SOIL FERTILITY STATUS AND SOYBEAN [Glycine max (L) Merr] PERFORMANCE FOLLOWING INTRODUCTION OF INDIGENOUS MYCORRHIZA COMBINED WITH VARIOUS NUTRIENT SOURCES INTO SANDY SOIL

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ABSTRACT
The experiment tested four treatments, namely inoculation of soil with mycorrhiza, inoculation of soil with mycorrhiza and cattle manure, inoculation of soil with mycorrhiza and rock phosphate, and inoculation of soil with mycorrhiza and inorganic fertilizers. Soil without any inoculation was provided as control. The treatments were arranged in a Completely Randomized Design with four replications. Soil fertility status was based on the concentrations of N, P, K, and organic-C as well as soil pH. Plant performance was determined based on its ability to uptake nutrients (N, P, K, and Ca), its growth and yield. Then, the activity of mycorrhiza was measured based on total population and percentage of root infection. Overall, results of the present study showed that, compared with other treatments, inoculation of mycorrhiza into soil and amended with cattle manure significantly performed higher concentrations of N, P, K, and organic-C. This soil condition caused soybean to absorb significantly higher nutrients, grew well with higher yield compared with plant performance in other treatments. Therefore, results of this study implies that the application of mycorrhiza into soil amended with organic matter is promising to sustain soil productivity under soybean cropping system.

Keywords: Arbuscular Mycorrhiza, soil fertility, soybean yield, dryland

INTRODUCTION
Shortage in availability of water, nutrients, and organic matter was a core problems in improving plant production in sandy soil of northern Lombok (Suwardji et al., 2007). Sandy soil with low organic matter content has low capacity in holding water and nutrients to support optimal soybean performance (Suzuki and Noble, 2007; Bastida et al., 2010). This character of sandy soil is considered as a specific problem in managing sandy soil in northern Lombok, especially to grow soybean. To overcome this particular problem, a strategy in managing soil, in long term, to gain improvement and stability of soil organic matters which then lead to the improvement of soil characteristics especially in holding capacity of water, plant performance and nutrients in the state of ready to be used by plants (Astiko et al., 2013). In addition, soil management by improving role of indigenous arbuscular mycorrhiza (AM) in sandy soil is one of best possible alternative solutions to improve plant productivity (Herrera et al., 1993 and Astiko, 2009). This was due to the role of AM in improving soil quality through the improvement of aggregate and colloid of the soil (Ijdo et al., 2011). This role became better in soil with adequate content of organic matter supplying carbón and micro nutrients required by AM to grow (Öpik et al., 2008; Smith and Read, 2008).

Optimizing role and beneficial characteristic of AM through application of biofertilizer to improve plant productivity in dry land is prospective enough (Sastrahidayat et al., 2001 and Astiko et al., 2005). Application of AM by introducing the organisms into soil is expected to be able to improve plant productivity significantly through role of AM in improving plant capacity to absorb N, P, K, Ca and other micro nutrients. Besides, with its external hypha, AM will improve plant resistance on drought, protect plant root from soil-born pathogen infection, stimulate activity of beneficial microorganisms, and improve soil texture and structure (Gianinazzi and Vosátka, 2004; Feldmann et al., 2009; Ijdo et al., 2011).

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Results of many previous studies have proven that nutrient absorption, growth and result of plant inoculated by AM were much higher than control (Azcón-Aguilar and Barea, 1997; Nogueira et al., 2007; Fisher and Jayachandran, 2008). Furthermore, it was reported that the use of indigenous AM had advantages as the microorganism was able to establish and develop well in situ environment as well as its better ability to compete with existing soil microbes compared with introduced AM (Turrini et al., 2008).

Incorporation of AM fungus on soybean in sandy soil of dry-land Northern Lombok was expected to have positive impact on soil characteristic improvement, nutrient absorption, and finally plant growth and yield. This hypothesis was constructed based on results of previous research in Vertisol soil (Astiko et al., 2005) proving that inoculation of AM on soybean improved absorption of P and plant yield was higher than that of plant without AM. The improvement of P absorption was due to AM activity in improving nutrient availability and root proliferation (Smith et al., 2010). This increase of plant yield was suggested due to the ability of AM to increase efficiency in water use, nutrient absorption, and to maintain turgor of plant cells. The external hypha of AM fungus were expected to be able to absorb soil pore water when plant roots enable to do so. In addition, wide distribution of external hypha caused the amount of water taken improved on the soil with low water content (Drew et al., 2003; Smith and Read, 2008). Base on the above mentioned, the aim of this study was to assess soil fertility status and soybean performance following introduction of indigenous mycorrhiza combined with various nutrient sources into sandy soil.

**MATERIALS AND METHODS**

**Experiment Preparation and Maintainance**

A series of pot experiments using sandy soil taken Northern Lombok was conducted in a glasshouse of Faculty of Agriculture, Mataram University using 10 kg polybag as experimental unit. Taken from upper layer up to 30 cm depth, soil was passed through 2-mm holes sieve, air dried, then filled into the polybags. The experiment was conducted in two cropping cycles, namely; the first cropping cycle in which soybean was grown and fertilized according to treatments, and the second cropping cycle in which soybean was grown on soil that had been used for the first cropping cycle without fertilization. The study tested four treatments, namely; soil was inoculated with AM (F1), soil was inoculated with AM and amended with cattle manure (F2), soil was inoculated with AM and amended with rock phosphate (F3), and soil was inoculated with AM and amended with inorganic fertilizers (F4). Soil without AM inoculation and amendment was provided as control (F0). The treatments were completely randomized designed with four replications. AM inoculum was mass produced in corn plants grown in pots. Pots were filled with mixture of sandy soil and sterile cattle manure (ratio 1:1). The soil was watered about field capacity and the plants were maintained in glass house for three months. Then the plants were harvested, dried, blended, and passed through 50-mesh sieve. The final form of the proliferation was powder. The AM inoculums was prepared by mixing spores, powder medium, and root residues. The AM inoculum (20 gram per pot) then was inoculated at sowing time by layering the inoculum at 10 cm depth. Soybean seeds of Kaba var. were sown (2 seeds per pot) at 3 cm depth and 14 days after sowing (das), only one seedling per pot was left to grow further. Nutrient sources applied according to treatments were cattle manure, rock phosphate, Urea, and SP36. Rock phosphate and cattle manure were applied at rate of 1.2 and 2.0 g per plant respectively, and inorganic fertilizers of Urea and SP-36 were applied at rate of 0.1 g and 0.2 g per plant, consecutively. The nutrient sources were buried 5 cm around the plant at depth of 7 cm. Weeding was done manually while watering plants in the afternoon. Pinat protection was done by applying organic pesticide 0.5% Azadirachtin (OrgaNeem TM) every three days. The plants were harvested 100 das.

**Parameters Observation**

Parameters dealing with soil fertility status (N, P, K, organic-C and soil pH) were measured before sowing and at 60 and 100 das. The agronomic parameters such as: top and roots dry weight biomass (60 das and 100 das), nutrients uptake (N, P, K, and Ca) at 60 das and component yield of soybean (cops, grain and 100 grain weight). The dry weight of the agronomic parameters were measured after being oven dried at 60 °C for 48 hours. Parameters related to AM activities including fungi population, roots
percentage infections at 60 das were also measured. Plant analysis for N was determined using Kjeldhal method, P using spectrophotometer, C-organic with colorimetric method according to Walkley and Black, K and Ca was recorded using Automatic Absorption Spectrophotometer (AAS).

**Soil, Plant Analysis and Mycorrhiza Observation**

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 AAS. Mycorrhiza population was observed using wet sieving technique according to Brundrett *et al.* (1996). The supernatant caught at 38 μm-sieve was added with 60% of sucrosa solution and subsequently centrifused 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 x 0.5 cm. Counting of mycoriza population was done using stereo microscope (extended 40 x). Calculation of roots percentage infections was conducted using modification of clearing and staining method (Kormanik and McGraw, 1982), counted with the Gridline Intersect technique (Giovenetti and Mosse, 1980) under stereo-microscope observation.

**Statistical analysis**

To know if there is any difference among treatments, a mathematic model below was applied:

\[ X_{ijk} = \mu + \rho_i + \beta_j + \epsilon_{ij} \]

**Remarks:**

- \( \mu \) = general average
- \( \rho_i \) = effect of replication-i
- \( \beta_j \) = effect of treatment-j
- \( \epsilon_{ij} \) = error

The model was applied when \( X_{ijk} = \mu + \rho_i + \beta_j \) linear and additive to \( \Sigma \rho_i = \Sigma \beta_j = 0 \), and \( \epsilon_{ij} \) was free and normally distributed with average and variance = \( (0, \sigma^2) \). Data were analysed by analysis of variance (ANOVA-MStat) and the effect of treatments was determined. When the variance ratio (F) was significant, means for each treatment were separated using a least significant difference test at 5% level.

**RESULTS AND DISCUSSION**

**Soil Chemical Properties**

In general, compared with control, all treatments significantly increased soil fertility status as indicated by concentration of total N, available P and K, and organic-C both at 60 das and 100 das (Table 1). The highest increases of soil fertility status were observed in sandy soil inoculated with arbuscular mycorrhiza (AM) and amended with cattle manure (CM). This indicated that AM performed well when combined with CM compared with other combinations. Compared with control, inoculation of AM amended with CM increased N, P, and org-C as much as 39%, 105%, 27%, and 85%, respectively on 60 das and 12%, 60%, 10%, and 11%, consecutively on 100 das at the first cropping cycle. Interestingly, these effects were also observed at the second cropping cycle as the total N, P, K, and org-C increased as much as 43%, 120%, 37%, and 36%, respectively on 60 das, and 44%, 53%, 14%, and 36%, respectively on 100 das. Data presented in Table 1 also indicated that at the second cropping cycle soybean performance on treatment of AM plus CM could improve soil fertility status as shown by nutrient concentration of P and N as much as 4 and 48% on 60 das, respectively, and on 100 das the treatment increased N and Org-C as much as 12 and 20%, consecutively.
Table 1. Soil fertility status (N, P, K, organic-C and soil pH) of sandy soil with various treatments after harvesting

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N (g kg⁻¹)</th>
<th>P (mg kg⁻¹)</th>
<th>K (cmol kg⁻¹)</th>
<th>org-C (g kg⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>1st</td>
<td>2nd</td>
<td>1st</td>
</tr>
<tr>
<td><strong>60 DAS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₀</td>
<td>1.15</td>
<td>0.87</td>
<td>16.97</td>
<td>16.54</td>
<td>0.69</td>
</tr>
<tr>
<td>F₁</td>
<td>1.34</td>
<td>1.15</td>
<td>23.60</td>
<td>23.40</td>
<td>0.75</td>
</tr>
<tr>
<td>F₂</td>
<td>1.60</td>
<td>1.25</td>
<td>34.83</td>
<td>36.54</td>
<td>0.88</td>
</tr>
<tr>
<td>F₃</td>
<td>1.44</td>
<td>1.10</td>
<td>26.34</td>
<td>27.01</td>
<td>0.83</td>
</tr>
<tr>
<td>F₄</td>
<td>1.40</td>
<td>0.93</td>
<td>20.59</td>
<td>30.97</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>100 DAS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₀</td>
<td>1.31</td>
<td>1.14</td>
<td>17.62</td>
<td>17.33</td>
<td>0.75</td>
</tr>
<tr>
<td>F₁</td>
<td>1.44</td>
<td>1.36</td>
<td>20.86</td>
<td>22.68</td>
<td>0.77</td>
</tr>
<tr>
<td>F₂</td>
<td>1.47</td>
<td>1.65</td>
<td>28.25</td>
<td>26.57</td>
<td>0.83</td>
</tr>
<tr>
<td>F₃</td>
<td>1.38</td>
<td>1.41</td>
<td>23.32</td>
<td>20.62</td>
<td>0.81</td>
</tr>
<tr>
<td>F₄</td>
<td>1.33</td>
<td>1.43</td>
<td>24.38</td>
<td>24.57</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Remarks: Means followed by the same letters within the same column are not significantly different (p=0.05): 1st and 2nd (first cycles and second cycles); F₀= Control, F₁= AM inoculation, F₂= AM inoculation plus CM, F₃= AM inoculation plus RP, and F₄= AM inoculation plus CF; 1) pre-treatment data

Results of this study were in accordance with those of done by Jeffries et al. (2003) and Gianinazzi et al. (2010) reporting that AM inoculation with organic matter amendment can recover soil fertility status on an ecosystem. Furthermore, Douds et al. (2006) reported that introduction AM inoculation combined with various nutrient sources into sandy soil could increase soil nutrient content. The same result was also reported by Astiko et al. (2013) evaluating contribution of indigenous AM combined with cattle manure to increase corn performance in sandy soil of northern Lombok. Combination of AM and cattle manure resulted in significant improvement on soil fertility status especially N, P, K, and organic-C. This increase was suggested due to activity of enzyme present in external hypha of AM in the rhizosphere able to catalize and hydrolyze unavailable nutrient complex into available nutrients (Widiastuti et al., 2003).

Previous results reported by Warnock et al., (2007) showed that enrichment of AM could be the escalation by the addition of organic matter and the combination has a positive effect on improving soil fertility status and therefore it is beneficial for soybean performance. This signify that better synergy of the inoculated AM-treatment in combination with CM, as previously confirmed by Kato and Miura (2008). Higher soil extractable-P found in this study was not only due to indirect contribution of CM in improving soil fertility status but also related to a positive contribution of mycorrhiza in producing phosphatase enzyme, for mineralization of organic-P in soil (Crowley and Rengel, 2000; Joner and Johansen, 2000) and resulted in enhancing insoluble-P in soils (Orcut and Nilsen, 2000).

**Nutrient Uptake, Growth and Yield**

Nutrient absorption by soybean is presented in Table 2. In general, treatments such AM inoculation alone or combined with other sources of nutrients significantly increased nutrient uptake by soybean, compared with control. The highest increases were observed at treatment AM inoculation amended with CM. At this treatment, absorption of N, P, K, and Ca increased as much as 214%, 185%, 342%, and 233%, respectively on the first cropping cycle, and 191%, 308%, 224%, and 214%, 185%, 342%, and 233%, respectively on the second cropping cycle.

This indicated an increase in soybean performance when grown in sandy soil amended with various nutrient sources.
The increase of P absorption could possibly cause such a new nutrient balance in plant that induced absorption of other nutrients such as N, K, and Ca. Sufficient availability of K created a condition in which the use of water was efficient as cell turgor was maintained. This condition leads to active metabolism process, K accumulation on the tips, buds, and roots, the accumulation that lead to formation of cortex tissue and cell elongation leading to improvement of soybean performance (Schweiger et al., 2007 and Smith et al., 2010). This result was in accordance with that of Kaschuk et al. (2010) stating that the increase of nutrient aborption by host plant lead to plant vitality to supply Carbon to rhizosphere to form AM external hypha. Mathur and Vyas (2000) stated that AM inoculation was also resulted in the increase of accumulation of amino acids, protein, chlorophyll, and sugar contents compared with non-AM plants. N status of shoot tip of plant with mycorrhiza at extreme condition was higher than that of plant without mycorrhiza (Subramanian and Charest, 1999).The same trends were also recorded for N, P, K, and Ca (Liu et al., 2000).

The inoculation of AM followed by CM also increased soybean performance, as observed on plant dry biomass measured by root and shoot dry weights on 60 and 100 das (Table 3). Dry weight of roots and shoots on 60 das increased as much as 164% and 136%, respectively; while on 100 das the increases were as much as 150% and 178%, respectively on the first cropping cycle. On the second cycle, on 60 das the increases were 337% and 718%, while on 100 das the increases were as much as 390% and 1102%, consecutively.

The improvement of biomass was probably due to AM role to influence soil fertility status, especially P. Sufficient availability of P can indirectly induce absorption of other nutrients leading to better plant growth (Carrenho et al., 2001). This positive effect was suggested due to suitability of AM type, plant, and soluble P (Nikolaou et al., 2002; Bhadalung et al., 2005).

This condition was due to good association between plant and AM to perform maximal activity. While AM received carbon from the plant, and the latter got P from the first. It was reported that each particular combination of AM and plant showed such a specific carbon translocation pattern that influenced production of plant biomass (Smith and Read, 2008; Smith et al., 2009). Inefficient symbiosis in use of carbon could decrease plant biomass. AM colonization could result in positive, neutral, or negative impact depending on AM types, plants, and growth environment (Johnson et al., 1997; Hoeksema et al., 2010).

Negative impact of mycorrhiza colonization on initial growth of plant was reported previously (Bethlenfalvay et al., 1982; Koide 1985; Johnson et al., 1997). Such sort of impact may be due to various factors, such as; high availability of P in soil (Mosse et al., 1973), competition for carbon between plant and AM in low light intensity condition (Buwalda and Goh 1982), and difference in biomass allocation pattern between plant with and without mycorrhiza (Smith and Read, 2008).
The increases of soil fertility status, plant performance and yield on the tretament of AM inoculation amended with CM were caused by the increase of AM activity in absorption of nutrient and water through its external hypha (EH). This was possibly due to EH can reach depletion zone that cannot be reached by plant roots (Zhu et al., 2001). The diameter of EH which is much smaller than that of roots makes the EH possibly to penetrate soil micro pores to take nutrient and water that cannot be reached by roots (Drew et al., 2003).

This ability causes plants with mycorrhiza be able to absorb nutrient, growth and perform better and resistance to drought stress (Smith and Read, 2008). In addition, AM is able to dilute phosphate tied in soil and fertilizer, to improve absorption of N, P, and K, to improve plant tolerance to drought, to improve plant ability to produce growth regulator, to stimulate activity of beneficial microbes, to improve soil structure and aggregation, and to enhance mineral cyclic (Cruz, 1990).

Decomposition and mineralization of organic matter were better with the presence of AM which positively affect physic, chemistry, and biology factors of the soil which in turn play key role in improving plant yield (Smith and Read, 2008).  All above facts indicated such a suitable functional among AM, host plants, and environment that are able to increase nutrient absorption, plant growth and yield as also reported earlier (Burleigh et al., 2002).

**Mycorrhiza Activity**

The inoculation of AM followed by CM amendment could increase AM activity as shown by numbers of spores per 100 g of soil and percentage of root infection (Tabel 4). The number of spores and percentage of infection on the first cropping cycle 60 das on sandy soil inoculated by AM and amended with CM increased as much as 179% and 266%, respectively, while on the second cycle the increases were 24% and 160%, respectively compared with control and were significantly different from other treatments.

**Table 3. Root and shoot dry weight of soybean grown in sandy soil with various treatments at 60 and 100 das**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Biomass dry weight (g. plant⁻¹)</th>
<th>60 DAS</th>
<th>100 DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First cycle</td>
<td>Second cycle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
<td>Roots</td>
</tr>
<tr>
<td>F₀</td>
<td>1.88</td>
<td>11.11</td>
<td>1.74</td>
</tr>
<tr>
<td>F₁</td>
<td>2.66</td>
<td>14.51</td>
<td>2.27</td>
</tr>
<tr>
<td>F₂</td>
<td>4.97</td>
<td>26.28</td>
<td>4.38</td>
</tr>
<tr>
<td>F₃</td>
<td>3.62</td>
<td>20.39</td>
<td>3.75</td>
</tr>
<tr>
<td>F₄</td>
<td>3.34</td>
<td>17.10</td>
<td>2.51</td>
</tr>
</tbody>
</table>

Remarks: Means followed by the same letters within the same column are not significantly different (p=0.05); F₀= Control, F₁= AM inoculation, F₂= AM inoculation plus CM, F₃= AM inoculation plus RP, and F₄= AM inoculation plus CF.
Figure 1. Yields of soybean (cobs, grain and 100 grain dry weight) grown in sandy soil with various treatments. A. the first cropping cycle, and B. the second cropping cycle. Bars with the same letters at the same category are not significantly different (p=0.05).

Table 4 showed that the number of spores and root infection were significantly high in all treatments when compared with control. Compared with before sowing, the number of spores and root infection 60 das increased significantly. This increases indicated that isolate MAA1 were able to compete with indigenous AM present in the rhizosphere of sandy soil Northern Lombok, particularly in colony forming inside roots. The other point that can be taken from these facts was that the isolate MAA1 used was able to produce abundance propagules in the form of spores and such colonized roots that they were able to live on dynamic and competitive habitat (Barrios, 2007; Doud and Johnson, 2007; Gianinazzi et al., 2010).
Result of a research on the role of indigenous mycorrhiza combined with cattle manure in improving yield of maize (*Zea mays* L.) on sandy loam of Northern Lombok showed that inoculation of AM combined with cattle manure resulted in higher number of spores and infected roots both in the first and the second growth season (Astiko et al., 2013). Similar results were also shown by Prasetya and Anderson (2011) on the assessment of the effect of long term tillage on the arbuscular mycorrhiza colonization of vegetable crops grown in andisols. This fact also indicated that isolate *M. aeruginosa* showed high effectiveness although they were inoculated on unsterile soil. Similar research on soybean inoculated with AM and the application of organic leaf fertilizer “greenstant” also showed similar results (Wangiyana et al., 2007).

Table 4. Biological activity of mycorrhiza (number of spores and percentage of infections) in sandy soil with various treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spores 100 g soil(^{-1}) and root infection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First cycle</td>
<td>Second cycle</td>
</tr>
<tr>
<td></td>
<td>Spores</td>
<td>Infection</td>
</tr>
<tr>
<td>60 DAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(_0)</td>
<td>1.06(^a)</td>
<td>21(^a)</td>
</tr>
<tr>
<td>F(_1)</td>
<td>2.159(^b)</td>
<td>41(^b)</td>
</tr>
<tr>
<td>F(_2)</td>
<td>2.960(^c)</td>
<td>77(^c)</td>
</tr>
<tr>
<td>F(_3)</td>
<td>2.343(^d)</td>
<td>54(^d)</td>
</tr>
<tr>
<td>F(_4)</td>
<td>2.215(^e)</td>
<td>46(^e)</td>
</tr>
</tbody>
</table>

Remarks: Means followed by the same letters within the same column are not significantly different (p=0.05); F\(_0\)= Control. F\(_1\)= AM inoculation. F\(_2\)= AM inoculation plus CM. F\(_3\)= AM inoculation plus RP. and F\(_4\)= AM inoculation plus CF; \(^1\) pre-treatment data

**CONCLUSION**

Inoculation of AM amended with cattle manure improved sandy soil fertility as shown by the increasing soil fertility status as observed on concentrations of N, P, K. and organic-C.

Soybean performance responded positively to the application of AM followed by cattle manure as indicated by plant improvement in nutrient absorption, plant growth and yield. The addition of cattle manure stimulated activity of AM leading to improvement of soil fertility and plant performance.

**ACKNOWLEDGEMENTS**


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