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Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands

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ABSTRACT

By improving the nutrient uptake and transport, an indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve crops' performance in sandy drylands of North Lombok (Indonesia) during dry seasons. A field experiment was designed with Randomized Complete Block Design and four replications to examine the benefits of mycorrhiza at varying doses of plant nutrition (nitrogen and phosphorus). Total of 1 kg of the AMF inoculum was applied to 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9 and 6 ton/ha) and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, soil N and P status and uptake, and dry biomass (root, shoot, and grain). The highest correspondence was observed in the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at 100 days after seeding. The number of AMF spores increased over the time where colonization rates were found higher in roots of sorghum (60-81%) than maize (55-75%). This study suggests that AMF inoculation increases the plant yield and improves soil nutrient availability

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which is very advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

Keywords: Arbuscular mycorrhizal fungi (AMF), cattle manure, maize-sorghum cropping sequence, plant nutrition, seed coating

INTRODUCTION

The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy soils texture. With a very short and low number of rainy days (December to April, 100-200 mm) per wet month or no rain during the long dry seasons (May to November), no food crops can be cultivated normally especially in the areas without deep wells. Moreover, inadequate phosphorus (P) availability is also one of the factors limiting the productivity of maize and other food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P_2O_5 and 76% of it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases nutrient uptake from soils and enhances growth and yield of the host plants although it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

The most common findings show that the external hyphae of AMF improve P uptake and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006). Although there is no growth improvement of the host plants due to the AMF symbiosis, the amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe and B when compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000; Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al., 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum (Plenchette et al., 1983), the results showed ~~a~~ high mycorrhizal dependency, both for maize (up to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013;

Tawaraya, 2003). The findings of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry matter is produced of the crops. With the high porosity and low water retention capacity on dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al., 2015).

The establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF inoculation in maize plants in sandy soils had positive implications for the improvement of soil properties by increasing the rates of nutrient uptake by maize crop from the soil and improving its grain yield. Our research group has also shown the benefit of this local inoculation in increasing the growth of soybean and its yield by improving P uptake from the soils, compared to those without AMF inoculation (Astiko et al., 2013b). However, as founded from other studies, the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of host plants could be influenced by the order of plant species cultivated in sequence in the cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson et al. (1992) as well as Vivekanandan and Fixen (1991) reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared to when maize crops were grown following maize or barley.

From the previous study on maize-soybean cropping patterns, it was found that the AMF inoculated in maize crops grown in the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crop in the cropping sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also found between cropping seasons or between crop species in the same cropping season in Central Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake by maize and subsequent sorghum crops as well as the growth and yield components of the maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok, Indonesia.

MATERIALS AND METHODS

Design of the Experiment

The field experiment of maize-sorghum cropping sequence in this study was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The study was carried out in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016. Treatments involving the use of five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The 100% NPK-only recommended dose (D_0) is the farmer's practice of dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle manure in varying fertilization packages (D_1, D_2, D_3, D_4), and added with AMF as listed in Table 1.

Table 1
The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D_0	NPK only at 100% the recommended doses (RD), i.e. 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea	No fertilizer applied
D_1	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D_2	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D_3	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D_4	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was of a sandy soil type (69% sand, 29% silt and 2% clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area. The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the land was splitted into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure), and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first cropping cycle.

An indigenous AMF inoculum, *Glomus mosseae* (the M_{AA01} mycorrhizal isolate including the hyphae and the mycorrhizal spores), which was originally isolated from dryland area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on the treatments (Table 1) was applied at the time of planting the maize seeds by burying the whole dose in the planting hole in the position below the seeds. The cattle manure variation in the fertilization package is to identify the optimum combinations to benefit the plant growth, increase the nutrient availability at the soils, and support the AMF development. The maximum combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses, followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered with soil.

After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum seeds were then planted in cropping cycle 2. Before the second sequence, the field was left fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was done at 100 DAS.

Measurement and Data Analysis

The variables measured were AMF development, N and P nutrition, and growth and yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total N and available P in the rhizosphere of maize and sorghum at the time when the vegetative and generative growth found optimum, respectively at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science, Faculty of Agriculture, University of Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with $(NH_4)_2SO_4$ and distillation with NaOH where the NH_4^+ was determined by indophenol blue colorimetric method and the NH_3 was defined by a titration with 0.05N of H_2SO_4 solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by $(NH_4)_2SO_4$ and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer (λ = 693 nm) after the extraction process using Bray and Kurt I solution (0.025 N HCl + NH4F 0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with $K_2Cr_2O_7$ in presence of sulphuric acid (H_2SO_4) following Walkley and Black's method (Horwitz, 2000).

AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after the final centrifugation were captured in a filter paper, which were then observed on a Petri dish using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil. The percentage of root colonization was determined using the Gridline Intersect technique (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using the clearing and staining method of Brundrett et al. (1996).

Data were analyzed using analysis of two way ANOVA and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

RESULTS AND DISCUSSION

AMF Development

In this study, however, maize and sorghum were grown in sequence, in which sorghum was directly seeded without treatments and without tillage following the harvest of maize plants. All treatments, including AMF inoculation, were applied only to maize plants in the first cropping cycle. Therefore, it means that sorghum in cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in cropping cycle 1. This is in line with the results reported in the previous study (Arihara & Karasawa, 2000) which revealed that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by the previous crop grown, whether they were host or non-host of AMF. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum. However, the P fertilization did not affect root colonization, especially on maize following AMF host plants.

Specifically, the degree of root colonization by AMF may be higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure) (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore production compared with conventional fertilization. It can also be seen from Table 2 that AMF spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D_2 treatment. This indicates a high buildup of AMF propagules for the

Table 2
Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence

Fertilization packages	AMF on maize (1st crop)			AMF sorghum (2nd crop)		
	Spore per 100 g soil		% colonization 60 DAS	Spore per 100 g soil		% colonization 60 DAS
	60 DAS	100 DAS		60 DAS	100 DAS	
D_0	764^{e}	3464^{d}	30^{d}	1231^{e}	3761^{d}	35^{e}
D_1	1059^{d}	3672^{c}	55^{c}	1343^{d}	4942^{b}	60^{d}
D_2	2119^{a}	4327^{a}	75^{a}	2981^{a}	5165^{a}	81^{a}
D_3	1690^{b}	3894^{b}	65^{b}	1881^{b}	4831^{c}	77^{b}
D_4	1294^{c}	3881^{b}	63^{b}	1769^{c}	4819^{c}	68^{c}
HSD 5%	231	13	2.0	109	12	6.5

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for descriptions of the packages

subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in roots of sorghum in the second cropping cycle compared with that of maize in the first cropping cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus mosseae* or *Glomus versiforme*, indicating that sorghum is more favorable for AMF development than maize plants (Guo et al., 2013).

There are many factors influencing the degrees of AMF colonization of crop roots as reported by Carrenho et al. (2007). In examining the effects of different phosphorus, liming, organic matter, and soil texture on root colonization of peanuts, sorghum, and maize in a factorial experiment, each factor significantly affected root colonization level, alone or in interaction with other factors (Carrenho et al., 2007). The most surprising results were the significant interaction effects of plant, phosphorous, and organic matter although there were no significant effects of phosphorous (simple superphosphate of 166.6 mg/L (w/v) soil) nor organic matter (22 mg/L (w/v) soil). As for the interaction effect involving maize, there was no significant effect of phosphorous and organic matter on AMF colonization of maize roots. On the other hand, the application of both phosphorous and organic matter significantly reduced AMF colonization on sorghum roots (Carrenho et al., 2007).

In terms of fertilization effects, AMF colonization rate was reported to be higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least was seen in exclusively mineral fertilized and conventional farming systems. Thus, it was concluded that the amount of soluble P in the soil was the most limiting factor for AMF colonization in roots of those crops (winter wheat, vetch-rye, and grassclover) (Mäder et al., 2000). Moreover, it was reported that the increasing P level in the growing media from 0.1 to 0.5 mM P significantly reduced AMF colonization in roots of lettuce plants especially with increasing levels of N contents (1, 5, or 9 mM N) (Azcón et al., 2003).

A negative impact on soil condition occur when the accumulation of soil P has increased beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that the amount of P input from the NPK fertilizer as applied in the D_2 treatment was most favorable for AMF development in maize crops. In the D_2 treatment, the NPK fertilizer was reduced to 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D_0 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF. Among the treatments with AMF in combination with cattle manure, the D_1 treatment had the highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the AMF infection and development in maize roots compared with those in the D_2 treatment, resulting in a higher AMF colonization rate on D_2 than on D_1 treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D_2 treatment, especially when compared with the D_1 or D_0 treatment. Using the same indigenous AMF applied to a pot experiment in which the soil for the growing media was taken from the same field in North Lombok, it was found that the highest level of AMF colonization of maize roots was in the treatment with AMF combined with cattle manure compared with AMF combined with NPK fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure combined with mineral fertilizers compared with those fertilized with mineral fertilizers only (Gryndler et al., 2006).

Soil Nutrient Status and Nutrient Sorption by Maize and Sorghum

There were significant effects of the different fertilizer packages on soil nutrient status (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient status, measured either at 60 or 100 DAS, are highest under the D_2 (60% NPK + 12 t/ha manure + AMF) or D_1 (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D_0 (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D_1 and D_2 treatments (Table 3).

At the present study, no infiltration data was measured, however it is important to note that since the NPK fertilizers applications, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have been loss through infiltration during the rainy season. Previous study shows that the sand content of the cultivated land had a positive correlation with infiltration rate, with an

Table 3
Mean concentration of total N and available P of soil in the rhizospheres of maize (1st crop) and sorghum (2nd crop) for each treatment of fertilization packages, measured at 60 and 100 DAS

Fertilization packages	Total N ($g.kg^{-1}$) at 60 DAS		Available P ($mg.kg^{-1}$) at 60 DAS		Total N ($g.kg^{-1}$) at 100 DAS		Available P ($mg.kg^{-1}$) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D_0	1.24c	1.10c	14.97d	11.41d	1.33d	1.23d	17.43d	10.15d
D_1	1.45a	1.29a	28.44b	16.25b	1.69b	1.38b	29.51b	21.58b
D_2	1.47a	1.25ab	35.02a	28.49a	1.86a	1.48a	36.56a	29.99a
D_3	1.39b	1.22ab	17.52c	14.59bc	1.47c	1.31c	19.37c	18.59c
D_4	1.33b	1.20b	16.29c	14.25c	1.42c	1.31c	18.53c	17.32c
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

r-value of +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK fertilizers through leaching was much higher in the D_0 than in the other treatments of fertilization packages due to dissolution by rain water during the rainy season. However, there were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres of maize crop, especially in those treated with manure and AMF application. This indicated a slow release of N and P nutrients from manure after application to the maize plants in the first cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can be seen from Table 2 that the highest average of AMF colonization levels in maize roots was in the D_2 treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D_2 treatment (Table 4).

In addition, soil contents of those nutrients corresponded well with the levels of N and P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not significant for P uptake, the highest values of N and P uptake were also seen in the D_2 treatment for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a value of r = + 0.970 (R-square = 94.1%, p = 0.006) for maize plants, and r = + 0.904 (R-square = 81.7%, p = 0.035) for sorghum plants. These indicate those fertilizers in the packages contribute to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

However, P status of the soil did not show a significant correlation with P uptake either by maize or sorghum crop. In spite of that, there is a significant positive correlation

Table 4
Mean N and P sorption (mg.g⁻¹ plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages

Fertilization packages	N and P uptake (mg.g^{-1} plant dry weight) by each crop at 60 DAS			
	Maize (1st cropping cycle)		Sorghum (2nd cropping cycle)	
	N	P	N	P
D_0	18.62^e	1.71^d	12.74^d	0.65^d
D_1	27.58^b	2.60^b	18.92^b	0.85^c
D_2	28.00^a	2.72^a	20.86^a	1.48^a
D_3	23.10^c	2.41^c	17.29^c	1.36^b
D_4	20.44^d	2.41^c	17.22^c	1.32^b
HSD 5%	0.41	0.11	0.07	0.04

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages

between the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with an r = + 0.909 (R-square = 82.6%, *p* = 0.032), and that of sorghum plants, with an r = + 0.940 (R-square = 88.4%, *p* = 0.017). This shows that there were significant contributions of AMF colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If soil soluble P has not exceeded crop requirements, AMF association will still result in significantly positive effects on P uptake and biomass production because of P requirements of the crops; for optimum growth and yields, crops require P since the early stage of their growth, either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

Although sorghum crop in the second cropping cycle was not fertilized with manure nor NPK fertilizers, direct seeding of sorghum after harvesting the maize plants without tillage seemed to be a favorable condition in establishing AMF association in the sorghum crop, which resulted in higher AMF colonization rates in sorghum than in maize roots for each fertilizer package (Table 2). The AMF associations seemed to enable the sorghum crops to absorb adequate P and other nutrients from soil and residues of the manure applied in the first cropping cycle even though these sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae that help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as other nutrients unavailable for uptake by plant roots (Menge, 1983; Smith & Read, 2010; Tawaraya et al., 2006). Guo et al. (2013) also reported significant effects of AMF inoculation on C, N, P, and K contents (mg/pot) in shoot and roots of maize and sorghum grown in pots filled with mixtures of mine tailing and topsoil, and between the AMF species used, *G. versiforme* showed higher colonization rates which resulted in higher nutrient sorption and biomass of maize and sorghum compared with *G. mosseae* (Guo et al., 2013).

Biomass and Yield Components of Maize and Sorghum

In terms of biomass production and yield components of maize and sorghum, there were also significant effects of the fertilization packages in which D_2 treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that the nutrient status of the soils corresponded well with the biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them were significant. The AMF applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with an r = + 0.912 (R^2 = 83.2%, p= 0.031) and shoot dry weight at maturity (100 DAS), with an r = + 0.892 (R^2 = 79.6%, p= 0.042). This could mean that during the vegetative growth, AMF colonization have focused on improving root growth to increase nutrient sorption during the vegetative growth of maize crops.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they did show a positive significant correlation with P uptake both in shoots of maize and sorghum, with an r = + 0.909 (R^2 = 82.6%, p = 0.032) for maize, and r = + 0.940 (R^2 = 88.4%, p = 0.017) for sorghum. These could be due to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based on the strength of the relationships between AMF colonization levels and P uptake, the value of R^2 is higher in sorghum (R^2 = 88.4%) than in maize (R^2 = 82.6%). This means that the contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not receive any fertilizers, and cattle manure is a

Table 5
Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages

Fertilization packages	Dry biomass weights (g/plant) of maize (1st crop) and sorghum (2nd crop)							
	Maize root		Maize shoot		Sorghum root		Sorghum shoot	
	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS	60 DAS	100 DAS
D_0	8.13^d	10.43^e	34.15^d	62.29^e	0.82^d	12.30^d	8.31^d	47.74^e
D_1	15.13^b	22.39^b	61.92^b	109.03^b	2.22^b	22.34^b	16.89^b	95.01^b
D_2	17.12^a	34.56^a	72.86^a	111.25^a	3.37^a	24.44^a	23.53^a	105.14^a
D_3	13.65^c	18.48^c	52.26^c	101.05^c	1.68^c	14.52^c	12.01^c	85.46^c
D_4	13.34^c	15.43^d	51.29^c	95.87^d	1.38^c	14.47^c	11.81^c	66.06^d
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages slow-release organic fertilizer, it could mean that most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping cycle, was mostly taken up from the manure under the contribution of AMF colonization in the roots. However, this still needs to be confirmed by further research. This view is supported by the conditions of the study area, which was dominated by sand, and if leaching happened during the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum crop in the second cropping cycle was the cattle manure applied to the maize crop in the first cropping cycle.

In relation to nitrogen nutrient, there was a very low and non-significant correlation between AMF colonization levels and N uptake in maize shoots with an R^2 of only 41.2%. However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status of the soil with an R^2 = 94.1% (p = 0.006). Moreover, the N soil availability also showed a significant correlation with biomass and grain yield of maize, i.e. with an R^2 = 84.1% (p = 0.029) with grain yield at 60 DAS, R^2 = 92.2% (p = 0.009) with shoot dry weight at 60 DAS, and R^2 = 90.6% (p = 0.012) with shoot dry weight at 100 DAS.

In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop where AMF colonization levels showed a significant and positive correlation with shoot dry weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any significant correlation with biomass weight or yield components of sorghum. However, AMF colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in sorghum shoots, with an R^2 = 71.9% (p = 0.069), and N uptake in sorghum shoots showed positive significant correlation with biomass and grain yield of sorghum, i.e. with an R^2 = 80.5% (p = 0.039) with grain

Table 6
Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1st cropping cycle		Sorghum in the 2nd cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D_0	9.60^e	22.48^d	3.57^d	2.73^d
D_1	17.40^b	26.94^b	5.05^b	3.01^b
D_2	22.80^a	28.12^a	6.65^a	3.61^a
D_3	15.60^c	25.98^c	4.43^c	2.90^c
D_4	10.20^d	24.61^c	4.17^c	2.81^{cd}
HSD 5%	0.59	1.37	0.26	0.09

Remarks: Mean values in each column followed by the same letters are not significantly different between treatments of fertilization packages; please refer to Table 1 for description of the packages yield, R^2 = 83.0% (p = 0.031) with shoot dry weight at 60 DAS and R^2 = 89.9% (p = 0.014) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure contributed from AMF colonization in sorghum roots. Many researchers have also shown the ability of AMF to utilize organic sources to supply N to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when the correlation analysis was done between averages of colonization levels, biomass and grain yield between maize in the first and sorghum in the second cropping cycle, in general the results show significant and positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum are highly significant, with an r = + 0.988 (R^2 = 97.6%, p = 0.002). This means that the pattern of differences in AMF colonization levels between roots of maize and sorghum is highly similar. In other words, AMF colonization levels in roots of maize in the first cropping cycle were carried over into the second cropping cycle where sorghum was grown as the rotation crop. This is because both maize and sorghum are hosts of AMF, and both crops have high mycorrhizal dependency (Guo et al., 2013).

In addition, when a t-test of 'Paired Two Sample for Means' was run on the averages of AMF colonization levels and spore number in soil at maturity of the crops between maize and sorghum in Table 2, higher significant values (p<0.01) were seen on sorghum than on maize. This could be due to the buildup of AMF in the soil after maize crop in the first copping cycle was harvested before sorghum was directly seeded without tillage. Even though both crops were grown simultaneously, it was also found that AMF colonization levels were higher on sorghum than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal conditions of the soil under a maize-sorghum cropping sequence.

CONCLUSION

Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping sequence system at the drylands in North Lombok of Indonesia, the D_2 package, consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was found to be the best fertilization package to improve crop yield and soil nutrient availability. This study noted that the AMF development was higher in the sorghum at the second cropping cycle compared to the growth in the maize at the first cropping cycle. This condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy dryland, with no additional fertilization and mycorrhizal propagules applied.

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