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Utilization of arbuscular mycorrhizal rhizosphere *Imperata cylindrica* to increase the yield of corn in podzolic soil: the indigenous inoculum effectiveness studies

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Abstract

Bladygrass (*Imperata cylindrica*) is the host for arbuscular mycorrhizal. Widespread deployment of bladygrass make arbuscular mycorrhizal inoculum easy to find. In this research examined the effectiveness of a indigenous inoculums. The inoculum obtained from rhizosphere bladygrass in the field, which is a mixture of soil containing spores and hyphae, and root hair pieces bladygrass. This research was carried out with arbuscular mycorrhiza culturing in plastic pots, using corn and bladygrass as a host plant. Observation variable were the root infection and the number of spores. .The results showed the development of arbuscular mycorrhizal in corn plant cells and bladygrass is quite good. This is indicated by an increase in root infection and the number of spores, which is consistent with the increasing age of the plant up to the age of 60 days after the plants inoculated. Root infection increase on the plant age of 60 day after inoculated was 28.48% and 31.33%, on corn plant and bladygrass respectively, compared with the plant age of 30 days after inoculated. Similarly with the number of spores on plant age 60 days after inoculated, an increase of 317.91% and 280.72%, on corn plant and bladygrass respectively, compared with the plant age of 15 days after inoculated. This is an indication that the indigenous inoculum, which taken from bladygrass rhizosphere in field, it was effectively used as arbuscular mycorrhizal inoculum on corn plant.

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Introduction

Research on the effectiveness of arbuscular mycorrhizal in corn plant have been reported, including reported by Almaca and Ortas (2010); Ananthi *et al.* (2010; 2011); Amanullah *et al.* (2011); Archana *et al.* (2012), it was using arbuscular mycorrhizal isolated from wild plants or from plants cultivated. The success rate of arbuscular mycorrhizal inoculation on cultivated plants is largely determined by the compatibility between plants which are inoculated and arbuscular mycorrhizal, beside influenced by environmental factors. Therefore, it is necessary to select the source (natural host), and host plant to arbuscular mycorrhizal trapping.

The form of inoculum also affect success of symbiosis arbuscular mycorrhizal with host plants. Various forms of inoculum has been studied, i.e. capsule, tablets, powders, granules, and mixed inoculum consisting of soil and arbuscular mycorrhizal propagules. Several studies have shown that the mixed inoculum is more effective in improving crop yields, compared with some other form of inoculum. Sastrahidayat (2011) reported that a mixed inoculum form is more effective in increasing the yield of soybean and melon. One important aspect to be studied is indigenous inoculum in form a mixed inoculum. It is inoculum that taken from host rhizosphere in field then applied directly to the cultured plants, without going through either the trapping, or pot culture process. The methode will be shorten and simplify the application process. However, should choose a natural host of arbuscular mycorrhizae which are widely scattered in various regions, is available, and its symbiosis with arbuscular mycorrhizal developing quite well.

Bladygrass (*Imperata cylindrica*) is one type of weeds that are hosts for arbuscular mycorrhizal, Simanungkalit *et al.* (1999) in Simanungkalit (2006) reported that in Jambi and Lampung arbuscular mycorrhizal species found in the land where the bladygrass grew with 10-11 species. Bladygrass is able to adapt to different types of soil with different climatic conditions that are less favorable for the plant growth. In addition to genetic ability, the ability to spread widely suspected to be supported by the arbuscular mycorrhizal symbiosis. In research Hasid *et al.* (2014) found roots infection of bladygrass 56.67 % - 86.67 %, arbuscular mycorrhizal found in the rhizosphere of bladygrass are the genus *Glomus, Gigaspora, Acaulospora, Entrophospora, Paraglomus.*

Bladygrass quite broad spreading in various area. This is the source of a very large indigenous inoculum. Indigenous inoculum effectiveness has been studied in this research, conducted in pot culture, host plant corn and bladygrass. This study was conducted to analyze the ability of arbuscular mycorrhizal infection on the roots of corn plants and bladygrass using indigenous inoculum, as early information for the utilization of indigenous inoculum in the development of the corn crop in the field.

Materials and methods

Place and time of research

The experiment was conducted in Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia. The analysis of root infection and the number of spores carried out at the Laboratory of Agrotechnology, University of Halu Oleo, Kendari, and the Laboratory Science Plant Pests and Diseases, University of Brawijaya, Malang, from February 2013 to April 2013.

Research implementation

Soil as a growing medium taken on topsoil depth of about 20 cm. The soil was sieved using a sieve pore size of 3.5 mm x 3.5 mm. Furthermore, in autotoclave sterilized at a temperature of 121^{o}C for one hour.

Preparation of indigenous inoculum was done by taking soil from bladygrass rhizosphere in the field and analyzed the root infected and density of the number of spores. The soil was sieved using a sieve pore size of 3.5 mm x 3.5 mm. Indigenous inoculum used on the pot culture in the form of a mixed inoculum (soil containing spores and hyphae, and hair roots of bladygrass are cut size about 0.5 cm).

Planting of seeds in plastic pots using a sterile soil medium as much as 400 g per pot. Seedlings of corn that planted were five days old, and the seedlings of bladygrass were a month old, the seedling were inoculated arbuscular mycorrhizal.

Arbuscular mycorrhizal observations

Observation of root infection and the number of spores carried out every 15 days up to 60 days after inoculation. 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). Root infection was observed according to the following formula:

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

Results and discussion

Climatic conditions and soil characteristics

The rainfall was around 22.5-177.9 mm per month, 7.0-5.9 mm per day during the observation. The temperature was 28.7-29.1°C, and 74-81% for the humidity.

Fig. 1. Infected root of corn plant in each plant age.

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. 2. Infected root of bladygrass in each plant age.

The indigenous inoculum effectiveness

Arbuscular mycorrhizal inoculation using indigenous inoculum on corn plant and bladygrass indicate that the ability of arbuscular mycorrhizal infection tend to increase, which is consistent with the increasing age of the plant up to the age of 60 days after the plants inoculated. (Figure 1 and Figure 2). In the age of corn plant 15 days after inoculated, root cell infected has not been seen, such as in bladygrass. Root infected has been seen in the age of the plant 30 days after inoculated, root infection was 46.7% and 53.3% respectively on corn plant and bladygrass. In the age of the plant 45 days after inoculated, root infection slightly increased in corn plants and bladygrass, respectively 7.07 % and 12.57%. Whereas 60 days after the plant inoculated, root infection increase was 28.48% and 31.33% on corn plant and bladygrass respectively, compared with 30 days after the plant inoculated.

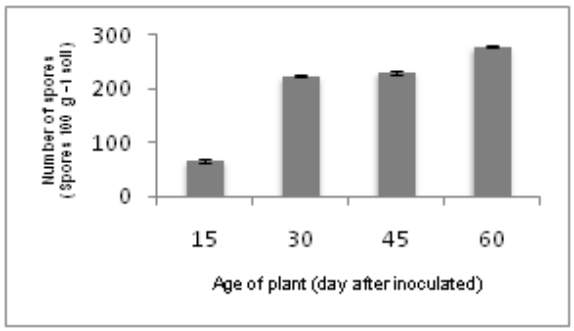

Fig. 3. Number of spores on soil of rhizosphere corn plant in each plant age.

The number of spores tend to increase, which is consistent with the increasing age of the plant (Figure 3 and Figure 4), as well as on root infection. In the

age of the plant 15 days after inoculated, the number of spores was 67 and 83 spores per 100 g soil rhizosphere respectively on corn and bladygrass. Compared with the age of the plant 15 days after inoculated, the increase the number of spores on the age of the plant 30 days after inoculated was 234.33% and 177.11%, whereas 45 days after the plant inoculated was 246.27% and 202.41%, and 60 days after the plant inoculated was 317.91% and 280.72%, respectively on corn and bladygrass.

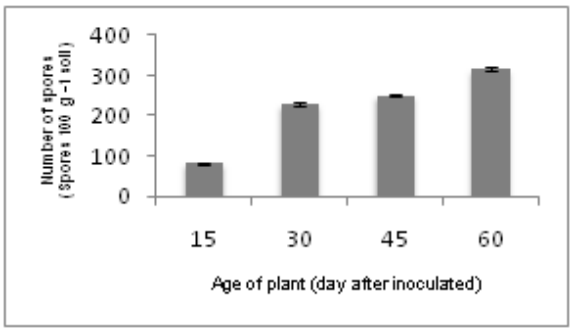

Fig. 4. Number of spores on soil of rhizosphere bladygrass in each plant age.

The results obtained in this study is slightly different from some earlier studies. In this study, infection of the roots into the plant cells of corn and bladygrass have not been found at 15 days after inoculation, whereas in some previous studies have root infections occur in this age, as well as on research Gravito and Miller (1998) to determine the potential of mycorrhizal indigenous in maize, suggesting that colonization occurred in 7 days after the emergency with the percentage of colonization ± 15%. This difference is thought to be caused due to differences in study conditions, differences in climate and soil, the source of inoculum, and the time of inoculation. Mycorrhizal infection is influenced by environmental factors, plant and inoculum used. An *et al.* (2010) reported that arbuscular mycorrhizal colonization of on corn varies, depending on the type of germplasm, origin (country and location), and year of release. Pairwise interactions among plants, arbuscular mycorrhizal and soil have shown that the effectiveness of these interactions depends on the origin of plants, soil, and fungi. Environmental conditions can influence the relative abundance of structures and level of colonization (Sikes *et al.*, 2010; Murray *et al.*, 2010). Root colonization varied from crop to crop, season to season and field to field (Bansal *et al.*, 2012). Amount of infection was varied from species to species of plant and ranging from 10 to 30% (Yeasmin *et al.*, 2007). Cavagnaro *et al.* (2001) reported that the percent of the total root length colonized in tomato varied with species of arbuscular mycorrhiza. 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).

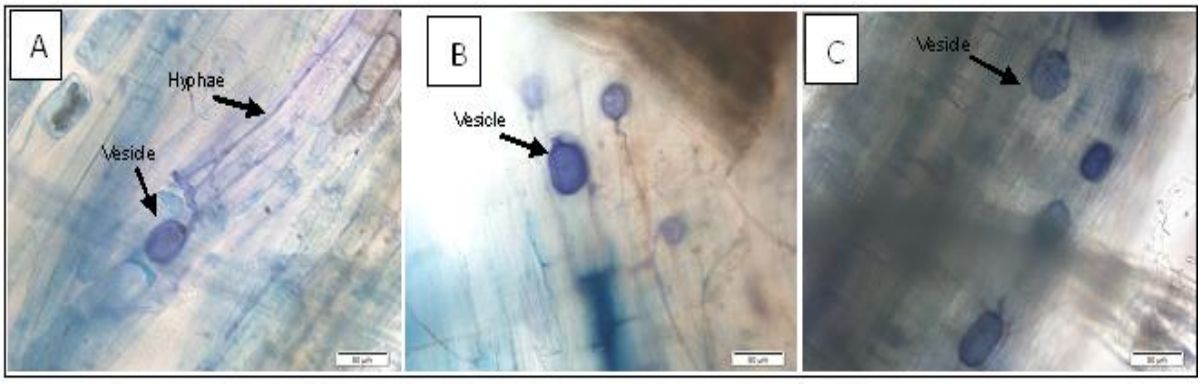

Fig. 5. Microscopic appearance of endomycorrhizal in roots of corn plants age 30 (A), 45 (B), and 60 (C) days after inoculation.

The form of inoculum may also affect infection of plant roots. Various forms of inoculum that can be inoculated at the plant in the form of tablets, capsules, granular, and a mixed inoculum. Sastrahidayat (2011) reported that the form of inoculum mixture gives the highest value compared

to other types (increasing the production of approximately 172 % and 108 % compared to control, respectively on soybean and melon). Noyd (1995) in Yeasmin *et al.* (2007) suggested that soil samples isolated from a place, which contains a mixture of arbuscular mycorrhizal spores and hyfa, and pieces colonized plant roots can be used as inoculum.

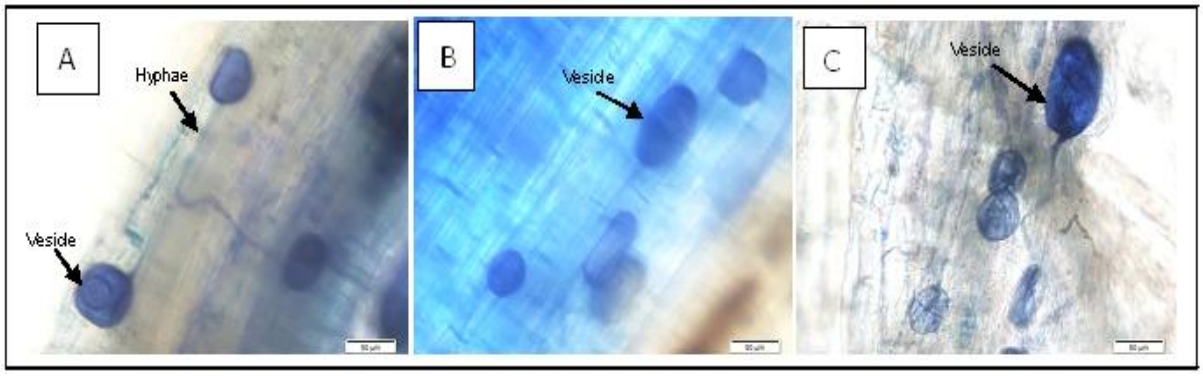

Fig. 6. Microscopic appearance of endomycorrhizal in roots of bladygrass age 30 (A), 45 (B), and 60 (C) days after inoculation.

The results obtained in this study indicate that soil (containing spores and hyphae) were taken directly in the field in the rhizosphere of bladygrass mixed with pieces of hair roots of bladygrass effectively used as arbuscular mycorrhizal inoculum. This is demonstrated by the ability of arbuscular mycorrhizal to infect of plant roots, as well as its ability to evolve in the root cells of plants inoculated. Arbuscular mycorrhizal infect roots of corn plants and bladygrass, and growing in the plant cells, forming arbuscular and vesicles. Microscopic appearance of endomycorrhizal at roots of corn plant and bladygrass aged 30, 45, and 60 days after inoculation in Figure 5 and Figure 6. Vesicles are round-oval and a means storage of food reserves. Vesicle formation in plant cells is one of the indications of symbiosis between plants and arbuscular mycorrhizae. In addition to the vesicles and arbuscular formed external hyphae that can help expand the area of nutrient absorption in plants. 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. Brundrett *et al.* (1996) suggested that mycorrhizal association mycorrhizal fungi in the soil begins when hypa response to the presence of roots. Root penetration occurs when one or more hypa penetrate the root epidermis cells, enter and penetrate into the hypodermis and exodermis also establish branches outside the cortex. Hypa scattered throughout the cortex in both directions from entry point to establish a colony (infection unit). Furthermore, the formation of arbuscular which is the development of sub-apical branches in the internal Hypa in cortical cells. 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 Hyphae).

Conclusion

Root infection and the number of spores increased with the increasing age of the plant up to the age of 60 days after inoculation, both on corn and in the bladygrass. Bladygrass can be used as host plants for arbuscular mycorrhizal fungal cultures, to be used as inoculum. Indigenous inoculum arbuscular mycorrhizal effectively used on corn.

Acknowledgements

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