Effect dual inoculation of *Azotobacter* and *Azospirillum* on the productive trait upland red rice cultivar

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Abstract. The aims of this research were to analyze the effect of dual inoculation of *Azotobacter* and *Azospirillum* on the productive trait upland red rice cultivar. The research was conducted in the field of experimental farm, department of agronomy, the faculty of agriculture, Halu Oleo University. This research arranged in split-plot design with dual inoculation treatment in the main plot and cultivar differences in the subplot, with three replications. The dual inoculation treatment consist of three levels as follows: b₀ = without inoculation, b₁ = treatment with *Azotobacter* 2.5 L ha⁻¹ + *Azospirillum* 2.5 L ha⁻¹ and b₂ = Treatment with *Azotobacter* 5.0 L ha⁻¹ + *Azospirillum* 5.0 L ha⁻¹. The type of cultivar used were (v₁) = Labandiri, (v₂) = Jangkobembe, (v₃) = Ranggohitam and (v₄) = Paedara. The result showed that the bacterial fixator application would (1) increase the productive tillers, and (2) increase rice productivity through the increase of productive tillers and grain weight.

1. Introduction

Rice is not the only source of carbohydrate but also a source of nutritive food [1]. Rice as a nutritive food because containing anthocyanins,[2]. Anthocyanin functions as an antioxidant [3,4] and plays an important role in human health [5]. As the functional food, rice, and especially red rice must be produced in an eco-friendly way and as much as minimize using of inorganic fertilizer [6].

The need for rice in Indonesia continues to increase in line with population growth, although food development other than rice also continues to increase [7]. Various attempts were made to increase rice production, including by developing upland rice [8,9], improving seed quality [10,11], and plant breeding through the irradiance [12,13] or hybridization [14–17].

Most of the efforts to increase production are made by providing inorganic fertilizer as one of the technologies are chosen [18]. The main choice the use of inorganic fertilizers because the fertilization effects have quickly seen [19]. However, continuous use of inorganic fertilizers can result in a decrease of soil fertility [20] and environmental sustainability [21,22].

Cultivation of red rice in the organic model is one of a good choice. That reduces as much as possible the use of synthetic materials and replacing them with improved organic fertilizer [23–25], that enriched plant growth promoter rhizobacteria (PGPR) as biological fertilizer. One of the PGPR
are root endophytic diazotrophic bacteria such as *Azotobacter* [25,26], and *Azospirillum*, [27,28]. *Azotobacter* sp. and *Azospirillum* sp., produce growth hormones, and it is able to carry out nitrogen fixation from the air [29,30]. Some research results show that the treatment of Azotobacter and Azospirillum can increase plant growth and production [31,32]. The use of organic fertilizer enriched with biological fertilizers had promised prospects and environmentally friendly.

2. **Materials and methods**

The research arranged in split plot design. The dual inoculation treatment placed it as main plot, consists of 3 level, include b0 = without inoculation; b1 = treatment with *Azotobacter* 2.5 L ha⁻¹ plus *Azospirillum* 2.5 L ha⁻¹; and b2 = treatment with *Azotobacter* 5.0 L ha⁻¹ plus *Azospirillum* 5.0 L ha⁻¹. The different of cultivar placed as subplot, i.e., (V₁) = Labandiri, (V₂) = Jangkobembe, (V₃) = Ranggohitam, (V₄) = Paedara.

Several generative characters are examined, such as the number of productive tillers, the number of filled grains, and the potential for grain production. The data processing in this study were performed using analysis of variance (ANOVA) with SPSS and further tested using Duncan’s Multiple Range Test (DMRT).

3. **Results**

3.1. **Effect of dual inoculation bacterial on productive tillers**

The results showed that dual inoculation treatment with *Azotobacter* and *Azospirillum* could increase the productive tillers, harvest date, and production (Table 1-3). The increase of productive tillers is in line with the increase of bacterial fixator dosage. It occurs in Labandiri, Ranggohitam, and Paedara cultivar. The highest number of productive tillers had on the Paedara Cultivar (18.69 clumps⁻¹), followed by Labandiri (18.61 clumps⁻¹), Ranggohitam (16.16 clumps⁻¹) and Jangkobembe (14.38 clumps⁻¹).

### Table 1. Effect application of dual bacterial *Azotobacter* and *Azospirillum* on productive tillers.

| Bacterial Treatment | Number of Productive Tiller |
|---------------------|-----------------------------|
| b₀ (Without bacterial treatment) | 17.13 15.40 14.53 18.40 16.37 |
| b₁ (*Azotobacter* 2.5 L ha⁻¹ + *Azospirillum* 2.5 L ha⁻¹) | 19.67 14.67 14.73 17.07 16.53 |
| b₂ (*Azotobacter* 5.0 L ha⁻¹ + *Azospirillum* 5.0 L ha⁻¹) | 19.00 13.07 19.20 20.60 17.97 |
| Average            | 18.60 14.38 16.16 18.69 16.96 |

Remarks : v₁=Labandiri, v₂=Jangkobembe, v₃=Ranggohitam, v₄=Paedara

3.2. **Effect of dual inoculation bacterial on harvest time and grain weight**

Variance analysis results showed that the treatment of fixator bacteria significantly affected the harvest date and grain weight (Table 2). The application of a bacterial fixator delayed the plant to reach the harvest date.

### Table 2. Effect application of dual bacterial *Azotobacter* and *Azospirillum* on harvest date and grain weight of local upland red rice.

| Bacterial Treatment | Harvest Date (Day) |
|---------------------|---------------------|
|                   | v₁     | v₂     | v₃     | v₄     | Average |
| b₀ (Without bacterial treatment) | 139.67 137.33 140.67 136.00 138.42 |
| b₁ (*Azotobacter* 2.5 L ha⁻¹ + *Azospirillum* 2.5 L ha⁻¹) | 140.33 138.00 141.33 135.67 138.83 |
| b₂ (*Azotobacter* 5.0 L ha⁻¹ + *Azospirillum* 5.0 L ha⁻¹) | 141.00 137.33 142.00 136.00 139.08 |
| Average            | 140.33 137.56 141.33 135.89 138.78 |

Weight of 1000-grain
In the grain weight character (Table 2), it increases with increasing dosage of application of fixative bacteria. The highest weight of one thousand grain founded in the Paedara cultivar (29.12 g), then followed by Labandiri (28.90 g), Jangkobembe (27.98 g), and Ranggohitam (20.85 g).

### Table 3. Effect application of dual bacterial *Azotobacter* and *Azospirillum* on the production of local upland red rice.

| Bacterial Treatment                                      | v1  | v2  | v3  | v4  | Average |
|----------------------------------------------------------|-----|-----|-----|-----|---------|
| b0 (Without bacterial treatment)                         | 28.64 | 27.70 | 20.84 | 29.46 | 26.66  |
| b1 (*Azotobacter* 2.5 L ha$^{-1}$ + *Azospirillum* 2.5 L ha$^{-1}$) | 28.98 | 29.27 | 19.97 | 28.56 | 26.69  |
| b2 (*Azotobacter* 5.0 L ha$^{-1}$ + *Azospirillum* 5.0 L ha$^{-1}$) | 29.07 | 26.99 | 21.75 | 29.35 | 26.79  |
| Average                                                  | 28.90 | 27.98 | 20.85 | 29.12 | 26.71  |

Remarks: v1 = Labandiri, v2 = Jangkobembe, v3 = Ranggohitam, v4 = Paedara

Fixator bacterial treatment, in general, can increase grain production per clump. The increase in grain production is in line with the increase in the application of fixator bacteria, and this occurs in the cultivars of Labandiri, Ranggohitam, and Paedara.

### 3.3. Effect of dual inoculation bacterial on potential production

The fixator bacterial treatment could also increase grain production (Table 3). The highest average grain production found in Labandiri cultivar (32.75 g), followed by Paedara (31.77 g), Jangkobembe (22.72 g), and Ranggohitam (20.94 g) respectively.

### 4. Discussion

In general, the treatment of fixative bacteria could increase the productive tiller, weight of 1000 grain and potential production. The bacterial application could delay the plant entering the generative phase. It indicated by the delays in flower formation and the delay in plants entering the age of harvest. It can be understood because the bacteria applied can carry out nitrogen fixation so that it can increase nitrogen supply and result in an extension of the vegetative phase [33] and the delay in the plant entering the generative phase.

While in some characters, bacterial fixator treatment could increase the productive tiller, grain weight, and production potential. This situation occurs because the bacteria used is a non-symbiotic bacterium that can carry out nitrogen fixation and is also able to extract other nutrients so that it can affect the increase in production. The application of bacterial fixator can increase production through its effect on increasing the number of tillers and increasing the grain weight.

### 5. Conclusion

It concluded that the treatment of fixative bacteria affected the extension of the vegetative phase and delayed the plants reaching flowering. The bacterial treatment could increase the potential production, and it influences through the increasing of productive tillers and grain weight.
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