Effects of mycorrhiza biofertilizer on anthocyanin contents and yield of various red rice genotypes under aerobic irrigation systems

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Abstract. Red color in red rice kernels is because of anthocyanins, making red rice to have high health values. Mycorrhizas are reported to affect anthocyanin contents of other crops. This research aimed to examine the effects of mycorrhiza biofertilizer on grain anthocyanin contents and yield of various promising lines of red rice under aerobic irrigation systems, by conducting field experiment in Beleke, West Lombok, Indonesia, from March to July 2018, in Split Plot design with three blocks and two treatment factors, namely red rice genotypes as main plots (G04, G10, G15, G21) and AMF bio-fertilizer as sub-plots (M0= without, M1= with biofertilizer application). Results of data analysis using ANOVA and Tukey’s HSD test indicated that anthocyanin contents and almost all yield components were significantly higher on red rice supplied with AMF biofertilizer than without AMF, while those variables were mostly non-significantly different between red rice genotypes, except for 100 grain weight. Despite no interaction effects, there was a significantly positive correlation between anthocyanin contents and harvest biomass weight on red rice fertilized with AMF biofertilizers, but not on those receiving no AMF, which indicates positive effects of AMF in increasing harvest biomass and grain anthocyanins of those red rice genotypes.

1. Introduction

Rice is staple food for nearly 50% of the world population, and more than 90% of the world rice is consumed in Asia [1,2]. The most common consumed rice is white rice, i.e. the color of the grain is white. Some types of specialty rice have different color of seed coat and endosperm, and based on the color of the seed coat, there are brown, red and black rice, depending on the intensity of the anthocyanin contents of the seed coat [2,3]. Compared with the common non-pigmented rice, red rice has a high health value due to the anthocyanins and proanthocyanidins contained in red rice as well as the antioxidant activities of the red rice [3]. Angraini et al. also reported that radical scavenging activities of some varieties of red rice were up to four times of those of white rice [4]. The economic value of red rice is also higher due to its higher price in the market than that of white rice.

In Indonesia, red rice varieties are mostly local varieties developed in many different areas of Indonesia, such as in Sumatera [4], West Nusa Tenggara [5] and Southeast Sulawesi [6]. In Lombok (West Nusa Tenggara Province), there have been various promising lines of red rice developed through hybridization and selection, which results were grouped into 11 upland and 12 amphibious promising
lines of red rice based on their final adaptation tests, and one of them shows an average dry grain yield of 7.8 tons/ha in irrigated riceland [5].

Application of mycorrhiza bio-fertilizer on the promising lines of red rice grown under aerobic irrigation system was reported to be able to increase tiller number, filled panicle number and grain yield per clump in pot culture in intercropping with peanut [7], or on permanent raised-beds even with reduced NPK fertilizer [8], or with full recommended doses of NPK fertilizer [9]. Many researchers have also reported that inoculation of rice plants with arbuscular mycorrhizal fungi (AMF) resulted in higher growth performance, nutrient uptake, and grain yield of AMF inoculated rice plants than those of non-inoculated ones, both under glasshouse and field experiments [10-13]. These mean that rice plants have some dependencies on symbiosis with AMF.

AMF inoculation on many other plant species has also been reported to be able to increase anthocyanin contents of the mycorrhizal plants. Scagel and Lee reported that increased P rates and inoculation of basil plants with AMF differentially enhanced production of several other minor polyphenolics resulting in plants with different polyphenolic profiles [14]. In strawberry, Cecatto et al. reported that AMF inoculation significantly affected the contents of anthocyanin and phenolics, and when the inoculation was performed during the transplantation, the fruits of the strawberry showed a high content of anthocyanin and total phenolics [15].

This study was aimed to examine the effect of application of the “Technofert” bio-fertilizer containing AMF on the grain anthocyanin content and yield of several promising lines of red rice grown on raised-beds in aerobic irrigation system.

2. Methods
In this study, the field experiment was conducted on farmers' ricefield in Beleke Village, Gerung District, West Lombok Regency, NTB Province, Indonesia (116°7’54” E; 8°39’29” S), from May to September 2018. Rice plants were grown on raised-beds under aerobic irrigation system.

2.1. Treatments and experimental design
The experiment was designed according to Split Plot design with 3 blocks and two treatment factors, namely red rice promising lines (G) as the main plot factor, consisting of 4 selected genotypes of red rice (G04 = MG4, G10 = MG10, G15 = AM4, and G21 = AM10), and application of AMF (M) as the sub-plot factor, consisting of 2 treatments (M0= without AMF application but the rice plants were fertilized with full recommended doses of N-P-K fertilizers; M1= AMF application at planting combined with full recommended doses of N-P-K fertilizer). Those promising lines of red rice were selected from previous research results, consisting of upland genotypes (MG4 and MG10) and amphibious genotypes (AM4 and AM10) [5]. The AMF inoculant used was the bio-fertilizer "Technofert", which contains mixed species of arbuscular mycorrhizal fungi (AMF) and zeolit particles, supplied by PT Mikata Sukses Mandiri, Serpong, Indonesia.

2.2. Implementation of the field experiment
The entire procedures for implementation of the field experiment, including the planting geometry, are as explained in Wangiyana et al., except for the treatments and anthocyanin analysis (no intercropping treatments in this study, and no anthocyanin analysis) [9]. The anthocyanin data of the husked red rice grains 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.

2.3. Observation variables and data analysis
Observation variables measured from four clumps of red rice plant samples per bed, which include filled panicle number at harvest, filled grain number, above-ground biomass weight (dry straw + grains), grain yield per clump, weight of 100 filled grains, 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 “CoStat for Windows” ver. 6.303.

3. Results and discussion

The ANOVA results summarized in Table 1 indicate that there was no interaction effect between genotypes of the promising lines of red rice and application of AMF on all observation variables. However, between the two treatment factors, AMF application shows significant effects on all variables but the genotypes of the red rice show differences among the promising lines only in filled panicle number per clump and weight of 100 filled grains.

Table 1. Average panicle number per clump, dry biomass weight per clump, percentage of unfilled grain number, grain yield per clump, weight of 100 grains, anthocyanin concentration of the grains, and anthocyanin yield in the grains for each level of treatment factor.

| Treatments                        | Panicle number per clump | Dry harvest biomass (g/clump) | Percentage of filled grain number (%) | Grain yield (g/clump) | Weight of 100 grains (g) | Anthocyanin concentration (ppm) | Anthocyanin yield in grain (mg/clump) |
|-----------------------------------|--------------------------|-------------------------------|---------------------------------------|-----------------------|--------------------------|---------------------------------|--------------------------------------|
| Mycorrhiza application (M):       |                          |                               |                                       |                       |                          |                                 |                                      |
| M0                                | 20.92 b                  | 67.50 b                       | 92.49 b                               | 37.84 b               | 2.56 b                   | 13.88 b                        | 0.53 b                              |
| M1                                | 23.46 a                  | 81.82 a                       | 95.78 a                               | 45.12 a               | 2.68 a                   | 16.36 a                        | 0.74 a                              |
| HSD 0.05                          | 1.62                     | 6.93                           | 2.19                                  | 6.00                  | 0.08                     | 1.11                           | 0.13                                 |
| Red rice genotypes (G):           |                          |                               |                                       |                       |                          |                                 |                                      |
| G04                               | 20.42 b                  | 67.36 a                       | 93.91 a                               | 36.66 a               | 2.51 b                   | 14.45 a                        | 0.54 a                              |
| G10                               | 23.50 a                  | 78.30 a                       | 94.33 a                               | 43.22 a               | 2.43 b                   | 15.13 a                        | 0.66 a                              |
| G15                               | 21.08 b                  | 72.48 a                       | 95.29 a                               | 40.68 a               | 2.79 a                   | 15.00 a                        | 0.61 a                              |
| G21                               | 23.75 a                  | 80.49 a                       | 93.00 a                               | 45.37 a               | 2.76 a                   | 15.91 a                        | 0.73 a                              |
| HSD 0.05                          | 1.84                     | 14.58                         | 2.99                                  | 11.64                 | 0.17                     | 2.28                           | 0.27                                 |

ANOVA results:

- Mycorrhiza: s s s s s s s s 2)
- Genotypes: ns ns ns ns s ns ns ns
- Interaction: ns ns ns ns ns ns ns ns

1) Same letters in each column indicate non-significant differences between levels of a treatment factor
2) ns = non-significant (p>=0.05); s = significant (p<0.05)

Based on the main effects of the treatment factors on yield components and anthocyanin contents, it can be seen from Table 1 that application of the bio-fertilizer containing AMF on red rice plants significantly increased mean values of all observation variables, compared with those in the non-biofertilized red rice plants. This means that AMF application increased yield potential of the red rice (filled panicle number per clump) and increased assimilate production and/or partition to the growing seeds in the filled panicles indicated by increased weight of 100 filled grains and percentage of filled grain number as well as grain yield per clump.

The higher average number of filled panicles 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, as has been previously reported by others, such as Dhillion and Ampornpan, who found that rice seedlings inoculated with AMF in the nursery contained higher amount of P, K, Ca, Fe, Cu, Na, B, Zn, Al, Mg and S, compared with the non-inoculated seedlings [10]. Solaiman and Hirata also reported that AMF inoculation significantly increased concentration of N, P and K in the unhulled rice grains, and increased concentration of N and K in shoots and roots of the rice plants [11]. From other experiment, Solaiman and Hirata also found that AMF inoculation increased uptake of micronutrients such as Zn, Cu, Fe and Mn [12]. It was also reported that AMF inoculation in dry nursery
was found to be more significant in increasing N, P, Cu and Zn uptake than in wet nursery, especially for rice plants grown in the field [13]. In addition to higher nutrient uptake of AMF inoculated rice plants, Solaiman and Hirata also suggested that AMF may accelerate N and P transfer from shoots and/or soil to grains of rice plants [11].

In addition to increased grain yield and some yield components of red rice, AMF biofertilizer application also significantly increased anthocyanin concentration in the husked red rice grains and total anthocyanin contained in the grains (anthocyanin yielded) per clump. Many previous researchers have also reported that AMF inoculation increased phytochemical contents including anthocyanins in several plant species, such as strawberry, especially in the Furtuna variety, and early inoculation [15], and basil plants [14]. Castellanos-Morales also found that AMF inoculation increased anthocyanin content in fruits of strawberry but only in the lower doses of N fertilizer application [16]. These all show the potential of AMF symbiosis in increasing quality of crop yield for human health, including anthocyanin concentration of the red rice genotypes in this study.

However, there are slight different responses between the red rice genotypes to application of the AMF biofertilizer, especially in terms of total above-ground biomass per clump (Fig. 1), percentage of filled grain number (Fig. 2), anthocyanin concentration of the husked grains (Fig. 3), and total anthocyanin yielded per clump (Fig. 4), in which response of both upland genotypes (i.e. G04 and G10) to application of the AMF biofertilizer was significant and was higher than that of both amphibious genotypes (i.e. G15 and G21) in terms of those observation variables, except for G21 in relation to anthocyanin concentration in the husked grains of the red rice.

Unlike experimental results reported by Rahman et al., who suggested that anthocyanin deposition in pericarp of black rice resulted in lower grain yield due to lower rates of photosynthesis of black rice compared with the white rice progenies, in this study, only the selected red rice genotypes were used among the progenies [17]. Results of regression analysis show significant positive correlation between total above-ground biomass weight and anthocyanin concentration, but only in the red rice plants fertilized with AMF (Fig. 5), whereas in those receiving no AMF, it was non-significant (Fig. 6). Since this significant relationship occurs only in the red rice plants fertilized with AMF, then the higher anthocyanin concentration accompanied by higher biomass and higher grain yield (Table 1) must be due to AMF application. These higher values of yield components of red rice plants supplied with AMF biofertilizer could be due to the potential of AMF in increasing nutrition of rice plants [10-13]. In addition, increased uptake of nutrients, such as N, P and K, due to AMF inoculation on rice plants were also reported to increase dry matter partitioning from straw to grains and increase grain yield [11].

**Figure 1.** Average (Mean ± SE) biomass weight (g/clump) for each combination of red rice genotype and AMF application.

**Figure 2.** Average (Mean ± SE) percentage of filled grain number for each combination of red rice genotype and AMF application.
The AMF biofertilizer in this study was applied at seeding. Therefore, the higher anthocyanin and biomass or grain yield in the AMF biofertilized red rice plants compared with in those receiving no AMF biofertilizer could be due to higher nutrient uptake, especially N, P and Mg, which could have been started since the seedling growth stage, as reported by Dhillion and Ampornpan [10]. According to experimental results reported by Tisarum et al., Mg-enrichment through MgSO₄ foliar fertilization of Thai black-pericarp grain rice plants at a concentration of 100 mM MgSO₄ could increase anthocyanin content and grain yield of the back rice compared with the control plants [18]. In soil, magnesium (Mg) and phosphorus (P) are immobile nutrients [19], and the most important contribution of AMF symbiosis is to increase nutrient uptake [20], especially the immobile nutrients [21,22]. Therefore, it is logical to conclude that the higher anthocyanin concentration accompanied by higher biomass and grain yield of those promising lines of the red rice fertilized with the “Technofert” biofertilizer containing AMF in this study could be due to better nutrition of the biofertilized red rice plants, including higher uptake of Mg²⁺ [10], which have been reported to increase anthocyanin content and grain yield of black rice [18].
4. Conclusion
It was concluded that AMF inoculation through application of the “Technofer” biofertilizer significantly increased biomass weight, grain yield, and anthocyanin content in the husked grains of various promising lines of red rice grown on raised-beds under aerobic irrigation system. Although the responses were not significantly different among the red rice genotypes tested, it appears that the responses to application of mycorrhiza biofertilizer were higher in both upland genotypes (i.e. G04 and G10) than in the amphibious genotypes (i.e. G15 and G21) of the promising lines of red rice, especially in terms of biomass weight, percentage of filled grain number, and total anthocyanin content in the husked grains.

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