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Mycorrhiza Diversity in Some Intercropping Systems of Potato (*Solanum tuberosum* L) and Faba Bean (*Vicia faba* L)

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) is the most widely distributed mycorrhizal fungi in the soil and can make a symbiosis with the roots of host plants to form arbuscular mycorrhizal symbionts. Intercropping is a practice of polyculture cropping where two or more plant species are simultaneously cultivated in the same field. The objective of this study was to define the effect of intercropping on the density and diversity of mycorrhizal spores. In this study, potatoes and faba beans, both of which have the ability to symbiosis with mycorrhizae, were intercropped. A randomized group design with 5 planting system treatments was employed in this study with 5 replications. The results concluded that density of mycorrhizal spores in the intercropping planting pattern was not statistically different from the density of mycorrhiza in the monoculture cultivation pattern. The types of mycorrhiza found included the genus of Glomus, Funneliformis, Scutellospora, Cetraspora, Septoglomus, and Entrophospora.

1. INTRODUCTION

Intercropping is a polycultural cropping practice in which at least two species of plants are cultivated in the same field at the same time to increase crop production, more efficient land use, and the intercrops have significant promise for improving the sustainability of current crop production (Bargaz *et al.*, 2021). Legumes are often used in intercropping due to the ability of their roots to form symbiosis with rhizobium which provides nitrogen (N) for plants (Liu *et al.*, 2020), and with Arbuscular Mycorrhizal fungi (AMF) as well which mediate the increase plant uptake of phosphorus (P) (Püschel *et al.*, 2017).

Intercropping affects the diversity of soil spores in the rhizosphere of plant roots (Johnson *et al.*, 2004; Muleta *et al.*, 2008; Chifflot *et al.*, 2009; Bainard *et al.*, 2011). Muleta *et al.* (2008) reported that the spore density of AMF in an agroforestry-based intercropping system between coffee and legumes had a much higher spore density and diversity of AMF spore populations compared to the coffee monoculture system, and the

greatest spore populations were discovered in the topsoil (0 – 30 cm). Chifflot *et al.* (2009), also reported the same thing, where tree-based intercropping between soybean plants and poplar trees had a higher diversity and abundance of AMF as compared to those of monoculture planting systems.

One form of intercropping that can be found in the highlands is potato and legume faba bean (*Vicia faba* L). Random intercropping of potatoes and faba beans can be found in Dieng, Central Java and has the potential to become an ecological cropping system that conserves soil quality (Banjarnahor, 2017). Faba bean interacts with mycorrhizae due to the presence of root exudate which supports the association of mycorrhizae to faba bean. In addition, the characteristics of faba bean as a legume plant, make the symbiosis with mycorrhiza more beneficial because it secretes more organic acid root exudate than that produced by other dicot plants (Li *et al.*, 2007). Root exudates are reported to play an important role in regulating AMF-plant interactions. The addition of root exudate can significantly stimulate the germination rate of AM fungal spores (Tian *et al.*, 2021).

Legume plants increase the plenty and diversity of mycorrhizae in the soil around their roots (Pivato *et al.*, 2007). Legumes can affect mycorrhizal associations with host plants by secreting more root exudates, even increasing when legumes are grown in intercropping (Liu *et al.*, 2019; Tian *et al.*, 2021). Faba bean grown in intercropping produced more root exudate than when grown in monoculture (Liu *et al.*, 2019; Li *et al.*, 2019). Root exudates such as strigolactone, another phytohormone, are reported to regulate; regulate spore germination (Foo *et al.*, 2013; Singh *et al.*, 2022). In addition, root exudates such as quercetin and strigolactone act as signals for spore germination (Marschner, 2011).

Based on the above exposition, study aims to determine the effect of monoculture cropping patterns and intercropping of potato and faba bean on AMF diversity in the root rhizosphere.

2. MATERIALS AND METHODS

The research was conducted at the end of the rainy season, namely April 2022 until the middle of the dry season in August 2022, at the Salaran Experimental Garden, Faculty of Agriculture and Business, Satya Wacana Christian University (SWCU), Wates Village, District of Getasan, Semarang Regency, Central Java Province, at the coordinate point -7.37524 S, 110.42564 E with an altitude of ± 1117 m and a daily average temperature of 29 °C. The amount of rainfall in the study area per month is in the range of 42 – 427 mm, with the highest rainfall in July and the lowest in August.

2.1. Experimental Design

The experiment was arranged in the form of a randomized block design (RBD) with 5 treatments namely 1) potato monoculture (KM), 2) intercropping of potato-fab bean with pattern 1 : 1 parallel (TS 1 : 1 parallel), 3) intercropping of potato-faba bean with pattern 1:1 top-down (TS 1 : 1 top-bottom), 4) intercropping potato-faba bean with pattern 2:1 top-bottom (TS 2 : 1 top-bottom), and 5) monoculture faba bean (FM). In each block there were 5 treatment cropping patterns which were applied to experimental units with a size of 5.5 m x 3.1 m. There were 7 beds measuring 150 cm x 40 cm in each treatment plot. The distance between the beds is 60 cm. Each bed is covered with plastic mulch.

2.2. Research Procedure

Potatoes in each treatment, both monoculture and intercropping, were planted in beds with a spacing of 30 cm. Faba beans are planted in beds or trenches between beds according to the type of treatment. The spacing between the beds and trenches wass 10 cm. Fertilizers in this study were manure, bokashi fertilizer, M-21 decomposer liquid fertilizer, and Pak Tani NPK 16-16-16 fertilizer. The dose of manure per planting hole is 25 g for potatoes and 15 grams for faba beans, and the M-21 decomposer liquid fertilizer is 3 M-21 bottle caps per 15 liters of water. The application of manure and M-21 decomposer liquid fertilizer is given at the beginning of planting by sowing and pouring in the planting hole. Fertilizers for bokashi and NPK 16-16-16 are given at a dose of 25 g/planting hole for potato plants and 8 g/planting hole for faba bean plants. Bokashi fertilizer and NPK 16-16-16 were applied in the middle of planting time by sowing in the planting hole. Application of contact and systemic fungicide namely Daconil 75 WP and folirfos 400 SL with active ingredients 75% chlortalonil and 400g/l phosphoric acid were given 30 days after planting, at doses of 1.5 g/l and 8 ml/l. The fungicide is applied by spraying it directly to the potato plants.

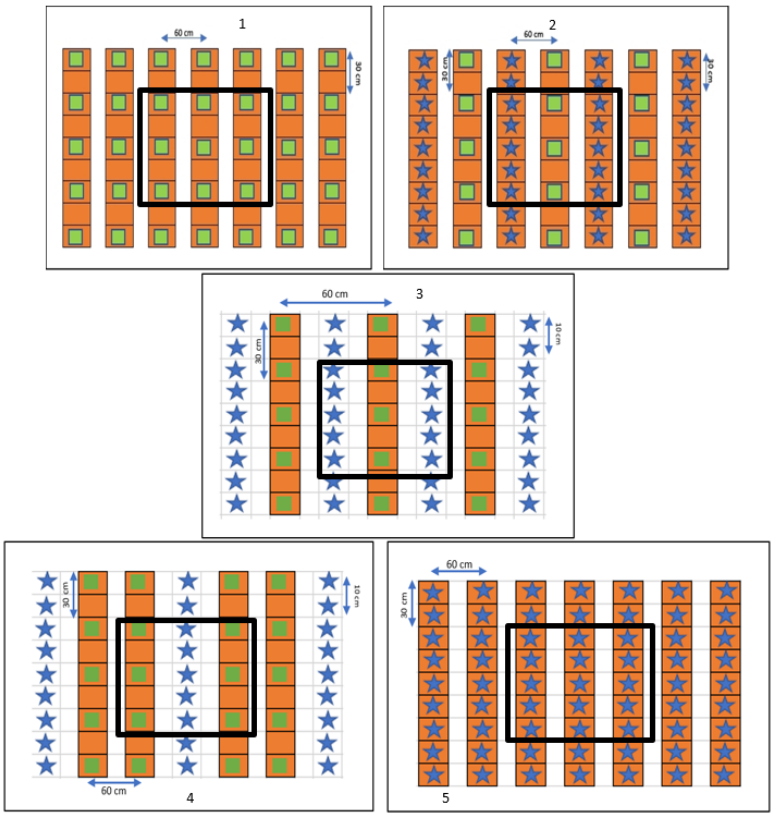

Figure 1. Sketch of cropping pattern with intercropping: 1) Monoculture of potato (KM), 2) Intercropping TS 1 : 1 parallel, 3) Intercropping TS 1 : 1 top-bottom, 4) Intercropping TS 2 : 1 top-bottom, and 5) faba bean monoculture (FB). (Black squares are soil sampling locations)

Mycorrhizal spore density was analyzed using a wet filter pour method (Pacioni, 1992) and followed by centrifugation (Brundrett *et al.*, 1996). Briefly, 1 kg of soil samples were taken compositely around the roots of the potato and faba bean plants, namely at a soil depth of 20 cm and a radius of 20 cm from the plant stem. Location of

plant and soil samples was determined based on the black squares in Figure 1. Steps in spore isolation were as the following: 1) 50 g of soil sample was added with water until 500 ml and stirred; 2) soil suspension was transferred into a multilevel filter from top to bottom with sizes of 400 µm (40 mesh), 250 µm (60 mesh), and 45 µm (325 mesh); 3) during the analysis, no mycorrhizal spores were found in the 250 µm (60 mesh) or 40 mesh filters in all cropping pattern treatments, both monoculture and intercropping, so that the soil suspension filtered on the 45 µm (325 mesh) filter was put into a test tube and then added with 60% sucrose solution up to the tera limit; 4) it was then centrifuged for 5 min at 5000 rpm; 5) the slightly clear liquid at the top of the tube (floats) was a transition from glucose to water solution, it was aspirated using a pipette to be washed and filtered with filter paper; 6) The results were filtered on filter paper and placed in a petri dish to be observed under a stereo microscope to calculate the spore density. To observe the number of spores and the diversity of spores in the intercropping pattern of potatoes and faba beans, samples were taken only from the soil around the roots of the potato plants.

AM spores was identified using direct observation analysis method on mycorrhizal spores. Spore specimens were taken in a petri dish with the help of a stereo microscope and a toothpick placed on a slide. Observations were made at a binocular light microscope by looking at the morphological characteristics of the spores, namely based on size, shape, color, number of spore walls, and were identified based on INVAM guidelines and comparisons of previous research journals.

The actual pH measurement was carried out by dissolving the soil sample in distilled water, then measuring it with a pH meter (Sulaeman *et al.*, 2005). In short, 5 grams of soil sample is put into the beaker glass. Then, 25 ml of distilled water was added. Next, the samples were shaken for 10 minutes using a shaker. The acidity level of the samples was measured using a pH-meter.

2.3. Data analysis

To see the effect of different intercropping treatments on spore density and soil pH, a one way ANOVA analysis test was performed using SPSS 25 with an analysis of variance performed for all test data. Data are expressed as means ± Standard deviation with n = 25 (spore density and soil pH). To identify the type and diversity of AM spores were analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1. Spore Density and Soil Acidity

Based on the test of variance, there was no significant difference in spore density and soil pH between the 5 treatments tested (Table 1). In all treatments, the spore density in all treatment cropping patterns ranged from 723-923 spores/50 g soil and the pH ranged from 6.98 to 7.10 (Table 2).

Table 1. Recapitulation of the results of the test of variance

Parameter	Unit	F calculated	KV %	F Table	
				5%	1%
Spore density	/50 gram	1.45	18%	2.59	3.88
Soil pH	pH	2.36	5%	2.59	3.88

Table 2. Spore density and soil pH for different cropping pattern

Treatment	Parameter	
	Spore density	pH
KM	853.80 ± 84,64	6.98 ± 0.42
TS 1 : 1 Parallel	722.60 ± 70,31	7.10 ± 0.25
TS 1 : 1 Top-bottom	884.20 ± 178,33	6.86 ± 0.27
TS 2 : 1 Top-bottom	918.00 ± 225,32	6.91 ± 0.42
FM	935.80 ± 139,35	6.84 ± 0.78

Note: data is shown as mean and standard deviation with *n* = cropping pattern treatment (*n* = 5)

Treatment of the cropping pattern both in the intercropping treatment and the monoculture treatment produced spore densities which were not statistically different among all treatments. This occurs because potato and faba bean plants are able to produce flavonoids, such as quercetin, in their roots (Ehiobu *et al.*, 2022; Y. Liu *et al.*, 2019; Maj *et al.*, 2010; Rashid *et al.*, 2017). From these results, it can be stated that potato and faba bean produce flavonoids, even though they are grown in monoculture or intercropping. However, these results are not in line with the research of Liu *et al.* (2019), who stated that more flavonoids are secreted by faba bean cultivated in intercropping than that of grown in monoculture. In addition, the amount of flavonoids secreted by faba bean roots cultivated in intercropping rose significantly by 31.7% at the insufficient N levels and by 35.6% in appropriate N levels, as compared to those of grown monoculturaly. Particularly, it is noted that flavonoids like quercetin and quercitrin can increase the germination bioactivity of AMF spores (Badri & Vivanco, 2009; Y. Liu *et al.*, 2019; Mandal *et al.*, 2010; Scervino *et al.*, 2005; Steinkellner *et al.*, 2007).

The spore density which was not statistically different from all treatments could be caused by seasonal changes in the study area, namely a change in season from the rainy season to the dry season, with an increase in spore density during the dry season as shown in Table 3.

Table 3. Differences in spore density in different seasons

Season	Spore density/50 gram
Rainy season	451 - 565
Dry season	545 – 1143

The spore density which was not significantly different among treatments could be caused by seasonal changes in the study area, namely a change in season from the rainy season to the dry season, with an increase in spore density during the dry season as shown in Table 3. From the results in Table 3, it was found that the density of spores in the dry season was higher than in the rainy season. Some studies have been reported and similar findings support the above hypothesis (Ramírez-Gómez *et al.*, 2019; Silva *et al.*, 2014; Sivakumar, 2013). In research from Ramírez-Gómez *et al.* (2019) it was stated that in the rainy season, there were anything from 20 to 120 spores per 10 g soil, while the dry season had the greatest spore numbers, ranging from 170 to 1531 spores per 10 g soil. Higher spore density in the dry season is considered an indication of root aging and available nutrients, stimulates development and growth of fungal sporulation due to the availability of groundwater, high soil temperature, decreased soil moisture, and increased oxygen concentration so that the

dry season is a period of spore production. The largest FMA (de Souza *et al.*, 2016; Jerbi *et al.*, 2020; Saravanakumar *et al.*, 2008). However, other studies contradict this result, among others are Oliveira & Oliveira (2005) and Khaekhum (2017) which state that the spore density of soil samples in the rainy season is higher than that of the dry season. The same was reported by Ramos-Zapata *et al*. (2011) and Luo *et al.* (2016) where the increases of spores amount during the rainy season is caused by flood that move spores to low-lying areas. In addition, many AMF propagules are transported by water in wetlands and continue to grow in the soil after flooding has passed.

The differences in crop patterns in this study did not affect soil pH where there was no significant change in pH in all cropping pattern treatments. The pH range in this study is included in neutral pH. A neutral soil pH reaction close to 7.0 is beneficial for AMF growth (Yu *et al*., 2020). Acidic soils create conditions that do not support AMF activity (Tahat & Sijam, 2012). The direct impact of the soil environmental pH reaction on AMF is restricted to the stages of sporulation and sprouting of AMF spores in the rhizosphere zone. Guo *et al*. (1996) reported that more spores were discovered in soil with a neutral pH as compared to that of soil with a acidic pH. The study of Liu *et al.* (2020) also said the same thing that the development and abundance of mycorrhizae is hampered in acid soil (pH 4.5) compared to that of soil with higher pH (6.5) due to inhibition of biosynthesis in mycorrhizae in two different ways, namely reducing mycorrhizal abundance and reducing alkaline phosphatase activity. The same thing also happens in basic or alkaline soils. Priya *et al.* (2014) reported that the spore density ranged higher, namely 420 - 820 spores at pH 7.8 - 7.4 and very few 36 - 180 spores at pH 8.8 - 8.2 which was caused by better spore development only in neutral to slightly alkaline soils than that in alkaline or saline soils. Higher pH also results in poor AMF diversity, caused by poor root development or vegetation, low soil fertility, and bad physicochemical conditions (Parihar *et al.*, 2019).

3.1. Spore Diversity

There were 8 spore genera found in each treatment, namely *Glomus*, *Funneliformis*, *Scutellospora*, *Cetraspora*, *Septoglomus* and *Entrophospora* in each treatment. The morphological characters used to identify the type of spore, size, shape, color, and number of spore walls, were identified based on INVAM guidelines and a comparison of previous research journals. The AMF spore genera found are listed in Figures 2. The mycorrhizal genera found in each treatment were similar to the mycorrhizal species in the literatures. Figure 3 shows the mycorrhizal genera collected from literatures and their resemblance to the mycorrhizae in our study.

Morphologically, the genus *Glomus* 1 (Figure 2A) is characterized by a sporocarp, spherical spores, 196.56 µm in diameter, yellow-brown or dark brown, smooth spore surface, and hyphae attached to the spore wall. The morphological of the genus *Glomus* 2 (Figure 2B) is characterized by a single spore, 202.53 µm in diameter, yellow-brown in color, rough spore surface, and spore wall consisting of two separate layers. According to Rodrigues & Rodrigues (2020), morphological characteristic of *Glomus* includes spores that grow at the ends of sporogenous hyphae or as the bulging of intercalary subglobose on the inside of sporogenous hyphae. Spores are typically attached to a single subtending hyphae that is simple, straight, or twisted, and infrequently swell. Spores are generated singly or in solid or loose sporocarps that often lack or contain peridium. Spores are generally globose to subglobose; seldom elliptical (Rodrigues & Thangavelu, 2009); hyaline, yellow, brown, or black with no less than two or three distinctive wall sheets (commonly inner wall sheets are laminated).

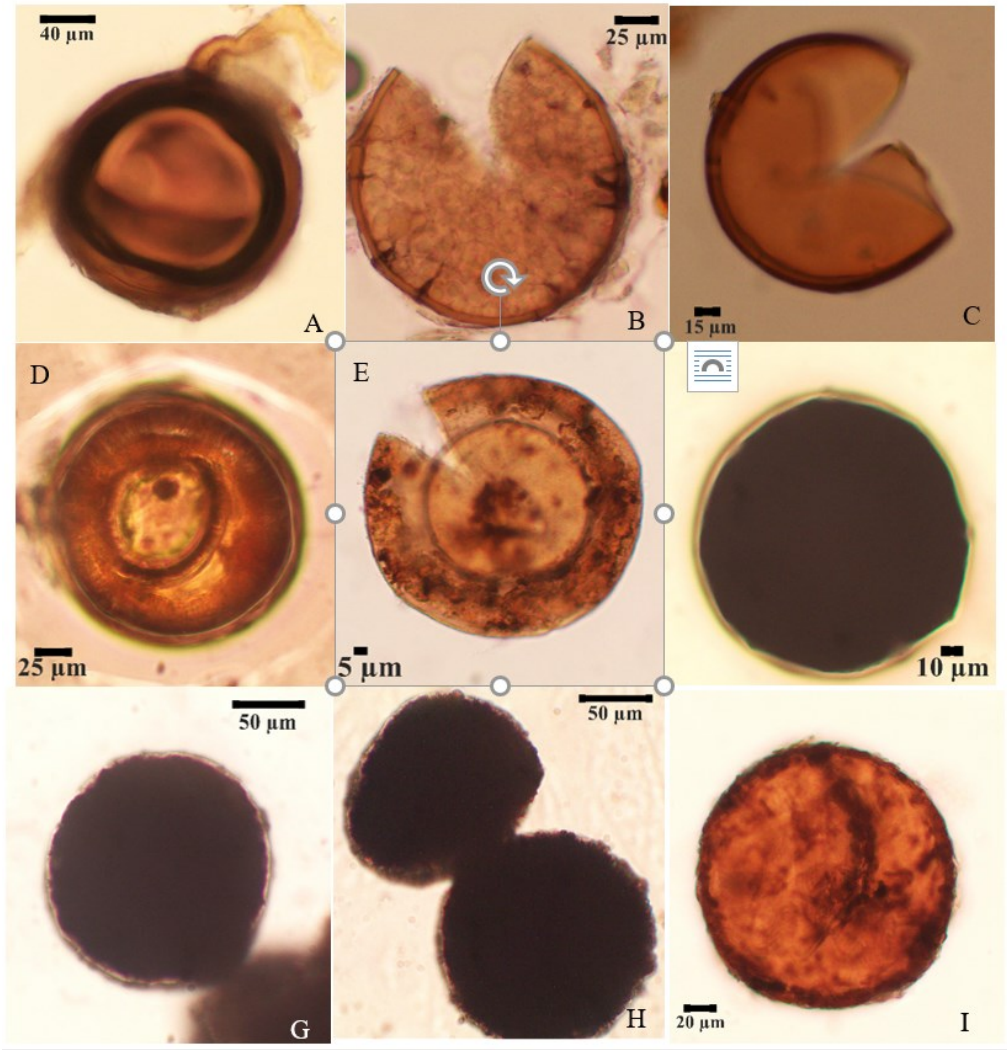

Figure 2. Genus of FMA spores found in each treatment: A – B: *Glomus*, C: *Funneliformis*, D: *Scutellospora*, E: *Cetraspora*, F: *Septoglomus*, and G – I: *Entrophospora*

The genus *Funneliformis* (Fig. 2C) is a single spore, 176.05 µm in diameter, yellow to dark brown, the spore surface is smooth, and the spore wall is composed of two separate layers. The genus *Scutellospora* (Figure 2D) is characterized by a single spore, 204.02 µm in diameter, orange-brown in color, has a layered spore wall, and a smooth spore surface. The genus *Scutellospora* in Figure 2D is similar to the species *Scutellospora* bionata similar to the study by Alimi *et al.* (2021). According to INVAM (2023b), the *Funneliformis* genus has a spore wall usually consisting of two or three layers. The outer layer is hyaline and often sloughs off with maturation of the spores. The genus *Cetraspora* (Figure 2E) is characterized by a single spore, 188.91 µm in diameter, dark brown yellow in color, rough spore surface, and has 3 layers of spore wall. The genus *Septoglomus* in Figure 2F is characterized by a single spore, 180.49 µm in diameter, dark brown in color, smooth spore surface, and has 1 layer of hyaline spore wall, while the second spore layer is fused with the inner layer of subtending hyphae. According to Palenzuela *et al.* (2013), the genus *Septoglomus* has morphological characteristics including spores with diameter of 132-205 µm, have a 2-3 layer spore wall, and produce spores singly or in groups in the soil. In addition, the

spores of *Septoglomus* have color of dark reddish brown to dark reddish black. The spore color of the three others *Septoglomus* spp. types are darker to black (Błaszkowski *et al*., 2014).

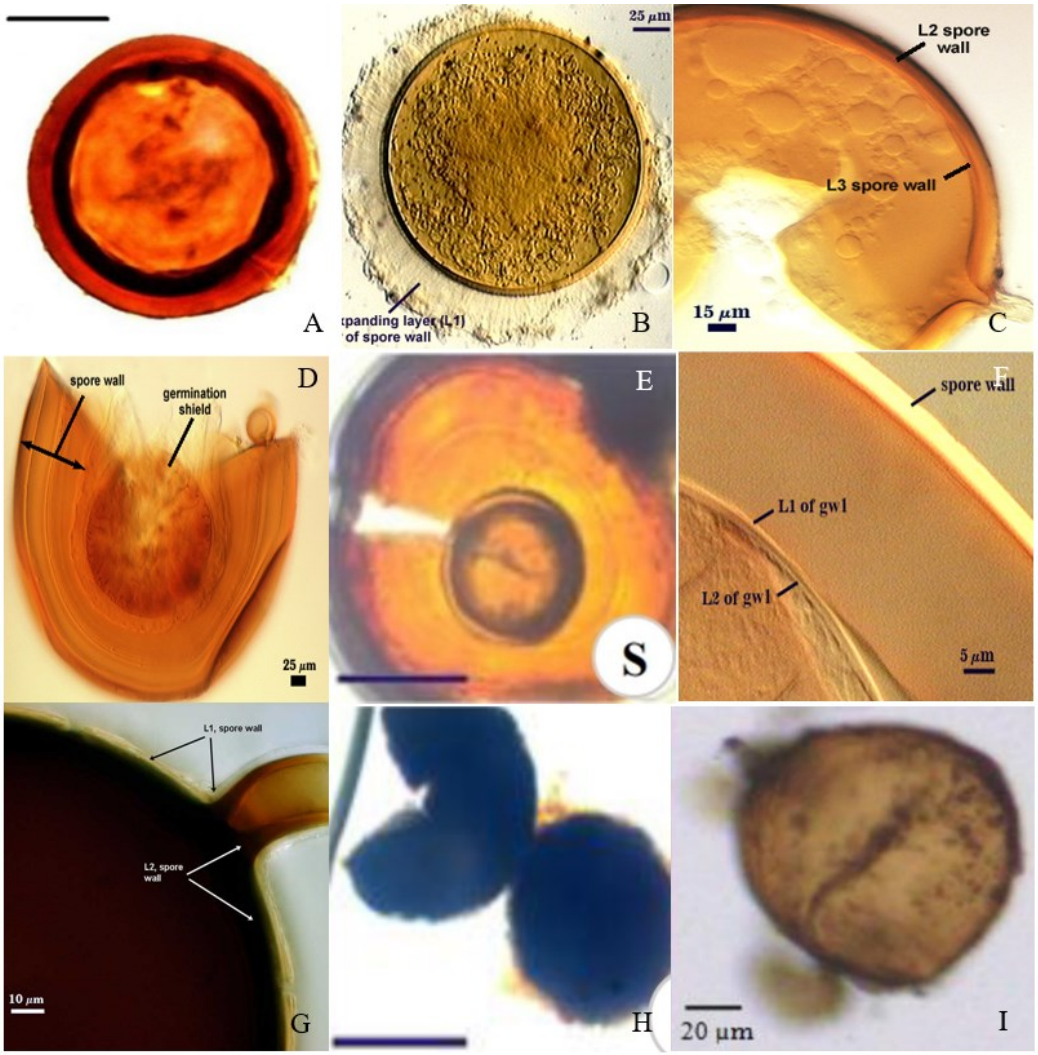

Figure 3. Mycorrhizal genera found in literatures and their similarity to our results. A: *Glomus macrocarpum* (Surendirakumar et al., 2019) similar to *Glomus* 1 (Fig. 2A); B: *Glomus pansihalos* (INVAM, 2023d) similar to *Glomus 2* (Fig. 2B); C: *Funneliformis verruculosus* (INVAM, 2023c) similar to *Funneliformis* (Fig. 2C); D: *Scutellospora bionata (*INVAM, 2023e) and E: *Scutellospora bionata (*Alimi et al., 2021) similar to *Scutellospora* (Fig. 2D); F: *Cetraspora pellucida* (INVAM, 2023a) similar to *Cetraspora (*Fig. 2E); G: *Septoglomus constrictum (*INVAM, 2023f) similar to Septoglomus (Fig. 2F); H: *Entrophospora infrequens* (Alimi et al., 2021) similar to Entrophospora 1-2 (Fig. 2G–H); and I: *Entrophospora infrequens (Rai et al., 2019)* similar to *Entrophospora* (Fig. 2I)

The *Entrophospora* genus (Figures 2G and 2H) is characterized by a round shape, 151.08 µm - 149.41 µm in diameter, dark brown in color, and the spore wall is not clearly visible. These fused spores are probably saccules and spores similar to *Entrophospores*. The *Entrophospora* genus in Figures 2G and 2H) is similar to the *Entrophospora infrequens* (Figure 3I) in the study of Alimi *et al*. (2021). The *Entrophospora* in Figure 2I has a diameter of 159.43 µm, orange-brown in color, the

spore wall is not clear and is similar to *Entrophospora* infrequency in the study by Rai *et al.* (2019). Spores of the genus *Entrophospora*, spring alone in the soil or in the roots. The spores are globose to subglobose with color of yellow-brown, orange-brown, to brown (Sieverding & Oehl, 2006). The spores grow in the neck of the sporiferous saccule either immediately at or close to the saccule. The sporiferous saccule originates from the intercalaries within the extraradical and intraradical hyphae through their swelling (INVAM, 2022).

According to several studies, agronomic arrangements on agricultural land in this study had a negative impact on the AMF community on the land. Agricultural soils that are cultivated intensively tend to prefer less diverse AMF communities because severe anthropogenic disturbances, such as plowing, tillage and fungicide treatments, can reduce the number and abundance of AM fungal species (Chagnon *et al.*, 2013; Vályi *et al.*, 2016). In addition, the changes in AMF communities can be driven by fertilizer application that acidify the soil and soil tillage that reduce organic carbon in the soil (Bouffaud *et al.*, 2016; Geisseler & Scow, 2014; Oehl *et al.*, 2017). In addition to tillage, the agricultural land also has a low number of AMF taxa due to very low plant diversity when crops consist of long-term monocultures over time (Guzman *et al.*, 2021; Strom *et al.*, 2020).

4. CONCLUSION

The results of this study indicated that the treatment of cropping patterns, both intercropping and monoculture, had no significant effect on spore density and soil pH. In addition, soil pH did not affect spore density because pH was included in the neutral range. Spore density and diversity in the study were influenced by seasonal differences in the study area and root exudates released by the two plants. It was found that there were 8 spore genera in each treatment, namely *Glomus*, *Funneliformis*, *Scutellospora*, *Cetraspora*, *Septoglomus* and *Entrophospora*. It is expected that future research will be able to carry out specific identification, namely identification of species and root exudates produced by the two plants in different cropping patterns.

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