Effect of Mycorrhiza Application with Reduced NPK Fertilizers on Growth and Yield of Several Promising Lines of Red Rice in Aerobic System

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Abstract: This study aimed to examine the effect of arbuscular mycorrhizal fungi (AMF) application with reduced doses of N-P-K fertilizers on growth and yield of several promising lines of red rice grown in aerobic rice systems on raised-beds. The field experiment, carried out on farmers' land in Beleke Village, West Lombok, Indonesia, from May to September 2018, was designed according to Split Plot design with 3 blocks and two treatment factors, i.e. red rice genotypes (G) as the main plot factor (G1=MG4; G2=MG10; G3=AM4; G4=AM10), and application of AMF (M) as the sub-plot factor (M0= full recommended doses of NPK fertilizers without AMF; M1= AMF application with only 60% recommended doses of NPK fertilizers). The pregerminated red rice seeds were dibbled on the raised-beds with a base spacing of 25 x 20 cm, which was modified into double-rows. The results indicated that although AMF application was combined with reduced doses of N-P-K fertilizers, AMF significantly increased tiller, filled panicle and percentage of panicle numbers, dry straw, total biomass, filled grain number and grain yield per clump while reducing percentage of unfilled grain number. Among the promising lines of red rice, there were differences in dry straw weight, total biomass weight, and grain yield per clump and weight of 100 grains, and the amphibious promising line "AM10" was the best genotype. Although interaction effect was significant only on dry straw, response to AMF application was also different between genotypes in terms of grain yield and percentage of unfilled grain number.

Keywords: Red rice; aerobic rice system; arbuscular mycorrhiza

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I. Introduction

Rice is the most common staple food for the largest number of people in the world, especially in the Asian countries. Because it is the most sustainable way of growing rice, especially in relation to the effort in reducing weed competition, rice is mostly cultivated under conventional technique, i.e. on flooded (submerged) soil conditions. In addition, flooding has beneficial effects on P, Fe and Zn availability in rice production (Bouman et al., 2007a). Unfortunately, there are many disadvantages of the flooded rice cultivation technique. The most obvious one is the wasteful use of irrigation water. Yaligar et al. (2017) reported that conventional techniques of growing rice required irrigation water of up to 20260 m^3 /ha, while dry seeded direct planting only required 4260 m^3 /ha.

In addition to wasting of irrigation water, flooded rice system causes a lot of disadvantages such as pollution of water in the downstream areas due to seepage, runoff and percolation from paddy water during growth of rice plants, especially during fertilizer and pesticide application. The anaerobic conditions due to flooding can cause rice fields to produce methane and N_2O gas emissions (Bouman et al., 2007b). N fertilization with Urea also becomes inefficient due to losses through seepage and percolation water in addition to loss of Urea N through volatilization to NH_3 gas, which can reach 50% of Urea fertilizer applied (Buresh and De Datta, 1990).

Growing rice under flooded conditions also reduces population of arbuscular mycorrhizal fungi (AMF) in paddy soil following flooded conditions of growing rice, when compared with following upland rice (Wangiyana et al., 2006). In fact, rice plants also need a good symbiosis with AMF for better nutrition, as has been reported by many researchers. Dhillion and Ampornpan (1992) for example reported that AMF inoculation in the nursery not just increased P uptake by rice seedlings but also increased uptake of other nutrients such as K, Ca, Fe, Cu, Na, B, Zn, Al, Mg and S. Solaiman and Hirata (1995) showed that AMF inoculation significantly increased grain and shoot dry matter as well as N, P and K concentration in the unhulled grains of rice. Solaiman and Hirata (1996) also indicated that AMF inoculation of rice seedlings in the nursery increased grain yield and contents of P and micronutrients in the grains such as Zn, Cu, Fe and Mn.

In order to reduce the use of irrigation water, or to improve irrigation water use efficiency, there have been several non-conventional techniques developed in more recently. One of which is the System of Rice Intensification (SRI), in which rice is irrigated under intermittent short flooding and drying during the vegetative growth stages followed with thin flooding during the reproductive growth stages, and this results in significantly higher grain yield under SRI compared with under conventional technique of growing rice (Uphoff, 2003). Another technique of growing rice is aerobic rice system (ARS), in which rice is grown under non-flooded, nonpuddled and non-saturated soil conditions (Bowman 2001; Prasad, 2011). However, grain yield of rice under ARS is normally lower than under conventional technique, especially when rice is grown in monoculture (Nie et al., 2012). In addition, growing rice under more aerobic conditions will decrease the availability of P, Fe and Zn for the rice plants. Fortunately, mycorrhizal symbiosis should be better under ARS than under conventional technique of growing rice, and this symbiosis will help improve nutrition and yields of rice grown under aerobic systems.

This research aimed to examine the effects of application of AMF with reduce N-P-K fertilizers, compared with the effects of application of full recommended doses of N-P-K fertilizers, on growth and yield of several promising lines of red rice under aerobic rice system.

II. Material And Methods

In this study, the field experiment was carried out on farmers' land in Beleke Village, Gerung District, West Lombok Regency, NTB Province, Indonesia, from May to September 2018. The experiment was designed according to Split Plot design with 3 blocks and two treatment factors, namely red rice promising lines (G) as the main plot factor, consisting of 4 selected genotypes (G1 = MG4, G2 = MG10, G3 = AM4, and G4 = AM10), and application of AMF (M) as the sub-plot factor, consisting of 2 treatments (M0= without AMF application but the rice plants were fertilized with full recommended doses of N-P-K fertilizers; M1= AMF application at planting combined with only 60% recommended doses of N-P-K fertilizers). The AMF inoculant used was the bio-fertilizer "Technofert", which contains mixed species of arbuscular mycorrhizal fungi (AMF) and zeolit particles. Pre-germinated red rice seeds were planted by dibbling them on permanent raised-beds, with a basic spacing of 25 x 20 cm, which was then modified into a double-row pattern.

Before plotting and making raised-beds, the land was cultivated with once plowing and once harrowing, then raised-beds were made with a bed surface size of 2.0 m x 1.0 m, separated by a furrow of 40 cm wide on the top of the beds (and 20 cm at the base of the furrow) with a bed height of 25 cm. At planting 3-4 pre-germinated seeds were dibbled at a distance of 20 cm within rows and 20 cm between each row of a double-row but 30 cm between double-rows (double-row modification of 25 x 20 cm planting distance). For the M0 treatment, only pre-germinated red rice seeds were laid in the planting hole, which the covered with thin layer of soil. For the M1 treatment, the base of planting hole was first filled with "Technofert" of 5 gram per planting hole, which was the covered with Bokashi (EM-4 fermented cattle manure) of 20 gram per planting hole, and then the pre-germinated red rice seeds were laid on the manure, which was then covered with thin layer of soil. The bio-fertilizer "Technofert" was supplied by PT Mikata Sukses Mandiri, Serpong, Indonesia. For application of N-P-K fertilizers in the M0 treatment, the Phonska (15-15-15) fertilizer was used, with a dose of 300 kg/ha applied by dibbling at 7 days after planting (DAP) rice seeds, followed with dibbling Urea (45% N) fertilizer at 45 DAP with a dose of 160 kg/ha, while for the M1 treatment, the N-P-K fertilizers applied were only 60% of the M0 doses. Irrigation water was supplied every 5-7 days by flowing water through the furrows surrounding the raised-beds. Harvest was done at 110 DAS.

Observation variables included growth variables and yield components of the rice plants measured from four clumps of rice plant samples per bed. The growth variables included plant height, tiller number, dry straw weight, and stem number. Harvest of rice was done at 110 days after seeding. The rice yield components measured were panicle number, panicle length, number of filled and unfilled grains, and weight of dry filled grains, total biomass per clump, weight of 100 seeds, and harvest index. Data were analyzed with analysis of variance (ANOVA) and HSD test at 5% level of significance, using the statistical software "CoStat for Windows" ver. 6.303.

III. Results And Discussion

The results of ANOVA summarized in Table 1 show that there was a significant interaction between the two treatment factors but only on panicle dry straw weight per clump. Between the two treatment factors, application of mycorrhiza seems to have stronger effects than the genotypes do. Although AMM application was combined with only 60% recommended doses of N-P-K fertilizers, AMF still significantly increased tiller number, panicle number, dry straw weight, total biomass weight of the above-ground parts, percentage of the panicle number, filled grain number, and grain yield per clump while decreased percentage of unfilled grains. However, there were also significant differences between genotypes in terms of weight of 100 seeds, grain yield, total biomass, and dry straw weight per clump. When the means test was conducted using the Tukey's HSD test,

it is clear from Table 2 that dry straw weight and total biomass weight were highest on the amphibious promising line AM10, which in the field looked to produce larger stems (highest total biomass weight per clump), and relatively longer individual panicle per clump, compared with other genotypes.

Table 1.	Summary	results	of	ANO	VA on	plant	height	and	tiller	number	at	anthesi	s, an	d nun	ıber	of	filled
	panicles,	stems,	straw	/ dry	weight	and j	percenta	ige n	umber	of fille	d pa	anicles	per d	clump	at h	arve	est of
	various pr	romising	g line	es of r	ed rice												

Observation variables:	Blocks	Genotypes	Mycorrhiza	Interaction
Plant height at anthesis	ns	ns	ns	ns
Tiller number at anthesis	ns	ns	*	ns
Panicle number per clump	ns	ns	**	ns
Stem number per clump	ns	ns	ns	ns
Dry straw weight per clump	ns	*	***	**
Biomass weight	*	***	***	ns
%-Panicle number	ns	ns	*	ns
Filled grain number per clump	ns	ns	***	ns
%-Unfilled grains	*	ns	*	ns
Dry grain yield per clump	ns	**	***	ns
Weight of 100 seeds	ns	**	ns	ns
Harvest index	ns	ns	ns	ns

Remarks: ns= non-significant; *, **, *** = significant respectively at p-value <0.05, p-value <0.01 and p-value <0.001

Table 2. Averages of plant height and tiller number per clump at anthesis; filled panicle number and stem number (clump size) per clump at harvest; dry straw weight and total biomass weight per clump

Treatments:	Plant height at anthesis (cm)	Tiller number at anthesis	Filled panicle number per clump at harvest	Stem number per clump at harvest	Dry straw (g/clump)	Total biomass (g/clump)
M0 (without AMF)	90.47 a	25.43 b	20.92 b	24.33 a	29.65 b	67.48 b ¹⁾
M1 (with AMF)	91.58 a	27.08 a	23.38 a	25.38 a	37.08 a	88.02 a
Tukey's HSD 0.05	5.92	1.34	1.33	1.12	0.96	5.13
G04 (MG4)	93.07 a	25.27 a	20.50 a	23.33 a	31.18 b	69.41 c
G10 (MG10)	89.37 a	27.17 a	23.25 a	25.83 a	32.90 ab	76.37 b
G15 (AM4)	88.17 a	25.62 a	21.33 a	24.08 a	33.14 ab	78.02 b
G21 (AM10)	93.50 a	26.97 a	23.50 a	26.17 a	36.24 a	87.21 a
Tukey's HSD 0.05	13.64	4.88	3.41	3.70	4.05	5.95

¹⁾ Mean values followed in each column by the same letters are not significantly different between levels of a treatment factor based on its Tukey's HSD value at 5% level of significance

Based on their yield components, it also can be seen from Table 3 that the amphibious promising lines, i.e. AM10 (G21) and AM4 (G15) genotypes produced higher weight of 100 grains than the upland promising lines (G04 and G10). Average grain yield per clump was also higher on the amphibious lines, especially on AM10, than on the upland lines. This could be due to the higher growth, i.e. total biomass weight on the G15 than the upland lines (Table 2). From the results of correlation analysis between variables, grain yield per clump showed significant positive correlation with tiller number, filled panicle number, stem number, dry straw weight, percentage of panicle number, number of filled grains, total biomass, and harvest index per clump with a correlation coefficient of 0.521, 0.842, 0.635, 0.765, 0.713, 0.948, 0.974, and 0.776, respectively (Table 4). This means that grain yield per clump was highly correlated with those observation variables, and when the R^2 values were calculated from the corresponding correlation (r_{XY}) coefficients, some high percentages of contribution

were obtained. For example, the R^2 value from correlation between total biomass and grain yield per clump is 0.949 or 94.9%, which means that the variation in grain yield per clump was 94.9% contributed by variation in total biomass per clump, in which the total biomass per clump was a combination of dry grain yield per clump and dry straw per clump. Similarly, variation in dry straw weight contributed 58.5% to variation in grain yield per clump (Table 4).

Table 3. Averages of percentage of panicle number, filled grain number, percentage of unfilled grain number, grain yield per clump, weight of 100 grains, and harvest index

Treatments:	%-panicle number	Filled grain number	%-unfilled grain number	Grain yield (g/clump)	Weight of 100 grains (g)	Harvest index (%)
M0 (without AMF)	85.83 b	1474.33 b	7.49 a	37.83 b	2.56 a	55.80 a ¹⁾
M1 (with AMF)	92.18 a	1966.50 a	5.49 b	50.95 a	2.59 a	57.70 a
Tukey's HSD 0.05	4.67	213.30	1.89	5.04	0.09	2.88
G04 (MG4)	87.87 a	1548.67 a	7.06 a	38.24 b	2.47 b	54.71 a
G10 (MG10)	90.08 a	1808.50 a	5.82 a	43.46 b	2.41 b	56.89 a
G15 (AM4)	88.27 a	1653.00 a	5.28 a	44.88 ab	2.71 a	56.90 a
G21 (AM10)	89.80 a	1871.50 a	7.80 a	50.98 a	2.73 a	58.51 a
Tukey's HSD 0.05	7.76	384.80	2.75	6.85	0.17	5.14

¹⁾ Mean values followed in each column by the same letters are not significantly different between levels of a treatment factor based on its Tukey's HSD value at 5% level of significance

Table 4. Correlation coefficient (rXY), its p-value and R² of correlation between grain yield per clump (Y) andtiller number, filled panicle number, stem number, dry straw weight, %-panicle number, filled grainnumber, total biomass, or harvest index per clump

Grain vield (Y)	Tiller number	Filled panicle	Stem number	Dry straw weight	%-panicle	Filled grain	Total biomass	Harvest index
Jiela (1)	number	number	number	weight	number	number	biointabb	mucA
r _{XY}	0.521	0.842	0.635	0.765	0.713	0.948	0.974	0.776
p-value	0.009	0.000	0.001	0.000	0.000	0.000	0.000	0.000
\mathbf{R}^2	0.271	0.709	0.403	0.585	0.508	0.899	0.949	0.602

In terms of the effects of AMF application on growth of the red rice promising lines, the AMF application significantly increased tiller number per clump followed by increased filled panicle number, percentage of panicle number, stem number, dry straw weight, and total biomass weight (Table 2 & Table 3). These could happen because of the capability of AMF in symbiosis with rice plants to increase the rates of water and nutrient uptake by rice plants, as has been reported by many previous researchers. Dhillion and Ampornpan (1992) reported higher uptake of P, K, Ca, Fe, Cu, Na, B, Zn, Al, Mg and S by inoculated rice seedlings compared with non-inoculated ones. Solaiman and Hirata (1995, 1996) also reported better nutrition, including N, P, K, Zn, Cu, Fe, Mn, and higher dry matter production and grain yield of AMF inoculated rice plants than the non-inoculated ones. As what has been done by Solaiman and Hirata (1995, 1996) in their research, in this research the AMF application or inoculation was also done at planting the seeds, so that AMF infection should have been started from the beginning of the vegetative growth stages. Therefore, the contribution of AMF to better growth of rice plants due to better nutrition resulted from AMF inoculation can be seen from the beginning of vegetative growth stages, for example in the forms of higher tiller number in the AMF inoculated rice plants, which was then followed by higher number of filled panicles, higher number filled grains, and finally higher grain yield per clump, when compared with the non-inoculated rice plants (as can be seen from Table 2 & Table 3).

Although there is a possibility that rice plants growing in more aerobic soils will face lower availability of P, Fe, and Zn for rice (Bowman et al., 2007a), AMF association with rice plants should be better in aerobic soil conditions than in flooded conditions, and this better AMF symbiosis would ensure better P nutrition of AMF inoculated rice plants (Solaiman and Hirata, 1995, 1996). The most common benefit of establishing a good symbiosis with AMF reported in the literature is better P nutrition for the host plants (Smith and read, 2008), and better P nutrition of the AMF inoculated rice plants in this case must have been the main factor in increasing grain yield per clump which could be resulted from higher rates of assimilate partition to the

developing seeds (lower percentage of unfilled grain number and/or higher number of filled grains per clump supported by higher filled panicle number per clump) due to AMF application at planting the pre-germinated rice seeds (Table 2). Dobermann et al. (1998) also reported that rice yield increase of IR72 cultivar can be up to 55% due to P addition in the fertilizers applied, in which P efficiency ranged from 220 to 900 kg grains per kg P applied in one site, and around 70% of the total P in above-ground biomass can be found in the grains. Although it was not stated how P application increased grain yield of the rice cultivar used by Dobermann et al. (1998), since 70% of the total P recovered in the seeds, it could be possible that the major function of P applied was to increase filled grains or to reduce unfilled grain number. It can be seen from Figure 1, grain yield response of the genotype G04 to AMF application was much higher than that of G10 although the filled grain number was the lowest on G04 (Table 3), which was much lower than that of G10. This was most probably due to the highest response of the genotype G04 to AMF application in relation to the percentage of unfilled grain number (Figure 2).



Figure 1. Average (Mean ± SE) of grain yield (g/clump) for each combination of red rice genotype and AMF application



Figure 2. Average (Mean \pm SE) of percentage of unfilled grain number for each combination of red rice genotype and AMF application

IV. Conclusion

It can be concluded that application of bio-fertilizer containing AMF at planting of pre-germinated rice seeds significantly increased grain yield of several promising lines of red rice grown in aerobic rice system on raised-beds most probably due to increases in tiller number, filled panicle number, total biomass, and filled grain number per clump while reducing percentage of unfilled grain number due to AMF application.

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