Exploration of Arbuscular Mycorrhizal Fungi from Sugarcane Rhizosphere in Marginal Land

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ABSTRACT

The exploration of arbuscular mycorrhiza fungi from sugarcane plantation in marginal land in South Sulawesi was carried out to find the source of inoculums showing effective infection. Soil samples were taken from four area with different characteristic of marginal land, namely land with low organic matter content, clay texture, limited irrigation, and undulating land. Mycorrhizae contained in the soil samples were then observed, and the spores obtained were used as the source of isolation by a single spore culture. The mycorrhizal spores were isolated by wet sieving and centrifugation method with 48% sucrose, which were observed under a compound microscope for spore details (100-1000x). Sugarcane root samples were taken to observe mycorrhizal infection in sugarcane root tissue by root staining method. The results of the study showed that the greatest diversity of mycorrhizal genera was found in soil samples of Jambua Block (Glomus, Gigaspora, and Sclerocistis) and AJ-5 Block area (Glomus, Acaulospora, and Sclerocistis). Single-spore isolates obtained were Glomus sp. and Acaulospora sp. Infection test result on four sugarcane varieties commonly grown in Takalar Sugar Factory showed that infectivity of mycorrhizal isolate of Acaulospora sp. was the highest (75%) and significantly different (LSD'test, p <0,05) compared to that of Glomus sp. (66%).

Keywords: Arbuscular Mycorrhizal Fungi, Rhizosphere, Sugarcane

ABSTRAK

Eksplorasi keragaman cendawan mikoriza arbuskular pada tanaman tebu di lahan marginal pada salah satu perusahaan perkebunan di Provinsi Sulawesi Selatan telah dilakukan untuk menemukan sumber inokulum yang memiliki kemampuan infeksi yang efektif. Sampel tanah diambil dari empat lahan dengan karakteristik lahan marginal yang berbeda, yaitu pada lahan dengan kandungan bahan organik rendah, tekstur lempung, irigasi terbatas, dan lahan bergelombang. Selanjutnya mikoriza yang terdapat pada sampel tanah diamati dan spora yang diperoleh digunakan sebagai sumber isolat melalui kultur spora tunggal. Isolasi spora mikoriza dilakukan dengan metode penyaringan basah dan sentrifugasi dengan sukrosa 48% kemudian diamati di bawah mikroskop compound untuk pengamatan spora dengan lebih teliti (100-1000x). Pengambilan sampel akar tebu untuk pengamatan infeksi mikoriza pada jaringan akar tebu dengan metode pewarnaan akar. Hasil penelitian menunjukkan bahwa keanekaragaman genus mikoriza terbesar terdapat pada sampel tanah daerah Blok Jambua, yaitu ***Glomus***, ***Gigaspora***, dan ***Sclerocistis*** dan pada daerah Blok AJ-5 yaitu ***Glomus***, ***Acaulospora***, dan ***Sclerocistis***. Isolat spora tunggal yang diperoleh merupakan ***Glomus*** sp. dan ***Acaulospora*** sp. Hasil uji infeksi pada empat varietas tebu yang ditanam di Pabrik Gula Takalar menunjukkan infektivitas isolat mikoriza ***Acaulospora*** sp. tertinggi (75%) dan berbeda nyata (uji LSD, p <0,05) dengan ***Glomus*** sp. (66%).

Kata kunci: Jamur Mikoriza, Rizosfer, Tebu

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is one of the essential industrial commodities categorized by World Trade Organization (WTO) that support world food security (Arifin, 2008). Through the sugar self-sufficiency program starting in 2002, the Indonesian government is trying to increase national sugarcane production, however the results have not been satisfactory so far. According to the roadmap of national self-sufficiency, sugar production in 2012-2014 is expected to reach 4 to 5.7 (million ton), yet there was a decline in production in 2012 and 2013 by 2.59 and 2.54, respectively (Directorate General of Plantation, 2012).

Takalar Sugar Factory (PG Takalar) is the largest sugar company in South Sulawesi established 35 years ago, with a total production area of approximately 5000 ha, located in three regencies, including Gowa, Takalar and Jeneponto (Pusat Informasi BUMN Perkebunan, 2012). The land conditions are classified as critical (pH <5, the low

level of fertility and soil moisture) (Rismaneswati, 2005), causing lower crop productivity (3 ton.ha^{-1}) compared to the national productivity (5.7 to 5.8 ton.ha^{-1}) (Toharisman, 2007; Anonymous, 2013). This problem is usually resolved using conventional practices that leads to the increase in the use of chemical fertilizers.

Sugarcane production is largely determined by the optimization of the production factors, particularly land quality and climates (Sabatier et al., 2015; Sulaiman et al., 2019). The climatic factors such as long dry season can cause widespread drought in the sugarcane land (Fahad et al., 2017). Low qualities of physical and morphological properties of the soil, along with low soil fertility, are limiting factors resulting in decreased plant growth and yield of the sugarcane. Efforts in increasing nutrient deficiency in sugarcane crops by continuous use of chemical fertilization, which are not always offset by increased production proportionally, can even cause damage to the soil structure. (Rossetto et al., 2014). According to Sugito et al. (1995), a decrease in land productivity due to the continuous use of inorganic fertilizers with higher dose resulted in the decreased soil organic matter content.

Therefore, it is necessary to apply technological innovation overcome the limitation of nutrition in sugarcane fields in various land conditions and to maintain the carrying capacity of the land, which is safe for the environment to achieve sustainable development of sugarcane production. One of the efforts that can be carried out is the use of arbuscular mycorrhizal fungi (AMF). AMF is the obligate fungi associated with most types of terrestrial plants (80%), which play an important role in nutrient uptake and plant growth (Harrison, 1997; Lone et al., 2017). AMF live and thrive in the plant rhizosphere region, and they can induce changes in plant physiology, affect microbial populations (Jakhar, 2017), and improve host plant resistance under abiotic stresses such as salinity, drought, heat and low nutrient levels (Setiadi, 2011; Latief, et al., 2016; Chen et al., 2018). Low nutrient availability, humidity pressure, soil erosion, phosphorus fixation, high acidity with aluminum toxicity, and low soil microbial biomass reduce soil quality and inhibit the sustainability of agricultural systems (Jakhar, 2017). The use of AMF is an effort to support the sustainability of the modern agricultural system due to its environmentally friendly characteristic (Kim et al., 2017; Begum, 2019). Research focus that needs to be developed in the future is the exploration of superior AMF strains that can be utilized to support plant growth (Latief et al., 2016).

The application of AMF can improve the growth and increase biomass of sugarcane (Ismayanti, 2013; Leovini, 2014), and its application at the early growth can increase the rate of mycorrhizal infection in sugarcane roots planted in dry land, thereby increasing the average leaf area and weight of sugarcane stems (Sulistiono, 2017). AMF can accelerate the growth rate, improve the quality and viability of plants, and increase plant growth and productivity in critical land (Wardhika, et al., 2015). Thus, the exploration and use of inoculant indigenous AMF species are potential to be developed to overcome drought and low fertility in an agricultural land (Nurhalimah et al., 2014). Under drought stress conditions, AMF can increase leaf proline, photosynthetic rate, leaf area index, crop growth rate, fresh weight, and seed dry weight of soybean (Pavithra and Yapa (2018). Meanwhile, the AMF type of *Glomus* spp. can increase leaf water absorption, plant biomass, and metabolic content, including phenols, ascorbic acid, glutathione, antioxidant enzymes, and fluorescence chlorophyll, in *Saccharum arundinaceum* Retz. (Mirshad dan Puthur (2016).

Several studies on the exploration of AMF have been carried out in various plant species, includ-

ing sugarcane. AMF genera found in the sugar cane plantation area of Kediri Regency, East Java Province are *Glomus, Gigaspora, Acaulospora, Scutellospora, Entrophospora,* and *Sclerocystis* (Wardhika, 2015). Some types of AMF were also found in South Sulawesi Province, including *Glomus, Gigaspora, Acaulospora*, and *Scutellospora* (Nurhalisyah and Rahmad, 2012), in which *Glomus, Gigaspora, Acaulospora*, and *Scutellospora* were found in Bone Regency (Nurhalisyah and Rahmad, 2012) and *Glomus, Gigaspora, Acaulospora*, and *Sclerocystis* were found in Takalar Regency (Kumalawati et al., 2014). However, indigenous species of these fungi with high infectivity in sugarcane has not been selected and tested to be specifically used in dry land. Therefore, it is necessary to explore and to study the use of indigenous mycorrhizal fungi with high level of infection and effectiveness in associating and contributing to the nutrient absorption of nutrients, which can support the growth and crop production.

MATERIALS AND METHODS

The research was conducted from June 2014 to October 2015. Soil analysis was carried out in chemical laboratories, Department of Soil Science, Faculty of Agriculture, Hasanuddin University, Makassar. Types of mycorrhizae were identified in the Cryptogamic laboratory, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Cibinong, Bogor, West Java. The isolation of mycorrhiza, single spore culture, root staining, and observation of mycorrhizal infection in root tissue were performed in microbiology laboratories, Forestry Research Center Makassar. The experiments to study the effects of mycorrhizal species on several sugarcane varieties were carried out in the experimental fields, Department of Plantation, Pangkep State Polytechnic of Agriculture, South Sulawesi.

Soil Sampling

Exploration was conducted in the sugarcane plantation of PT. Perkebunan Nusantara XIV Takalar Sugar Factory based on four main criteria of marginal land, which are clay soil texture, low organic matters, dryland (non-irrigated), and land with a slope of > 8%. The first and second soil samples were taken from the land with low organic matter of Block AJ 5 and AA 13 located in Jeneponto (05°17'843" S and 119°33'500" E). The third soil sample was taken from the land with limited-irrigation of Block Pattalikang located in Takalar (05°18'765" S and 119°33'481"E). The fourth soil sample was taken from the land with a slope of > 8% of Block Jambua located in Gowa (05°19'843" S and 119°35'355" E). Other soil sample points were determined at each field site, then the composite of soil samples was taken as much as two kilograms.

Soil Analysis

The soil analysis was performed on the soil physical, chemical and fertility properties, consisting of texture (Bouyoucos, 1962), pH and organic C (Rayment and Higginson, 1992), total P (Olsen et al., 1954), CEC, and EC (Hajek et al., 1972).

Isolation and Identification Mycorrhizal Spores

Isolation of mycorrhizal spores was conducted by wet sieving and centrifugation method with 48% sucrose (Walker et al., 1982). Mycorrhizal spores obtained in a petri dish were separated based on their types and moved into a petri dish (5 cm diameters). The population of each type of spore in 100 g soil was calculated. Then, preparations were made according to each type of spore, by taking the spores (using a pipette or tweezers spores), which then were transferred onto an object glass, etched with a solution of PVLG and closed with a cover glass. The mixture of mycorrhizal spores were then

observed under a microscope with a magnification of 100-400 times. The identification was based on morphological characteristics of spores, including shape, color, size, and ornaments on the walls of spores.

Trapping and Single Spore Culture

Trapping culture of mycorrhizae was done to multiply the population of mycorrhizal spores present in the soil samples using corn plant host. A total of 70-80 g of each soil sample from sugar cane plant rhizosphere was put into a plastic cup (volume 250 g) containing a third of sterile sand and planted with corn, then preserved for three months and given with liquid fertilizer once a week.

To obtain mycorrhizal spores, each soil sample from the culture trapping was isolated by wet sieving method (Walker et al., 1982). The spores obtained were accommodated in a petri dish with a diameter of 8 cm containing distilled water and labeled in according to the origin of soil samples, and then observed under a dissecting microscope. The spores were then separated based on the type, shape, color, and size, and each was transferred to a smaller petri dish (5 cm diameter). Mycorrhizal spores were inoculated on the roots of host plants (sorghum) and placed in the axillary roots of host plants. The sorghum plants were maintained for three months and given with liquid fertilizer once a week.

Root Staining

Root staining was conducted following the procedures of Kormanik and Mc. Graw (1982). Root preparations were made to calculate the percentage of mycorrhizal infection. A total of ten roots with a length of 1 cm were arranged on the object glass, covered with cover glass, and labeled according to the treatments. The preparations compound was observed under a microscope with a magnification of 200-400 times.

The percentage of mycorrhizal colonization was calculated based on the following formula (Giovanetti and Mosse, 1980):

$$Mycorrhizal\ infection\ (\%) = \frac{Number\ of\ infected\ root\ area}{Total\ area\ of\ the\ roots\ observed} \times 100\%$$

Data Analysis

This research was arranged in a split plot design. The main plot is sugarcane varieties, consisting of CM 2012, PSJK 922, PS 862, and Cenning. The subplot is type of mycorrhizal inoculants, consisting of *Glomus* sp and *Acaulospora* sp. Both of these mycorrhizae were used as inoculants applied to sugarcane because they show good sporulation ability and meet the number of spore requirements in a single spore culture (70-100) (Delvian, 2006). The data obtained were analyzed by using ANOVA and continued with a Least Significant Differences (LSD) test at the level of 5%.

RESULTS AND DISCUSSION

Soil Analysis

The analysis of soil physical and chemical properties showed that the lowest values in the soil chemical properties, including pH, organic C content, and base saturation were found in the block of AJ 5. The P content was the lowest in the Block AA 13, while the lowest value of soil CEC was in Block Pattalikang. Meanwhile, the organic C content and CEC in Block Jambua were categorized as slightly low. Previous soil analysis on each sugarcane planting blocks showed that all study sites were categorized as marginal land, with lower values of organic C, P content and CEC (Table 1).

The analysis of soil texture showed that the four samples had different texture classes, causing the physical and chemical properties of the soil to be different. In accordance with the characteristics of the sampling location, the first and second samples

Table 1. Soil Analysis of Four Sugarcane Field Locations

No	Soil Property Physics/Chemical	Field Location (Block)			
		AJ- 5	AA-13	Pattalikang	Jambua
1	Texture Class	Clay	Clay Loam	Sandy Clay Loam	Loam
2	pH	6.2	5.9	6.4	6.3
3	% Organic C	1.25	2.34	2.44	2.00
4	P2O5 content	15.0	14.6	15.2	15.3
5	CEC	26.33	24.31	21.69	22.63
6	Base Saturation	40	41	54	52

Table 2. Types of Vesicular Arbuscular Mycorrhiza found in the Rhizosphere of Sugarcane in PG. Takalar Sugar Factory

No	Soil Sample (Block)	Field Location (Block)		
		AJ- 5	AA-13	Pattalikang
1	AJ-5	Single	*Acaulospora scrobiculata* Trappe	-
		Single	*Scutellospora c.f .erythropus (Koske & C. Walker) C. Walker & F.E. Sanders*	Reddish brown
		Single	*Scutellospora c.f .erythropus* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	Clear
		Single	*Glomus* s p.1	Yellow, tawny
2	AA-13	Single	*Acaulospora scrobiculata* Trappe	Tawny with white and thick outer walls
		Single	*Glomus* s p.2	-
3	Pattalikang	Single	*Acaulospora scrobiculata* Trappe	-
		Sporocarp	*Glomus* s p.1	Yellow, tawny
4	Jambua	SIngle	*Glomus* s p.1	Yellow, tawny
		Single	*Glomus* s p.3	Reddish dark brown, thick walls hyaline
		Sporocarp	*Glomus* s p.4	-
		Single	*Gigaspora* sp.1	Bright yellow
		Sporocarp	*Sclerocystis rubiformis* Gerdemann & Trappe	-

showed a higher clay content than others, classified into clay and clay loam texture.

Based on the assessment referring to the criteria by the Soil Research Institute (Eviati and Sulaeman, 2009), all samples showed low acidity level (table 1). The first soil sample had low organic C content and base saturation, but the CEC value was high. The second and third soil samples showed that all of the soil chemical properties were classified as medium. Meanwhile, the fourth soil sample had a low organic C content, with other chemical properties classified as moderate.

The assessment of the soil chemical properties showed different levels of soil fertility based on the criteria by Mutert et al. (2000). The first soil sample from the land with low organic matter indicated that low fertility . The soil samples from the land with clay texture and limited irrigation (the second and third samples) had medium soil fertility. Meanwhile, the fourth soil sample from land with a slope of more than 8% had a low soil fertility.

Identification of Mycorrhizal spores

The results of the identification of sugarcane symbiotic mycorrhizal fungi in marginal land are presented in Table 2.

Observation on the population and characteristics of the types of arbuscular mycorrhiza in sugarcane rhizosphere showed a similar diversity in the soil samples taken from all locations. Genera of *Glomus* and *Acaulospora* were found in all research sites. This shows that the both types of genera are spread widely and capable of associating with sugarcane. These results are similar to the research by Nurhalisyah and Rahmat (2012), exploring the mycorrhiza on sugarcane land in Arasoe and Camming Sugar Factories. In addition, a previous study that explored the abundance and diversity of fungi in normal land type of sugarcane plantation in Takalar Sugar Factory (Kumalawati, 2014) also found that the most widely spread genera of the fungi was *Glomus,* followed by *Gigaspora* and *Acaulospora*.

In the area of Khuzestan, Iran, *Glomus* is the most commonly found mycorrhizal fungi genus associated with four varieties of sugarcane generally grown in local sugarcane fields. In addition, the genus of *Acaulospora* was also found associated with sugarcane crops with limited numbers and distribution as suggested by Rokni and Goltapeth (2011). Other research conducted by Soenartiningsih (2007) in the rhizosphere of corn and beans plants in four districts in East Java also indicated that *Glomus* sp. showed the greatest average abundance in the research sites.

The diversity of mycorrhizal fungi varies in each marginal land location according to its characteristics. Arbuscular mycorrhizal fungi showed is higher diversity (three types of genus) in land types with low fertility and a slope of more than 8% when compared to in other types of land (two types of genus). The difference in mycorrhizal diversity is caused by the differences in specific ecosystems in a land (Arezlvarez-Sánchez et al., 2012; Castillo et al., 2016) that affect the state of mycorrhizal associations with sugarcane as its host (Lee et al., 2013).

The mycorrhizal genus showing the highest rate of spread was *Glomus* as it was found in all marginal land types, followed by Acaulospora. Meanwhile, Scutellospora, Gigaspora and Sclerocystis had the lowest spread rate. These results are supported by Datta and Kulkarni (2012), reporting five types of mycorrhizal genus associated with sugarcane plants in 10 regions of Maharashtra, India, including *Glomu,* which has the highest rate of spread with the greatest abundance (75.39%), *Acaulospora* (8,62%), *Scutellospora* (8,47%), *Gigaspora* (5,83%), and *Sclerocystis* (1,69%). In addition, the spread of the *Glomus* was widely enough to be recommended as bio-inoculant to increase the plant productivity, especially sugarcane.

Single-spore Culture of the Mycorrhiza

Isolation of single spore of mycorrhiza from four different types of marginal land resulted in two types of isolates, which were *Glomus* sp. and *Acaulospora* sp. Both types of these mycorrhizal isolates are eligible (70-100 spores) to be reproduced, producing 55-191 and 82 spores, respectively. Meanwhile, other single spore isolates of the mycorrhizal fungi failed to meet the required number of population of spores after single spores were grown in culture for three months (Figure 1).

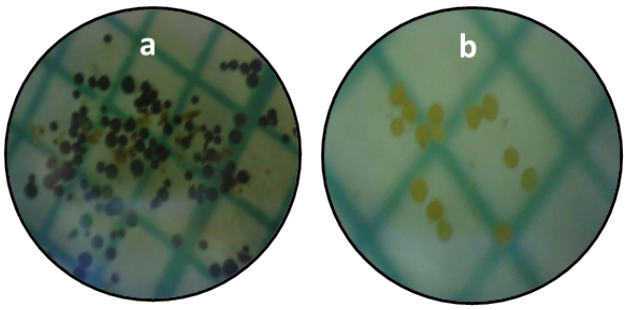

Figure 1. Set of mycorrhizal spores derived from a single spore culture: Glomus sp. (a); Acaulospora sp. (b).

The isolated mycorrhizal fungi of *Glomus* sp. were from the rhizosphere of sugarcane variety of TK 386 taken from Gowa Regency. Meanwhile, the mycorrhizal isolates of *Acaulospora* sp. were obtained from trapping the soil samples from

the rhizosphere of sugarcane variety of PS 864 in Takalar Regency. *Glomus* sp. and *Acaulospora* sp. managed to survive and produce spores optimally in single spore culture. The level of development and symbiosis of mycorrhizal mutualism depend on the type of host plant, the genotype of the mycorrhizal fungi, and the biotic and abiotic environment of the mycorrhizal strains (Jones and Smith, 2004; Silva et al., 2018). *Glomus* sp. is the dominant and most adaptive type of mycorrhizal fungi in various soil ecosystem conditions, which are related to the sporulation pattern. They dominate habitats in cold to tropical regions and thrive better in neutral and slightly alkaline soils (Suresh and Nelson, 2015). In addition, the abundance of spores and their colonization of roots are better in soil conditions with available Cu, Zn, and Fe concentrations (Datta and Kulkarni, 2012). However, compared to *Glomus* sp., the development and sporulation of *Acaulospora* sp. are better in a slightly clay soil texture (Vieira et al., 2020) and more acidic (pH 4.9-5.3) soil (Veresoglou et al., 2013), such as the soil conditions found in Takalar Regency.

Percentage of Mycorrhizal Infection in Several Sugarcane Varieties

The highest mycorrhizal infection level in this study was shown by *Acaulospora* sp. from the rhizosphere of sugarcane variety of PSJK 922 (Table 3). This means that the application of mycorrhizal inoculants significantly increased the spread of hyphae, either internal or external, in the root cortex of the plant. The external, intercellular and extracellular hyphae in the root tissue form a typical structure of mycorrhizae as revealed by Brundrett et al. (1996) that hyphae, spores and mycorrhizal vesicles found on roots showed a high degree of symbiosis between mycorrhizae and roots. This mechanism allows some life cycle of the mycorrhizae in the root of sugarcane. Furthermore, the vesicular arbuscular mycorrhizal fungi form a typical mycelia system on the plant roots and also in the soil. The structure serves as propagules for the spread and defense of the fungus in the soil.

Table 3. Average percentage of the mycorrhizal infection in root of several sugarcane varieties

Variety	Mycorrhiza (%)		Mean
	Glomus sp.	*Acaulospora* sp.	
CM 2012	73.16 **a x**	67.78 a x	70.47 y
PSJK 922	60.74 a x	86.48 b y	73.61 y
PS 862	70.56 a x	78.33 a xy	74.44 y
Cenning	57.78 a x	67.96 a x	62.87 y
Mean	65.56 a	75.14 b	

Remarks: Means followed by the same letters in the same row or column are not significantly different according to LSD´s test, $p < 0.05$.

The AMF generally infect the plant root, including sugarcane, and form an advantage symbiotic relationship (Kelly et al., 2005). The main benefit of this symbiotic is to increase the absorption of water, P nutrient and other nutrients. The AMF hyphae produce a wider and more effective root surface, resulting in bigger root volume, thereby increasing the zone of water and nutrient absorption (Jamal et al., 2004).

Higher effectiveness of the *Acaulospora* sp. (75.14%) in the symbiosis with sugarcane plant when compared to the *Glomus* sp. (65.56%) might be due to a greater infectivity level of this genera in the colonized sugarcane roots. The more infectious nature of the *Acaulospora* sp. is enabling more intra-radical and extra-radical hyphae to be formed. Extra-radical phase of mycorrhizal fungi acts as an extension of the root system to absorb mineral nutrients from the soil and helps release nutrients from the soil sorption complex, especially P, Cu, and Zn. Meanwhile, nitrogen is absorbed by the extra-radical mycorrhizal mycelia in the form of NH_4^+ or NO_3 and also in the form of amino acids. Nutrients are then transported into the intraradical structure and absorbed by the root cells in

the root cortex (Linderman, 2000; Neumann and George, 2010). Soluble nutrients like N, K, and S will be carried along with the mass flow in the process of absorption of water by the external hyphae. When released by hyphae, nutrients transported through the membrane into the cytoplasm are adjacent to the structure of mycorrhizal hyphae, which is usually a membrane peri-arbuscular (Neumann and George, 2010).

CONCLUSION

The greatest diversity of mycorrhizal genera was found in the soil samples of Jambua Block area (*Glomus*, *Gigaspora*, and *Sclerocistis*) and AJ-5 Block area (*Glomus*, *Acaulospora*, and *Sclerocistis*). The mycorrhizal genera obtained by single-spore isolation were *Glomus* sp. and *Acaulospora* sp. The results of the infection test on four sugarcane varieties commonly grown in Takalar Sugar Factory showed the infectivity level of *Acaulospora* sp. was the highest (75%) compared to that of *Glomus* sp. (66%). However, mycorrhizal association with plants also involves the formation of metabolites produced by both primary metabolites (sugars, organic acids, and amino acids) and secondary metabolites (Carotenoids, Phenylpropanoids, etc.). Therefore, to learn more about the status of mycorrhizal symbiosis, it is important to study the metabolites formed which are definitely influenced by the type of host plant, mycorrhizal strains and the condition of the soil ecosystem in which they live.

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