Increasing Yield Components of Several Promising Lines of Red Rice through Application of Mycorrhiza Bio-Fertilizer and Additive Intercropping with Soybean in Aerobic Irrigation System

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***Abstract*— *This study aimed to examine the effect of application of a bio-fertilizer containing arbuscular mycorrhizal fungi (AMF) and additive intercropping with soybean relay-planted between double rows of rice on growth and yield components of various promising lines of red rice grown in aerobic irrigation systems on permanent raised-beds. The field experiment, carried out on farmers' riceland in Beleke village of Gerung district in West Lombok, Indonesia, from May to September 2018, was designed according to Split Split-Plot design with three blocks and three treatment factors, i.e. intercropping (T0= without; T1= with soybean) as the main plot factor, red rice promising lines with 4 genotypes (G1= MG4, G2= MG10, G3= AM4, G4= AM10) as the sub-plot factor, and AMF bio-fertilizer (M0= without; M1= with AMF) as the sub-sub-plot factor. The pre-germinated red rice seeds were planted on permanent raised-beds, with a base spacing of 25x20 cm, which was then modified into a double-row pattern of 20x20 cm within the double-row and 30 cm between double-rows. The results indicated that application of the bio-fertilizer "Technofert" containing AMF significantly increased growth and yield components of various promising lines of red rice, especially in relation to filled grain number and grain yield per clump, but significantly reduced percentage of unfilled grain number. Additive intercropping with soybean that was relay planted between double-rows of rice (one week after seeding rice) also significantly increased grain yield of the red rice genotypes. Among the four selected genotypes, grain yield of AM10 was the highest, especially when bio-fertilized with AMF and intercropped with soybean.*

***Keywords*— *red rice, aerobic rice system, arbuscular mycorrhiza, intercropping, soybean*.**

I. INTRODUCTION

In general, rice is grown on irrigated rice fields with a flooded system (conventional technique of growing rice), and even farmers in Indonesia in fully irrigated areas generally provide water for their rice crop by continuous flow of irrigation water. Because the land is flooded, and the irrigation water even flows between rice paddocks, then the need for irrigation water for rice production using the conventional technique is very high. For example, from the results of Yaligar *et al*. [1], in rice cultivation with conventional techniques, i.e. the inundated irrigation system commonly practiced by farmers, the total use of irrigation water reached 20260 m^3/ha, while with dry seeded direct planting it was only 4260 m^3/ha.

In addition to inefficient or high use of irrigation water in rice production systems under flooded irrigation or conventional cultivation techniques, the inundated system could cause a lot of disadvantages. These could include pollution of water bodies in the downstream area due to seepage, runoff and percolation from paddy water during the maintenance process for the rice plants, such as after spraying pesticides to control pests / diseases / weeds, and after applying fertilizers. Because of the anaerobic condition due to flooding, rice fields of the conventional cultivation techniques also result in emissions of methane and N_2O gasses [2]. Nitrogen fertilization, which is generally done using Urea fertilizer, is also inefficient in flooded rice because of high N loss through ammonia volatilization, denitrification, and leaching, while most rice soils are acute N deficiency [3]. According to Buresh *et al.* [4], the total gaseous N loss from Urea broadcasted to flooded rice was 34% in Thailand and 31% in Indonesia after 10 days of application, and the majority of the N loss from Urea applied was through ammonia volatilization, which reached up to 88% of the total N loss [5]. The conventional technique of rice cultivation is also a source of P leaching in addition to N leaching, which all contributes to pollution of downstream areas [6].

To avoid those disadvantages of flooding in growing rice, a less water-consuming cultivation technique of growing rice has been developed, i.e. the aerobic rice systems (ARS), which term was coined at the IRRI; and with the ARS, rice is grown under nonflooded, nonpuddled and nonsaturated soil conditions [7]. Unfortunately, when using irrigated rice varieties, grain yields are normally reduced under the ARS; therefore, specific varieties must be developed to have high yielding rice varieties under the aerobic rice systems, such as those developed in North China, which yield up to 6-7.5 t/ha [7]. However, when rice is grown in monoculture under aerobic rice systems, grain yields normally declined; so that it is suggested to have some rotations with legume crops [8].

Because rice is grown under nonpuddled and nonflooded soil conditions in aerobic rice systems, then it is also possible to grow rice plants in intercropping with legume crops. Many have reported that intercropping cereal crops with legume crops can provide some benefits for both crops. Inal *et al.* [9] reported that intercropping maize with peanut significantly increased chlorophyll and P content of maize. Chu *et al.* [10] also reported that chlorophyll and N contents as well as weights of dry matter per plant, spikelet per plant and 1000 seeds were higher in rice plants intercropped with peanut compared with rice plants in monocrop, and they measured significant transfer of N from peanut to rice in the intercropping systems.

In addition to intercropping with legume crops, there could be much better opportunities of using crop symbiosis with beneficial soil microbes such as arbuscular mycorrhizal fungi (AMF) and *Rhizobium* sp to manage plant nutrients for better crop nutrition under aerobic rice systems, in which soil is not flooded, when compared with under the conventional technique of growing rice, in which soil is normally flooded. Solaiman and Hirata [11] reported that AMF development (infection, colonization, and sporulation) was much better in rice inoculated under dry nursery than under wet nursery, which resulted in higher grain number and grain yield per clump on AMF inoculated rice plants than the uninoculated ones, especially those inoculated in the dry nursery. Wangiyana *et al.* [12] also reported that AMF inoculation significantly increased filled panicle number and grain yield of a promising red rice line grown together with several varieties of peanut in pot under aerobic rice system, and the magnitudes of the AMF inoculation effects were different between different varieties of peanut growing together with the red rice plant.

The aim of this research was to examine the effects of AMF inoculation on several promising lines of red rice additively intercropped with soybean plants inserted between double rows of rice plants grown on permanent raised beds under aerobic irrigation system.

II. MATERIALS AND METHOD

The field experiment was conducted on farmers' rice-land in Beleke Village of Gerung District of West Lombok Regency (Indonesia), from May to September 2018. The experiment was designed according to Split Split-Plot design with 3 blocks, to test three treatment factors, namely intercropping (T) with soybeans as the main plot factor, consisting of two levels of treatment (T0 = without, and T1 = relay planting of one soybean row planted between the double-rows of rice plants one week after planting pre-germinated rice seeds); promising lines of red rice (G) as the subplot factor, consisting of 4 genotypes selected from previous research (G04= MG4, G10= MG10, G15= AM4, and G21= AM10); and application of “Technofert” bio-fertilizer containing mixed species of AMF (M) as the sub-sub-plot factor, consisting of 2 levels of treatment (M0 = without, and M1 = with “Technofert” application at the time of planting the pre-germinated rice seeds). Red rice plants were grown on permanent raised-beds, with a base plant spacing of 25 x 20 cm, which was then modified into double-row patterns, as also has been explained in Wangiyana *et al.* [13], and the spatial arrangement of the row patterns for the double rows of rice plants and inserted single rows of soybean is as sown in Figure 1.

Formation of raised-beds, planting of pre-germinated red rice seeds, fertilizer application, mycorrhiza application, application of irrigation water, and harvest of the red rice were done exactly the same as those explained in Wangiyana *et al*. [13], except for the dose of Phonska (NPK 15-15-15) fertilizer, which was full doses in this experiment. The red rice genotypes used were also the same as those explained in Wangiyana *et al.* [13], which were previously selected from 11 promising lines of upland red rice genotypes and 12 promising lines of amphibious red rice genotypes [14]. The other difference was that there was an intercropping treatment in this experiment, i.e. additive intercropping those red rice genotypes with soybean var. Dering-1, which seeds were supplied by “Balitkabi” research institute in Malang, East Java, Indonesia. Soybean was relay-planted 7 days after sowing (DAS) of red rice, between double-rows of rice plants as sown in Fig. 1.C. After emergence, soybean plants were tinned by leaving only two soybean plants per planting hole, and after that soybean was fertilized with Phonska by dibbling the fertilizer beside the soybean plants within the soybean row with a recommended dose of 200 kg/ha.

Observation variables included the growth of rice plants consisting of plant height, number of tillers and panicles per clump, percentage of panicle number and weight of dry straw per clump, and rice yield components consisting of number of spikelet and filled grains per clump, percentage of unfilled grain number, weight of 100 filled grains, and filled grain weight (or grain yield) per clump. Data were analyzed with Analysis of Variance (ANOVA) and Tukey's HSD at 5% level of significance, using the statistical software CoStat for Windows ver. 6.303. In addition, correlation and regression analyses were also conducted to examine the degree of relationship between selected observation variables, using Minitab for Windows Rel. 13.0.

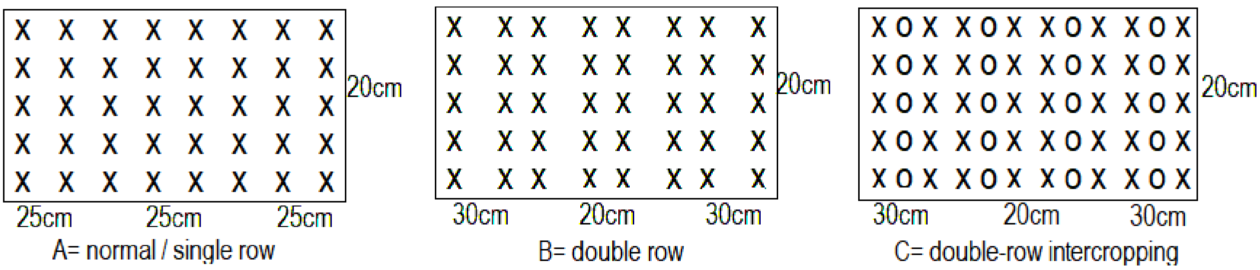

Fig.1. The schematic planting geometry of rice and soybean in the additive relay intercropping treatment (x = rice plants; o = soybean plants)

III. RESULTS AND DISCUSSION

Results of data analysis of growth variables (Table 1) and yield components (Table 2) of the red rice using ANOVA indicate that application the “Technofert” bio-fertilizer containing arbuscular mycorrhizal fungi (AMF) had the strongest effects, in which this treatment factor shows significant effects on all observation variables. Intercropping treatment also shows significant effects but only on straw dry weight, grain number, filled grain number, and grain yield per clump, while genotypes of the red rice show differences only in panicle number and dry straw weight per clump of the growth variables (Table 1), and in all yield components (Table 2). However, the interaction effects among the treatment factors were significant only on some observation variables, including three-way interaction effects on weight of dry straw, %-unfilled grain number, and grain yield per clump.

Table 1. Summary of ANOVA results on the effects of intercropping with soybean and mycorrhiza application on growth of red rice (plant height and tiller number per clump at anthesis; panicle number, %-panicle number, and weight of dry straw per clump)

Treatment factors and their interactions	Plant height at anthesis	Tiller number per clump at anthesis	Panicle number per clump	%-panicle number per clump	Weight of dry straw (g/clump)
Main effects:					
Intercropping	ns	ns	ns	ns	*
Genotypes	ns	ns	***	ns	***
Mycorrhiza	**	**	***	***	***
Interaction effects:					
Genotype x Intercrop	ns	ns	ns	ns	*
Mycorrhiza x Intercrop	ns	ns	*	ns	**
Mycorrhiza x Genotype	ns	ns	ns	ns	***
Myc x Geno x Intercrop	ns	ns	ns	ns	***

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

Table 2. Summary of ANOVA results on the effects of intercropping with soybean and mycorrhiza application on yield components of red rice (spikelet number, filled grain number, %-unfilled grain number, weight of 100 grains, and grain yield per clump)

Treatment factors and their interactions	Spikelet number per clump	Filled grain number per clump	%_Unfilled grain number	Weight of 100 filled grains (g)	Grain yield (g/clump)
Main effects:					
Intercropping	*	*	ns	ns	*
Genotypes	**	*	*	***	**
Mycorrhiza	***	***	***	***	***
Interaction effects:					
Genotype x Intercrop	ns	ns	ns	ns	ns
Mycorrhiza x Intercrop	**	**	ns	ns	**
Mycorrhiza x Genotype	ns	ns	ns	ns	ns
Myc x Geno x Intercrop	ns	ns	**	ns	*

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

Based on the main effects on the mean values of the observation variables, it can be seen from Table 3 and Table 4 that application of the AMF bio-fertilizer significantly increased growth and yield components of the red rice while reducing percentage of unfilled grains. This can occur because of the ability of AMF in helping their host plants, which in this case the red rice genotypes, to increase nutrient uptake and absorption of water from the soil [15], [16], [17]. Solaiman and Hirata [16] also indicated that rice plants inoculated with AMF increased dry matter partitioning from straw to grains, which increased filled grain number, when compared with uninoculated rice plants, as can also be seen from our results (Table 4) that AMF inoculated red rice resulted in higher number of spikelet and filled grains per clump but lower percentage of unfilled grain number, compared with those uninoculated with AMF bio-fertilizer.

In terms of the effect of intercropping red rice with legume crop by inserting one row of soybean plants (additive series) between double-rows of red rice one week after seeding rice, it can be seen that additive intercropping with soybean of Dering-1 variety increased dry matter production of the red rice plants (straw dry weight) (Table 3) and several yield components such as spikelet number and filled grain number, as well as grain yield per clump (Table 4), when compared with those of red rice plants grown in monocrop. From a previous pot experiment [18], it was clear that leaves of red rice plants that were planted together with soybean plants in one pot were much greener than those of red rice plants in monocrop. This indicated N contribution by soybean plants to red rice plants grown together in an intercropping system. Some researchers have also reported N contribution from legume crops to cereal crops in intercropping systems, such as from peanut to maize [9], from peanut to rice [10], and from soybean to sorghum [19], and the rates of N transfer from legumes to non-legumes will be greater when there is an involvement of AMF hyphae infecting roots of both legume and non-legume crops grown in an intercropping system [20], [21].

In relation to the interaction effects between the treatment factors, Fig. 2 shows the three-way interaction effect of AMF application, intercropping with soybean, and different genotypes of rice on grain yield of the red rice tested. It can be seen from Fig. 2 that grain yield of the G21 per clump was highest when the rice plants were bio-fertilized with AMF and additive intercropped with soybean, but when it was neither bio-fertilized nor intercropped with soybean, its grain yields were low. This indicates the interaction effects of application of AMF bio-fertilizer and intercropping with soybean, which was significant on grain yield of the red rice tested (Table 2). This could be due to the ability of soybean in establishing tripartite symbiosis, i.e. with AMF and *Rhizobium* bacteria, due to the application of AMF bio-fertilizer to the rice plants adjacent to the soybean plants, with a distance of only 15 cm between soybean and rice plant. According to the results obtained by Fujita *et al.* [19], the closer the distance between sorghum and soybean plants in intercropping, i.e. among four levels of planting distances tested (12.5 cm, 17.7 cm, 25 cm, and 50 cm), then the higher the rates of N transfer from soybean to sorghum. According to other previous research, this kind of N transfer is facilitated by the involvement of AMF that infect roots of both legume and non-legume crops in the intercropping system [20], [21].

Table 3. Average plant height, tiller number per clump at anthesis, panicle number, %-panicle number, and weight of dry straw per clump for each level of the treatment factors

Treatments	Plant height (cm) at anthesis	Tiller number per clump at anthesis	Panicle number per clump	%-panicle number per clump (%)	Weight of dry straw (g/clump)
M0: no AMF	89.90 b	25.93 b	21.23 b	87.40 b	30.00 b [1)]
M1: with AMF	95.23 a	27.63 a	24.88 a	93.87 a	39.05 a
HSD 0.05	3.33	1.09	0.99	3.23	1.36
G04	93.12 a	25.54 a	21.13 c	88.40 a	33.09 bc
G10	91.10 a	27.55 a	24.04 ab	91.73 a	35.35 ab
G15	90.03 a	26.47 a	22.71 b	90.59 a	32.15 c
G21	96.00 a	27.55 a	24.33 a	91.83 a	37.51 a
HSD 0.05	6.57	2.63	1.52	5.22	2.41
T0: monocrop	93.33 a	26.28 a	22.19 a	89.04 a	33.17 b
T1: intercropping	91.79 a	27.28 a	23.92 a	92.23 a	35.88 a
HSD 0.05	6.46	0.11	3.42	6.19	2.15

[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 at 5% level of significance

Table 4. Average grain number per clump, filled grain number per clump, %-unfilled grain number, weight of 100 grains, and grain yield per clump for each level of the treatment factors

Treatments	Spikelet number per clump	Filled grain number per clump	%_Unfilled grain number	Weight of 100 filled grains (g)	Grain yield (g/clump)
M0: no AMF	1774.2 b	1622.6 b	8.52 a	2.55 b	41.34 b [1)]
M1: with AMF	2120.7 a	2027.5 a	4.34 b	2.69 a	54.64 a
HSD 0.05	124.6	127.2	1.28	0.07	3.35
G04	1810.6 b	1710.1 ab	5.76 a	2.54 b	43.62 b
G10	2074.3 ab	1962.5 a	5.53 a	2.44 b	48.01 ab
G15	1805.3 b	1688.4 b	6.47 a	2.74 a	46.29 b
G21	2099.5 a	1939.3 ab	7.96 a	2.77 a	54.04 a
HSD 0.05	273.9	265.7	2.44	0.12	7.39
T0: monocrop	1678.4 b	1581.8 b	5.87 a	2.62 a	41.48 b
T1: intercropping	2216.4 a	2068.3 a	6.99 a	2.62 a	54.49 a
HSD 0.05	282.5	312.9	5.04	0.07	7.97

[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 at 5% level of significance

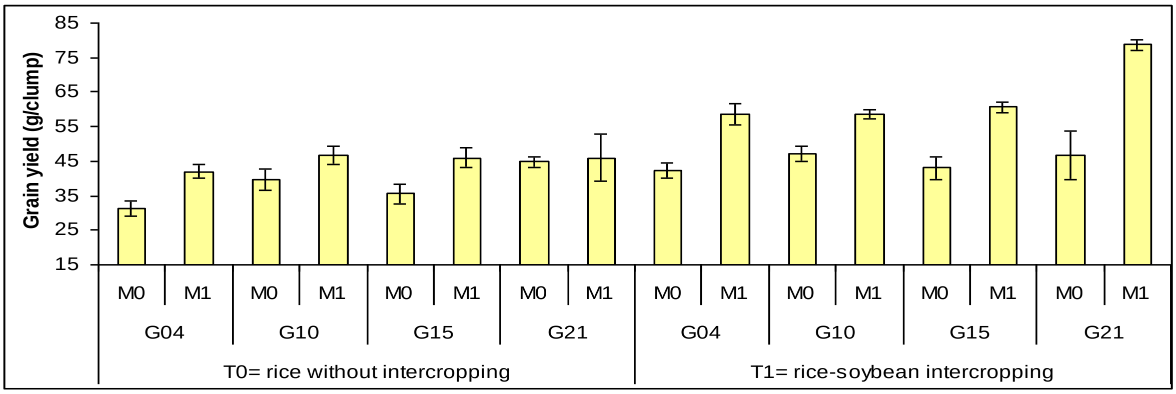

Fig. 2. Average (Mean ± SE) grain yield (gram/clump) of several promising lines of red rice as affected by application of bio-fertilizer containing AMF and additive intercropping with soybean in aerobic system

IV. CONCLUSION

It can be concluded that application of the bio-fertilizer "Technofert" containing AMF significantly increased growth and yield components of various promising lines of red rice, especially in relation to increased grain yield and decreased percentage of unfilled grain number. Relay intercropping of soybean between double-rows of red rice plants also significantly increased grain yield of red rice. Among the four selected genotypes, grain yield of AM-10 was the highest, especially when inoculated with AMF and intercropped with soybean.

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