the soil from the Rhizosphere of chili plants in Nigeria. From the results of this study, the location of Cilubang Mekar was found in two types of mycorrhizal spores, namely Gigaspora spp and Glomus sp2, as well as in Cikoneng two types of spores were found, Acaulospora spp and Glomus sp2 compared to two other locations, which were found only one type of mycorrhizal spores. Mycorrhizal diversity in a region is caused by the response of different types of mycorrhiza to soil properties, such as pH (Sjoberg [6]).

The method of spore density analysis refers to Brundrett [2] using wet sieving decanting or wet filtering, only counting intact spores and thought to still contain droplet lipids. The type of Glomus sp.1 had the highest number of spores, namely 22 spores from soil samples located in Kampung Baru (Table 1). This shows that the genus Glomus still has a fairly high adaptation compared to the genera Gigaspora and Acaulospora. This is in line with the study of Soenartiningsih [7], finding mycorrhizal Glomus sp. which has the greatest abundance average in the rhizosphere of corn and legumes. According to Ulfa [8], that mycorrhizal genus Glomus.sp. able to demonstrate its existence to survive and develop in an environment formed due to landfill without topsoil in nutrient-poor areas such as former coal mines compared to the genus Gigaspora sp. and Acaulospora sp.

The genus Acaulospora spp was only found in soil samples from the location of Cikoneng, namely one spore, and was the genus with the least amount of spores compared to the genera Gigaspora spp and Glomus sp. Based on research (Nurhalisyah [9]), showed that on sugarcane land in Arouseo PG and PG Camming genus Glomus had the most widely spread, followed by the genus Gigaspora, while Acaulospora spread was limited.

Table 1 Density of Mycorrhizal Fungus spores from Rizosphere of Chili Plants

No	Location	Types of mycorrhizal fungus spores	The amount of Spore in 50 g of land
1	Cilubang Mekar	*Gigaspora* spp.	2
		Glomus sp. 2	4
2	Gunung Picung	*Gigaspora* spp.	5
3	Cikoneng	*Acaulospora* spp.	1
		Glomus sp. 2	2
4	Kampung Baru	*Glomus* sp. 1	22
		Glomus sp. 3	6

Based on its spore shape, the types of mycorrhizae obtained from several rhizosphere soil samples of chili plants are 3 genera, namely: Mycorrhizal Genus, Gigaspora, Acaulospora, and Glomus. The Mycorrhizae Gigaspora and Acaulospora only consist of one species, namely Gigaspora spp. and Acaulospora spp. While glomus mycorrhizae consisted of 3 different types based on the morphological characteristics of the spores are namely Glomus sp.1, Glomus sp.2, and Glomus sp.3 (Figure 2). The grouping of the types of mycorrhizae based on the characteristics of each microscopic spore morphology was identified by key identification according to (Brundrett [2]).

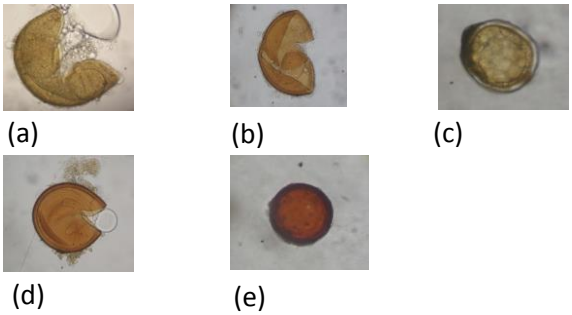

Figure 2 Mycorrhizal spores from the rhizosphere soil of chili plants
(a) *Gigaspora* spp., (b) *Acaulospora* spp., (c) *Glomus* sp. 1, (d) *Glomus*sp. 2, (e) *Glomus* sp. 3

Each spore genus has different characteristics and sizes. Mycorrhizal Gigaspora spp. has a yellowish spore with a boulbus suspensory which has a short, thin spore-wall and the terminal wall with a size of 160x150 µm. Spores of the genus Acaulospora spp. are yellow with orange in the spore-wall in the form of a small hole evenly distributed. Acaulospora spp spore size namely 210 x 230 µm. Glomus has more than one spore wall, Glomus spores which are found have round shapes, yellowish spore walls, slightly reddish yellow, to brown with spore sizes between species not much different (Table 2).

Table 2 Characteristics of mycorrhizae from the rhizosphere of chili plants

No	Mycorrhiza	Characteristic
1	*Gigaspora* spp.	Yellowish Gigaspora spores measure 160x150 µm. The spores have a boulbus suspensor that is short, spore-wall thin and has a germinal-wall.
2	*Acaulospora* spp.	The spores of the genus Acaulospora with organisms in the spore-wall are small evenly distributed holes. Spores measure 210 x 230 µm, yellow.
3	*Glomus* sp.1	Spore Glomus sp.1 is yellowish with a size of 200x230 µm, has a clear, thick, and layered spore-wall.
4	*Glomus* sp.2	Spom Glomus sp.2 yellow is slightly reddish with a size of 250x250 µm, has a dense and clear spore-wall.
5	*Glomus* sp.3	Glomus sp.3 spores are 150x150 µm in size, brown in color and clear in the spore-wall layer.

Volume 01, Number 02, September 2018, Page 50-53
p-ISSN: 2615-3769 ; e-ISSN: 2615-3750

According to Nurhalimah [10], Gigaspora spores are formed from a rounded bulbous suspensor, then small spheres that appear to grow larger become single spores formed in the soil. Glomus spores are formed from the development of hyphae (chlamydospores) which sometimes branch out and form a sporocarp. When the adult spores are separated from the adhesive hyphae. According to Ulfa [8], that the mycorrhizal genus Glomus sp. is able to demonstrate its existence to survive and develop in an environment formed due to landfill without topsoil in nutrient-poor areas such as former coal mines compared to the genus Gigaspora sp. and Acaulospora sp. This means that CMA has each characteristic to adapt to changes that occur in the environment.

Based on Matsetio's [11] research mycorrhiza is able to increase plant growth and resistance. Mycorrhizal origin from the land after the coal mine was able to increase the growth of maize plants, namely the best plant height in the 3rd week by Glomus sp.1 of 10.75%, canopy dry weight by Gigaspora sp. at 8.70%, root wet weight by Glomus sp.1 at 67.39%, and root dry weight by Glomus sp.2 of 12.09%. The administration of mycorrhizae is able to induce the resistance of corn plants to stem rot disease Fusarium sp. Mycorrhizal Gigaspora sp. able to delay 66.67% disease incubation period, Glomus sp.1 mycorrhiza, Glomus sp.2, and Gigaspora sp. can reduce the percentage of disease attack by 20%, and Glomus sp.1 and 2 can reduce disease intensity by 30%, and Glomus sp.3 has a higher level of colonization (73.33%) compared to other types of mycorrhizal treatment.

IV. CONCLUSION

In soil samples taken from the rhizosphere or around the roots of chili plants in four locations, 3 genera of arbuscular mycorrhizal fungi spores, namely mycorrhizal genus, Gigaspora, Acaulospora, and Glomus were obtained. The dominant genus is Glomus spore which has the highest number of spores, which are 22 spores in 50 grams of chili rhizosphere soil located in Kampung Baru. Mycorrhizae Gigaspora and Acaulospora consist of only one species each, namely Gigaspora spp. and Acaulospora spp., while Glomus mycorrhizae consisted of 3 different types based on the morphological characteristics of spores, namely Glomus sp.1, Glomus sp.2, and Glomus sp. 3.

Suggestion

Based on the results, it is necessary to study molecular spore identification which identifies clearly the species and characteristics of spores.

Acknowledgement

Acknowledgments the authors say to the Pakuan Siliwangi Foundation that has provided financial assistance so that this research can run smoothly.

REFERENCES

[1] Kumar,Tata SS. 2009. Ascorbic Acid Contents in Chili Peppers (*Capsicum* L.)Available online at www.notulaebiologicae.ro Print ISSN 2067-3205; Electronic ISSN 2067-3264*Not Sci Biol* 1 (1): 50-52

[2] Brundrett M., Bougher N., Dell B., Grove T. and Malajczuk N. 1996.Working with Mycorrhizas in Forestry and Agriculture. Canberra: AClAR Monograph.

[3] Phillips, J.M. and D.S. Hayman, 1970.Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Brit. Mycol. Soc., 55: 158–161.

[4] Sukmawaty E, Hafsan, Asriani. Identifikasi Cendawan Mikoriza Arbuskula Dari Perakaran Tanaman Pertanian. Biogenesis Vol 4, No. 1, Juni 2016, hal 16-20

[5] McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. and Swan, J. A., 1990, A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495-501.

[6] Sjoberg, J. (2005). Arbuscular Mycorrhiza Fungi.Occurence in Sweden and Interaction with a Plant Pathogenic Fungus in Barley.Acta Universitais Agriculturae Sueciae. Uppsala.

[7] Soenartiningsih, 2007.Peranan Jamur Mikoriza Arbuskular dalam Mengendalikan Penyakit Busuk Pelepah (*Rhizoctonia solani*) pada Jagung.Disertasi Univ. Gadjah Mada, Yogyakarta.

[8] Ulfa, M., A. Kurniawan, Sumardi, dan I. Sitepu. 2011. Populasi fungi mikoriza arbuskula (FMA) lokal pada lahan pasca tambang batubara. Jurnal Penelitian Hutan dan Konservasi Alam 8(3): 301-309.

[9] Nurhalisyah dan D. Rahmad, 2012.Identifikasi fungi mikoriza arbuskular di lahan tebu PTPN XIV serta efektifitasnya untuk meningkatkan serapan fosfat dalam menunjang produksi tebu. Jurna Agrisistem seri hayati 8 (2): 62-69

[10] Nurhalimah, S., S. Nurhatika, A. Muhibuddin. 2014. Eksplorasi mikoriza vesicular arbuskular (MVA) Indigenous pada tanah regosol di Pamekasan, Madura. Jurnal Sains dan Seni Pomits 3(1):30-34.

[11] Matsetio A. 2014. Jenis dan Potensi Fungi Mikoriza asal Tanah Pasca Tambang Batubara dalam Mengendalikan Penyakit Busuk Batang Fusarium sp. pada Tanaman Jagung [Skripsi]. Fakultas Pertanian Universitas Bengkulu.