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ARBUSCULAR MYCORRHIZAE EXPLORATION AND IDENTIFICATION ON SUGARCANE PLANTATIONS IN HUMID TROPIC AREA OF INDONESIA

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ABSTRACT

The presence of arbuscular mycorrhizae can vary in type and quantity which is determined by the environment. This study aims to explore and identify vesicular arbuscular mycorrhizae (VAM) in sugarcane plantations in tropical area of Indonesia. The method used was a quantitative survey that described the VAM characteristics and its morphology in sugarcane plantation. The Soil sampling were located at 3 provinces in Indonesia, namely, Takalar (South Sulawesi), Bombana (Southeast Sulawesi), and Gorontalo. The isolation of mycorrhizal fungi was carried out by extracting spores using the pour-filter according to Pacioni method, followed by centrifugation based on Brundrett method. The results of the soil samples from 3 different sugarcane plantations in Sulawesi island found 3 types of VAM namely, *Acaulospora* sp., *Glomus* sp., and *Gigaspora* sp. The highest number of VAM spores belonging to Acaulospora sp genus with small round shape was found in Takalar (South Sulawesi), while the lowest number was found in the soil samples of Bombana (Southeast Sulawesi) and Gorontalo sugarcane plantations. The VAM spores number and types on sugarcane plantation were commonly found in soil with high fertility than in nutrient-poor soil.

Keywords: Arbuscular mycorrhizae; sugarcane plantation; humid tropical area of Indonesia.

INTRODUCTION

Mycorrhizae is a symbiosis form between fungi and plants which plays an important role in sugarcane growth, and improves its root system. Use of VAM had positive impact on sugarcane growth and production by enhancing the nutrient and water uptake in the plant hyphae [1]. Foth [2] also stated that the increased absorption of nutrients and water from the soil allow plants to

produce new cells and growth hormones namely, auxins and gibberellins [3-5]. The Wright and Uphadhyaya [6], in the Musfal (2010) theory stated that the VAM's external roots produces glomalin glycoproteins and organic acids that bind soil grains into micro aggregates, which further form macro clusters. Furthermore, mycorrhizae also protect plants living in unfavorable environment such as, soil contaminated with petroleum, heavy metals, low pH, water stress, and others [7-9]. The use of arbuscular mycorrhizal fungi as biological agents is an environmentally friendly approach in the fields of forestry, agriculture, and plantations (Nova, 2005; Sausa et al. 2013). The advantages of arbuscular mycorrhizal fungi includes, safe to use, does not cause environmental pollution, and improves nutrient cycle [10-12].

Mycorrhizae generally varies according to climate, environment, and the soil type [13-15]. It has great potential as bio fertilizer and sources of soil micro-organisms which aid nutrient cycle by facilitating the nutrients absorption to increase plant growth. Mycorrhizal associations do not always benefit the host plant and depends on environmental factors such as, temperature, soil pH, moisture, phosphorus, nitrogen and potassium content [16-18]. These conditions show that mycorrhizal fungi is cosmopolitan in nature (i.e., possess the ability to exist in any soil condition).

The utilization of indigenous Vesicular Arbuscular Mycorrhizae (VAM) is an input of microbial technology, developed to overcome the drought problem and low fertility [19-21]. The natural geophysical conditions allow the mycorrhizae presence in the soil to vary. Therefore, soil physical and chemical properties play an important role in the VAM development in plant roots [22-24]. Meanwhile, some plants do not grow without mycorrhizae presence at the roots, for example, pine seedlings usually fail to grow after transplanting in the absence of mycorrhizal tissue around the roots [25].

Agricultural land, especially sugarcane plantation, has begun to be displaced towards suboptimal lands, due to the construction of modern facilities. Besides being constrained by poor soil, the water availability is also limited in the suboptimal soil. This condition is found in humid tropical areas with average rainfall of 2,000 mm/year, and >22°C which allow the weathering of organic matter and more intensive nutrients leaching. The soil improvement with chemical fertilization does not last long and also ineffective, therefore an idea of using microbes emerged which were considered as efficient soil repair agents [26-28]. However, the direct application of microbes either by spraying or sprinkling with fertilizers requires professional knowledge. The VAM used in sugarcane plantations is an alternative soil improver for overcoming the problem of fertilization inefficiency, increase inoculation efficiency, and, rise the productivity level on marginal land such as, dry and nutrient-poor areas. As an initial step, it is necessary to identify the amount and type of VAM at 3 sugarcane plantations that have different agroecological conditions (mainly climate and soil) in Sulawesi Island, Indonesia, namely, Takalar (South Sulawesi), Bombana (Southeast Sulawesi), and Gorontalo Sugarcane Plantation (Gorontalo). This study purposes to explore and identify the characteristics and morphology of indigenous Vesicular Arbuscular Mycorrhizae (VAM) on dry land in sugarcane plantation area of Sulawesi Island, Indonesia.

METHODOLOGY

This research was conducted using quantitative survey method to identify and explore the characteristics and morphology of VAM in sugarcane plantation areas. The soil samples were obtained from three different locations in Sulawesi Island, Indonesia, namely, Takalar (South Sulawesi), Bombana (Southeast Sulawesi), and Gorontalo Sugarcane Plantation (Gorontalo). The locations for taking soil samples were shown in Fig. 1. The analyses of the soil physical and chemical properties were carried out at the Chemical and Soil Fertility Laboratory, Department of Soil Science, Hasanuddin University. The soil parameters analyzed and the methods used were shown in Table 1.

The analysis of soil samples for VAM identification were conducted at the Laboratory of Indonesian Center for Environmental & Forestry Research & Development, and the Laboratory of Soil, Fertilizer, Water, Agricultural Research &

Development Agency Bogor, Indonesia. 1 kg of soil samples were taken randomly from sugarcane rhizosphere at 20 cm radius and 0-25 cm depth, then, dried with air, and packed in a clear plastic bag, which was taken to the laboratory for mycorrhizae isolation and identification. The isolation process was carried out by extracting spores using the pour-filter, based on the Pacioni (1992) method in the Yassir & Mulyana (2006) theory, by mixing 50 g of soil sample with 500 ml of water and stirring until the grains were suspended, then poured into filter set with mesh numbers of 10, 20, 40, 60, 80, and 100. The soil taken was in the filter 20 and 40 mesh, then followed by centrifugation technique based on the Brundrett et al. [29] method in the Yassir & Mulyana (2006) theory. The filtrates were added to 30 ml distilled water and centrifuged at 2,000 rpm for 5 mins, after this, 15 ml of 80% sucrose was added, then centrifuged again at 2,000 rpm for 1 min. The supernatant was placed on a petri-dish and observed using a microscope. The mycorrhizal identification included the spores' colour, their number (population) in 100 g of soil, the ornament on their wall, and their size in diameter.

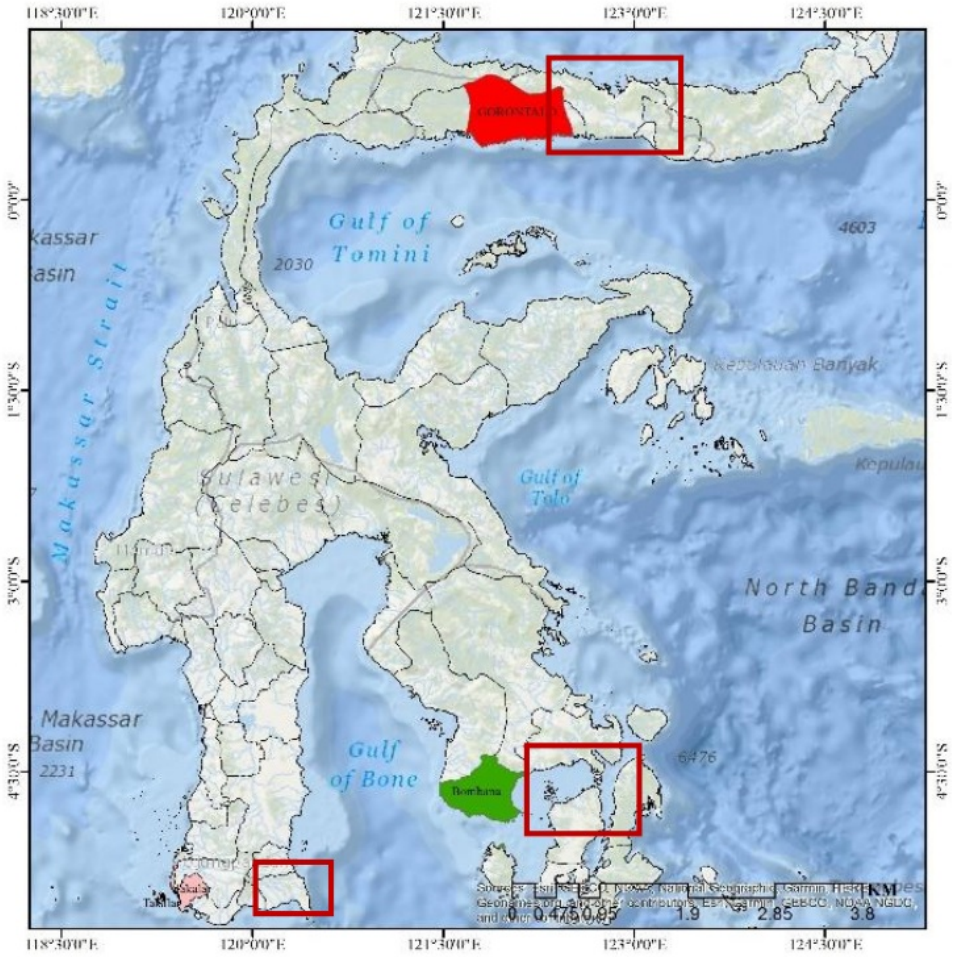


Fig. 1. Soil sampling location (Takalar, Bombana, and Gorontalo)

Table 1. Physical and chemical properties of the soil analyzed and the method used

Soil properties	Method of analysis
Soil texture	Hydrometer
pH	pH H_2O ratio soil:water 1: 2.5
C-Organik	Walkey & Black
N	Kjehdahl
P_2O_5	Bray-1 & Olsen
Ca	Destilation, Ammonium asetat pH 7.0
Mg	Destilation, Ammonium asetat pH 7.0
K	Destilation, Ammonium asetat pH 7.0
Na	Destilation, Ammonium asetat pH 7.0
Cation Exchange Capacity (CEC)	Destilation, Ammonium asetat pH 7.0

RESULTS

Characteristics of Soil Research Location

The analysis results of the soil chemical and physical properties in the 3 research locations were shown in Table 2. The criteria for the chemical properties of soil fertility in the Takalar, Bombana, and Gorontalo locations were generally moderate to low level. However, among the three locations, land originating from Bombana had the lowest fertility compared to the other 2. The Takalar sugarcane plantation had chemical properties and soil fertility relatively better than others, especially in the aspect of cation exchange capacity (CEC), the number of bases exchanged (Ca, Mg, K, Na), and the base saturation (BS).

The identification of VAM's number and types in 3 sugarcane plantations on Sulawesi island was the preliminary findings from the studies on exploration and characterization in tropical dry land. The results of initial identification and isolation showed that mycorrhizal spores were found at three research locations. The VAM types found were from the spore genus of *Acaulospora, Glomus*, and *Gigaspora* (Table 3).

The dominant species of mycorrhizal spores found in the Takalar plantations were the genus of *Acaulospora* (small round yellow and green in colour), and *Gigaspora* (large round shape). While in Bombana sugarcane plantation, they were the genus of *Acaulospora* (small round green), and *Glomus* (small round yellow). Finally, in Gorontalo sugarcane plantation, they were genus of *Gigaspora* (large round shape) and *Acaulospora* (small round yellow and green). The morphological features of VAM genus found in 3 locations were shown in Figs. 2, 3, and 4, respectively.

Table 2. Analysis results of the soil physical and chemical properties in the research location

Soil properties	Takalar	Criteria *	Bombana	Criteria*	Gorontalo	Criteria *
Soil texture	Clay loam	-	Clay loam	-	Clay loam	-
pH H_2O	6.01	Slightly acid	5.5	Acid	6.32	Slightly acid
C-Organic (%)	2.04	Moderate	1.89	Low	2.14	Moderate
N (%)	0.13	Low	0.12	Low	0.16	Low
P_2O_5 (ppm P)	12.25	Moderate	10.24	Low	11.85	Moderate
Ca (cmol (+)kg^{-1})	7.21	Moderate	5.14	Low	6.54	Moderate
Mg (cmol (+)kg^{-1})	2.26	High	2.21	High	0.89	Low
K (cmol (+)kg^{-1})	0.35	Low	0.25	Low	0.52	Moderate
Na (cmol (+)kg^{-1})	0.56	Moderate	0.3	Low	0.54	Moderate
Cation Exchange Capacity (cmol (+)kg^{-1})	21.96	Moderate	21.07	Moderate	20.41	Moderate
Base saturation (cmol (+) kg^{-1})	47	Moderate	37	Low	42	Moderate

*Source: Department of Agriculture Soil Research Institute (2009)

Table 3. Symptoms of microorganism symbiotic with sugar cane cultivation in 3 study locations

Location	Morphotype	Genus	Sum of spores per 100 g soil sample	Spore diameter (µm)
Takalar	Small, green, round	*Acaulospora sp.3*	3	133
	Small, clear, round	*Acaulospora sp.1*	14	128
	Small, yellow, round	*Gigaspora sp.*	1	233
	Small, clear, round	*Acaulospora sp.2*	3	113
Bombana	Small, green, round	*Acaulospora sp.2*	4	107
	Small, yellow, round	*Glomus sp.1*	2	98
	Small, clear, round	*Acaulospora sp.1*	1	105
Gorontalo	Big, clear, round	*Gigaspora sp*	1	221
	Small, yellow, round	*Acaulospora sp.3*	2	152
	Small, black, round	*Glomus sp.2*	1	176
	Small, green, round	*Acaulospora sp.1*	1	76

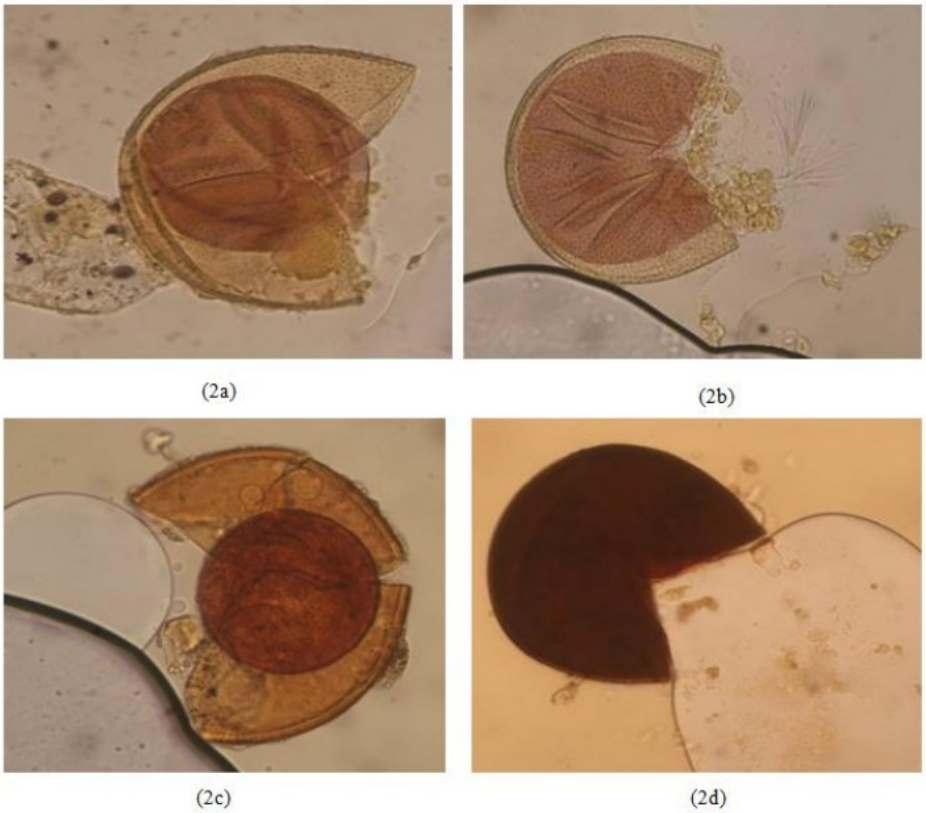
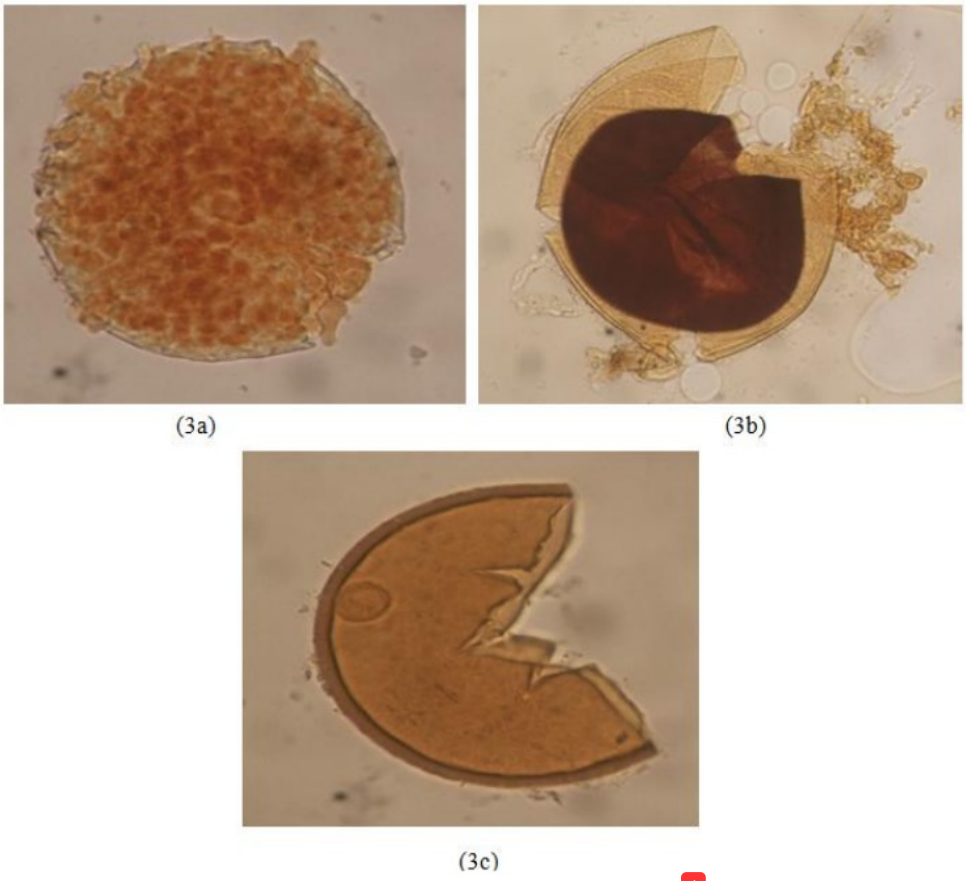


Fig. 2. VAM spores morphology in Takalar sugarcane plantations *Acaulospora sp.1* (2a), *Acaulospora sp.3* (2b), *Acaulospora sp.2* (2c), *Gigaspora sp.1* (2d)

The highest number of soil mycorrhizal spores (21 genus) per 100 g was obtained from Takalar sugarcane plantations (South Sulawesi), followed by Bombana (Southeast Sulawesi) with 7 genus, and Gorontalo with 5 genus. The comparison of mycorrhizal spores at each location was shown in Fig. 5.



(3a) (3b)

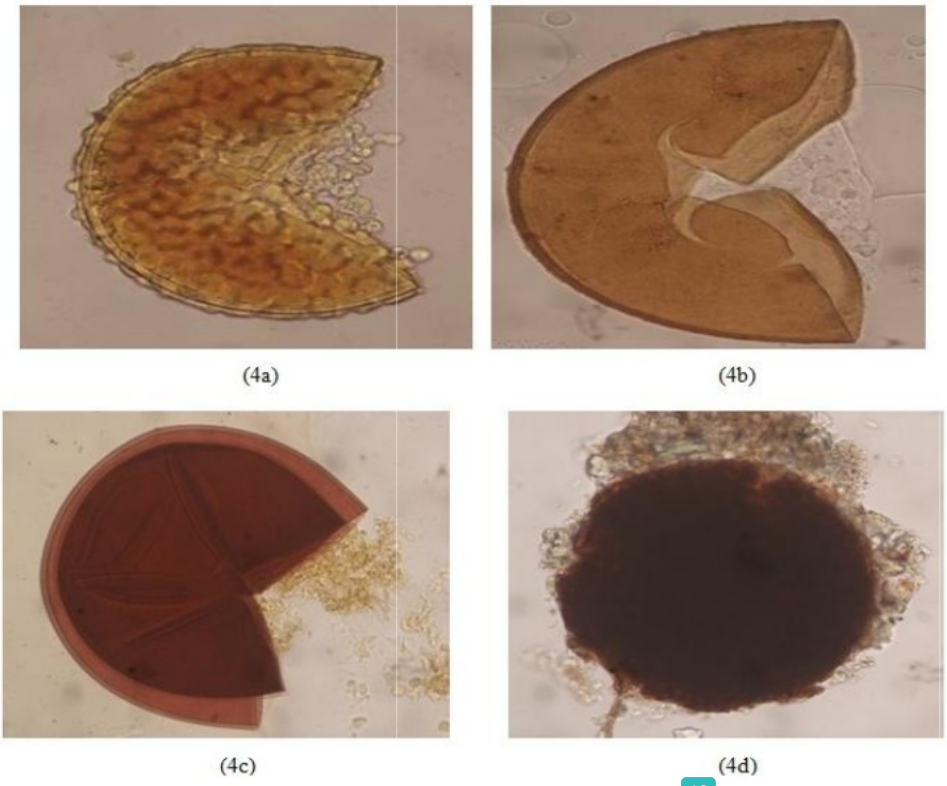
(3c)

Fig. 3. VAM spores morphology in Bombana sugarcane plantations, *Acaulospora sp.1* (3a), *Acaulospora sp.2* (3b), *Glomus sp.2* (3c)

DISCUSSION

The soil in the Bombana was classified as Inceptisols, according to Subagyo et al. [30], it possessed various fertility levels ranging from low to high, slightly acidic to neutral in reaction, with low to moderate organic matter content, and moderate to high base saturation. The soil types in 2 other locations namely, Takalar and Gorontalo were classified as Alfisols with base saturation> 35%, and neutral acidic reaction, as well as the presence of clayey accumulation horizons (argillic) [30]. The average annual rainfall in Takalar, Bombana, and Gorontalo were 1,986 mm per year, 1,232 mm per year, and 1,460 mm per year respectively. Therefore, they were classified as relatively dry tropical areas with a period of 3-4 dry months.

The physical and chemical properties of the soil in 3 sugarcane plantations indicated that the Takalar soil had a clayey texture with a pH of H_20, medium C-organic, P_2O_5, Ca, Na, CEC, and BS, high Mg, as well as low N and K. The soil from Gorontalo had similar chemical property with that of Takalar, except for the low Mg and medium K content. Soil analysis showed that the level of soil fertility in Takalar was relatively better than those in Gorontalo and Bombana.



(4a) (4b) (4c) (4d)

Fig. 4. VAM spores morphology in Gorontalo sugarcane plantations *Acaulospora sp.1* (4a), *Acaulospora sp.3* (4b), *Gigaspora sp.* (4c), *Glomus* sp.2 (4d)

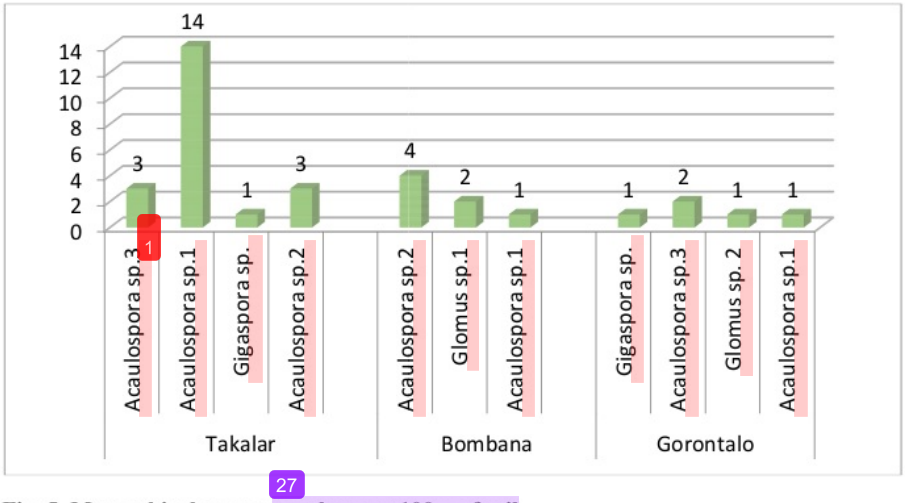


Fig. 5. Mycorrhizal spores number per 100 g of soil

The VAM exploration and identification carried out on 3 dry land areas located in the sugarcane plantations of Takalar, Bombana, and Gorontalo showed that the common type of VAM was *Acaulospora sp*. Meanwhile, based on the number of mycorrhizal spores in the three sugarcane plantations per 100 g soil, Takalar area had the highest spores (21), followed by Bombana (7), and Gorontalo (5) with their soil types classified as clayey. According to Sunarko (2014) and Nursyamsi et al. (2012) soils with high clay content and acidic to neutral soil pH produced relatively high MAV spores. This was in accordance with the hypothesis of Widiastuti and Kramadibrata (1992) which stated that the soil dominated by clay fractions was very suitable for VAM spore growth. Simangunsong (2006) also stated that spore easily develop in an acidic to neutral pH range. The number and type of VAM spores were influenced by the following factors namely, the physical and chemical properties in the sugarcane planting area, acid-reacting soil, clayey texture, moderate CEC, exchangeable base content, C-organic, N and P medium. Therefore, the high nutrient content in Takalar and Gorontalo soil was preferred by VAM compared to soils with less nutrient (Bombana). This was indicated by the lack of VAM types and the lower number of spores in Bombana compared to Takalar.

Acidic mineral soils in Indonesia were generally dominated by the following genus namely, *Glomus*, *Acaulospora*, *Gigaspora*, and *Scutellospora* [31]. The *Glomus* and *Gigaspora* were known as vesicular-arbuscular fungi found in various soil types in coconut and clove plantations (Soputan, 2003). Safir and Duniway (1988) in the Nurhalimah et al. [32] theory stated that the distribution of mycorrhizae was influenced by many factors such as, soil type and structure, P and N nutrients, water, pH, and soil temperature. Widiastuti et al. [33] stated that, generally, VAM formed external hyphae that extended the reach of plant roots to absorb nutrients, especially the P nutrient which improved the root system of oil palm plants. The VAM's role in acidic soil was for plant growth enhancement due to P nutrient availability. However, Suhardi [34] stated that phosphorus was one of the growth limiting elements of root seeds and young plants.

CONCLUSION

The exploration and identification of VAM on three sugarcane plantations in Sulawesi Island showed three dominant spore genus namely, *Acaulospora sp*, *Glomus sp*, and *Gigaspora sp*. The highest number of VAM spores belonging to the genus *Acaulospora sp*. was found in Takalar (South Sulawesi), while the lowest number was found in the soil samples of Bombana (Southeast Sulawesi) and Gorontalo sugarcane plantations. Furthermore, the number and types of VAM spores were influenced by the following factors such as, the physical and chemical properties of the soil, the soil acidity, clayey texture, moderate CEC and exchangeable base content, C-organic content, and N and P medium. Takalar and Gorontalo soil were more preferable for VAM's growth compared to those in Bombana with less nutrients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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