Diversity of Arbuscular Mycorrhiza Fungi (AMF) in the rhizosphere of sugarcane

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Abstract. Exploration of AMF types in sugarcane cropping areas is an important and necessary initial study to be able to isolate and identify specific types of AMF that exist. Utilization of AMF is one alternative in overcoming problems in acid soils, because AMF can help plants absorb P elements and other nutrients from the soil. This study aims to identify the types of AMF in the rhizosphere of sugarcane. The research was conducted from June to October 2015, soil sampling was carried out in the community sugar cane garden in Ngemplak Plantation, district Pati (KP. Muktiharjo). While the isolation, identification and capture of spores is carried out in the Ecophysiology Laboratory and the greenhouse of the Indonesian Spices and Medicinal Crops Research Institute, Bogor. The identification results obtained 2 arbuscular mycorrhizal fungi in the sugarcane rhizosphere, namely Glomus sp, (3 species) and Acaulospora sp (1 species). The amount of initial spore density was 120-130 spores per 50 g of soil samples and after trapping, the number increased to 407 spores / 20 g soil samples or increased 6.8 times.

1. Introduction
The government targets sugarcane production to reach 2.97 million tons in 2015. The number continues to increase to 3.27 million tons in 2016 and subsequently is targeted at 3.82 million tons in 2019 [1]. Efforts to accelerate the achievement of these targets can be done through efforts to improve the efficiency of smallholder sugarcane cultivation. At present, the average productivity of people's sugarcane plantations is 72 tons / ha with a yield of 7.69%. To achieve self-sufficiency, the productivity that needs to be achieved is a minimum of 120 tons / ha with a yield above 9% [2].

The presence of mycorrhiza important for an ecosystem resilience, stability and maintenance of biological diversity of plants. The role of mycorrhiza in preserving biodiversity and ecosystems is now beginning to be recognized, mainly due to the influence of mycorrhiza to maintain plant diversity and increase productivity [3]. Arbuscular Mycorrhiza Fungi (AMF) can help plants in increasing the absorption of nutrients and water [4].

Arbuscular mycorrhizal fungi are known to be mutually symbiotic with plants, antagonistic to parasites and naturally free living in the rhizosphere. AMF is also known to be able to colonize almost all the roots of agricultural crops. The ability of AMF associations with various types of plants reaches 90% [5]. The role of AMF is very important especially in terms of conservation of the nutrient cycle,
helping to improve soil structure, carbon transportation in root systems, addressing degradation of soil fertility and protecting plants from disease, as well as phytoremediation agents [6].

In general, sugarcane cultivation in Indonesia is carried out in sub-optimal lands, such as dry land and acid land. Only in certain areas carried out on irrigated rice fields. Water limitations and poor soil chemical properties will certainly affect plant growth and development. Acid soils which generally fix most of the phosphorus given causes low P availability in the soil [7]. States that one characteristic of acid mineral soils is low P content and high P fixation [8].

Mycorrhizae can also function as biological protectors for root colonization. This protection mechanism can be explained as follows: (1) the presence of hyphae (mantle) layers can function as physical protectors for the entry of toxic substances; (2) mycorrhizae use almost all of the excess carbohydrates and other root exudates, so they are not suitable for pathogens; and (3) mycorrhizal fungi can release antibiotics that can inhibit the development of toxicity [9].

Information about AMF and its use in sugarcane so far is still limited. Considering the potential of AMF that can increase plant growth and productivity as well as fertilizer efficiency, AMF research on sugarcane needs to be carried out. To study the potential of an organism, the first thing to know is the existence and diversity of the organism. Likewise the potential for its use in acid soils. Exploration of AMF types in sugarcane planting areas is an important preliminary study and is needed to be able to identify and map the existing dominant and specific AMF types. This study aims to isolate and identify the types of AMF in the rhizosphere of sugarcane.

2. Materials and methods

Soil and roots sample plants was carried out at the Ngemplak Sugarcane Plantation (KP. Muktiharjo, Pati Regency) Spore extraction, identification and of AMF colonization in the roots of the sample plants were carried out at the Department of Ecophysiology Laboratory, Indonesian Spices and Medicinal Crops Research Institute from June-October 2015. Soil samples are taken from the root zone with a depth of 5-20 cm (rhizosphere area) in a composite from 10 points of soil sampling, each point as much as 500 g, then put into a plastic bag and labeled. Isolation of spores from soil samples was carried out by a wet filter pour method, then counts were carried out on the spores obtained under a microscope. If the observations show a small number of spores, trapping activities are carried out. Isolated spores from the field are not optimal as material for identification and determination [10]. Spore trapping is one technique for obtaining intact spores by manipulating the spore production environment [11]. Preliminary analysis is very necessary to obtain information on the presence of AMF roots in the sugarcane area. AMF extraction is carried out to separate the spores from soil samples so that AMF identification can be done to determine the genus of AMF spores. The technique used is the filter pouring technique (Gedermann & Nicolson 1963) and continued with centrifugation techniques [12]. Observation of AMF colonization in plant root samples was carried out using root staining techniques. Root colonization is characterized by hyphae, vesicles and arbuscules or one of the three. Because the anatomical characteristics that characterize the presence or absence of AMF colonization cannot be seen directly. The method used in the sample root staining technique is the staining method from [13].

Making AMF spore preparations is intended to assist in the identification process, from these preparations it is expected that morphological information and sub-cellular structure of spores can determine the genus AMF. Preparation makers use Melzer’s coloring agent and preservative PVLG (polyvinyl lactoglycerol). PVLG preserves do not change the original color of the spores and have a permanent shelf life, if added Melzer will change color in certain genus AMF. With the help of a microscope and spore tweezers, collect the spores obtained by size, color and shape. Next drop on the slide preparation of each PVLG and Melzer and cover with a glass cover and press a little on the Melzer solution so that the spores break and the reaction occurs. Analysis of AMF spore types according to the morphology of size, color and sub-cellular structure.
3. Results and discussion

The results of isolation from the initial soil sampling location obtained several types of FMA spores in the sugar cane plant rhizosphere after observing under a microscope obtained that FMA was found with a total spore density of 120-130 spores per 50 g of soil samples and after trapping the number increased by 407 spores / 20 g soil sample or 6.8 fold (Table 1). The number of initial spores obtained is much higher than the number of initial spores isolated from gotu kola plants [14]; banana [10] and jatropha plants that get limited numbers and types of FMA spores because they are not sporulating, and contain more propagules such as hyphae [15]. While the yield of trapping is also much higher compared to the spore density of trapping results by who found 1–474 spores / 100 g of soil [16], 161–173 spores / 50 g of soil in the oil palm rhizosphere [17], and 2–57 spores / 50 g of soil in banana rhizosphere [10].

Table 1. Amount of FMA spores of initial soil samples before and after trapping from sugarcane rhizosphere

| AMF type (Mix spora) | Number of spores / 50 g soil sample | AMF type (Mix spora) | Number of spores / 20 g soil sample |
|----------------------|-----------------------------------|----------------------|-----------------------------------|
| Glomus sp, Acaulospora sp, Scutelospora sp | 130 | Glomus sp-1, Glomus sp-2, Glomus sp-3, dan Acaulospora sp | 407 |

These results indicate that the rhizosphere environment of sugarcane plant is good enough to support the growth and development of spores and can be an indicator that the environment of sugarcane roots is safe and compatible for FMA inoculation. In general, in soils contaminated with synthetic materials (pesticides and or inorganic fertilizers) mycorrhizae cannot grow and develop properly.

An individual, each AMF has an intrinsic factor that will respond to environmental or seasonal changes. Although there are spore types that are not affected by season or environment, there are spore types that are affected by seasonal or environmental changes. This confirms that the influence of seasonal changes on AMF activity depends on the type of spore as the identity of intrinsic factors [18]. State that differences in diversity and number of spores are determined by the environment and land management and land type [19].

All AMF do not have the same morphological and physiological characteristics, therefore it is very important to know their identity. The process of identifying FMA up to the species level requires a thorough introduction of 11 spore characters, namely (1) spore arrangement, (2) spore shape, (3) spore development, (4) spore size, (5) spore wall layer, (6) spore color, (7) spore germination, (8) spore content, (9) surface texture, (10) auxiliary cell, and (11) subtending hyphae [20].

The results of initial identification of the spores obtained showed that there were several genes of AMF in soil samples, namely the genus Glomus Sp, followed by the genus Acaulospora Sp, and the genus Scutelospora Sp. Spore conditions observed were mostly in a healthy and living condition, and only a small proportion of spores died. While the results obtained after trapping showed that the genus Glomus is dominant compared to other genera, even the genus Scutelospora is no longer found.

The results of the identification of the type of spore isolated on the basis of morphological characteristics and response to Melzer's solution with magnification 100-200 times showed the dominance of the genus Glomus and only one type of Acaulospora. Melzer's solution is one of the tools in the identification process to distinguish the type of FMA spores to the level of the genus, Genus Glomus does not react to Melzer's solution, on the contrary the genus Acaulospora gives a reaction that is indicated by the change in spore color. The names of FMA spore species identified by identification and characterization based on the trapping results can be seen in Table 2.

The results of research by [21] in the Maharashtra Province of India, obtained five (5) genus FMA, namely Glomus, Gigaspora, Acaulospora, Scutelospora and Sclerocystis in the rhizosphere of sugar
cane also shows that in the sugarcane area Genus *Glomus* is the most dominant FMA and can be used individually or as a consortium as a bioinoculant for increasing sugarcane productivity.

The same results were obtained in the study of [22], the dominance of the genus Glomus in the sugar cane rhizosphere in the Tamil Nadu region, in 10 different agroecosystems. Glomus is represented by 16 species forming the dominant genus followed by Acaulospora, Gigaspora, and Scutellospora, which are represented by three species each. Species identified were *Glomus aggregatum*, *G. fasciculatum*, *G. mosseae*, *G. macrocarpum*, *G. ambisporum*, *G. fulvum*, *G. multisubstatum*, *G. maculosum*, *G. geosporum*, *G. scintillans*, *G. deserticola*, *G. constrictum*, *G. clarium*, *G. palvinatum*, *G. reticulatum*, *G. flavisporum*, *Acaulospora bireticulata*, *A. spinosa*, *A. elegans*, *Gigaspora margarita*, *G. candida*, *G. decipiens*, *Scutellospora nigra*, *S. minuta*, *S. calospora*.

**Table 2.** The type of spores trapped by spores from the sugar cane planting rhizosphere from the soil sampling

| Number | Spore type | Morphological characteristics |
|--------|------------|------------------------------|
| 1.     | *Acaulospora sp* | Spores are round, small, yellow, ornament like orange peel, spores measuring 57.6 µm. Yellow walls, diameter (6.25-7µm) |
| 2.     | *Glomus sp-1*   | Oval spores, brownish yellow. Spores measuring (100-120) x (50-80) µm. Older spore walls (light brown), 5-7.5 µm in diameter. Yellow hyphae with a diameter of 8.75 µm thicken at the attachment site |
| 3.     | *Glomus sp-2*   | Oval spores, brownish yellow. Spores measuring (100-120) x (50-80) µm. Older spore walls (light brown), 5-7.5 µm in diameter. Yellow hyphae with a diameter of 8.75 µm thicken at the attachment site |
| 4.     | *Glomus sp-3*   | Spores are rather oval, reddish brown in color. Ringing spores are relatively thin and measuring 140 µm. Brown spore walls, with a diameter of 5-7.5 µm |

Ten types of AMF were found in three districts in the province of South Sulawesi, namely *Glomaceae*, *Acaulosporaceae*, and *Gigasporaceae*. The genus *Glomus* and *Gigaspora* have the same abundance and characteristics of spores in three districts and show that this genera has a wide distribution and has a high ability associated with sugar cane. The greatest diversity of mycorrhizae is in Gowa district with four types of mycorrhizal genus found, namely *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis* [23].

The results of these observations provide hope and potential to explore the richness of microbes in the area of sugarcane root, especially AMF to be explored and subsequently developed and utilized as one of the specific local biological assets. Considering that AMF is a type of microbial which is very potential in helping plants to increase nutrient uptake, resistance to drought, prevention of pest attacks, increase production of primary and secondary metabolites, and fertilizing efficiency.
The presence of AMF in sugarcane rhizosphere provides data and information on potential soil microorganisms, which can then be collected and utilized as an innovation in the use of biological agents that can increase growth, plant productivity and fertilizer efficiency, especially phosphorus fertilizer. Several studies on the use of AMF indicate that AMF inoculation can increase available P 529% higher than without AMF in chili plants in Andosol [24], the use of FMA combined with rhizobium can increase P nutrient uptake by 854% in soybean plants [25]. Inoculation of AMF alone or in combination with rhizobacteria can significantly increase the availability and uptake of P [26].

The results of the study of the effect of AMF on the agronomic characteristics of sugarcane showed that the number of roots produced was more than the treatment without AMF, so the fresh root stover weight of sugarcane doubled from 0.85 g to 1.79 g. AMF infection affected water availability for plants, sugar cane plants that are colonized with AMF water content is about 48% higher compared to those without AMF. Overall the use of arbuscular mycorrhizal fungi in the single bud planting propagation system of sugarcane significantly affected the growth of seedlings, especially plant height, leaf width, and plant root system [27].

The research results of [28] showed that the of mycorrhizae was able to increase the level of P nira, by 38.84% - 71.65%. Increased levels of P nira, followed by an increase in sugarcane yield of 4.76% -21.15%. Mycorrhiza can increase sugar productivity (crystal) by 13.66% - 67.90%. The increase in crystal productivity in soils with "low" available P is higher by 27.80% - 40.11% than in soils with "very high" available P. The use of mycorrhizal fertilizer can reduce the level of SP-36 fertilizer amount by 25 - 50%.

Sugarcane is proven to be symbiotic with AMF, seen from the root morphology of plants on root staining results, where the structure of vesicles and hyphae is formed and seen in plant roots (Figure 1). Each type of AMF may vary in its ability to form hyphae in the soil, both the distribution and quantity of the hyphae. Besides that, it has been ascertained that the development of AMF infection is related to the ability of AMF in increasing plant growth [18].

![Figure 1. AFM infected of Sugarcane roots showed form shape of vesicle (1), arbuscula (2) and internal hypha structure (3).](image-url)

Soil samples in the Sugar Cane Plantation owned by PTPN 2 Kebun Sei Semayang, North Sumatra, obtained a percentage of root colonization by AMF of 45.4% of the total field of view, it shows that, AMF infection in the roots of sugarcane shows a positive direction, although the degree of infection does not reach 50% [9]. Identified 23 AMF species and observed that root colonization ranged from 60 to 89% in 14 sugarcane fields [29].

The colonization of AMF plays a role in changes in plant physiology in terms of resistance to bacterial and fungal pathogens, increasing the rate of photosynthesis and increasing stomata under water stress conditions [30]. Arbuscular mycorrhizal fungi increase seedling growth capacity, number of tillers, stem diameter and sugarcane yield. as well as being able to increase the percentage of brix and fertilizing efficiency of P up to 25% without affecting sugarcane yield [31].

For plants that grow in arid regions, the presence of AMF is beneficial because it can increase the ability of plants to grow and survive under conditions that are less water. The presence of AMF can improve and increase the water absorption capacity of host plants. According to [32] there are a number of allegations as to why the mycorrhizal plants are more resistant to drought including: (1) the
presence of mycorrhizal root resistance to decreased water movement so that water transfer to the roots increases, (2) P deficiency plants are more sensitive to drought, the presence of AMF causes P status of plants increases so that the resistance to drought increases as well, (3) The presence of external hyphae causes AMF plants to be able to get more water than non-AMF but if this mechanism occurs means the metal content decreases faster. An interesting recent discovery is the relationship between groundwater potential and mycorrhizal activity. In mycorrhizal plants the amount of water needed to produce 1 gram of dry weight of plants is less than plants that do not have mycorrhizae, (4) Mycorrhizal plants are more resistant to drought because mycorrhizae can extend hyphae to get water, and (5) Indirect effects due to the presence of myelin externally causes AMF to be effective in aggregating soil grains so that the ability of the soil to store water increases.

4. Conclusion
Type of AMF that was successfully isolated and identified in the rhizosphere of sugarcane in the Ngemplak KP Muktiharjo garden before trapping there were 3 genera namely *Glomus*, *Acaulospora*, and *Scutellospora* and after trapping, only 2 genera were left, namely *Glomus* (3 types) and *Acaulospora* (1 type). The number of spores obtained before trapping is relatively high 120-130 spores per 50 g of soil samples and after trapping the number increases by 407 spores / 20 g of soil samples or an increase of 6.8 times. Symbiosis occurs between AMF and sugar cane, as seen from observations at the root of the plant there has been an infection by AMF with the formation of vesicle, arbuskula and hyphae structures.

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