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Effects of Inoculation with Arbuscular Mycorrhizal Fungi on Growth and Yield of Promising Line of Red Rice Grown together with Several Peanut Varieties

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Abstract: *This study aimed to examine whether inoculation with Arbuscular Mycorrhizal Fungi (AMF) and planting together with several peanut varieties affect growth and yield of red rice grown in an aerobic system. The pot culture experiment was carried out in a plastic house, from June to September 2017, designed according to Completely Randomized Design with factorial arrangement of the two treatment factors tested, i.e.: AMF inoculation (M0 = without or M1 = with AMF inoculation) and peanut varieties (V1= Biawak, V2= Hypoma-1, V3= G300-II promising line, V4= Wajik, and V5= local Bima). Each treatment combination was made in three replications. The results indicated that AMF inoculation had significant effect in increasing rice growth, panicle number, number and weight of filled grains per pot as well as decreasing the percentage of unfilled grains, while peanut varieties only had a significant effect on panicle number, number and weight of filled grains per pot. However, there were significant interactions between AMF inoculation and peanut varieties on panicle number, panicle length, as well as number and weight of filled grains of rice per pot. On the AMF inoculated pots, the highest grain yield was on the red rice grown together with the peanut V3 ("G300-II" promising line) while on the uninoculated pots, there was a tendency of grain yield to be highest on the red rice grown together with the peanut V4 ("Wajik" variety).*

Keywords: *Red rice, intercropping, peanut, arbuscular mycorrhiza*

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I. Introduction

Red rice is known as functional food because of its anthocyanin content having a high antioxidant activity essential for maintaining human health, and as daily food ingredients because of its nutritional content including carbohydrates, lipids, proteins and minerals (Rohman et al., 2014; Murdifin et al., 2015). The red color in red rice is due to the anthocyanin pigment content (Fasahat et al., 2012; Anggraini et al., 2015). However, most of the red rice varieties are varieties of upland rice generally grown on dry land with mostly low in average grain productivity (Aryana and Wangiyana, 2016). Therefore, it is necessary to create red rice production technology that is more productive and capable of maintaining the sustainability of production systems, one of which is the application of aerobic rice system (ARS), which is recently developed (Prasad, 2011). Unlike the conventional technology of growing rice crops where rice in grown in continuously flooded previously puddled soil, in ARS rice is grown on non-puddled soil without flooded conditions (Prasad, 2011).

Since the system is not flooded, then one of the advantages of applying ARS in growing rice is that rice crop can be intercropped with legume crops, which have the potential to fix N_2 from atmosphere through biological nitrogen fixation (BNF), and according to Peoples et al. (1995), BNF can be primary source of N for agriculture, and through establishment of symbiosis with *Rhizobium* bacteria, legume crops are capable of increasing soil N content via BNF. Therefore, maintaining or increasing yield could be achieved by increasing the use of BNF in agriculture systems (Figueiredo et al., 2013). Since the source of N in the BNF by legume-*Rhizobium* symbiosis is N_2 from the atmosphere, then the legume crops need to be grown in aerobic soil conditions in order for the N_2 gas to reach the legume root nodules. Therefore, aerobic rice system is one of the potential technologies in maintaining or increasing rice production while maintaining soil fertility when compared with the use of conventional techniques of growing rice. However, to be efficient in fixing N_2, the nitrogen fixation process in root nodules requires high amount of ATP so that higher P supply, either through fertilization or mycorrizal association, is required by legume crops (Ibijbijen et al., 1996; Figueiredo et al., 2013).

From the results of a pot experiment, rice crops grown together with soybean (Anjasmoro variety) in an aerobic system produced much higher grain yield compared with the flooded rice system in the conventional rice growing technique, in addition to soybean seed yield (Dulur et al., 2016). Farida et al. (2016) also reported that red rice crop grown in an aerobic system on raised beds relay-planted with groundnut plants (Hypoma-1 variety) that were inserted 2 or 3 weeks after planting of rice between double or triple-row of red rice plants produced higher numbers of tiller and panicles per clump compared with rice plants grown in aerobic system but without intercropping with peanut plants. This means that insertion of one row of peanut plants between the double or triple rows of rice plants has a significant contribution to better growth and higher panicle production of the rice crops grown in an aerobic system.

This study aimed to examine the effect of inoculation with propagules of arbuscular mycorrhizal fungi (AMF) on growth and yield of red rice crops grown together with several varieties of peanut in an aerobic system, in order to find the best peanut variety for intercropping with red rice.

II. Material And Methods

In this research, the pot culture experiment was carried out from early June to end of September 2017, in a plastic house built on farmer's land in Kediri village (West Lombok, Indonesia). The experiment was designed according to Completely Randomized Design, with factorial arrangement of the treatments, consisting of two treatment factors, i.e. AMF inoculation (M0 = without, or M1 = with AMF inoculation), and peanut varieties grown together with rice cops (V1 = Biawak, V2 = Hypoma-1, V3 = G300-II promising line, V4 = Wajik, and V5 = local Bima). Each treatment combination was made in three replications.

Rice plants were grown in pots from plastic buckets filled with 6 kg of air-dried soil that has been sieved with a 2 mm sieve-opening diameter. The soil was taken from the farmer's paddy field on which the plastic house was built. Before being filled with the air-dried soil, the pots were punctured from the side of the pots about 1 cm above the bottom of the pot as many as 4 holes around for use as the entry holes of irrigation water (sub-irrigation). The red rice line used was the promising amphibious red rice line G4 (F2BC4A86-3) or Am-G4 (Aryana and Wangiyana, 2016). Two days before planting, soil in the pots was watered up to field capacity. The pre-germinated rice seeds were dibbled in a planting hole made in the center of the soil surface in the pot (3-4 seeds per hole), in which 5 g of Technofert (a biofertilizer containing mixed species of AMF in zeolit growing media) was placed in the bottom of the planting hole (for the M1 treatment only), and pre-germinated rice seeds were placed on it, then thin covered with soil from the pot. After planting rice seeds, all pots were placed in a plastic-sheet-covered wooden tub filled with standing water with water surface reaching 1-2 cm above those side irrigation holes of the pots (sub-irrigation holes).

Rice crops were fertilized with "Phonska" fertilizer (containing NPKS 15-15-15-10) of 300 kg/ha (or 1.2 g/pot) at 1 WAP and followed with Urea (45% N) application of 100 kg/ha (or 0.4 g/pot) only at 50 WAP, to show the effect of intercropping with peanut (normally at 30 and 50 WAP with 100 kg/ha each). Peanut seeds (3-4 seeds) were planted 3 weeks after planting (WAP) of rice seeds, with a distance of 5 cm beside the rice plant, on the opposite side of the Phonska position, in the same way of seeding rice (either inoculated or not inoculated with AMF). Unlike rice, peanut plants were fertilized only with Phonska fertilizer of 200 kg/ha at 1 week after planting peanut, by dibbling the fertilizer on the furthest side of rice clump in the pot, at 5 cm distance from the peanut plant around 7 cm deep. On the day of Phonska application, thinning was done by leaving only 2 plants per pot either for rice or peanut.

Observations were made on plant height, leaf number, and tiller number, measured weekly. Harvest of rice was done after panicle achieved physiological maturity, which was at the age of 100 days after seeding. The yield components measured were panicle number, panicle length, number of filled and unfilled grains, and weight of dry filled grains per pot. Data were analyzed with analysis of variance (ANOVA) and HSD test at 5% level of significance, using CoStat for Windows ver. 6.303.

III. Results And Discussion

The results of ANOVA summarized in Table 1 show that there were significant interaction between the two treatment factors but only on panicle number per pot, panicle length, number of filled grains, and weight of filled grains per pot. The results in Table 1 show that the AMF inoculation factor had more dominant effects on growth and yield components of the red rice plants compared with the peanut varieties used, but overall, there were significant interactions between the two factors, especially on the yield components of the red rice crops. This means that, in terms of those yield components, the response of rice plants to AMF inoculation depends on the varieties of peanuts grown together with the rice plants in an aerobic system.

The existence of those interaction effects can be clearly seen in the results of measurement of panicle number per pot at harvest (Fig. 1) and weight of filled grains per pot (Fig. 2). From Fig. 1 it can be seen that the number of red rice panicles per pot was significantly different between the peanut varieties grown together with the rice plants if the pots for growing rice and peanut plants were inoculated with AMF, whereas in the treatments without inoculation with AMF, the number of panicles per pot was not significantly different among peanut varieties grown together with the rice plants. In contrast, in relation to the weight of filled grain per pot, there were differences in rice grain yields among peanut varieties grown together with the red rice crop and between AMF inoculation treatments. In the AMF inoculated pots, rice grain yield was highest on red rice plants grown together with the peanut variety V3 (i.e. the peanut promising line "G300-II") while in the pots without AMF inoculation, there was a tendency for rice grain yield to be highest on the red rice plants grown together with the peanut variety V4 ("Wajik" variety). However, it is clear that the effect of mycorrhiza (AMF) inoculation was much more dominant than that of peanut varieties, so that rice grain yields are generally higher on the rice plants inoculated with AMF compared with on the non-inoculated rice plants.

Table 1. Summary of ANOVA results on all observation variables

Observation variables	Mycorrhiza (AMF)		Peanut varieties		Interaction	
	p-value	sig.	p-value	sig.	p-value	sig.
AGR (average growth rate) of plant height	0.0001	***	0.2979	ns	0.8840	ns
Maximum plant height	0.0116	*	0.4529	ns	0.1297	ns
AGR of tiller number	0.0001	***	0.9389	ns	0.5177	ns
Tiller number at flowering	0.0000	***	0.8784	ns	0.8905	ns
AGR of leaf number	0.0000	***	0.5788	ns	0.2656	ns
Leaf number at flowering	0.0000	***	0.2824	ns	0.2980	ns
Panicle number per pot at maturity	0.0000	***	0.0236	*	0.0040	**
Average panicle length (cm)	0.8186	ns	0.0501	ns	0.0053	**
Total filled grains per pot	0.0000	***	0.0037	**	0.0013	**
Percentage of unfilled grains	0.0014	**	0.6659	ns	0.3207	ns
Weight of dry 100 filled grains (g)	0.5517	ns	0.1130	ns	0.5201	ns
Total weight of dry filled grains per pot (g)	0.0000	***	0.0108	*	0.0015	**

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

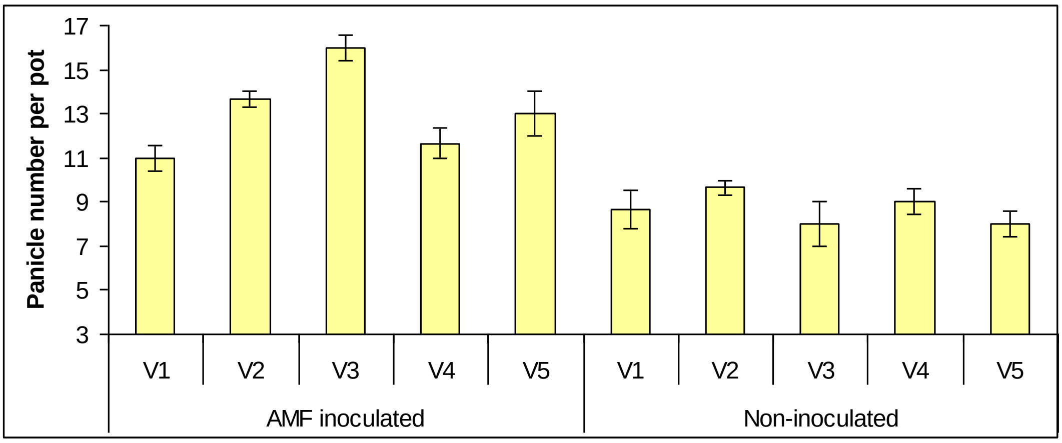

Fig. 1. Average (Mean ± SE) number of rice panicles per pot at harvest between treatment combinations

In terms of the main effects of AMF inoculation in which no significant effects of interaction, it can be seen from Table 2 that inoculation of red rice plants with AMF inoculant significantly increased red rice growth. In contrast, AMF inoculation significantly reduced percentage of unfilled grains, in which red rice plants inoculated with AMF inoculant produced significantly lower percentage of unfilled grains compared with those not inoculated with AMF (Table 3). This means that AMF inoculated rice plants produced significantly more percentage number of filled grains.

Based on regression analysis to show the relationship between percentage of panicle number per pot as the predictor and percentage number of unfilled grain per pot, the results show a negative relationship, with an R^2 = 25.12% and regression equation Y= 9.46 – 0.39 X (p-value = 0.005). This means that the higher the panicle number per pot then the higher the percentage of unfilled grain. Fortunately, AMF inoculation reduced the percentage of unfilled grains. This could be related to nitrogen supply to the rice plants during their grain filling stage. Wei et al. (2011) suggested that delaying N-dressing for longer photosynthesis could increase grain yield of the aerobic rice variety HD297. This is in line with Sinclair and de Wit (1975), who found that grain yield of a grain producing crop is related to N supply to the developing seeds during the grain filling stage.

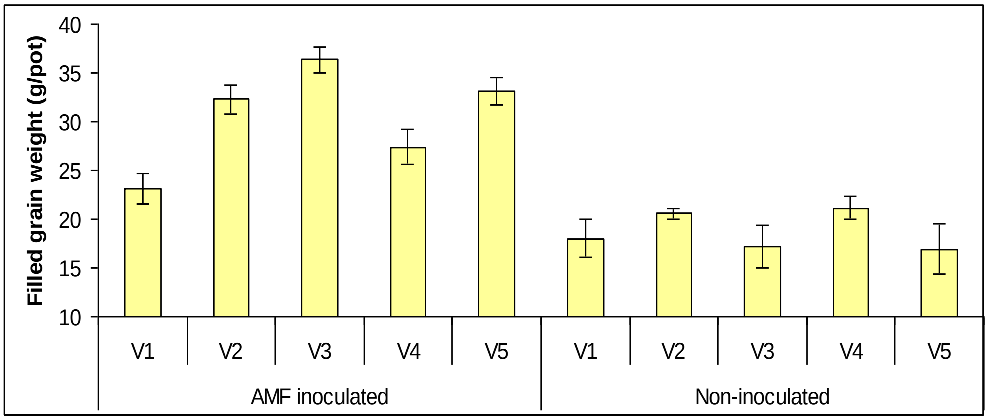

Fig. 2. Average (Mean ± SE) weight (g) of rice filled grains per pot between treatment combinations

Table 2. Main effects of AMF inoculation on growth of red rice plants growing together with several varieties of peanut in aerobic system

AMF inoculation	AGR of plant height (cm/week)		Plant height 11 WAS (cm)		AGR of tiller number per week		Tiller number 10 WAS		AGR of leaf number per week		Leaf number at flowering (10 WAS)	
M0	12.25	b	92.96	b	1.87	b	12.07	b	6.25	b	47.87	b
M1	14.02	a	99.35	a	3.31	a	18.33	a	12.76	a	76.87	a
HSD 5%	0.77		4.80		0.59		2.27		1.68		9.44	

Remarks: Means in the same column followed by the same letters are not significantly different between AMF inoculation treatments

Table 3. Main effects of AMF inoculation on yield components of red rice plants growing together with several varieties of peanut in aerobic system

AMF ino-culation	Panicle number per pot		Average panicle length (cm)		Filled grain number per pot		% unfilled grain number		Weight of 100 filled grains (g)		Grain yield (g/pot)	
M0	8.67	b	19.44	a	720.87	b	6.44	a	2.60	a	18.78	b
M1	13.07	a	19.39	a	1180.73	a	3.96	b	2.58	a	30.44	a
HSD 5%	0.91		0.46		76.57		1.39		0.09		2.22	

Remarks: Means in the same column followed by the same letters are not significantly different between AMF inoculation treatments

In addition, in relation to mycorrhizal symbiosis, mycorrhizal plants are capable of exploring and absorbing more nutrients from soil, compared with non-mycorrhizal plants, due to the capability of AMF external hyphae to explore more volume of soil and to absorb more nutrients (Smith and Read, 2008). Mycorrhizal rice plants are also reported to be capable of absorbing more P, N, K (Solaiman and Hirata, 1995), as well as some micronutrients such as Zn, Cu, Fe and Mn from soil compared with uninoculated rice plants (Solaiman and Hirata, 1996). Since the rice plants, in this research, grew together with peanut plants, which were also AMF inoculated in the M1 treatments, and peanut is a leguminous crop, which is capable of establishing a tripartite symbiosis like soybean (Antunes and Goss, 2005), then the AMF inoculated pots, where rice and peanut plants grew together, could have higher N nutrition, in addition to higher P nutrition.

In an intercropping between rice and peanut plants, many have reported that there is N transfer from peanut to rice (Chu et al., 2004; Shen and Chu, 2004). With a tripartite symbiosis or dual inoculation, i.e. symbiosis with AMF for more nutrient absorption, especially Phosphorous and other immobile nutrients, and with *Rhizobium* bacteria for N_2 fixation through formation of root nodules, then it is expected that those peanut plants were able to absorb more nutrients and fix more N_2 from the atmosphere, compared with those growing on the pots with no AMF inoculation, as has been reported by Khan et al. (1995). With rice and peanut plants growing together in the pots inoculated with AMF, the presence of AMF could more facilitate N transfer from peanut to rice plants because of a high possibility for roots of both plants to grow in a very close proximity, and this can facilitate infection of roots from both plants to build hyphal bridges, and those types of AMF hyphae were reported to facilitate interspecific N-transfer (Hamel and Smith, 1991; Smith and Read, 2008). Even without AMF inoculation, Chu et al. (2004) reported that 11.9% of the total N accumulated in rice plants was from N transfer from peanut plants grown in intercropping with the rice plants. As can be seen from Fig. 2, grain yield of the red rice was significantly different between varieties of peanut grown together with the rice plants, in which grain yield of red rice was highest on rice plants intercropped with the V3 peanut (G300-II promising line) on the pots inoculated with AMF. Therefore, further research needs to be carried out on the combinations of these crop species.

IV. Conclusion

It can be concluded that AMF inoculation significantly increased growth and grain yield of red rice crops grown together with several varieties of peanut when compared with those without AMF inoculation, and the highest rice grain yield was obtained on rice plants grown together with the peanut promising line "G300-II". Therefore, further research need to be carried out on the combinations of these crop species in order to formulate better production technologies for those crops, especially for the red rice.

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