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The Presence Of Arbuscular Mycorrhizal (AM) Fungi In Some Agricultural Plants

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ABSTRACT. The objective of this study was to determine the number of spores, root colonization and the type of AM Fungi in some agricultural plants in Oransbari District. Soil and root samples were collected across the agricultural land to isolate AM Fungi propagules using wet sieving method and root colonization using gridline method. AM Fungi spores subsequently identified by the Gedermann and Trappe identification manuals, some soil characteristics were also analyzed. The results showed that the number of spores at each sampling location ranged from 5.0 to 35 spores/50 gr of soil. The highest number of spores is found in the rhizosphere of Lycopersicum esculentum and the lowest is in Oryza sativa. The percentage of roots infected ranged from 15,3 to 76,38%, the highest of infections found in Oryza sativa and the lowest in Capsicum annuum. There were four AM Fungi morphotypes on agricultural soil of Oransbari i.e Glomus, Acaulospora, Scutellospora and Gigaspora. Soil pH and water content influenced the presence of AM Fungi, while Nitrogen and Phosphorus content did not influence the spore numbers in the soil as well as in the root. This might be related to the routine and highly use of inorganic fertilizers and pesticides in these areas.

Keywords: *Agricultural Crops, Spores, Roots Infected*

INTRODUCTION

Arbuscular Mycorrhizal (AM) Fungi are abundant in the soil and play an important role in the growth, productivity and quality of agricultural crops. Arbuscular mycorrhizal Fungi are able to associate with almost 90 % of vascular plants (Fitter, 2005; Parniske, 2008) and over 80% of all extant terrestrial plants (Jefries et al. 2003; Wang and Qui, 2006), including agricultural crops in various types of soil. Population of AM Fungi and its distribution in the soil are strongly influenced by several factors such as land use, climate, temperature, chemical, physical and biological properties of soil (Djuuna, 2007), crop rotation, monoculture, tillage, organic amendments, and application of biocides (Kaur and Mukerji, 1999). Furthermore, the occurrence of AM Fungi in some soil types is a key factor determining AM Fungi community composition (Oehl et al. 2010).

AM Fungi can be associated with almost 90% of agriculture plants, where each type of plant can be associated with one or more types of AM Fungi. However, not all types of AM Fungi are effective in increasing plant growth. AM Fungi plays an important role` in maintaining the stability of plant diversity by transferring nutrients from one plant root to another adjacent plant roots through a structure called "bridge hypha" (Setiadi, 2004). The presence of AM Fungi in plant roots can increase the plant growth (Cavagnaro et al. 2006; Nunes et al. 2010) due to the ability of AM Fungi in increasing the absorption of nutrients and water (Rillig and Mummey, 2006). Moreover, in low P soil, AM Fungi can improve nutrients uptake of Phosphorus (Barea, 1991), Nitrogen uptake (MacFarland et al. 2010) by plants and also accumulate some micronutrients (Zn, Cu and Co) (Feber et al. 1990). These broad roles of AM Fungi in plant growth requires the identification of Arbuscular Mycorrhizal Fungi as an initial step in the exploration and utilization of local AM Fungi. Research on the diversity of the AM Fungi genus in the rhizosphere of agricultural crops in the Oransbari District has not been done. Therefore, through this research it is expected that this will be the beginning of the use of AM Fungi as a suitable biological agent for the local area.

The purpose of this study was to determine the number of spores and percent of root colonization by AM Fungi, as well as the diversity of AM Fungi types in the soil and roots of some agricultural crops in the Oransbari District agricultural land. The results of this study are expected to be used as a source of information regarding the number and types of AM Fungi in Oransbari District agricultural land and the results of isolation from the AM Fungi can be used as a source of inoculum for plants that will be planted on the Oransbari District agricultural land and as a source of biological fertilizer in the future.

MATERIAL AND METHODS

Location and Time of Research.

This research was conducted in March to September 2017. Sampling of soil from the rhizosphere and plant roots was carried out on agricultural land in 4 (four) villages (Muari, Margomulyo, Sidomulyo, and Sindangjaya) of Oransbari Districts Manokwari Region, Papua Barat.

Soils and Roots Sampling.

Composite soil and root samples were taken in the rhizosphere of the crop (0-20 cm). At each location, 5 sample points were taken and the plant root samples are taken on each type of agricultural plant that grows on agricultural land in Oransbari District. The total of soil and root samples are 15 samples at all the sampling areas.

Isolation and extract of AM Fungi propagules.

Isolation and extraction of AM Fungi spores from soil samples was carried out using the Wet Sieving and Decanting method (Gerdemann and Nicholson, 1963). 50 gram of soil was mixed with 100 ml of water in 500 ml conical flask, the mixture of soil and water was agitated vigorously to free the AM Fungi spores from soil and allowed to settle for 15-30 minutes, then the supernatant was decanted through the standard sieves, then followed by centrifugation technique (Brundrett et al. 1996). Spores were picked by means of needle or tweezers using a dissecting microscope. Spore calculation is performed to determine spore density (number of spores/50 grams of soil).

Root colonization by AM Fungi.

Root samples were cleared and stained with Trypan Blue (Abbptt and Robson, 1981). The stained roots were assessed for % root infection by examination under dissecting microscope. The presence or absence of AM fungi that intersected the grid in the field of view was recorded for 100 intercepts (Newman, 1966). Morphotypes of AM Fungi that exist at each interception identified up to the genus level according to (Abbott, 1982).

AM Fungi identification.

Identification of AM Fungi was carried out by making a specimen or AM Fungi preparation using the PVLG (poly venyl lacto gycerol) technique. The identification of AM Fungi spores was carried out on genus level based on the identification handbook of Gedermann and Trappe (1974).

Soil Analysis.

Some related soil properties were analyzed such as pH (1:5), soil moisture content and soil Phosphorus content (Bray II).

Data Analysis.

The data obtained from the results of the analysis in the laboratory are then presented in the form of tables using the Microsoft Excel software program.

RESULT AND DISCUSSION

Number of spores, percentage root of infected, and spore types of AM Fungi

The number of spores, the percentage of roots infected, types of spores on several types of plants growing on the Oransbari agricultural land are presented in Table 1. The result showed that the number of spores at all sampling locations of various plant roots is in the range of 5 to 35 spores/50 gr of soil. The number of spores found in the study area was relatively low, however the numbers varied at all sampling points. Daniels and Skipper (1982) mentioned that soils have spore density about 2000 spores per 100 grams of soil are categorized as high number of AM Fungi spores. The low number of spores is probably due to the influence of soil properties in the study area which generally have a slightly acidic pH. Spore and extra radical mycelium are low at the lower pH value (Hegalson and Fitter (2009). The highest number of spores are found in the rhizosphere of tomato plants (31-35 spores/50 gr soil) and the lowest in paddy rice rhizosphere of Margomulyo (5 spores/50 gr soil). The difference in the number of spores is thought to be due to differences in the ability of plants to associate with AM Fungi.

Root exudates play an important role of AM Fungi to establish the symbiosis (Vierheiling et al. 2003). Indirect root exudates also contribute to the amount of spore density, where differences in root exudates produced between plants in each study location affect the rhizosphere which stimulates differences in spore germination.

Tabel 1. Number of spores, percentage root of infected, and spore types of AM Fungi at each sampling location

Sampling Location/ Root Plants	Number of Spores/ 50 gram soil	Percentage root infection (%)	Morphotypes of AM Fungi
1. Muari			
Tomatto (*Lycopersicum esculentum*)	21	58,33	Glomus, Acaulospora
Paddy rice (*Oryza sativa*)	23	76,38	Glomus, Acaulospora
Chilli (*Capsicum annum*)	12	31,8	Glomus, Acaulospora
2. Margomulyo			
Paddy rice (*Oryza sativa*)	5	68,05	Glomus, Scutellospora, Gigaspora
Tomatto (*Lycopersicum esculentum*)	35	65,2	Glomus
Chilli (*Capsicum annum*)	23	35,3	Glomus, Scutellospora
3. Sidomulyo			
Beans (*Phaseolus vulgaris*)	14	59,5	Glomus
Long bean (*Vigna sinensis*)	21	46	Glomus, Scutellospora
Cabbage (*Brassica oleracea*)	17	46,4	Glomus, Scutellospora
Corn (*Zea mays*)	20	57,5	Glomus, Acaulospora
Tomatto (*Lycopersicum esculentum*)	31	34,1	Glomus, Scutellospora
4. Sindangjaya			
Cabbage (*Brassica oleracea*)	28	53,5	Glomus
Tomatto (*Solanum lycopersicum*)	27	56,6	Glomus
Onions (*Allium* cepa var *aggregatum*)	15	74	Glomus, Scutellospora, Gigaspora
Chilli (*Capsicum frutescens*)	19	15,3	Glomus, Scutellospora

The percentage of root infected by AM Fungi ranged from 15,5 to 76,38% (Table 1), which classified as moderate to high (Setiadi, 1990). All of the plants formed colonization of AM Fungi in each study location. This shows that the association between AM Fungi and plant roots that grow on agricultural land in Oransbari District developed very well, even though the percentage of infection of each plant is different from one to another. The highest percentage of infection was found in roots of paddy rice (76,38%) of Muari and the lowest was in chili roots (15,3%) of Sindang Jaya. The presence of spores and the percentage of root infections by AM Fungi at all sampling locations showed that Oransbari's agricultural land is suitable habitat for growth and development of AM Fungi. Brundrett et al. (1996) mentioned that infection of the roots is the formation of hyphae, arbuscular and vesicles which are present simultaneously. This results showed that the rhizosphere of agricultural plants with the high percentage of infections is not followed by the high number of spores of AM fungi. However, there are some samples of plant roots that showed of root tendencywith a high percentage of infections in the rhizosphere. This is supported by the statement of Ragupathy and Mahadevan (1991) that there is no correlation between the number of spores and the percentage of root infections in host plants, certainly plants with a high percentage of roots also have a high number of spores in their rhizosphere or vice versa. There are three factors that influence AM Fungi infection, namely host pressure on infection, climate and soil factors, including fertilization, soil nutrition and pesticide treatment (Fakura, 1988). This is supported by Setiadi 1990 that infection is strongly influenced by the sensitivity of the host to infection, climate and soil factors. Some factors that stimulate or inhibit the process of root infection will also resist the formation of AM Fungi spores (Brundrett et al. 1996). Although root infection and spore formation have a close relationship, these two phenomenas are not always have a positive relationship. Root infection and spore production are influenced by many factors both in soil and its environment. Agricultural land in Oransbari District is an agricultural area that often experiences with routine and highly dose of fertilizer and pesticide use. This might be a factor that caused the decrease in root infection and spore number by AM Fungi in the rhizosphere of agricultural crops in Oransbari District.

Based on the identification of AM Fungi spores, there were 4 (four) AM Fungi morphotypes accross the observation sites, mostly from the Glomus and some from the Acaulospora, Scutellospora and Gigaspora (Table 1). Glomus is the most dominant genus in every rhizosphere of agricultural crops in Oransbari District. According to Wanda et al., (2015) Glomus spores are the most dominant spore type found in several ecosystems, because this type has a broad host range. The study of Hartoyo et al. (2011) found 14 species of AM Fungi of Glomus in the *Centela asiatica* rhizosphere. Delvian (2006) reported that there were 8 genus of Glomus from a total of 13 spores of AM Fungi in their study. In line with the report of Douds and Milner (1999), the results of this study also show that agricultural intensification not only affects the AM Fungi population in the soil, but also affects the diversity of the genus.

Soil Characteristics

The result of the soil analysis is shown in Table 2, it showed that the moisture content ranged from 23,80 to 96,31%. The highest moisture content of soil was obtained in the soil sample of Sindang Jaya 2 (96,31%), followed by Sidomulyo 2 (88,30%), Margomulyo 2 (81,39%), and Muari 2 (77,89%). The moisture content can affect the growth and infection of AM Fungi in plant roots.

Table 2. Soil properties of sampling location in Muari, Margomulyo, Sidomulyo and Sindang Jaya of Oransbari

Sampling Location	Soil Moisture Content (%)	Soil pH (1:5)		P-available (ppm)	
Muari 1	34,62	4,90	Acidic	5,18	Low
Muari 2	77,89	6,22	Slightly Acid	70,77	Very High
Margomulyo 1	41,67	5,22	Acidic	2,96	Very Low
Margomulyo 2	81,39	6,32	Slightly Acid	43,92	Very High
Sidomulyo 1	43,47	5,08	Acidic	3,41	Very Low
Sidomulyo 2	88,30	5,98	Slightly Acid	15,14	Very High
Sindang Jaya 1	23,80	4,50	Acidic	5,79	Low
Sindang Jaya 2	96,31	5,24	Acidic	5,26	Low

The moisture content of soil have a direct or indirect effect on infection and mycorrhizal fungal growth. Menge's (1984) reported that good germination of *Glomus epigaeus* if the groundwater content was in between field capacities. The presence of AM Fungi is very beneficial for plants that grow in dry areas, because it can increase the ability of plants to grow and survive in conditions that are less water absorption of host plants, AM Fungi can improve the host plants resistance to drought condition (Gruemberg et al. 2015). Soil pH ranged from 4,50 to 6,32 (Table 2), the highest soil pH values were obtained in Margomulyo 2 (6,32) and the lowest in Sindang Jaya 1 (4,50). AM Fungi is influenced by the host and edafic factors such as soil pH. Microbes including AM Fungi are tolerant of soil pH that is near neutral, changes in soil pH can interfere the availability of nutrients and metabolism that can disrupt the work of enzymes which caused death for soil organisms including AM Fungi. In general, AM Fungi are more resistant to the changes of soil pH. However, the adaptability of each AM Fungi species to soil pH varies because soil pH affects germination, development and mycorrhizal's role in plant growth (Mosse, 1981). Moreover, soil pH can affect the work activity of enzymes that play a role in germination of mycorrhizal fungal spores. The soil P-available ranged from 2,96 to 70,77 ppm (very low to very high) (Table 2). The highest P content is in agricultural land of Muari 2 (70,77 ppm), followed by Margomulyo 2 (43,92 ppm) and Sidomulyo 2 (15,14 ppm), and the lowest is in Sindang Jaya soil. It showed that the low soil pH content is in line with the low of P-avaliable in the soil. A study of Effendy et al. (2006) found that there are positive relationship between soil P availability and AM Fungi spores. Available soil P may regulate the abundance and diversity of AM Fungi as well as their symbiotic function in intensive cropping systems (Zefeng et al. 2020). However, in this study, these two soil properties are not closed related to the number of spore and percentage of infection of AM Fungi.

CONCLUSION

The number of spores at all sampling location ranged from 5 – 35 spores per 50 grams of soil and the percentage of root infection ranged from 15,5 to 76,38%. All the plant root samples are infected by AM fungi. There are four morphotype of AM Fungi i.e Glomus, Acaulospora, Scutellospora and Gigaspora, whereas Glomus is the most dominant genus compared to other. The soil is classified as acidic to slightly acidic, soil moisture content ranged from 23,80 to 96,31%, and soil P availability ranged from 2,96 to 70,77 ppm (very low to very high). The presence of AM fungi in Oransbari Agricultural soil is early information for the future management of agricultural in this area. The routine and highly use of anorganic fertilizer in this area might be influenced the presence of AM Fungi in the soil as well as their infection in plant roots.

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