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RESEARCH ARTICLE

Synergistic effect of straw-mulch and mycorrhiza-biofertilizer in increasing yield of black rice intercropped with soybean on raised-beds in aerobic irrigation system

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ABSTRACT

Application of mycorrhiza-biofertilizer and intercropping with legume crops could improve performance and yield of red-rice grown on raised-beds under aerobic irrigation systems, but weeds became a serious problem, which needs more frequent weeding. A Split Split-Plot (SSP) experiment was designed to examine synergistic effects of rice-straw mulch and mycorrhiza-biofertilizer application on yield of several black rice promising-lines that were additively intercropped with soybean on aerobically irrigated raised-beds. Three treatment factors including black-rice genotypes (G3, G9, G4/15), straw mulching (S0 = without; S1 = with mulch), and mycorrhiza-biofertilizer (M0 = without; M1 = with biofertilizer) were arranged as the main, subplots and sub-subplots respectively in three blocks (replications). The results indicated that straw mulch and biofertilizer application significantly increased growth and yield of the black rice plants, while the genotypes were also significantly different in several growth and yield variables. However, there were significant interaction effects of mycorrhiza-genotypes (M*G), mycorrhiza-straw (M*S), and mycorrhiza-straw-genotype (M*S*G) on several growth and yield variables, indicating variation of responses to mycorrhiza among the genotypes. The M*S*G interaction also indicated synergistic effects between straw mulch and mycorrhiza biofertilizer in increasing the black rice yield, in which the S1M1 treatment resulted in the highest grain yield on the G4/15 line (58.2 g/clump) while the S0M0 treated G9 line showed the lowest yield (29.8 g/clump).

Keywords: Aerobic irrigation; Black rice; Straw mulch; Mycorrhiza; Biofertilizer; Soybean

INTRODUCTION

Rice (*Oryza sativa* L.), due to the main use of its grains as staple food, is the most important food crops in the world, especially in Indonesia (Datta et al., 2017). As staple food, it is highly advantageous for human health due to the various types of biomolecules contained in the grains, especially in the colored grain rice (Sen et al., 2020). In addition to white grain rice that is the common rice consumed by most people, there are yellow (golden), brown, red, and purple or black whole-grain color of rice, whose color depends on the levels of anthocyanin content of the whole grains. Those colored grain rice is more interesting than the common rice (white rice) due to its unique color and flavor, as well as health benefits for human because of its anthocyanins and other bioactive compounds in the grains (Kushwaha, 2016).

In Indonesia, black rice is mostly used as source of functional food products (Pratiwi and Purwestri, 2017). Almost all of the cultivars are upland rice cultivars with low yield potential, but after hybridization of some cultivars of black rice with national varieties of red and white rice cultivars, followed with bulk and Pedigree selections for several generations, some promising lines of black rice were obtained, and some of them have a relatively high yield potential (6-7 ton/ha) under upland conditions during a rainy season (Aryana et al., 2020).

Rice is mostly grown under conventional (flooded) cultivation technique, and flooded rice has much lower weed competition compared with growing rice under upland or aerobic conditions. However, growing rice under flooded conditions has several disadvantages, including wasting a lot of irrigation water, which uses up

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to 20260 m^3/ha, compared with only 4260 m^3/ha under dry seeded rice growing technique (Yaligar et al., 2017). Other disadvantages include high loss of N from soil and N-fertilizers, mostly through ammonia volatilization (Choudhury and Kennedy, 2005). It was reported that the total gaseous N loss was 34% in Thailand and 31% in Indonesia after 10 days of Urea application to flooded rice (Buresh et al., 1991). P leaching in addition to N leaching from flooded rice is also very common (Peng et al., 2011).

In addition to reducing soil pH (Lv et al., 2020), flooded rice also depletes populations of arbuscular mycorrhizal fungi (AMF), which are highly beneficial for both rice and non-rice food crops cultivated following rice (Wangiyana et al., 2006). Soybean grown on vertisol soil following flooded rice when fertilized with NPK fertilizer under 25 x 25 cm planting distances, soybean of "Anjasmoro" variety produced dry grain yield only 10.6 g/clump while under bio-fertilization with Rhizobium and AMF inoculants it produce dry grain yield of 27.3 g/clump but with Rhizobium only it yield 16.09 g/clump (Wangiyana and Farida, 2019), which indicate the importance of AMF in flooded rice and non-rice cropping pattern. Dulur et al. (2019b) also reported that waxy maize direct seeded following flooded red rice only produced grain yield on average of only 58.4 g/plant while following aerobic irrigated red rice on raised-bed intercropped with peanut, the average grain yield was 183.2 g/plant, which indicate positive impact of growing red rice on raised beds in intercropping with peanut on the subsequent non-rice crops in irrigated rice growing areas.

In addition to that positive impact, intercropping an irrigated aerobic-red-rice with peanut resulted in significantly higher grain yield than conventional or monocropped red rice (Dulur et al., 2019a; Wangiyana et al., 2021c). Red rice relay-planted with soybean also increased its grain anthocyanin content in addition to increased grain yield, compared with its monocrop (Wangiyana et al., 2021b). Application of mycorrhiza biofertilizer on several red rice promising lines also increased grain anthocyanin and yield (Wangiyana et al., 2021a).

However, from our experience, growing aerobic-irrigated red rice on raised-beds required a high frequency of weeding, which can be up to 6 times of weeding during the vegetative growth of the rice plants. Devasinghe et al. (2011) reported that rice straw mulching significantly reduced weed dry weight at 50% anthesis in direct wet-seeded rice and direct dry-seeded rice, especially during the dry season, and mulching significantly increased rice yield compared with unweeding. El-Beltagi et al. (2022) also recently reviewed the advantages of mulching such as reduced soil moisture loss, enriched soil fauna, improved soil properties and nutrient availability. In addition, Nyamwange et al. (2021) found that mulching of maize growing system significantly increased soil microbial biomass C and N, but it was better under minimum tillage than conventional tillage, while NPK fertilization reduced them. In contrast, increasing N fertilizer dose from the three treatments of 0, 60 and 120 kg/ha N, under zero-tillage and application of 3 ton/ha rice straw mulch, was reported to significantly increase carbon balance of upland rice field due to higher potential C inputs from higher above-ground and root biomass of the rice plants, and under the lowest N dose, much soil carbon loss was due to heterotrophic respiration (Dossou-Yovo et al., 2016). This means that under rice straw mulching, the above-ground and root biomass of rice plants can be increased by increasing the availability of N in the soil. In this study, black rice was intercropped with soybean, which has the ability to increase soil N through N-rhizodeposition (Fustec et al., 2010). Therefore, this study was carried out to examine the effects of application of straw-mulch and mycorrhiza biofertilizer on growth, yield and yield components of several promising lines of black rice grown on raised-beds in additive intercropping with soybean under an aerobic irrigation system, in irrigated growing areas of paddy rice. It was also aimed to examine if there is an interaction effect between straw mulching and mycorrhiza-biofertilizer on yield performance of back rice.

MATERIALS AND METHODS

The three promising lines of black rice tested were G3, G9 and G4/15 (Aryana et al., 2020); the mycorrhiza biofertilizer applied was "MycoGrow"; the soybean variety was "Dena-1"; the experiment was carried out on an irrigated paddy rice-field in Dasan Tebu (-8.653912, 116.130813), Indonesia, from April to August 2021; and the entire procedures for conducting this experiment are as previously explained (Wangiyana et al., 2021d), based on planting geometry of Wangiyana et al. (2019). The SSP design was applied to arrange the three treatment factors tested, i.e. black-rice genotypes (G3, G9, G4/15), straw mulching (S0= without; S1= with mulch), and mycorrhiza-biofertilizer (M0= without; M1= with biofertilizer), which were arranged as the main, subplots and sub-subplots respectively in three blocks (replications).

The growth and yield variables as explained in Wangiyana et al. (2021d) were analyzed with ANOVA and Tukey's HSD using CoStat for Windows ver. 6.303, while bar-chart presentation of interaction effects was based on Riley (2001) using the mean values and SE. Regression and correlation analyses were done using Minitab for Windows Rel. 13.

RESULTS AND DISCUSSION

In this study, soybean (Dena-1 variety) was relay-planted between double-rows of rice at two weeks after seeding pre-germinated seeds of black rice on the raised-beds. Based on the official description of soybean, this Indonesian soybean variety is categorized as tolerant to shading. When the rice plants were at 24 days after seeding (DAS), the soybean plants were nearly as tall as the rice plants (Fig. 1). However, at 49 days of rice seeding, there was a slight difference in plant height, in which rice plants were slightly taller under straw mulching than under without mulching and under straw mulching, rice plants were taller than soybean plants (Fig. 2).

Concerning the results of data analysis, as listed in Table 1, most variables show significant effects of mycorrhiza biofertilizer, which means that it significantly increased mean values of those variables while decreased the percentage of unfilled grain number as can be seen further on the mean values summarized in Table 2 and Table 3. The rice-straw mulching also improved performance of several growth and yield variables but decreased the proportion or percentage of unfilled grains, while those genotypes of black rice showed significant differences only in several growth and yield variables. However, there were significant interaction effects on several growth and yield variables, in which interaction between mycorrhiza and genotypes (M*G interaction) had significant effects on more variables than the M*S and M*S*G interactions, while the S*G interaction did not show significant effect on all variables (Table 1).

Concerning the positive contribution of biofertilizer, the calculated increased yield was up to an average of 32.3% due to the biofertilizer application (Table 3). Based on the best subset regression (BSR) analysis (Table 4), the most closely related growth and yield variables that determined the amount of grain yield per clump was biomass weight with a determinant coefficient (R^2) of 58.3% but if biomass weight was excluded, the most closely related variable (R^2 = 55.9%) was filled grain number. However, the two most closely related variables to grain yield were biomass weight and straw dry weight with an R^2 of close to 100.0% followed by biomass weight and harvest index with an R^2 of 97.8%. These mean that to increase grain yield, the above-ground biomass is the best variable to be increased followed with increasing harvest index, which also means increasing the rates of remobilization of biomass from shoot to the developing grains at the seed filling stage. When biomass weight was excluded from the BSR analysis, the two most closely related variables (R^2 of 94.2%) were panicle number

Table 1: The probability (*p-values*) of the ANOVA results for all observation variables measured for the black rice plants

Variables	p-values of the treatment effects						
	Genotype	Straw	S*G	Mycorrhiza	M*G	M*S	M*S*G
Plant height	0.0555	0.0660	0.3816	**0.0219**	**0.0032**	0.3814	**0.0110**
Leaf number	0.1185	**0.0013**	0.6488	**0.0001**	0.0970	**0.0442**	0.2759
Tiller number	0.0859	0.3262	0.6297	**0.0003**	**0.0001**	1.0000	**0.0012**
Dry straw weight	**0.0002**	**0.0139**	0.3623	**0.0211**	0.1480	0.0600	0.7374
Panicle length	0.3398	0.2576	0.3751	0.1124	0.0675	0.3917	0.6475
Biomass weight	**0.0005**	**0.0006**	0.2600	**0.0000**	**0.0142**	**0.0485**	0.0543
Panicle number	**0.0080**	0.1417	0.4619	**0.0000**	**0.0002**	0.7431	**0.0002**
Filled grains/panicle	**0.0022**	**0.0007**	0.2614	**0.0000**	**0.0157**	0.1019	0.0627
%-unfilled grains	**0.0035**	**0.0028**	0.5267	**0.0000**	**0.0005**	**0.0061**	0.6254
Weight of 100 grains	0.5953	0.0652	0.9022	0.4941	0.9047	0.3773	0.3811
Grain yield/clump	0.0712	**0.0000**	0.2443	**0.0000**	**0.0218**	0.5456	0.0093
Harvest index (%)	**0.0023**	0.8783	0.8301	**0.0004**	0.3838	**0.0474**	0.3789

Fig 1. Raised-beds covered with straw mulch immediately after seeding the pre-germinated black rice seeds (Left); conditions of rice and soybean plants 24 days after seeding black rice

Table 2: Effect of the treatment factors on growth-related variables of black rice plants in additive intercropping with soybean on raised-beds

Treatments	Plant height (cm)	Leaf number per clump	Tiller number per clump	Dry straw weight (g/clump)	Panicle length (cm)	Biomass weight (g/clump)
M0: no myc	91.88[b]	81.08[b]	18.56[b]	39.33[b]	22.34[a]	76.44[b]
M1: myc	93.44[a]	97.17[a]	19.89[a]	43.01[a]	22.74[a]	92.11[a]
HSD 0.05	1.30	6.01	0.57	3.03	ns	3.40
S0: no mulch	90.46[a]	84.36[b]	18.89[a]	36.42[b]	22.37[a]	74.43[b]
S1: straw	94.86[a]	93.89[a]	19.56[a]	45.91[a]	22.71[a]	94.12[a]
HSD 0.05	ns	4.14	ns	6.76	ns	7.37
G1(G3)	91.13[a]	86.67[a]	19.08[a]	32.08[b]	22.35[a]	73.68[b]
G2(G9)	95.21[a]	95.83[a]	18.25[a]	30.74[b]	22.89[a]	72.76[b]
G4/15	91.65[a]	84.88[a]	20.33[a]	60.68[a]	22.39[a]	106.39[a]
HSD 0.05	ns	ns	ns	7.51	ns	10.07

Table 3: Effect of the treatment factors on yield components of black rice plants in additive intercropping with soybean on raised-beds

Treatments	Panicle number/clump	Filled grain number/clump	%-unfilled grain number	100-grain weight (g)	Dry grain yield (g/clump)	Harvest index (%)
M0: no myc	16.86[b]	97.24[b]	15.54[a]	2.27[a]	37.12[b]	49.72[b]
M1: myc	19.17[a]	111.46[a]	10.36[b]	2.30[a]	49.10[a]	55.03[a]
HSD 0.05	0.54	3.29	1.47	ns	1.87	2.38
S0: no mulch	17.61[a]	95.56[b]	15.41[a]	2.25[a]	38.01[b]	52.26[a]
S1: straw	18.42[a]	113.15[a]	10.49[b]	2.32[a]	48.21[a]	52.49[a]
HSD 0.05	ns	6.71	2.47	ns	1.91	ns
G1(G3)	17.75[b]	102.59[b]	10.95[b]	2.28[a]	41.60[a]	56.54[a]
G2(G9)	16.71[b]	110.99[a]	12.15[b]	2.26[a]	42.02[a]	57.87[a]
G4/15	19.58[a]	99.48[b]	15.75[a]	2.32[a]	45.71[a]	42.72[b]
HSD 0.05	1.62	4.69	2.23	ns	ns	6.68

Fig 2. The conditions of rice and soybean plants at 49 days after seeding black rice (black rice slightly taller on straw mulching than on no-mulching raised-beds

per clump and average number of filled grains in each panicle, followed by harvest index and weight of dry straw, which means the rates of remobilization of biomass from shoot to the growing grains at the seed filling stage.

Based on the coefficients of correlation between various variables (X) and dry grain yield (Y1) and harvest index (Y2) showing a significant coefficient (r_{xy}) of correlation, it can be seen from Table 5 that there are slight differences between black rice with M0 and M1 treatments. Under M0 treatment (without biofertilizer), grain yield was significantly correlated with only three variables, i.e. positively correlated with numbers of leaves and filled grains and negatively correlated with percentage of unfilled grain number, but under M1 treatment (with biofertilizer), grain yield was significantly correlated with almost all variables, except panicle length as well as number of leaves. Harvest index also had stronger negative correlation with dry straw weight under application of mycorrhiza biofertilizer (M1) than under M0 (Table 5). This could mean that the rate of biomass remobilization from shoot to grains was higher under M1 than M0. This fact is also supported by other data, such as higher above-ground biomass weight (Table 3), number of filled grains and harvest index, but lower percentage of unfilled grains, and these all together with higher panicle number would support higher yield under M1 than M0 treatment (Table 4). Under M0 treatment, those three significantly correlated variables with grain yield (Table 5) could also mean that lower grain yield in the M0 than M1 treatment was determined by lower number of leaves at anthesis (Table 2), lower number of filled grains while higher percentage of unfilled grains (Table 3). These indicate lower grain filling potential of the black rice plants under M0 than M1 treatment.

In relation to biomass and nutrient remobilization from shoot to the developing grains, Solaiman and Hirata (1995)

Table 4: Results of best subset regression of 1-3 X-variables with grain yield per clump (including or excluding above-ground biomass weight per clump

X-vars	R-Square	Plant height	Leaf no.	Tiller no.	Straw DW	Panicle length	Panicle no.	Filled grain no.	%-un-filled grains	100-grain wt.	HI (%)	Bio-mass wt.
					---------------------------------- including Biomass weight --------------------------------							
1	58.3											X
1	55.9							X				
1	50.1						X					
2	100.0				X							X
2	97.8										X	X
2	94.2						X	X				
3	100.0				X					X		X
3	100.0				X	X						X
3	100.0				X						X	X
					---------------------------------- excluding Biomass weight --------------------------------							
1	55.9							X				
1	50.1						X					
1	42.0		X									
2	94.2						X	X				
2	91.8				X						X	
2	87.1			X				X				
3	99.2						X	X		X		
3	95.1			X				X		X		
3	95.0				X		X	X				

Table 5: Coefficients of correlation of various variables (X) with grain yield (Y1) and harvest index (Y2), which show a significant coefficient (rxy) of correlation under with or without mycorrhiza biofertilizer

Y-variables	Leaf number	Tiller number	Dry straw weight	Panicle length	Panicle number	Filled grain number	%-unfilled grains	100-grain weight
			------------------ black rice with no application of mycorrhiza biofertilizer (M0) ---------------					
Dry grain yield	0.734	0.378	0.401	0.176	0.450	0.716	-0.516	0.214
p-value	**0.001**	0.122	0.099	0.486	0.061	**0.001**	**0.028**	0.393
Harvest index	0.385	-0.031	-0.867	0.443	-0.182	0.318	-0.707	-0.199
p-value	0.115	0.902	0.000	0.066	0.470	0.199	0.001	0.429
			------------ black rice with application of mycorrhiza biofertilizer at planting (M1) ----------					
Dry grain yield	0.122	0.578	0.675	0.447	0.667	0.601	-0.517	0.654
p-value	0.629	0.012	**0.002**	0.063	**0.002**	**0.008**	**0.028**	**0.003**
Harvest index	0.472	-0.409	-0.932	-0.266	-0.579	0.109	-0.079	-0.308
p-value	**0.048**	0.092	**0.000**	0.287	**0.012**	0.668	0.755	0.213

also indicated that mycorrhizal rice plants had higher nutrient remobilization from shoot and soil to the grains compared with the non-mycorrhizal ones. The facts that average number of filled grains and harvest index were higher with lower percentage of unfilled grains in M1 than in M0 black rice (Table 3) could also mean that the black rice plants, which were intercropped with soybean in additive series in this study, had a significantly higher grain filling potential under M1 than under M0 treatment. This could be due to a higher photosynthetic capacity of the black rice plants under M1 than under M0 as indicated by a higher number of leaves (19.8% higher) at anthesis under M1 treatment (Table 2). In addition, AMF inoculated rice seedlings were reported to have higher uptake of various nutrients, compared with non-inoculated seedlings (Dhillion and Ampornpan, 1992), which could support for higher photosynthetic rates resulted in higher dry weight of AMF inoculated rice seedlings. Application of mycorrhiza biofertilizer on red rice grown together in one pot with mungbean also showed higher number of filled panicles and filled grains, and higher yield (Wangiyana et al., 2018). Mycorrhiza-biofertilized red rice plants on raised-beds was also reported to result in higher number of panicle, filled grains, biomass weight, and grain yield, compared with no biofertilizer application (Wangiyana et al., 2021b).

Unlike the biofertilizer treatments, which increased grain yield per clump by 32.3% due to biofertilizer, covering the raised-beds with increased grain yield by only 26.6%, but yield difference between with and without straw mulch was also significant (Table 3). From the correlation coefficients summarized in Table 6 it can be seen that dry straw weight

Table 6: Coefficients of correlation of various variables (X) with grain yield (Y1) and harvest index (Y2), which show a significant coefficient (r_{xy}) of correlation under with or without straw mulch

Y-variables	Leaf number	Tiller number	Dry straw weight	Panicle length	Panicle number	Filled grain number	%-unfilled grains	100-grain weight
----------------------- black rice without straw mulch application (S0) -----------------------								
Dry grain yield	0.643	0.696	0.360	0.331	0.827	0.703	-0.564	0.061
p-value	**0.004**	**0.001**	0.142	0.179	**0.000**	**0.001**	**0.015**	0.811
Harvest index	0.625	0.065	-0.828	0.047	0.034	0.472	-0.665	-0.269
p-value	**0.006**	0.798	**0.000**	0.853	0.894	**0.048**	**0.003**	0.281
----------------------- black rice with straw mulch application (S1) -----------------------								
Dry grain yield	0.520	0.430	0.444	0.302	0.674	0.534	-0.443	0.460
p-value	**0.027**	0.075	0.065	0.223	**0.002**	**0.023**	0.066	0.055
Harvest index	0.428	-0.283	-0.903	0.374	-0.379	0.399	-0.552	-0.175
p-value	0.076	0.255	**0.000**	0.127	0.121	0.101	**0.018**	0.487

was stronger negatively correlated with harvest index in S1 than in S0 treatment, which also means that the rate of biomass remobilization from shoot to the grains was higher under S1 than S0 treatment. Under S0 treatment, grain yield was significantly positive correlated with numbers of leaves, tillers, panicles and filled grains, and these all were lower ($p<0.05$) in S0 than S1 treatment. The proportion of unfilled grains was negatively correlated with grain yield and this correlation was stronger under S0 than S1 (Table 6). Therefore, these all could also mean that lower grain yield of black rice with no application of straw mulch was strongly determined by lower leaf number per clump (Table 2), as well as lower number of tillers and panicles while higher proportion of unfilled grains (Table 3). These indicated lower production and grain filling potentials of black rice under S0 than S1 treatment.

In our experiences, one of the disadvantages in growing aerobic rice is competition with weeds. Application of straw mulch, in addition to its function to suppress growth of and competition by weeds (Ghosh et al., 2006; Devasinghe et al., 2011; El-Beltagi et al., 2022), also increases soil moisture (Akhtar et al., 2019), soil carbon balance (Dossou-Yovo et al., 2016), soil microbial biomass C and N (Nyamwange et al., 2021) and decreases soil temperature (Akhtar et al., 2019), as well as increases the availability various nutrients in the long-term (Ghosh et al., 2006; Devasinghe et al., 2011; Akhtar et al., 2019). Weed competition could decrease rice yield by 49.88% during dry season in dry direct-seeded rice and by 22.24% during wet season (Devasinghe et al., 2011).

In this study, black rice was dry direct seeded and because black rice was intercropped with soybean on raised-beds, irrigation was done using an aerobic irrigation system by irrigating through the furrows every 3-4 days. Therefore, during application of irrigation water, at least 10 cm depth of the bed surface is under aerobic conditions, and so during the majority of the time. Due to no flooding, the application of aerobic irrigation systems in growing rice on raised-beds is favorable for weed growth. However, by application of straw mulch spread on the surface of the raised-beds, due to its ability in suppressing weed growth, maintaining soil moisture, and decreasing soil temperature (Akhtar et al., 2019), then application of straw mulch would increase grain yield per clump through increased number of leaves and biomass (Table 2) in addition to increased number of filled grains but decreased proportion of unfilled grains (Table 3).

Those genotypes of black rice also showed significant differences, especially in dry straw and biomass weight (Table 2). The number of panicles per clump (Table 3) was also significantly different, which was highest in the G4/15 promising line, but because of the lowest number of filled grains and highest proportion of unfilled grains, then harvest index of the G4/15 promising line became the lowest among those genotypes of black rice. This lowest rate of biomass partition to the grains will need to be improved in the future generations. However, there are significant interaction effects on various observation variables, especially the M*G interactions that shows significant interaction on the highest number of variables, which means that response of those genotypes to application of mycorrhiza biofertilizer significantly different among those genotypes, so that the significant differences between genotypes cannot be generalized.

Based on the patterns of M*G interactions, it is clear from Figs. 3-9 that responses of those genotypes to application of mycorrhiza biofertilizer varied between genotypes, which were also different between the observation variables. Numbers of tillers (Fig. 4) as well as panicles (Fig. 5) showed similar pattern of interaction, in which both variables were increased by biofertilization of G9 and G4/15 except G3, but plant height was increased by biofertilizer application in G3 and G4/15 except on G9. However, biofertilization increased number of filled grains, above-ground biomass and grain yield. In contrast,

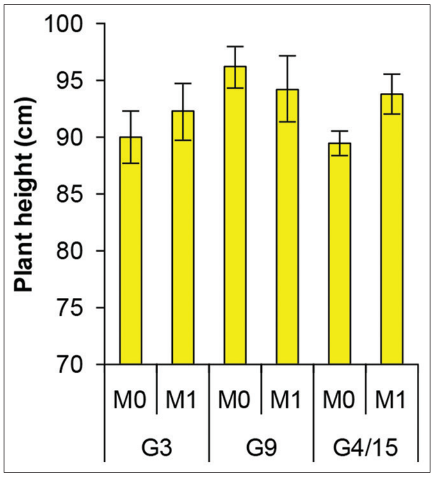

Fig 3. The M*G interaction effect on plant height

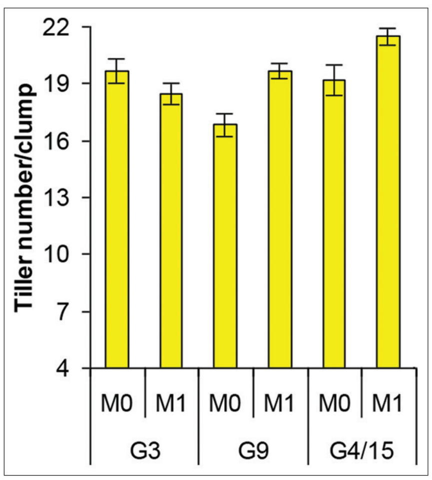

Fig 4. The M*G interaction effect on tiller number per clump

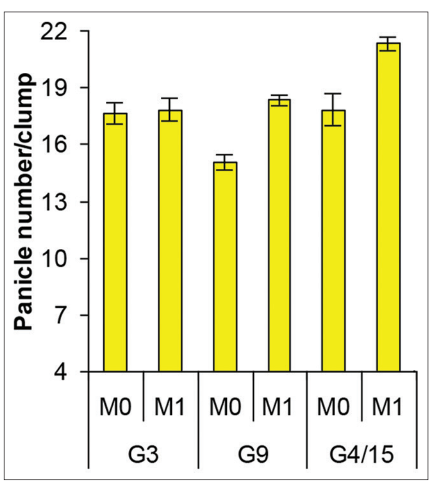

Fig 5. The M*G interaction effect on panicle number per clump

biofertilization reduced the proportion of unfilled grains. In fact, the highest proportion of unfilled grains in G4/15 line in Table 3 was most probably due to the highest value under no application of mycorrhiza biofertilizer (Fig. 7).

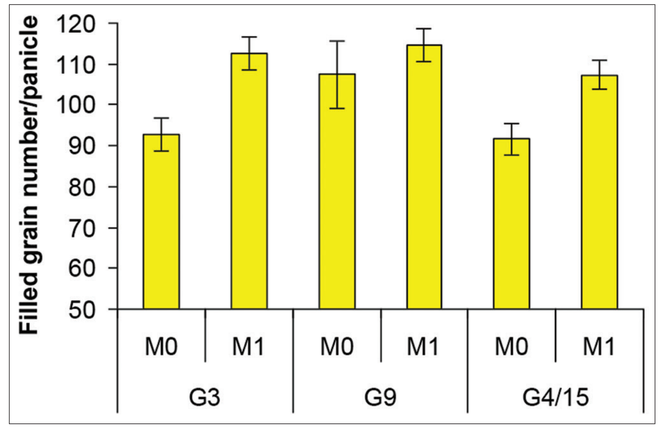

Fig 6. The M*G interaction effect on filled grain number per clump

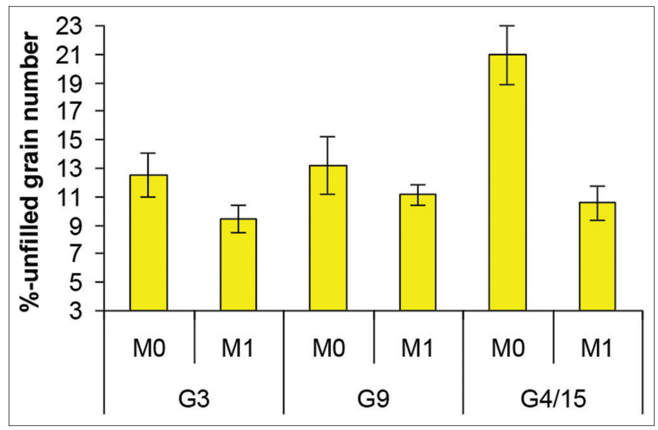

Fig 7. The M*G interaction effect on percentage of unfilled grain number

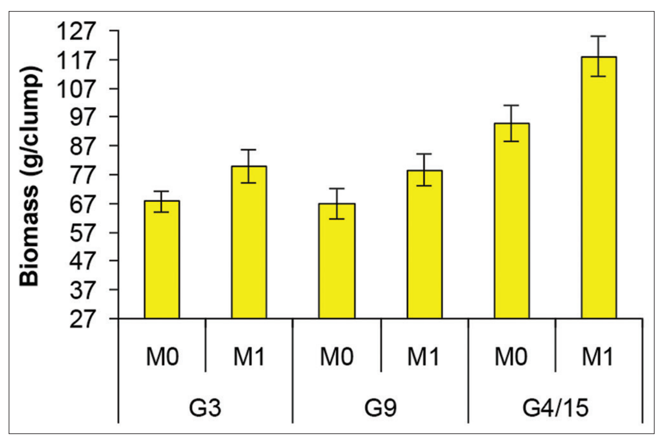

Fig 8. The M*G interaction effect on biomass weight per clump

This indicates the strong response of the G4/15 line to biofertilization in relation to proportion of unfilled grains. However in general, it is clear that all the promising lines of black rice showed positive response to application of mycorrhiza biofertilizer, and among those lines, G4/15 showed the highest response.

In relation to the significant S*M interaction effects, Figs.10-13 show that application of mycorrhiza biofertilizer

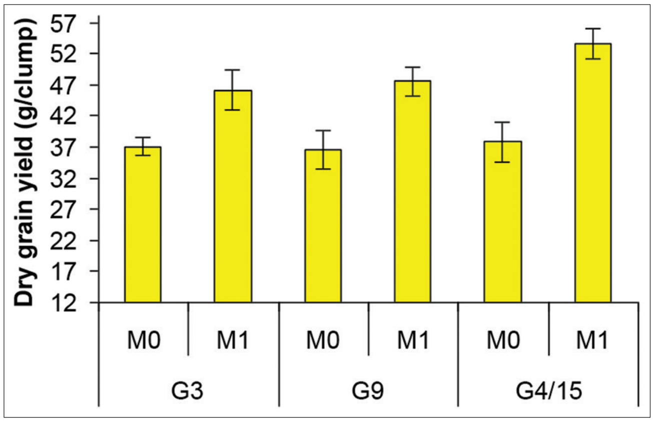

Fig 9. The M*G interaction effect on dry grain yield per clump

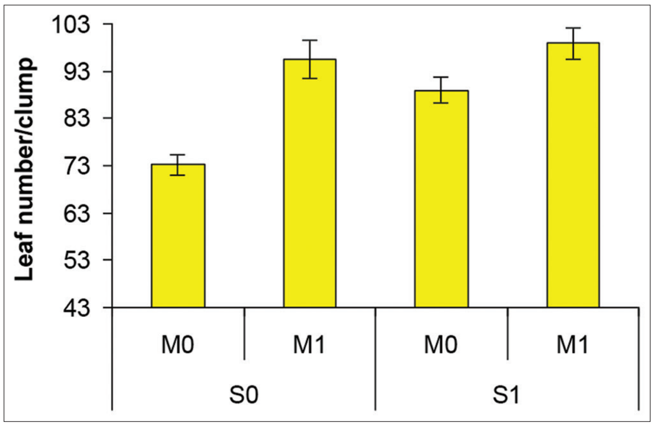

Fig 10. The M*S interaction effect on leaf number per clump

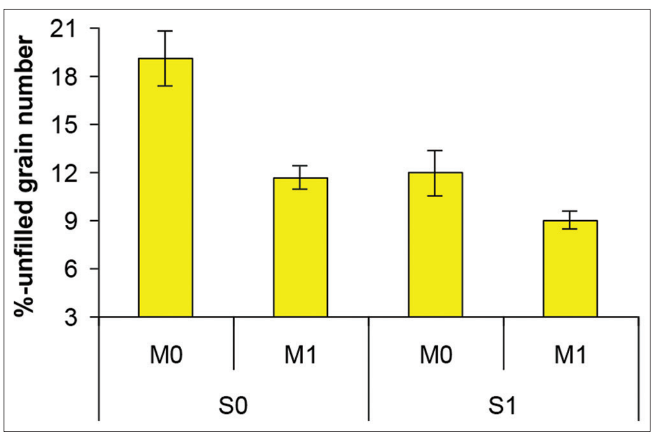

Fig 11. The M*S interaction effect on the proportion of unfilled grains

in general support higher growth and yield both under without and with application of straw mulch, and the best results were obtained on black rice plants supplied with mycorrhiza biofertilizer (M1) on the raised-beds covered with straw mulch (S1). Without application of straw mulch (S0), black rice plants under M0 treatment (S0M0) showed the proportion of unfilled grains more than twice as much as that in S1M1 treatment. On the contrary, the above-ground biomass weight was highest on black rice

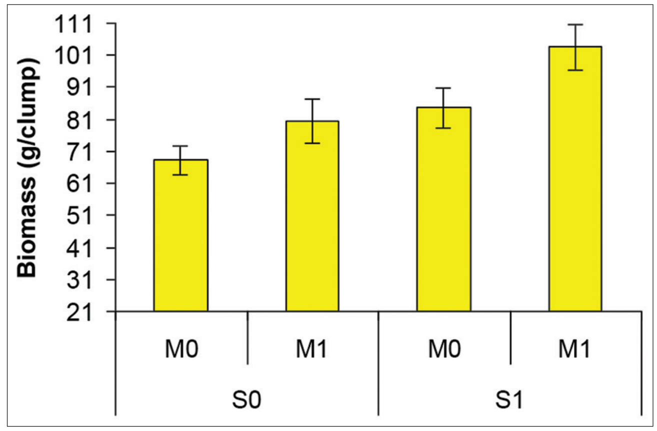

Fig 12. The M*S interaction effect on above-ground biomass weight per clump

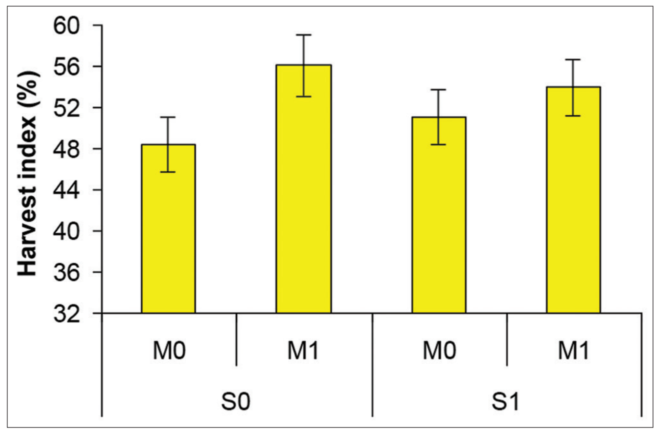

Fig 13. The M*S interaction effect on harvest index

plants under S1M1 treatment. This indicates a synergistic effect of straw mulching and mycorrhiza biofertilization in increasing the above-ground biomass (weight of dry straw + dry grain per clump) (Fig. 12) and number of leaves per clump (Fig. 10), while decreasing the proportion of unfilled grains (Fig. 11). This synergistic effect also occurred in the M*S*G interactions, especially on grain yield (Fig. 14), as well as on number of panicles (Fig. 15) and tillers (Fig. 16) except on G3 line, whereas on plant height, there seems no strong synergistic effect of mycorrhiza biofertilizer and straw-mulch application (Fig. 17).

Based on the M*S*G interaction, the interaction showing the most significant differences between M0 and M1 was the interaction effect on grain yield (Fig. 14), which significantly increased due to biofertilization either in S0 or S1 treatment, and Fig. 14 also clearly shows that there was a synergistic effect of straw mulching and biofertilization on the rice yield. From Table 4, it can be seen that when the above-ground biomass weight was excluded from the correlation analysis, panicle number per clump was the second most closely related to grain yield. However, Fig. 15 shows that on the G3 line, the S0M0 treatment had a higher

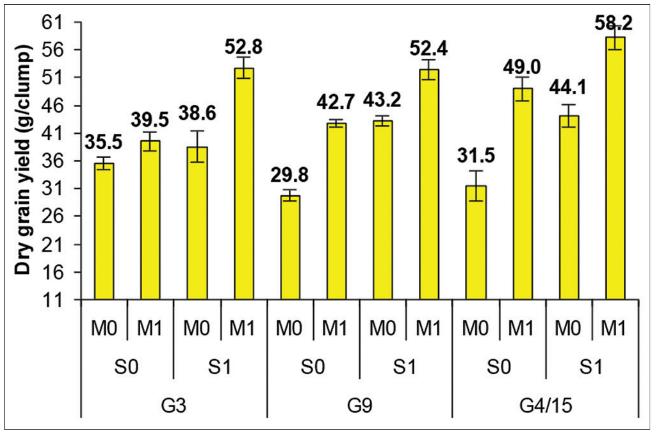

Fig 14. The M*S*G interaction effect on dry grain yield per clump

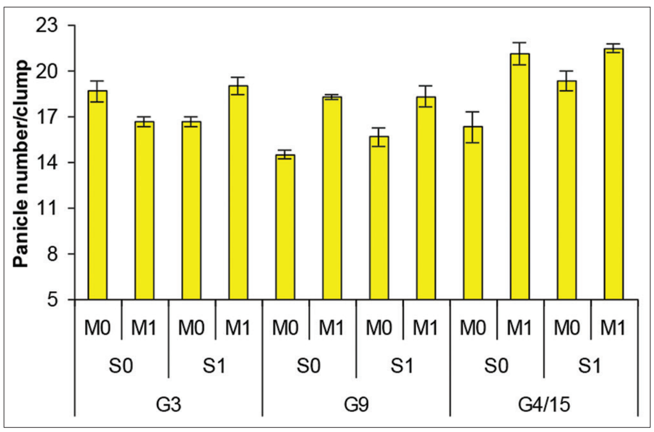

Fig 15. The M*S*G interaction effect on panicle number per clump

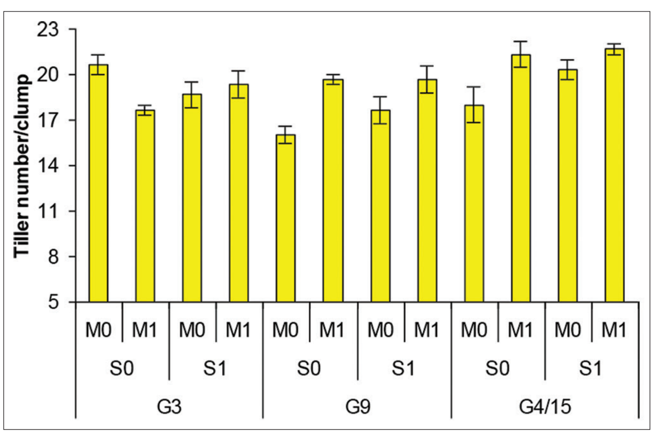

Fig 16. The M*S*G interaction effect on tiller number per clump

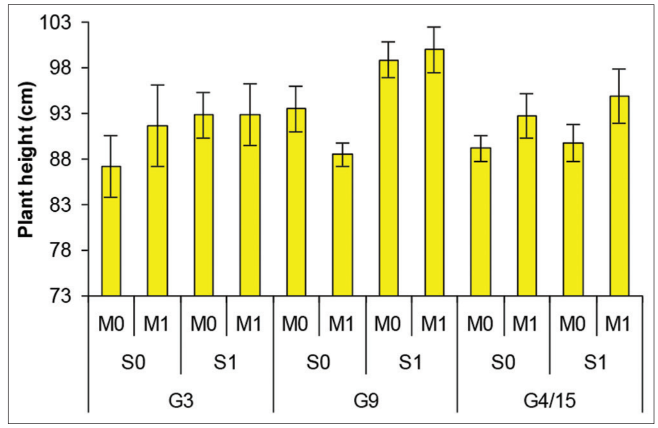

Fig 17. The M*S*G interaction effect on plant height

panicle number per clump compared with the S0M1 but grain yield was on the contrary to the panicle number between these treatment combinations. These trends were similar to the tiller number per clump in Fig. 16. This means that in this genotype, higher tiller or panicle number resulted in lower grain yield specifically in the G3 line of black rice under S0M0 treatment. The possible reasons for these contrasting results are harvest index (Fig. 13) and leaf number per clump (Fig. 10), which were the lowest on S0M0 treatment, as well as the proportion of unfilled grains, which was highest on S0M0 treatment (Fig. 11), when compared with those in the S0M1 treatment. This clearly indicates the low potential of the black rice plants in remobilization of dry weight from shoot to the grains, and this was increased by the application of mycorrhiza biofertilizer. Solaiman and Hirata (1995) also indicated similar conclusion, in which mycorrhizal rice plants showed a higher potential in remobilization of nutrients from shoot and/or soil to the grains compared with the non-mycorrhizal ones. Wangiyana et al. (2021a, b) also reported higher percentage of filled grain number on mycorrhiza-biofertilized red rice than on non-biofertilized plants.

Regarding to the genotypes, G4/15 line seems to be the most responsive to straw mulching and biofertilization, while G3 seems to be the least responsive, because when the G3 black rice plants were no supplied with mycorrhiza biofertilizer at planting (G3M0), application of straw mulch (S1) did not seem to significantly increase grain yield compared with the S0 treatment (Fig. 14). The black rice in this study was planted on raised-beds additively intercropped with soybean. With the presence of soybean plants, i.e. one row between the double-rows of black rice, application of mycorrhiza biofertilizer could facilitate N transfer from soybean to the adjacent black rice, and this kind of N transfer waas reported to happen between soybean and maize plants in intercropping systems (Bethlenfalvay et al., 1991; Meng et al., 2015). The application of straw mulch could be more beneficial for this kind of nutrient transfer as well as for better growth of the black rice because straw mulching resulted in a significant increase in number of leaves (Table 2), as well as number of filled grains while decreasing the proportion of unfilled grains (Table 3), which could be due to better growing environment, such as higher soil moisture content, lower weed competition, and lower soil temperature (Devasinghe et al., 2011; Akhtar et al., 2019; El-Beltago et al., 2022).

Hence, it's logical to conclude that straw mulching and mycorrhiza biofertilization would result in a synergistic effect on yield of black rice additively intercropped with soybean planted on aerobic-irrigated raised-beds.

CONCLUSION

It was concluded that straw mulching and mycorrhiza biofertilization significantly increased growth and yield of black rice additively intercropped with soybean on aerobic irrigated raised-beds, whereas between the promising lines of black rice, there were also significant differences in several growth and yield variables. However, there were significant interaction effects, especially between mycorrhiza and genotypes (M*G), mycorrhiza and straw (M*S), and the three-way interaction (M*S*G) on several growth and yield variables, which indicates different responses to the application of mycorrhiza biofertilizer among different genotypes of black rice, and there were synergistic effects of straw mulching and mycorrhiza biofertilization in increasing black rice yield while decreasing the percentage of unfilled grain number.

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Authors' contributions

All authors had participated in running the field experiment, field measurement, data compilation, and preparation of the manuscript. Author #3 did the field plant measurements and data tabulation; Author #2 conducted the field experiment and crop maintenance; Author #1 designed the entire research project including the field experiment and layout, conducted the field experiment, ran statistical data analyses, and wrote the English manuscript, submitted the manuscript, and completed the revisions.

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