

**Running Title:**

**Mycorrhizal seed-coating on maize-sorghum cropping sequence**

**Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands**

Wahyu Astiko<sup>1\*</sup>, Wayan Wangiyana<sup>1</sup>, Lolita Endang Susilowati<sup>2</sup>

<sup>1)</sup> Study Program of Agroecotechnology Faculty of Agriculture, University of Mataram

<sup>2)</sup> Department of Soil Science Faculty of Agriculture, University of Mataram

Jalan Majapahit No. 62, Mataram, Lombok, Indonesia, 83125

E-mail addresses:

astiko@unram.ac.id (Wahyu Astiko)

w.wangiyana@unram.ac.id (Wayan Wangiyana)

lolitaabas37@unram.ac.id (Lolita Endang Susilowati)

<sup>\*)</sup> Corresponding author

The List of Number of Tables

Table 1. <i>The mycorrhizal-based fertilization packages tested, and applied to maize only in the maize-sorghum cropping sequence</i> .....	6
Table 2. <i>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</i> .....	9
Table 3. <i>Mean concentration of total N and available P of soil in the rhizospheres of maize (1<sup>st</sup> crop) and sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100 DAS</i> .....	11
Table 4. <i>Mean N and P sorption (mg.g<sup>-1</sup> plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages</i> .....	12
Table 5. <i>Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages</i> .....	13
Table 6. <i>Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages</i> .....	14

## **Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands**

### **ABSTRACT**

An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to the maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 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 only was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). Sorghum seeds were planted, subsequently, at the cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P uptake, and dry biomass (root, shoot, and grain). The highest corresponding was observed at the crops which utilized a combination of 60% NPK and 12 ton/ha cattle manure, and the performance was higher at the 100 days-after-seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than on maize root (55-75%). This study suggests the AMF inoculation higher the yield of maize, and improves the soil nutrient availability which was very advantageous for the growth of the following crop.

Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition.

## INTRODUCTION

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 per wet month or no rain during the long dry seasons, consequently, no food crops can normally be cultivated especially in the areas having no deep wells. Moreover, an 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. The requirement of maize crop for P is very high, i.e. 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 only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope the unavailability of soil water and P and other essential nutrients in drylands of North Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis is expected to improve performance of food crops especially during the dry seasons. Many have reported that the soil fungi, in symbiosis with their host plants, can help the plants to improve water relation and makes their host plants more tolerant to drought (Augé, 2004). The AMF colonization also increases nutrient uptake from soils and enhance 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 improves the 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 the movement through the host roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts in increasing 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 (George et al., 1995), and with the external hyphae, the mycorrhizal roots can explore further, 10-100 times more volume of soils compare the non-mycorrhizal roots (Sieverding et al., 1991). 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 study indicates that the higher the dependent rate on AMF symbiosis, the more the dry matter 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).

Establishment of AM symbiosis can be done through inoculation with AMF propagules. Our previous study orchestrates that the indigenous AMF inoculation in maize plants in sandy soils presents 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 (Astiko et al., 2013a). Our research group have also shown the benefit of this local inoculation in increasing the soybean crops grown and yield by improving the P uptake from the soils, compared with those without AMF inoculation (Astiko et al., 2013b). However, as found 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). Those two studies reported that the P uptake and the AMF colonization were higher on maize crops grown following soybean compared with those 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 at the first cycle increased root colonization and AMF sporulation which was very advantageous for the growth of the following crops 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). This present study examines 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 by the subsequent sorghum crops, as well as 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 established in the Akar-Akar village located in North Lombok regency, Indonesia, from January to August 2016, which was arranged according to the Randomized Complete Block Design (RCBD) with four replications (blocks). The treatments were five fertilizer packages consisting of different combinations of organic, inorganic and indigenous AMF bio-fertilizer, which were applied only to the maize crop in the first cropping cycle of the maize-sorghum cropping sequence. The details of the treatments or fertilization packages are 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 <sub>0</sub>	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 <sub>1</sub>	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>2</sub>	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>3</sub>	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>4</sub>	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

A piece of land used in this study was 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 the geographic position of -8.221650, 116.350283. After being cultivated using minimum tillage and cleaned from weeds, the land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The different fertilization packages are consisting of the 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, i.e. the M<sub>AA01</sub> mycorrhizal isolate, which was originally isolated from dryland area 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 charcoal powder and 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 the 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 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 and then were covered with soil.

For the cropping cycle 2, the plots were cleaned from the maize crop debris and weeds; then seeds of sorghum (“Numbu” variety) were direct seeded using dibbling 2 seeds per planting holes made around the maize stubbles. The sorghum plants were not fertilized nor inoculated with AMF inoculum. For both crops, the young maize and sorghum plants were thinned 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. This OrgaNeem solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at 3 days intervals. 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 sorghum. The AMF development includes spore counts at 60 and 100 DAS, and root colonization levels at 60 DAS. The N and P nutrition include concentration of total N and available P in the rhizosphere of maize and sorghum at 60 and 100 DAS. The crop variables include dry weight (shoots and roots) and yield components (grain).

Determination of N was by Kjeldhal, and P by spectrometer. AMF spore extraction from soil (100 g soil sample) was done using 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 variance (ANOVA) and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

## RESULTS AND DISCUSSION

### *AMF development*

In terms of root colonization rates by AMF, there were slight differences between maize and sorghum between treatments of fertilizer packages. This can be seen from Table 2 orchestrating the levels of root colonization by the indigenous AMF are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment, it was 77% on sorghum and 65% on maize roots. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). In this study, however, maize and sorghum were grown in sequence, in which sorghum was direct seeded without treatments and without tillage following 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 the cropping cycle 2 was grown after AMF propagules have built up in the soil during the growth of the maize plants in the cropping cycle 1. This is in line with the results reported in previous study revealing that AMF root colonization and shoot dry weight of maize at 58 DAS were affected by previous crops, whether they were host or non-host of AMF (Arihara & Karasawa, 2000), however the P fertilization did not affect root colonization, especially on maize following AMF host plants.

In more details, the degree of root colonization by AMF may higher in the roots of maize fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium sulfate and Urea), in the other hand, the length of extra-radical mycelium and number of AMF spores were significantly higher in maize fertilized with organic fertilizer (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 were higher at harvest of the maize crop (100 DAS) compared with those at 60 DAS, especially on the D<sub>2</sub> treatment, indicating a high build up of AMF propagules for the subsequent sorghum crop. 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 higher than in maize or bean roots (Alguacil et al., 2008). AMF colonization rates may also higher in roots of sorghum than maize, either inoculated with *Glomus mosseae*



or *G. 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). In this interaction effect, for 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).

**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 (1 <sup>st</sup> crop)			AMF sorghum (2 <sup>nd</sup> crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D <sub>0</sub>	764 <sup>e</sup>	3464 <sup>d</sup>	30 <sup>d</sup>	1231 <sup>e</sup>	3761 <sup>d</sup>	35 <sup>e</sup>
D <sub>1</sub>	1059 <sup>d</sup>	3672 <sup>c</sup>	55 <sup>c</sup>	1343 <sup>d</sup>	4942 <sup>b</sup>	60 <sup>d</sup>
D <sub>2</sub>	2119 <sup>a</sup>	4327 <sup>a</sup>	75 <sup>a</sup>	2981 <sup>a</sup>	5165 <sup>a</sup>	81 <sup>a</sup>
D <sub>3</sub>	1690 <sup>b</sup>	3894 <sup>b</sup>	65 <sup>b</sup>	1881 <sup>b</sup>	4831 <sup>c</sup>	77 <sup>b</sup>
D <sub>4</sub>	1294 <sup>c</sup>	3881 <sup>b</sup>	63 <sup>b</sup>	1769 <sup>c</sup>	4819 <sup>c</sup>	68 <sup>c</sup>
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 description of the packages.

In terms of fertilization effects, AMF colonization rate was reported higher on crop roots in unfertilized soils, followed by organic and biodynamic, and the least in exclusively mineral fertilized and conventional farming systems; and they 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 grass-clover) (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 of soil condition will start to occur when the accumulation of soil P has increased beyond requirement of the crops cultivated (Grant et al., 2005). In the study we report here, it seems that the amount of P input from the NPK fertilizer as applied in the D<sub>2</sub> treatment is most favorable for AMF development in maize crops. In D<sub>2</sub> 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<sub>0</sub> 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<sub>1</sub> 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<sub>2</sub> treatment, resulting in a higher AMF colonization rates on D<sub>2</sub> than on D<sub>1</sub> treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D<sub>2</sub> treatment, especially when compared with the D<sub>1</sub> or D<sub>0</sub> 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 the 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<sub>2</sub> (60% NPK + 12 t/ha manure + AMF) or D<sub>1</sub> (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments are higher than those in D<sub>0</sub> (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D<sub>1</sub> and D<sub>2</sub> treatments (Table 3).

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

Fertilization packages	Total N (g.kg <sup>-1</sup> ) at 60 DAS		Available P (mg.kg <sup>-1</sup> ) at 60 DAS		Total N (g.kg <sup>-1</sup> ) at 100 DAS		Available P (mg.kg <sup>-1</sup> ) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D <sub>0</sub>	1.24 <sup>c</sup>	1.10 <sup>c</sup>	14.97 <sup>d</sup>	11.41 <sup>d</sup>	1.33 <sup>d</sup>	1.23 <sup>d</sup>	17.43 <sup>d</sup>	10.15 <sup>d</sup>
D <sub>1</sub>	1.45 <sup>a</sup>	1.29 <sup>a</sup>	28.44 <sup>b</sup>	16.25 <sup>b</sup>	1.69 <sup>b</sup>	1.38 <sup>b</sup>	29.51 <sup>b</sup>	21.58 <sup>b</sup>
D <sub>2</sub>	1.47 <sup>a</sup>	1.25 <sup>ab</sup>	35.02 <sup>a</sup>	28.49 <sup>a</sup>	1.86 <sup>a</sup>	1.48 <sup>a</sup>	36.56 <sup>a</sup>	29.99 <sup>a</sup>
D <sub>3</sub>	1.39 <sup>b</sup>	1.22 <sup>ab</sup>	17.52 <sup>c</sup>	14.59 <sup>bc</sup>	1.47 <sup>c</sup>	1.31 <sup>c</sup>	19.37 <sup>c</sup>	18.59 <sup>c</sup>
D <sub>4</sub>	1.33 <sup>b</sup>	1.20 <sup>b</sup>	16.29 <sup>c</sup>	14.25 <sup>c</sup>	1.42 <sup>c</sup>	1.31 <sup>c</sup>	18.53 <sup>c</sup>	17.32 <sup>c</sup>
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98
Before planting <sup>1)</sup>	1.20	-	12.28	-	-	-	-	-

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.

Since the NPK fertilizers applied, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, are all easy to get dissolved in water, significant amount of its nutrients could have been loss through infiltration during rainy season. Previous study shows the sand content of the cultivated land has a positive correlation with infiltration rate, with an 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 has been much higher in the D<sub>0</sub> than in the other treatments of fertilization packages due to dissolution by the rain water during that 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, indicating some 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 have reported that AMF can mobilize and take N and P from organic matter 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<sub>2</sub> treatment. Therefore, N and P uptake in shoots of maize and sorghum are highest in the D<sub>2</sub> treatment (Table 4).

In addition, soil contents of those nutrients were also in a good corresponding 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, but the highest values of N and P uptake are also in the D<sub>2</sub> treatment, both for maize and sorghum (Table 4). Based on correlation analysis of the mean values obtained at 60 DAS, there is 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 some contributions of those fertilizers in the packages to nutrient contents of the crops, which are significantly different between treatments of fertilization packages (Table 4).

**Table 4. Mean N and P sorption ( $\text{mg.g}^{-1}$  plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages**

Fertilization packages	N and P uptake ( $\text{mg.g}^{-1}$ plant dry weight) by each crop at 60 DAS			
	Maize (1 <sup>st</sup> cropping cycle)		Sorghum (2 <sup>nd</sup> cropping cycle)	
	N	P	N	P
D <sub>0</sub>	18.62 <sup>e</sup>	1.71 <sup>d</sup>	12.74 <sup>d</sup>	0.65 <sup>d</sup>
D <sub>1</sub>	27.58 <sup>b</sup>	2.60 <sup>b</sup>	18.92 <sup>b</sup>	0.85 <sup>c</sup>
D <sub>2</sub>	28.00 <sup>a</sup>	2.72 <sup>a</sup>	20.86 <sup>a</sup>	1.48 <sup>a</sup>
D <sub>3</sub>	23.10 <sup>c</sup>	2.41 <sup>c</sup>	17.29 <sup>c</sup>	1.36 <sup>b</sup>
D <sub>4</sub>	20.44 <sup>d</sup>	2.41 <sup>c</sup>	17.22 <sup>c</sup>	1.32 <sup>b</sup>
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.

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 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%,  $p = 0.017$ ). These mean 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; and 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 seems 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). These AMF associations seem 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 sorghum crops were not fertilized. This could occur because of the potential of AMF external hyphae to help the host plants in absorbing and dissolving nutrients from soil and manures and other nutrient pools, as well as absorbing 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 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 which D<sub>2</sub> treatment presents the highest values of biomass production (Table 5) and yield components (Table 6). This means that nutrient status of the soils was in a good corresponding with biomass weight of the crops, indicated by the positive correlation coefficients, although only some of them are significant. The AMF applications to maize crop, observed at the 60 DAS, orchestrate 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 show 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 maybe focused to improve root growth in order to increase nutrient sorption during the vegetative growth of maize crop.

**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 (1 <sup>st</sup> crop) and sorghum (2 <sup>nd</sup> 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 <sub>0</sub>	8.13 <sup>d</sup>	10.43 <sup>e</sup>	34.15 <sup>d</sup>	62.29 <sup>e</sup>	0.82 <sup>d</sup>	12.30 <sup>d</sup>	8.31 <sup>d</sup>	47.74 <sup>e</sup>
D <sub>1</sub>	15.13 <sup>b</sup>	22.39 <sup>b</sup>	61.92 <sup>b</sup>	109.03 <sup>b</sup>	2.22 <sup>b</sup>	22.34 <sup>b</sup>	16.89 <sup>b</sup>	95.01 <sup>b</sup>
D <sub>2</sub>	17.12 <sup>a</sup>	34.56 <sup>a</sup>	72.86 <sup>a</sup>	111.25 <sup>a</sup>	3.37 <sup>a</sup>	24.44 <sup>a</sup>	23.53 <sup>a</sup>	105.14 <sup>a</sup>
D <sub>3</sub>	13.65 <sup>c</sup>	18.48 <sup>c</sup>	52.26 <sup>c</sup>	101.05 <sup>c</sup>	1.68 <sup>c</sup>	14.52 <sup>c</sup>	12.01 <sup>c</sup>	85.46 <sup>c</sup>
D <sub>4</sub>	13.34 <sup>c</sup>	15.43 <sup>d</sup>	51.29 <sup>c</sup>	95.87 <sup>d</sup>	1.38 <sup>c</sup>	14.47 <sup>c</sup>	11.81 <sup>c</sup>	66.06 <sup>d</sup>
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.

In relation to nutrient uptake, although AMF colonization levels did not show significant correlation with N uptake in shoots of maize or sorghum, they showed 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 matter 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 ( $R^2 = 82.6\%$ ), which means that 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 nutrient slow-releasing organic matter, 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 contribution of AMF colonization in the roots, although this still needs to be confirmed with further research. This view is supported by the conditions of the study area, which is dominated by sand, and if leaching happened during rainy season, the loss of the 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 is cattle manure applied to the maize crop in the first cropping cycle.

**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 1 <sup>st</sup> cropping cycle		Sorghum in the 2 <sup>nd</sup> cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D <sub>0</sub>	9.60 <sup>e</sup>	22.48 <sup>d</sup>	3.57 <sup>d</sup>	2.73 <sup>d</sup>
D <sub>1</sub>	17.40 <sup>b</sup>	26.94 <sup>b</sup>	5.05 <sup>b</sup>	3.01 <sup>b</sup>
D <sub>2</sub>	22.80 <sup>a</sup>	28.12 <sup>a</sup>	6.65 <sup>a</sup>	3.61 <sup>a</sup>
D <sub>3</sub>	15.60 <sup>c</sup>	25.98 <sup>c</sup>	4.43 <sup>c</sup>	2.90 <sup>c</sup>
D <sub>4</sub>	10.20 <sup>d</sup>	24.61 <sup>c</sup>	4.17 <sup>c</sup>	2.81 <sup>cd</sup>
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.

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, 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 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 nor 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 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. These could mean that for maize crop, most nutrients derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the residues of manure with contribution from AMF colonization in sorghum roots. Many researchers have also showed 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 resulted in significantly higher N status of the mycorrhizal than non-mycorrhizal hosts (Hawkins et al., 2000).

However, when 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, the results mostly show significant positive coefficients of correlation. For example, correlation of AMF colonization levels between roots of maize and sorghum is highly significant, with an  $r = + 0.988$  ( $R^2 = 97.6\%$ ,  $p = 0.002$ ), which 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 a 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, there were higher significant values ( $p < 0.01$ ) on sorghum than on maize. This could be due to some build up of AMF in the soil after harvest of the maize crop in the first cropping cycle before sorghum was direct seeded without tillage. Even both crops were grown simultaneously, it was also found that AMF colonization level was 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<sub>2</sub> 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 for improving the 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 the following crops, at the sandy and dry land, with no additional fertilization and mycorrhizal propagules applied.

## REFERENCES

- Alguacil, M., Lumini, E., Roldan, A., Salinas-Garcia, J., Bonfante, P., & Bianciotto, V. (2008). The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecological Applications*, 18(2), 527-536.
- Arihara, J., & Karasawa, T. (2000). Effect of previous crops on arbuscular mycorrhizal formation and growth of succeeding maize. *Soil Science and Plant Nutrition*, 46(1), 43-51.
- Astiko, W. (2013). *The role of indigenous mycorrhizae in different plant patterns to increase soybean yield in sandy soil (Case studies on dryland North Lombok)*. (Unpublished doctoral thesis), Universitas Brawijaya Malang, Indonesia.
- Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013a). The role of indigenous mycorrhiza in combination with cattle manure in improving maize yield (*Zea mays* L) on sandy loam of northern Lombok, eastern of Indonesia. *Journal of Tropical soils*, 18(1), 53-58.
- Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013b). Soil Fertility Status and Soybean [*Glycine Max* (L) Merr] Performance Following Introduction of Indigenous Mycorrhiza Combined with Various Nutrient Sources Into Sandy Soil. *AGRIVITA, Journal of Agricultural Science*, 35(2), 127-137.
- Augé, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science*, 84(4), 373-381.
- Azcón, R., Ambrosano, E., & Charest, C. (2003). Nutrient acquisition in mycorrhizal lettuce plants under different phosphorus and nitrogen concentration. *Plant Science*, 165(5), 1137-1145.
- Bautista-Cruz, A., Cruz-Domínguez, G., Rodríguez-Mendoza, M. d. I. N., Pérez-Pacheco, R., & Robles, C. (2014). Effect of compost and slow-release fertilizers addition on soil biochemistry and yield of maize (*Zea mays* L.) in Oaxaca, Mexico. *Rev. FCA UNCUYO*, 46, 181-193.
- Bruand, A., Hartmann, C., & Lesturgez, G. (2005). *Physical properties of tropical sandy soils: A large range of behaviours*. Paper presented at the Management of Tropical Sandy Soils for Sustainable Agriculture. A holistic approach for sustainable development of problem soils in the tropics.
- Brundrett, M., Bougher, N., Dell, B., & Grove, T. (1996). Working Ylith Mycorrhizas in Forestry and Agriculture.



- Calderón-Vázquez, C., Alatorre-Cobos, F., Simpson-Williamson, J., & Herrera-Estrella, L. (2009). Maize under phosphate limitation. In *Handbook of Maize: Its Biology* (pp. 381-404): Springer.
- Carrenho, R., Trufem, S. F. B., Bononi, V. L. R., & Silva, E. S. (2007). The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta Botanica Brasilica*, 21(3), 723-730.
- Cavagnaro, T. R., Bender, S. F., Asghari, H. R., & van der Heijden, M. G. (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, 20(5), 283-290.
- Dhillon, S. S., & Ampornpan, L.-a. (1992). The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant rice (*Oryza sativa* L.) plants. *Biology and Fertility of Soils*, 13(2), 85-91.
- Drew, E., Murray, R., Smith, S., & Jakobsen, I. (2003). Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant and Soil*, 251(1), 105-114.
- Feng, G., Song, Y., Li, X., & Christie, P. (2003). Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Applied Soil Ecology*, 22(2), 139-148.
- George, E., Marschner, H., & Jakobsen, I. (1995). Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, 15(3-4), 257-270.
- Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3), 489-500.
- Grant, C., Bittman, S., Montreal, M., Plenchette, C., & Morel, C. (2005). Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science*, 85(1), 3-14.
- Gryndler, M., Larsen, J., Hřselová, H., Řezáčová, V., Gryndlerová, H., & Kubát, J. (2006). Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. *Mycorrhiza*, 16(3), 159-166.
- Guo, W., Zhao, R., Zhao, W., Fu, R., Guo, J., Bi, N., & Zhang, J. (2013). Effects of arbuscular mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) grown in rare earth elements of mine tailings. *Applied Soil Ecology*, 72, 85-92.
- Hawkins, H.-J., Johansen, A., & George, E. (2000). Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil*, 226(2), 275-285.
- Johnson, N. C., Copeland, P. J., Crookston, R. K., & Pflieger, F. (1992). Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. *Agronomy Journal*, 84(3), 387-390.
- Koide, R., & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist*, 148(3), 511-517.
- Leigh, J., Hodge, A., & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist*, 181(1), 199-207.
- Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., & Niggli, U. (2000). Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of Soils*, 31(2), 150-156.

- Marulanda, A., Azcon, R., & Ruiz-Lozano, J. M. (2003). Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. *Physiologia Plantarum*, *119*(4), 526-533.
- Menge, J. (1983). Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture. *Canadian Journal of Botany*, *61*(3), 1015-1024.
- Patle, G. T., Sikar, T. T., Rawat, K. S., & Singh, S. K. (2018). Estimation of infiltration rate from soil properties using regression model for cultivated land. *Geology, Ecology, and Landscapes*, 1-13.
- Plenchette, C., Fortin, J., & Furlan, V. (1983). Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. *Plant and Soil*, *70*(2), 199-209.
- Sieverding, E., Friedrichsen, J., & Suden, W. (1991). Vesicular-arbuscular mycorrhiza management in tropical agrosystems. *Sonderpublikation der GTZ (Germany)*.
- Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*: Academic press.
- Supardi, G. (1996). Mining the Synergistic Effects towards Strong Agriculture. *HITI News*, *4*, 10-13.
- Tarafdar, J., & Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry*, *26*(3), 387-395.
- Tawaraya, K. (2003). Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition*, *49*(5), 655-668.
- Tawaraya, K., Naito, M., & Wagatsuma, T. (2006). Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant Nutrition*, *29*(4), 657-665.
- Vivekanandan, M., & Fixen, P. (1991). Cropping systems effects on mycorrhizal colonization, early growth, and phosphorus uptake of corn. *Soil Science Society of America Journal*, *55*(1), 136-140.
- Wangiyana, W., Cornish, P. S., & Morris, E. (2006). Arbuscular mycorrhizal fungi dynamics in contrasting cropping systems on vertisol and regosol soils of Lombok, Indonesia. *Experimental agriculture*, *42*(4), 427-439.
- Zhu, Y.-G., Cavagnaro, T. R., Smith, S. E., & Dickson, S. (2001). Backseat driving? Accessing phosphate beyond the rhizosphere-depletion zone. *Trends in Plant Science*, *6*(5), 194-195.



**Journal of Tropical Agricultural Science** <onbehalfof@manuscriptcentral.com>

kepada saya, w.wangiyana, lolitaabas37 ▾

Jum, 8 Feb 2019 19:33



🗨️ Inggris ▾ > Indonesia ▾ Terjemahkan pesan

Nonaktifkan untuk: Ingg

08-Feb-2019

Dear Assoc. Prof. Astiko:

Your manuscript entitled "Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands" has been successfully submitted online and is presently being given full consideration for publication in the Journal of Tropical Agricultural Science.

Your manuscript ID is JTAS-1651-2018.R1.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts <https://mc.manuscriptcentral.com/upm-itas> and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to <https://mc.manuscriptcentral.com/upm-itas>.

Thank you for submitting your manuscript to the Journal of Tropical Agricultural Science.

Sincerely,

Journal Officer

Journal of Tropical Agricultural Science Editorial Office

- 1 **Running Title:**
- 2 **Mycorrhizal seed-coating on maize-sorghum cropping sequence**
- 3
- 4

5 **Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-**  
 6 **uptake and availability on maize-sorghum cropping sequence in Lombok's drylands**

7

8 Wahyu Astiko<sup>1\*</sup>, Wayan Wangiyana<sup>1</sup>, Lolita Endang Susilowati<sup>2</sup>

9 <sup>1)</sup> Study Program of Agroecotechnology, Faculty of Agriculture, University of Mataram

10 <sup>2)</sup> Department of Soil Science Faculty of Agriculture, University of Mataram

11 Jalan Majapahit No. 62, Mataram, Lombok, Indonesia, 83125

12 E-mail addresses:

13 astiko@unram.ac.id (Wahyu Astiko)

14 w.wangiyana@unram.ac.id (Wayan Wangiyana)

15 lolitaabas37@unram.ac.id (Lolita Endang Susilowati)

16 <sup>\*)</sup> Corresponding author

17

18 The List of Number of Tables

19 Table 1. *The mycorrhizal-based fertilization packages tested and applied to maize only in the*  
 20 *maize-sorghum cropping sequence* ..... 6  
 21 Table 2. *Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization*  
 22 *rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence*..... 9  
 23 Table 3. *Mean concentration of total N and available P of soil in the rhizospheres of maize (1<sup>st</sup>*  
 24 *crop) and sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100*  
 25 *DAS*..... 11  
 26 Table 4. *Mean N and P sorption (mg.g<sup>-1</sup> plant dry weight) by each crop at 60 DAS for each*  
 27 *treatment of fertilization packages* ..... 12  
 28 Table 5. *Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of*  
 29 *fertilization packages* ..... 14  
 30 Table 6. *Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each*  
 31 *treatment of fertilization packages* ..... 15

32

33

34 **Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-**  
35 **uptake and availability on maize-sorghum cropping sequence in Lombok's drylands**

36  
37

**ABSTRACT**

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

59

60 Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum ~~,~~ cropping  
61 sequence, cattle manure, plant nutrition.

62

63

## 64 INTRODUCTION

65 The northern part of North Lombok regency (Indonesia) is dominated by drylands with  
66 sandy soils texture. With a very short and low number of rainy days (December to April, 100-  
67 200 mm) per wet month or no rain during the long dry seasons (May to November), no food  
68 crops can be cultivated normally especially in the areas without deep wells. Moreover,  
69 inadequate phosphorus (P) availability is also one of the factors limiting the productivity of  
70 maize and other food crops in the dryland of North Lombok. Maize crop requires very high P;  
71 for example, for the production of 10 ton/ha, maize crops need 102 kg/ha of P<sub>2</sub>O<sub>5</sub> and 76% of  
72 it is transported into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take  
73 up P at only about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope  
74 with the unavailability of soil water and P and other essential nutrients in drylands of North  
75 Lombok, arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to  
76 improve the performance of food crops especially during the dry seasons. Many have reported  
77 that the soil fungi, in symbiosis with their host plants, can help plants to improve water retention  
78 and make their host plants more tolerant to drought (Augé, 2004). AMF colonization also  
79 increases nutrient uptake from soils and enhances growth and yield of the host plants although  
80 it depends on the species of AMF colonizing the host plants (Marulanda et al., 2003).

81 The most common findings show that the external hyphae of AMF improve P uptake  
82 and transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003;  
83 George et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006).  
84 Although there is no growth improvement of the host plants due to the AMF symbiosis, the  
85 amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host  
86 roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase  
87 the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe, and B when  
88 compared with non-mycorrhizal plants (Dhillon & Ampornpan, 1992; Hawkins et al., 2000;  
89 Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the  
90 extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al.,  
91 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the  
92 external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying  
93 the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum  
94 (Plenchette et al., 1983), the results showed a high mycorrhizal dependency, both for maize (up  
95 to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings  
96 of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

97 matter is produced of the crops. With the high porosity and low water retention capacity on  
98 dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop  
99 dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with  
100 AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al.,  
101 2015).

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

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

## 126 **MATERIALS AND METHODS**

### 128 *Design of the experiment*

129 The field experiment of maize-sorghum cropping sequence in this study was arranged  
130 according to the Randomized Complete Block Design (RCBD) with four replications (blocks).



131 The study was carried out in the Akar-Akar village located in North Lombok regency,  
 132 Indonesia, from January to August 2016. Treatments involving the use of five fertilizer  
 133 packages consisting of different combinations of organic, inorganic and indigenous AMF bio-  
 134 fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum  
 135 cropping sequence. The details of the treatments or fertilization packages are listed in Table 1.  
 136 The 100% NPK-only recommended dose (D0) is the farmer's practice of dose for maize by the  
 137 locals. The NPK's doses were decreased and had been replaced by cattle manure in varying  
 138 fertilization packages (D1, D2, D3, D4), and added with AMF as listed in Table 1.

139 **Table 1. The mycorrhizal-based fertilization packages tested and applied to maize only in the**  
 140 **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 <sub>0</sub>	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 <sub>1</sub>	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>2</sub>	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>3</sub>	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>4</sub>	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

141  
 142 A piece of land used in this study was of a sandy soil type (69% sand, 29% silt, and 2%  
 143 clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area.  
 144 The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82  
 145 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and  
 146 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the  
 147 land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The  
 148 different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure),  
 149 and inorganic fertilizer (NPK and urea), were applied only to the maize crop in the first cropping  
 150 cycle.

151 An indigenous AMF inoculum, i.e. *Glomus mosseae* (the M<sub>AA01</sub> mycorrhizal isolate  
 152 including the hyphae and the mycorrhizal spores), which was originally isolated from dryland  
 153 area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied  
 154 through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF  
 155 inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier  
 156 materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were  
 157 coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds  
 158 ("Bisma" variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant  
 159 spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on

160 the treatments (Table 1) was applied at the time of planting the maize seeds by burying the  
161 whole dose in the planting hole in the position below the seeds. The cattle manure variation in  
162 the fertilization package is to identify the optimum combinations to benefit the plant growth,  
163 increase the nutrient availability at the soils, and support the AMF development. The maximum  
164 combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure  
165 applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of  
166 available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic  
167 (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300  
168 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with  
169 the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were  
170 fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses,  
171 followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers  
172 were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered  
173 with soil.

174 After harvest of maize at 100 days after seeding (DAS) and field cleaning from maize  
175 debris, sorghum seeds were then planted in cropping cycle 2. In cropping cycle 2, the plots were  
176 cleared from maize crop debris and weeds before. Before the second sequence, the field was left  
177 fallow (rest) for 10 days. Seeds of sorghum ("Numbu" variety) were directly seeded by dibbling  
178 2 seeds per planting holes made around the maize stubbles. These sorghum plants were not  
179 fertilized nor inoculated with additional AMF inoculum, but only the chopped maize roots  
180 containing the AMF mycorrhizal and spread throughout the plot. For both crops, the young  
181 maize and sorghum plants were tinned at 7 DAS by leaving one maize or sorghum plant per  
182 planting hole. Weeding and soil piling of the maize and sorghum plant base were done at 15  
183 and 30 DAS. Plant protection was done by spraying "OrgaNeem" (an organic pesticide of plant  
184 origin containing Azadirachtin extracted from neem leaves) at a concentration of 5 ml  
185 OrgaNeem per liter of water. The OrgaNeem solution was applied to both crops (maize or  
186 sorghum) from 10 to 60 DAS at a 3-day interval. Harvesting of maize or sorghum crops was  
187 done at 100 DAS.

#### 188 ***Measurement and data analysis***

189 The variables measured were AMF development, N and P nutrition, and growth and  
190 yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS,  
191 and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total  
192 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, Universitas Mataram. Soil pH and texture were measured by standard procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by destruction with  $(\text{NH}_4)_2\text{SO}_4$  and distillation with NaOH where the  $\text{NH}_4^+$  was determined by indophenol blue colorimetric method and the  $\text{NH}_3$  was defined by a titration with 0.05N of  $\text{H}_2\text{SO}_4$  solution (Page et al., 1982). Total N in plants was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by  $(\text{NH}_4)_2\text{SO}_4$  and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer ( $\lambda = 693 \text{ nm}$ ) after the extraction process using Bray and Kurt I solution (0.025 N HCl +  $\text{NH}_4\text{F}$  0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with  $\text{K}_2\text{Cr}_2\text{O}_7$  in presence of sulphuric acid ( $\text{H}_2\text{SO}_4$ ) following Walkley and Black's method (Horwitz, 2000).

Determination of N was done using the Kjeldhal method and P by using a spectrometer. 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  $\mu\text{m}$  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 variance (ANOVA) and the Tukey's HSD (Honestly Significant Difference) means tested at 5% level of significance.

## RESULTS AND DISCUSSION

### AMF development

In terms of root colonization rates by AMF, this study acknowledged that the maximum rate was found at the 60 DAS and there is no significant growth observed at the 100 DAS. there were slight differences between maize and sorghum between treatments fertilizer packages. As This can be seen from Table 2, which displays the levels of root colonization by the indigenous AMF which are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment; where it was 77% on sorghum and 65% on maize roots. When

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Superscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

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

236 **Table 2. Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization**  
 237 **rates (%-colonization) on maize and sorghum in a maize-sorghum cropping sequence**  
 238

Fertilization packages	AMF on maize (1 <sup>st</sup> crop)			AMF sorghum (2 <sup>nd</sup> crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D <sub>0</sub>	764 <sup>e</sup>	3464 <sup>d</sup>	30 <sup>d</sup>	1231 <sup>e</sup>	3761 <sup>d</sup>	35 <sup>e</sup>
D <sub>1</sub>	1059 <sup>d</sup>	3672 <sup>c</sup>	55 <sup>c</sup>	1343 <sup>d</sup>	4942 <sup>b</sup>	60 <sup>d</sup>
D <sub>2</sub>	2119 <sup>a</sup>	4327 <sup>a</sup>	75 <sup>a</sup>	2981 <sup>a</sup>	5165 <sup>a</sup>	81 <sup>a</sup>
D <sub>3</sub>	1690 <sup>b</sup>	3894 <sup>b</sup>	65 <sup>b</sup>	1881 <sup>b</sup>	4831 <sup>c</sup>	77 <sup>b</sup>
D <sub>4</sub>	1294 <sup>c</sup>	3881 <sup>b</sup>	63 <sup>b</sup>	1769 <sup>c</sup>	4819 <sup>c</sup>	68 <sup>c</sup>
HSD 5%	231	13	2.0	109	12	6.5

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

241 Specifically, the degree of root colonization by AMF may be higher in the roots of maize  
 242 fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium  
 243 sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF  
 244 spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure)  
 245 (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore  
 246 production compared with conventional fertilization. It can also be seen from Table 2 that AMF  
 247 spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60  
 248 DAS, especially on the D<sub>2</sub> treatment. This indicates a high buildup of AMF propagules for the  
 249 subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in  
 250 roots of sorghum in the second cropping cycle compared with that of maize in the first cropping  
 251 cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than

252 the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates  
253 may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus*  
254 *mosseae* or *G. versiforme*, indicating that sorghum is more favorable for AMF development  
255 than maize plants (Guo et al., 2013).

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

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

275 A negative impact on soil condition occur when the accumulation of soil P has increased  
276 beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that  
277 the amount of P input from the NPK fertilizer as applied in the D<sub>2</sub> treatment was most favorable  
278 for AMF development in maize crops. In the D<sub>2</sub> treatment, the NPK fertilizer was reduced to  
279 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D<sub>0</sub>  
280 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF.  
281 Among the treatments with AMF in combination with cattle manure, the D<sub>1</sub> treatment had the  
282 highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the  
283 AMF infection and development in maize roots compared with those in the D<sub>2</sub> treatment,  
284 resulting in a higher AMF colonization rate on D<sub>2</sub> than on D<sub>1</sub> treatment. Therefore, AMF

285 colonization and spore numbers in maize were highest in the D<sub>2</sub> treatment, especially when  
 286 compared with the D<sub>1</sub> or D<sub>0</sub> treatment. Using the same indigenous AMF applied to a pot  
 287 experiment in which the soil for the growing media was taken from the same field in North  
 288 Lombok, it was found that the highest level of AMF colonization of maize roots was in the  
 289 treatment with AMF combined with cattle manure compared with AMF combined with NPK  
 290 fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a  
 291 significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure  
 292 combined with mineral fertilizers compared with those fertilized with mineral fertilizers only  
 293 (Gryndler et al., 2006).

#### 294 *Soil nutrient status and nutrient sorption by maize and sorghum*

295 There were significant effects of the different fertilizer packages on soil nutrient status  
 296 (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and  
 297 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient  
 298 status, measured either at 60 or 100 DAS, are highest under the D<sub>2</sub> (60% NPK + 12 t/ha manure  
 299 + AMF) or D<sub>1</sub> (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient  
 300 status in these treatments were higher than those in D<sub>0</sub> (100% NPK only) treatment, although  
 301 the dose of NPK fertilizers was reduced in the D<sub>1</sub> and D<sub>2</sub> treatments (Table 3).

302 **Table 3. Mean concentration of total N and available P of soil in the rhizospheres of maize (1<sup>st</sup>**  
 303 **crop) and sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100**  
 304 **DAS**

Fertilization packages	Total N (g.kg <sup>-1</sup> ) at 60 DAS		Available P (mg.kg <sup>-1</sup> ) at 60 DAS		Total N (g.kg <sup>-1</sup> ) at 100 DAS		Available P (mg.kg <sup>-1</sup> ) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D <sub>0</sub>	1.24 <sup>c</sup>	1.10 <sup>c</sup>	14.97 <sup>d</sup>	11.41 <sup>d</sup>	1.33 <sup>d</sup>	1.23 <sup>d</sup>	17.43 <sup>d</sup>	10.15 <sup>d</sup>
D <sub>1</sub>	1.45 <sup>a</sup>	1.29 <sup>a</sup>	28.44 <sup>b</sup>	16.25 <sup>b</sup>	1.69 <sup>b</sup>	1.38 <sup>b</sup>	29.51 <sup>b</sup>	21.58 <sup>b</sup>
D <sub>2</sub>	1.47 <sup>a</sup>	1.25 <sup>ab</sup>	35.02 <sup>a</sup>	28.49 <sup>a</sup>	1.86 <sup>a</sup>	1.48 <sup>a</sup>	36.56 <sup>a</sup>	29.99 <sup>a</sup>
D <sub>3</sub>	1.39 <sup>b</sup>	1.22 <sup>ab</sup>	17.52 <sup>c</sup>	14.59 <sup>bc</sup>	1.47 <sup>c</sup>	1.31 <sup>c</sup>	19.37 <sup>c</sup>	18.59 <sup>c</sup>
D <sub>4</sub>	1.33 <sup>b</sup>	1.20 <sup>b</sup>	16.29 <sup>c</sup>	14.25 <sup>c</sup>	1.42 <sup>c</sup>	1.31 <sup>c</sup>	18.53 <sup>c</sup>	17.32 <sup>c</sup>
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

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

307 At the present study, no infiltration data was measured, however it is important to note  
 308 that since the NPK fertilizers application, i.e. Phonska and Urea at 7 DAS and Urea at 21  
 309 DAS, at sandy lands, are easily dissolved in water, and significant amount of the nutrients could  
 310 have been loss through infiltration during the rainy season. Previous study shows that the sand  
 311 content of the cultivated land had a positive correlation with infiltration rate, with an r-value of  
 312 +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that

313 at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK  
 314 fertilizers through leaching was much higher in the D<sub>0</sub> than in the other treatments of  
 315 fertilization packages due to dissolution by rain water during the rainy season. However, there  
 316 were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres  
 317 of maize crop, especially in those treated with manure and AMF application. This indicated a  
 318 slow release of N and P nutrients from manure after application to the maize plants in the first  
 319 cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF  
 320 can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins  
 321 et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can  
 322 be seen from Table 2 that the highest average of AMF colonization levels in maize roots was  
 323 in the D<sub>2</sub> treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in  
 324 the D<sub>2</sub> treatment (Table 4).

325 In addition, soil contents of those nutrients corresponded well with the levels of N and  
 326 P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not  
 327 significant for P uptake, the highest values of N and P uptake were also seen in the D<sub>2</sub> treatment  
 328 for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at  
 329 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a  
 330 value of  $r = + 0.970$  (R-square = 94.1%,  $p = 0.006$ ) for maize plants, and  $r = + 0.904$  (R-square  
 331 = 81.7%,  $p = 0.035$ ) for sorghum plants. These indicate those fertilizers in the packages  
 332 contribute to nutrient contents of the crops, which are significantly different between treatments  
 333 of fertilization packages (Table 4).

334 **Table 4. Mean N and P sorption ( $mg.g^{-1}$  plant dry weight) by each crop at 60 DAS for each**  
 335 **treatment of fertilization packages**

Fertilization packages	N and P uptake ( $mg.g^{-1}$ plant dry weight) by each crop at 60 DAS			
	Maize (1 <sup>st</sup> cropping cycle)		Sorghum (2 <sup>nd</sup> cropping cycle)	
	N	P	N	P
D <sub>0</sub>	18.62 <sup>e</sup>	1.71 <sup>d</sup>	12.74 <sup>d</sup>	0.65 <sup>d</sup>
D <sub>1</sub>	27.58 <sup>b</sup>	2.60 <sup>b</sup>	18.92 <sup>b</sup>	0.85 <sup>c</sup>
D <sub>2</sub>	28.00 <sup>a</sup>	2.72 <sup>a</sup>	20.86 <sup>a</sup>	1.48 <sup>a</sup>
D <sub>3</sub>	23.10 <sup>c</sup>	2.41 <sup>c</sup>	17.29 <sup>c</sup>	1.36 <sup>b</sup>
D <sub>4</sub>	20.44 <sup>d</sup>	2.41 <sup>c</sup>	17.22 <sup>c</sup>	1.32 <sup>b</sup>
HSD 5%	0.41	0.11	0.07	0.04

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

338 However, P status of the soil did not show a significant correlation with P uptake either  
 339 by maize or sorghum crop. In spite of that, there is a significant positive correlation between  
 340 the degree of AMF colonization in the roots and P sorption in the shoot of maize plants, with

341 an  $r = + 0.909$  ( $R^2 = 82.6\%$ ,  $p = 0.032$ ), and that of sorghum plants, with an  $r = + 0.940$   
342 ( $R^2 = 88.4\%$ ,  $p = 0.017$ ). This shows that there were significant contributions of AMF  
343 colonization in the roots to the amount of P taken up by the plants, both maize and sorghum. If  
344 soil soluble P has not exceeded crop requirements, AMF association will still result in  
345 significantly positive effects on P uptake and biomass production because of P requirements of  
346 the crops; for optimum growth and yields, crops require P since the early stage of their growth,  
347 either from soil, fertilizer application, or from AMF associations (Grant et al., 2005).

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

### 363 ***Biomass and yield components of maize and sorghum***

364 In terms of biomass production and yield components of maize and sorghum, there were  
365 also significant effects of the fertilization packages in which D<sub>2</sub> treatment presents the highest  
366 values of biomass production (Table 5) and yield components (Table 6). This means that the  
367 nutrient status of the soils corresponded well with the biomass weight of the crops, indicated  
368 by the positive correlation coefficients, although only some of them are significant. The AMF  
369 applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot  
370 weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization  
371 levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with  
372 an  $r = + 0.912$  ( $R^2 = 83.2\%$ ,  $p = 0.031$ ) and shoot dry weight at maturity (100 DAS), with an  $r$   
373  $= + 0.892$  ( $R^2 = 79.6\%$ ,  $p = 0.042$ ). This could mean that during the vegetative growth, AMF



374 colonization have focused on improving root growth to increase nutrient sorption during the  
375 vegetative growth of maize crops.

376 **Table 5. Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of**  
377 **fertilization packages**  
378

Fertilization packages	Dry biomass weights (g/plant) of maize (1 <sup>st</sup> crop) and sorghum (2 <sup>nd</sup> 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 <sub>0</sub>	8.13 <sup>d</sup>	10.43 <sup>c</sup>	34.15 <sup>d</sup>	62.29 <sup>c</sup>	0.82 <sup>d</sup>	12.30 <sup>d</sup>	8.31 <sup>d</sup>	47.74 <sup>c</sup>
D <sub>1</sub>	15.13 <sup>b</sup>	22.39 <sup>b</sup>	61.92 <sup>b</sup>	109.03 <sup>b</sup>	2.22 <sup>b</sup>	22.34 <sup>b</sup>	16.89 <sup>b</sup>	95.01 <sup>b</sup>
D <sub>2</sub>	17.12 <sup>a</sup>	34.56 <sup>a</sup>	72.86 <sup>a</sup>	111.25 <sup>a</sup>	3.37 <sup>a</sup>	24.44 <sup>a</sup>	23.53 <sup>a</sup>	105.14 <sup>a</sup>
D <sub>3</sub>	13.65 <sup>c</sup>	18.48 <sup>c</sup>	52.26 <sup>c</sup>	101.05 <sup>c</sup>	1.68 <sup>c</sup>	14.52 <sup>c</sup>	12.01 <sup>c</sup>	85.46 <sup>c</sup>
D <sub>4</sub>	13.34 <sup>c</sup>	15.43 <sup>d</sup>	51.29 <sup>c</sup>	95.87 <sup>d</sup>	1.38 <sup>c</sup>	14.47 <sup>c</sup>	11.81 <sup>c</sup>	66.06 <sup>d</sup>
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

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

381 In relation to nutrient uptake, although AMF colonization levels did not show significant  
382 correlation with N uptake in shoots of maize or sorghum, they did show a positive significant  
383 correlation with P uptake both in shoots of maize and sorghum, with an  $r = +0.909$  ( $R^2 = 82.6\%$ ,  
384  $p = 0.032$ ) for maize, and  $r = +0.940$  ( $R^2 = 88.4\%$ ,  $p = 0.017$ ) for sorghum. These could be due  
385 to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters  
386 for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based  
387 on the strength of the relationships between AMF colonization levels and P uptake, the value  
388 of  $R^2$  is higher in sorghum ( $R^2 = 88.4\%$ ) than in maize ( $R^2 = 82.6\%$ ). This means that the  
389 contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at  
390 the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not  
391 receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that  
392 most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping  
393 cycle, was mostly taken up from the manure under the contribution of AMF colonization in the  
394 roots. However, this still needs to be confirmed by further research. This view is supported by  
395 the conditions of the study area, which was dominated by sand, and if leaching happened during  
396 the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first  
397 cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum  
398 crop in the second cropping cycle was the cattle manure applied to the maize crop in the first  
399 cropping cycle.

400

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

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1 <sup>st</sup> cropping cycle		Sorghum in the 2 <sup>nd</sup> cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D <sub>0</sub>	9.60 <sup>e</sup>	22.48 <sup>d</sup>	3.57 <sup>d</sup>	2.73 <sup>d</sup>
D <sub>1</sub>	17.40 <sup>b</sup>	26.94 <sup>b</sup>	5.05 <sup>b</sup>	3.01 <sup>b</sup>
D <sub>2</sub>	22.80 <sup>a</sup>	28.12 <sup>a</sup>	6.65 <sup>a</sup>	3.61 <sup>a</sup>
D <sub>3</sub>	15.60 <sup>c</sup>	25.98 <sup>c</sup>	4.43 <sup>c</sup>	2.90 <sup>c</sup>
D <sub>4</sub>	10.20 <sup>d</sup>	24.61 <sup>c</sup>	4.17 <sup>c</sup>	2.81 <sup>cd</sup>
HSD 5%	0.59	1.37	0.26	0.09

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

405 In relation to nitrogen nutrient, there was a very low and non-significant correlation  
 406 between AMF colonization levels and N uptake in maize shoots with an  $R^2$  of only 41.2%.  
 407 However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status  
 408 of the soil with an  $R^2 = 94.1\%$  ( $p = 0.006$ ). Moreover, the N soil availability also showed a  
 409 significant correlation with biomass and grain yield of maize, i.e. with an  $R^2 = 84.1\%$  ( $p = 0.029$ )  
 410 with grain yield at 60 DAS,  $R^2 = 92.2\%$  ( $p = 0.009$ ) with shoot dry weight at 60 DAS, and  $R^2 =$   
 411  $90.6\%$  ( $p = 0.012$ ) with shoot dry weight at 100 DAS.

412 In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS  
 413 showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop  
 414 where AMF colonization levels showed a significant and positive correlation with shoot dry  
 415 weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any  
 416 significant correlation with biomass weight or yield components of sorghum. However, AMF  
 417 colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in  
 418 sorghum shoots, with an  $R^2 = 71.9\%$  ( $p = 0.069$ ), and N uptake in sorghum shoots showed  
 419 positive significant correlation with biomass and grain yield of sorghum, i.e. with an  $R^2 = 80.5\%$   
 420 ( $p = 0.039$ ) with grain yield,  $R^2 = 83.0\%$  ( $p = 0.031$ ) with shoot dry weight at 60 DAS, and  $R^2 =$   
 421  $89.9\%$  ( $p = 0.014$ ) with shoot dry weight at 100 DAS. **No additional fertilizers and inoculums**  
 422 **were applied at the cropping cycle 2.** These could mean that for maize crop, most nutrients were  
 423 derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the  
 424 residues of manure contributed from AMF colonization in sorghum roots. Many researchers  
 425 have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g.  
 426 Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did

427 not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal  
428 hosts (Hawkins et al., 2000).

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

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

#### 447 **CONCLUSION**

448         Among the mycorrhiza-based fertilization packages tested on the maize-sorghum  
449 cropping sequence system at the drylands in North Lombok of Indonesia, the D<sub>2</sub> package,  
450 consisting of 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF  
451 inoculation, was found to be the best fertilization package to improve crop yield and soil  
452 nutrient availability. This study noted that the AMF development was higher in the sorghum at  
453 the second cropping cycle compared to the growth in the maize at the first cropping cycle. This  
454 condition led to the higher NP-uptake and soil nutrient availability in subsequent crops, both at  
455 the sandy ~~and~~ dry-land, with no additional fertilization and mycorrhizal propagules applied.

456

457

458 **REFERENCES**

- 459 Alguacil, M., Lumini, E., Roldan, A., Salinas-Garcia, J., Bonfante, P., & Bianciotto, V.  
460 (2008). The impact of tillage practices on arbuscular mycorrhizal fungal diversity in  
461 subtropical crops. *Ecological Applications*, 18(2), 527-536.
- 462 Arihara, J., & Karasawa, T. (2000). Effect of previous crops on arbuscular mycorrhizal  
463 formation and growth of succeeding maize. *Soil Science and Plant Nutrition*, 46(1),  
464 43-51.
- 465 Astiko, W. (2013). *The role of indigenous mycorrhizae in different plant patterns to increase*  
466 *soybean yield in sandy soil (Case studies on dryland North Lombok)*. (Unpublished  
467 doctoral thesis), Universitas Brawijaya Malang, Indonesia.
- 468 Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013a). The role of  
469 indigenous mycorrhiza in combination with cattle manure in improving maize yield  
470 (*Zea mays* L) on sandy loam of Northern Lombok, Eastern of Indonesia. *Journal of*  
471 *Tropical soils*, 18(1), 53-58.
- 472 Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013b). Soil Fertility Status  
473 and Soybean [*Glycine Max* (L) Merr] Performance Following Introduction of  
474 Indigenous Mycorrhiza Combined with Various Nutrient Sources Into Sandy Soil.  
475 *AGRIVITA, Journal of Agricultural Science*, 35(2), 127-137.
- 476 Augé, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal*  
477 *of Soil Science*, 84(4), 373-381.
- 478 Azcón, R., Ambrosano, E., & Charest, C. (2003). Nutrient acquisition in mycorrhizal lettuce  
479 plants under different phosphorus and nitrogen concentration. *Plant Science*, 165(5),  
480 1137-1145.
- 481 Bautista-Cruz, A., Cruz-Domínguez, G., Rodríguez-Mendoza, M. d. I. N., Pérez-Pacheco, R.,  
482 & Robles, C. (2014). Effect of compost and slow-release fertilizers addition on soil  
483 biochemistry and yield of maize (*Zea mays* L.) in Oaxaca, Mexico. *Rev. FCA*  
484 *UNCUYO*, 46, 181-193.
- 485 Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of  
486 phosphorus in soils. *Soil science*, 59(1), 39-46.
- 487 Bruand, A., Hartmann, C., & Lesturgez, G. (2005). *Physical properties of tropical sandy*  
488 *soils: A large range of behaviours*. Paper presented at the Management of Tropical  
489 Sandy Soils for Sustainable Agriculture. A holistic approach for sustainable  
490 development of problem soils in the tropics, Khon Kaen, Thailand. [https://hal-](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)  
491 [insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand\\_keynote\\_paper.pdf](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)  
492 (Retrieval Date: 26 February 2019)
- 493 Brundrett, M., Bougher, N., Dell, B., & Grove, T. (1996). Examining mycorrhizal  
494 associations. In *Working with Mycorrhizas in Forestry and Agriculture* (pp. 173-193).  
495 Canberra, Australia Australian Centre for International Agricultural Research  
496 Retrieved from [http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-](http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf)  
497 [content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf](http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf)  
498 (Retrieval Date: 26 February 2019).
- 499 Calderón-Vázquez, C., Alatorre-Cobos, F., Simpson-Williamson, J., & Herrera-Estrella, L.  
500 (2009). Maize under phosphate limitation. In *Handbook of Maize: Its Biology* (pp.  
501 381-404). 233 Spring Street, New York, NY 10013, USA: Springer.
- 502 Carrenho, R., Trufem, S. F. B., Bononi, V. L. R., & Silva, E. S. (2007). The effect of different  
503 soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize.  
504 *Acta Botanica Brasilica*, 21(3), 723-730.
- 505 Cavagnaro, T. R., Bender, S. F., Asghari, H. R., & van der Heijden, M. G. (2015). The role of  
506 arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, 20(5),  
507 283-290.

- 508 Dhillon, S. S., & Ampornpan, L.-a. (1992). The influence of inorganic nutrient fertilization  
509 on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization  
510 of pretransplant rice (*Oryza sativa* L.) plants. *Biology and Fertility of Soils*, *13*(2), 85-  
511 91.
- 512 Drew, E., Murray, R., Smith, S., & Jakobsen, I. (2003). Beyond the rhizosphere: growth and  
513 function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes.  
514 *Plant and Soil*, *251*(1), 105-114.
- 515 Feng, G., Song, Y., Li, X., & Christie, P. (2003). Contribution of arbuscular mycorrhizal  
516 fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil.  
517 *Applied Soil Ecology*, *22*(2), 139-148.
- 518 George, E., Marschner, H., & Jakobsen, I. (1995). Role of arbuscular mycorrhizal fungi in  
519 uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, *15*(3-  
520 4), 257-270.
- 521 Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular  
522 arbuscular mycorrhizal infection in roots. *New Phytologist*, *84*(3), 489-500.
- 523 Grant, C., Bittman, S., Montreal, M., Plenchette, C., & Morel, C. (2005). Soil and fertilizer  
524 phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian*  
525 *Journal of Plant Science*, *85*(1), 3-14.
- 526 Gryndler, M., Larsen, J., Hřselová, H., Řezáčová, V., Gryndlerová, H., & Kubát, J. (2006).  
527 Organic and mineral fertilization, respectively, increase and decrease the development  
528 of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment.  
529 *Mycorrhiza*, *16*(3), 159-166.
- 530 Guo, W., Zhao, R., Zhao, W., Fu, R., Guo, J., Bi, N., & Zhang, J. (2013). Effects of  
531 arbuscular mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor*  
532 L. Moench) grown in rare earth elements of mine tailings. *Applied Soil Ecology*, *72*,  
533 85-92.
- 534 Hawkins, H.-J., Johansen, A., & George, E. (2000). Uptake and transport of organic and  
535 inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil*, *226*(2), 275-285.
- 536 Horwitz, W. (2000). *Official Methods of Analysis of AOAC International* (17th ed. Vol. 1).  
537 Maryland, USA: Agricultural Chemicals, Contaminants, Drugs. AOAC International.
- 538 Imam, S. M. H., & Didar, M. A. (2005). A handbook on analysis of soil, plant and water.  
539 *Bangladesh-Australia Centre for Environmental Research (BACER-DU), Dhaka*, 31-  
540 40.
- 541 Johnson, N. C., Copeland, P. J., Crookston, R. K., & Pflieger, F. (1992). Mycorrhizae:  
542 possible explanation for yield decline with continuous corn and soybean. *Agronomy*  
543 *Journal*, *84*(3), 387-390.
- 544 Koide, R., & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus*  
545 intraradices can hydrolyse organic phosphate. *New Phytologist*, *148*(3), 511-517.
- 546 Leigh, J., Hodge, A., & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer  
547 substantial amounts of nitrogen to their host plant from organic material. *New*  
548 *Phytologist*, *181*(1), 199-207.
- 549 Lisle, L., Gaudron, J., & Lefroy, R. (1990). *Laboratory Techniques for Plant and Soil*  
550 *Analysis*. Armidale, Australia: UNE-ACIAR- Crawford Fund. Department of  
551 Agronomy and Soil Science, University of New England, and Australian Centre for  
552 International Agricultural Research.
- 553 Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., & Niggli, U. (2000). Arbuscular  
554 mycorrhizae in a long-term field trial comparing low-input (organic, biological) and  
555 high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of*  
556 *Soils*, *31*(2), 150-156.

- 557 Marulanda, A., Azcon, R., & Ruiz-Lozano, J. M. (2003). Contribution of six arbuscular  
558 mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought  
559 stress. *Physiologia Plantarum*, 119(4), 526-533.
- 560 Menge, J. (1983). Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture.  
561 *Canadian Journal of Botany*, 61(3), 1015-1024.
- 562 Page, A. L., Miller, R. H., & Keeney, D. R. (1982). *Methods of Soil Analysis, Part 2.*  
563 *Chemical and microbiological properties, 2nd Edition.* Madison, Wisconsin:  
564 American Society of Agronomy.
- 565 Patle, G. T., Sikar, T. T., Rawat, K. S., & Singh, S. K. (2018). Estimation of infiltration rate  
566 from soil properties using regression model for cultivated land. *Geology, Ecology, and*  
567 *Landscapes*, 1-13.
- 568 Plenchette, C., Fortin, J., & Furlan, V. (1983). Growth responses of several plant species to  
569 mycorrhizae in a soil of moderate P-fertility. *Plant and Soil*, 70(2), 199-209.
- 570 Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis.* Great Britain, United Kingdom:  
571 Academic press.
- 572 Supardi, G. (1996). Mining the Synergistic Effects towards Strong Agriculture. *HITI News*, 4,  
573 10-13.
- 574 Tarafdar, J., & Marschner, H. (1994). Phosphatase activity in the rhizosphere and  
575 hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic  
576 phosphorus. *Soil Biology and Biochemistry*, 26(3), 387-395.
- 577 Tawaraya, K. (2003). Arbuscular mycorrhizal dependency of different plant species and  
578 cultivars. *Soil Science and Plant Nutrition*, 49(5), 655-668.
- 579 Tawaraya, K., Naito, M., & Wagatsuma, T. (2006). Solubilization of insoluble inorganic  
580 phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant*  
581 *Nutrition*, 29(4), 657-665.
- 582 Vivekanandan, M., & Fixen, P. (1991). Cropping systems effects on mycorrhizal  
583 colonization, early growth, and phosphorus uptake of corn. *Soil Science Society of*  
584 *America Journal*, 55(1), 136-140.
- 585 Wangiyana, W., Cornish, P. S., & Morris, E. (2006). Arbuscular mycorrhizal fungi dynamics  
586 in contrasting cropping systems on vertisol and regosol soils of Lombok, Indonesia.  
587 *Experimental agriculture*, 42(4), 427-439.
- 588 Zhu, Y.-G., Cavagnaro, T. R., Smith, S. E., & Dickson, S. (2001). Backseat driving?  
589 Accessing phosphate beyond the rhizosphere-depletion zone. *Trends in Plant Science*,  
590 6(5), 194-195.
- 591

## Reviewer's attachment



**Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands**

Journal:	<i>Journal of Tropical Agricultural Science</i>
Manuscript ID	JTAS-1651-2018.R1
Manuscript Type:	Regular Article
Scope of the Journal:	Crop nutrition < Crop and pasture production < AGRICULTURAL SCIENCES, Soil fertility < Crop and pasture production < AGRICULTURAL SCIENCES, Physicochemical assimilation < Plant physiology < AGRICULTURAL SCIENCES, Plant nutrition < Soil and water sciences < AGRICULTURAL SCIENCES, Soil biology < Soil and water sciences < AGRICULTURAL SCIENCES, Micropropagation techniques < Biotechnology < BIOLOGICAL SCIENCES, Microbiology < BIOLOGICAL SCIENCES
Keywords:	Seed coating, Arbuscular Mycorrhizal Fungi, AMF, maize-sorghum, cropping sequence, plant nutrition
Abstract:	<p><b>ABSTRACT</b></p> <p>An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 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). Sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P 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 day 100 after seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than the rate seen on maize root (55-75%). This study suggests that AMF inoculation maize yield and improves soil nutrient availability which is very advantageous for the growth of the subsequent crop.</p> <p>Keywords: Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition</p>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





**Running Title:**

**Mycorrhizal seed-coating on maize-sorghum cropping sequence**

For Review Only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands**

The List of Number of Tables

Table 1. <i>The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-sorghum cropping sequence</i> .....	6
Table 2. <i>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</i> .....	9
Table 3. <i>Mean concentration of total N and available P of soil in the rhizospheres of maize (1<sup>st</sup> crop) and sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100 DAS</i> .....	11
Table 4. <i>Mean N and P sorption (mg.g<sup>-1</sup> plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages</i> .....	12
Table 5. <i>Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization packages</i> .....	13
Table 6. <i>Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment of fertilization packages</i> .....	14

## Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands

### ABSTRACT

An indigenous arbuscular mycorrhizal fungal (AMF) is expected to improve performance of food crops in sandy and drylands of North Lombok (Indonesia) in dry seasons. To examine the benefits of mycorrhiza to maize yield at varying doses on plant nutrition (nitrogen and phosphorus), 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). Sorghum seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum applied. Results indicated that the AMF applications to the maize-sorghum cropping sequence increased the AMF colonization rate, the N and P nutrition at the soils, N and P 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 day 100 after seeding. When grown simultaneously, mycorrhizal colonization rate on sorghum roots (60-81%) was slightly higher than the rate seen on maize root (55-75%). This study suggests that AMF inoculation maize yield and improves soil nutrient availability which is very advantageous for the growth of the subsequent crop.

*Keywords:* Seed coating, arbuscular mycorrhizal fungi (AMF), maize-sorghum, cropping sequence, plant nutrition

## 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 per wet month or no rain during the long dry seasons, 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

1  
2  
3 matter is produced of the crops. With the high porosity and low water retention capacity on  
4 dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop  
5 dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with  
6 AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al.,  
7 2015).  
8  
9

10  
11 The establishment of AM symbiosis can be done through inoculation with AMF  
12 propagules. Our previous study (Astiko et al., 2013a) showed that the indigenous AMF  
13 inoculation in maize plants in sandy soils had positive implications for the improvement of soil  
14 properties by increasing the rates of nutrient uptake by maize crop from the soil and improving  
15 its grain yield. Our research group has also shown the benefit of this local inoculation in  
16 increasing the growth of soybean and its yield by improving P uptake from the soils, compared  
17 to those without AMF inoculation (Astiko et al., 2013b). However, as found from other studies,  
18 the development of AMF in the soil and its contribution to growth, yield and nutrient uptake of  
19 host plants could be influenced by the order of plant species cultivated in sequence in the  
20 cropping system (Johnson et al., 1992; Vivekanandan & Fixen, 1991). The studies by Johnson  
21 et al. (1992) and Vivekanandan and Fixen (1991) reported that the P uptake and the AMF  
22 colonization were higher on maize crops grown following soybean compared to when maize  
23 crops were grown following maize or barley.  
24  
25

26 From the previous study on maize-soybean cropping patterns, it was found that the AMF  
27 inoculated in maize crops grown in the first cycle increased root colonization and AMF  
28 sporulation which was very advantageous for the growth of the following crop in the cropping  
29 sequence (Astiko, 2013). Different AMF populations (colonization and spore counts) were also  
30 found between cropping seasons or between crop species in the same cropping season in Central  
31 Lombok, Indonesia (Wangiyana et al., 2006). The present study examined the effects of several  
32 combinations of fertilizer packages consisting of indigenous AMF bio-fertilizer and varying  
33 doses of organic and NPK fertilizers applied to maize crops on N and P status in soil, and uptake  
34 by maize and subsequent sorghum crops as well as the growth and yield components of the  
35 maize and sorghum crops in a maize-sorghum cropping sequence on drylands in North Lombok,  
36 Indonesia.  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 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 details of the treatments or fertilization packages are 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 <sub>0</sub>	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 <sub>1</sub>	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>2</sub>	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>3</sub>	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>4</sub>	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. After being cultivated using minimum tillage and cleared of weeds, the land was split 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, i.e. the M<sub>AA01</sub> mycorrhizal isolate, which was originally isolated from dryland area 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 charcoal powder and 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 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,

1  
2  
3 i.e. at 7 DAS (days after seeding) and at 21 DAS, with the amount applied depending on the  
4 treatments (Table 1). At 7 DAS, the maize plants were fertilized with a mixture of the entire  
5 Phonska and one third of the Urea treatment doses, followed by application of the remaining  
6 Urea treatment doses at 21 DAS. The NPK fertilizers were applied in a furrow of 5 cm alongside  
7 the maize plant row at 5-7 cm depth before covered with soil.  
8  
9

10  
11  
12 In cropping cycle 2, the plots were cleared from maize crop debris and weeds before  
13 seeds of sorghum (“Numbu” variety) were directly seeded by dibbling 2 seeds per planting  
14 holes made around the maize stubbles. These sorghum plants were not fertilized nor inoculated  
15 with AMF inoculum. For both crops, the young maize and sorghum plants were thinned at 7  
16 DAS by leaving one maize or sorghum plant per planting hole. Weeding and soil piling of the  
17 maize and sorghum plant base were done at 15 and 30 DAS. Plant protection was done by  
18 spraying “OrgaNeem” (an organic pesticide of plant origin containing Azadirachtin extracted  
19 from neem leaves) at a concentration of 5 ml OrgaNeem per liter of water. The OrgaNeem  
20 solution was applied to both crops (maize or sorghum) from 10 to 60 DAS at a 3-day interval.  
21 Harvesting of maize or sorghum crops was done at 100 DAS.  
22  
23  
24  
25  
26  
27  
28  
29

### 30 *Measurement and data analysis*

31  
32 The variables measured were AMF development, N and P nutrition, and growth and  
33 yield of maize and sorghums. The AMF development includes spore counts at 60 and 100 DAS,  
34 and root colonization levels at 60 DAS. The N and P nutrition includes concentration of total  
35 N and available P in the rhizosphere of maize and sorghum at 60 and 100 DAS. The crop  
36 variables include dry weight (shoots and roots) and yield components (grain).  
37  
38  
39  
40

41 Determination of N was done using the Kjeldhal method and P by using a spectrometer.  
42 AMF spore extraction from soil (100 g soil sample) was done using the wet sieving and  
43 decanting technique (Brundrett et al., 1996). The spores collected from the 38 µm sieve after  
44 the final centrifugation were captured in a filter paper, which were then observed on a Petri dish  
45 using a stereo microscope at 40 x magnification, and counted as spore number per 100 g of soil.  
46 The percentage of root colonization was determined using the Gridline Intersect technique  
47 (Giovannetti & Mosse, 1980) under a stereo microscope after the root pieces were stained using  
48 the clearing and staining method of Brundrett et al. (1996).  
49  
50  
51  
52  
53  
54

55 Data were analyzed using analysis of variance (ANOVA) and the Tukey’s HSD  
56 (Honestly Significant Difference) means tested at 5% level of significance.  
57  
58  
59

## 60 **RESULTS AND DISCUSSION**

### *AMF development*

In terms of root colonization rates by AMF, there were slight differences between maize and sorghum between treatments fertilizer packages. This can be seen from Table 2 which displays the levels of root colonization by the indigenous AMF which are slightly higher on sorghum roots than on maize roots in each treatment, especially in the D3 treatment where it was 77% on sorghum and 65% on maize roots. When grown simultaneously, Carrenho et al. (2007) found higher colonization rates on maize than on sorghum (Carrenho et al., 2007). 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. 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<sub>2</sub> treatment. This indicates a high buildup of AMF propagules for the 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 *G. 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).

**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 (1 <sup>st</sup> crop)			AMF sorghum (2 <sup>nd</sup> crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D <sub>0</sub>	764 <sup>c</sup>	3464 <sup>d</sup>	30 <sup>d</sup>	1231 <sup>e</sup>	3761 <sup>d</sup>	35 <sup>e</sup>
D <sub>1</sub>	1059 <sup>d</sup>	3672 <sup>c</sup>	55 <sup>c</sup>	1343 <sup>d</sup>	4942 <sup>b</sup>	60 <sup>d</sup>
D <sub>2</sub>	2119 <sup>a</sup>	4327 <sup>a</sup>	75 <sup>a</sup>	2981 <sup>a</sup>	5165 <sup>a</sup>	81 <sup>a</sup>
D <sub>3</sub>	1690 <sup>b</sup>	3894 <sup>b</sup>	65 <sup>b</sup>	1881 <sup>b</sup>	4831 <sup>c</sup>	77 <sup>b</sup>
D <sub>4</sub>	1294 <sup>c</sup>	3881 <sup>b</sup>	63 <sup>b</sup>	1769 <sup>c</sup>	4819 <sup>c</sup>	68 <sup>c</sup>
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.

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 grass-clover) (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<sub>2</sub> treatment was most favorable for AMF development in maize crops. In the D<sub>2</sub> 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<sub>0</sub>

1  
2  
3 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF.  
4 Among the treatments with AMF in combination with cattle manure, the D<sub>1</sub> treatment had the  
5 highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the  
6 AMF infection and development in maize roots compared with those in the D<sub>2</sub> treatment,  
7 resulting in a higher AMF colonization rate on D<sub>2</sub> than on D<sub>1</sub> treatment. Therefore, AMF  
8 colonization and spore numbers in maize were highest in the D<sub>2</sub> treatment, especially when  
9 compared with the D<sub>1</sub> or D<sub>0</sub> treatment. Using the same indigenous AMF applied to a pot  
10 experiment in which the soil for the growing media was taken from the same field in North  
11 Lombok, it was found that the highest level of AMF colonization of maize roots was in the  
12 treatment with AMF combined with cattle manure compared with AMF combined with NPK  
13 fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a  
14 significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure  
15 combined with mineral fertilizers compared with those fertilized with mineral fertilizers only  
16 (Gryndler et al., 2006).

### 27 ***Soil nutrient status and nutrient sorption by maize and sorghum***

28  
29 There were significant effects of the different fertilizer packages on soil nutrient status  
30 (N and P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and  
31 100 DAS (Table 3). In general, there is a tendency that the highest values of the soil nutrient  
32 status, measured either at 60 or 100 DAS, are highest under the D<sub>2</sub> (60% NPK + 12 t/ha manure  
33 + AMF) or D<sub>1</sub> (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient  
34 status in these treatments were higher than those in D<sub>0</sub> (100% NPK only) treatment, although  
35 the dose of NPK fertilizers was reduced in the D<sub>1</sub> and D<sub>2</sub> treatments (Table 3).  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

Fertilization packages	Total N (g.kg <sup>-1</sup> ) at 60 DAS		Available P (mg.kg <sup>-1</sup> ) at 60 DAS		Total N (g.kg <sup>-1</sup> ) at 100 DAS		Available P (mg.kg <sup>-1</sup> ) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D <sub>0</sub>	1.24 <sup>c</sup>	1.10 <sup>c</sup>	14.97 <sup>d</sup>	11.41 <sup>d</sup>	1.33 <sup>d</sup>	1.23 <sup>d</sup>	17.43 <sup>d</sup>	10.15 <sup>d</sup>
D <sub>1</sub>	1.45 <sup>a</sup>	1.29 <sup>a</sup>	28.44 <sup>b</sup>	16.25 <sup>b</sup>	1.69 <sup>b</sup>	1.38 <sup>b</sup>	29.51 <sup>b</sup>	21.58 <sup>b</sup>
D <sub>2</sub>	1.47 <sup>a</sup>	1.25 <sup>ab</sup>	35.02 <sup>a</sup>	28.49 <sup>a</sup>	1.86 <sup>a</sup>	1.48 <sup>a</sup>	36.56 <sup>a</sup>	29.99 <sup>a</sup>
D <sub>3</sub>	1.39 <sup>b</sup>	1.22 <sup>ab</sup>	17.52 <sup>c</sup>	14.59 <sup>bc</sup>	1.47 <sup>c</sup>	1.31 <sup>c</sup>	19.37 <sup>c</sup>	18.59 <sup>c</sup>
D <sub>4</sub>	1.33 <sup>b</sup>	1.20 <sup>b</sup>	16.29 <sup>c</sup>	14.25 <sup>c</sup>	1.42 <sup>c</sup>	1.31 <sup>c</sup>	18.53 <sup>c</sup>	17.32 <sup>c</sup>
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98
Before planting <sup>1)</sup>	1.20	-	12.28	-	-	-	-	-

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.

Since the NPK fertilizers applied, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS, are easily dissolved in water, 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 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<sub>0</sub> 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<sub>2</sub> treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in the D<sub>2</sub> 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<sub>2</sub> 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).

**Table 4. Mean N and P sorption ( $\text{mg.g}^{-1}$  plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages**

Fertilization packages	N and P uptake ( $\text{mg.g}^{-1}$ plant dry weight) by each crop at 60 DAS			
	Maize (1 <sup>st</sup> cropping cycle)		Sorghum (2 <sup>nd</sup> cropping cycle)	
	N	P	N	P
D <sub>0</sub>	18.62 <sup>e</sup>	1.71 <sup>d</sup>	12.74 <sup>d</sup>	0.65 <sup>d</sup>
D <sub>1</sub>	27.58 <sup>b</sup>	2.60 <sup>b</sup>	18.92 <sup>b</sup>	0.85 <sup>c</sup>
D <sub>2</sub>	28.00 <sup>a</sup>	2.72 <sup>a</sup>	20.86 <sup>a</sup>	1.48 <sup>a</sup>
D <sub>3</sub>	23.10 <sup>c</sup>	2.41 <sup>c</sup>	17.29 <sup>c</sup>	1.36 <sup>b</sup>
D <sub>4</sub>	20.44 <sup>d</sup>	2.41 <sup>c</sup>	17.22 <sup>c</sup>	1.32 <sup>b</sup>
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.

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 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<sub>2</sub> 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 are 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.

**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 (1 <sup>st</sup> crop) and sorghum (2 <sup>nd</sup> 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 <sub>0</sub>	8.13 <sup>d</sup>	10.43 <sup>e</sup>	34.15 <sup>d</sup>	62.29 <sup>e</sup>	0.82 <sup>d</sup>	12.30 <sup>d</sup>	8.31 <sup>d</sup>	47.74 <sup>e</sup>
D <sub>1</sub>	15.13 <sup>b</sup>	22.39 <sup>b</sup>	61.92 <sup>b</sup>	109.03 <sup>b</sup>	2.22 <sup>b</sup>	22.34 <sup>b</sup>	16.89 <sup>b</sup>	95.01 <sup>b</sup>
D <sub>2</sub>	17.12 <sup>a</sup>	34.56 <sup>a</sup>	72.86 <sup>a</sup>	111.25 <sup>a</sup>	3.37 <sup>a</sup>	24.44 <sup>a</sup>	23.53 <sup>a</sup>	105.14 <sup>a</sup>
D <sub>3</sub>	13.65 <sup>c</sup>	18.48 <sup>c</sup>	52.26 <sup>c</sup>	101.05 <sup>c</sup>	1.68 <sup>c</sup>	14.52 <sup>c</sup>	12.01 <sup>c</sup>	85.46 <sup>c</sup>
D <sub>4</sub>	13.34 <sup>c</sup>	15.43 <sup>d</sup>	51.29 <sup>c</sup>	95.87 <sup>d</sup>	1.38 <sup>c</sup>	14.47 <sup>c</sup>	11.81 <sup>c</sup>	66.06 <sup>d</sup>
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.

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 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.

**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 1 <sup>st</sup> cropping cycle		Sorghum in the 2 <sup>nd</sup> cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D <sub>0</sub>	9.60 <sup>e</sup>	22.48 <sup>d</sup>	3.57 <sup>d</sup>	2.73 <sup>d</sup>
D <sub>1</sub>	17.40 <sup>b</sup>	26.94 <sup>b</sup>	5.05 <sup>b</sup>	3.01 <sup>b</sup>
D <sub>2</sub>	22.80 <sup>a</sup>	28.12 <sup>a</sup>	6.65 <sup>a</sup>	3.61 <sup>a</sup>
D <sub>3</sub>	15.60 <sup>c</sup>	25.98 <sup>c</sup>	4.43 <sup>c</sup>	2.90 <sup>c</sup>
D <sub>4</sub>	10.20 <sup>d</sup>	24.61 <sup>c</sup>	4.17 <sup>c</sup>	2.81 <sup>cd</sup>
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.

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

1  
2  
3 where AMF colonization levels showed a significant and positive correlation with shoot dry  
4 weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any  
5 significant correlation with biomass weight or yield components of sorghum. However, AMF  
6 colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in  
7 sorghum shoots, with an  $R^2 = 71.9\%$  ( $p = 0.069$ ), and N uptake in sorghum shoots showed  
8 positive significant correlation with biomass and grain yield of sorghum, i.e. with an  $R^2 = 80.5\%$   
9 ( $p = 0.039$ ) with grain yield,  $R^2 = 83.0\%$  ( $p = 0.031$ ) with shoot dry weight at 60 DAS, and  $R^2 =$   
10  $89.9\%$  ( $p = 0.014$ ) with shoot dry weight at 100 DAS. These could mean that for maize crop,  
11 most nutrients were derived from NPK fertilizers while for sorghum, N and P nutrients were  
12 derived from the residues of manure contributed from AMF colonization in sorghum roots.  
13 Many researchers have also showed the ability of AMF to utilize organic sources to supply N  
14 to their host (e.g. Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF  
15 colonization did not necessarily result in significantly higher N status of the mycorrhizal than  
16 non-mycorrhizal hosts (Hawkins et al., 2000).

17  
18 However, when the correlation analysis was done between averages of colonization  
19 levels, biomass and grain yield between maize in the first and sorghum in the second cropping  
20 cycle, in general the results show significant and positive coefficients of correlation. For  
21 example, correlation of AMF colonization levels between roots of maize and sorghum are  
22 highly significant, with an  $r = + 0.988$  ( $R^2 = 97.6\%$ ,  $p = 0.002$ ). This means that the pattern of  
23 differences in AMF colonization levels between roots of maize and sorghum is highly similar.  
24 In other words, AMF colonization levels in roots of maize in the first cropping cycle were  
25 carried over into the second cropping cycle where sorghum was grown as the rotation crop.  
26 This is because both maize and sorghum are hosts of AMF, and both crops have high  
27 mycorrhizal dependency (Guo et al., 2013).

28  
29 In addition, when a t-test of 'Paired Two Sample for Means' was run on the averages of  
30 AMF colonization levels and spore number in soil at maturity of the crops between maize and  
31 sorghum in Table 2, higher significant values ( $p < 0.01$ ) were seen on sorghum than on maize.  
32 This could be due to the buildup of AMF in the soil after maize crop in the first cropping cycle  
33 was harvested before sorghum was directly seeded without tillage. Even though both crops were  
34 grown simultaneously, it was also found that AMF colonization levels were higher on sorghum  
35 than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal  
36 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<sub>2</sub> 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 and dry land, with no additional fertilization and mycorrhizal propagules applied.

## REFERENCES

- Alguacil, M., Lumini, E., Roldan, A., Salinas-Garcia, J., Bonfante, P., & Bianciotto, V. (2008). The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecological Applications*, *18*(2), 527-536.
- Arihara, J., & Karasawa, T. (2000). Effect of previous crops on arbuscular mycorrhizal formation and growth of succeeding maize. *Soil Science and Plant Nutrition*, *46*(1), 43-51.
- Astiko, W. (2013). *The role of indigenous mycorrhizae in different plant patterns to increase soybean yield in sandy soil (Case studies on dryland North Lombok)*. (Unpublished doctoral thesis), Universitas Brawijaya Malang, Indonesia.
- Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013a). The role of indigenous mycorrhiza in combination with cattle manure in improving maize yield (*Zea mays* L) on sandy loam of Northern Lombok, Eastern of Indonesia. *Journal of Tropical soils*, *18*(1), 53-58.
- Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013b). Soil Fertility Status and Soybean [*Glycine Max* (L) Merr] Performance Following Introduction of



- 1  
2  
3 Indigenous Mycorrhiza Combined with Various Nutrient Sources Into Sandy Soil.  
4  
5 *AGRIVITA, Journal of Agricultural Science*, 35(2), 127-137.  
6  
7  
8 Augé, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal*  
9  
10 *of Soil Science*, 84(4), 373-381.  
11  
12 Azcón, R., Ambrosano, E., & Charest, C. (2003). Nutrient acquisition in mycorrhizal lettuce  
13  
14 plants under different phosphorus and nitrogen concentration. *Plant Science*, 165(5),  
15  
16 1137-1145.  
17  
18  
19 Bautista-Cruz, A., Cruz-Domínguez, G., Rodríguez-Mendoza, M. d. I. N., Pérez-Pacheco, R.,  
20  
21 & Robles, C. (2014). Effect of compost and slow-release fertilizers addition on soil  
22  
23 biochemistry and yield of maize (*Zea mays* L.) in Oaxaca, Mexico. *Rev. FCA UNCUYO*,  
24  
25 46, 181-193.  
26  
27  
28 Bruand, A., Hartmann, C., & Lesturgez, G. (2005). *Physical properties of tropical sandy soils:*  
29  
30 *A large range of behaviours*. Retrieved February 26, 2019, from [https://hal-](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)  
31  
32 [insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand\\_keynote\\_paper.pdf](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)  
33  
34  
35 Brundrett, M., Bougher, N., Dell, B., & Grove, T. (1996). *Examining mycorrhizal associations.*  
36  
37 *in working with Mycorrhizas in forestry and agriculture*. Retrieved February 26, 2019,  
38  
39 from, [http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-](http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf)  
40  
41 [Brundrett-et-al-Mycorrhizal-Methods-Book.pdf](http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf)  
42  
43  
44 Calderón-Vázquez, C., Alatorre-Cobos, F., Simpson-Williamson, J., & Herrera-Estrella, L.  
45  
46 (2009). Maize under phosphate limitation. In *Handbook of Maize: Its Biology* (pp. 381-  
47  
48 404). 233 Spring Street, New York, NY: Springer.  
49  
50  
51 Carrenho, R., Trufem, S. F. B., Bononi, V. L. R., & Silva, E. S. (2007). The effect of different  
52  
53 soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize.  
54  
55 *Acta Botanica Brasilica*, 21(3), 723-730.  
56  
57  
58  
59  
60

- 1  
2  
3 Cavagnaro, T. R., Bender, S. F., Asghari, H. R., & van der Heijden, M. G. (2015). The role of  
4  
5 arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, 20(5),  
6  
7 283-290.  
8  
9
- 10 Dhillion, S. S., & Ampornpan, L.-a. (1992). The influence of inorganic nutrient fertilization on  
11  
12 the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of  
13  
14 pretransplant rice (*Oryza sativa* L.) plants. *Biology and Fertility of Soils*, 13(2), 85-91.  
15  
16
- 17 Drew, E., Murray, R., Smith, S., & Jakobsen, I. (2003). Beyond the rhizosphere: growth and  
18  
19 function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant*  
20  
21 *and Soil*, 251(1), 105-114.  
22  
23
- 24 Feng, G., Song, Y., Li, X., & Christie, P. (2003). Contribution of arbuscular mycorrhizal fungi  
25  
26 to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Applied*  
27  
28 *Soil Ecology*, 22(2), 139-148.  
29  
30
- 31 George, E., Marschner, H., & Jakobsen, I. (1995). Role of arbuscular mycorrhizal fungi in  
32  
33 uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, 15(3-  
34  
35 4), 257-270.  
36  
37
- 38 Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular  
39  
40 arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3), 489-500.  
41  
42
- 43 Grant, C., Bittman, S., Montreal, M., Plenchette, C., & Morel, C. (2005). Soil and fertilizer  
44  
45 phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal*  
46  
47 *of Plant Science*, 85(1), 3-14.  
48  
49
- 50 Gryndler, M., Larsen, J., Hřselová, H., Řezáčová, V., Gryndlerová, H., & Kubát, J. (2006).  
51  
52 Organic and mineral fertilization, respectively, increase and decrease the development  
53  
54 of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment.  
55  
56 *Mycorrhiza*, 16(3), 159-166.  
57  
58  
59  
60

- 1  
2  
3 Guo, W., Zhao, R., Zhao, W., Fu, R., Guo, J., Bi, N., & Zhang, J. (2013). Effects of arbuscular  
4 mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench)  
5 grown in rare earth elements of mine tailings. *Applied Soil Ecology*, 72, 85-92.  
6  
7  
8  
9  
10 Hawkins, H.-J., Johansen, A., & George, E. (2000). Uptake and transport of organic and  
11 inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil*, 226(2), 275-285.  
12  
13  
14 Johnson, N. C., Copeland, P. J., Crookston, R. K., & Pflieger, F. (1992). Mycorrhizae: possible  
15 explanation for yield decline with continuous corn and soybean. *Agronomy Journal*,  
16  
17 84(3), 387-390.  
18  
19  
20  
21 Koide, R., & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus*  
22 intraradices can hydrolyse organic phosphate. *New Phytologist*, 148(3), 511-517.  
23  
24  
25  
26 Leigh, J., Hodge, A., & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer  
27 substantial amounts of nitrogen to their host plant from organic material. *New*  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- Patle, G. T., Sikar, T. T., Rawat, K. S., & Singh, S. K. (2018). Estimation of infiltration rate  
from soil properties using regression model for cultivated land. *Geology, Ecology, and*  
*Landscapes*, 1-13.

- 1  
2  
3 Plenchette, C., Fortin, J., & Furlan, V. (1983). Growth responses of several plant species to  
4 mycorrhizae in a soil of moderate P-fertility. *Plant and Soil*, 70(2), 199-209.  
5  
6  
7 Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Great Britain, United Kingdom:  
8 Academic Press.  
9  
10  
11  
12 Supardi, G. (1996). Mining the Synergistic Effects towards Strong Agriculture. *HITI News*, 4,  
13 10-13.  
14  
15  
16 Tarafdar, J., & Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere  
17 of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology*  
18 *and Biochemistry*, 26(3), 387-395.  
19  
20  
21  
22  
23 Tawarayana, K. (2003). Arbuscular mycorrhizal dependency of different plant species and  
24 cultivars. *Soil Science and Plant Nutrition*, 49(5), 655-668.  
25  
26  
27  
28 Tawarayana, K., Naito, M., & Wagatsuma, T. (2006). Solubilization of insoluble inorganic  
29 phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant*  
30 *Nutrition*, 29(4), 657-665.  
31  
32  
33  
34  
35 Vivekanandan, M., & Fixen, P. (1991). Cropping systems effects on mycorrhizal colonization,  
36 early growth, and phosphorus uptake of corn. *Soil Science Society of America Journal*,  
37 55(1), 136-140.  
38  
39  
40  
41  
42 Wangiyana, W., Cornish, P. S., & Morris, E. (2006). Arbuscular mycorrhizal fungi dynamics  
43 in contrasting cropping systems on vertisol and regosol soils of Lombok, Indonesia.  
44 *Experimental agriculture*, 42(4), 427-439.  
45  
46  
47  
48  
49 Zhu, Y.-G., Cavagnaro, T. R., Smith, S. E., & Dickson, S. (2001). Backseat driving? Accessing  
50 phosphate beyond the rhizosphere-depletion zone. *Trends in Plant Science*, 6(5), 194-  
51 195.  
52  
53  
54  
55  
56  
57  
58  
59  
60



**Journal of Tropical Agricultural Science** <onbehalf@manuscriptcentral.com>  
kepada saya ▾

Sen, 15 Apr 2019 13:41 ☆ ↶ ⋮

🌐 Inggris ▾ > Indonesia ▾ [Terjemahkan pesan](#)

[Nonaktifkan untuk: Inggris x](#)

15-Apr-2019

Dear Assoc. Prof. Astiko:

Recently, you received a decision on Manuscript ID JTAS-1651-2018.R1, entitled "Indigenous mycorrhizal seed-coating inoculation on plant growth and yield, and NP-uptake and availability on maize-sorghum cropping sequence in Lombok's drylands." The manuscript and decision letter are located in your Author Center at <https://mc.manuscriptcentral.com/uom-itas>.

This e-mail is simply a reminder that your revision is due in two weeks. If it is not possible for you to submit your revision within two weeks, we will consider your paper as a new submission.

You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.

\*\*\* PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. \*\*\*

[https://mc.manuscriptcentral.com/uom-itas?URL\\_MASK=8c2ef767c0504fbb8dbe2f77ef68babf](https://mc.manuscriptcentral.com/uom-itas?URL_MASK=8c2ef767c0504fbb8dbe2f77ef68babf)

Sincerely,  
Journal Officer  
Journal of Tropical Agricultural Science Editorial Office  
[journal\\_officer-1@uom.my](mailto:journal_officer-1@uom.my)

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Corresponding Author

this paper is a good paper, but can be improved with additional characterisation data and details of methods etc to improve the understanding of the work and the results.

Reviewer: 2

Comments to the Corresponding Author

Since cattle manure is part of the fertilization packages, it will be good to include the nutrient content of the manure in the in the manuscript.



**PUSAT PEMAJUAN KOMPETENSI BAHASA**  
CENTRE FOR THE ADVANCEMENT OF LANGUAGE COMPETENCE (CALC)

Reference: UPM/CALC/Editing and Translation/2019

Date: 7 February 2019

To Whom It May Concern:

**SUBMISSION OF A JOURNAL ARTICLE FOR EDITING**


I hereby confirm that the following journal article had been sent for editing at the Centre for the Advancement of Language Competence (CALC), Universiti Putra Malaysia.

The details for the editing service provided are as follows:

DETAILS OF CLIENT	TITLE OF DOCUMENT(S) SUBMITTED
<p><b>NAME:</b> ASSOC. PROF. DR WAHYU ASTIKO</p> <p><b>ADDRESS:</b> STUDY PROGRAM OF AGROECOTECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITY OF MATARAM JALAN MAJAPAHIT NO. 62, MATARAM, LOMBOK, INDONESIA, 83125</p> <p><b>PHONE NO.:</b> +62 812-3788-910</p> <p><b>E-MAIL:</b> astiko@unram.ac.id</p>	<p>'INDIGENOUS MYCORRHIZAL SEED-COATING INOCULATION ON PLANT GROWTH AND YIELD, AND NP-UPTAKE AND AVAILABILITY ON MAIZE-SORGHUM CROPPING SEQUENCE IN LOMBOK'S DRYLANDS'</p>

Thank you.

**'WITH KNOWLEDGE WE SERVE'**

  
Associate Professor Dr. Arshad Abd Samad. PhD (Applied Linguistics)  
Director  
Centre for the Advancement of Language Competence (CALC)  
*Pusat Pemanjuaan Kompetensi Bahasa*  
Universiti Putra Malaysia  
43400 UPM Serdang  
Selangor Darul Ehsan

c.c.: *Coordinator, Editing and Translation Unit*  
*File - CALC*

1 Mycorrhizal Seed-coating on Maize-sorghum Cropping Sequence

2  
3 **Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-**  
4 **uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands**

5  
6 **Wahyu Astiko<sup>1\*</sup>, Wayan Wangiyana<sup>1</sup> and Lolita Endang Susilowati<sup>2</sup>**

7 <sup>1</sup>*Study Program of Agroecotechnology, Faculty of Agriculture, University of Mataram, Jalan*  
8 *Majapahit No. 62, 83125 Mataram, Lombok, Indonesia*

9 <sup>2</sup>*Department of Soil Science Faculty of Agriculture, University of Mataram, Jalan Majapahit*  
10 *No. 62, 83125 Mataram, Lombok, Indonesia*

11  
12 *E-mail addresses:*

13 astiko@unram.ac.id (Wahyu Astiko)

14 w.wangiyana@unram.ac.id (Wayan Wangiyana)

15 lolitaabas37@unram.ac.id (Lolita Endang Susilowati)

16 \* Corresponding author

17  
18 The List of Number of Tables

19 Table 1. *The mycorrhizal-based fertilization packages tested and applied to maize only in the*  
20 *maize-sorghum cropping sequence* ..... 5

21 Table 2. *Mean spore number (spores per 100 g soil) at 60 and 100 DAS, and colonization rates*  
22 *(%-colonization) on maize and sorghum in a maize-sorghum cropping sequence*..... 8

23 Table 3. *Mean concentration of total N and available P of soil in the rhizospheres of maize (1<sup>st</sup>*  
24 *crop) and sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100*  
25 *DAS*..... 10

26 Table 4. *Mean N and P sorption (mg.g<sup>-1</sup> plant dry weight) by each crop at 60 DAS for each*  
27 *treatment of fertilization packages* ..... 11

28 Table 5. *Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of*  
29 *fertilization packages* ..... 13

30 Table 6. *Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each*  
31 *treatment of fertilization packages* ..... 14

32  
33

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

36

37 **ABSTRACT**

38 By improving the nutrient uptake and transport, an indigenous arbuscular mycorrhizal fungal  
39 (AMF) is expected to improve crops' performance in sandy drylands of North Lombok  
40 (Indonesia) during dry seasons. A field experiment was designed with Randomized Complete  
41 Block Design and four replications to examine the benefits of mycorrhiza at varying doses of  
42 plant nutrition (nitrogen and phosphorus). Total of 1 kg of the AMF inoculum was applied to  
43 20 kg maize seeds in different fertilization packages of cattle manure (15, 12, 9 and 6 ton/ha)  
44 and inorganic fertilizers (80, 60, 40 and 20% NPK recommended dose). A 100% NPK  
45 recommended dose was used as the control (200 kg/ha Phonska and 300 kg/ha Urea). After  
46 harvest of maize at 100 days after seeding (DAS) and field cleaning from maize debris, sorghum  
47 seeds were then planted in cropping cycle 2 with no additional fertilization and inoculum.  
48 Results indicated that the AMF applications to the maize-sorghum cropping sequence increased  
49 the AMF colonization rate, soil N and P status and uptake, and dry biomass (root, shoot, and  
50 grain). The highest correspondence was observed in the crops which utilized a combination of  
51 60% NPK and 12 ton/ha cattle manure, and the performance was higher at 100 days after  
52 seeding. The number of AMF spores increases over the time where colonization rates were  
53 found higher in roots of sorghum (60-81%) than maize (55-75%). This study suggests that AMF  
54 inoculation increases the plant yield and improves soil nutrient availability which is very  
55 advantageous for the growth of the maize-sorghum subsequent crop in Lombok's drylands.

56

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

59

60



## 61 INTRODUCTION

62 The northern part of North Lombok regency (Indonesia) is dominated by drylands with sandy  
63 soils texture. With a very short and low number of rainy days (December to April, 100-200  
64 mm) per wet month or no rain during the long dry seasons (May to November), no food crops  
65 can be cultivated normally especially in the areas without deep wells. Moreover, inadequate  
66 phosphorus (P) availability is also one of the factors limiting the productivity of maize and other  
67 food crops in the dryland of North Lombok. Maize crop requires very high P; for example, for  
68 the production of 10 ton/ha, maize crops need 102 kg/ha of P<sub>2</sub>O<sub>5</sub> and 76% of it is transported  
69 into the seeds (Calderón-Vázquez et al., 2009). In contrast, plant roots can take up P at only  
70 about 8-13% of the amount of P fertilizer applied (Supardi, 1996). Thus, to cope with the  
71 unavailability of soil water and P and other essential nutrients in drylands of North Lombok,  
72 arbuscular mycorrhizal fungal (AMF) symbiosis has been used as it is expected to improve the  
73 performance of food crops especially during the dry seasons. Many have reported that the soil  
74 fungi, in symbiosis with their host plants, can help plants to improve water retention and make  
75 their host plants more tolerant to drought (Augé, 2004). AMF colonization also increases  
76 nutrient uptake from soils and enhances growth and yield of the host plants although it depends  
77 on the species of AMF colonizing the host plants (Marulanda et al., 2003).

78  
79 The most common findings show that the external hyphae of AMF improve P uptake and  
80 transport the nutrient to root cortex of their host (Azcón et al., 2003; Feng et al., 2003; George  
81 et al., 1995; Koide & Kabir, 2000; Tarafdar & Marschner, 1994; Tawaraya et al., 2006).  
82 Although there is no growth improvement of the host plants due to the AMF symbiosis, the  
83 amount of P uptake through AMF (mycorrhizal pathway) can be higher than through the host  
84 roots or the direct pathway (Smith & Read, 2010). AMF can also help their hosts to increase  
85 the uptake of other essential nutrients such as N, K, Ca, Mg, S, Cu, Zn, Mn, Fe and B when  
86 compared with non-mycorrhizal plants (Dhillion & Ampornpan, 1992; Hawkins et al., 2000;  
87 Leigh et al., 2009; Menge, 1983). Most of these capabilities of the AM plants are due to the  
88 extensive growth of AMF external hyphae beyond the host roots (Drew et al., 2003; Zhu et al.,  
89 2001). The length ratio of AMF hyphae to roots in soil can be 100:1 or higher and with the  
90 external hyphae, the mycorrhizal roots can explore further (George et al., 1995). By applying  
91 the Plenchette's mycorrhizal dependency formula to the shoot dry weight of maize and sorghum  
92 (Plenchette et al., 1983), the results showed a high mycorrhizal dependency, both for maize (up  
93 to 70.12%) and for sorghum (up to 79.42%) (Guo et al., 2013; Tawaraya, 2003). The findings  
94 of the study indicate that the higher the dependent rate is on AMF symbiosis, the more the dry

95 matter is produced of the crops. With the high porosity and low water retention capacity on  
96 dryland and sandy areas (Bruand et al., 2005) in most areas of North Lombok, the levels of crop  
97 dependence on symbiosis with AMF could be higher. Moreover, the symbiosis of crops with  
98 AMF could significantly reduce nutrient loss from soil through leaching (Cavagnaro et al.,  
99 2015).

100

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

114

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

126

## 127 **MATERIALS AND METHODS**

### 128 **Design of the Experiment**

129 The field experiment of maize-sorghum cropping sequence in this study was arranged  
 130 according to the Randomized Complete Block Design (RCBD) with four replications (blocks).  
 131 The study was carried out in the Akar-Akar village located in North Lombok regency,  
 132 Indonesia, from January to August 2016. Treatments involving the use of five fertilizer  
 133 packages consisting of different combinations of organic, inorganic and indigenous AMF bio-  
 134 fertilizer were applied only to the maize crop in the first cropping cycle of the maize-sorghum  
 135 cropping sequence. The 100% NPK-only recommended dose (D<sub>0</sub>) is the farmer's practice of  
 136 dose for maize by the locals. The NPK's doses were decreased and had been replaced by cattle  
 137 manure in varying fertilization packages (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>), and added with AMF as listed in  
 138 Table 1.

139

140 Table 1

141 *The mycorrhizal-based fertilization packages tested and applied to maize only in the maize-*  
 142 *sorghum cropping sequence*

Fertilization packages	Doses of the packages applied to maize plants only (in the cropping cycle 1)	Sorghum (cropping cycle 2)
D <sub>0</sub>	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 <sub>1</sub>	NPK at 80% RD + 15 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>2</sub>	NPK at 60% RD + 12 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>3</sub>	NPK at 40% RD + 9 ton/ha cattle manure + AMF	No fertilizer applied
D <sub>4</sub>	NPK at 20% RD + 6 ton/ha cattle manure + AMF	No fertilizer applied

143

144 A piece of land used in this study was of a sandy soil type (69% sand, 29% silt and 2%  
 145 clay, containing an average of 421 AMF spores per 100 g soil), typical of North Lombok area.  
 146 The land is located at a geographic position of -8.221650, 116.350283, and measured with 13.82  
 147 mg/kg of available P, 0.01% Total N, 0.57 cmol/kg of available K, 7.31 cmol/kg of Ca, and  
 148 1.21% of C-organic. After being cultivated using minimum tillage and cleared of weeds, the  
 149 land was split into 20 treatment plots of 7m x 5m based on the RCBD plotting layout. The  
 150 different fertilization packages consisted of AMF inoculum, organic fertilizer (cattle manure),  
 151 and inorganic fertilizer (NPK and Urea), were applied only to the maize crop in the first  
 152 cropping cycle.

153

154 An indigenous AMF inoculum, *Glomus mosseae* (the M<sub>AA01</sub> mycorrhizal isolate  
 155 including the hyphae and the mycorrhizal spores), which was originally isolated from dryland  
 156 area (1,500 spores per 20 g of soils) in Akar-Akar village of North Lombok, was applied  
 157 through seed-coating of maize seeds prior to seeding. Before application, 1 kg of the AMF  
 158 inoculum was mixed with 300 g of charcoal powder and 90 g of tapioca flour as the carrier

159 materials. Then this inoculum mixture was mixed with 20 kg maize seeds so that the seeds were  
160 coated by the inoculum mixture. For cropping cycle 1, the uncoated or AMF coated maize seeds  
161 (“Bisma” variety) were planted by dibbling 2 seeds per planting hole at 70 cm x 20 cm plant  
162 spacing. The entire dose of cattle manure (organic fertilizer) at different doses depending on  
163 the treatments (Table 1) was applied at the time of planting the maize seeds by burying the  
164 whole dose in the planting hole in the position below the seeds. The cattle manure variation in  
165 the fertilization package is to identify the optimum combinations to benefit the plant growth,  
166 increase the nutrient availability at the soils, and support the AMF development. The maximum  
167 combination of cattle manure was 15 ton/ha, the limit for transportation. The cattle manure  
168 applied in the package was measured with 3.08% Total Nitrogen, pH 6.66, 17.70 mg/kg of  
169 available P, 2.31 cmol/kg of available K, 10.45 C/N ration, and 32.2% C-organic. The inorganic  
170 (NPK) fertilizers with the recommended doses of 200 kg/ha Phonska (NPK 15:15:15) and 300  
171 kg/ha Urea (46% N) were applied twice, i.e. at 7 DAS (days after seeding) and at 21 DAS, with  
172 the amount applied depending on the treatments (Table 1). At 7 DAS, the maize plants were  
173 fertilized with a mixture of the entire Phonska and one third of the Urea treatment doses,  
174 followed by application of the remaining Urea treatment doses at 21 DAS. The NPK fertilizers  
175 were applied in a furrow of 5 cm alongside the maize plant row at 5-7 cm depth before covered  
176 with soil.

177

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

191

192 **Measurement and Data Analysis**

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

199

200 Laboratory analysis was conducted at Laboratory of Chemistry and Soil Science,  
201 Faculty of Agriculture, University of Mataram. Soil pH and texture were measured by standard  
202 procedures (Imam & Didar, 2005). Determination of total nitrogen in soil was done by  
203 destruction with  $(\text{NH}_4)_2\text{SO}_4$  and distillation with NaOH where the  $\text{NH}_4^+$  was determined by  
204 indophenol blue colorimetric method and the  $\text{NH}_3$  was defined by a titration with 0.05N of  
205  $\text{H}_2\text{SO}_4$  solution (Page et al., 1982). Total N in plants was measured using spectrophotometric  
206 indophenol blue methods with wave length 636 nm after destruction by  $(\text{NH}_4)_2\text{SO}_4$  and  
207 distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available  
208 Phosphorus in soil and plant was measured using spectrophotometer ( $\lambda = 693$  nm) after the  
209 extraction process using Bray and Kurt I solution (0.025 N HCl +  $\text{NH}_4\text{F}$  0.03 N) (Bray & Kurtz,  
210 1945). Total organic C was measured by oxidation with  $\text{K}_2\text{Cr}_2\text{O}_7$  in presence of sulphuric acid  
211 ( $\text{H}_2\text{SO}_4$ ) following Walkley and Black's method (Horwitz, 2000).

212

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

220

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

223

## 224 **RESULTS AND DISCUSSION**

### 225 **AMF Development**

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

237

238 Table 2

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

Fertilization packages	AMF on maize (1 <sup>st</sup> crop)			AMF sorghum (2 <sup>nd</sup> crop)		
	Spore per 100 g soil		% colonization	Spore per 100 g soil		% colonization
	60 DAS	100 DAS	60 DAS	60 DAS	100 DAS	60 DAS
D <sub>0</sub>	764 <sup>e</sup>	3464 <sup>d</sup>	30 <sup>d</sup>	1231 <sup>e</sup>	3761 <sup>d</sup>	35 <sup>e</sup>
D <sub>1</sub>	1059 <sup>d</sup>	3672 <sup>c</sup>	55 <sup>c</sup>	1343 <sup>d</sup>	4942 <sup>b</sup>	60 <sup>d</sup>
D <sub>2</sub>	2119 <sup>a</sup>	4327 <sup>a</sup>	75 <sup>a</sup>	2981 <sup>a</sup>	5165 <sup>a</sup>	81 <sup>a</sup>
D <sub>3</sub>	1690 <sup>b</sup>	3894 <sup>b</sup>	65 <sup>b</sup>	1881 <sup>b</sup>	4831 <sup>c</sup>	77 <sup>b</sup>
D <sub>4</sub>	1294 <sup>c</sup>	3881 <sup>b</sup>	63 <sup>b</sup>	1769 <sup>c</sup>	4819 <sup>c</sup>	68 <sup>c</sup>
HSD 5%	231	13	2.0	109	12	6.5

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

243

244 Specifically, the degree of root colonization by AMF may be higher in the roots of maize  
 245 fertilized with highly soluble mineral fertilizers (NPK, diammonium phosphate, ammonium  
 246 sulfate and Urea). On the other hand, the length of extra-radical mycelium and number of AMF  
 247 spores were significantly higher in maize fertilized with organic fertilizers (Bokashi manure)  
 248 (Bautista-Cruz et al., 2014). This indicates that organic fertilization is more favorable for spore  
 249 production compared with conventional fertilization. It can also be seen from Table 2 that AMF  
 250 spore numbers are higher at harvest of the maize crop (100 DAS) compared with those at 60  
 251 DAS, especially on the D<sub>2</sub> treatment. This indicates a high buildup of AMF propagules for the  
 252 subsequent sorghum crops. This condition seems to favor higher AMF colonization levels in  
 253 roots of sorghum in the second cropping cycle compared with that of maize in the first cropping

254 cycle. With no tillage treatment, the AMF colonization levels in sorghum may be higher than  
255 the levels in maize or bean roots (Alguacil et al., 2008). In addition, AMF colonization rates  
256 may be higher in roots of sorghum than in the roots of maize, either inoculated with *Glomus*  
257 *mosseae* or *Glomus versiforme*, indicating that sorghum is more favorable for AMF  
258 development than maize plants (Guo et al., 2013).

259

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

271

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

280

281         A negative impact on soil condition occur when the accumulation of soil P has increased  
282 beyond the requirement of the crops cultivated (Grant et al., 2005). In the study, it seemed that  
283 the amount of P input from the NPK fertilizer as applied in the D<sub>2</sub> treatment was most favorable  
284 for AMF development in maize crops. In the D<sub>2</sub> treatment, the NPK fertilizer was reduced to  
285 120 kg/ha Phonska (60% of the recommended NPK dose of 200 kg/ha Phonska in the D<sub>0</sub>  
286 treatment) combined with an application of 12 ton/ha cattle manure and inoculation with AMF.  
287 Among the treatments with AMF in combination with cattle manure, the D<sub>1</sub> treatment had the

288 highest NPK fertilizer dose (Table 1), and this level of NPK fertilization seems to limit the  
 289 AMF infection and development in maize roots compared with those in the D<sub>2</sub> treatment,  
 290 resulting in a higher AMF colonization rate on D<sub>2</sub> than on D<sub>1</sub> treatment. Therefore, AMF  
 291 colonization and spore numbers in maize were highest in the D<sub>2</sub> treatment, especially when  
 292 compared with the D<sub>1</sub> or D<sub>0</sub> treatment. Using the same indigenous AMF applied to a pot  
 293 experiment in which the soil for the growing media was taken from the same field in North  
 294 Lombok, it was found that the highest level of AMF colonization of maize roots was in the  
 295 treatment with AMF combined with cattle manure compared with AMF combined with NPK  
 296 fertilizer or phosphate rock (Astiko et al., 2013a). The previous study also reported a  
 297 significantly higher AMF spore number in plots fertilized with cattle manure or cattle manure  
 298 combined with mineral fertilizers compared with those fertilized with mineral fertilizers only  
 299 (Gryndler et al., 2006).

300

### 301 **Soil Nutrient Status and Nutrient Sorption by Maize and Sorghum**

302 There were significant effects of the different fertilizer packages on soil nutrient status (N and  
 303 P contents of the soil) of the rhizosphere of both maize and sorghum crops at 60 and 100 DAS  
 304 (Table 3). In general, there is a tendency that the highest values of the soil nutrient status,  
 305 measured either at 60 or 100 DAS, are highest under the D<sub>2</sub> (60% NPK + 12 t/ha manure +  
 306 AMF) or D<sub>1</sub> (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient  
 307 status in these treatments were higher than those in D<sub>0</sub> (100% NPK only) treatment, although  
 308 the dose of NPK fertilizers was reduced in the D<sub>1</sub> and D<sub>2</sub> treatments (Table 3).

309

310 Table 3

311 *Mean concentration of total N and available P of soil in the rhizospheres of maize (1<sup>st</sup> crop) and*  
 312 *sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100 DAS*

Fertilization packages	Total N (g.kg <sup>-1</sup> ) at 60 DAS		Available P (mg.kg <sup>-1</sup> ) at 60 DAS		Total N (g.kg <sup>-1</sup> ) at 100 DAS		Available P (mg.kg <sup>-1</sup> ) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D <sub>0</sub>	1.24 <sup>c</sup>	1.10 <sup>c</sup>	14.97 <sup>d</sup>	11.41 <sup>d</sup>	1.33 <sup>d</sup>	1.23 <sup>d</sup>	17.43 <sup>d</sup>	10.15 <sup>d</sup>
D <sub>1</sub>	1.45 <sup>a</sup>	1.29 <sup>a</sup>	28.44 <sup>b</sup>	16.25 <sup>b</sup>	1.69 <sup>b</sup>	1.38 <sup>b</sup>	29.51 <sup>b</sup>	21.58 <sup>b</sup>
D <sub>2</sub>	1.47 <sup>a</sup>	1.25 <sup>ab</sup>	35.02 <sup>a</sup>	28.49 <sup>a</sup>	1.86 <sup>a</sup>	1.48 <sup>a</sup>	36.56 <sup>a</sup>	29.99 <sup>a</sup>
D <sub>3</sub>	1.39 <sup>b</sup>	1.22 <sup>ab</sup>	17.52 <sup>c</sup>	14.59 <sup>bc</sup>	1.47 <sup>c</sup>	1.31 <sup>c</sup>	19.37 <sup>c</sup>	18.59 <sup>c</sup>
D <sub>4</sub>	1.33 <sup>b</sup>	1.20 <sup>b</sup>	16.29 <sup>c</sup>	14.25 <sup>c</sup>	1.42 <sup>c</sup>	1.31 <sup>c</sup>	18.53 <sup>c</sup>	17.32 <sup>c</sup>
HSD 5%	0.08	0.07	1.31	1.99	0.08	0.06	1.0	2.98

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

315



316 At the present study, no infiltration data was measured, however it is important to note  
 317 that since the NPK fertilizers applications, i.e. Phonska and Urea at 7 DAS and Urea at 21 DAS,  
 318 at sandy lands, are easily dissolved in water, and significant amount of the nutrients could have  
 319 been loss through infiltration during the rainy season. Previous study shows that the sand  
 320 content of the cultivated land had a positive correlation with infiltration rate, with an r-value of  
 321 +0.75 (Patle et al., 2018). Since cattle manure is slow in releasing nutrients, it is possible that  
 322 at the time of measurement (60 and 100 DAS), the loss of released nutrients from the NPK  
 323 fertilizers through leaching was much higher in the D<sub>0</sub> than in the other treatments of  
 324 fertilization packages due to dissolution by rain water during the rainy season. However, there  
 325 were small increases in nutrient status of the soil from 60 to 100 DAS around the rhizospheres  
 326 of maize crop, especially in those treated with manure and AMF application. This indicated a  
 327 slow release of N and P nutrients from manure after application to the maize plants in the first  
 328 cropping cycle, which could be due to AMF inoculation. Many studies have reported that AMF  
 329 can mobilize and take N and P from organic matters for their hosts (Feng et al., 2003; Hawkins  
 330 et al., 2000; Koide & Kabir, 2000; Leigh et al., 2009; Tarafdar & Marschner, 1994), and it can  
 331 be seen from Table 2 that the highest average of AMF colonization levels in maize roots was  
 332 in the D<sub>2</sub> treatment. Therefore, N and P uptake in shoots of maize and sorghum was highest in  
 333 the D<sub>2</sub> treatment (Table 4).

334

335 In addition, soil contents of those nutrients corresponded well with the levels of N and  
 336 P uptake of the crops, i.e. all showing positive correlation coefficients. Although it is not  
 337 significant for P uptake, the highest values of N and P uptake were also seen in the D<sub>2</sub> treatment  
 338 for both maize and sorghum (Table 4). The correlation analysis of the mean values obtained at  
 339 60 DAS showed a significant correlation between soil N and N sorption in the shoots, with a  
 340 value of  $r = + 0.970$  (R-square = 94.1%,  $p = 0.006$ ) for maize plants, and  $r = + 0.904$  (R-square  
 341 = 81.7%,  $p = 0.035$ ) for sorghum plants. These indicate those fertilizers in the packages  
 342 contribute to nutrient contents of the crops, which are significantly different between treatments  
 343 of fertilization packages (Table 4).

344

345 Table 4

346 *Mean N and P sorption (mg.g<sup>-1</sup> plant dry weight) by each crop at 60 DAS for each treatment of*  
 347 *fertilization packages*

Fertilization packages	N and P uptake (mg.g <sup>-1</sup> plant dry weight) by each crop at 60 DAS			
	Maize (1 <sup>st</sup> cropping cycle)		Sorghum (2 <sup>nd</sup> cropping cycle)	
	N	P	N	P
D <sub>0</sub>	18.62 <sup>e</sup>	1.71 <sup>d</sup>	12.74 <sup>d</sup>	0.65 <sup>d</sup>

D <sub>1</sub>	27.58 <sup>b</sup>	2.60 <sup>b</sup>	18.92 <sup>b</sup>	0.85 <sup>c</sup>
D <sub>2</sub>	28.00 <sup>a</sup>	2.72 <sup>a</sup>	20.86 <sup>a</sup>	1.48 <sup>a</sup>
D <sub>3</sub>	23.10 <sup>c</sup>	2.41 <sup>c</sup>	17.29 <sup>c</sup>	1.36 <sup>b</sup>
D <sub>4</sub>	20.44 <sup>d</sup>	2.41 <sup>c</sup>	17.22 <sup>c</sup>	1.32 <sup>b</sup>
HSD 5%	0.41	0.11	0.07	0.04

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

350

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

361

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

377

378 **Biomass and Yield Components of Maize and Sorghum**

379 In terms of biomass production and yield components of maize and sorghum, there were also  
 380 significant effects of the fertilization packages in which D<sub>2</sub> treatment presents the highest values  
 381 of biomass production (Table 5) and yield components (Table 6). This means that the nutrient  
 382 status of the soils corresponded well with the biomass weight of the crops, indicated by the  
 383 positive correlation coefficients, although only some of them are significant. The AMF  
 384 applications to maize crops, observed at 60 DAS, showed a significant correlation to dry shoot  
 385 weight, N and P status of the soil, and the N and P sorption. In contrast, AMF colonization  
 386 levels of maize roots only showed significant correlation with root dry weight at 60 DAS, with  
 387 an  $r = + 0.912$  ( $R^2 = 83.2\%$ ,  $p = 0.031$ ) and shoot dry weight at maturity (100 DAS), with an  $r$   
 388  $= + 0.892$  ( $R^2 = 79.6\%$ ,  $p = 0.042$ ). This could mean that during the vegetative growth, AMF  
 389 colonization have focused on improving root growth to increase nutrient sorption during the  
 390 vegetative growth of maize crops.

391  
 392 Table 5  
 393 *Mean dry biomass weight (g/plant) of maize and sorghum for each treatment of fertilization*  
 394 *packages*

Fertilization packages	Dry biomass weights (g/plant) of maize (1 <sup>st</sup> crop) and sorghum (2 <sup>nd</sup> 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 <sub>0</sub>	8.13 <sup>d</sup>	10.43 <sup>e</sup>	34.15 <sup>d</sup>	62.29 <sup>e</sup>	0.82 <sup>d</sup>	12.30 <sup>d</sup>	8.31 <sup>d</sup>	47.74 <sup>e</sup>
D <sub>1</sub>	15.13 <sup>b</sup>	22.39 <sup>b</sup>	61.92 <sup>b</sup>	109.03 <sup>b</sup>	2.22 <sup>b</sup>	22.34 <sup>b</sup>	16.89 <sup>b</sup>	95.01 <sup>b</sup>
D <sub>2</sub>	17.12 <sup>a</sup>	34.56 <sup>a</sup>	72.86 <sup>a</sup>	111.25 <sup>a</sup>	3.37 <sup>a</sup>	24.44 <sup>a</sup>	23.53 <sup>a</sup>	105.14 <sup>a</sup>
D <sub>3</sub>	13.65 <sup>c</sup>	18.48 <sup>c</sup>	52.26 <sup>c</sup>	101.05 <sup>c</sup>	1.68 <sup>c</sup>	14.52 <sup>c</sup>	12.01 <sup>c</sup>	85.46 <sup>c</sup>
D <sub>4</sub>	13.34 <sup>c</sup>	15.43 <sup>d</sup>	51.29 <sup>c</sup>	95.87 <sup>d</sup>	1.38 <sup>c</sup>	14.47 <sup>c</sup>	11.81 <sup>c</sup>	66.06 <sup>d</sup>
HSD 5%	0.31	3.04	0.97	2.21	0.30	0.05	0.20	9.54

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

397  
 398 In relation to nutrient uptake, although AMF colonization levels did not show significant  
 399 correlation with N uptake in shoots of maize or sorghum, they did show a positive significant  
 400 correlation with P uptake both in shoots of maize and sorghum, with an  $r = + 0.909$  ( $R^2 = 82.6\%$ ,  
 401  $p = 0.032$ ) for maize, and  $r = + 0.940$  ( $R^2 = 88.4\%$ ,  $p = 0.017$ ) for sorghum. These could be due  
 402 to the ability of AMF extra-radical hyphae in mobilizing and absorbing P from organic matters  
 403 for their hosts (Feng et al., 2003; Koide & Kabir, 2000; Tarafdar & Marschner, 1994). Based  
 404 on the strength of the relationships between AMF colonization levels and P uptake, the value  
 405 of  $R^2$  is higher in sorghum ( $R^2 = 88.4\%$ ) than in maize ( $R^2 = 82.6\%$ ). This means that the  
 406 contribution of AMF colonization in roots to P uptake in shoots was higher in sorghum crop at  
 407 the second cropping cycle than in maize in the first cropping cycle. Since sorghum did not

408 receive any fertilizers, and cattle manure is a slow-release organic fertilizer, it could mean that  
 409 most P nutrient contained in the shoots, especially in shoots of sorghum in the second cropping  
 410 cycle, was mostly taken up from the manure under the contribution of AMF colonization in the  
 411 roots. However, this still needs to be confirmed by further research. This view is supported by  
 412 the conditions of the study area, which was dominated by sand, and if leaching happened during  
 413 the rainy season, the loss of dissolved nutrients from the NPK fertilizers applied in the first  
 414 cropping cycle could be high. Therefore, the main source of nutrients, especially for sorghum  
 415 crop in the second cropping cycle was the cattle manure applied to the maize crop in the first  
 416 cropping cycle.

417

418 Table 6

419 *Mean weights of total dry grains (kg/plot) and 100 dry grains for each crop and each treatment*  
 420 *of fertilization packages*

Fertilization packages	Mean dry grain yield (kg/plot) and weight of 100 dry grains (g)			
	Maize in the 1 <sup>st</sup> cropping cycle		Sorghum in the 2 <sup>nd</sup> cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D <sub>0</sub>	9.60 <sup>c</sup>	22.48 <sup>d</sup>	3.57 <sup>d</sup>	2.73 <sup>d</sup>
D <sub>1</sub>	17.40 <sup>b</sup>	26.94 <sup>b</sup>	5.05 <sup>b</sup>	3.01 <sup>b</sup>
D <sub>2</sub>	22.80 <sup>a</sup>	28.12 <sup>a</sup>	6.65 <sup>a</sup>	3.61 <sup>a</sup>
D <sub>3</sub>	15.60 <sup>c</sup>	25.98 <sup>c</sup>	4.43 <sup>c</sup>	2.90 <sup>c</sup>
D <sub>4</sub>	10.20 <sup>d</sup>	24.61 <sup>c</sup>	4.17 <sup>c</sup>	2.81 <sup>cd</sup>
HSD 5%	0.59	1.37	0.26	0.09

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

423

424 In relation to nitrogen nutrient, there was a very low and non-significant correlation  
 425 between AMF colonization levels and N uptake in maize shoots with an R<sup>2</sup> of only 41.2%.  
 426 However, the N uptake of maize at 60 DAS was highly significantly correlated with the N status  
 427 of the soil with an R<sup>2</sup> = 94.1% ( $p = 0.006$ ). Moreover, the N soil availability also showed a  
 428 significant correlation with biomass and grain yield of maize, i.e. with an R<sup>2</sup> = 84.1% ( $p =$   
 429 0.029) with grain yield at 60 DAS, R<sup>2</sup> = 92.2% ( $p = 0.009$ ) with shoot dry weight at 60 DAS,  
 430 and R<sup>2</sup> = 90.6% ( $p = 0.012$ ) with shoot dry weight at 100 DAS.

431

432 In contrast, for sorghum in the second cropping cycle, the soil N status at 60 DAS  
 433 showed a significant correlation only with shoot dry weight at 100 DAS. Unlike in maize crop  
 434 where AMF colonization levels showed a significant and positive correlation with shoot dry  
 435 weight at maturity (100 DAS), AMF colonization levels in sorghum roots did not show any

436 significant correlation with biomass weight or yield components of sorghum. However, AMF  
437 colonization rates in sorghum roots showed a sufficiently high correlation with N uptake in  
438 sorghum shoots, with an  $R^2 = 71.9\%$  ( $p = 0.069$ ), and N uptake in sorghum shoots showed  
439 positive significant correlation with biomass and grain yield of sorghum, i.e. with an  $R^2 = 80.5\%$   
440 ( $p = 0.039$ ) with grain yield,  $R^2 = 83.0\%$  ( $p = 0.031$ ) with shoot dry weight at 60 DAS and  $R^2$   
441  $= 89.9\%$  ( $p = 0.014$ ) with shoot dry weight at 100 DAS. No additional fertilizers and inoculums  
442 were applied at the cropping cycle 2. These could mean that for maize crop, most nutrients were  
443 derived from NPK fertilizers while for sorghum, N and P nutrients were derived from the  
444 residues of manure contributed from AMF colonization in sorghum roots. Many researchers  
445 have also showed the ability of AMF to utilize organic sources to supply N to their host (e.g.  
446 Hawkins et al., 2000; Leigh et al., 2009) although it was also found that AMF colonization did  
447 not necessarily result in significantly higher N status of the mycorrhizal than non-mycorrhizal  
448 hosts (Hawkins et al., 2000).

449  
450 However, when the correlation analysis was done between averages of colonization  
451 levels, biomass and grain yield between maize in the first and sorghum in the second cropping  
452 cycle, in general the results show significant and positive coefficients of correlation. For  
453 example, correlation of AMF colonization levels between roots of maize and sorghum are  
454 highly significant, with an  $r = + 0.988$  ( $R^2 = 97.6\%$ ,  $p = 0.002$ ). This means that the pattern of  
455 differences in AMF colonization levels between roots of maize and sorghum is highly similar.  
456 In other words, AMF colonization levels in roots of maize in the first cropping cycle were  
457 carried over into the second cropping cycle where sorghum was grown as the rotation crop.  
458 This is because both maize and sorghum are hosts of AMF, and both crops have high  
459 mycorrhizal dependency (Guo et al., 2013).

460  
461 In addition, when a t-test of 'Paired Two Sample for Means' was run on the averages of  
462 AMF colonization levels and spore number in soil at maturity of the crops between maize and  
463 sorghum in Table 2, higher significant values ( $p < 0.01$ ) were seen on sorghum than on maize.  
464 This could be due to the buildup of AMF in the soil after maize crop in the first cropping cycle  
465 was harvested before sorghum was directly seeded without tillage. Even though both crops were  
466 grown simultaneously, it was also found that AMF colonization levels were higher on sorghum  
467 than on maize (Guo et al., 2013). This means that sorghum can be used to improve mycorrhizal  
468 conditions of the soil under a maize-sorghum cropping sequence.

469

**470 CONCLUSION**

471 Among the mycorrhiza-based fertilization packages tested on the maize-sorghum cropping  
472 sequence system at the drylands in North Lombok of Indonesia, the D<sub>2</sub> package, consisting of  
473 60% NPK recommended dose, 12 t/ha cattle manure, and the indigenous AMF inoculation, was  
474 found to be the best fertilization package to improve crop yield and soil nutrient availability.  
475 This study noted that the AMF development was higher in the sorghum at the second cropping  
476 cycle compared to the growth in the maize at the first cropping cycle. This condition led to the  
477 higher NP-uptake and soil nutrient availability in subsequent crops, both at the sandy dryland,  
478 with no additional fertilization and mycorrhizal propagules applied.

479

**480 ACKNOWLEDGEMENT**

481

482 The authors would like to thank the Directorate of Research and Community Service, the  
483 Directorate General for Research and Development at the Ministry of Research, Technology  
484 and Higher Education (DRPM RISBANG KEMRISTEKDIKTI), and to the University of  
485 Mataram, for the research grants with Number: 030/SP2H/LT/DRPM/II/2016.

486

487

488

489

490

491

492

493

494

495

496

497

498

499

500

501

502

503

504

505

506

507

508

509

510

511

512

513

514  
515  
516  
517

518 **REFERENCES**

519 Alguacil, M., Lumini, E., Roldan, A., Salinas-Garcia, J., Bonfante, P., & Bianciotto, V. (2008).

520       The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical  
521       crops. *Ecological Applications*, 18(2), 527-536.

522 Arihara, J., & Karasawa, T. (2000). Effect of previous crops on arbuscular mycorrhizal  
523       formation and growth of succeeding maize. *Soil Science and Plant Nutrition*, 46(1), 43-  
524       51.

525 Astiko, W. (2013). *The role of indigenous mycorrhizae in different plant patterns to increase*  
526       *soybean yield in sandy soil (Case studies on dryland North Lombok)* (Unpublished  
527       Doctoral thesis), University of Brawijaya, Indonesia.

528 Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013a). The role of  
529       indigenous mycorrhiza in combination with cattle manure in improving maize yield  
530       (*Zea mays* L.) on sandy loam of Northern Lombok, Eastern of Indonesia. *Journal of*  
531       *Tropical Soils*, 18(1), 53-58.

532 Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013b). Soil fertility status  
533       and soybean [*Glycine max* (L.) Merr.] performance following introduction of  
534       indigenous mycorrhiza combined with various nutrient sources into sandy soil.  
535       *AGRIVITA, Journal of Agricultural Science*, 35(2), 127-137.

536 Augé, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal*  
537       *of Soil Science*, 84(4), 373-381.

538 Azcón, R., Ambrosano, E., & Charest, C. (2003). Nutrient acquisition in mycorrhizal lettuce  
539       plants under different phosphorus and nitrogen concentration. *Plant Science*, 165(5),  
540       1137-1145.

- 541 Bautista-Cruz, A., Cruz-Domínguez, G., Rodríguez-Mendoza, M. d. I. N., Pérez-Pacheco, R.,  
542 & Robles, C. (2014). Effect of compost and slow-release fertilizers addition on soil  
543 biochemistry and yield of maize (*Zea mays* L.) in Oaxaca, Mexico. *Revista de la*  
544 *Facultad de Ciencias Agrarias*, 46(1), 181-193.
- 545 Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of  
546 phosphorus in soils. *Soil Science*, 59(1), 39-46.
- 547 Bruand, A., Hartmann, C., & Lesturgez, G. (2005). *Physical properties of tropical sandy soils:*  
548 *A large range of behaviours*. Retrieved February 26, 2019, from [https://hal-](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)  
549 [insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand\\_keynote\\_paper.pdf](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)
- 550 Brundrett, M., Bougher, N., Dell, B., & Grove, T. (1996). *Examining mycorrhizal associations*.  
551 Retrieved February 26, 2019, from [http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-](http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf)  
552 [content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf](http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf)
- 553 Calderón-Vázquez, C., Alatorre-Cobos, F., Simpson-Williamson, J., & Herrera-Estrella, L.  
554 (2009). Maize under phosphate limitation. In *Handbook of maize: Its biology* (pp. 381-  
555 404). New York, NY: Springer.
- 556 Carrenho, R., Trufem, S. F. B., Bononi, V. L. R., & Silva, E. S. (2007). The effect of different  
557 soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize.  
558 *Acta Botanica Brasilica*, 21(3), 723-730.
- 559 Cavagnaro, T. R., Bender, S. F., Asghari, H. R., & van der Heijden, M. G. (2015). The role of  
560 arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, 20(5),  
561 283-290.
- 562 Dhillon, S. S., & Ampornpan, L. A. (1992). The influence of inorganic nutrient fertilization on  
563 the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of  
564 pretransplant rice (*Oryza sativa* L.) plants. *Biology and Fertility of Soils*, 13(2), 85-91.



- 565 Drew, E., Murray, R., Smith, S., & Jakobsen, I. (2003). Beyond the rhizosphere: Growth and  
566 function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant*  
567 *and Soil*, 251(1), 105-114.
- 568 Feng, G., Song, Y., Li, X., & Christie, P. (2003). Contribution of arbuscular mycorrhizal fungi  
569 to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Applied*  
570 *Soil Ecology*, 22(2), 139-148.
- 571 George, E., Marschner, H., & Jakobsen, I. (1995). Role of arbuscular mycorrhizal fungi in  
572 uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, 15(3-  
573 4), 257-270.
- 574 Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular  
575 arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3), 489-500.
- 576 Grant, C., Bittman, S., Montreal, M., Plenchette, C., & Morel, C. (2005). Soil and fertilizer  
577 phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal*  
578 *of Plant Science*, 85(1), 3-14.
- 579 Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H., & Kubát, J. (2006).  
580 Organic and mineral fertilization, respectively, increase and decrease the development  
581 of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment.  
582 *Mycorrhiza*, 16(3), 159-166.
- 583 Guo, W., Zhao, R., Zhao, W., Fu, R., Guo, J., Bi, N., & Zhang, J. (2013). Effects of arbuscular  
584 mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench)  
585 grown in rare earth elements of mine tailings. *Applied Soil Ecology*, 72, 85-92.
- 586 Hawkins, H. J., Johansen, A., & George, E. (2000). Uptake and transport of organic and  
587 inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil*, 226(2), 275-285.
- 588 Horwitz, W. (2000). *Official methods of analysis of AOAC International* (17th ed., Vol. 1).  
589 Gaithersburg, USA: AOAC International.

- 590 Imam, S. M. H., & Didar, M. A. (Eds.) (2005). *A handbook on analysis of soil, plant and water*.  
591 Dhaka, Bangladesh: Bangladesh-Australia Centre for Environmental Research  
592 (BACER-DU).
- 593 Johnson, N. C., Copeland, P. J., Crookston, R. K., & Pflieger, F. (1992). Mycorrhizae: Possible  
594 explanation for yield decline with continuous corn and soybean. *Agronomy Journal*,  
595 84(3), 387-390.
- 596 Koide, R., & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus*  
597 intraradices can hydrolyse organic phosphate. *New Phytologist*, 148(3), 511-517.
- 598 Leigh, J., Hodge, A., & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer  
599 substantial amounts of nitrogen to their host plant from organic material. *New*  
600 *Phytologist*, 181(1), 199-207.
- 601 Lisle, L., Gaudron, J., & Lefroy, R. (1990). *Laboratory techniques for plant and soil analysis*.  
602 Armidale, Australia: UNE-ACIAR- Crawford Fund.
- 603 Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., & Niggli, U. (2000). Arbuscular  
604 mycorrhizae in a long-term field trial comparing low-input (organic, biological) and  
605 high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of*  
606 *Soils*, 31(2), 150-156.
- 607 Marulanda, A., Azcon, R., & Ruiz-Lozano, J. M. (2003). Contribution of six arbuscular  
608 mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought  
609 stress. *Physiologia Plantarum*, 119(4), 526-533.
- 610 Menge, J. (1983). Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture.  
611 *Canadian Journal of Botany*, 61(3), 1015-1024.
- 612 Page, A. L., Miller, R. H., & Keeney, D. R. (1982). *Methods of soil analysis, Part 2: Chemical*  
613 *and microbiological properties* (2nd Ed.). Madison, USA: American Society of  
614 Agronomy.

- 615 Patle, G. T., Sikar, T. T., Rawat, K. S., & Singh, S. K. (2018). Estimation of infiltration rate  
616 from soil properties using regression model for cultivated land. *Geology, Ecology, and*  
617 *Landscapes*, 3(1), 1-13.
- 618 Plenchette, C., Fortin, J., & Furlan, V. (1983). Growth responses of several plant species to  
619 mycorrhizae in a soil of moderate P-fertility. *Plant and Soil*, 70(2), 199-209.
- 620 Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. New York, NY: Academic Press.
- 621 Supardi, G. (1996). Mining the synergistic effects towards strong agriculture. *HITI News*, 4,  
622 10-13.
- 623 Tarafdar, J., & Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere  
624 of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology*  
625 *and Biochemistry*, 26(3), 387-395.
- 626 Tawarayama, K. (2003). Arbuscular mycorrhizal dependency of different plant species and  
627 cultivars. *Soil Science and Plant Nutrition*, 49(5), 655-668.
- 628 Tawarayama, K., Naito, M., & Wagatsuma, T. (2006). Solubilization of insoluble inorganic  
629 phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant*  
630 *Nutrition*, 29(4), 657-665.
- 631 Vivekanandan, M., & Fixen, P. (1991). Cropping systems effects on mycorrhizal colonization,  
632 early growth, and phosphorus uptake of corn. *Soil Science Society of America Journal*,  
633 55(1), 136-140.
- 634 Wangiyana, W., Cornish, P. S., & Morris, E. (2006). Arbuscular mycorrhizal fungi dynamics  
635 in contrasting cropping systems on vertisol and regosol soils of Lombok, Indonesia.  
636 *Experimental Agriculture*, 42(4), 427-439.
- 637 Zhu, Y. G., Cavagnaro, T. R., Smith, S. E., & Dickson, S. (2001). Backseat driving? Accessing  
638 phosphate beyond the rhizosphere-depletion zone. *Trends in Plant Science*, 6(5), 194-  
639 195.



# ACCEPTANCE OF MANUSCRIPT ID. JTAS-1651-2018 FOR PUBLICATION

Kotak Masuk x



**JOURNAL OFFICER TEE / UPM** <journal.officer-1@upm.edu.my>  
kepada saya, MSchons., lolitaabas37, PERTANIKA ▾

Sen, 15 Jul 2019 14:56 ☆ ↶ ⋮

🗨 Inggris ▾ > Indonesia ▾ [Terjemahkan pesan](#)

[Nonaktifkan untuk: Inggris](#) x

Dear Author(s),

I am writing to you in reference to an article entitled, "**Indigenous Mycorrhizal Seed-coating Inoculation on Plant Growth and Yield, and NP-uptake and Availability on Maize-sorghum Cropping Sequence in Lombok's Drylands**" with author(s): **Wahyu Astiko, Wayan Wangiyana and Lolita Endang Susilowati**, submitted to *Pertanika* on 26 February 2019 for intended publication in JTAS.

Your paper has been anonymously peer-reviewed by two to three referees competent in the specialized areas appropriate to your manuscript independently evaluating the scientific quality of the manuscript.

I am pleased to tell you that based on the clarity, technical approach and scientific validity presented, your paper **has been accepted** by the Editorial Board on 23 May 2019, and is **tentatively** scheduled for publication in **JTAS Vol. 42(3) Aug. 2019**.

Henceforth, your manuscript will be undergoing the publication process. You shall receive the proof of your manuscript from our Pre-Press section, in due course of time. Please review the proof carefully for accuracy and consistency before returning it to the Pre-Press officer.

I thank you for considering *Pertanika* as your preferred Journal.

Best regards,

—

*(Ms. Slyn Ying, Tee on behalf of CEE)*

Please cc your email to [executive\\_editor,pertanika@upm.edu.my](mailto:executive_editor,pertanika@upm.edu.my); [mzamri@upm.edu.my](mailto:mzamri@upm.edu.my)

---

Chief Executive Editor (UPM Journals)

JOURNAL DIVISION | PERTANIKA

Office of the Deputy Vice Chancellor (R&I)

Universiti Putra Malaysia

Email 1: [jurnal.officer-1@upm.my](mailto:jurnal.officer-1@upm.my) (Pertanika JTAS)

Email 2: [executive\\_editor,pertanika@upm.edu.my](mailto:executive_editor,pertanika@upm.edu.my) (Chief Executive Editor)

Email 3: [mzamri@upm.edu.my](mailto:mzamri@upm.edu.my) (Editor-in-Chief - JTAS)

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

**Wahyu Astiko<sup>1\*</sup>, Wayan Wangiyana<sup>1</sup> and Lolita Endang Susilowati<sup>2</sup>**

*<sup>1</sup>Study Program of Agroecotechnology, Faculty of Agriculture, University of Mataram, Jalan Majapahit No. 62, 83125 Mataram, Lombok, Indonesia*

*<sup>2</sup>Department of Soil Science Faculty of Agriculture, University of Mataram, Jalan Majapahit No. 62, 83125 Mataram, Lombok, Indonesia*

### **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

### **ARTICLE INFO**

#### *Article history:*

Received: 26 February 2019

Accepted: 23 May 2019

Published: 19 August 2019

#### *E-mail addresses:*

[astiko@unram.ac.id](mailto:astiko@unram.ac.id) (Wahyu Astiko)

[w.wangiyana@unram.ac.id](mailto:w.wangiyana@unram.ac.id) (Wayan Wangiyana)

[lolitaabas37@unram.ac.id](mailto:lolitaabas37@unram.ac.id) (Lolita Endang Susilowati)

\* Corresponding author

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<sub>2</sub>O<sub>5</sub> 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 (Dhillon & 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  $(\text{NH}_4)_2\text{SO}_4$  and distillation with NaOH where the  $\text{NH}_4^+$  was determined by indophenol blue colorimetric method and the  $\text{NH}_3$  was defined by a titration with 0.05N of  $\text{H}_2\text{SO}_4$  solution (Page et al., 1982). Total N in plants

was measured using spectrophotometric indophenol blue methods with wave length 636 nm after destruction by  $(\text{NH}_4)_2\text{SO}_4$  and distillation with NaOH following the Conway instruction (Lisle et al., 1990). The available Phosphorus in soil and plant was measured using spectrophotometer ( $\lambda = 693 \text{ nm}$ ) after the extraction process using Bray and Kurt I solution (0.025 N HCl +  $\text{NH}_4\text{F}$  0.03 N) (Bray & Kurtz, 1945). Total organic C was measured by oxidation with  $\text{K}_2\text{Cr}_2\text{O}_7$  in presence of sulphuric acid ( $\text{H}_2\text{SO}_4$ ) 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  $\mu\text{m}$  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  $\text{D}_2$  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 (1 <sup>st</sup> crop)			AMF sorghum (2 <sup>nd</sup> 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 <sub>0</sub>	764 <sup>c</sup>	3464 <sup>d</sup>	30 <sup>d</sup>	1231 <sup>c</sup>	3761 <sup>d</sup>	35 <sup>c</sup>
D <sub>1</sub>	1059 <sup>d</sup>	3672 <sup>c</sup>	55 <sup>c</sup>	1343 <sup>d</sup>	4942 <sup>b</sup>	60 <sup>d</sup>
D <sub>2</sub>	2119 <sup>a</sup>	4327 <sup>a</sup>	75 <sup>a</sup>	2981 <sup>a</sup>	5165 <sup>a</sup>	81 <sup>a</sup>
D <sub>3</sub>	1690 <sup>b</sup>	3894 <sup>b</sup>	65 <sup>b</sup>	1881 <sup>b</sup>	4831 <sup>c</sup>	77 <sup>b</sup>
D <sub>4</sub>	1294 <sup>c</sup>	3881 <sup>b</sup>	63 <sup>b</sup>	1769 <sup>c</sup>	4819 <sup>c</sup>	68 <sup>c</sup>
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 grass-

clover) (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<sub>2</sub> treatment was most favorable for AMF development in maize crops. In the D<sub>2</sub> 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<sub>0</sub> 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<sub>1</sub> 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<sub>2</sub> treatment, resulting in a higher AMF colonization rate on D<sub>2</sub> than on D<sub>1</sub> treatment. Therefore, AMF colonization and spore numbers in maize were highest in the D<sub>2</sub> treatment, especially when compared with the D<sub>1</sub> or D<sub>0</sub> 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<sub>2</sub> (60% NPK + 12 t/ha manure + AMF) or D<sub>1</sub> (80% NPK + 15 t/ha manure + AMF) treatment. The values of the soil nutrient status in these treatments were higher than those in D<sub>0</sub> (100% NPK only) treatment, although the dose of NPK fertilizers was reduced in the D<sub>1</sub> and D<sub>2</sub> 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 (1<sup>st</sup> crop) and sorghum (2<sup>nd</sup> crop) for each treatment of fertilization packages, measured at 60 and 100 DAS

Fertilization packages	Total N (g.kg <sup>-1</sup> ) at 60 DAS		Available P (mg.kg <sup>-1</sup> ) at 60 DAS		Total N (g.kg <sup>-1</sup> ) at 100 DAS		Available P (mg.kg <sup>-1</sup> ) at 100 DAS	
	maize	sorghum	maize	sorghum	maize	sorghum	maize	sorghum
D <sub>0</sub>	1.24 <sup>c</sup>	1.10 <sup>c</sup>	14.97 <sup>d</sup>	11.41 <sup>d</sup>	1.33 <sup>d</sup>	1.23 <sup>d</sup>	17.43 <sup>d</sup>	10.15 <sup>d</sup>
D <sub>1</sub>	1.45 <sup>a</sup>	1.29 <sup>a</sup>	28.44 <sup>b</sup>	16.25 <sup>b</sup>	1.69 <sup>b</sup>	1.38 <sup>b</sup>	29.51 <sup>b</sup>	21.58 <sup>b</sup>
D <sub>2</sub>	1.47 <sup>a</sup>	1.25 <sup>ab</sup>	35.02 <sup>a</sup>	28.49 <sup>a</sup>	1.86 <sup>a</sup>	1.48 <sup>a</sup>	36.56 <sup>a</sup>	29.99 <sup>a</sup>
D <sub>3</sub>	1.39 <sup>b</sup>	1.22 <sup>ab</sup>	17.52 <sup>c</sup>	14.59 <sup>bc</sup>	1.47 <sup>c</sup>	1.31 <sup>c</sup>	19.37 <sup>c</sup>	18.59 <sup>c</sup>
D <sub>4</sub>	1.33 <sup>b</sup>	1.20 <sup>b</sup>	16.29 <sup>c</sup>	14.25 <sup>c</sup>	1.42 <sup>c</sup>	1.31 <sup>c</sup>	18.53 <sup>c</sup>	17.32 <sup>c</sup>
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<sub>0</sub> 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<sub>2</sub> treatment. Therefore, N and P

uptake in shoots of maize and sorghum was highest in the D<sub>2</sub> 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<sub>2</sub> 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 ( $\text{mg}\cdot\text{g}^{-1}$  plant dry weight) by each crop at 60 DAS for each treatment of fertilization packages

Fertilization packages	N and P uptake ( $\text{mg}\cdot\text{g}^{-1}$ plant dry weight) by each crop at 60 DAS			
	Maize (1 <sup>st</sup> cropping cycle)		Sorghum (2 <sup>nd</sup> cropping cycle)	
	N	P	N	P
D <sub>0</sub>	18.62 <sup>c</sup>	1.71 <sup>d</sup>	12.74 <sup>d</sup>	0.65 <sup>d</sup>
D <sub>1</sub>	27.58 <sup>b</sup>	2.60 <sup>b</sup>	18.92 <sup>b</sup>	0.85 <sup>c</sup>
D <sub>2</sub>	28.00 <sup>a</sup>	2.72 <sup>a</sup>	20.86 <sup>a</sup>	1.48 <sup>a</sup>
D <sub>3</sub>	23.10 <sup>c</sup>	2.41 <sup>c</sup>	17.29 <sup>c</sup>	1.36 <sup>b</sup>
D <sub>4</sub>	20.44 <sup>d</sup>	2.41 <sup>c</sup>	17.22 <sup>c</sup>	1.32 <sup>b</sup>
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 ( $\text{mg}/\text{pot}$ ) 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<sub>2</sub> 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 (1 <sup>st</sup> crop) and sorghum (2 <sup>nd</sup> 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 <sub>0</sub>	8.13 <sup>d</sup>	10.43 <sup>c</sup>	34.15 <sup>d</sup>	62.29 <sup>c</sup>	0.82 <sup>d</sup>	12.30 <sup>d</sup>	8.31 <sup>d</sup>	47.74 <sup>c</sup>
D <sub>1</sub>	15.13 <sup>b</sup>	22.39 <sup>b</sup>	61.92 <sup>b</sup>	109.03 <sup>b</sup>	2.22 <sup>b</sup>	22.34 <sup>b</sup>	16.89 <sup>b</sup>	95.01 <sup>b</sup>
D <sub>2</sub>	17.12 <sup>a</sup>	34.56 <sup>a</sup>	72.86 <sup>a</sup>	111.25 <sup>a</sup>	3.37 <sup>a</sup>	24.44 <sup>a</sup>	23.53 <sup>a</sup>	105.14 <sup>a</sup>
D <sub>3</sub>	13.65 <sup>c</sup>	18.48 <sup>c</sup>	52.26 <sup>c</sup>	101.05 <sup>c</sup>	1.68 <sup>c</sup>	14.52 <sup>c</sup>	12.01 <sup>c</sup>	85.46 <sup>c</sup>
D <sub>4</sub>	13.34 <sup>c</sup>	15.43 <sup>d</sup>	51.29 <sup>c</sup>	95.87 <sup>d</sup>	1.38 <sup>c</sup>	14.47 <sup>c</sup>	11.81 <sup>c</sup>	66.06 <sup>d</sup>
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 1 <sup>st</sup> cropping cycle		Sorghum in the 2 <sup>nd</sup> cropping cycle	
	Grain yield	100 grains	Grain yield	100 grains
D <sub>0</sub>	9.60 <sup>c</sup>	22.48 <sup>d</sup>	3.57 <sup>d</sup>	2.73 <sup>d</sup>
D <sub>1</sub>	17.40 <sup>b</sup>	26.94 <sup>b</sup>	5.05 <sup>b</sup>	3.01 <sup>b</sup>
D <sub>2</sub>	22.80 <sup>a</sup>	28.12 <sup>a</sup>	6.65 <sup>a</sup>	3.61 <sup>a</sup>
D <sub>3</sub>	15.60 <sup>c</sup>	25.98 <sup>c</sup>	4.43 <sup>c</sup>	2.90 <sup>c</sup>
D <sub>4</sub>	10.20 <sup>d</sup>	24.61 <sup>c</sup>	4.17 <sup>c</sup>	2.81 <sup>cd</sup>
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 cropping 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<sub>2</sub> 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.

## ACKNOWLEDGEMENT

The authors would like to thank the Directorate of Research and Community Service, the General Directorate of Research and Development at the Ministry of Research, Technology and Higher Education (DRPM RISBANG KEMRISTEKDIKTI), and to the University of Mataram, for the research grants with the number of 030/SP2H/LT/DRPM/II/2016.

## REFERENCES

- Alguacil, M., Lumini, E., Roldan, A., Salinas-Garcia, J., Bonfante, P., & Bianciotto, V. (2008). The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecological Applications*, 18(2), 527-536.
- Arihara, J., & Karasawa, T. (2000). Effect of previous crops on arbuscular mycorrhizal formation and growth of succeeding maize. *Soil Science and Plant Nutrition*, 46(1), 43-51.
- Astiko, W. (2013). *The role of indigenous mycorrhizae in different plant patterns to increase soybean yield in sandy soil (Case studies on dryland North Lombok)* (Unpublished Doctoral thesis), University of Brawijaya, Indonesia.
- Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013a). The role of indigenous mycorrhiza in combination with cattle manure in improving maize yield (*Zea mays* L.) on sandy loam of Northern Lombok, Eastern of Indonesia. *Journal of Tropical Soils*, 18(1), 53-58.
- Astiko, W., Sastrahidayat, I. R., Djauhari, S., & Muhibuddin, A. (2013b). Soil fertility status and soybean [*Glycine max* (L.) Merr.] performance following introduction of indigenous mycorrhiza combined with various nutrient sources into sandy soil. *AGRIVITA, Journal of Agricultural Science*, 35(2), 127-137.
- Augé, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science*, 84(4), 373-381.
- Azcón, R., Ambrosano, E., & Charest, C. (2003). Nutrient acquisition in mycorrhizal lettuce plants under different phosphorus and nitrogen concentration. *Plant Science*, 165(5), 1137-1145.
- Bautista-Cruz, A., Cruz-Domínguez, G., Rodríguez-Mendoza, M. d. I. N., Pérez-Pacheco, R., & Robles, C. (2014). Effect of compost and slow-release fertilizers addition on soil biochemistry and yield of maize (*Zea mays* L.) in Oaxaca, Mexico. *Revista de la Facultad de Ciencias Agrarias*, 46(1), 181-193.
- Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59(1), 39-46.
- Bruand, A., Hartmann, C., & Lesturgez, G. (2005). *Physical properties of tropical sandy soils: A large range of behaviours*. Retrieved February 26, 2019, from [https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand\\_keynote\\_paper.pdf](https://hal-insu.archives-ouvertes.fr/file/index/docid/79666/filename/Bruand_keynote_paper.pdf)
- Brundrett, M., Bougher, N., Dell, B., & Grove, T. (1996). *Examining mycorrhizal associations*. Retrieved February 26, 2019, from <http://irt-pw-cp1.irt.csus.edu/jbaxter/wp-content/uploads/2016/02/1996-Brundrett-et-al-Mycorrhizal-Methods-Book.pdf>
- Calderón-Vázquez, C., Alatorre-Cobos, F., Simpson-Williamson, J., & Herrera-Estrella, L. (2009). Maize under phosphate limitation. In *Handbook of maize: Its biology* (pp. 381-404). New York, NY: Springer.
- Carrenho, R., Trufem, S. F. B., Bononi, V. L. R., & Silva, E. S. (2007). The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta Botanica Brasilica*, 21(3), 723-730.

- Cavagnaro, T. R., Bender, S. F., Asghari, H. R., & van der Heijden, M. G. (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, 20(5), 283-290.
- Dhillon, S. S., & Ampornpan, L. A. (1992). The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant rice (*Oryza sativa* L.) plants. *Biology and Fertility of Soils*, 13(2), 85-91.
- Drew, E., Murray, R., Smith, S., & Jakobsen, I. (2003). Beyond the rhizosphere: Growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant and Soil*, 251(1), 105-114.
- Feng, G., Song, Y., Li, X., & Christie, P. (2003). Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Applied Soil Ecology*, 22(2), 139-148.
- George, E., Marschner, H., & Jakobsen, I. (1995). Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, 15(3-4), 257-270.
- Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3), 489-500.
- Grant, C., Bittman, S., Montreal, M., Plenchette, C., & Morel, C. (2005). Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science*, 85(1), 3-14.
- Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H., & Kubát, J. (2006). Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. *Mycorrhiza*, 16(3), 159-166.
- Guo, W., Zhao, R., Zhao, W., Fu, R., Guo, J., Bi, N., & Zhang, J. (2013). Effects of arbuscular mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) grown in rare earth elements of mine tailings. *Applied Soil Ecology*, 72, 85-92.
- Hawkins, H. J., Johansen, A., & George, E. (2000). Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil*, 226(2), 275-285.
- Horwitz, W. (2000). *Official methods of analysis of AOAC International* (17th ed., Vol. 1). Gaithersburg, USA: AOAC International.
- Imam, S. M. H., & Didar, M. A. (Eds.) (2005). *A handbook on analysis of soil, plant and water*. Dhaka, Bangladesh: Bangladesh-Australia Centre for Environmental Research (BACER-DU).
- Johnson, N. C., Copeland, P. J., Crookston, R. K., & Pflieger, F. (1992). Mycorrhizae: Possible explanation for yield decline with continuous corn and soybean. *Agronomy Journal*, 84(3), 387-390.
- Koide, R., & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist*, 148(3), 511-517.
- Leigh, J., Hodge, A., & Fitter, A. H. (2009). Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist*, 181(1), 199-207.
- Lisle, L., Gaudron, J., & Lefroy, R. (1990). *Laboratory techniques for plant and soil analysis*. Armidale, Australia: UNE-ACIAR- Crawford Fund.
- Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., & Niggli, U. (2000). Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop

- rotation. *Biology and Fertility of Soils*, 31(2), 150-156.
- Marulanda, A., Azcon, R., & Ruiz-Lozano, J. M. (2003). Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. *Physiologia Plantarum*, 119(4), 526-533.
- Menge, J. (1983). Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture. *Canadian Journal of Botany*, 61(3), 1015-1024.
- Page, A. L., Miller, R. H., & Keeney, D. R. (1982). *Methods of soil analysis, Part 2: Chemical and microbiological properties* (2nd Ed.). Madison, USA: American Society of Agronomy.
- Patle, G. T., Sikar, T. T., Rawat, K. S., & Singh, S. K. (2018). Estimation of infiltration rate from soil properties using regression model for cultivated land. *Geology, Ecology, and Landscapes*, 3(1), 1-13.
- Plenchette, C., Fortin, J., & Furlan, V. (1983). Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. *Plant and Soil*, 70(2), 199-209.
- Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. New York, NY: Academic Press.
- Supardi, G. (1996). Mining the synergistic effects towards strong agriculture. *HITI News*, 4, 10-13.
- Tarafdar, J., & Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry*, 26(3), 387-395.
- Tawarayaya, K. (2003). Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition*, 49(5), 655-668.
- Tawarayaya, K., Naito, M., & Wagatsuma, T. (2006). Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *Journal of Plant Nutrition*, 29(4), 657-665.
- Vivekanandan, M., & Fixen, P. (1991). Cropping systems effects on mycorrhizal colonization, early growth, and phosphorus uptake of corn. *Soil Science Society of America Journal*, 55(1), 136-140.
- Wangiyana, W., Cornish, P. S., & Morris, E. (2006). Arbuscular mycorrhizal fungi dynamics in contrasting cropping systems on vertisol and regosol soils of Lombok, Indonesia. *Experimental Agriculture*, 42(4), 427-439.
- Zhu, Y. G., Cavagnaro, T. R., Smith, S. E., & Dickson, S. (2001). Backseat driving? Accessing phosphate beyond the rhizosphere-depletion zone. *Trends in Plant Science*, 6(5), 194-195.