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Nutrient Status and Mycorrhizal Population on Various Food Crops Grown Following Corn Inoculated with Indigenous Mycorrhiza on Sandy Soil of North Lombok, Indonesia

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ABSTRACT

This study was aimed to determine the nutrient status and population of arbuscular mycorrhizal fungi (AMF) on the second cropping cycle of corn-based cropping patterns which utilized indigenous mycorrhizal fungi on sandy soil. The experiment was conducted at the Akar-Akar village in Bayan district of North Lombok, in a Randomized Block Design, with 4 replications and 6 treatments of cropping cycles (P0 = corn-soybean as a control, in which the corn plants were not inoculated with AMF; P1 = corn-soybean, P2 = corn-peanut, P3 = corn-upland rice, P4 = corn-sorghum, and P5 = corn-corn, in which the first cycle corn plants were inoculated with AMF). Results indicated that the status of N, P, K and organic-C increased significantly up to 112%, 148%, 88%, 88% at 60 DAS and 66%, 135%, 54%, 60% at 100 DAS, respectively in the second cropping cycle of sorghum compared to control. Uptake of N, P, K and Ca the sorghum plants at 60 DAS of the second cropping cycle reached 200%; 550%; 120% and 490%, respectively a higher than in the control. Mycorrhizal populations (spore number and infection percentage) were highest in the second cycle sorghum, achieving 335% and 226% respectively, which were significantly higher than those in the control.

Keywords: Arbuscular mycorrhizal fungi, corn, cropping pattern, dryland, NPK

INTRODUCTION

The limiting factors such as water availability, poor nutrient and soil organic matter are the root of the problems in an effort to increase maize yield on sandy soil in dryland area of North Lombok. Another constraint is the highly dependency of the implementation of agriculture intensification on the use of inorganic fertilizers. The practice of fertilization in this way is not efficient. Of the N fertilizer applied, at most only 50% is absorbed by the crop roots and the rest is left behind or lost from the soil. The most inefficient fertilizer is P fertilizer, which is absorbed by the roots only about 8-13% (Supardi 1996). Problem solving can be done by studying the behavior of the limiting factors as well fixing them through land management actions to improvement of the soil characteristics which support the improve the water system and adequate soil nutrients for plant growth (Zuzuki and Noble 2007). One of the ways for solving the problems is to utilize arbuscular mycorrhizal fungi (AMF) for improving crop growth and yield.

Inoculation of AMF on maize in sandy soil is expected to have positive implications to the improvement of soil properties, nutrient uptake and yield. This method is an alternative to bio-fertilizer that has a high efficiency because it can catalyze the hydrolysis of adsorbed nutrients in the soil through enzymatic reaction by the AMF to be available to plants (Widiastuti *et al*. 2003). This has been proven from the results of previous studies that AMF inoculation management of cropping patterns accompanied by manure application can increase P uptake, which result in higher crop yield than without AMF. Increased in nutrient uptake occurs as a resulted of AMF activities in increasing the availability of nutrients and improving root proliferation (Smith *et al*. 2010).

Management of the cropping patterns by arrangement of the sequences of different crop species planted in the period a year can lead to different levels of enrichment in AMF populations in the next cropping cycle. As an example, a maize crop planted in the first cycle of a corn-soybean cropping pattern can improve AMF sporulation and

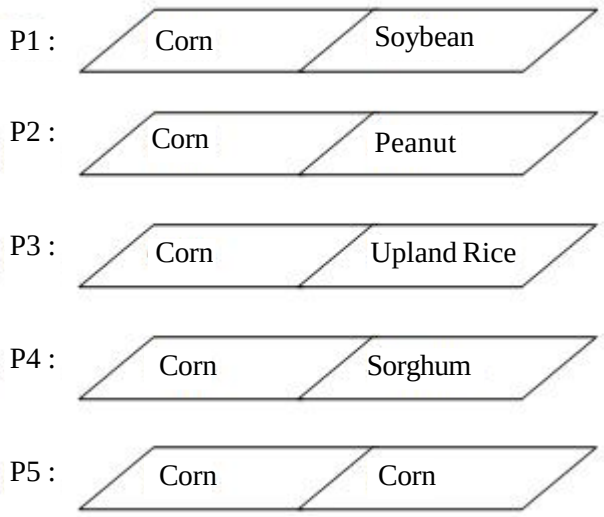
infection on the plant roots. This leads to the enrichment of AMF population in the soil, which is very favorable for growing the next crop cycle (Sylvia *et al*. 2005; Muhibuddin 2006). Wangiyana *et al.* (2006) also reported different dynamics of AMF populations (AMF colonization and spore counts) between cropping patterns on two dominant soil types in Lombok, Indonesia.

However, how much increases in the nutrient status and AMF population in the second cycle of a certain cropping patterns commonly cultivated by the farmers after growing their corn crop has not been revealed. Since different cropping patterns would indicate different increases in the nutrient status and populations of AMF, this study has revealed nutrient status and AMF population on some crops of the second cycle of cropping patterns after the first cycle of AMF inoculated and uninoculated corn crops had been harvested.

MATERIALS AND METHODS

Study Site and Design

This research based on a field experiment conducted on sandy soil in the dryland area of Akar-Akar Village of Bayan District in North Lombok, and the soil characteristics are presented in Table 1. The experiment tested five cropping patterns and one control, and each pattern consisted of two cropping cycles. The corn plants in the first growing cycle were fertilized using the recommended fertilizer dosage, while those in the second cycle were grown on the same land but without fertilizer. The experiments were designed using a Randomized Complete Block Design with four replications. The cropping pattern as the control treatment was corn-soybean without AMF inoculation (P_0). The other treatments of cropping patterns in which corn in the first cycle was inoculated with AMF were as follows:



Implementation of the Experiment and Observation

The land was prepared using minimum soil tillage, cleared from weeds, and plots were subsequently created in blocks and in each block smaller plots of 7 m × 5 m were created as the treatment plots.

Inoculum of indigenous AMF used in this experiment was the result of a private collection of the mycorrhizal isolate M_{AA01} which was the best isolate of indigenous mycorrhiza of North Lombok. The final form of the inoculum is a powder. Inoculation with the mycorrhizal isolates M_{AA01} was done through seed-coating, i.e. by mixing thoroughly the seeds with the mycorrhizal inoculum with a help of glue made from tapioca flour, with a dose of 1 kg of inoculum for 20 kg of seeds (Astiko 1995; Feldmann *et al*. 2009). AMF inoculation was done only on corn in the first cycle, except for the control crops, and no inoculation was done on the second-cycle crops.

Seeding was done by dibbling 2 seeds of the 2nd cycle crops per planting hole of 2 cm. At 7 days after seeding (DAS), tinning was done by leaving 1 plant per planting hole. For crops in the cycle 2nd cycle, direct seeding was done after harvest of the crops in the 1st cycle, by firstly clearing the plots from the remains of plants from the 1st cycle, followed by shallow harrowing the surface of the plots. Measurement on the plants was done on destructive plant samples at 60 DAS on each treatment.

The planting distance for corn and sorghum was 70 cm between rows and 20 cm within row, while for soybeans and peanuts was 30 cm between rows and 20 cm within row, whereas for upland rice it was 20 cm within and between rows. The varieties used were Bisma for corn, Kaba for soybean, Bison for peanuts, Inpago Unram 1 for upland rice, and Numbu varieties for sorghum.

Fertilization for corn was performed twice, with the recommended dose of 300 kg ha^{-1} of urea and 200 kg ha^{-1} of Phonska (NPK 15-15-15). The first fertilization was done at 1 week after seeding (WAS) at a dose of 100 kg ha^{-1} of urea and 200 kg ha^{-1} of Phonska fertilizer, and the second fertilization with the rest of the urea fertilizer at 1 month after seeding (MAS). Fertilizers were applied only to corn plants grown in the first cropping cycle, while those in the second cycle were grown on the same land but without fertilizer application.

Maintenance of the plants includes several activities such as replanting, weeding and soil-piling. Replanting for non-emerging maize and sorghum seedlings was done after 4-7 DAS, whereas for soybeans, peanuts and upland rice it was done between 5-10 DAS. Weeding and soil-piling were done twice; first at 15 DAS, while the second weeding is done at 30 DAS prior to subsequent fertilization. In the second weeding, soil-piling was also done by tilling and piling the soil around the stems. For soybeans and peanuts, the last soil-piling was done after flowering, *i.e*. at 40 DAS.

Protection of the plants was done using organic pesticides Azadirachtin under the trade name of OrgaNeem, with a concentration of 5 ml per liter of water, which was sprayed every 3 days.

Harvesting of corn, sorghum and upland rice were done about 100 DAS, when the corn husks color turned to brownish yellow. Soybean and peanuts are also harvested at 100 DAS.

Observation of all parameters was done on the crops in the second cycle. Parameters related to soil fertility status (N, P, K, organic-C and soil pH) were measured before sowing and at 60 and 100 DAS while nutrients uptake (N, P, K, and Ca) was measured at 60 DAS. Parameters related to AMF activities including fungal population and percentage of root infections at 60 DAS.

Analyses for N, P, and organic-C were done by using Kjeldhal method, spectrophotometer, and colorimetric method according to Walkley and Black, respectively. K and Ca were analyzed by using Automatic Absorption Spectrophotometer (AAS). Mycorrhiza population was measured using wet sieving technique according to Brundrett *et al.* (1996). The supernatant caught at 38 µm-sieve was transferred to centrifuge tubes and added with 60% of sucrose solution and subsequently centrifuged at 3000 rpm for 10 minute (Daniel and Skipper 1982). The harvested spore were stored on the Whatman paper with permanent ink marked of 0.5 × 0.5 cm. Counting of mycorrhiza population was done using stereo microscope (40 × magnification). Measurement of root infection percentage was conducted using modification of clearing and staining method (Kormanik and McGraw 1982), followed by counting using the *Gridline Intersect* technique (Giovannetti and Mosse 1980) under stereo-microscope observation.

Table 1. Characteristics of the sandy soil in the Akar-Akar Village of Bayan District in North Lombok.

Soil Characteristic	Value	Category
pH (H_2O)	6.25	Rather neutral
N Total (%)	0.01	Very low
Available P (mg kg^{-1})	13.82	High
Available K (cmol kg^{-1})	0.57	Moderate
Available Ca (cmol kg^{-1})	7.38	Moderate
Organic C (%)	1.21	Low
Texture:		
- Sand (%)	69	-
- Silt (%)	29	-
- Clay (%)	2	-
Texture class		Sandy loam

Data Analysis

Data were analyzed using analysis of variance (ANOVA), followed by means comparison using the Least Significant Difference test at 5% level when the ANOVA showed a significant effect.

RESULTS AND DISCUSSION

Soil Characteristics

The soil used in this experiment is rough-textured (sandy loam), so it is relatively low in water holding capacity and high porosity (Soil Survey Staff 1988). The N fertilizer applied to the land (generally in the form of urea) could be lost along the percolation water. It is marked by very low levels of N-total (<0.01%). Soil reaction (pH) is rather neutral, high levels of available P, medium available K, medium available Ca, and low organic C (Table 1). The high levels of P could be caused by accumulated residue from the previous P fertilization. It may occur due to the application of agricultural intensification program with a high doses of inorganic P fertilizer (100-200 kg SP-36 per ha) in each planting season. The oretically, high soil P content is potentially able to meet P requirements of the plants. However, P in the soil is easy to form complex compounds, making P unavailable to plants (Priyono 2005). The complex P compounds need to be transformed first into phosphate ions through the mineralization process catalyzed by the phosphatase enzyme (Sylvia *et al*. 2005).

Soil Nutrient Status

Cropping pattern that contributed the highest and significant to the improvement of soil nutrient status of N, P, K and C-organic content at 60 and 100 DAS was the cropping pattern of corn - sorghum (P_4), in which sorghum was grown in the second cycle of the cropping pattern, after harvest of corn plants in the first cycle. The increases in the status of N, P, K, and C-organic on sorghum in the second cycle at 60 and 100 DAS, when compared with control (P_0) were up to 112%, 148%, 88%, 88%

and 66%, 135%, 54%, 60%, respectively (Table 2). The tendency of improvement in nutrient status seems to be controlled by the role of AMF, the suitability of the host plant and the supply of nutrients derived from inorganic fertilizer applied at the beginning of the experiment.

Those facts similar to the results of research by Astiko *et al*. (2013), which suggests that application of AMF combined with manure on corn planting pattern significantly contribute to the improvement of soil nutrient status. The high nutrient status in the 2nd planting cycle of sorghum compared with the 2nd cycles of other crops is indicative of a positive contribution from the AMF role in improving the status of the soil nutrient availability. The positive contribution of AMF includes the increased activities of phosphatase enzymes in solubilization of adsorbed phosphates to make them available in the soil (Widiastuti *et al*. 2003). Increased AMF activities can also increase hydrolysis of unavailable P complex to be available in the soil (Khade *et al*. 2010). The addition of inorganic fertilizer in the beginning of planting with a low dose in the first growing cycle also significantly contributed the compatibility between host plants and AMF which has a positive impact on the improvement of soil nutrient status (Astiko *et al*. 2012).

Nutrient Uptake

The highest nutrient uptake and significantly different from the control treatment (P0) at 60 DAS was achieved by sorghum in the planting cycles two on the corn - sorghum pattern (P4). Increased uptake of N, P, K and Ca in the sorghum crop at 60 DAS reached 200%; 550%; 120% and 490% higher when compared to the control (Table 3).

The differences in nutrient uptake between plant species in the 2nd cycle of cropping patterns seem to be more influenced by the different responses between crops. These could be the main reason for the differences in nutrient uptake between crops in the 2nd cropping cycle. The facts that the sorghum crop nutrient uptake in the 2nd cycle of maize - sorghum cropping pattern was higher and significantly different from other rops in 2nd cycle of the other cropping patterns, might be related to the suitability of the role of AMF with the sorghum host plant in improving the absorption of nutrients. Angel *et al*. (2007) and Muchane *et al*. (2010) suggested that AMF role can increase nutrient uptake of N and P as well as Cu and Zn in plant tissue of suitable hosts. Furthermore, Knapp *et al*. (2010) stated that the role of AMF can enhance nutrient uptake when coupled with an appropriate fertilizer application to the soil. The increased nutrient uptake might be caused by the activities of the external hyphae that extend beyond the depletion zone that is not accessible for plant roots (Joner *et*

Table 2. Soil nutrient status (N, P, K, organic-C and soil pH) of sandy soil on with second cyles various treatments after harvesting.

Cropping pattern	N (g kg^{-1})	P (mg kg^{-1})	K (cmol kg^{-1})	org-C (g kg^{-1})	pH
60 DAS					
P_0	0.86 a	10.22 a	0.51 a	12.1 a	6.25 a
P_1	1.41 b	14.12 b	0.77 b	13.3 b	6.46 b
P_2	1.61 c	18.86 c	0.79 c	12.2 c	6.47 b
P_3	1.41 b	14.76 b	0.77 b	13.3 b	6.43 c
P_4	1.77 d	25.39 d	0.96 d	22.8 d	6.49 d
P_5	1.68 e	22.15 e	0.95 d	20.7 e	6.47 b
LSD 5%	0.06	3.25	0.01	1.0	0.01
100 DAS					
P_0	1.14 a	11.30 a	0.63 a	17.5 a	6.50 a
P_1	1.41 b	14.30 b	0.75 b	19.5 b	6.80 b
P_2	1.66 c	19.68 c	0.85 c	17.3 c	6.60 c
P_3	1.41 b	22.57 d	0.82 d	19.7 d	6.70 d
P_4	1.90 d	26.62 e	0.97 e	28.0 e	6.90 e
P_5	1.75 e	25.57 e	0.86 c	25.5 f	6.80 b
LSD 5%	0.08	2.88	0.01	0.1	0.09

Remarks: Means followed by the same letters within the same column are not significantly different using LSD test (at p=0.05); P_0= control, P_1= corn-soybean, P_2= corn-peanut, P_3= corn-upland rice, P_4= corn-sorghum, and P_5= corn-corn

Table 3. Plant nutrient uptake (N, P, K and Ca) in the second cyles crop grown in sandy soil with various treatments.

Cropping pattern	Nutrient uptake (mg g^{-1} plant)			
	N	P	K	Ca
60 DAS				
P_0	11.37 a	0.32 a	12.63 a	2.01 a
P_1	18.20 b	1.17 b	21.44 b	2.03 a
P_2	22.40 c	1.22 b	23.09 b	2.71 b
P_3	11.38 a	0.34 a	19.07 c	1.68 c
P_4	34.43 d	2.09 c	28.26 d	11.67 d
P_5	30.45 e	1.61 d	25.14 e	7.79 e
LSD 5%	3.99	0.05	2.04	0.34

Remarks: Means followed by the same letters within the same column are not significantly different ($p<0.05$); P_0= cntrol, P_1= corn-soybean, P_2= corn-peanut, P_3= corn-upland rice, P_4= corn-sorghum, and P_5= corn-corn.

al. 2000; Drew *et al*. 2003 and Zhu *et al*. 2003). The AMF inoculation at the beginning of the growing season becomes a necessity in the systems of planting patterns practiced by the farmers in sandy soil (sandy loam) of North Lombok (Astiko *et al*. 2013). However, in order for the AMF inoculation to be highly successful, there should be a host plant suitability to AMF species, the status of soil nutrients supporting plant growth, and adequate AMF inoculum potential (Corkidi *et al*. 2008).

Population of Mycorrhiza

The number of spores and infection of the roots of sorghum in the corn - sorghum cropping pattern (P_4) were consistently and convincingly higher compared with those in other cropping patterns. The increase in spore number and infection of the roots of sorghum in the second cycle at 60 DAS compared with control were up to 335% and 226%, respectively (Table 4). This indication reveals that sorghum in the cropping pattern P4 can increase the population of AMF in the soil.

The accelerated increase in population of AMF on sorghum in the maize - sorghum cropping pattern (P_4) might be caused by environmental suitability and anatomical and physiological compatibility between the host plants and AMF (Kato and Miura. 2008). Maize and sorghum crops are favored AMF host plants, which may trigger AMF sporulation. The phenomenon of increased AMF sporulation around rhizosphere of maize and sorghum crops, as shown in the P4 treatment, is an indication of increased AMF activity (Astiko *et al*. 2013a). With the increased AMF sporulation, then enrichment of AMF population in the soil will occur. Maize and sorghum are plants that have coarse roots with low number of root hairs, so that they are preferable AMF host plants. This fact also reported by Van

Table 4. Population of mycorrhiza (number of spores and percentage of infections) second cycles crop in sandy soil with various treatments.

Cropping pattern	Population of mycorrhiza	
	Spores (100 g $soil^{-1}$)	Root Infection (%)
60 DAS		
P_0	1.071 a	27 a
P_1	3.231 b	71 b
P_2	3.343 c	75 c
P_3	2.981 d	61 d
P_4	4.881 e	88 e
P_5	4.369 f	81 f
LSD 5%	110.5	2.29

Remarks: Means followed by the same letters within the same column are not significantly different using LSD test (at p=0.05); P_0= control, P_1= corn-soybean, P_2= corn-peanut, P_3= corn-upland rice, P_4= corn-sorghum, and P_5= corn-corn

der Heijden *et al*. (2001), which suggests that plants having magnoloid type of roots (coarse roots with few or even no hairy roots), such as maize and sorghum crops, are more sensitive and responsive to the AMF infection resulting in increased AMF populations in the soil.

CONCLUSIONS

Status of N, P, K and organic-C in sorghum of the second cropping cycle, when compared to control at 60 and 100 DAS, increased significantly by 112%, 148%, 88%, 88% and 66%, 135%, 54%, 60 %, respectively. Increased uptake of N, P, K and Ca at 60 DAS reached 200%; 550%; 120%, and 490% higher in the sorghum crop compared with controls. Mycorrhizal populations (spore number and infection percentage) were the highest (335% and 226%) in sorghum of the second cropping cycle, which were significantly higher than those in the control treatment.

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