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Yield performance of several promising lines of black rice as affected by application of mycorrhiza biofertilizer and additive intercropping with soybean under aerobic irrigation system on raised-beds

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Abstract. Mycorrhiza-biofertilizer and legume intercropping were reported to increase yield and anthocyanin content of red-rice. This study was conducted to investigate the effects of mycorrhiza-biofertilizer and soybean-intercropping in additive series on yield of black-rice of different genotypes in irrigated aerobic-systems on raised-beds. The experiment, conducted on farmer's ricefield from April to August 2021, was designed using SSP design, with black-rice genotypes (G3, G9, G4/15) as main plots; intercropping (T0= monocrop; T1= intercropping) as subplots, and mycorrhiza-biofertilizer (without and with mycorrhiza) as the sub-subplots. The results indicated that mycorrhiza-biofertilizer showed the most significant effects in increasing yield of black-rice followed by soybean-intercropping and genotypes. However, the significant interaction effects especially between mycorrhiza-biofertilizer and genotypes (M*G) indicated that G3 was not responsive but both G9 and G4/15 were highly responsive to application of mycorrhiza-biofertilizer with the highest grain yield (56.79 g/clump or 10.54 t/ha) was on G4/15 with biofertilizer and the lowest (27.48 g/clump or 5.5 t/ha) was on G9 without biofertilizer. Mycorrhiza-biofertilizer was also resulted in higher increase in grain yield under soybean-intercropping (31.9%) compared with under monocropped black-rice (15.5%).

1. Introduction

Black-rice is a functional food material that can help improving human health due to its anthocyanin content [1], and black-rice is the highest in anthocyanin content compared with red, golden and white rice [2]. In Indonesia, most varieties of black-rice are upland rice, and are mostly local cultivar [1]. Upland rice varieties normally yield less than irrigated rice [3]. However, grain yield of upland red-rice was higher under soybean-intercropping in additive series compared with under rice monocropping in irrigated aerobic systems on raised-beds [4, 5]. Additive intercropping with peanut on raised-beds in irrigated aerobic system also significantly increased yield of red-rice [6]. Anthocyanin content of whole grains in some red-rice genotypes was also higher under additive intercropping with soybean than under monocropped rice [5]. Black-rice-peanut intercropping in 2:2 row replacement series also significantly increased grain yield of black-rice [7].

Increases in growth, yield and nutrient contents of such crops as rice, maize, sorghum in legume-intercropping such as with soybean and peanut were mostly attributed to significant nutrient transfer, especially N, from legume rhizosphere to cereal plants, for example in sorghum-soybean intercropping [8], in rice-peanut intercropping [9, 10], in maize-peanut intercropping [11], and in maize-soybean



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intercropping [12]. Nutrient availability was higher in maize-peanut rhizosphere than in rhizosphere of monocropped maize or peanut resulting in increased uptake of those nutrients by the intercrops [11]. Mycorrhiza-biofertilized sweetcorn was much greener with more number of green leaves and higher weight of harvested fresh cobs when intercropped with peanut compared with monocropped sweetcorn plants without application of mycorrhiza-biofertilizer [13]. Many have also reported that symbiosis with mycorrhizal fungi increases nutrient transfer rates through hyphal bridge infecting roots of adjacent plants [14, 15, 12].

Mycorrhizal fungi association with roots of rice also reported to have beneficial effect on rice plants. Even in the seedling growth period, arbuscular mycorrhizal fungi (AMF) inoculation was reported to result in higher uptake of various nutrients by the rice seedlings and better seedling growth [16]. Solaiman and Hirata [17, 18, 19] also reported that mycorrhizal inoculation in the nursery and in the field significantly increased growth, yield, and nutrient uptake (N, P, Zn) by flooded and non-flooded rice plants, although the effects were more significant in non-flooded than flooded rice. These all indicated that rice plants are hosts of AMF. Biofertilizer containing AMF applied in the seed holes at planting also significantly increased red-rice growth and yield [4, 5, 20], as well as those of black-rice [7] under irrigated aerobic systems. This study was conducted to investigate the effects of mycorrhiza-biofertilizer application and intercropping different genotypes of black-rice in additive series with soybean on growth and yield of the black-rice under irrigated aerobic-systems on raised-beds.

2. Materials and Method

This experiment was carried out on an irrigated rice growing area located in Dasan Tebu, Ombe Baru village (-8.653912, 116.130813), South Kediri, West Lombok, Indonesia, from April to August 2021. Different genotypes of black-rice were planted on permanent raised-beds under irrigated aerobic-system, with bed design, planting geometry, and the cultivation techniques of the black-rice and soybean are as previously described [4], but in this study red-rice was replaced with black-rice. In the intercropping beds, soybean was relay-planted by dibbling soybean seeds of Dena-1 variety in additive series between black-rice twin-rows at two weeks after seeding of black-rice.

For treatment arrangement the SSP experimental design was used, in which the black-rice genotypes (G) were randomly placed in main plots (G3, G9, G4/15), intercropping (T) as subplots (T0= monocrop and T1= rice-soybean-intercropping), and mycorrhiza-biofertilizer (M) as sub-subplots consisting of without biofertilizer (M-) and with biofertilizer (M+). The mycorrhiza-biofertilizer applied was a commercial-biofertilizer with the trademark "Mycogrow" containing several AMF species.

The variables measured were growth and yield components, including height of rice plants and number of tillers at anthesis, dry straw weight, length and number of panicles, filled and %-unfilled grain number, 100-grain weight, and grain yield of black-rice per clump. ANOVA and Tukey's HSD at $p=0.05$ were used to analyze the data using CoStat Windows 6.303.

3. Results and Discussion

According to the ANOVA results in Table 1, mycorrhiza-biofertilizer affected on most variables measured, followed by soybean-intercropping, but the black-rice genotypes tested were different only in number of tillers and panicles as well as grain yield. However, interaction effect was also significant although it was only between mycorrhiza and genotypes on number of tillers and panicles, and grain yield, and between mycorrhiza and intercropping on grain yield of black-rice per clump. Table 2 further shows higher number of tillers and dry straw weight due to mycorrhiza-biofertilizer and soybean-intercropping. Black-rice plant height was also higher under intercropping than in monocropped black-rice. However, those genotypes were significantly different in average tiller number, which was highest in G4/15 (22.83 tiller/clump) than in G3 (18.17 tiller/clump) or in G9 (17.67 tiller/clump). Average panicle number per clump was also the highest in G4/15 (20.92 panicles)

than in G3 (16.58 panicles) on in G9 (16.08 panicles). Although they were not significantly different, however, %-unfilled grains tended to be the highest among those black-rice genotypes. Fortunately the average grain yield of black-rice per clump was still highest in G4/15 (Table 3).

Table 1 Summary of the effect of mycorrhiza-biofertilizer (M), intercropping (T), and genotypes (G), and their interaction effects (T*G; M*G; M*T; M*T*G) on the black-rice measurement variables

Variables	ANOVA p-values						
	G	T	T*G	M	M*G	M*T	M*T*G
Anthesis plant height	0.8955	0.0016	0.3748	0.5425	0.1963	0.4769	0.8180
Anthesis tiller number	0.0067	0.0003	0.0703	0.0006	0.0001	0.2358	0.2504
Dry straw weight	0.1196	0.0019	0.1924	0.0078	0.6550	0.1719	0.0514
Panicle length	0.4737	0.1341	0.3290	0.8750	0.1246	0.4201	0.4012
Number of panicles	0.0039	0.0000	0.1591	0.0008	0.0003	0.1522	0.2817
Number of filled-grains per panicle	0.7944	0.0453	0.0684	0.0088	0.2569	0.2223	0.2211
%-unfilled grain number	0.0548	0.4415	0.2193	0.0002	0.1404	0.0562	0.1664
Weight of 100 grains	0.1265	0.3412	0.7238	0.0003	0.0800	0.7663	0.6402
Dry grain yield per clump	0.0079	0.0019	0.2148	0.0001	0.0018	0.0473	0.7420

Table 2 Mean anthesis plant height, number of tillers, dry straw weight per clump, and panicle length for each treatment

Treatment	Anthesis plant height (cm)	Tillers (number/clump)	Dry straw weight (g/clump)	Average panicle length (cm)
M-: without Myc	85.06 a	18.61 b	24.46 b	22.11 a
M+: with Mycorrhiza	86.03 a	20.50 a	27.90 a	22.05 a
HSD	3.38	0.91	2.36	0.85
T0: monocrop	79.28 b	18.50 b	23.81 b	21.75 a
T1: intercropping	91.81 a	20.61 a	28.55 a	22.41 a
HSD	5.64	0.69	2.22	0.94
G3 line	85.21 a	18.17 b	29.03 a	21.88 a
G9 line	84.92 a	17.67 b	24.46 a	22.62 a
G4/15 line	86.50 a	22.83 a	25.04 a	21.75 a
HSD	12.61	3.07	6.45	2.49

The highest average grain weight per clump in the G4/15 genotype seems to be supported most strongly by the highest number of panicle per clump in G4/15. When Best Subset Regression (BSR) was done, grain yield showed the highest R² in the regression between grain yield and panicle number with an R² of 75.4% (p<0.001), followed with regression between grain yield and tiller number with an R² of 72.1% (p<0.001). When two X-variables were included in the BSR, the highest R² was obtained from the regression between grain yield (Y) and panicle number (X1) and filled grain number per panicle (X2) with an R² of 96.2% (p<0.001), with the regression equation $Y = -38.28 + 2.25 X1 + 0.39 X2$. These indicate that the correlation was highly significant between yield and both the X-variables. This means that those two X-variables are the most significant determinants for grain yield of black-rice in this study.

Based on the mean values in Table 3, these two variables are significantly higher in the black-rice supplied with mycorrhiza-biofertilizer at seeding compared with those without biofertilizer application, which means that application of mycorrhiza-biofertilizer was significant treatment for

increasing number of both panicles and filled-grains. Soybean-intercropping in additive series also shows similar effect. In addition, mycorrhiza-biofertilizer also increased 100-grain weight while reducing %-unfilled grains, which further supported for higher yield of black-rice per clump (Table 3).

Table 3 Mean numbers of panicles and filled-grains, %-unfilled grains, 100-grain weight, and grain yield for each treatment

Treatment	Panicles (number/clump)	Filled grains (number/panicle)	%-unfilled grains	100 grain weight (g)	Gain yield per clump (g)
M-: without Myc	16.97 b	89.82 b	14.91 a	2.23 b	34.79 b
M+: with Myc	18.75 a	100.07 a	11.25 b	2.38 a	43.21 a
HSD	0.87	7.16	1.53	0.07	3.33
T0: monocrop	16.47 b	91.63 b	13.40 a	2.32 a	35.15 b
T1: intercropping	19.25 a	98.25 a	12.76 a	2.29 a	42.85 a
HSD	0.62	6.43	1.89	0.07	3.57
G3 line	16.58 b	96.39 a	10.43 a	2.30 a	36.77 b
G9 line	16.08 b	94.58 a	14.39 a	2.27 a	34.27 b
G4/15 line	20.92 a	93.85 a	14.42 a	2.35 a	45.96 a
HSD	2.44	13.35	4.52	0.12	6.85

Table 4 Regression for yield (Y) vs number of panicles (X1) and filled grains per panicle (X2)

Parameter	Coefficient	SE	t-calc	Prob.
Intercept	-38.28	2.9212282	-13.1045	1.25E-14
Number of panicles per clump (X1)	2.25	0.1070212	21.0432	1.08E-20
Number of filled-grains per panicle (X2)	0.39	0.0290254	13.4472	6.07E-15

However, some interaction effects were also significant despite only M*G interaction on number of panicles and filled-grains per panicle and grain yield, and M*T interaction on grain yield (Table 1). The pattern of the M*G interaction in Fig. 1, Fig. 2 and Fig. 3 indicates different responses of black-rice genotypes to the treatment with mycorrhiza-biofertilizer, in which G3 was not responsive but both G9 and G4/15 showed significantly positive responses to application of mycorrhiza-biofertilizer. The pattern of the M*T interaction in Fig. 4 also indicates different responses of black-rice to mycorrhiza and intercropping, in which mycorrhiza-biofertilizer resulted in more increase in grain yield of black-rice under soybean intercropping (31.9%) compared with under monocropped black-rice (15.5%). Likewise, with mycorrhiza-biofertilizer, soybean-intercropping significantly increased grain yield of black-rice, while without mycorrhiza-biofertilizer, soybean-intercropping did not have a significant effect on yield of black-rice (Fig. 4).

The significantly positive effect of the mycorrhiza-biofertilizer on number of tillers (Table 2) must be associated with higher nutrient uptake by black-rice plants from the beginning of their vegetative growth stages and even in the nursery stage [16]. Rice plants inoculated with mycorrhizal fungi also had higher uptake of various nutrients compared with the uninoculated rice plants, and mycorrhizal fungi supported higher rice growth and nutrient uptake under non-flooded than flooded conditions [17, 18, 19]. In this study, black-rice was grown on non-flooded raised-beds, which means the rhizosphere conditions are favourable to development of mycorrhizal fungi. Previous studies also reported that with mycorrhiza-biofertilizer, the number of red-rice tillers and panicles per clump was higher than those without mycorrhiza-biofertilizer [4, 5, 20]. Table 3 also shows that higher number of panicles in the black-rice supplied with mycorrhiza-biofertilizer than without it is associated with higher number of filled-grains per panicle, higher 100-grain weight, and lower %-unfilled grains, and these all associated with higher grain yield of black-rice per clump as the consequence. This was confirmed by

the multiple regression analysis showing that grain yield of black-rice per clump was significantly correlated with its number of tillers and filled-grains per panicle (Table 4).

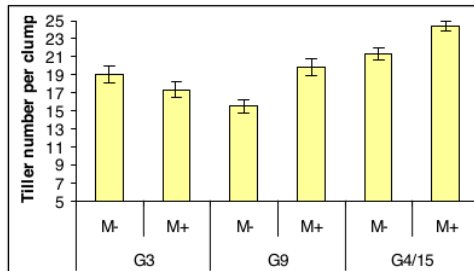


Fig. 1. The pattern M*G interaction effects on number of the black-rice tillers per clump

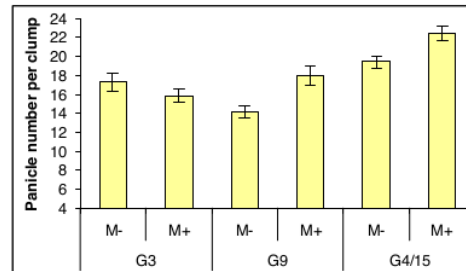


Fig. 2. The pattern M*G interaction effects on number of the black-rice panicles per clump

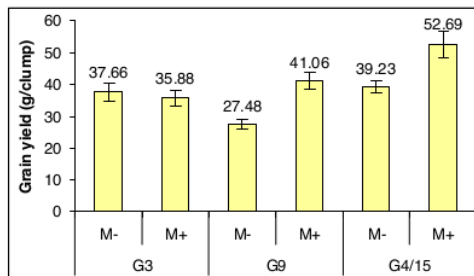


Fig. 3. The pattern M*G interaction effects on the black-rice-grain yield per clump

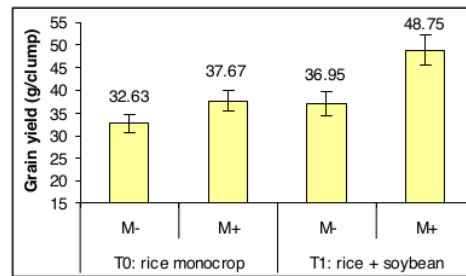


Fig. 4. The pattern M*T interaction effects on the black-rice grain yield per clump

Intercropping black-rice in additive series with soybean also significantly increased tiller number of rice per clump (Table 2). This could also be associated with higher uptake of nutrients by rice plants additively intercropped with soybean because many have reported that intercropping with legume crops increased nutrient uptake by cereal crops as a result of nutrients transfer, especially N-transfer from adjacent legumes to cereal plants, such as in sorghum-soybean intercropping [8], and in rice-peanut intercropping [9, 10]. Higher uptake of nutrients by intercrops in maize-peanut intercropping compared with their monocrops was also reported [11]. Higher number of red-rice panicles and grain yield per clump in rice-soybean intercropping than in monocropped rice was also reported [4, 5].

However, the pattern of significant M*T interaction effect on grain yield of black-rice per clump in Fig. 4 clearly indicates that under rice+soybean intercropping, mycorrhiza-biofertilizer resulted in higher increase in black-rice grain yield compared with under monocropped black-rice. The reason for this significant effect could be higher nutrient uptake by the rice plants resulted from the application of arbuscular mycorrhizal fungi [16, 17, 18, 19], and involvement of mycorrhiza in a legume-cereal intercropping system was reported to increase nutrient transfer from legume to cereal crops [14, 15, 12]. From Fig 4, it can also be seen that under rice-soybean intercropping, the application of mycorrhiza-biofertilizer increases grain yield by 31.9%, but under monocropped black-rice, the grain yield increase was only by 15.5%.

4. Conclusion

Mycorrhiza-biofertilizer was the most significant treatment to increase yield performance of back-rice followed by intercropping and genotypes. However, the interaction effects indicated that G3 was not responsive but both G9 and G4/15 were highly responsive to mycorrhiza-biofertilizer application, with the highest grain yield (56.79 g/clump or 10.54 t/ha) was on G4/15 with biofertilizer and the lowest (27.48 g/clump or 5.5 t/ha) was on G9 without biofertilizer. Mycorrhiza-biofertilizer also resulted in higher increase in the black-rice grain yield under rice+soybean intercropping (31.9%) than under monocropped black-rice (15.5%).

5. Acknowledgment

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