Vegetative growth response of upland rice to *Actinomycetes, Azospirillum and Azotobacter*

Agung Gunawan¹, Yusminah Halaf, Alimuddin Ali¹, Oslan Jumadi², Muhammad Junda²

Biology Dept, Math and Natural Science Faculty, Makassar University State, Indonesia
Email: agungunawann97@gmail.com

Abstract. The research aims is to determine the suitability of nitrogen fixing bacteria, namely Actinomycetes, Azospirillum and Azotobacter with upland rice seeds to the speed of radicle formation and growth of upland rice plants. Upland rice plant growth measurement parameters include; speed of formation of radicle length, upland rice plant height, number of upland rice tillers, dry weight of the top of upland rice plants and roots of upland rice plants, wet weight of upland rice plants and roots of upland rice plants, and total N of upland rice plants and upland roots. Testing the application of N₂ fixing bacteria on upland rice plants on a laboratory scale was carried out to determine the suitability of microbes with upland rice plant seeds in vitro. The pot test was carried out to determine the suitability of the N₂ fixing bacteria with the vegetative growth of upland rice plants in vivo. Data were analyzed using ANOVA with Duncan's advanced test. The results showed that upland rice plants inoculated with Actinomycetes, Azospirillum and Azotobacter showed significantly different growth from upland rice plants without nitrogen fixing bacteria treatment, namely the radicle formation speed and radicle length, plant height, number of tillers, wet weight, dry weight, and total N (%) plants. It can be concluded that the inoculation of nitrogen-fixing bacteria on upland rice plants has a significant effect on plant vegetative growth parameters and plant nitrogen content.

1. Introduction
The type of rice plants that correspond to dry land conditions is the upland rice type [1]. The upland rice plants require high nitrogen nutrients, but the nitrogen content in almost all upland rice crop soils is low. The provision of inorganic nitrogen fertilizer on upland rice farm soil is less efficient because the response of plants to fertilizer is low. This is because the groundwater content is so low that it inhibits the collection of nitrogen by plants and possibly also due to the underdeveloped rooting upland rice.

The availability of nitrogen for upland rice plants can be increased with the help of N₂-anchoring bacteria that live on the roots. This N₂ tethering bacteria have a nitrogen-fastening enzyme known as nitrogenous that can work to catalyze N₂'s altered reactions into ammonia compounds (₂NH₃). Ammonia then changes to ammonium (NH₄⁺) and nitrates (NO₃⁻) which are inorganic forms of nitrogen that can be absorbed by plants.
Application of Endophytic Bacteria Tether N\textsubscript{2} and Inorganic N Fertilizer In Saline Soil can increase the N Content of Plants and Rice Yields Upland [2]. The provision of bacteria-based biological fertilizers to grow in sour soils provides high growth and production of upland rice [3]. In inceptisol soil derived from Rawa Lebak, inoculation of various consortiums of nutrient-contributing bacteria provides high rice growth and yield [4]. The most commonly used N\textsubscript{2} mooring bacteria that exhibit good growth associations with upland rice plants are Bacillus sp., Pseudomonas sp., Azospirillum sp., and Azotobacter sp.

The ability of nitrogen-fastening bacteria produce growth regulators (ZPT) and stimulate root development causes upland rice plants inoculated with nitrogen-fastening bacteria to have twice as much root biomass as plants without being treated with nitrogen-inoculating bacteria. This will cause the upland rice plant to absorb much greater nutrients from the plant without being given the inoculation treatment of nitrogen-fastening bacteria in this case of control.

The increase in the dry weight of the roots is thought to be due to the role of bacteria as a growth booster of plants that are producing growth hormone. Azotobacter in addition to being able to bind nitrogen in the air, also produces indole acetic acid (IAA) in amounts directly proportional to its population density [5]. Azospirillum also has the ability to produce IAA [6]. IAA content is useful to stimulate root growth through the increase in length or surface area, so that the roots are able to bind water and gain wet weight [7].

The genus Azospirillum is one of the genus that is considered important among rhizobacteria in supporting plant growth (PGPR). This microbial potential is used as a biological fertilizer because Azospirillum can increase plant growth, such as nitrogen tethering, phosphate dissolving and production of phytohormones indole 3-acetic acid (IAA), cytokinin, abscess acid (ABA), ethylene, gibereic acid (GA3) and zeatin [8].

Nitrogen-fastening bacteria are often called diazotropic bacteria that are able to use nitrogen from the air as a source of nitrogen for their growth. The role of bacteria in fixing air, nitrogen has a major effect on the economic value of agricultural land [2]. The use of these bacteria has the potential to reduce the need for synthetic nitrogen, increase the production and income of farmers with cheaper inputs.

2. Methods

2.1. Test the suitability of N\textsubscript{2} mooring bacteria with upland rice seeds
Actinomycetes SP12, Azospirillum and Azotobacter ATH39 bacteria are inoculated to upland rice seeds to test the ability to associate with upland rice in vitro. The upland rice seeds are disinfected with 70% (v/v) ethanol for 3 minutes, washed three times in sterile distilled water (aquadest), soak 2% sodium hypochlorite for 5 minutes, washed three times with sterile aquadest, then winded in laminar air flow cabinet for an hour. The seed that has been drained is then treated. Treatment of seeds with isolates of N\textsubscript{2}-anchoring bacteria. This test was conducted by soaking the seeds with all three isolates of N\textsubscript{2}-tethering bacteria. Seed priming was performed against 50 upland rice seeds soaked for 24 hours in pure isolate suspension Actinomycetes SP12, Azospirillum and Azotobacter ATH39 with concentrations of 10\textsuperscript{8}-10\textsuperscript{9} cfu/ml. In this test, the effect of Actinomycetes SP12, Azospirillum and Azotobacter ATH39 inoculation was observed on the rate of radicle formation and radicle length.

2.2. Test the ability of N\textsubscript{2} fastening bacteria in planta (greenhouse) on upland rice growth
Azotobacter SP12, Azospirillum and Actinomycetes ATH39 bacteria were suspended by mixing NaCl 0.9% with several bacterial colonies taken using sterile ose. Incubated for 48 hours to obtain bacteria with a concentration of 10\textsuperscript{8} – 10\textsuperscript{9} cfu/ml. Culture is grown aerobically with shaker to optical density (OD\textsubscript{600}) in accordance with 3 x 10\textsuperscript{8} cfu / ml, then applied to upland rice seeds. Seeds directly planted in the planting media polybag tugal with 10 seeds per polybag with a distance of 2 cm and planting depth of 2 cm.
2.3. Processing Of Planting Media
Planting media used is soil. The origin of the land taken comes from the rice fields. The soil is first dried to reduce the moisture content, the soil is sifted by using a sieve sand with a size of 3x2 mm. Weigh the soil weighing 2 kg per polybag of plants. The soil is put in plastic to be sterilized into the autoclave at a temperature of 121°C for 20 minutes.

2.4. Upland Rice Plant Maintenance
Upland rice plants are watered 5 times a week adjusted to the level of soil drought. Upland rice plants are done weed weeding on a weekly basis by plucking the grass between the planting distance by hand is done at 3 weeks after planting, 5 weeks after planting, and 7 weeks after planting. Suspension of nitrogen-fastening bacteria is carried out at the age of 15 days after planting with a concentration of $10^8$ cfu/ml.

2.5. Measurement of Vegetative Parameters of Upland Rice Plants
a. Height of rice plants (cm)
The height of the plant is measured at the age of 7 weeks after planting. Measurement starts from the ground level to the highest tip of the leaf, by straightening the leaves upwards.

b. Number of rice saplings
The number of rice saplings is calculated at the age of 7 weeks after planting.

c. Wet weight of roots and top of rice plant (gram)
Wet weight is measured at 7 weeks after planting. Each sample family is carefully separated from the ground, then weighed with analytical scales.

d. Biomass (dry weight) roots and the top of rice plants (gram)
The plant is put in aluminum foil and dried in the oven at a temperature of 60°C until its weight is constant. Once dry, the sample is weighed with an analytical scale.

2.6. Total N Content on Upland Rice Plants
The testing of total N content on Upland Rice Plants is done on the top of the plant and the root part of the plant. Each part of the plant is tested by the kjeldahl method to find out the total N contained in the plant. The Kjeldahl method is a method used to determine nitrogen levels in organic compounds as well as inorganic compounds. The working principles include; distillation, titration and calculation. The shifted stage is the nitrogen decomposition stage in the sample using concentrated acids. This stage is enhanced by boiling the sample on concentrated sulfuric acid. Distillation is the stage of adding excess bases into a solution shifted to convert ammonium into ammonia followed by heating and condensation of ammonia gas in the receiving solution. Titration aims to determine the amount of ammonia in the receiving solution. The amount of nitrogen can be calculated from the amount of ammonia ions in the receiving solution.

2.7. Soil Physical and Chemical Properties Analysis
a. Determination of soil texture can be done in a field by taking wet soil then placed between the forefingers, rubbed and when the twist feels very clay and attached, the sign is a lot of clay levels (clay). Testing of soil N levels by kjeldahl method, namely with three working principles; gesi, distillation.

b. Determination of P-levels is available by Olsen method which is to weigh 1,000 gram of soil sample <2 mm, put in a whipped bottle, plus 20 ml of Olsen extractor, then shaken for 30 minutes. Strain and 18 when the cloudy solution is returned to the original sieve. The extract is piped 2 ml into the test tube and then together with the standard series added 10 ml of phosphate dye reaction, beat until homogeneous and leave for 30 minutes. The absorption of the solution is measured by a spectrophotometer at a wavelength of 693 nm.

c. Determination of K-levels is available by Bray method which is weighing 2,500 gram of soil sample <2 mm, plus extractor Bray and Kurt I as much as 25 ml, then shaken for 5 minutes. Strain and when the turbid solution is returned to the original sieve (maximum filtration process 5 minutes). Pickpocketed 2 ml clear extract into the test tube. Examples and standard series are each plus a
phosphate dye reaction of 10 ml, shaken and left for 30 minutes. Measured its absorbance with a spectrophotometer at a wavelength of 693 nm.

d. Calculation of soil pH levels by H2O method is to weigh 10.00 gram of soil sample twice, each put in a whipped bottle, plus 50 ml of ion-free water to one bottle (pH H2O). Shake with the whisking machine for 30 minutes. Ground suspension is measured by a calibrated pH meter using a pH buffer solution of 7.0 and pH of 4.0. Report the pH value in 1 decimal place. All analysis of soil chemical properties is conducted in laboratory tests at the Soil Laboratory Hall, Maros, South Sulawesi.

2.8. Parametric measurement.
The upland rice plant growth response can be seen by using the research parameters namely the number of tillers, plant height, plant and root dry weight. Measurement of plant height and maximum tillering were done when the plant grows 60 days after transplanting. The dry weight of the top and roots of plants is measured by weighing after the plants are cleaned and dried. Analysis of the total N content of plants was carried out using the Kjeldahl method [9]. Production of total plant N is the total amount of N contained in plants (g/plant) which is the result of multiplying the total plant N content (%) with the plant dry weight (g). The research results data were analysed using the analysis of variances method (F test)/ANOVA with a confidence level $\alpha = 0.05\%$. Treatments which showed a significant effect were further analysed with the Duncan test.

3. Results and Discussion

3.1. Inoculation effect of Actinomyces SP12, Azospirillum and Azotobacter ATH39 on the speed of formation of radiculate seeds of Upland Rice plants

The speed of formation of upland rice seed radiculas can be seen at radiculal length at 5 days after inoculation. Radiculate at 5 days after the longest inoculation in the treatment of Azotobacter ATH39 bacteria (Table 1) this indicates that Azotobacter ATH39 bacteria can stimulate the speed of formation of radiculas faster than other treatments.

Table 1. The average radiculal length of the seeds of the Upland Rice plant after bacterial inoculation Actinomyces SP12, Azospirillum and Azotobacter ATH39

| Number | Treatment          | Length radiculal rice seed upland (mm) | Average |
|--------|--------------------|----------------------------------------|---------|
|        |                    | I          | II         | III         |          |
| 1      | Control            | 19,12      | 19,79      | 19,93       | 19,61c    |
| 2      | Actinomyces SP12   | 41,68      | 40,97      | 41,71       | 41,45b    |
| 3      | Azospirillum       | 41,86      | 41,20      | 42,99       | 42,02b    |
| 4      | Azotobacter ATH39  | 45,65      | 46,41      | 47,30       | 46,45a    |

a) Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$

Coating soybean seeds with biological agents that have often been done by farmers is the provision of Azotobacter spp. inoculant. Use of Azotobacter spp. It has another advantage, which is able to increase the ability of roots to absorb nutrients [10]. Azotobacter not only fixates N2 from the air but also improves root development. The ability of Azotobacter spp. to improve root development because it produces phytohormones auxin and cytokinin [11].

3.2. Inoculation effect of Actinomyces SP12, Azospirillum and Azotobacter ATH39 on high upland rice plants

The results of high measurements of gogo rice plants at the age of 7 weeks after planting showed that
the height of gogo rice plants that were treated by nitrogen-fastening bacteria differed markedly from the controls. Treatment with *Azotobacter* ATH39 bacteria gives the highest gogo rice crop height compared to 2 treatment of *Actinomycetes* SP12 bacteria and *Azospirillum* bacteria (Table 2).

Table 2. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on high upland rice plants

| Number | Treatment           | Upland Rice Plant Height (cm) | Average |
|--------|---------------------|-----------------------------|---------|
|        |                     | I   | II  | III |               |
| 1      | Control             | 76.5| 73.8| 75.5| 75.3<sup>b</sup>|
| 2      | *Actinomycetes* SP12| 81  | 81  | 87.5| 83.2<sup>a</sup>|
| 3      | *Azospirillum*      | 88.8| 86.5| 85.5| 86.9<sup>a</sup>|
| 4      | *Azotobacter* ATH39 | 83  | 88.5| 89.4| 87.0<sup>a</sup>|

<sup>a</sup>Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$

3.3. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the number of upland rice tillers

The number of upland rice tillers at the age of 7 weeks after planting the highest value in the treatment of *Azotobacter* ATH39 bacteria but this value was not significantly different from the treatment of *Actinomycetes* SP12 and *Azospirillum* bacteria. Upland rice plants inoculated with *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 bacteria had a significantly different number of tillers from the control (Table 3).

Table 3. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the number of upland rice tillers

| Number | Treatment           | Number of upland rice tillers | Average |
|--------|---------------------|------------------------------|---------|
|        |                     | I   | II  | III |               |
| 1      | Control             | 12  | 12.6| 13.5| 12.7<sup>b</sup>|
| 2      | *Actinomycetes* SP12| 22  | 22.5| 22.7| 22.4<sup>a</sup>|
| 3      | *Azospirillum*      | 23  | 22.7| 22.9| 22.9<sup>a</sup>|
| 4      | *Azotobacter* ATH39 | 22.9| 23  | 23.3| 23.1<sup>a</sup>|

<sup>a</sup>Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$

The treatment of biological fertilizers has a real influence on the total number of saplings with the highest average value of 2.58 pot-1 rods for the *Azotobacter* sp. formula [6]. Better results on *Azotobacter* sp. formula, possibly related to the ability of microbes in helping to provide nutrients, especially N and P for rice plants. N-tethering microorganisms and phosphate solvents have the ability to produce reductase urea and phosphatase enzymes that play an important role in air-free N tethering and P dissolving of elusive P compounds [12].

3.4. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the top dry weight of upland rice plant

The dry weight of the top of the upland rice plant is calculated at the age of 7 weeks after planting highest in plants treated by *Azotobacter* ATH39 bacteria, but this number is no different from the treatment of 2 bacteria, namely *Actinomycetes* SP12 and *Azospirillum*. Upland rice plants inoculated with *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 bacteria treatment have a dry weight on the top of the upland rice plant which differs markedly by control (Table 4).
Table 4. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the top of the upland rice dry weight

| Number | Treatment            | Heavy dry top of gogo rice plant (gram) | Average |
|--------|----------------------|----------------------------------------|---------|
|        |                      | I   | II          | III       |         |
| 1      | Control              | 4,70| 5,37        | 5,39      | 5,15b    |
| 2      | *Actinomycetes* SP12 | 5,49| 8,00        | 10,34     | 7,94a    |
| 3      | *Azospirillum*       | 5,65| 8,12        | 10,48     | 8,09a    |
| 4      | *Azotobacter* ATH39  | 7,91| 9,74        | 12,75     | 10,13a   |

Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$.

The dry weight of the plant reflects the growth of the plant and the amount of nutrients absorbed per unit of biomass weight produced. The higher the dry weight value of the resulting plant, the better the growth of plants and more absorbed nutrients [13].

3.5. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the heavy dried roots of upland rice plant

The ability of nitrogen-fastening bacteria to produce growth regulators (ZPT) and stimulate root development causes gogo rice plants inoculated with nitrogen-fastening bacteria to have twice as much root biomass as plants without being treated with nitrogen-inoculating bacteria. This will cause the gogo rice plant to absorb much greater nutrients from the plant without being given the inoculation treatment of nitrogen-fastening bacteria in this case of control. The dry weight of the roots of upland rice plants measured at the age of 7 weeks after planting the highest in plants treated by *Azotobacter* ATH39 bacteria, but this number is no different from 2 other bacterial treatments, namely *Actinomycetes* SP12 bacteria and *Azospirillum* bacteria. Upland rice plants inoculated with the bacteria *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 have a real different dry weight plant roots by control (Table 5).

Table 5. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the heavy dried roots of Upland Rice plant

| Number | Treatment            | Heavy dried roots of upland rice plants (gram) | Average |
|--------|----------------------|-----------------------------------------------|---------|
|        |                      | I   | II          | III       |         |
| 1      | Control              | 1,76| 3,85        | 3,93      | 3,18b    |
| 2      | *Actinomycetes* SP12 | 4,11| 12,67       | 10,99     | 9,26a    |
| 3      | *Azospirillum*       | 9,54| 7,96        | 11,08     | 9,53a    |
| 4      | *Azotobacter* ATH39  | 11,62| 12,74      | 19,10     | 14,48a   |

Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$.

The increase in the dry weight of the roots is thought to be due to the role of bacteria as a growth booster of plants that are producing growth hormone. *Azotobacter* in addition to being able to bind to N$_2$ in the air, also produces indole acetic acid (IAA) in amounts directly proportional to its population density [5]. *Azospirillum* also has the ability to produce IAA [6]. IAA content is useful to stimulate root growth through the increase in length or surface area, so that the roots are able to bind water and gain wet weight [7].

3.6. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the heavy wet top of Upland Rice plant
The wet weight of the top of upland rice plants measured at the age of 7 weeks after planting the highest on upland rice plants that are treated by *Azotobacter* ATH39 bacteria, but the wet weight of the top of the upland rice plant is no different from the treatment of other 2 bacteria, namely *Actinomycetes* SP12 and *Azospirillum* bacteria. Upland rice plants inoculated with *Actinomycetes* bacteria SP12, *Azospirillum* and *Azotobacter* ATH39 have a wet weight over the top of the upland rice plant that differs markedly by control (Table 6).

**Table 6. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the heavy wet top of Upland Rice**

| Number | Treatment          | Heavy wet top of upland rice plant (gram) | Average |
|--------|--------------------|------------------------------------------|---------|
|        |                    | I            | II          | III         |          |
| 1      | Control            | 13,52        | 21,68       | 25,37       | 20,19b   |
| 2      | *Actinomycetes* SP12 | 45,09       | 48,70       | 42,31       | 45,37a   |
| 3      | *Azospirillum*     | 39,85        | 47,83       | 48,87       | 45,52a   |
| 4      | *Azotobacter* ATH39 | 43,59        | 42,05       | 55,83       | 47,16a   |

Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$

3.7. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the heavy wet top of upland rice plant

The wet weight of upland rice plant roots measured at the age of 7 weeks after planting the highest on upland rice plants that are treated by *Azotobacter* ATH39 bacteria, but the wet weight of the roots of upland rice plants is no different from the other two bacterial treatments, namely *Actinomycetes* SP12 bacteria and *Azospirillum* bacteria. Upland rice plants inoculated with *Actinomycetes* SP12 bacteria, *Azospirillum* and *Azotobacter* ATH39 have wet weight roots of upland rice plants that differ markedly by control (Table 7).

**Table 7. Inoculation Effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the heavy wet top of upland rice plants**

| Number | Treatment          | Wet weight of upland rice roots (gram) | Average |
|--------|--------------------|----------------------------------------|---------|
|        |                    | I            | II          | III         |          |
| 1      | Control            | 4,52        | 14,47       | 17,41       | 12,13b   |
| 2      | *Actinomycetes* SP12 | 20,17       | 26,05       | 28,71       | 24,98a   |
| 3      | *Azospirillum*     | 27,68        | 28,17       | 30,46       | 28,77a   |
| 4      | *Azotobacter* ATH39 | 26,41        | 25,95       | 36,73       | 29,69a   |

Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level $\alpha = 0.05$

3.8. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the total N upper part of upland rice plants

The total N of the top of the upland rice plant is calculated at the age of 7 weeks after the highest planting on plants treated by *Azotobacter* ATH39 bacteria, and the total N of the upper part of this plant is significantly different from the treatment of 2 bacteria, namely *Actinomycetes* SP12 and *Azospirillum*. Upland rice plants inoculated with *Actinomycetes* SP12 bacteria treatment, *Azospirillum* and *Azotobacter* ATH39 have a total N upper part of upland rice plant which is significantly different from the control (Table 8).
Table 8. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the total N upper part of upland rice plants

| Number | Treatment         | Total N upper part of Upland Rice plant (%) | Average |
|--------|-------------------|---------------------------------------------|---------|
|        |                   | I   | II  | III |                   |
| 1      | Control           | 1,05| 1,09| 1,13| 1,09c             |
| 2      | *Actinomycetes* SP12 | 1,31| 1,29| 1,30| 1,30b             |
| 3      | *Azospirillum*     | 1,27| 1,29| 1,35| 1,30b             |
| 4      | *Azotobacter* ATH39 | 1,39| 1,36| 1,40| 1,38a             |

\[\text{Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level } \alpha = 0.05\]

Bacteria from a different species, associated with upland rice roots, tend to differ in the formation of plant biomass. Inoculation with bacteria increases root dry weight which causes an increase in dry weight of the upper part of the plant and total N production [14].

3.9. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the total N root upland rice plant

Total N roots of upland rice plants are calculated at the age of 7 weeks after the highest planting in plants treated by *Azotobacter* ATH39 bacteria, but the total N roots of this plant is no different from the treatment of *Azospirillum* bacteria. Upland rice plants inoculated with *Azospirillum* and *Azotobacter* ATH39 bacteria have a total N root of upland rice plants that are significantly different from *Actinomycetes* SP12. Upland rice plants inoculated with N2 mooring bacteria treatment differ markedly with control (Table 9).

Table 9. Inoculation effect of *Actinomycetes* SP12, *Azospirillum* and *Azotobacter* ATH39 on the total N root upland rice plant

| Number | Treatment         | Total N roots of Upland Rice plant (%) | Average |
|--------|-------------------|----------------------------------------|---------|
|        |                   | I   | II  | III |                   |
| 1      | Control           | 1,19| 1,25| 1,21| 1,22c             |
| 2      | *Actinomycetes* SP12 | 1,45| 1,48| 1,55| 1,49b             |
| 3      | *Azospirillum*     | 1,55| 1,58| 1,60| 1,58a             |
| 4      | *Azotobacter* ATH39 | 1,59| 1,63| 1,64| 1,62a             |

\[\text{Data come from the average of three replicate. The same letter indicates that there is no significant difference based on the results of the Duncan test with confidence level } \alpha = 0.05\]

Two genus, *Azospirillum* and *Azotobacter* are active in soil fertility maintenance and vegetation productivity. Nitrogen binding capacity is estimated by the Kjeldhal method. Results showed both *Azosirillum sp.* and *Azotobacter sp.* have the efficiency to repair nitrogen. Among other factors, the binding capability of *Azospirillum* and *Azotobacter* isolate nitrogen is dependent on the activity of nitrogenase enzymes [15].

3.10. Analysis of the physical and chemical properties of soil before planting and after planting

Analysis of Soil Physical and Chemical Properties is a supporting factor in the provision of nutrients (fertility) in the soil for the growth of upland rice plants. Parameters of analysis of the nature and characteristics of this soil become one of the conditions of growth of upland rice plants. Analysis of soil properties and characteristics is sampling only on soils treated with nitrogen-fastening bacteria.
Table 10. Soil Quality before and after planting

| Number | Parameters          | Content       | Category       |
|--------|---------------------|---------------|----------------|
| 1      | Soil texture        | -             | Clay           |
| 2      | pH (H2O)            | 5.6           | Low (sour)     |
| 3      | N total (%)         | 0.35          | Low            |
| 4      | P- available (ppm)  | 5.76          | Low            |
| 5      | K- available (ppm)  | 7.20          | Low            |
|        |                     | 19.43         | Height         |
|        |                     | 14.66         | Height         |

This soil analysis is done to determine the role of nitrogen-fastening bacteria to soil quality. Soil samples were only taken on soil previously treated with nitrogen-ing bacteria. Soil analysis results before planting showed that the characteristics of soil with clay texture, pH 5.6 (sour), N total 0.35 (low), P-available 5.76 (low) and K-available 7.20 (low). While the results of soil analysis after planting showed that the characteristics of soil with clay texture, pH 6.7 (neutral), N total 1.29 (height), P-available 19.43 (height) and K-available 14.66 (height). Analysis of soil physical and chemical properties is very influential on the addition of nitrogen-fastening bacteria to the soil so as to improve the quality of soil fertility, namely from pH parameters and NPK elements that are indispensable for the growth process of upland rice plants.

4. Conclusion
Nitrogen-inoculated bacteria against upland rice seeds can accelerate radicle discharge and stimulate the formation and lengthen radiculate. Nitrogen-inoculated bacteria with upland rice plants have the highest crop nitrogen content. Nitrogen-fastening bacteria inoculated to upland rice seeds greatly affected the number of tillers, plant height, wet weight and dry weight of the upper plant, and roots of upland rice plants.

References
[1] BB-Padi. 2017. Tiga Varietas Baru Padi Gogo Hasil Konsorsium Telah dilepas Tahun Ini. Balai besar penelitian tanaman padi Balitbangtan kementerian pertanian.
[2] Setiawati, M.R., H. Dedeh, P. Suryatmana and R. Hudaya. 2008. Aplikasi bakteri endofitik penambat N2 untuk meningkatkan populasi bakteri endotifik dan hasil tanaman padi sawah. J. Agrikultura. 19: 13-19.
[3] Aryanto, A., Triadiati and Sugiyanta. 2015. Pertumbuhan dan produksi padi sawah dan gogo dengan pemberian pupuk hayati berbasis bakteri pemacu tumbuh dan diolah tanah masam. J. Ilmu Pertanian Indonesia. 20: 229-235.
[4] Wuriesyliane, W., N. Gofar, A. Madjid, H. Widjajanti and S.R. Ni Luh Putu. 2013. Pertumbuhan dan hasil padi pada inceptisol asal rawa lebak yang diikulasi berbagai konsorsium bakteri penyumbang unsur hara. J. Lahan Suboptimal. 2: 18-27.
[5] Lestari, P., D.N. Susilowati and E.I. Riyanti. 2007. Pengaruh hormon asam indol asetat yang dihasilkan Azospirillum sp. terhadap perkembangan akar padi. J. Agrobiogen. 3: 66-72.
[6] Mezuan, I.P. Handayani and E. Inoriah. 2002. Penerapan formulasi pupuk hayati untuk budidaya padi gogo: studi rumah kaca. J. Ilmu-Illu Pertanian Indonesia. 4: 27 – 34.
[7] Ni Putu, R. 2015. Isolasi dan identifikasi bakteri penambat nitrogen non simbiosis dari dalam tanah. Proc. Seminar Nasional FMIPA UNDIKSHA 5: 230-235.
[8] Tien, T.M., M.H. Gaskins, D.H. Hubbell. 1979. Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisetum americanum L.). J. Appl. Environ Microbiol 37: 1016-1024.
[9] Setiawati, M.R., P. Suryatmana and R. Hudaya. 2016. Peningkatan kandungan N tanaman dan hasil padi gogo akibat aplikasi bakteri endofitik penambat N\textsubscript{2} dan pupuk N anorganik pada tanah salin. \textit{Unpad Open Repository}. https://pustaka.unpad.ac.id/archives/29109

[10] Milic, V., Nastasija Mrkovacki, M. Popovic and Dorde Malencic. 2002. Nodule efficiency of three soybean genotypes inoculated by different methods. \textit{J. Rostlinna Vyroba} 48: 356-360.

[11] Suryatmana, Pujawati, Mieke Rochimi Setiawati and I.K. Susanti. 2008. Aplikasi \textit{Azotobacter vinelandii} dan \textit{Azolla piñata} untuk bioremediasi limbah minyak bumi. \textit{Proc Seminar dan Kongres Nasional MKTI, Bogor} pp 1-8.

[12] Goenadi, H.D. dan Herman. 1999. Manfaat dan prospek pengembangan industri pupuk hayati di Indonesia. \textit{J. Litbang Pertanian} 29: 91-97.

[13] Musfal. 2010. Potensi cendawan mikoriza \textit{Arbuskula} untuk meningkatkan hasil tanaman jagung. \textit{J. Litbang Pertanian} 16: 154-158.

[14] Hala, Y. 2020. The effect of nitrogen-fixing bacteria towards upland rice plant growth and Nitrogen content. \textit{J. ICFST 2019} 484: 1-6.

[15] Sulaiman, K.H., F.N. Al-barakah, A.M. Assaeed and B.A.M. Dar. 2019. Isolation and identification of \textit{Azospirillum} and \textit{Azotobacter} species from \textit{Acacia spp}. at Riyadh, saudi arabia. \textit{J. Bangladesh J. Bot} 48: 239-251.