Exploration Of Arbuscular Mycorrhizal Fungi From Sugarcane Rhizosphere In South Sulawesi

Zahraeni Kumalawati, Yunus Musa, Nur Amin, Laode Asrul, Ifayanti Ridwan

Abstract: A survey considering the occurence of arbuscular mycorrhiza (AM) fungi in sugarcane plantation field in South Sulawesi were conducted with the aim to exploit the site spesific genetic resources in relation to plant growth promotion. Eleven soil samples were collected from sugarcane rhizosphere in three districts of South Sulawesi province, Indonesia. Six samples of sugarcane varieties CM 2012, PS 862, PS 864, TK 386, BL, and Triton were taken from Takalar district; two samples of sugarcane varieties, CM 2012 and TK 386 were from Jeneponto district; and three samples taken from Gowa district were varieties of PS 862 and TK 386. The study informed that ten different types of AM fungi were found in three districts of South Sulawesi province included three families of the fungi *i.e. Glomaceae, Acaulosporaceae*, and *Gigasporaceae*. *Glomus* and *Gigaspora* genera have similiar abundance and spores characteristics in three districts and indicate that these genera have a wide spread in and have high capability associated with sugarcane. The greatest mycorrhizal diversity was in Gowa district with four types of mycorrhizal genera found *i.e. Glomus, Gigaspora, Acaulospora* and *Sclerocystis*.

Index Terms: *Arbuscular Mycorrhizal Fungi, Rizosphere, Sugarcane, diversity.*

1. INTRODUCTION

Sugarcane in South Sulawesi has been maintained intensively since 31 years ago in conjunction with establishment of sugar industry that supervised by PT. Perkebunan Nusantara XIV in 1981 (Pusat Informasi BUMN, 2012)[1]. Sugarcane land area of about 7000 ha located in three districts, *i.e.* Gowa, Takalar, and Jeneponto. The land is generally acidic (pH <5) with low soil fertility (C-organic and % saturation) and limited irigation systems (Rismaneswati, 2005)[2] led to low crop productivity (Toharisman, 2007)[3]. This condition is often overcome by the increasingly use of conventional chemical fertilizers. Monoxenic cultivation technologies are now a reality and provide significantly high and economically attractive options to chemical fertilizations (Adholeya *et. al.*, 2005)[4]. The use of mycorrhiza is considered one of the effective ways to improve and enhance the nutrient status of plants and environmentally safe to use. Mycorrhizal fungi, particularly Arbuscular Mycorrhiza (AM), are ubiquitous in soil and create symbiotic associations with most terrestrial plants including agricultural crops, cereals, vegetables, and horticultural plants (Smith and Read, 1998; Siddiqui and Pichtel, 2008)[5,6].

- *Zahraeni Kumalawati: Department of Plantation, Polytechnic of Pangkep State Agricultural, South Sulawesi, Indonesia, Email: zahraeni.km@gmail.com*
- *Yunus Musa: Department of Agronomy, Agricultural Faculty Hasanuddin University, Makassar, Indonesia*
- *Nur Amin: Department of Pests and Plant Diseases Agricultural Faculty Hasanuddin University, Makassar, Indonesia*
- *Laode Asrul: Department of Agronomy, Agricultural Faculty Hasanuddin University, Makassar, Indonesia*
- *Ifayanti Ridwan: Department of Agronomy, Agricultural Faculty Hasanuddin University, Makassar, Indonesia*

In agriculture, several factors such as host crop dependency to mycorrhizal colonization, tillage system, fertilizer application, and the potential of mycorrhizal fungi inoculant, affect plant responses and plant benefits from mycorrhiza. Interest in AM fungi propagation to be used for sustainable agriculture is increasing due to its role in the promotion of plant health, and improvements in soil fertility and soil aggregate stability. This fungi can be utilized effectively for increasing yields while minimizing use of pesticides and inorganic fertilizers (Siddiqui and Pichtel, 2008)[6]. Studies on the presence of arbuscular mycorrhizal fungi on sugarcane rhizosphere in Indonesia are still limited. Adinurani and Hendroko (2005)[7] reported some types of indigenous AM fungi found in PG Tolanghula sugarcane planting area, Gorontalo in the northern part of Sulawesi. These fungus indentified as *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp. A further study on use of these fungus shows that among the three species of mycorrhizas applied, *Glomus* sp. gave the best effect in increasing sugarcane production. The exploration of natural plant root colonization of the AM fungi is essential to address the problem of nutritional management for stressed soil where crop rotation system is absence for many years (Datta and Kulkarni, 2012)[8] Therefore, it is important to undertake an assesment to identify the occurence of AM fungi associated to sugarcane plant in order to obtain isolates to be utilized to improve the uptake of plant nutrients particularly P.

2. MATERIAL AND METHODS

Soil samples were collected from eleven sites located in three districts of South Sulawesi province, *i.e.* Takalar, Jeneponto and Gowa. The soil samples consisted of six samples from Takalar region with sugarcane varieties of CM 2012, PS 862, PS 864, TK 386, BL, and Triton; two samples from varieties of CM 2012 and TK 386 taken in Jeneponto district, and three samples from two varieties, PS 862 and TK 386 were taken from Gowa district. A total weight of about one kilogram of four to five soil sub samples was taken sistematically from each site at a depth of 0-20 cm and then mixed evenly. From the mix, three to five samples were taken to a total weight of one kilogram and air dried. Hence, 100 g of soil from each sample was used for spore identification. Isolation of mycorrhizal spores was conducted by wet sieving and centrifugation method with 48% sucrose (Walker *et. al*.,

1982)[9]. Identification of the AM fungi content in the soil sample was carried out at Laboratory of Cryptogame, Biology department of Indonesian Science Institute. Mycorrhizal spores obtained were prepared by preserving them on slides in polyvinyl alcohol-lactic acid-glycerol (PVLG) and then observed under a compound microscope for spore details (100-1000x). Identification of species based on morphological characteristics and size of the spores. In addition, the numbers of spores were counted for each sample. Observation was also made for mycorrhizal infection in sugarcene root tissue. Sugarcane root samples taken from the field were washed in situ with water then placed in a 80 ml bottle filled before with alcohol 50%. The samples were taken to the laboratory to examine the colonization of the fungi on the plant rhizosphere by a modified root staining method (Koske and Gemma, 1989)[10].

3. RESULTS AND DISCUSSION

In this study, eleven different types of AM fungi were found in three districts of South Sulawesi province that consisted of three fungi families, *Glomaceae, Acaulosporaceae*, and *Gigasporaceae* (Table 1). The types associated with sugarcane varieties of CM 2012, PS 864, PS 862, TK 386, BL and Triton found in Takalar district were indentified as *Glomus* sp.1, *Gigaspora* sp.1, and genus *Acaulospora* that consisted of three types: *Acaulospora scrobiculata, A. tuberculata*, and *A. foveata.* In Jeneponto region, several types of mycorrhiza fungi associated with sugarcane varieties of CM 2012 and TK 386 identified as *Glomus* sp.2, *Gigaspora* sp.2, and *Acaulospora* genus from only one species *i.e. Acaulospora tuberculata*. Similarly, types of mycorrhiza fungi found in the sugarcane area in Gowa district were *Glomus* sp.,*Gigaspora* sp*., G. sensulatu*, *Acaulospora scrobiculata* and *Sclerocystis rubiformis.* These types are associated with sugarcane varieties of PS 862 and TK 386. Observation on population and characteristics of types of mycorrhiza found in the rhizosphere of sugarcane in this study shows similarity in almost three regencies. Mycorrhiza from *Glomus, Gigaspora* and *Acaulospora* genus were found in all research sites. This shows that the three genus have a wide spread and capable to associate with sugarcane plant.

Table 1. Population and characteristics of mycorrhiza spores associated with sugarcane from three districts in South Sulawesi Province.

No.	The Origin of Soil Samples	Species	Population (spores/100 g of soil)	Colour	Diameter (µm)
1.	Takalar district	*Glomus* sp. 1	>100	Hyaline, yellow, brownish yellow	29-150 x 36-150
		Gigaspora sp.1	50-100	Yellow, yellowish-brown	90-225 x 210-286
		Acaulospora tuberculata	10-50	Brown	186 x 186
		A. Scrobiculata	<10	Brownish yellow	90-96 x 108-120
2.	Jeneponto district	*A. Foveata*	<10	Brown	180 x 180
		Glomus sp. 2	50-100	Hyaline, yellow, brownish yellow	43-90 x 86-157
		Gigaspora sp. 2	10-50	Golden brown	210-215 x 229-240
3.	Gowa district	*Acaulospora tuberculata*	10-50	Brown	150 x 150
		Glomus sp.3	50-100	Hyaline, yellow	75-90 x 90-243
		Gigaspora sp.3	50-100	Yellow, yellowish-brown	143-240 x 240-243
		Gigaspora sensulatu	<10	Brown	180 x 240
		Sclerocystis rubiformis	35	Yellowish brown	180-195 x 210-225
		A. scrobiculata	<10	Yellow	105 x 105

The greatest population found is from genus *Glomus* which similar to study by Widiastuti and Karmadibrata (1992)[11] that explored the AM fungi occurence in acid soil in West Java. The *Glomus* population was found as the highest compare to other mycorrhizal genus in the acid soil where reeds, corn and little bit cocoa are generally overgrow the land. On the other hand, *Acaulospora* genus also found in all the three districts, but with a limited number of spore population, whereas *Gigaspora sensulatu* only found in Gowa district with a population of 35 spores. This finding support the previous study that detected the indigenous fungi in soil of sugarcane planting area in North Sulawesi province (Adinurani and Hendroko, 2005)[7]. Mycorrhizal diversity was greatest in Gowa district where four types of mycorrhizal genera found in the soil sample taken from this region. Those types are *Glomus, Gigaspora, Acaulospora* and *Sclerocystis* genera, whereas in Takalar and Gowa region the genera found only from 3 to 4 kinds of the fungi. This diversity might be caused by soil characteristics as distribution and abundance among species and strain of AM fungi is known to be differed in response to soil properties (Sjoberg, 2005)[12]. This could be related to the characteristic of the soil in Gowa regency such as pH. The acidity level in the sugarcane planted area is reported to be low within the range of 4.7 - 5.5 while higher figures are correspond to the other two regencies, 5,9 - 6,2 and 5 – 7 for Takalar and Jeneponto, respectively. Great variability of the AM fungi also found by Chairani *et. al.* (2002)[13] in the rhizosphere of Durian in the acid soil with pH of 4.2. In addition, variability of mycorrhiza in this region is similar with Kramadibrata *et. al.* (1985)[14] that isolated mycorrhiza fom the rhizosphere of Maize in Lampung and West Java. The previous research also found four types of different mycorrhiza with more varied species for each genus.

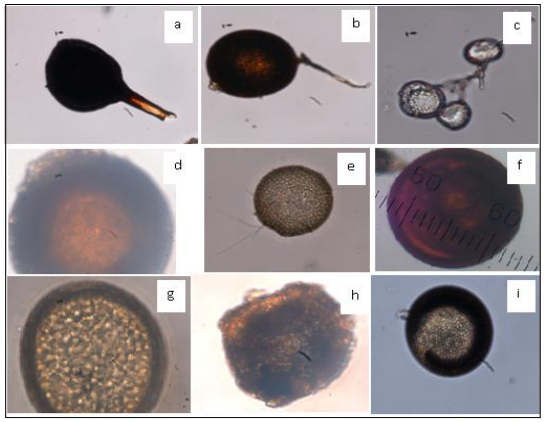

Fig. 1 Spores of Mycorrhiza from Sugarcane rhizosphere in South Sulawesi. a) *Glomus* sp.1; b) *Gigaspora* sp.; c) *Glomus* sp.2)(hyaline);d) *Acaulospora tuberculata*; e) *A. scrobiculata; f) A. foveata*; g) *A. tuberculata*; h) *Sclerocystis rubiformis*; i) *A. scrobiculata*

The spores of *A. foveata* succesfully isolated in the recent study (Table 1 and Fig. 1) has similar colour and shape to the spores found by Janos and Trappe (1982)[15] in the sites planted with sugarcane only much smaller (180 x 180 µm) in size compare to those found before (185–310 x 215-350 µm).

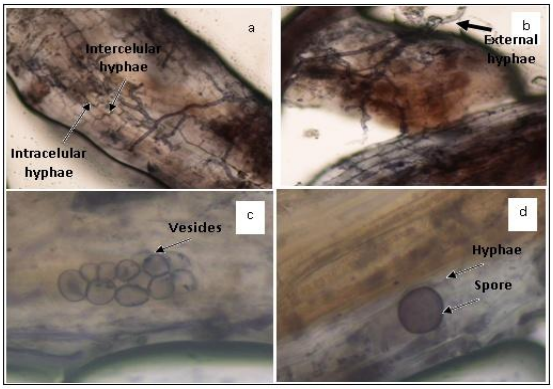

Fig. 2 Sugar root infection of mycorrhizal arbuscular a) internal hyphae; b) external hyphae; c) vesicles; d) hyphae and spore found in rhizosphere of sugarcane

In addition to the occurence of several type of AM fungi spores, infection of AM fungi in the root tissue of sugarcane was also observed. Fig. 2 shows eksternal, interselular and ekstraselular hyphae inside the root tissue and form a typical mycorrhizal structure (Brundrett *et al.*1996)[16]. Mycorrhiza spores and vesicle in the root shown in the figure indicates a highly simbiotic activities of the fungi in fungi-plant interaction and some life cycles of the fungi in the plant root system Result of the recent study implies the chance for isolating the indigenous mycorrhizal fungi from the soil of sugarcane planting area and use it for promoting the plant growth. A further study needs to be undertaken to study the ability of those fungi species found in this study to interact with sugarcane root system and establish a simbiotic plant-fungi association that in turn will give advantage to the plant as in promoting plant growth.

4. CONCLUSION

The study informed that eleven different types of arbuscular mycorrhizal fungi was found in three districts of South Sulawesi province that includes three families, *Glomaceae, Acaulosporaceae*, and *Gigasporaceae*. *Glomus* and *Gigaspora* genera have similar abundance and spores characteristics in three districts and indicate these genera have a wide spread high capability associated with sugarcane. The greatest mycorrhizal diversity was found in Gowa district with four types of mycorrhizal genera they are *Glomus, Gigaspora, Acaulospora* and *Sclerocystis*.

REFERENCES

[1]. Pusat informasi BUMN Perkebunan (2012). ProfilPTPNXIVMakassar(http://www.lpp.ac.id/ptpn.php?id=251&act=view&ptpn=14) Access date: August 17th 2013

[2]. Rismaneswati (2005). Analisis Kesesuaian Lahan Sebagai Dasar Optimalisasi Penggunaan Sumberdaya Lahan Perkebunan Tebu (Studi Kasus PG Takalar). Thesis, Postgraduate Program, Universitas Hasanuddin, Makassar. pp. 92.

[3]. Toharisman A, (2007). Kinerja Industri Gula Indonesia, Pusat Penelitian dan Pengembangan Gula Indonesia, Pasuruan.

[4]. Adholeya A, P Tiwari, R Singh (2005). Large- Scale Inoculum Production of Arbuscular Mycorrhizal Fungi on Root Organs and Inoculation Strategies, In: Declerck S, Strullu DG, Fortin A (Eds.). In Vitro Culture of Mycorrhizas, Soil Biology 4, Springer-Verlag Berlin Heidelberg:315-338.

[5]. Smith DE, DJ Read (1998).Mycorrhizal symbiosis. Academic Press, London.

[6]. Siddiqui ZA, Pichtel J (2008). Mycorrhizae: an overview. In: Siddiqui ZA, MS Akhtar, K Futai (Eds.). Mycorrhizae : Sustainable Agriculture And Forestry, Springer Science and Business Media BV. 1-35

[7]. Adinurani PG, M Mataburu, R Hendroko (1999). Unjuk Kerja Cendawan Mikoriza Arbuskula (CMA) Pada Tanaman Tebu di Tanah Masam PG Tolanghula. Eugenia 9(2):109-118

[8]. Datta P, M Kulkarni (2012). Arbuscular Mycorrhizal Fungal Diversity in Sugarcane Rhizosphere in Relation with Soil Properties. Not Sci Biol 4(1):66-74

[9]. Walker C, CW Maize, HS McNabb Jr (1982). Populations Of Endogonaceous Fungi at Two Locations In Central Iowa. Canadian Journal of Botany 60:2518-2529

[10]. Koske, R.E. and J.N Gemma, 1989. A Modified Procedure for Staining roots to detect VA mycorrhizas (short communications). Mycol. Res. 92 (4):486-505

[11]. Widiastuti H., K Kramadibrata (1992). Jamur Mikoriza Bervesikula Arbuskula di Beberapa Tanah Masam dari Jawa Barat. Menara Perkebunan 60 (1):9-19.

[12]. Sjoberg, J. (2005). Arbuscular Mycorrhiza Fungi. Occurence in Sweden and Interaction with a Plant Pathogenic Fungus in Barley. Acta Universitais Agriculturae Sueciae. Uppsala.

[13]. Chairani, A.W. Gunawan, dan K. Kramadibrata (2002). Mikoriza Durian di Bogor dan Sekitarnya. Jurnal Mikrobiologi Indonesia, Vol 7 No.2. 44-46

[14]. Kramadibrata K, EJ Riyanti,RDM Simanungkalit (1995). Arbuscular Mycorrhizal Fungi from the Rhizospheres of Soybean Crops in Lampung and West Java. Biotropia 8:30-38.

[15]. Janos DP, JM Trappe (1982). Two New *Acaulospora* Species from Tropical America. Mycotaxon 15:512-522.

[16]. Brundrett M, N Bougher, B Dell, T Grove, N Malajczuk (1996). Working with Mycorrhizas in Forestry and Agriculture.ACIAR Monograph 32, Canberra, Australia.