The bacterial mixture effect of *Azotobacter* and *Azospirillum* on nitrogen content and harvest date of upland red rice cultivar

Muhidin1, E Syam’un2, Kaimuddin2, Y Musa2, G R Sadimantara1, S Leomo1, D N Yusuf1 and T C Rakian1

1Department of Agrotechnology, Faculty of Agriculture, Universitas Halu Oleo, Kendari, Southeast Sulawesi 93212 Indonesia
2Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, 90245 Indonesia

E-mail: muhidinunhalo@gmail.com

**Abstract.** The research aims were to analyse the effect of bacterial mixture of *Azotobacter* and *Azospirillum* on the nitrogen content and harvest date of upland red rice. This research was conducted in Agriculture Farm of Agriculture Faculty in Halu Oleo University. The research was arranged in a split plot design. The bacterial mixture dosage as main plot and different of cultivar as sub plot. The bacterial mixture dosage were without application bacterial mixture, Bacterial combination mixture of *Azotobacter* 2.5 L ha\(^{-1}\) + *Azospirillum* 2.5 L ha\(^{-1}\), and Bacterial combination mixture of *Azotobacter* 5.0 L ha\(^{-1}\) + *Azospirillum* 5.0 L ha\(^{-1}\). The cultivar tested were Labandiri, Jangkobembe, Ranggohitam, and Paedara. This research show that application of bacterial mixture had no significant effect on nitrogen leaf content, but had a significant effect on flowering time and harvesting. It was found that flowering time tended to accelerate, but on harvesting-date parameters tended to be prolonged.

1. Introduction
Rice is a dominant staple food and the needed always to be increased every year follow the population increase [1]. Rice is not only as main source of carbohdrat but in the form of red rice, also source of anthocyanins [2], antioxidant [3-4] that good for healthy life [5]. Production of red rice always coincidence with the production of upland rice [6]. It must be developed in a healthy and eco friendly manner [7] as organic rice. To fulfill the rice need, various program introduced to maintain the rice sustainability. It is starting from the increase of seed quality [8-9], using plant breeding approach through the irradiance [10] or hybridization [11-14], development alternative farming system [15-19], using hybrid variety [20-21] and decreasing level rice consumption [22].

Most of the production-enhancing effort is achieved through the provision of inorganic fertilizer as one of the preferred technologies [23]. The main alternative is the use of inorganic fertilizers, as it is easy to observe the effects of fertilization [24]. Continued use of inorganic fertilizers can, however, lead to decrease in soil fertility [25] and environmental sustainability [26-27].

Another option that can be used and more secure is by fertilizing using organic fertilizer. Application of biological fertilizers non symbiotic fixing-nitrogen as *Azotobacter* sp [28-29] and *Azospirillum* sp [30-31] were able to reduce the use of inorganic fertilizer and preventing a decrease in
soil organic matter and reduce pollution [32-34]. *Azotobacter* and *Azospirillum* produce growth hormones and have ability to fix nitrogen from the air [35-36]. The earlier researcher found that *Azotobacter* and *Azospirillum* can increase plant growth [37-38] and production [39-40]. The application of organic fertilizer that enriched with biological fertilizers had good prospects to increase growth of plant and also environmentally sound. The research aims were to analyse the effect of bacterial mixture of *Azotobacter* and *Azospirillum* on the nitrogen content and harvest date of upland red rice.

2. Materials and methods

The research was arranged in split plot design with three replication. The main plot was the different doses of bacterial fixator, consisting of three levels such as $b_0$ = without bacterial fixator, $b_1 = Azotobacter$ $2.5$ L ha$^{-1} + Azospirillum$ $2.5$ L ha$^{-1}$, and $b_2 = Azotobacter$ $5.0$ L ha$^{-1} + Azospirillum$ $5.0$ L ha$^{-1}$. While in subplot was the difference of upland red rice cultivar i.e., ($v_1$) = Labandiri, ($v_2$) = Jangkobembe, ($v_3$) = Ranggohitam, ($v_4$) = Paedara. A study was conducted in which different variables were measured including plant height, stem diameter, leaf area, and root length. The experimental data were analyzed by analysis of variance (ANOVA). If the study showed significant differences, then multiple comparisons were performed using Duncan’s Multiple Range Test (DMRT) to determine if those differences are significant at the 5%.

3. Results and discussion

3.1. Effect of bacterial fixator on leaf nitrogen

Results of analysis of variance show that the treatment of a bacterial mixture of *Azotobacter* and *Azospirillum* did not significantly affect the leaf nitrogen. Leaf nitrogen levels in each cultivar were relatively the same, and there was no significant increase due to the increased dose of nitrogen application (table 1).

### Table 1. Effect of bacterial mixture of *Azotobacter* and *Azospirillum* on leaf nitrogen [1].

| Bacterial treatment | $v_1$ | $v_2$ | $v_3$ | $v_4$ | Average |
|---------------------|-------|-------|-------|-------|---------|
| $b_0$               | 41.54 | 44.06 | 43.20 | 43.02 | 42.96   |
| $b_1$               | 41.02 | 42.39 | 43.17 | 36.83 | 40.85   |
| $b_2$               | 41.07 | 43.61 | 43.01 | 42.77 | 42.62   |
| Average             | 41.21 | 43.35 | 43.13 | 40.87 | 42.14   |

Remarks:
- $v_1$ = Labandiri Cultivar
- $v_2$ = Jangkobembe Cultivar
- $v_3$ = Ranggohitam Cultivar
- $v_4$ = Paedara Cultivar
- $b_0$ = Without bacterial mixture
- $b_1$ = *Azotobacter* $2.5$ L ha$^{-1} + Azospirillum$ $2.5$ L ha$^{-1}$
- $b_2$ = *Azotobacter* $5.0$ L ha$^{-1} + Azospirillum$ $5.0$ L ha$^{-1}$

3.2. Flowering time

The results show that the bacterial mixture application had a significant effect on flowering age (table 2). Based on table 2, it appears that the Labandiri cultivar had the fastest time to reach flowering, followed by Paedara, Jangkobembe, and Ranggohitam cultivar.
Table 2. Effect of bacterial mixture of *Azotobacter* and *Azospirillum* on Flowering Time [1].

| Bacterial treatment | v1  | v2  | v3  | v4  | Average  |
|---------------------|-----|-----|-----|-----|----------|
| b0                  | 112.00 | 109.00 | 112.00 | 108.00 | 110.25  |
| b1                  | 108.33 | 112.00 | 117.67 | 107.00 | 111.25  |
| b2                  | 108.00 | 108.67 | 115.33 | 107.33 | 109.83  |
| Average             | 109.44 | 109.89 | 115.00 | 107.44 | 110.44  |

Remarks:

- v1 = Labandiri Cultivar
- v2 = Jangkobembe Cultivar
- v3 = Ranggohitam Cultivar
- v4 = Paedara Cultivar
- b0 = Without bacterial fixator
- b1 = *Azotobacter* 2.5 L ha⁻¹ + *Azospirillum* 2.5 L ha⁻¹
- b2 = *Azotobacter* 5.0 L ha⁻¹ + *Azospirillum* 5.0 L ha⁻¹

Based on further tests with DMRT, it appears that there is a significant difference in the flowering age between Ranggohitam cultivar and the other three cultivars. Then there was a significant difference in the Labandiri and Jangkobembe cultivars' flowering time, but not significantly different from the Paedara cultivars. Likewise, there was a significant difference in flowering age between Jangkobembe and Ranggohitam cultivars, but not significantly different from Paedara (table 3).

Table 3. Effect of bacterial mixture of *Azotobacter* and *Azospirillum* on flowering time [1].

| Cultivar               | Flowering time (Day) |
|------------------------|-----------------------|
| Labandiri (v1)         | 107.67 a              |
| Paedara (v4)           | 108.28 ab             |
| Jangkobembe (v2)       | 109.78 b              |
| Ranggohitam (v3)       | 112.67 c              |

Remarks: The numbers followed by the same letter are not significantly different in the DMRT 95%.

3.3. Harvest date

The application of bacterial mixture has a significant effect on the harvest date. It appears that the Paedara cultivar has the fastest harvesting date compared to the other three cultivars (figure 1).

Figure 1. Effect of bacterial mixture application on harvest date.
The different cultivar has a significant effect on harvest date (table 4). The Paedara cultivar had the fastest harvest date, compared to the other three cultivars. Paedara cultivar average age of 135.89 days followed by Jangkobembe with a harvest date of 137.56 days and Labandiri with 140.33 days. Meanwhile, the cultivar Ranggohitam had the most prolonged harvest of 141.33 days.

Table 4. Effect of difference cultivar on harvest date of upland red rice [1].

| Different cultivar | Harvest date (Day) |
|--------------------|--------------------|
| Paedara (v4)       | 135.89 a           |
| Jangkobembe (v2)   | 137.57 a           |
| Labandiri (v1)     | 140.33 b           |
| Ranggohitam (v3)   | 141.33 b           |

Remarks: The numbers followed by the same letter are not significantly different in the DMRT 95%.

Based on further tests using DMRT, it appears that there was no significant difference in the harvest age between Paedara and Jangkobembe cultivar, and also between Labandiri and Ranggohitam. Paedara and Jangkobembe have a significant difference with Labandiri dan Ronggohitam cultivar.

In general, it appears that the application of a bacteria mixture can accelerate flowering in the Labandiri, Jangkobembe, and Paedara cultivars (table 2 and 3). The acceleration of flowering is in line with the increasing doses of the bacterial mixture application. On the other hand, the bacterial mixture application tended to prolong the harvest date, and correspondingly with the increase of the bacterial with the increasing doses of the bacterial mixture application (table 4). This situation can occur because the bacteria used is a non-symbiotic type of bacteria. Which besides having the ability to fix nitrogen also can extract other nutrient elements. It can affect the increase in production; in general, these bacteria's ability to improve plant growth increases with higher concentrations.

4. Conclusion
The research concluded that the application of bacterial mixture Azotobacter sp. and Azospirillum sp has no significant effect on nitrogen leaf content, but has a significant effect on flowering time and harvesting. It found that flowering time tends to accelerate, but on harvesting-date parameters tends to be prolonged.

References
[1] Muhidin 2015 Morphological Characterisation and the Quality of Red Rice Irradiated with Gamma Rays in Various Kinds of Shade and the Application of Bacterial Fixation in Dry Land in Southeast Sulawesi (Hasanuddin University)
[2] Gunaratne A, Wu K, Li D, Bentota A, Corke H and Cai Y-Z 2013 Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins Food Chem. 138 1153–61
[3] Shao Y and Bao J 2015 Polyphenols in whole rice grain: Genetic diversity and health benefits Food Chem. 180 86–97
[4] Shao Y, Xu F, Sun X, Bao J and Beta T 2014 Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (Oryza sativa L.) J. Cereal Sci. 59 211–8
[5] Bhat F M and Riar C S 2015 Health benefits of traditional rice varieties of temperate regions Med. Aromat. Plants 4
[6] Suliantini N W S, Wijayanto T, Madiki A, Boer D, Muhidin and Juniawan 2018 Relationship of some upland rice genotype after gamma irradiation IOP Conf. Ser. Earth Environ. Sci. 122
[7] Sutariati G A K, Bande L O S, Khaeruni A, Muhidin, Mudi L and Savitri R M 2018 The effectiveness of preplant seed bio-invigoration techniques using Bacillus sp. CKD061 to improving seed viability and vigor of several local upland rice cultivars of Southeast Sulawesi *IOP Conf. Ser. Earth Environ. Sci.* **122**

[8] Sutariati G A K, Muhidin, Rakian T C, Afa L O, Made Widanta I, Mudi L, Sadimantara G R and Leomo S 2018 The effect of integrated application of pre-plant seed bio-invigoration, organic and inorganic fertilizer on the growth and yield of local upland rice *Biosci. Res.* **15** 160–5

[9] Sutariati G A K, Arif N, Muhidin, Rakian T C, Mudi L and Nuralam 2017 Persistency and seed breaking dormancy on local upland rice of Southeast Sulawesi, Indonesia *Pakistan J. Biol. Sci.* **20** 563–70

[10] Sulistanti N W S, Wijayanto T, Madika A, Boer D, Muhidin and Tufaila M 2018 Yield potential improvement of upland red rice using gamma irradiation on local upland rice from southeast sulawesi Indonesia *Biosci. Res.* **15** 1673–8

[11] Sadimantara G R, Muhidin, Sri Sulartiini N W, Nuraida W, Sadimantara M S, Leomo S and Ginting S 2018 Agronomic and yield characteristics of new superior lines of amphibious rice derived from paddy rice and local upland rice crossbreeding in konawe of Indonesia *Biosci. Res.* **15** 893–9

[12] Sadimantara G R, Alawyah T, Sulartiini N W S, Febrianti E and Muhidin 2019 Growth performance of two superior line of local upland rice (Oryza sativa L.) from SE Sulawesi on the low light intensity *IOP Conference Series: Earth and Environmental Science* vol 260 (IOP Publishing) p 12145

[13] Sadimantara G R, Kadidaa B, Suaib, Safuan L O and Muhidin 2018 Growth performance and yield stability of selected local upland rice genotypes in Buton Utara of Southeast Sulawesi *IOP Conference Series: Earth and Environmental Science* vol 122

[14] Sadimantara G R, Muhidin, Ginting S and Sulartiini N W S 2016 The potential yield of some superior breeding lines of upland rice of Southeast Sulawesi Indonesia *Biosci. Biotechnol. Res. Asia* **13** 1867–70

[15] Kadidaa B, Sadimantara G R, Suaib, Safuan L O and Muhidin 2017 Genetic diversity of local upland rice (Oryza sativa L.) genotypes based on agronomic traits and yield potential in North Buton, Indonesia *Asian J. Crop Sci.*

[16] Sadimantara G R, Febrianti E, Sulartiini N W S, Sutariati G A K and Yusuf D N 2020 Grain yield and yield attributes response of four upland rice (Oryza sativa L.) promising lines to shade stress *E&ES* **454** 12188

[17] Nuraida W O, Pitra Pradipita R, Sri Sulartiini N W, Wijayanto T, Muhidin and Sadimantara G R 2020 Production and quality of upland red rice under the shade stress *Int. J. Sci. Technol. Res.* **9** 5016–9

[18] Muhidin, Syam’un E, Kaimuddin, Musa Y, Sadimantara G R, Usman, Leomo S and Rakian T C 2018 Shading effect on generative characters of upland red rice of Southeast Sulawesi, Indonesia *IOP Conference Series: Earth and Environmental Science* vol 157 p 012017

[19] Muhidin, Syam’un E, Kaimuddin, Musa Y, Sadimantara G R, Usman, Leomo S and Rakian T C 2018 The effect of shade on chlorophyll and anthocyanin content of upland red rice *IOP Conf. Ser. Earth Environ. Sci.* **122** 012030

[20] Afa L O, Purwoko B S, Junaedi A, Haridjaja O and Dewi I S 2018 Simulation of hybrid rice tolerance to drought stress on nutrients culture in seedling phase *Biosci. Res.* **15** 530–9

[21] Afa L O, Purwoko B S, Junaedi A, Haridjaja O and Dewi I S 2018 Screening of hybrid rice tolerance through stimulated condition of drought stress in rainfed lowland *Biosci. Res.* **15** 1630–7

[22] Muhidin, Leomo S, Alam S and Wijayanto T 2016 Comparative studies on different agroecosystem base on soil physicochemical properties to development of Sago Palm on Dryland *Int. J. ChemTech Res.*
[23] Chen J, Lü S, Zhang Z, Zhao X, Li X, Ning P and Liu M 2018 Environmentally friendly fertilizers: A review of materials used and their effects on the environment Sci. Total Environ. 613 829–39

[24] Singh Brar B, Singh J, Singh G and Kaur G 2015 Effects of long term application of inorganic and organic fertilizers on soil organic carbon and physical properties in maize–wheat rotation Agronomy 5 220–38

[25] Bora R, Pandey P C, Singh D K, Yadav S K and Chilwal A 2018 Assessment of soil fertility status under long term balance fertilizer application on rice (Oryza sativa L.) JCS 6 1696–9

[26] Sabiha N-E, Salim R, Rahman S and Rola-Rubzen M F 2016 Measuring environmental sustainability in agriculture: A composite environmental impact index approach J. Environ. Manage. 166 84–93

[27] Rahman K M and Zhang D 2018 Effects of fertilizer broadcasting on the excessive use of inorganic fertilizers and environmental sustainability Sustainability 10 759

[28] Ding W, Xu X, He P, Ullah S, Zhang J, Cui Z and Zhou W 2018 Improving yield and nitrogen use efficiency through alternative fertilization options for rice in China: A meta-analysis F. Crop. Res. 227 11–8

[29] Banik A, Dash G K, Swain P, Kumar U, Mukhopadhyay S K and Dangar T K 2019 Application of rice (Oryza sativa L.) root endophytic diazotrophic Azotobacter sp. strain Avi2 (MCC 3432) can increase rice yield under green house and field condition Microbiol. Res. 219 56–65

[30] Zhang J, Hussain S, Zhao F, Zhu L, Cao X, Yu S and Jin Q 2018 Effects of Azospirillum brasilense and Pseudomonas fluorescens on nitrogen transformation and enzyme activity in the rice rhizosphere J. soils sediments 18 1453–65

[31] Hahn L, Sá E L S de, Osório Filho B D, Machado R G, Damasceno R G and Giongo A 2016 Rhizobial Inoculation, Alone or Coinoculated with Azospirillum brasilense, Promotes Growth of Wetland Rice Rev. Bras. Ciência do Solo 40

[32] Syaiful S A, Syam’un E, Dachlan A, Kamaruzaman J and Haerani N 2013 The effect of inoculating nitrogen fixing bacteria on production of rice. World Appl. Sci. J. 26 94–9

[33] Zhao J, Ni T, Li J, Lu Q, Fang Z, Huang Q, Zhang R, Li R, Shen B and Shen Q 2016 Effects of organic–inorganic compound fertilizer with reduced chemical fertilizer application on crop yields, soil biological activity and bacterial community structure in a rice–wheat cropping system Appl. soil Ecol. 99 1–12

[34] Zhang M, Yao Y, Tian Y, Ceng K, Zhao M, Zhao M and Yin Q 2018 Increasing yield and N use efficiency with organic intensive rice cropping systems F. Crop. Res. 227 102–9

[35] Fukami J, Cerezini P and Hungria M 2018 Azospirillum: benefits that go far beyond biological nitrogen fixation AMB Express 8 73

[36] Wani S A, Chand S, Wani M A, Ramzan M and Hakeem K R 2016 Azotobacter chroococcum—a potential biofertilizer in agriculture: an overview Soil Science: Agricultural and Environmental Prospectives (Springer) pp 333–48

[37] Muhidin, Syam’un E, Kaimuddin, Musa Y, Sadimantara G R, Rakian T C, Leomo S and Yusuf D N 2020 Effect of root endophytic diazotrophic azotobacter and azospirillum on the vegetative growth of local upland red rice Int. J. Sci. Technol. Res. 9 3345–8

[38] Muhidin, Syam’un E, Musa Y, Sadimantara G R, Leomo S, Sutariati G A K, Yusuf D N and Rakian T C 2020 Effect dual inoculation of Azotobacter and Azospirillum on the productive trait upland red rice cultivar IOP Conference Series: Earth and Environmental Science vol 575 (IOP Publishing) p 12093

[39] Nurmas A, Anwar, Karimuna L, Sabaruddin L, Khaeruni A and Muhidin 2018 The role of azotobacter sp. In reducing inorganic fertilizer of nitrogen on growth of local maize (zea mays L.) In ultisol Biosci. Res. 15 428–36

[40] Nurmas A, Karimuna L, Sabaruddin L, Khaeruni A, Muhidin, Rahayu M, Hasid R and
Adawiyah R 2018 The effectiveness of azotobacter sp. In increasing grown of local maize and sorghum in the intercropping system in ultisols Biosci. Res. 15 1645–4652