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Trap Culture and Colonization of Arbuscular Mycorrhizal Fungi from Corn Roots in Tidal Swamps Using Several Host Plants

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Abstract

Arbuscular mycorrhizal fungi (AMF) are mycorrhizal from the endomycorrhizal group. The fungi live on higher plants' roots by performing symbiotic mutualism. This study aimed to identify AMF spores after trapping in corn roots and the degree of root infection by AMF in several host plants. The study was conducted using tidal swamps soil samples taken from Mulyasari Tanjung Lago Village, Banyuasin Regency, South Sumatra. The experiment used a randomized block design and three replicates with four host plants as a treatment: corn, soybean, sugarcane (monoculture) and sugarcane-soybean (combination). The results showed that AMF spores found on corn roots after trapping were from the *Acaulospora* sp. and *Glomus* sp. groups. Hyphae, vesicles, arbuscules and spores are AMF structures found in the roots of host plants infected by AMF. The percentage of AMF infection in host plant roots ranged from 1.11% to 77.44% where the highest was in maize host plant roots at 77.44% in the form of internal hyphae. The maize host plant has a high potential to be colonized by AMF compared to a mixture of soybean, sugarcane and sugarcane-soybean.

Keywords: AMF spore propagation; corn; soybean; sugarcane; tidal swamps

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INTRODUCTION

As one of the agricultural countries, Indonesia has large areas of suboptimal land that can be used for agriculture with good management. The tidal swamp is a suboptimal land and is still quite prominent in Indonesia but has a low fertility rate. The widest tidal swamp land is in Sumatra, around 3.02 million ha. This land must be optimally utilized due to low land productivity, cropping intensity and unused idle land (Ritung et al., 2015). Problems in tidal swamp land occur especially in layers of pyrite or sulfidic materials, which when oxidized will cause an acidification process and are toxic to plants (Fahmid et al., 2022). Other constraints of tidal swamps are the low availability of macronutrients such as N, P and K even though the total nutrient amouant is high, especially P. The total P nutrient content in type B tidal soil in Banyuasin Regency, South Sumatra, is 106.2 ppm, while the available P is only 13.1 ppm (Sefrila et al., 2021). Continuous use of inorganic fertilizers with excessive doses on infertile land is usually one-way farmers obtain high production yields. The negative impact on the soil is that the soil becomes compact and there is little activity of soil microorganisms.

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Soil microorganisms are found in many plant roots, including mycorrhiza. Mycorrhizal fungi are cosmopolitan and almost certainly exist in soil conditions, including suboptimal land. Arbuscular mycorrhizal fungi (AMF) are mycorrhizae of the endomycorrhizal group. It lives on higher plant roots by performing mutualism symbiosis and is obligate facultative (Brundrett, 2004). In suboptimal fields, the presence of AMF will help the host plant obtain many nutrients, especially P, to increase plant growth. In addition, proper mycorrhizal management can improve soil quality and sustainability, reduce production costs and lessen environmental pollution (Muis, 2021). AMF can significantly improve the absorption of essential nutrients such as K, Ca and Mg and reduce the damaging effects of drought stress. The antioxidant defense system and osmolyte synthesis maintain phytohormone levels in the *Ephedra foliata* Boiss (Al-Arjani et al., 2020). AMF affect nutrient availability and absorption, increase the rate of photosynthesis, antioxidant activity, and tolerance to environmental stresses (Piliarová et al., 2019; Khan et al., 2022).

AMF can also protect the root from pathogen attacks and increase the tolerance of the host plant to unfavorable environmental conditions such as drought and flooding (Piliarová et al., 2019; Campo et al., 2020). In return, the host plant will provide AMF resulting from photosynthesis in the form of sugars and lipids (Keymer and Gutjahr, 2018). AMF is the main biotic component of soil, and if AMF is lost or reduced, it can cause imbalances in the ecosystem (Berruti et al., 2016). The existence of AMF in both types and quantities in each ecosystem is not always the same. Depending on the chemical conditions of the soil (environment) and the type of host plant. AMF in the form of AMF inoculants is often used as a natural biological fertilizer. The application of AMF biofertilizers can reduce the use of chemical fertilizers. The demand for AMF as a biofertilizer is gradually increasing to maintain intensive agricultural growth (Oviatt and Rillig, 2021). Efforts that can multiply AMF are by trapping culture. The trapping culture is one of the efforts made to increase the diversity of AMF spores, one of the biotic components in an ecosystem, where its presence directly plays a role in plant growth. AMF entrapment aims to multiply AMF spores by preparing pots containing host plants to which soil composites or roots of plants infected with AMF have been added. Trapping culture is usually done in a greenhouse and maintained for three months (Brundrett et al., 1996). Corn plants grown on soil-zeolite media showed the highest infection percentage and many spores compared to sorghum, citronella and scallions (Prasetia et al., 2012). The success and efficiency of the trapping culture depend mainly on the interaction that occurs with plants, the environment, AMF and agroecosystem management (Busby et al., 2017).

The selection of AMF host plants determines the success of AMF propagation in the silting culture. Fast-growing C4 plants such as maize are commonly used as host plants in trapping culture. Corn can grow well on all soil types with sufficient water and nutrient availability. However, the most significant decline in yield occurs if the plant experiences a lack of water in the flowering phase, where male and female flowers that appear will dry out, thus inhibiting the pollination process and resulting in a reduced number of seeds in the cob (Herlina and Prasetyorini, 2020). The percentage of AMF colonization on corn plant roots showed a high percentage of colonization on saline soil, former coal mining soil and forest soil (Liu and Wang, 2003).

The consortium of host plants (maize-sorghum) is more efficient in the propagation of AMF spores through trapping culture when compared to monoculture host plants of corn and sorghum (Tenzin et al., 2022). The combination of two or three host plants in a trapping culture produced more spores than one type of plant, with colonization proportions ranging from 54.5% to 68.6% (Yao et al., 2010). Host plants from the Graminae group are more suitable for AMF propagation because they have a high percentage of root colonization and a higher number of spores than plants from the Leguminosae group (Rini and Rozalinda, 2010). AMF inoculants from chilli and lemon grass plants can increase the growth of sandalwood plants (Pareira and Agu, 2021). On this basis, tests were carried out on several plants to be used as host plants, monoculture and combination in trapping culture, using tidal soil type B. This study aimed to identify AMF spores from tidal soil type B after trapping and the level of root infection by AMF on some host plants.

MATERIALS AND METHOD

The study was conducted from September to December 2020. The source of the inoculant was soil from tidal land type B, with the physical and

Table 1. Analysis of physical and chemical properties of soil samples on corn rhizosphere on tidal swamp

Analysis variables	Method	Results	Criteria
pH	H_2O	3.78	Very acidic
	KCl	3.47	Very acidic
Organic matter	C Walkley & Black	5.49%	High
	N Kjeldahl	0.24%	Medium
	C/N	22.88%	High
P_2O_5	HCl 25%	106.2 ppm	Very high
	Bray 1	13.1 ppm	High
CEC		34.80 cmol(+) kg^{-1}	High
Al^{3+}		4.94 cmol(+) kg^{-1}	
Fe		925.6 ppm	
H^+		0.80 cmol(+) kg^{-1}	
Texture (pipette method)	Sand	4.61%	
	Silt	53.21%	
	Clay	42.18%	

Source: Sefrila et al. (2021)

chemical properties of the soil as shown in Table 1. Soil samples were taken from 2 months old corn roots in Mulyasari Tanjung Lago Village, Banyuasin Regency, South Sumatra, Indonesia, which is located in 2°40'25.9" S 104°44'43.0" E.

AMF trapping used three host plants: corn, soybean and sugarcane. The experiment used a randomized block design and three replicates with four host plants as a treatment: corn, soybean, sugarcane (monoculture) and sugarcane-soybean (combination). Each treatment consisted of 10 pots. The isolation and analysis of AMF spore types were carried out in the Biosystems and Landscape Management laboratory of SEAMEO BIOTROP.

Preparation of the host plant

Three host plants were used to test mycorrhiza multiplication: maize, soybean and sugarcane. The sugarcane plant came from the bud chip-shaped PS 862 variety sugarcane seeds that had been seeded first for four weeks on sand media. The corn and soybean seeds used as host plants were first soaked in a sodium hypochlorite solution (5%) for 5 minutes to sterilize the surface, then rinsed with water. Next, the seeds were soaked in warm water (40 °C) for 24 hours to stimulate germination. Then, the corn and soybean seeds were seeded in a seedbed filled with sand at a distance of 1 cm x 1 cm and watered two times a day using a sprayer up to 2 weeks of age. The sugarcane, corn and soybean seedlings were transferred into the culture pot.

AMF trapping

The trapping culture followed the Brundrett method (Brundrett et al., 1996) by using a culture pot with a diameter of 10 cm. The planting medium was a sample of 50 g soil taken from tidal swamps and zeolite rocks with a size of 1 mm, as much as 150 g. Before use, zeolite was sterilized by washing it with water until it was clear, soaked in boiling water and dried.

The planting media in the culture pot were prepared through the following methods. The culture pot was filled with 100 g of zeolite, added with tidal swamps soil of as much as 50 g, and finally covered with zeolite as much as 50 g so that the planting medium was composed of zeolite-soil-zeolite examples. The maintenance of the culture pot includes manual watering, fertilizing and pest control. Fertilizing using compound fertilizers (20-15-15) with a concentration of 1 g per liter of water was given once every week, as much as 50 ml per culture pot. After the plant was 90 days old, harvesting was carried out. Harvesting was done by cutting off the roots of the plant and then washing it thoroughly in running water, and then staining was carried out using the method of Brundrett et al. (1996).

Root coloring was performed using the Vierheilig et al. (1998) method modified by Nusantara et al. (2012). The roots were washed thoroughly under running water, then soaked in KOH 10% for 12 to 24 hours. If the roots remained dark in color and a few drops of alkaline

H_2O_2 were added, then washed under running water 3 to 5 times using a tea filter as a container. The washed roots were soaked in a 5% ink-vinegar solution for 24 to 72 hours. Next, the roots were immersed in a destaining solution to remove the excess color solution, and the roots were then cut 1 cm long and placed in a row on the glass of the object. After the roots were colored, they were under a Nikon eclipse microscope with 40x magnification or Xiamen binocular microscope (Nusantara et al., 2012). Morphological identification of AMF spores was carried out up to the genus level to the criteria proposed by The International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) and several journal literature from the International Mycological Association (IMA) (Oehl et al., 2011). Data analysis was performed using descriptive analysis through data tabulation. The percentage (%) of roots colonized by AMF used the Equation 1.

$$\% = \frac{\text{Number of infected roots}}{\text{Number of observed roots}} \text{ x } 100\% \quad (1)$$

The resulting data were analyzed using analysis of varians (ANOVA) to determine if there was an actual effect, followed by the least significant different (LSD) test at a basic level of 5%. Observing data were processed using statistical tool for agricultural research (STAR) and Microsoft Excel 2021.

RESULTS AND DISCUSSION

Identification of AMF spore after trapping culture on corn root

AMF trapping with corn root showed the *Acaulospora* sp. and *Glomus* sp. groups dominate AMF spores. They are mainly *A. mellea*, *A. scrobiculata*, *Glomus* sp., *Acaulospora* sp., *G. manohotis* and *G. monosporum* (Figure 1). The type of host plant influences AMF diversity has been reported (Lu et al., 2022). AMF spores of *Glomus* sp. are the dominant spores found on mineral and peat soils (Yuwati et al., 2020).

Acaulospora has a spore wall with 2 to 3 relatively thick layers, yellow to brownish color, and round and oval shapes. In contrast, *Glomus* has a round shape, with many spore walls consisting of more than one layer. The color of the spores of the genus *Glomus* is clear, yellow, to brownish-yellow. Spores of *A. mellea* are brownish-yellow, 87.5 to 135 µm in size, and three layered. The first layer is thin and transparent and round to oval in shape. *A. scrobiculata* has yellow spores with blackish strokes in the middle, spore size of 80 to 165 µm, and three layers with a rounded spore shape (Straker et al., 2010).

AMF colonization of several host plants root in tidal swamps

The results of observations of the roots of the host plant showed the colonization of AMF with the presence of vesicular, arbuscular, spore

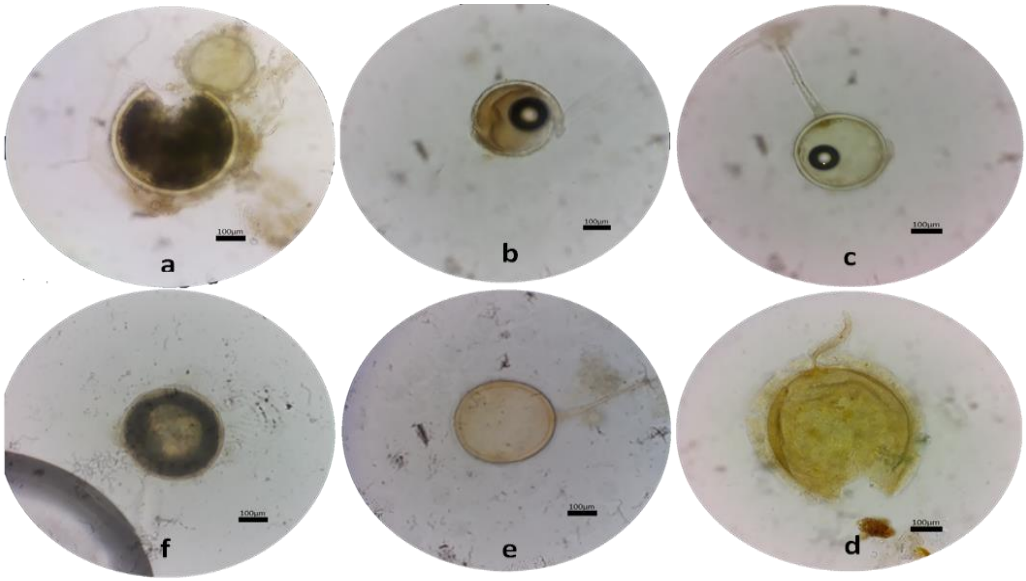

Figure 1. AMF spore type diversity after trapping on corn root a) *A. mellea*; b) *A. scrobiculata*; c) *Glomus* sp.; d) *Acaulospora* sp.; e) *G. manihotis*; f) *G. monosporum*

Table 2. Percentage of root infection and spore density of corn, soybean, sugarcane and sugarcane-soybean crops

Host type	Root infection (%)					Spore density (per 10 g soil)		
	Ih	Eh	Ve	Sp	Ar	76 DAP	83 DAP	90 DAP
Corn (*Zea mays* ssp.)	77.44±4.30^{a} high	0.33±0.58 low	35.00±5.78^{a} high	43.44±4.55^{b} high	40.33±9.02^{a} high	317.6±168.58	372.00±301.69	62.3±35.79
Soybean (*Glycine max* Merr.)	64.11±1.26^{a} high	1.78±0.38 low	13.00±4.91^{b} medium	67.22±2.83^{a} high	31.17±19.81^{ab} high	580.6±215.35	149.6±88.62	113.3±121.39
Sugarcane (*Saccharum officinarum* Linn.)	19.22±2.59^{b} medium	2.00±2.31 low	1.22±0.51^{c} low	25.44±3.60^{c} medium	2.67±0.88^{c} low	166.3±36.95	124.0±60.09	122.6±129.51
Sugarcane-soy (*Saccharum officinarum* Linn-*Glycine max* Merr.)	35.17±16.20^{b} high	1.11±0.63 low	1.44±1.07^{c} low	41.94±3.77^{b} high	10.67±2.29b^{c} medium	177.3±213.60	32.6±14.18	21.3±14.15

Note: Numbers followed by the same letter in the same column are not significantly different based on the LSD test at $\alpha = 5\%$. The mean value is followed by the standard error (SE) value. Ih = internal hyphae; Eh = external hyphae; Ve = vesicles; Sp = spore; Ar = Arbuscular; DAP = days after planting; high (> 30); medium (10 to 30); low (< 10); non-colonized (< 0)

and hyphae structures at the roots of host plants (Figure 2). The discovery of the main structure of AMF, namely arbuscular and vesicular, in plant roots indicates that colonized root host plant were characterized by internal hyphae, external hyphae, vesicular, arbuscular and spore AMF in the roots host plant. A previous study found that the infected roots of several arabica coffee varieties colonized by AMF have spores, hyphae and vesicle structures (Syahputra et al., 2021).

The colonization structure can be different among locations. The structure of AMF colonization in mineral soils is in the form of vesicles and internal hyphae, while in peat soils in the form of vesicles, internal hyphae and spores (Yuwati et al., 2020). Vesicular and arbuscular are the main structures of AMF to aid nutrient absorption for plants (Diastama et al., 2015).

The results of the analysis of variants showed that the type of host showed a significant effect on the infection of host plant roots by AMF in the form of internal hyphae, vesicles, spores and arbuscles, but had no significant effect on the percentage of infection of host plant roots by AMF in the form of external hyphae; the number of spores was 76, 83 and 90 days after planting (DAP) (Table 2). The percentage of plant root colonization can be seen from the presence of AMF structures, namely internal hyphae, external hyphae, vesicular, arbuscular and spores. In the propagation process of AMF (trapping) corn, soybeans, sugar cane, and a mixture of sugarcane-soybeans are used with different infection percentages and AMF spore densities.

Host plant bases corn plants have a high potential to be infected by AMF, especially 83 DAP. Because corn at that age can produce more spores than other host plants, the corn root structure can be more easily infected by AMF and has root fibers. Corn and sorghum plants showed increased sporulation dynamics compared to other host plants, such as soybeans. This increase occurred 80 DAP and then continued to decrease (Muis et al., 2016).

The percentage of root infection of monoculture host plants (maize and soybean) was more significant, when compared to sugarcane monoculture and combination hosts (sugarcane-soybean). The percentage of AMF infection in host plant roots ranged from 1.11% to 77.44%; the highest (77.44%) was found in maize host plant roots in the form of internal hyphae. The structure of sugar cane roots is harder than the structure of corn and soybean roots. In addition, there is a competition between

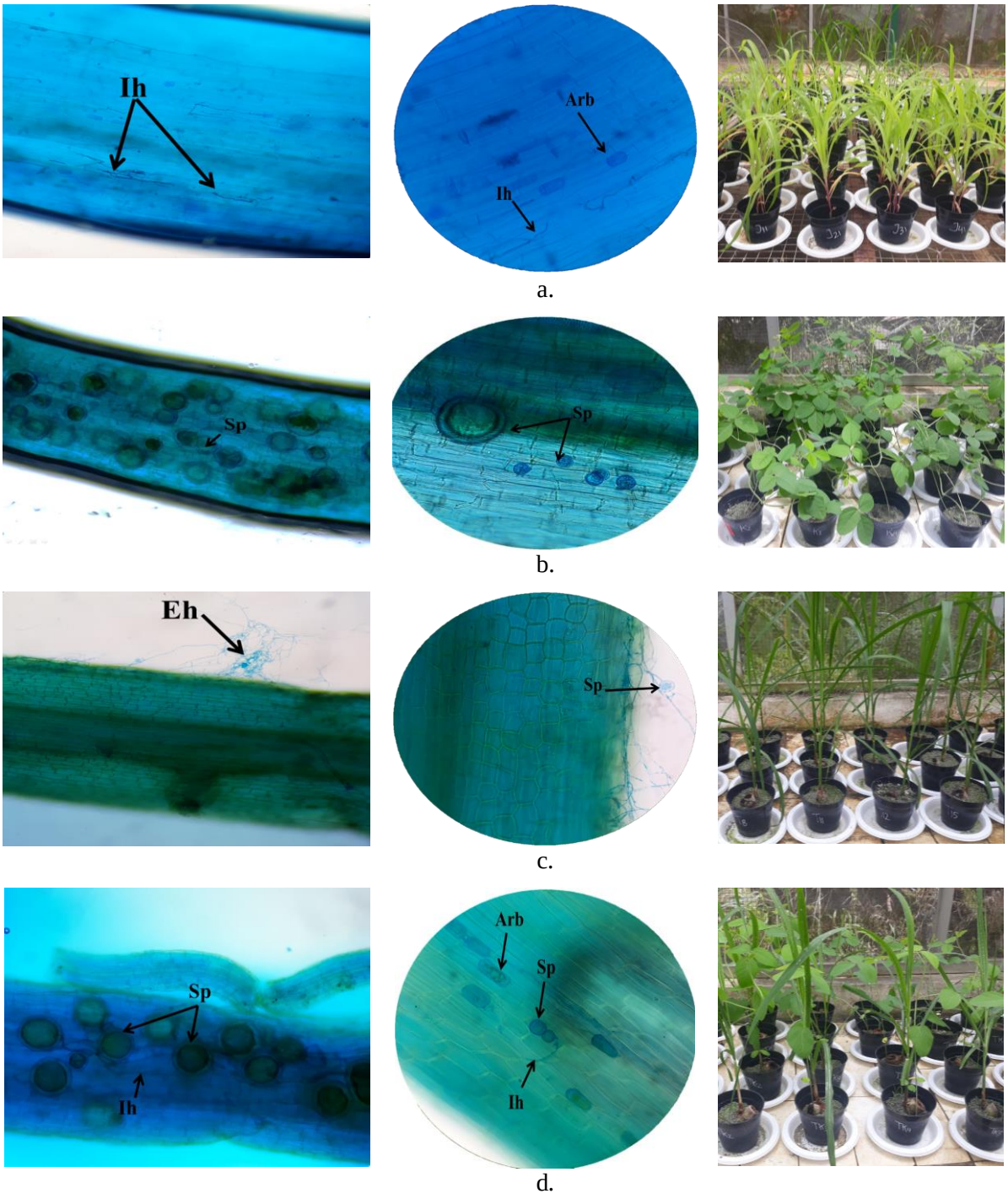

Figure 2. Colonization of AMF on a) corn root; b) soybean root; c) sugarcane root; d) sugarcane-soybean root. Ih = internal hyphae; Eh = external hyphae; Sp = spore; Ve = vesicle; Arb = arbuscular

two different species, sugarcane and soybeans. Presumably, there is competition in absorbing nutrients between the two species, sugarcane and soybean. The success of the propagation of AMF spores depends on the interaction between plant species and the environment. In addition, the combination-cultivation of corn and sorghum results in interspecific competition between hosts (Tenzin et al., 2022). There is an interaction between the host plant type and the inoculum source against the degree of infection and P uptake of the plant. The AMF of the rhizosphere origin of the host plant, which is the same as the host plant type, is more compatible than

the AMF of the rhizosphere origin of the host plant, which is different from the host plant type (Nurhayati, 2019).

Host plants of maize, soybean, sugarcane, and sugarcane-soybean combinations have different colonization responses and AMF spore densities; each plant has a different metabolic reaction. Root exudates of different plant species can affect the germination and growth of AMF species (Douds et al., 1996). C4 host plants such as maize and sugarcane have the potential to be used as host plants for the multiplication of AMF spores and sources of inoculants. Although both are classified as C4 plant species, maize and sugarcane have different responses to AMF, as seen from the number of spores and the percentage of AMF colonization. They are different in anatomy and life cycle. After the first (120 DAP) and second (240 DAP) cycles, maize plants had a higher percentage of AMF colonization than sorghum (Selvakumar et al., 2016). *Echinochloa crus-galli* (monocotyledon) is a better host than *Vigna radiata* (dicotyledon). It can maximize AMF propagation (Abd Rahim et al., 2016). The number of spores after trapping will increase compared to before trapping with different plants (Zulfita et al., 2020). The trapping culture causes changes in spore type and increases spore density in soil samples from forest cloves (Mahulette et al., 2021). High spore density and arbuscular mycorrhizal spore colonization during trapping have led to increased growth and biomass of maize and sorghum host plants (Husein et al., 2022).

In addition to the type of host plant, the amount and diversity of AMF are influenced by environmental factors, including soil acidity (pH) of 3.47 to 3.78 and nutrient content. The results of the chemical analysis of tidal soil in Mulyasari Tanjung Lago Banyuasin village have a high organic C and C/N content of 5.49% with a C/N ratio of 22.88% (Table 1). The highest number of AMF spores was found at low pH, total P and organic C, whereas a low number of AMF spores was found in soils with high water content and total P values (Yusriadi et al., 2018; Sukmawati et al., 2021). The degree of AMF infection in coffee roots is influenced by the height of the place and the organic content of the soil (3.79% and 3.56%) (Sinaga et al., 2015). AMF spore populations are closely related to soil organic carbon (Salim et al., 2019). Soil organic carbon is one of the abiotic factors in ecosystems affecting the number of spores in plant roots (Aji et al., 2021). A sufficiently large population of AMF spores can only sometimes increase root colonization. The chemical properties of the soil can affect the colonization of roots by AMF, especially Mg (Salman et al., 2020). Spore density affects the level of infection at plant roots; when the number of spores is higher the roots of plants that are colonized AMF will also increase. Besides that, the depth of the soil will affect the spore density; the more profound the soil, the lower (Rasyid et al., 2016) the vegetation type and distance affect the number of AMF spores (Natalia et al., 2016). Changes influence the abundance, activity, and impact of AMF on ecosystems in soil conditions and host plant types (Soka and Ritchie, 2015).

CONCLUSIONS

It can be concluded that AMF spores found on corn roots after trapping were from *Acaulospora* sp. and *Glomus* sp. groups. Hyphae, vesicles, arbuscules and spores are AMF structures found in the roots of host plants infected by AMF. The percentage of AMF infection in host plant roots ranged from 1.11% to 77.44% where the highest was in maize host plant roots of 77.44% in the form of internal hyphae. The host plant of maize is a plant that has a high potential to be colonized by AMF compared to a mixture of soybean, sugarcane and sugarcane-soybean, especially in the early generative phase. It is necessary to carry out molecular tests on the roots of plants infected with AMF by comparison with plants that are not infected; it may be identified that metabolite compounds also influence the occurrence of infection in host plants.

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