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Treating Radiation-induced Aceh Rice Germplasm with Arbuscular Mycorrhizal Fungi has been Shown to Effectively Address Bacterial Leaf Blight

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ABSTRACT

Background: Bacterial leaf blight can damage upland rice and reduce yield by 30-40%. Chemical pesticides harm ecosystems and can increase pathogen resistance. The objective of this research was to explore the potential of AMF, a biological agent, to enhance the resistance of local Acehnese-radiated mutant rice against bacterial leaf blight.

Methods: Two-factor factorial design with three levels of AMF dosage (0 g, 10 g and 20 g per plant) and three upland rice lines (UA-01, UA-09 and UA-10). There were 9 treatment combinations replicated 3 times, resulting in 81 experimental units.

Result: The intensity of the disease decreased significantly as the dosage of AMF increased and the maximum number of tillers increased substantially up to 20 g plant^{-1} AMF application. UA-10 strains exhibited the highest number of tillers per clump, while UA-09 strains had the lowest. The interaction between local Aceh rice varieties and AMF did not impact bacterial leaf blight intensity, maximum number of tillers and percentage of filled grain, except for AMF colonization on roots. The significance of AMF colonization varied among lines, with the highest AMF colonization observed in the UA-10 strain (20.64%) at an AMF dose of 20 g plant^{-1}. AMF inoculation appeared to affect the percentage of filled grain positively.

Key words: Aceh local rice line, Bacterial leaf blight, Biocontrol, Mycorrhiza, Radiation mutation.

INTRODUCTION

Bacterial leaf blight is a serious disease that affects upland rice in Indonesia, resulting in a loss of up to 30-40% in yield (Yanti *et al*., 2018). The pathogen penetrates the plant through wounds on the leaves or the stomata, causing damage to the leaf chlorophyll and reducing the plant's ability to photosynthesize. During the vegetative phase, the rice plant will perish and during the generative phase, the grain filling will be sub-optimal (Department of Agriculture, 2015).

The farmers of West-South Aceh, Indonesia, are renowned for their skill in cultivating a variety of rice, including the ever-popular Sigupai rice from Nagan Raya. Unfortunately, this particular type of rice is susceptible to leaf blight - a common disease caused by *Xanthomonas oryzae* pv. *oryzae* bacteria. To combat this issue, the Aceh Rice Research Institute has created hybrid rice varieties by cross-breeding Sigupai rice with the IRBB27 rice variety - which has proven to be resistant to leaf blight and possesses other desirable traits. However, it remains unclear whether these new hybrids are effective in preventing leaf blight (Bakhtiar *et al*., 2011).

The extensive use of chemical pesticides has caused harmful effects on farmland ecosystems, leading to an increase in the resistance of fungi, bacteria, nematodes and other pathogens to pesticides, which has resulted in the rapid spread of diseases (Huang *et al*., 2011). Due to the improvement in people's living standards, food safety concerns arising from the use of pesticides and chemical fertilizers have become a widespread issue (Razak and Gange, 2021). As a result, environmental scientists and plant pathologists are actively looking for green and eco-friendly technologies to control plant diseases (Begum *et al*. 2019; Van Driesche *et al*., 2010).

Arbuscular mycorrhizal fungi (AMF) have been shown to regulate secondary metabolites in host plants, which can enhance the physical appearance of plants and the chemical properties of the soil. Additionally, AMF can compete with pathogens for resources, activate the plant's disease resistance and defense systems and serve as an effective biological control agent. Studies have demonstrated that AMF can reduce the damage caused by various pathogens in crops (Razak and Gange, 2021; Begum *et al*., 2019; Awad-Allah *et al*., 2023). More than 30 species of AMF are effective in controlling plant soil-borne

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diseases (Jung *et al*., 2012). By utilizing AMF as a biological control agent, we can reduce the need for pesticides and improve the resistance of local Aceh rice lines to bacterial leaf blight. Overall, the study of AMF as a biological control agent is both theoretically and practically important.

MATERIALS AND METHODS

Research design

This research was conducted in 2022, at the Laboratory of Plant Pathology and Experiment Station of Syiah Kuala University and utilized a completely randomized design (CRD) with a two-factor factorial pattern. The first factor was AMF and included three dosage levels: 0 g FMA.plant^{-1}, 10 g AMF. plant^{-1} and 20 g AMF. plant^{-1}. The second factor consisted of upland rice lines of local Acehnese varieties, UA-01, UA-09 and UA-10, resulting from radiation mutation. As a result, there were 9 treatment combinations. Each treatment was repeated 3 times and consisted of 3 experimental pots, resulting in a total of 81 experimental units.

Preparation of *Xanthomonas oryzae* pv. *oryzae* (Xoo) isolates

Xanthomonas oryzae pv. *oryzae* (Xoo) isolates were from the Plant Disease Laboratory at Syiah Kuala University. They were grown on Nutrient Agar (NA) media and prepared into a 10^{-6} dilution suspension after 4 days of growth.

Preparation of planting media

Soil samples were collected from IeSuum in Aceh Besar District. The soil, identified as inseptisol, underwent a thorough process of drying, crushing and passing through a 2-mesh sieve. To create the planting media, the soil was blended with mature cow manure in a 2:1 ratio. The resulting mixture was then deposited into experimental pots (polybags) weighing 10 kg each. Before planting, the media was carefully assessed to ensure it was at field capacity and this measurement was maintained employing the weighing method.

Application of AMF and planting of upland rice

AMF (provided by PT Agrofarm Mycogrow), consisted of various spores including *Glomus manihotis, G. intraradices, G. aggregatum, Acaulospora* sp. and *Gigaspora* sp. The spore count was 33 per gram and the medium used was a zeolite carrier. The rice seeds (provided by the Plant Breeding and Biotechnology Laboratory) were F9 inbred, resulting from a cross between Sigupai and IRBB27 varieties. Some of the seeds (UA-01, UA-09 and UA-10) were mutated using Gamma-ray technology. The AMF treatment was applied according to protocol; the spores were inserted into a 3 cm hole and covered with soil. Three 2-day-old rice seedlings were then transplanted into the holes. Thinning was carried out by removing two poorly growing plants when the seedlings reached 7 days old.

Inoculation of xoo suspension

Xoo inoculation was performed on rice leaves 30 days after planting (HST) using the sterile scissor-cutting technique. The leaf 3 cm from the tip was cut and then dipped into the *Xoo* isolate suspension.

Observation variable

Disease intensity (%)

The disease intensity of rice was evaluated in the third week after inoculation (WAI) using the standard evaluation formula for rice (IRRI, 2014).

$$\text{Disease intensity} = \frac{\text{Length of infected leaves}}{\text{Whole leaf length}} \times 100\%$$

AMF colonization on roots (%)

The ability of AMF to colonize the roots was examined at the age of 50 HST through the root staining technique (Nusantara, 2011). Ten cuttings of roots approximately 1 cm in length were placed in a row on a glass slide and then observed under a microscope. The colonization ability of arbuscular mycorrhizal fungi on the roots was calculated using the following formula (Hapsoh *et al*., 2006):

$$\text{Root colonized} = \frac{\text{Number of infected roots}}{\text{Total number of root pieces}} \times 100\%$$

Maximum number of tillers per clump

The number of tillers per clump was counted 60 days after planting (DAP) for each treatment.

Percentage of full grain per clump (%)

The percentage of full grain and hulled grain were calculated using different formulas after drying the seeds at room temperature

$$\text{Percentage of full grain} = \frac{\text{Number of full grain}}{\text{Total grain}} \times 100\%$$

Data analysis

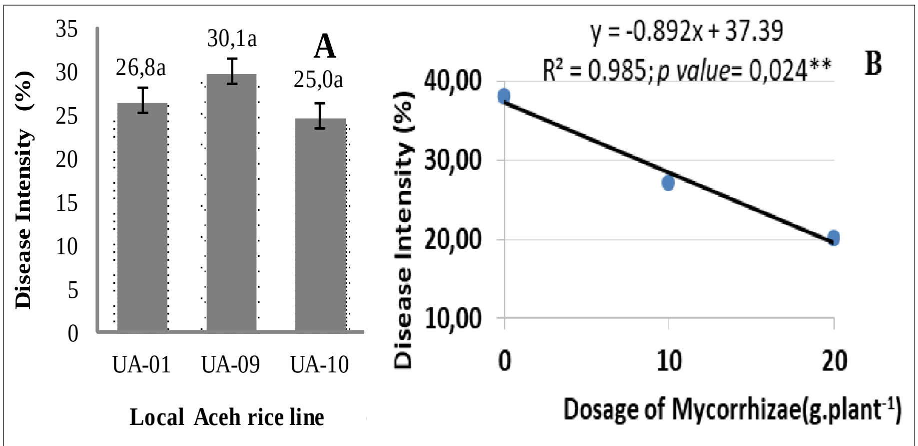
The ANOVA test was used to analyze data from observations on each variable. If a variable significantly influenced the F count, the analysis continued with the least significant difference test (LSD) at the 0.05 level (Gomez and Gomez, 1995).

RESULTS AND DISCUSSION

There was no interaction between AMF and the Aceh local rice line, resulting from gamma radiation mutation, on disease intensity, number of tillers and percentage of full grain, except for AMF colonization on plant roots. It can be concluded that the intensity of bacterial leaf blight in the rice line was determined independently by each factor.

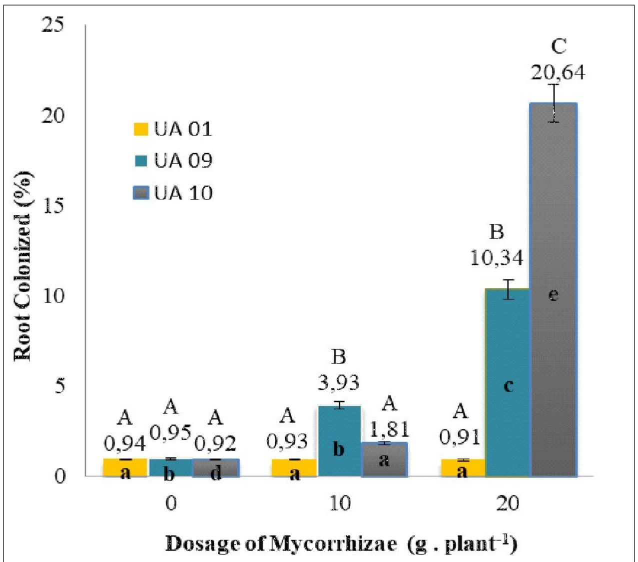
Disease intensity

UA-09 exhibited a slightly higher incidence of 30.10% (Fig 1 A). The application of AMF had an impact on the severity of bacterial leaf blight disease. As the quantity of mycorrhiza



Notes: Numbers followed by the same letter indicate no significant difference on the LSD test (α = 0.05).

Fig 1: The intensity of bacterial leaf blight disease in upland rice lines of local .Aceh varieties resulting from gamma radiation mutation (A) and in the presence of AMF (B).



Notes: Small letters within the same block of the line at the same dose do not show any significant difference in the LSD test (with an alpha value of 0.05).

Fig 2: Mycorrhizal colonization on the roots of local rice variety line resulting from radiation mutation.

used increased, there was a noticeable decrease in the intensity of bacterial leaf blight disease (Fig 1 B).

FMA colonization on roots (%)

AMF given up to 10 g. plant^{-1} of AMF, gave no significant difference in root AMF colonization among the three lines compared to the line without AMF. UA-09 exhibited the highest root colonization (3.93%), which was significantly different from UA-01 (0.93%) and UA-10 (1.81%). When the mycorrhiza application was increased to 20 g.plant^{-1}, the UA-10 strain displayed the highest root colonization (20.64%), which was significantly higher than root colonization in the other two lines. At a dose of 20 g.plant^{-1}, root colonization in both UA-09 (10.34%) and UA-10 (20.64%) was significantly higher than root colonization in the same line at a mycorrhizal dose of 10 g.plant^{-1} and without AMF application (Fig 2).

Maximum number of tillers

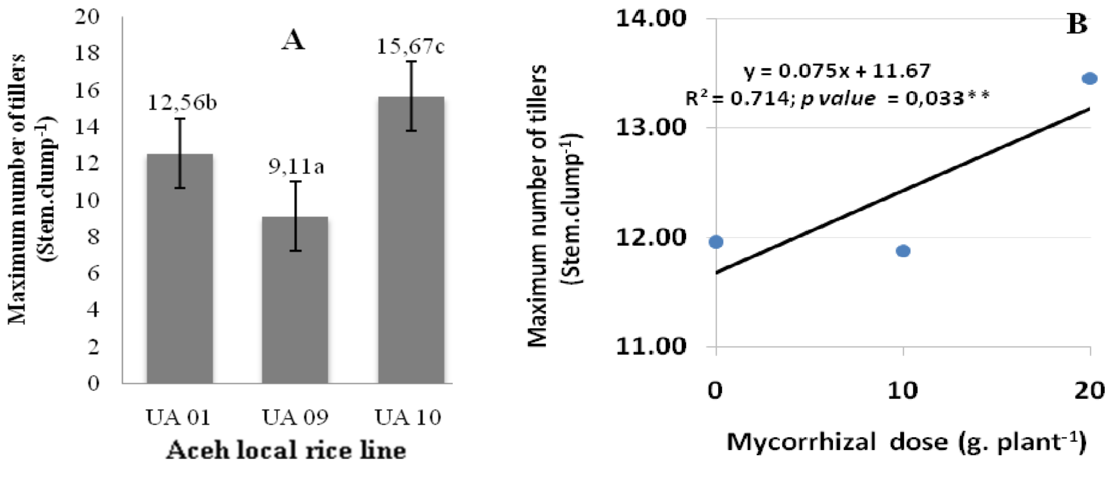
The local Acehnese rice lines UA-01, UA-09 and UA-10, which were produced through radiation mutation, displayed varying maximum numbers of tillers per clump. UA-10 had a significantly higher number of tillers per clump compared to UA-01 and both had significantly higher numbers than UA-09 (Fig 3A). The application of mycorrhiza was found to have a significant impact on increasing the maximum number of tillers per clump in the local Acehnese rice line resulting from radiation mutation, as suggested by regression analysis, as shown in (Fig 3B). The p-value was less than 0.05. The introduction of mycorrhiza significantly affects the intensity of bacterial leaf blight in the upland rice line of local Acehnese rice, which resulted from radiation mutation. p-value<0.05 = 0.024** in the regression equation y = 37.39 - 0.892 X; R^2 = 0.985 (Fig 1 B).

Full grain per clump (%)

There were significant differences in the percentage of parched grain among these three lines (Fig 4 A). UA-09 had the highest percentage of full grain per clump (38.55%), followed by UA-01 (35.84%) and UA-10 had the lowest (30.77%). The regression analysis indicated that mycorrhizal inoculation on the local Acehnese rice line resulting from radiation mutation did not have a significant effect on the percentage of full grain per clump in the regression equation y = 34.42+ 0.356x with a value of pÃ0.05, but did show an increasing trend (Fig 4 B).

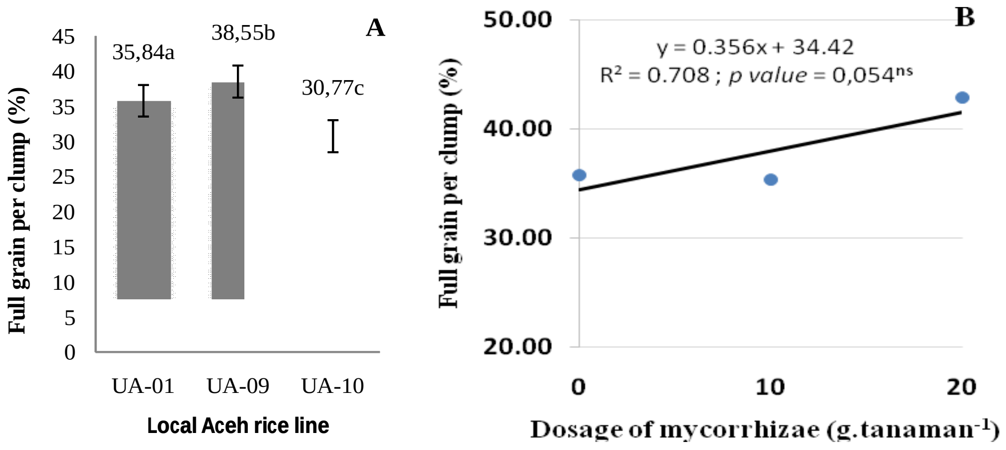
The introduction of mycorrhiza significantly affects the intensity of bacterial leaf blight. Arbuscular mycorrhizal fungi (AMF) play a crucial role, in enhancing plant nutrition, changing the morphological structure of plant roots, regulating the synthesis of secondary metabolites and promoting plant disease resistance and defense system formation. Plants benefit from root endophytes that extend their zone of activity beyond the rhizosphere (Hohman *et al*., 2012). The mechanism analysis mainly includes the improvement of plant nutrition, changes in the morphological structure of plant roots, regulation of the synthesis of secondary metabolites, improvement of the plant rhizosphere microenvironment, direct competition with pathogenic microorganisms for invasion sites and nutrients and promotion of plant disease resistance and defense system formation (Chen *et al*. ,2021; Song *et al*., 2011). The symbiosis of AMF can lead to the growth, thickening and branching of the host plant's root system, which effectively slows down the process of root infection by pathogens (Basyal and Emery, 2021). Boutaj *et al*. (2019) reported that *Gossypium hirsutum* symbiotic with *G. mosseae* and *G. etunicatum* showed palisade tissue increase, vessel deformation, a gelatinous substance produced in vessels, cell deformation and shrinkage and significant cell wall thickening. These changes are all advantageous in enhancing the host plant's resistance to *Verticillium dahliae*.

Colonization by native or indigenous AMF species on cereal crops in general and rice, in particular, has been reported previously (Campos-Soriano *et al*., 2010; Cosme *et al*., 2014; Chen *et al*., 2021). To assess symbiotic relationships, the level of mycorrhizal colonization in cereal crops, including rice, is a widely accepted index. Our experiment revealed that the mycorrhizal colonization rate of Aceh local upland rice lines UA 01, UA 09 and UA 10 varied, ranging from an average of 0.92% to 20.64%. UA 09 and UA 10 had a significantly higher colonization rate than



Notes: Numbers followed by different letters indicate significantly different according to the LSD test (α = 0.05).

Fig 3: The maximum number of tillers per clump of Aceh local rice resulting from mutation radiation by strain (A) and mycorrhiza dose (B).



Notes: Numbers followed by different letters indicate significantly different according to the LSD test (á = 0.05).

Fig 4: Percentage of full grain per clump based on upland rice line local variety (A) and mycorrhiza dose B).

UA 01. The number of spores produced by the host plant is directly related to the percentage of colonization. The differences in spore production could be attributed to the host plant's adaptability to growth conditions such as soil temperature, pH and moisture.

Plants that grow faster and are more rigid in their growth such as maize plants provide suitable conditions for higher sporulation. Plants that are more stable in growth and larger in root size, often have more extensive root systems that allow for higher spore formation which in turn increases the percentage of colonization on roots compared to smaller hosts (Sinegani and Sharifi, 2007). The current data shows that extensive colonization of host plant roots results in healthy plant growth and better root system development (Tahat *et al*., 2008). Associated with the high percentage of root colonization in the UA-10 and UA-09 lines indicates that the growth of these two lines is faster, more rigid, or sturdy with a wider root system that supports higher colonization.

The variation in plant colonization of lines UA-01, UA-09 and UA-10 inoculated with mycorrhiza at different levels or doses, may be related to the specific nature of protection in each strain induced by mycorrhiza. Thus, it was hypothesized that the low AMF colonization in the roots of local rice varieties Aceh UA-01 and UA-10 is due to the low synthesis of JA in these two lines (Hause *et al*., 2007).

Regression analysis of the maximum number of tillers in our study found that the maximum number of tillers in the rice line of local Acehnese varieties resulting from radiation mutation (UA 01, UA 09 and UA 10) increased significantly in line with the increase in the dose of mycorrhiza applied ($y = 11.67 + 0.075x$; $R^2 = 0{,}714$; p-value = 0.033**. Fig 3 B). These results in turn will have an impact on increasing the yield of rice plants, especially local Acehnese rice varieties resulting from radiation mutations.

Higher grain yield indicates that the genotype has high productivity. The grain yield is highly determined by the availability of nutrients, the guarantee of plant photosynthesis and the number of panicles on each tiller. Fully grain is strongly influenced by the results of photosynthate which comes from two sources of assimilation. One is before fertilization which is stored in the stem tissue and then converted into substances and lifted to the seeds, the other is, made during ripening (Yoshida, 1981). This is also due to the colonization process of AMF on plant roots that can expand nutrient absorption to plant roots with the help of AMF external hyphae that grow and develop through plant roots. The use of AMF as a biofertilizer can improve plant growth and plant quality.

CONCLUSION

The utilization of AMF in upland rice, particularly in Aceh local varieties resulting from radiation mutation, can have a positive ecological impact as a biocontrol solution. The establishment of AMF in the roots is crucial in protecting against bacterial leaf blight. In the UA 10 line, AMF colonization increased significantly, up to 20 grams per mycorrhiza plant. All three lines exhibited comparable resistance to bacterial leaf blight. Although the UA 09 line had a slightly higher ability to generate full grain, it was not significantly different from the other lines.

Conflict of interest

All authors declare that they have no conflicts of interest.

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