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Utilization of arbuscular mycorrhizal rizosphere Imperata cylindrica to increase the yield of corn in podzolic soil: Study of arbuscular mycorrhizal diversity

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Abstract

Bladygrass (*Imperata cylindrica*) are categorised as weeds whose range of proliferation is wide. Bladygrass are capable of adapting to any types of soil and inclement climate. Only a few researches identify mycorrhizal symbiosis with the bladygrass roots. This research is aimed to explore the infecting capability, population of spores, and diversity of arbuscular mycorrhiza in the vegetation of bladygrass in arid soil in Kendari, the Province of southeast Sulawesi, Indonesia. Research activity involved observation and laboratory analysis. The research results are expected to be applicable in developing the application for the utilisation of arbuscular mycorrhiza to grow plants. From research results, it was obtained that on vegetation of bladygrass was found in several genuses of arbuscular mycorrhiza, with the infecting capability ranging from 56.67 – 86.67%, the number of spores ranging from 359 to 401 per 100 g soil.. Arbuscular mycorrhizal were found to be dominated by the genus of *Glomus* and *Acaulospora*.

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Introduction

Arbuscular mycorrhiza is classified as a fungus growing on host plant on obligate biotropic soil. This type of fungus is characterised by its vesicles and/or arbuscules. Due to this particular characteristic, this fungus is commonly known as arbuscular mychorrhiza (Brundrett *et al.*, 1996).

Mycorrhiza is the most essential component in plant ecosystem, because it has extensive distribution in varied environment (Smith and Read, 2008). This type of fungus is able to grow in more than 80% of plants from liverworts to ferns, from gymnospermae to angiospermae (Bonfante *et al.*, 2009), it has no specificity of host plant (Remy *et al.*, 1994).

Bladygrass (*Imperata cylindrica*) is one of plant types which can serve as host plant for mycorrhiza. Bladygrass is weed with its ability to spread extensively. Its ability to spread is supported by its symbiosis with mycorrhiza. Subiksa (2002) in Pudjiharta (2008), agrees that reeds are tolerant to extreme condition, for such plant is capable of associating with soil microbes helping with the plant growth. Bladygrass are found to be associated with various types of arbuscular mychorrhizal fungi (AMF) from the genus of *Glomus*, *Acaulospora*, and *Gigaspora*.

The ability of bladygrass to extensively spread over all kinds of soil give a chance for mycorrhiza to naturally grow on the bladygrass. The soil of rhizosphere could serve as inoculum of arbuscular mycorrhiza. However, the diversity of mycorhiza on rhizosphere of bladygrass and their ability to infect is not generally known, especially for the bladygrass growing on arid soil or sub-optimal lands. In this research, the diversity of arbuscular mycorrhiza in the vegetation of bladygrass was identified and studied in term of their level of infection and the density of spore population. This research was aimed to study the diversity of mycorrhiza on rhizosphere of bladygrass, the population of spores, and roots infection.

Methods

This research was carried out by exploring the area where the bladygrass were growing, in Halu Oleo University, Kendari, Southeast Sulawesi, from January to February 2013. The exploration was done by randomly obtaining soil samples and the root of bladygrass from ten points, referring to soil biological analysis method (Anonym, 2007).

Furthermore, isolation of spore, the cleaning and dyeing of the bladygrass roots were done. The identification in order to find the type of mycorrhiza was done in the Laboratory of Pest Science and Plant Disease, The University of Brawijaya, Malang. The identification methods followed the steps introduced by Sastrahidayat (2011), while the classification guide of *Glomeromycata* was used to determine the type of mycorrhiza (Anonym, 2013; 2014).

Research implementation

Samples were taken according to the following procedures: (1) determining the points where the samples were taken from by using 1m x 1m quadrant, (2) the sample of the root and soil weighing ± 1 kg in each sample point, (3) Samples were inserted into plastic bags and labelled according to the time they were taken, the location, soil type, height above sea level, and the type of vegetation. There was only one sample point in each quadrant, making up 10 sample points for the total numbers of sample points.

Spores of arbuscular mycorrhizal extracted from soil by wet sieving and decanting method from Gerdemann and Nicolson (1963). Clearing and staining of roots were done by applying the method of DR. I. HSLL's (1982) in Setiadi *et. al.*, 1992).

Infected roots were observed based on the slide method by Giovannetti and Mosse (1980). Infected root was observed according to the following formula:

$$\% \text{ root infection} = \frac{number\ of\ infected\ roots}{number\ of\ observed\ roots}\ x\ 100\%$$

The pattern of infection, the size of spore, the colour of spore, the sub-cellular structure (the number of walls) of spore were identified based on the classification guide of *Glomeromycota* (Anonym,

2013; 2014).

Results and discussion

Sampling site

Samples were taken from Regency of Kambu, sub-district of Kambu, Kendari, Province of Southeast Sulawesi at ± 320 m above sea level. Geographically, the location lies to the south of equator, in 03°45'–04°15' of south latitude and 122°15'-122°54' East Longitude. The rainfall was around 7.6 mm per day during the observation. The temperature was 33°C, and 81% for the humidity.

Table 1. Criteria of dominant genus of arbuscular mycorrhiza found on rhizosphere of bladygrass on arid land.

Criterion	*Glomus spp.*	*Glomus spp.*	*Acaulospora sp.*
Colour	Orange-reddish brown	Brown	Yellow - light brown
Shape	spherical	Spherical– rather spherical	Spherical
Size	102 – 105 µm	107 – 108 µm	80 – 89 µm
No. of Layer Wall	2	2	1
Wall thickness	L1 = 3.82 L2 = 4.16	L1 = 1.15 L2 = 6.98	L = 3-22 µm

The chemical analysis results of the soil revealed that the reaction characteristic was a bit acid, and the C-organic content and the N-total were quite low. The ratio of C/N was moderate. Moreover, the available P was very low, so was the available K; the available Na was moderate, but the available Ca was very low, so was the available Mg. The value of Cation Exchange Capacity (CEC) was high and the percentage of base saturation was low. The soil texture was dusty; it was classified as rather light, easily cultivated, and having good drainage.

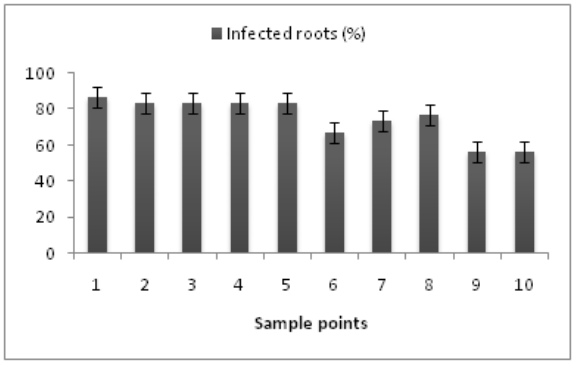

Fig. 1. Infected roots of bladygrass on arid land in each sample points.

Roots infection

The percentage of root infection in each sample point ranged from 56.67 – 86.67 % (Fig. 1). The difference in the rate of infection can be caused by the length of time the infection has been undertaken (starting from the initiation of infection), arbuscular mycorrhizal types, plant species, climate and soil. Environmental conditions can influence the relative abundance of structures and level of colonization (Sikes *et al.*, 2010; Murray *et al.*, 2010). Bansal *et al.* (2012) reported that root colonization varied from crop to crop, season to season and field to field. The percent of the total root length colonized in tomato varied with species of arbuscular mycorrhiza (Cavagnaro *et al.*, 2001). Arbuscular mycorrhiza communities can be very different depending on their host plants, even within the same ecosystem. These differences are significant not only in terms of species composition, but also in their seasonal dynamics (Santos – Gonz'alez *et al.*, 2007).

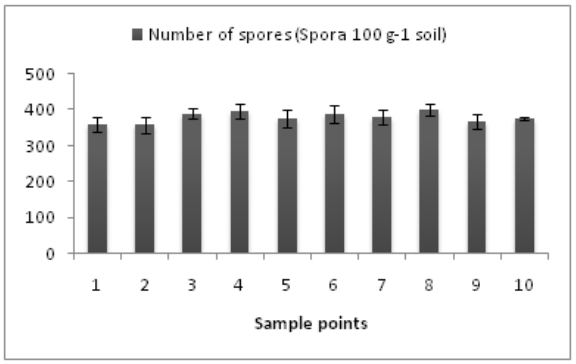

Fig. 2. Number of spores on rhizosphere of bladygrass on arid land in each sample points.

The infection caused by arbuscular mycorrhizal could be in the form of hypa, vesicle, and or arbuscule. Based to the classification guide of Glomeromycota

(Anonym, 2013; 2014), the infection found on the bladygrass roots was in the form of extraradical hypha, intraradical hyphae, entry point, intraradical vesicles, arbuscule, and or coil. These results were consistent to those found by Moreira-Souza *et al.* (2003) and Moreira *et al.* (2006) in *Araucaria angustifolia*. Microscopic appearance of endomycorrhizal is presented in Fig. 3. Intraradical vesicles were found but not in every sample point, neither were arbuscular and coiled mycorrhizae. The fact that arbuscules were not found possibly because the formed arbuscules had fallen, or because the infecting michorrhizal fungi had not formed arbuscules yet. Brundertt *et al.* (1996) suggested that the growth of arbuscules as a result of subapical parts in internal hyphae followed the growth of hyphae, which was the further development of entry point. Meanwhile, the initiation of vesicles occurred soon after the initiation of the first arbuscules, but their growth continued after arbuscules of *senescens* initiated. At a later stage collapse arbuscular (progress, starting again with the best branch of Hypa). An arbuscule is formed between cellular wall and plasma membrane. Despite the short duration, arbuscules could appear in numbers on roots for a long time as long as the fungi kept growing and reproducing. In INVAM (Anonym, 2013), it was stated that the differentiation in arbuscules seemed to be longer (especially in planting pot) on the infected plant root of subordo *Gigasporineae* (3-4 months) than that of subordo Glomineae (2-3 months). The morphology of arbuscules was also different among fungal species (*Scutellospora heterogama* is different from *Glomus diaphanum* or from *Acaulospora denticulata*). Chaudhry *et al.* (2012) reported that the extraradical mycorrhizal association comprised hyphae showing various types of structural differences. Two distinctive types of hyphae were observed i.e., runner and absorbing hyphae. The runner hyphae were running parallel along the axis of root and were mostly darkly stained with thick or double walls, with or without septa and variation of diameters. The absorbing hyphae were mostly aseptate, much branched, less stained and thin walled.

Number of spores

The spore isolation brought results showing that the average number of spores of each sample point ranged from 359-401 spores per 100 g soil (Fig. 2). The number of spore in each sample point was not far different. The growing of spores was influenced by the infection period since the time of initiation, species of plants, climate, and soil, as reported in several previous studies. Significantly more arbuscular mycorrhizal spores were observed in soil from no-tilled wheat field than from the tilled field following the rapeseed season. However, the total spore count was not significantly affected by soil tillage following the maize season (Jansa *et al.*, 2002).

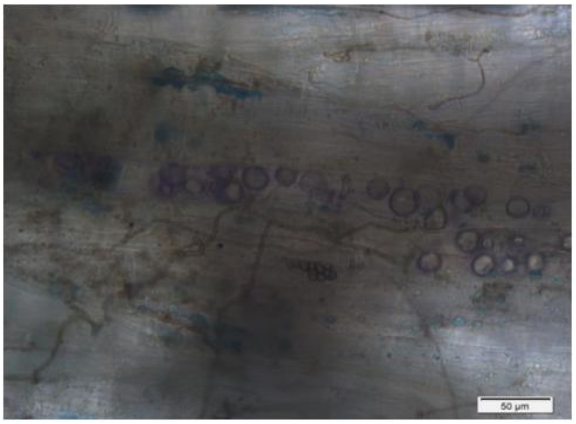

Fig. 3. Microscopic appearance of endomycorrhizal at roots of bladygrass on arid land.

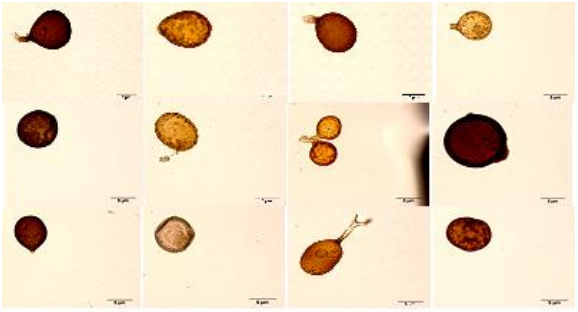

Fig. 4. The microscopic appearance of single spores isolated from rhizosphere of bladygrass

Influence of plant rotation on arbuscular mycorrhizae is significant and should be considered in field management, in which the number of spores in soil decreased significantly by planting *Triticum aestium*, *Satureja hotensis*, and *Raphanus sativa* cultures, and increased significantly by planting *Zea mays* and *Allium cepa*. Colonization and arbuscular mycorrhiza spores occur in different habitats. The number of

arbuscular mycorrhiza spores in soils differed among plant species of the same region. The number of spores in *Alopecurus arundinaceus* rhizosphere was about twice that of *Verbascum cheiranthifolium* and three times that of *Hyoscyamus niger* (Sinegani and Sharifi, 2007). In this study, the correlation between infection level and the number of spores on rhizosphere of bladygrass was not found. Sample point with the highest population of spores did not always have the highest infection level, and vice versa. The results of this study were consistent with findings by Khakpour and Khara (2012) on several species of plants. Moreira *et al*. (2006) reported that arbuscular mycorrhiza sporulation at the same sampling point is very dynamic in relation to different seasons, numbers of spores are not always reliable as parameters to determine the composition of the arbuscular mycorrhiza community in an ecosystem. There are fungi that sporulate more, while others sporulate less (perhaps never), and others yet, only sporulate during certain periods of the year. Sampling sites several times throughout the year is indispensable.

Fig. 5. Dominant spores of arbuscular mycorrhiza associated with bladygrass

Identification of arbuscular mycorrhizal

The identification of the genus of arbuscular mycorrhizal having symbiosis with the bladygrass according to the infection (mycorrhiza) and the structure of sub-cellular spore referred to INVAM (Anonym, 2013). The microscopic appearance of single spores isolated from rhizosphere of bladygrass is presented in Fig. 4. The results of identification showed that there were some genuses of arbuscular mycorrhizal found on bladygrass. Several genuses found on rhizosphere of bladygrass in one of any arid lands in Kendari, the Province of Southeast Sulawesi were *Glomus, Gigaspora, Acaulospora, Entrophospora, and Paraglomus*. Microscopic appearance of infected roots is shown in Fig. 3. Based on the criteria of spores identified (Table 1), the genus of arbuscular mycorrhizal was dominated by the genus of *Glomus* and *Acaulospora*, with the population of spores higher than that of the genus of *Glomus*. The dominant spores are presented in Fig. 5. According to Simanungkalit *et al*., (1999) in Simanungkalit (2006) in Jambi and Lampung, the species of arbuscular mycorrhiza was obtained in the forest ecosystem, agricultural ecosystem, and the land where the bladygrass grew with 7-10 species, 8-11 species, 10-11 species, respectively. Jansa *et al* (2002) reported 14 species of arbuskular mycorrhizal were detected from rhizosphere of four-year crop-rotation consisted of a rapeseed (*Brassica napus* L.), winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.). Schalamuk *et al*. (2006) had identified 24 species in soil samples from rhizosphere of wheat, the dominant species were *A. excavata, A. mellea, G. claroideum, G. clarum, G. etunicatum, G. mosseae and Scutellospora aff. dipapillosa.*

Conclusion

Arbuscular mycorrhizal such as *Glomus, Gigasphora, Acaulospora, Entrophospora*, and *Paraglomus*, were found on rhizosphere of bladygrass on arid land in Kendari, Southeast Sulawesi. Arbuscular mycorrhizal were found to be dominated by the genus of *Glomus*

and *Acaulospora*. The case of infected roots caused by arbuscular mycorrhiza ranged from 56.67-86.67 % with the number of spores ranging from 359 to 401 per 100 g soil.

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