Mycorrhizal diversity in the rhizosphere of sugarcane and grass on different soil types

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Abstract. Mycorrhiza has been known well as beneficial microbiota for supporting plant growth and production. Understanding of the variability and the consistency of the mycorrhizal diversity on various habitats is important for developing mycorrhizal utilization. Mycorrhizal diversity in the rhizosphere of sugarcane from 4 (four) soil types and the rhizosphere of grass from 3 (three) soil types were investigated in the present study. The results showed that *Glomus* indicated as a versatile genus because it was found as a common and dominant genus in the sugarcane rhizosphere on all of four soil types (Alfisol, Andisol, Inceptisol, Vertisol) and in the grass rhizosphere on all of three soil types (Ultisol, Oxisol, Histosol). In addition, *Acaulospora* was found as a common genus in grass rhizosphere. Statistical analysis indicated that P availability in the rhizosphere of sugarcane had a significantly negative correlation with mycorrhizal spore density, in which decreasing P availability significantly related with increasing spore density.

1. Introduction

Mycorrhiza has been known well as beneficial microbiota for supporting plant growth and production. It also protects plants against abiotic stresses such as drought, acidic or alkalic soil. Mycorrhiza establish broadly distributed potentially symbiotic association with the majority of higher plants and has a role for absorbing nutrient, especially phosphor (P) for plants [1-4]. It also improves drought [5] and freezing tolerance [6], acidic or alkalic soil and salinity tolerance [7-9], protects plant from soil-borne pathogen [10], improves plants phytohormone [11].

The existence of arbuscular mycorrhiza widely found in rhizosphere area, including sugarcane [12-15] and grass [16]. Indigenous mycorrhiza shows better potential than commercial mycorrhiza [17]. Most of the sugarcane field found in agriculture plantation, while grass field found in the nonproductive land. Many factors including host types, soil, and environmental conditions can affect mycorrhizal diversity, infectivity and effectivity [18]. Many studies reported that mycorrhizal diversity determines plant biodiversity, ecosystem variability, and productivity [19-21].

The purpose of the present study was to examine mycorrhizal diversity in the rhizosphere of sugarcane and grass on different soil types. Examining the diversity of mycorrhiza on sugarcane lands and grass lands can extend and deepen the understanding of the variability and the consistency of the existence of mycorrhiza in the rhizosphere of the two types of plants which grown continuously on various habitats. Knowledge of mycorrhizal diversity is expected to be the basis within categorizing various mycorrhiza types based on its ability for supporting plants growth and fitness [22].
2. Materials and Methods

2.1. Materials
Rhizosphere soils of sugarcane were collected from 4 soil types: Alfisol taken from Jumantono (07°37’47″S 110°56’51″E), Andisol taken from Tengaran (07°15’51″ S 110°26’57″ E), Inceptisol taken from Delanggu (07°39’20″ S 110°44’21″E), Vertisol taken from Jatikuwung (07°31’06″S 110°50’44″ E). Rhizosphere soils of grass were collected from 3 soil types: Oxisol taken from Tuntang (07°21’08″S 110°28’43″E), Ultisol taken from Jatinangor (06°55’49″S 107°45’38″ E), and Histosol taken from Rawa Pening (07°15’48″S 110°27’00″E).

2.2. Sampling of Rhizosphere Soils
Rhizosphere soil of sugarcane and grass were taken in about 500 g from each location with three replications. Nonrhizosphere soils were randomly taken from the bulk soil of nonplant area for the same each soil type as rhizosphere soils at a depth of 0-20 cm with three replications. Soil samples then were analyzed for chemical characteristics of pH H₂O and P availability.

2.3. Isolation and Identification of Mycorrhiza
Isolation of mycorrhiza from rhizosphere and nonrhizosphere soil of sugarcane and grass were conducted using a modified method of wet-sieving and decantation [23] followed by adding sugar solution [24]. A suspension of 100 g of each soil samples and 250 ml aquadest were stirred and allowed a moment for heavier particle settling. Then, the suspension was filtered using a series of sieves with three different diameter sizes of 250 μ, 90 μ, 45 μ. The suspension of filtrate was transferred and divided to several test tube with a balanced weight then added 60% of sugar solution and centrifuged at 2000 rpm for 3 minutes. The sugar solution was pipetted on 45 μ sieves then sprayed with aquadest to petri dish and observed under the microscope. Particle retained in the last sieve 45 μ were collected in the petri dish, and re-suspend in a shallow of standing aquadest then observed using the binocular microscope for spore details (40-400x).

As for mycorrhizal identification, some mycorrhizal spores from petri dish were transferred to slide glass and observed by dropping Melzer solution then covered with cover glass. Identification of spores morphology was using Species Description of INVAM (International Culture Collection (Vesicular) Arbuscular Mycorrhizal) [25]. Spore density was calculated from total spores found per 100 g soil. Statistical analysis was done to determine the correlation between mycorrhizal spore density and soil chemical characteristics.

3. Results and Discussion
The characteristic of chemical soil content was divided into two parameters, pH and P availability (Figure 1). The effect of soil pH is an important factor toward the development and efficiency of mycorrhiza in various crops that affected mycorrhizal diversity [26-27]. The result of pH measurement was shown in Figure 1 that Alfisol has pH 6.1 which belonged to acidic soil, Andisol has pH 7.1 which belonged to neutral soil, Inceptisol has pH 6.5 which belonged to acidic soil, and Vertisol has pH 7.3 which belonged to alkalic soil.

Figure 1 shows soil type which higher soil acidity or higher soil alkalinity tended to have a lower P availability, while Andisol as a neutral soil in pH had a lowest P availability, this results were from the effect of alophan besides of pH. Figure 1 also showed that all of the soil types on grass rhizosphere were belong acidic soil. P availability of Histosol from grass rhizosphere known as the highest P availability compared to the other soil types both of grass rhizosphere and sugarcane rhizosphere.
Figure 1. pH \( \text{H}_2\text{O} \) and P availability of sugarcane rhizosphere (A) and grass rhizosphere (B).

Spore density from sugarcane rhizosphere was higher and more diverse than spore density from grass rhizosphere (Table 1) and (Table 2). In sugarcane rhizosphere with acidic and neutral soil types (Alfisol, Inceptisol, and Andisol) had lower spore density than spore density of alkalic soil type (Vertisol). But, spore diversity of acidic and neutral soil were more diverse than spore diversity of alkalic soil type. Sugarcane rhizosphere on Alfisol contained spore density of 109 spores/100 g soil which consisted of Acaulospora, Gigaspora, Glomus, and Scutellospora. Sugarcane rhizosphere of Andisol had 142 spores/100 g soil which consisted of Gigaspora, Glomus, and Scutellospora. Sugarcane rhizosphere on Inceptisol had 114 spore/100 g soil which consisted of Acaulospora, Gigaspora, and Glomus. Whereas, sugarcane rhizosphere on Vertisol had 152 spores/100 g soil which consisted of Acaulospora and Glomus.

### Table 1. Mycorrhizal spore density and diversity in the sugarcane rhizosphere

| Soil Types | Spore density (spores 100\(^7\)g soil) | Spore diversity based on Genus |
|------------|---------------------------------------|------------------------------|
| Alfisol    | 109                                   | Acaulospora                  |
| Andisol    | 142                                   | Gigaspora                    |
| Inceptisol | 114                                   | Scutellospora                |
| Vertisol   | 152                                   | Glomus                       |

### Table 2. Mycorrhizal spore density and diversity in the grass rhizosphere

| Soil Types | Spore density (spores 100\(^7\)g soil) | Spore diversity based on Genus |
|------------|---------------------------------------|------------------------------|
| Ultisol    | 174                                   | Acaulospora                  |
| Oxisol     | 286                                   | Glomus                       |
| Histosol   | 68                                    | Gigaspora                    |

In the grass rhizosphere on Ultisol had 174 spores/100 g soil which consisted of Acaulospora, Gigaspora, and Glomus. The highest spore density was shown by Oxisol which reached 286 spores/100 g soil than Ultisol that had 174 spores/100 g soil, and the lowest was 68 spores/100 g soil from Histosol. Mycorrhiza spore density and diversity of sugarcane rhizosphere were higher and more diverse than grass rhizosphere. The matter was suspected because soil type of sugarcane rhizosphere had more diverse of pH. The value of soil pH affected an existence of mycorrhiza [28].
In this study, the correlation was found between spore density with pH and between spore density with P availability. The results of statistical correlation analysis between pH and spore density in the rhizosphere of sugarcane and the rhizosphere of grass were \((r=0.478)\) and \((r=0.487)\). The correlation analysis between P availability and spore density in the rhizosphere of sugarcane and the rhizosphere of grass were \((r=-0.917**\)) and \((r=-0.218)\). The results indicated that decreasing P availability in the sugarcane rhizosphere significantly related with increasing mycorrhizal spore density.

### Table 3. Mycorrhizal spore morphology from the rhizosphere of sugarcane (magnification 400x)

| Shape     | Color         | Spore surface texture | Special characteristics | Reaction with Melzer |
|-----------|---------------|-----------------------|-------------------------|----------------------|
| Round     | Brown         | Smooth                | Subtending hyphae       | -                    |
|           |               |                       |                         |                      |
| Glomus    |               |                       |                         |                      |
| Round     | Dark yellow   | Smooth                | -                       | Slight change from dark yellow to light yellow |
|           |               |                       |                         |                      |
| Gigaspora |               |                       |                         |                      |
| Round     | Hyaline       | Rough                 | Have an ornament        | A color change inside spore |
|           |               |                       |                         |                      |
| Acaulospora |             |                       |                         |                      |
| Round     | Dark brown    | Smooth                | -                       | None                 |
|           |               |                       |                         |                      |
| Scutellospora |         |                       |                         |                      |

Table 3 shows that spore of sugarcane rhizosphere consisted of four genera; there were *Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora*. *Glomus* from sugarcane rhizosphere was round, had a smooth surface, there was subtending hyphae, and did not react with Melzer solution. *Acaulospora* of sugarcane rhizosphere was hyaline, and when it was dropped by Melzer solution the color changed from hyaline into light brown, and in center part of the spore changed darker than side part, it has a rough surface as its ornament. *Scutellospora* of sugarcane was dark brown, had a smooth surface, and no reaction after dropping Melzer solution, it had three layers of the cell wall, and had special characteristic germinal shield within the spore. *Gigaspora* in this study was dark yellow with a smooth surface, had no special characteristic, and had reacted with Melzer then turned from dark yellow to
light yellow. *Acaulospora* of sugarcane rhizosphere was hyaline; there was an ornament on its spores surface, it had three layers of the cell wall, after adding a drop of Melzer solution, the color of spore was changed, in the center part of the spore turned into more darker.

The present study shows similar finding as reported by Kumalawati [14] that *Glomus, Acaulospora, Gigaspora* were found as a common mycorrhiza of the sugarcane rhizosphere in three districts of South Sulawesi province. The difference is that South Sulawesi, especially in Gowa, found *Sclerocystis* besides the three genera, and they did not found *Scutellospora*. The other study that found similar result was reported by Srikumar [12] that *Glomus* and *Gigaspora* were found in four study sites areas in India.

**Table 4. Mycorrhizal spore morphology from the rhizosphere of grass (magnification 400x)**

| Shape        | Color   | Spore surface texture | Special characteristics | Reaction with Melzer                |
|--------------|---------|-----------------------|-------------------------|-------------------------------------|
| Round        | Hyaline | Smooth                | -                       | Slight change from hyaline to pale yellow |
| *Acaulospora*|         |                       |                         |                                     |
| Round        | Brown   | Smooth                | Subtending hyphae       | None                                |
| *Glomus*     |         |                       |                         |                                     |
| Subglobose   | Red     | Rough                 | Have an ornament        | React thoroughly                     |
| *Gigaspora*  |         |                       |                         |                                     |

Table 4 shows that on the grass rhizosphere there were only found three genera; there were *Acaulospora, Glomus, and Gigaspora*. In Ultisol had 174 spores/100 g soil that consisting of *Acaulospora, Gigaspora* and *Glomus*. Whereas, grass rhizosphere in Oxisol contained spores density of 286 spores/100 g soil and in Histosol 68 spores/100 g soil, consisting of *Acaulospora*, and *Glomus*. *Acaulospora* in this study was hyaline with the surface structure was smooth, had no special characteristic, and reacted with Melzer from hyaline to pale yellow. *Glomus* in this study was brown with the surface structure was smooth, had subtending hyphae, and had no reacted with Melzer. *Gigaspora* in this study was red with the surface structure was rough, had an ornament, and reacted with Melzer from red to orange. Another study [15] reported the same genera that found in the several rhizosphere of grasses. *Acaulospora, Glomus, and Gigaspora* were found on the grass rhizosphere in Penelokan village of Bali.
Conclusion

Glomus was only found as a common and dominant genus on all of four soil types (Alfisol, Andisol, Inceptisol, Vertisol) in the sugarcane rhizosphere. The rhizosphere of sugarcane from Vertisol indicated the highest of mycorrhizal spore density of 152 spores/100 g with the lowest of mycorrhizal diversity. The rhizosphere of sugarcane from Alfisol indicated the lowest of mycorrhizal spore density of 109 spores/100 g with the highest of mycorrhizal diversity.

Acabulospora and Glomus were found as a common genera on all of three soil types (Ultisol, Oxisol, Histosol) in the grass rhizosphere. The rhizosphere of grass from Oxisol indicated the highest of mycorrhizal spore density of 286 spores/100 g and from Histosol indicated the lowest of mycorrhizal spore density of 68 spores/100 g. In addition, the genus of Gigaspora was only found in the rhizosphere of grass on Ultisol.

It was found that P availability in the sugarcane rhizosphere had a negative correlation significantly with mycorrhizal spore density, in which decreasing P availability significantly related with increasing spore density.

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References

[1] Cahyani VR 2009 Proceeding of International Seminar of Upland for Food Security. 7-8 November 2009, Faculty of Agriculture Jenderal Soedirman University. ISBN: 978-979-99046-1-4, 254-260.
[2] Papilaya PM 2013 International Journal of Sciences and Research (IJSR) 4: 1935-1942.
[3] Syafria H, Jamarun N, Zein M and Yani E 2015 International Journal of Agriculture Science IJAC 1:47-54.
[4] Al-Karaki GN 2016 Agrofor International Journal. 1: 154-161. doi: 10.7251/AGRENG1602154A.
[5] Sulistiono W, Taryono, Yudono P and Irham 2017 Journal of Agricultural Science 9: 95-108.
[6] Otgonsuren B, Lee JT and Lee MJ 2015 International Journal of Advanced Research in Biological Sciences 2:191-204.
[7] Shinde SK, Shinde BP, and Patale SW 2013 Int. J. Life Sci & Pharma Res. 3: 11-15.
[8] Cahyani VR, Ariyanto DP, Qodri BM, Prameshi GPA, Purwani RH, Purba SM, Asakawa S and Kimura M 2014 Proceedings of 11th International Conference of the East and Southeast Asia Federation of Soil Science Societies, 21-24 October 2013, IPB, Bogor, ISBN 978-979-19904-1-7, 434-435.
[9] Elhindi KM, El-Din AS and Elgoban AM 2016 Saudi Journal of Biological Sciences 24: 170-179. Sjs.2016.02.010.
[10] Newsham KK, Fitter AH and Watkitason AR 1995 Journal of Ecology 83: 991-1000.
[11] Pozo MJ, Lopez-Raez JA, Azcon-Aguilar C and Garcia-Garrido JM 2015 New Phytologist 205: 1431–1436. doi: 10.1111/nph.13252.
[12] Srikumar R, Thangaraj R and Murugaian P 2009 Agricultural Science Digest 9: 1-4.
[13] Prabudoss V 2011 International Journal of Current Research 3: 228-234.
[14] Kumalawati Z, Musa Y, Asrul L and Ridwan I 2014 International Journal of Scientific & Technology Research IJSTR 3: 201-203.
[15] Srinivasan M, Kumar K, Kumutha K and Marimuthu P 2014 Journal of Applied and Natural Sciences 6: 29-293.
[16] Dewi NKS, Susrama IGK, Sriatmin M, Adnyana M and Wirawan IGP 2014 International Journal of Biosciences and Biotechnology 2: 26-31.
[17] Berruti A, Lumin E, Balestrini R and Bianciotto V 2016 Front. Microbiol. 6:1559. doi: 10.3389/fmicb.2015.01559.
[18] Moreira M, Baretta D, Tsai SM, Gomes-daCosta SM and Cardoso EJBN 2007 Sci. Agric. 64 4: 393-399.
[19] Van der Heijden MGA, Klironomos J, Ursic M and Sanders IR 1998 Nature 396.6706 69.
[20] Van der Heijden MGA 2002 Mycorrhizal Ecology 157: 243-265.
[21] Klironomos J, McCune J, Hart M, Neville J 2000 Ecology Letters 3 2: 137-141
[22] Alarcon A, Hernandez-Cuevas LV, Ferrera-Cerrato R and Franco-Ramirez A 2012 J. Biofertil Biopestici 3 1 p 1000115.
[23] Gerdemann JW, Nicolson TH 1963 Trans. Brit. Mycol. Soc. 46, 336-338.
[24] Walker C, Mize W and McNabb Jr HS 1982 Can. J. Bot. 60, 2518-2529.
[25] INVAM [International Name of Vesicle Arbuscular Mycorrhizae] 2016 http://www.invam.wvu.edu/the-fungi. Accessed on June 2016.
[26] Singh G, Goyne KW and Kabrick JM 2015 Geoderma Regional 5: 117-126.
[27] Singh S 2004 Mycorrhiza News 16 2: 2-23.
[28] Kluber LA, Carrino-Kyker Sr, Coyle KP, DeForest JL, Hewins CR, Shaw AN, Smemo KA, Burke DJ 2012 PLoS ONE 7(11): e48946. doi:10.1371/journal.pone.0048946.