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Mycorrhiza biofertilizer and intercropping with soybean increase anthocyanin contents and yield of upland red rice under aerobic irrigation systems

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Abstract. Red rice kernels are known to have high antioxidant properties due to its anthocyanins, and application of mycorrhizal fungi was reported to increase grain anthocyanins. This study aimed to examine the effects of intercropping with soybean and mycorrhiza biofertilizer on grain anthocyanins and yield of upland red rice under aerobic irrigation system. The experiment was conducted in Beleke, West Lombok, Indonesia, under Split Split-Plot design with three treatment factors, i.e. upland red rice genotypes as the main plots (G04, G10), intercropping as the sub-plots (monocrop or intercropping with soybean), and mycorrhiza as the sub-sub-plots (without or with mycorrhiza). Results indicated that intercropping and mycorrhiza application significantly increased grain anthocyanin contents and yield, but rice genotypes showed differences only in grain yield per clump. However, there was a three-way interaction on percentage of filled grain number, and a two-way interaction on grain anthocyanins, in which mycorrhiza application resulted in more significant increases in anthocyanin contents and grain yield of the red rice under intercropping with soybean than under monocrop. Therefore, application of mycorrhiza biofertilizer and intercropping with soybean, besides increasing grain yield, are also capable of increasing health values of the red rice grown on raised-beds in aerobic irrigation system.

1. Introduction

Rice (*Oryza sativa* L.) is grown in all the six continents of the world (Asia, Africa, Australia, Europe, North America, South America) where field crop production is practiced, and is the staple food for nearly half of the world's population [1]. In Indonesia, based on the total area harvested, rice is the most important staple food crop and most widely planted by the farmers, with the total harvested areas in 2015 based on the national statistical crop production data (<https://bps.go.id/subject/53/tanaman-pangan.html#subjekViewTab3>), was 14,116,638 ha, with the total production in 2015 was 75,397,841 ton. However, this total rice production is still unable to meet domestic needs for rice, so that Indonesia still imports rice from various countries, including Asian countries, such as Vietnam and Thailand (<https://www.antaranews.com/berita/1223248/kebijakan-impor-beras-dan-ketahanan-pangan-indonesia>). Therefore, rice production needs to be increased significantly, and the most feasible ways for increasing rice production is to extend rice cultivation to dryland areas because the current availability of irrigated areas for rice production so far still unable to meet domestic needs for rice. To be able to utilize dryland areas for rice production, it requires suitable rice genotypes for dry



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environments with a reasonably high yield potential. For that, several upland rice varieties need to be developed for adaptation to the dry growing environment.

Unlike paddy rice cultivars, which are almost all known as white rice, i.e. the color of the husked grains is white, except for the Inpari-24 variety that produces red grains, there are different color of upland rice genotypes based on the color of the seed coat and the endosperm, i.e. brown, red and black rice, depending on the intensity of the anthocyanin contents of the seed coat [2,3]. Red rice grains have a high health value due to the anthocyanins and proanthocyanidins contained in red rice grains as well as the antioxidant activities of the red rice [3,4]. Red rice is also more expensive than white rice, so that production of red rice could be more profitable than white rice. However, the use of dryland areas for production of upland rice is normally resulted in low productivity of upland rice compared with production of rice in irrigated paddy field, especially if better technologies of rice production are not applied. One of the technologies that can be applied to increase grain yield of rice under aerobic systems, including upland growing conditions, is the application of biofertilizers containing arbuscular mycorrhizal fungi (AMF) [5,6].

Many other researchers have also reported that AMF inoculation on rice, especially under upland or dryland conditions, could significantly increase growth and grain yield of rice, such as reported by Solaiman and Hirata [7–9]. In addition, application of mycorrhiza biofertilizer was also found to significantly increase growth and yield of soybean during the dry season, which was direct seeded following flooded (conventional) rice [10] or following rice of different techniques of cultivation, either conventional or SRI (systems of rice intensification) techniques [11], indicating the importance of establishing mycorrhizal symbiosis for crops grown in dry soil environments. In addition to influencing grain yield, application of mycorrhiza biofertilizer was also reported to be able to increase anthocyanin concentration of the husked grains and dry biomass weight of several promising lines of red rice, but the different lines seem to show different response to the biofertilizer application [12]. In addition to application of mycorrhiza biofertilizer, intercropping rice with legume crops was also reported to be able to increase grain yield of rice, such as those reported by Chu et al [13], Wangiyana et al [14], and Dulur et al [15] under intercropping with peanut; Wangiyana et al [16] and Arifuddin et al [17] under intercropping with mungbean; and Wangiyana et al [6] under intercropping with soybean.

This study aimed to examine the effect of mycorrhiza biofertilizer and intercropping with soybean on yield components and anthocyanin contents of two genotypes of upland red rice grown on raised-beds under aerobic irrigation systems on riceland usually used to grow paddy rice under flooded conditions (conventional rice) year by year.

2. Materials and methods

In this study, the field experiment was carried out from May to September 2018, on a farmers' ricefield previously used to grow rice under conventional (flooded) technique of rice cultivation year after year, which is located in Beleke Village, Gerung District, West Lombok Regency, NTB Province, Indonesia. However, the upland red rice genotypes were grown on raised-beds under aerobic irrigation systems by flooding the furrows between the raised-beds for several hours until the bed surfaces were saturated, and then the irrigation water inflow was stopped. This technique of irrigation was done every 7 days until one week before harvest date of the rice grains at 110 days after seeding (DAS).

2.1. Treatments and experimental design

The experiment was designed according to Split Split-Plot design with 3 blocks and three treatment factors, namely upland red rice genotypes (G) as the main plot factor, consisting of two selected genotypes of upland red rice promising lines (G04 = MG4 and G10 = MG10), intercropping treatment (T) as the subplot factor, consisting of two treatments (T0= without intercropping or monocrop rice and T1= additive intercropping red rice with soybean), and application of mycorrhiza biofertilizer (M) as the sub-subplot factor, consisting of two treatments (M0= without biofertilizer but the rice plants were fertilized with full recommended doses of N-P-K fertilizers; M1= biofertilizer application at

planting combined with full recommended doses of N-P-K fertilizer). The biofertilizer "Technofert", which contains mixed species of arbuscular mycorrhizal fungi (AMF) and zeolite particles, was supplied by PT Mikata Sukses Mandiri, Serpong, Indonesia.

2.2. Implementation of the field experiment

Pre-germinated red rice seeds were dibbled on the raised-beds with double row patterns and soybean seeds of Dena-1 variety were dibbled between the double-rows. The entire procedures for implementation of the field experiment from the beginning until harvest of the rice plants, including the planting geometry, were as explained in Wangiyana et al [6], except for the anthocyanin analysis and the rice genotypes used, i.e. in this study, only upland genotypes of the red rice promising lines were used. The anthocyanin data of the husked red rice grains of the upland rice genotypes under intercropping with soybean were obtained in more recently from the Testing Laboratory of Food Quality and Food Safety, the Faculty of Agricultural Technology, University of Brawijaya, Malang, Indonesia, where the samples were sent for anthocyanin analysis. Previous study on the effects of mycorrhiza biofertilization on anthocyanin contents of both upland and amphibious promising lines of red rice have also been done by Wangiyana et al [12], but without intercropping treatments.

2.3. Observation variables and data analysis

Observation variables included several yield components and anthocyanin content of the husked grains of the upland red rice measured from four clumps of red rice plant samples per bed, which included percentage of filled panicle number at harvest, percentage of filled grain number, above-ground biomass weight (dry straw + grains), grain yield per clump, anthocyanin concentration and total anthocyanin yielded in the husked grains per clump. Data were analyzed with analysis of variance (ANOVA) and Tukey's HSD test at 5% level of significance, using the statistical software "CoStat for Windows" ver. 6.303.

3. Results and discussion

The ANOVA results shown in Table 1 indicated that there was a significant three-way interaction effect on the percentage of filled grain number, and two-way interaction effects between mycorrhiza and intercropping on the percentage of filled grain number, and between mycorrhiza and genotypes of rice on the grain anthocyanin concentration. Among the three factors tested, mycorrhiza showed the strongest effect, showing significant effects on all observation variables, followed by intercropping, showing non-significant effect only on the percentage of filled grain number, whereas genotypes only show significant different in grain yield per clump. Therefore, based on the main effects of application of mycorrhiza biofertilizer and intercropping with soybean, it can be concluded that both treatment factors are capable of increasing grain yield, grain anthocyanin contents and yield components of the upland promising lines of red rice grown on raised-beds under aerobic irrigation systems (Table 2). This means that AMF application increased yield potential of the upland red rice (percentage of filled panicle number per clump) and increased assimilate production and/or partition to the growing seeds in the filled panicles indicated by increased percentage of filled grain number per clump as well as grain yield per clump, and increased anthocyanin contents (concentration and total anthocyanin harvested in the grains per clump) in the husked grains of the upland red rice genotypes, without considering which genotype, and whether they were grown in monocropping or in intercropping with soybean.

The higher average of the percentages of filled panicle number per clump on the red rice fertilized with the "Technofert" bio-fertilizer containing AMF could be due to the ability of AMF in symbiosis with the red rice plants to take up more nutrients. According to results of previous researches, AMF inoculated rice plants or seedlings took up more nutrients, especially N, P, K, Ca, Zn, Cu, and other nutrients, compared with the uninoculated rice plants or seedlings [7–9,18]. Solaiman and Hirata [7] also suggested acceleration of N and P transfer from shoots and/or soil to grains due to AMF, which is very crucial for the growing seeds during the grain filling period, such as indicated by higher

percentage of filled grain number per clump on rice plants fertilized with the mycorrhiza biofertilizer in this study. In relation to higher concentration and total contents of anthocyanin in the husked grains of the red rice per clump, the result obtained here for the upland red rice is also consistent with those for the amphibious red rice lines, but there were different responses of the amphibious red rice lines to application of mycorrhiza biofertilizer in relation to increasing anthocyanin contents of the husked grains [12]. However, many researchers have also reported that AMF application significantly increased anthocyanin content in strawberry fruits, as reported by Cecatto et al [19] and Castellanos-Morales [20].

Table 1. Summary of ANOVA results on the effects of red rice genotypes, intercropping with soybean and mycorrhiza application on all observation variables

Treatment factors and their interactions	Percentage of panicle number	Percentage of filled grain number	Biomass weight per clump	Dry grain yield per clump	Grain anthocyanin concentration	Grain anthocyanin yield
Main effects:						
Genotypes	ns	ns	ns	*	ns	ns
Intercropping	**	ns	***	**	***	***
Mycorrhiza	*	***	***	***	**	***
Interaction effects:						
Genotype x Intercrop	ns	ns	ns	ns	ns	ns
Mycorrhiza x Genotype	ns	ns	ns	ns	*	ns
Mycorrhiza x Intercrop	ns	*	ns	ns	ns	ns
Myc x Intercrop x Geno	ns	**	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. Main effects of each treatment factor on all observation variables

Treatments	Percentage of panicle number per clump	Percentage of filled grain number per clump	Dry biomass weight (g/clump)	Dry grain yield (g/clump)	Grain anthocyanin concentration (ppm)	Grain anthocyanin yield (mg/clump)
M0: no AMF	87.08 ^b	92.17 ^B	70.18 ^b	40.19 ^b	14.51 ^b	0.59 ^b
M1: with AMF	93.05 ^a	96.38 ^A	89.88 ^a	51.44 ^a	16.85 ^a	0.88 ^a
HSD 0.05	5.33	1.22	4.43	3.84	1.17	0.10
G04	88.48 ^b	93.95 ^A	72.83 ^b	39.94 ^b	14.79 ^b	0.60 ^b
G10	91.65 ^a	94.59 ^A	87.23 ^a	51.68 ^a	16.57 ^a	0.87 ^a
HSD 0.05	1.89	2.47	4.49	3.89	0.52	0.08
T0: monocrop	88.40 ^a	94.24 ^A	76.71 ^a	43.62 ^b	15.29 ^a	0.68 ^a
T1: intercropping	91.73 ^a	94.31 ^A	83.36 ^a	48.01 ^a	16.08 ^a	0.79 ^a
HSD 0.05	15.78	4.84	7.78	3.73	2.62	0.15

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

In relation to the effects of intercropping the upland red rice with soybean on the yield potential of the red rice, many have previously reported significant positive effects of intercropping with soybean in increasing yield potential of rice, as those reported by Wangiyana et al [6]. However, in these upland red rice genotypes, intercropping with soybean only significantly increased grain yield per clump, but the increase in dry biomass weight, as well as concentration and total content of anthocyanin in the husked grains was not significant. However, there were significant three-way and two-way interaction effects on both percentage of filled grain number and anthocyanin concentration of the grains (Table 1), and the patterns of their interactions can be seen in Figure 1, Figure 2 and Figure 3.

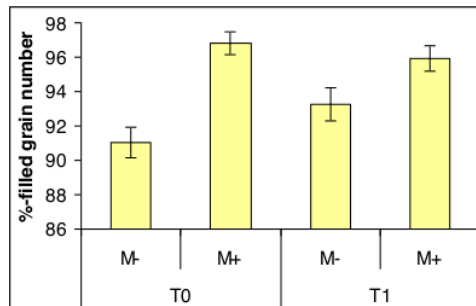


Figure 1. Average (Mean \pm SE) percentage of filled grain number as affected by interaction between intercropping with soybean and mycorrhiza

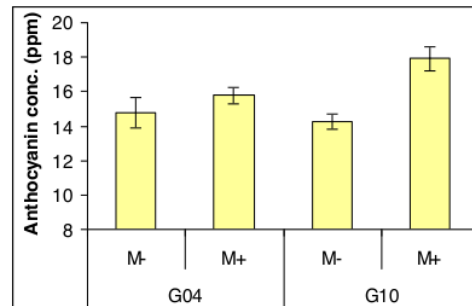


Figure 2. Average (Mean \pm SE) anthocyanin concentration (ppm) of husked grains as affected by interaction between genotypes and mycorrhiza

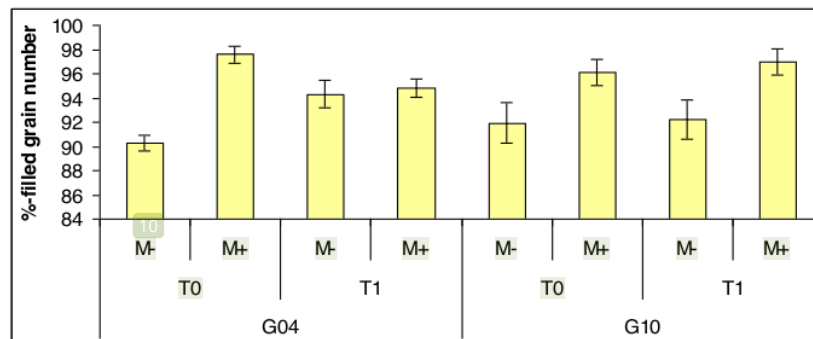


Figure 3. Average (Mean \pm SE) percentage of filled grain number as affected by three-way interactions between genotypes, intercropping with soybean, and mycorrhiza biofertilizer

It can be seen from Figure 1 that application of mycorrhiza biofertilizer resulted in more significant effects in increasing the percentage of filled grain number per clump compared with the effect of intercropping with soybean. The effect of biofertilizer application is more significant in increasing the percentage of filled grain number in monocropping than in intercropping systems. This could be due to the significant effect of intercropping with soybean in increasing the percentage of filled grain number on the rice plants that were not fertilized with mycorrhiza biofertilizer. In relation to anthocyanin concentration in the husked grains, there were also differences between the genotypes in responding to application of mycorrhiza biofertilizer. This could mean that G10 is more responsive to application of mycorrhiza biofertilizer than G04, as can also be seen from the significantly higher grain yield of the G10 than G04 (Table 2). What the causes of this different responsiveness, needs further investigation. However, in relation to the development of new red rice varieties for upland conditions, such as in the dryland, it can be seen that G10 is much better than G04.

In relation to the three-way interaction, it is clear that the responses of both genotypes to the treatments were different (Figure 3). In G04, responses of the rice plants to application of mycorrhiza biofertilizer were different between monocropping and intercropping with soybean, in which the positive significant response was higher in monocropping system than in intercropping with soybean, due to the significant positive effect of intercropping in the rice plants with no application of the

mycorrhiza biofertilizer. On the other hand, in the G10, the rice plants seem to be more responsive to application of mycorrhiza biofertilizer than to intercropping with soybean. Since intercropping with legume crops was reported to be able to increase nutrient status of the rhizosphere and grain yield of rice [13], further investigation needs to be done to find out which species of legumes is more suitable for intercropping with this G10, which shows a higher yield potential than G04. In a pot experiment, Arifuddin et al [17] found that different legume species resulted in different yield potential of red rice, in which among soybean, mungbean and peanut, it was found that intercropping with mungbean of “Kenari” variety resulted in the highest grain yield of red rice in aerobic irrigation systems.

4. Conclusion

It can be concluded that application of mycorrhiza biofertilizer resulted in more significant effect than intercropping with soybean in increasing grain yield and anthocyanin contents in the husked grains of the upland red rice genotypes. Since both factors resulted in positive and significant effects, then application of mycorrhiza biofertilizer and intercropping with soybean, in addition to increasing grain yield, are also capable of increasing health values of the husked grains of the upland red rice grown on raised-beds in aerobic irrigation system.

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