Arbuscular Mycorrhizas in Various Rice Growing Environments and their Implication for Low Soybean Yields on Vertisol Soil in Central Lombok, Indonesia

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Abstract: This paper reports AMF (arbuscular mycorrhizal fungi) dynamics in 16 sites of vertisol rice growing environments existing in Central Lombok, Indonesia, for the 1999 non-rice dry season cropping to the end of the subsequent (1999/2000) rainy season rice cropping. The results showed that among the types of rice growing environments surveyed, the rainfall environments either with no flooding (upland rice) or only a short period of flooding (Gora rice) had higher AMF colonization levels and transparent spore number, compared with the irrigated environments in which rice crops are grown under flooded conditions (the once-rice or twice-rice cropping systems), indicating adverse effects of flooding on AMF colonization of rice. The transparent (viable) spore number was lowest and the percentage of black (dead) spores was highest in the twice-rice at the end of the rainy season rice crop indicating bad effects of flooded rice in maintaining AMF propagules in the soil. This may have an implication for the low yield of soybean normally direct seeded following harvest of the rainy season rice crops or following the second cycle rice crops in the twice-rice cropping systems, so that better production technologies need to be developed for sustainable crop production in those environments.

Keywords: arbuscular mycorrhiza, colonization, dynamics, rice growing environment, vertisols

I. Introduction

Since the success of “Gora” rice (or dry direct seeded then flooded rice) in the mid 1980s until the beginning of the third millennium, there were four types of rice growing environments existing in Central Lombok (Indonesia), in which vertisol soil types are dominating the rice growing areas. Those environments differ principally in water regimes, especially the duration of flooded conditions, and in cropping patterns. In the upland rice environment there is no flooded condition, in which rice is grown during the rainy season on the sloping land areas (rainfed agriculture). In the lowland rainfed environments, “Gora” rice is practiced, in which rice is dry direct-seeded and grown as upland rice before the end of the vegetative phase, then flooded when rainfall is sufficient. In the irrigated environments, there is either twice or once flooded rice cropping practiced in the rainy season.

During the non-rice crop season, in the dry season, soybean is the most widely grown non-rice food crop in Lombok, although peanut, mungbean, maize and sweet potato are also grown (Arsyad and Sembiring, 2003). The farmers normally do not fertilize their non-rice food crops (Penninkhoff and Suyamto, 1992), and from a survey in Central Lombok, it was reported that soybean yields were very low, with an average of 0.35 ton/ha (Murni, 2000). Even with complete fertilization, from field experiments on the vertisol areas in Lombok, Adisarwanto et al. (1992) found no effect of N, P and K fertilization on soybean yields. Whilst the authors suggested that these soils were still fertile, the average yields were only 1.29 versus 1.21 t/ha in Sengkol, and 1.48 versus 1.47 t/ha in Keruak from fully fertilized versus non-fertilized plots. In these types of cropping systems in which fertilizer application is low or no fertilizer application for the non-rice food crops in rotation with rice crops, arbuscular mycorrhizal fungi (AMF) are expected to play an important role since arbuscular mycorrhizal (AM) symbiosis can increase the availability of phosphates to upland non-rice crops (Arthara and Karasawa, 2001). In addition, drying flooded rice soil for growing non-rice crops increases P sorption by the soil making P less available for uptake by upland non-rice crops (Muirhead and Humphreys, 1996). From a survey in other areas of Lombok covering upland and irrigated lands with two types of soil (regosols and vertisols), it was also found that flooded rice significantly reduced AMF populations (Wangiyana et al., 2006).

This paper reports an extensive field survey in Central Lombok on vertisol soil types for two cropping seasons to quantify dynamics of AMF colonization levels and spore counts between rice growing environments, including Gora rice, and to find out its implication for the low yields of soybean normally grown in the dry seasons following rice crops (which are mostly grown in the rainy season).
II. Materials And Methods

2.1. Design of Field Survey, Site Selection, and Sampling

Fields were sampled three times over two consecutive cropping seasons, and this sampling time (T) was assigned as factor 1 with three levels (T1, T2 and T3), which was designed to follow AMF population dynamics with time over the cropping cycles: T1 was in the non-rice crop season of 1999 (dry season) prior to rice; T2 was in the early growth of rice in the subsequent rice season of 1999/2000 (wet season of the monsoon); and T3 was at maturity or harvest of rice. Factor 2 was the type of rice growing environments (E) or cropping systems, i.e. upland rice (UR), once-rice (OR), twice-rice (TR), and “Gora” rice (GR) cropping systems. Four representative sites, scattered in two districts, were surveyed in each type of rice growing environment (Table 1). In each site, five replicates of field samples (as site replicates) were taken at each sampling time, which were taken from a diagonal line across the selected paddock.

Each of the five field samples (site replicates) taken from farmers’ fields was taken from the topsoil of plots (20 cm x 20 cm) up to 15 cm depth to include plant (crop or weed) roots and the rhizosphere soil. The roots from each sample were separated from the soil for inspection of AMF colonization after staining them in the laboratory following a modified procedure used by Wangiyana (2004). The soil was air-dried and sieved using a 2 mm mesh sieve for spore count and soil analysis. AMF colonization and spore number were quantified from each site replicate. Soil chemical analyses were done on the bulked replicates of each site. Soil pH was determined at each sampling time (T1, T2 and T3) but for available P and organic C, the analysis was performed at T1 only.

AMF spores were extracted from soil using a modified wet sieving procedure used by Wangiyana (2004), by wet sieving 20 g air-dried soil for each site replicate. The data were reported as spore number per gram oven-dried soil (after adjusting for moisture content of the air-dried soil samples). The sieves used had aperture sizes of 38, 105 and 750 µm respectively from bottom to top.

2.2. Variables and Measurement

The variables measured were AMF colonization in the roots of crops sampled from the fields and number of spores extracted from the soil of the field samples. Soil properties were also measured including soil pH in water (Anderson and Ingram, 1993), available (extractable) P content using the Bray-1 method (Kuo, 1996), and organic C content using the Walkley-Black method (Nelson and Sommers, 1996), which were analyzed in the Laboratory of Soil Chemistry in the Faculty of Agriculture, University of Mataram, Mataram, Lombok, Indonesia.

AMF colonization was measured as percentage length of root colonized by hyphae or other AMF structures in each root fragment observed under a compound microscope at a magnification of 100 x. Up to 25 to 150 root fragments were observed per site replicate depending on the total stained root fragments obtained. Root colonization level for each sample was expressed as the average percentage length of root colonized, which was measured from all root fragments in that sample.

Both transparent (viable) and black (presumably dead) spores were counted. Spores that were still colored from hyaline to dark brown were categorized as transparent spores, and were assumed to be viable, because when they were cracked, they still had cell content. Black spores in this study had cell contents that were black, dry, and was easily to crack; hence, they were assumed to be dead. Spores captured on stamp-
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gridded filter papers from the sieving-fraction of 105 µm were counted over the entire area of the filter papers. Spores from the sieving-fraction of 38 µm were counted using a sub-sampling technique developed using regression analysis (Wangiyana, 2004).

2.3. Data Analysis
To enable data analysis using Analysis of Variance (ANOVA) with a full post-hoc mean comparison, data from site replicates for AMF variables were averaged to obtain the site mean. These mean values were then used as site data and became replicates of each combination of the two factors (i.e. sampling time and types of rice growing environments). Before subjecting them to ANOVA, these data were examined for their normal distribution using Kolmogorov-Smirnov test in Minitab 13 for Windows, and otherwise they were transformed to become normally distributed. Based on the pre-analyses, those site mean data were transformed into hyperbolic arcsine [Asinh(x+0.5)] for root colonization (AshCol), and Ln(x) for transparent spore number (LnTS), while percentage of black to total spore (BkT) did not need transformation. The analysis aimed to determine if there was an interaction between rice-based cropping systems and sampling times, which were designed to follow cropping seasons or crop growth stages. Data were analyzed using analysis of variance (ANOVA) and Least Significant Difference (LSD) test at 5% level of significance, using the statistical software “CoStat for Windows ver. 6.303”.

The data for soil properties were analyzed using one-way ANOVA, because available P and organic C were measured only at T1. Soil pH, although it was measured at each sampling time, was also analyzed using one-way ANOVA (at every sampling time). For analysis of variance, data were transformed into Ln(x+1.3) for available P and hyperbolic arcsine (Asinh(x)) for organic C, while pH data did not require transformation. Relationships between variables were analyzed using the Best Subset Regression (BSR) in Minitab for Windows Release 13.

III. Results And Discussion

3.1. Soil Properties
The results of ANOVA performed for each sampling time showed that only pH at T2 was significantly different between rice growing environments (p =0.043) (Fig. 1); the others were not significantly different (p=0.138 at T1, and p=0.654 at T3). Gora rice showed the highest pH, which was significantly higher than in the once-rice and twice-rice, but was not significantly different from that in upland rice. With sampling times, the trend of changes in soil pH seems to be similar between rice growing environments, i.e. increasing from T1 to T2, then decreasing at T3 (after growing rice).

![Fig. 1. Averages (Mean ± SE) of soil pH in water between sampling times ( UR = T1, TR = T2, GR = T3, respectively) for each rice-growing environment](image1)

![Fig. 2. Averages (Mean ± SE) of transformed means of available P ( ; Ln(x+1.3) transformed) and organic C ( ; hyperbolic arcsine transformed) for each rice-growing environment](image2)

Although differences in available P (p=0.547) and organic C (p=0.811) were not significant, they tended to vary similarly between environments (Fig. 2). The available P concentrations ranged from 1 to 3.5 mg/kg in the twice-rice, 2.5 to 7.6 mg/kg in the once-rice, 1 to 23.8 mg/kg in the upland rice, and 1 to 25.7 mg/kg in the Gora rice growing environments. Thus according to the classification by Olsen and Sommers (1982) the available P concentration of the soil were mostly low (<3 mg/kg) and medium (3-7 mg/kg), especially for the two irrigated rice growing environments.

3.2. AMF Data between Rice Growing Environments and Sampling Times
The results of ANOVA showed highly significant main effects of rice growing environments and sampling times on AMF colonization and transparent spore number but interaction was significant only on AMF
Arbuscular Mycorrhizas in Various Rice Growing Environments and their Implication for Low colonization, while for the percentage of black spores, only rice growing environments had a significant main effect (Table 3). These mean that the levels of AMF colonization, averaged over growing environments, changed significantly ($p<0.05$) with sampling time, being highest at T1 (35%), i.e. in the non-rice crop season before planting the rainy season rice, and lowest at sampling 2 (T2), i.e. the early stage of rice (12%), but increasing slightly (14%) but not significantly ($p = 0.055$) at rice maturity (Fig. 3). The effect of the rice growing environments was also significant ($p<0.05$), with the Gora and upland rice systems (rainfed environments) having significantly higher AMF colonization levels than the once or twice rice systems (flooded irrigated rice environments). The highest average of AMF colonization was in the Gora rice system (26.2%) and the lowest average was in the twice-rice system (11.4%) (Fig. 4).

Table 3. Summary of the ANOVA results for AMF colonization (%), transparent spore number, and percentage number of black spores

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Colonization (%)</th>
<th>Transparent spore number</th>
<th>Percentage of black spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environments</td>
<td>6.13 ***</td>
<td>6.60 ***</td>
<td>8.36 ***</td>
</tr>
<tr>
<td>Sampling time</td>
<td>12.09 ***</td>
<td>19.85 ***</td>
<td>0.83 ns</td>
</tr>
<tr>
<td>Time x Env. Interaction</td>
<td>3.85 ***</td>
<td>0.85 ns</td>
<td>1.67 ns</td>
</tr>
</tbody>
</table>

1) Data were transformed into hyperbolic arcsine [Asinh(x+0.5)].
2) Data were transformed into natural log [Ln(x)].

Fig 3. Averages (Mean ± SE) of AMF colonization data (AshCol), black spore number (BkT/20), and transparent spore number (LnTS) between sampling times (T1, T2, T3, respectively).

Fig 4. Averages (Mean ± SE) of AMF colonization data (AshCol), black spore number (BkT/20), and transparent spore number (LnTS) between rice systems (U = upland rice, O = once-rice, T = twice-rice, G = Gora rice).

The effect of sampling time on AMF colonization was different between types of rice growing environments (Table 4). Sampling time had no significant effect on AMF colonization in both Upland and Gora rice, but the effect was significant in both the once-rice and twice-rice systems. In these rice cropping systems, the root colonization by AMF in the field in the non-rice season of 1999 (T1) was much higher than at T2 (Table 4). The small increase in mean colonization levels between the sampling times T2 and T3 (Fig. 3) was evidently due to an increase in the AMF colonization of Gora rice (Table 4).

Although the Gora rice system involves a short period of flooded conditions, it appeared to maintain higher levels of AMF colonization and spore counts compared with the more fully flooded environments in the conventional rice, especially the twice rice cropping system. The higher colonization levels in upland rice and Gora rice systems compared with the once-rice and twice-rice systems were most probably due to the shorter duration of flooded conditions. These results showed that AM fungi in Gora rice systems (with their short period of flooding in the reproductive growth stage) behaved similarly to upland rice. Solaiman and Hirata (1995, 1996) also found much lower colonization levels in rice grown in flooded compared with dry conditions or changed from flooded to non-flooded. However, they also showed considerable AMF colonization in flooded conditions, indicating that rice is a host. Since rice plants, especially in flooded conditions, acidify their rhizosphere (Hinsinger, 2001), the lower colonization in flooded conditions could also be due to lower soil pH, which was lower in fully irrigated rice compared with Gora rice, especially at T2 (Fig. 1). Rohyadi et al. (2004) also found significantly lower colonization by the AMF *Glomus etunicatum* on cowpea (*Vigna anguiculata* L. Walp.) in growing medium with low pH. However, in more recently, Vallino et al. (2014) reported that there was a negative correlation between AMF colonization and the amount of aerenchyma in roots of rice under flooding, and longer flooding increases aerenchyma, which can occupy up to 90% of the cortex.
were relatively large decreases in transparent spores. Solaiman and Hirata (1995, 1996) also reported that colonization and sporeization, the population of transparent spores continued to decline throughout the growing season, especially in the twice-flooded conditions in the early stages of growth are required for successful infection by AMF propagules on Gora rice (18.3) and the twice-flooded rice system (Fig. 5), while the percentage numbers of black spores increased (Fig. 6).

Unlike root colonization (by AMF), spore data did not show significant interaction between sampling times and types of rice growing environments. However, there were relatively large decreases in transparent spore number from non-rice to the subsequent rice season, as well as from the beginning to the end of the rice season, especially in the twice-rice system (Fig. 5). In fact, the main effect of sampling time was significant on transparent spore number (Table 3), and the averages decreased significantly with sampling times (Fig. 3), especially from T2 to T3 in the twice-rice cropping systems (Fig. 5), while the percentage numbers of black spores increased (Fig. 6).

In addition to high AMF colonization levels, the rainfed rice growing environments, especially Gora rice system, were also associated with higher numbers of transparent spores, and on average, the Gora rice system had the highest transparent spore number (32.8) compared with the once-rice (18.3) and the twice-rice systems (17.6 spores/g soil). Ilag et al. (1987) from their survey in the Philippines, and Wangiyana et al. (2006) from a survey in other areas in Lombok, similarly reported a higher population of AMF propagules at rainfed sites compared with irrigated sites. Solaiman and Hirata (1995, 1996) also reported that colonization and spore production on rice were higher on crops transplanted from a dry nursery compared with wet nursery seedlings. These were attributed to the higher initial colonization levels on the dry nursery seedlings compared with wet nursery ones, in which the colonization levels persisted in the field or pots after transplanting (Solaiman and Hirata, 1996). These authors also reported that non-flooded (NF) conditions resulted in the highest colonization levels and spore number at 60 days after planting, compared with non-flooded-flooded (NF-F) or flooded-non-flooded (F-NF) or fully flooded (F) systems (Solaiman and Hirata, 1995). Thus both the timing and duration of flooding seem to be important determinants of the level of colonization by AMF in rice. The data for the Gora rice system (Table 4), also suggest that the non-flooded conditions in the early stages of rice growth are required for successful infection by AMF propagules on Gora rice roots.

Unlike AMF colonization levels, transparent spore number decreased significantly with sampling times in all types of rice growing environments (Fig. 3). Thus in all rice crops, even those in the rainfed environments having relatively high AMF colonization, the population of transparent spores continued to decline throughout the life of rice crop, even at maturity when spore number might be normally expected to increase. This decline could be related to an increase in the percentage of black (dead) spores with sampling times, which could be due to ageing, flooded conditions (in some environments), reduction in soil pH or germination with unsuccessful

### Table 4. The averages of AMF colonization levels (%) for each combination of sampling times and types of rice growing environments

<table>
<thead>
<tr>
<th>Sampling times / sequences</th>
<th>Averages of AMF colonization level (%) for each rice growing environment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upland rice</td>
</tr>
<tr>
<td>T1 (non-rice)</td>
<td>33.0 (3.84)</td>
</tr>
<tr>
<td>T2 (early rice)</td>
<td>22.6 (3.29)</td>
</tr>
<tr>
<td>T3 (maturity of rice)</td>
<td>14.6 (3.14)</td>
</tr>
<tr>
<td>LSD _{0.05}</td>
<td>(1.01)</td>
</tr>
</tbody>
</table>

1) For ANOVA, data were transformed into hyperbolic arcsine [Asinh(x+0.5)].
2) Figures in the same row or column followed by the same letters, are not significantly different between environments or between sampling times, based on the LSD _{0.05}, using the transformed data.

**Note:** Figures in bracket are mean (or LSD) values of the transformed data.

![Fig 5. Averages (Mean ± SE) of transparent spore number (LnTS) for each combination of sampling time (T1 = T2, T2 = T3) and rice systems (UR = upland rice, OR = once-rice, TR = twice-rice, GR = Gora rice systems)](image)

![Fig 6. Averages (Mean ± SE) of percentage number of black spore for each combination of sampling time (T1 = T2, T2 = T3) and rice systems (UR = upland rice, OR = once-rice, TR = twice-rice, GR = Gora rice systems)](image)
infection, although these need further investigations. Whatever the reason for the fall in transparent spore numbers what is significant is that the numbers fell in all systems, even those in which the rice roots were relatively well colonized with hyphae (upland and Gora rice plants). Thus, whilst rice may be a good host for AM fungi in terms of root colonization, it appears to be a poor host in terms of maintaining inoculum levels in soil, or at least spore populations, which can have some implications on non-rice crops following rice, especially flooded rice.

3.3. Relationship between Soil Properties and AMF Variables

The results of the BSR analysis using the factorial dataset showed that only soil pH had a relatively high R² value. When simple regression analysis was performed on the dataset, soil pH showed a significantly negative relationship with percentage of black spore (BKT), with a regression equation of \( \text{BKT} = 89.8 - 4.65 \text{pH} \) \( (R^2 = 0.155, p = 0.006) \), while with transparent spore number (LnTS; LN transformed), there was a positive relationship, with a regression equation of \( \text{LnTS} = 1.69 + 0.216 \text{pH} \) \( (R^2 = 0.081, p = 0.049) \). LnTS also showed a significant relationship with colonization percentage \( \text{AshCol}; \) hyperbolic Arcsine transformed), with a regression equation of \( \text{LnTS} = 2.34 + 0.216 \text{AshCol} \) \( (R^2 = 0.112, p = 0.020) \). Colonization in general, using this factorial dataset, did not show a significant relationship (although it was positive) with soil pH. However, per sampling time basis, colonization levels at T2 showed a significantly positive relationship with soil pH at T2, with a regression equation of \( \text{AshCol} (T2) = -5.54 + 1.21 \text{pH(T2)} \) \( (R^2 = 0.341, p = 0.017) \).

Based on those results of regression analyses, in relation to AMF data, since flooded rice normally reduces pH of its rhizosphere (Hinsinger, 2001), it could mean that in an environment having lower pH, there would be higher percentages of black spores, lower number of transparent spores, and maybe lower AMF colonization. As can be seen from Table 3, there was a significant different in percentage of black spores between types of rice growing environments, and from Fig. 6, it can be seen that the twice-rice suffered a large apparent increase in percentage of black spores after having flooded conditions, and because of the negative correlation between soil pH and percentage of black spores, it could mean that the lower soil pH of flooded soils was deleterious to spore survival. Since longer flooding can reduce rhizosphere pH (Hinsinger, 2001); also increases aerenchyma and reduces AMF colonization (Vallino et al., 2014), it seems appropriate to conclude that rice growing environments or rice cropping systems with longer periods of flooding suffered higher rates of spore mortality compared with those that undergo little flooding or have rainfed conditions. In addition, in the twice-rice cropping systems, there are two cycles of flooded rice cropping cultivated per year, which could further reduce AMF propagules at the end of the second rice crop. However, since in the conventional (flooded) rice cropping system, rice crop is normally transplanted from seedlings initiated from a nursery, which normally prepared outside the paddy fields, it could also possible that rice seedlings are infected by AMF in the nursery, which can survive in the paddy fields after transplanting, as has been proven by Solaiman and Hirata (1996).

3.4. Possible Implications for the Low Yield of Soybean in Rotation with Flooded Rice

Because AMF spore populations are reduced in irrigated rice, especially in the twice-rice cropping systems, it is possible that the low yields of soybean achieved by farmers in Central Lombok in the rice-based cropping systems, reported by Murni (2000), were due to low levels of AMF colonization on soybean following flooded rice in the irrigated systems, especially in the systems having two cycles of flooded rice crops. In addition, as can be seen from Fig. 2, levels of available P contents of the soil were mostly low, especially in the twice-rice cropping systems, ranging from 1 to 3.5 mg/kg. Ellis (1998) also reported a significantly lower colonization on soybean grown following flooded conditions, even after only 4 weeks of flooding, indicating lower AMF infection potential of the soil following flooded conditions.

Many researchers also reported a high dependency of soybean to AMF symbiosis for normal growth and yield. Research results reported by Ross (1971) clearly indicated the critical importance of AMF symbiosis for soybean yield, which may not be replaced with P fertilization, since the results show that grain yield was still higher on soybean inoculated with AMF although without P fertilizer, in which the average yield was 801 g/m², while without AMF inoculation, the yields were lower, i.e. only 360 g/m² at 0 kg/ha P, 467 g/m² at 44 kg/ha P, and 627 g/m² at 176 kg/ha P fertilization. According to research results by Fernandez et al. (2009), even under high P fertilization, soybean is still dependent on AMF symbiosis with mycorrhizal dependency (MD) of 3.11%, while under medium P, the MD was 46.89%, and under low P the MD was 71.0%. In more recent experiment conducted in one site of vertisol soil in Central Lombok, in which two varieties of soybean (Wilis and Grobogan varieties) grown following flooded rice, Wangiyana et al. (2009) found that there was a highly significant positive correlation between levels of AMF colonization in the soybean roots at 44 days after planting and their grain yield, with an overall correlation coefficient of 0.688 \( (p-value= 0.003) \), which strongly indicates the importance of AMF symbiosis for soybean grown following irrigated rice.

DOI: 10.9790/2402-1012035157  www.iiosjournals.org  56 | Page
IV. Conclusion
Since the survey results showed that different types of rice growing environments resulted in different levels of mycorrhizal dynamics, in which rice grown under conventional technique with fully flooded conditions, especially the twice-rice cropping systems depleted AMF populations after flooded conditions, it is then necessary to find out food production technologies that favor high yield of food crops but never destroy the populations of beneficial microorganisms in the soil, especially arbuscular mycorrhizal fungi (AMF). This is because many food crops have a high dependency on AMF symbiosis for normal growth and yield, especially soybean.

Acknowledgements
The authors (WW) gratefully thanks Mr. Abdul Hamid Kule for his consistent help on the laboratory work, the University of Mataram for the study leave permission, and AusAID for the Ph.D scholarship at UWS, Australia, and others involved during the field samplings and other laboratory activities.

References