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Research Article
Existence Arbuscula Mycorrhiza and Its Application Effect to Several Variety of Corn Plant (*Zeal mays* L.) in Marginal Dry Land

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Abstract

Background and Objective: Arbuscula mycorrhiza (AM) have a very large function in symbiosis with plant roots, it's very important to be studied further because AM utilization is an alternative solution to improve the yield of corn plant in poor land. Until now the productivity of corn plant, especially in Southeast Sulawesi, Indonesia is lower than it's genetic potential, one of the causes is the cultivation of many plant done in sub optimal land with low technology applications especially the use of organic and biological fertilizer very low. This study aimed to observe the presence of AM and evaluate the growth and productivity of corn plant that AM inoculated. **Materials and Methods:** The study consisted two series of experiments, namely (1) Existence arbuscula mycorrhiza in rhizosphere of dominant weed (bladygrass) in dry land in Kendari, Southeast Sulawesi, Indonesia, was observed descriptively, (2) Effect of arbuscula mycorrhiza to several variety of corn plant (*Zea mays* L.) in Marginal dry land. The experiment was arranged based Randomized Block Design (RBD) consisted of eight treatments corn variety. **Results:** The results showed that spore populations were found in rhizosphere of dominant weed was 792-901 spores 100 g^{-1} soil, 70-90% roots infection. Some genera found are *Glomus*, *Gigasphora*, *Acaulospora*, *Entrophospora*, *Scutellospora*. Symbiosis effect of arbuscula mycorrhiza with plant growth indicates that Phosphorus uptake was highest in Batu Putih variety. However the highest in yield was show in Dana variety. Compared with the lowest production, the production difference was higher in Dana 47.70%. **Conclusion:** Existence of arbuscula mycorrhiza in dominant weed rhizosphere in dry land is very high. The response of various varieties of maize plants to arbuscula mycorrhiza indicates that local varieties have a higher adaptability compared with introduction varieties.

Key words: Dana variety, indigenous, Kendari, local corn, phosphorus, root infection, spore population, biological fertilizers and Batu Putih

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Arbuscula mycorrhiza (AM) is a group of obligate biotrophic soil fungi that cannot grow and develop without host plants. This fungus is characterized by the presence of vesicles and/or arbuscules. Based on the specific characteristics of this mushroom is known as arbuscula mycorrhiza fungi. The arbuscula mycorrhiza is grouped in *Zygomycota* Phylum, *Glomeromycota* order, having sub order *Glomineae* and *Gigasporinea*e[1]. Arbuscula mycorrhiza is easily developed in the rhizosphere of various plant species, both cultivated and wild plants[2]. This fungus can also survive in various climatic conditions and various types of soil with conditions varying fertility rates. Arbuscula mycorrhiza is commonly found in plant rhizosphere, including weeds that grow in dry land of Kendari, Sulawesi Tenggara, Indonesia. Various types of weeds that are found dominant on dry land include *Imperata cylindrica* and *Chromolaena odorata*. The diversity of AM has not been widely known although this type of fungus has been widely proven that it is able to increase the growth and yield of cultivated plants.

Arbuscula mycorrhiza (AM) has a very large function in symbiosis with plant roots. Various research results of arbuscula mycorrhiza application in corn plant showed improvement of metabolism, growth and crop production. Arbuscula mycorrhiza can increase nutrient status and uptake[3,4], growth[5-7], reduce the effects of soil compaction stress[8,9], increased crop resistance in competition with weeds[10].

In plant ecosystems, mycorrhiza fungi are responsible for most acquisitions of P by plants. In fact, many reports clearly illustrate the increasing mobilization of P by arbuscula mycorrhiza (AM). Given the magnitude of the role of arbuscula mycorrhiza in supporting the growth and yield of plants, then this fungus needs to be studied further. In this study an arbuscula mycorrhiza exploration was conducted to study its existence in dominant weed rhizosphere in dry land and its application effect to corn plant in dry land.

MATERIALS AND METHODS

Research design: This study was conducted in Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University from February to July, 2017. The study consisted two series of experiments, namely (1) Existence arbuscula mycorrhiza in rhizosphere of dominant weed in dry land in Kendari, Southeast Sulawesi, Indonesia. Observation existence arbuscula mycorrhiza in rhizosphere of dominant weed (bladygrass) was observed descriptively using material: Plastic bag, aquadest, glucose, glycerine, KOH, H_2O_2, HCl, Lactophenol Cotton Blue and tools: filter, scales, petri dish, glass backer, heater, microscope, spray bottle, centrifuge. Observation did several weeks until get data of spore population and root infection (%) of bladygrass, (2) Application Effect of arbuscula mycorrhiza to several variety of corn plant. The experiment did by application of mycorrhiza arbuscula inoculum to corn plant by inserting the inoculum into the planting hole. The experiment was arranged based Randomized Block Design (RBD) consisted of eight treatments, namely variety of corn: Ereke (V_1), Rumba-Rumba (V_2), Batu Putih (V_3), Dana (V_4), Sidamangura (V_5), Bisma (V_6), Arjuna (V_7) and Pertiwi 3 (V_8). Each treatment was replicated 3 times, therefore, overall there were 24 experimental units.

Sample preparation of arbuscula mycorrhiza: Sample preparation did by taking samples of soil and roots of host plants randomly at 10 samples. Sampling based on Soil Biological Analysis Method[11], performed by procedure as follows: (1) Determination of sampling point by using quadrant, measuring 1×1 m, (2) Soil sampling, as deep as 10-15 cm, as much as ±1 kg per sample and taken root of bladygrass, (3) The sample is put into a plastic bag, (4) Spore isolation to count of spores, cleansing and root coloring to count infection of root (%) and identification of arbuscula mycorrhiza genus.

Observation of arbuscula mycorrhiza: Spores of arbuscula mycorrhiza extracted from soil by wet sieving and decanting method[12]. Clearing and staining of roots were done by applying the method of DR. I. HSLL's[13]. Infected roots were observed based on the slide method[14]. Infected root was observed according to the following Eq.:

$$\text{Root infection (\%)} = \frac{\text{Number of infected roots}}{\text{Number of observed roots}} \times 100$$

Identification of genus is based on the form of the infection, referring to the *Glomeromycota* classification guidelines[15,16].

Application inoculum of arbuscula mycorrhiza to plant: The indigenous AM inoculum is isolated from bladygrass (*Imperata cylindrica*) with spore population densities ranging ±500 spores per 100 g of soil. Inoculation of indigenous MA inoculum treatment was done simultaneously planting, by inserting the inoculum into the planting hole before the seeds

were planted. The planting of maize using plant spacing of 75×20 cm, as much as 1 grain of seed per planting hole.

Evaluation growth and yield some variety of corn plant: Evaluation was conducted of plant variable growth at 15 and 30 day after planting (DAP): (1) Plant height, measured from the base of the root to the tip of the stem, (2) Diameter of the stem, measured in the middle of the stem by using the sliding term, (3) Number of leaves, calculated all the leaves that are formed, (4) Leaf area, measured in length (L) and width (W) of leaves then calculated with the formula: Leaf area = L×W×C (constanta), (5) Dry weight of stover (in oven at 80°C for 2×24 h and then weighed) and seed production and (6) Phosphor uptake of plant tissue was analysis at 30 DAP.

Statistical analysis: Statistical analysis was performed using two-way analysis of variances (ANOVA). If there is a significant difference in the analysis of variance, further testing is done with Honestly Significant Difference (HSD) at $\alpha = 0.05$.

RESULTS

Spore populations, root infection and genus identification of arbuscula mycorrhiza: The spore populations were found to be quite dense, the number of spores at each sample ranging from 792-901 spores per 100 g of dry soil (Table 1). The rate of progression of the spore population is relatively homogeneous among host plant sample, indicated by differences in spore populations at each relatively small sample. Arbuscula mycorrhiza infections (%) found in the roots of host plants at each sample point ranged from 70-90% (Table 1). Consistency between root infection (%) and number of spores in host plant rhizosphere was not found.

The forms of infection found in host plant roots in the form of extra radical hyphae, intra radical hyphae, entry point, extra radical vesicles, intra radical vesicles external, arbuscula and/or coiled (Table 2). Vesicles were found not at all sample points, as were arbuscules and coiled. The results of this research showed that in the dominant weed (bladygrass) rhizosphere in dry land, there were various arbuscula mycorrhiza genuses. Some genera found were *Glomus*, *Gigasphora*, *Acaulospora*, *Entrophospora*, *Scutellospora*. Types of arbuscula mycorrhiza were found to be predominantly of the genus *Glomus*.

Application effect of arbuscula mycorrhiza to several variety of corn plant: Based on the observation of plant growth, eight varieties of maize plants have a fairly diverse response in symbiosis with mycorrhiza arbuscula. Significant differences were observed in plants 15 days after planting where Arjuna varieties had higher plant height and stem diameter. Plant height (Table 3) and stem diameter (Table 4) of plants 30 days after planting, number and leaf area, dry weight of stalk (Table 5) of plants 15 and 30 days after planting were not significantly different.

Phosphorus uptake of corn plant different variety (Table 5) very varies where the Batu Putih variety has the highest phosphorus absorption, while the lowest is the Pertiwi 3. However the difference in yields achieved by each varieties has low diversity, five of the eight variety are not

Table 1: Existence of arbuscula mycorrhiza at rhizosphere dominant weed (Bladygrass) in dry land Kendari, Southeast Sulawesi, Indonesia

Samples	Populasi of spora AM (Spora)^{ns}	Root infection of host plant (%)^{ns}
1	865	80
2	857	70
3	901	78
4	897	85
5	857	90
6	894	87
7	792	90
8	871	80
9	805	83
10	796	90

ns: Not significantly different

Table 2: Types of arbuscular mycorrhizal infections at the host roots

Sample number	Extraradical hyphae	Intraradical hyphae	Entry points	Extraradical vesicles	Intraradical vesicles	Arbuscula	Coiled
1	+	+	+	+	+	+	+
2	+	+	+	+	+	-	-
3	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-
5	+	+	+	+	+	-	-
6	+	+	+	-	+	+	-
7	+	+	+	-	+	+	-
8	+	+	+	+	+	-	+
9	+	+	+	-	-	-	-
10	+	+	+	-	+	-	-

Identification of infectious form refers to the identification key in website INVAM: http://invam.caf.wvu.edu/fungi/fungindex.html

Table 3: Stem growth of local corn plant of several variety after applicated of inoculums indigenous arbuscula mycorrhiza

	Plant height (cm)		Stem diameter (cm)	
Variety	15 DAP	30 DAP^{ns}	15 dap	30 DAP^{ns}
Ereke	8.20^{b}	17.57	0.47^{ab}	0.73
Rumba-rumba	8.90^{b}	17.17	0.53^{ab}	0.80
Batu putih	9.37^{ab}	19.70	0.50^{ab}	0.83
Dana	7.63^{b}	16.57	0.40^{b}	0.65
Sidamangura	7.83^{b}	17.07	0.43^{ab}	0.65
Bisma	8.10^{b}	16.30	0.47^{ab}	0.63
Arjuna	11.83^{a}	20.43	0.60^{a}	0.73
Pertiwi 3	9.20^{b}	17.03	0.40^{b}	0.65

Means in the same column suffixed with different lower case letters are different at 5% levels of significance according to HSD, ns: Not significantly different

Table 4: Leaves growth of local corn plant of several variety after applicated of inoculums indigenous arbuscula mycorrhiza

	Number of leave (strands)		Area of leaf (cm)	
Variety	15 DAP^{ns}	30 DAP^{ns}	15 DAP^{ns}	30 DAP^{ns}
Ereke	4.33	6.67	110.00	521.82
Rumba-rumba	4.33	7.00	121.29	514.17
Batu putih	4.33	7.33	118.18	448.83
Dana	4.33	6.33	103.05	368.59
Sidamangura	4.33	6.67	105.84	490.65
Bisma	4.33	7.00	108.08	436.08
Arjuna	4.33	7.33	146.91	495.43
Pertiwi 3	4.33	7.33	130.70	335.29

ns: Not significantly different

Table 5: Dry weight of stover, phosphorus uptake and yield of local corn plant of several variety after applicated of inoculums indigenous arbuscular mycorrhiza

	Dry weight of stover (g)			
Variety	15 DAP^{ns}	30 DAP^{ns}	P uptake of plant (mg)	Yield (t ha^{-1})
Ereke	0.43	3.07	23.03^{c}	2.66^{bc}
Rumba-rumba	0.46	3.43	15.36^{e}	2.81^{ab}
Batu putih	0.56	3.93	47.19^{a}	3.18^{ab}
Dana	0.38	1.90	23.08^{c}	3.53^{a}
Sidamangura	0.37	2.60	18.07^{d}	2.39^{c}
Bisma	0.43	2.76	24.20^{b}	2.78^{ab}
Arjuna	0.54	3.17	10.09^{f}	2.39^{c}
Pertiwi 3	0.49	2.62	8.46^{g}	2.83^{ab}

Means in the same column suffixed with different lower case letters are different at 5% levels of significance according to HSD, ns: Not significantly different

significantly different, the highest in yield is shown in Dana Variety while the lowest varieties are Sidamangura and Arjuna. Compared with the lowest production (in Sidamangura and Arjuna), (Table 5).

DISCUSSION

The spore populations were found to be quite dense, the rate of progression of the spore population is relatively homogeneous among host plant sample, indicated by differences in spore populations at each relatively small sample. Forms of infection found in host plant roots in the form of extra radical hyphae, intra radical hyphae, entry point, extra radical vesicles, intra radical vesicles external, arbuscula and/or coiled. Arbuscula mycorrhiza (%) infections found in the roots of host plants at each sample point high enough, differences in infection rates were thought to be due caused by differences in plant age in the field and differences in soil conditions at each sample point, as reported that root colonization varies from plant to plant, season to season and field to field[17] and the environmental conditions can affect the relative density of structures and levels of colonization[18,19]. Consistency between root infection (%) and number of spores in host plant rhizosphere was not found, the sample points with the highest spores population did not always have the highest percentage of root infections and vice versa, as find in some plant species[20]. Arbuscula mycorrhiza sporulation is not always reliable as a parameter to determine the composition of the arbuscula mycorrhiza community in the ecosystem. There are fungi that sporulation more, while others are sporulation less (probably never) and others just sporulation for a certain period of the year. Sampling sites multiple times throughout the year is indispensable[21,22].

The results achieved in this study indicate that the presence of AM in nature is quite high, i.e., 792-901 spores per 100 g of dry soil with a percentage of root infections in host plants reaching 70-90%. This is very helpful in the development of biological fertilizers, which can be applied among others to corn crops. The corn plant that cultivated on marginal land with technological input in the form of AM usage showed the result that all tested corn varieties can be symbiotic with AM although there is a different response between local varieties and national superior varieties. Varietal responses showed that local varieties (Ereke, Batu Putih, Rumba-Rumba, Sidamangura and Dana) have a higher adaptability in symbiosis with arbuscula mycorrhiza compared with introduction variety/superior national varieties (Bisma, Arjuna and Pertiwi 3). This is indicated by the absence of significant differences in the performance of local and national superior varieties on various observation benchmarks, although based on descriptions of national superior varieties have greater performance than local varieties. Even the highest phosphorus absorption ability in local varieties of Batu Putih and the highest production on local varieties of Dana. This is an indication that local varieties more adaptability in marginal soil.

Differences in varieties or cultivar of plant may cause respond differently to the environment, that in macadamia plants (*Macadamia tetraphylla* L.) there is a difference between "660" cultivars and "H2" cultivars in symbiosis with mycorrhiza in a state of water deprivation. The cultivar "H2" showed higher growth in the inoculated plant than without inoculation, whereas the "660" cultivar showed no difference. Higher local varieties' responses are thought to be due to local varieties that have long adapted to the research sites and thus have the potential to grow and develop better locally (in their home regions)[23]. The same results are shown in experiments using indigenous arbuscula mycorrhiza, that native (local) plants tend to grow better than that introduction plant[24].

The indigenous arbuscula mycorrhiza symbiotic with local varieties of corn plant is compact, it is capable to higher yield than the introduction varieties. This ability is among others supported by higher phosphorus uptake. In symbiosis with corn plants, arbuscula mycorrhiza are connected to plant roots, helping plants to absorb more water along with phosphorus and other nutrients, beside that the plant exudes organic acids and phosphatase enzymes that spur the process of mineralization[25]. Epidermal PAPS (Potential Absorbing Plasmalemma Surface Area) in *Zea mays*, greater significance in arbuscula mycorrhiza inoculated plants (37.3 cm^2 per cm root length) than non inoculated plants (11.2 cm^2 per cm length of root)[26].

CONCLUSION

The presence of mycorrhiza in dominant weed rhizosphere in dry land is very high, spore population reaches 900 spores per 100 of soil, root infection (%) in host plants ranges from 70-90%, with dominant spores of the genus *Glomus*. The response of various varieties of maize plants varies considerably in symbiosis with arbuscula mycorrhiza, where local varieties have a higher adaptability compared with introduction varieties.

SIGNIFICANCE STATEMENT

This study discovered that local corn plant provide a significantly higher response in it's association with indigenous arbuscula mycorrhiza in marginal drylands than with national superior varieties. Based on the results of this research, it can be explained that in the rhizosphere of weeds that grows dominant on dry land there are many arbuscular mycorrhizal. The soil isolated from the weed rhizosphere can be used as an inoculum as an arbuscular mycorrhizal source that can be applied to corn crops.

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