The effect of nitrogen-fixing bacteria towards upland rice plant growth and nitrogen content

Yusminah Hala
Biology Dept, Math and Natural Science Faculty, Makassar University State, Indonesia
Email: yushala@unm.ac.id

Abstract. This study was performed to observe the associative effect of Nitrogen-fixing bacteria and combination of each with nitrogen dosage on Nitrogen content and upland rice plant growth. *Klebsiella pneumonia* and *Azospirillum brasilense* were applied, while urea used as fertilizer on 0, 25, 50, and 75 kg N/Ha. The result revealed that inoculation of nitrogen-fixing bacteria to upland rice can increase tiller formation, shoot and root dry weight and plant N Total production. The effect of *A. brasilense* on increase tiller formation, biomass dry weight (shoot and root) and N total production revealed to tendency to increase compare to *K. pneumonia*. Differences in nitrogen fertilizer did not cause a significant effect on all parametric measured, except plant N content. Bacteria and nitrogen fertilizer interaction did not cause a significant effect on all parametric measured. From the regression analysis, there was a correlation between root dry weight with shoot dry weight; and the correlation between root dry weight with plant N total production.

1. Introduction
Upland rice plants are rice plants that live in dry or upland soils that do not require standing water on their land such as lowland rice. The development of upland rice so far has not given encouraging results. Production of upland rice is still far below the production of lowland rice. Upland rice plants need high nitrogen nutrients, but the nitrogen content of almost all upland rice is low. The application of inorganic nitrogen fertilizer on upland rice farming is less efficient because the plant's response to fertilizer is low. This is because the groundwater content is so low that it inhibits nitrogen uptake by plants and may also be due to underdeveloped upland rice roots. Nevertheless, productivity of upland rice can be increased through improved land management [1], use of soil enhancers [2], and the use of improved varieties [3].

The availability of nitrogen for upland rice plants can be increased with the help of N2-fixing bacteria that live in the roots and rhizosphere [4]. Some bacteria can live in the Gramineae plant rhizosphere and are able to fix free nitrogen from the air [5]. In addition, nitrogen-fixing bacteria can produce growth regulators that can stimulate the growth of plant roots [6]. The dominant bacteria were *Azospirillum sp.*, *Enterobacter sp.*, *Klebsiella sp.*, and *Pseudomonas sp* [7]. This allows the use of *Azospirillum* as a substitute for chemical fertilizers for rice, corn and other Gramineae.

Besides having the ability to fix N, nitrogen-fixing bacteria can produce growth regulators that can stimulate plant root growth. The rhizosphere of rice plants has higher N2-fixing ability in lowland rice compared to upland rice because the exudates released by the roots are also different. In addition, the number of bacteria in the base of rice paddy stalks is 10 times higher than upland rice [8].

The life of N2-fixing bacteria in the soil depends on the host plant because most of the energy source is provided by the plant through the exudation process. Some carbohydrates and amino acids
excreted by rice roots will be used by N2-fixing bacteria [9]. The removal of such substances is considered to vary according to species or strain, therefore the composition of bacteria in the rhizosphere will vary according to plant genotype.

The association of bacteria with plant roots is characteristic and requires some specificity between plants and bacteria [10]. Azospirillum which is inoculated in plants from different strains will give different results. Azospirillum isolated from rice plants can only stimulate exudation of rice roots if planted in the same environment as the origin. Therefore, inoculation of N2-inhibiting bacteria to upland rice plants needs to be done to determine the ability of the association to increase growth and N content of upland rice plants.

2. Methods

2.1. Bacterial strains and culture conditions.

The bacterial strains used, Azospirillum brasilense SP7, Azospirillum brasilense SP27 and Klebsiella pneumoniae, were grown at 30 °C. All strains were kept and restreaked every three weeks on fresh plates of Luria-Bertani (LB) medium. A. brasilense SP7 and SP27 inoculated to 100 ml N-free Malat Broth (NFb) and K. pneumonia inoculated to N-free Medium [11]. Cultures were grown aerobically in a rotary shaker to an optical density (OD 600) of 1.0, corresponding to 3 x 10^8 bacteria ml^-1, as determined by plating.

2.2. Cultivation of upland rice seedlings and preparation of roots.

Seeds of Upland rice cv. Sentani (Food Crops Research Institute, Indonesia) were surface-sterilized by immersion in 70% (v/v) ethanol for 3 min, washing three times in sterile distilled water, soaking three times for 30 min in a solution of sixfold diluted commercial bleach containing 0-5 % sodium dodecyl sulphate, rinsing with sterile water for 60 min, soaking in fresh bleach/ sodium dodecyl sulphate solution of the same composition for 60 min, and finally rinsing five times with sterile distilled water. Sterilized seeds were germinated in a sterile medium of a mixture of sand and soil 1: 1. The seeds are allowed to grow for up to 20 days and were individually and aseptically transferred to the experiment pot for 3 plants/pot. Before transferred, the seedling was inoculated with bacterial suspension first.

2.3. Inoculation Bacteria to Plant root.

Inoculation of 3 x 10^8 bacteria ml^-1 to plant root was done twice. First, the root plant portion was immersed in the bacterial suspension. Ten days after transplanting, about 5 ml bacterial suspension inoculated by syringe to the rhizosphere.

2.4. 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 [12]. 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 α = 0.05%. Treatments which showed a significant effect were further analysed with the Duncan test.

3. Results and Discussion

3.1. Upland Rice Growth

The effect of N2-fixing bacterial inoculation treatment and its combination with nitrogen fertilizer on upland rice plants can be determined by measuring the number of tillers, plant height and biomass. Inoculation of N2- fixing bacteria to upland rice plants could increase the number of tillers. The number of tillers was significantly different from plants that were not inoculated (Table 1). This result is fit with the research of Tien et al (1979) [6] that is nitrogen fixing bacteria can produce growth
regulators and can stimulate the growth of plant roots and finally produce high tillers number. Statistically different fertilization doses do not affect the number of tillers, even there is a tendency that the use of N in plants inoculated with *A. brasilense* SP7 and with *K. pneumonia* inhibits the formation of tillers. The tendency of increasing or decreasing the number of saplings of plants treated with inoculation with different bacteria does not correlate with an increase in fertilizer dosage.

**Table 1.** Inoculation effect of *A. brasilense* and *K. pneumonia*, and N fertilizer on upland rice seedling formation

| Fertilizer dose (Kg N/Ha) | Number of tillers | Average |
|---------------------------|-------------------|---------|
|                           | C                 | SP7     | SP27 | Kp  |
| 0                         | 6.0               | 7.3     | 9.3  | 9.7 | 8.1<sup>a</sup> |
| 25                        | 5.3               | 8.3     | 8.0  | 8.0 | 7.4<sup>a</sup> |
| 50                        | 9.0               | 9.7     | 7.7  | 7.0 | 8.4<sup>a</sup> |
| 75                        | 7.0               | 9.0     | 9.0  | 7.0 | 8.0<sup>a</sup> |
| Average                   | 6.8<sup>a</sup>   | 8.6<sup>b</sup> | 8.6<sup>b</sup> | 7.9<sup>b</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 α = 0.05

Inoculation with N2-fixing bacteria, the use of N fertilizer and the interaction between the two treatments had no effect on plant height. Although it appears that the height of inoculated plants tends to be smaller compared to plants that are not inoculated. The response of upland rice plants in the form of plant height was not correlated with an increase in the dose of nitrogen fertilizer.

**Table 2.** Inoculation effect of *A. brasilense* and *K. pneumonia*, and N fertilizer on upland rice plant height

| Fertilizer dose (Kg N/Ha) | Plant height (cm)<sup>a</sup> | Average |
|---------------------------|------------------------------|---------|
|                           | C                            | SP7     | SP27 | Kp  |
| 0                         | 104.3                        | 102.3   | 101.7| 100.7| 102.3<sup>a</sup> |
| 25                        | 100.0                        | 105.7   | 104.0| 104.0| 102.8<sup>a</sup> |
| 50                        | 110.7                        | 103.0   | 105.3| 105.3| 105.3<sup>a</sup> |
| 75                        | 103.0                        | 102.3   | 104.3| 97.7 | 101.8<sup>a</sup> |
| Average                   | 104.5                        | 103.3<sup>a</sup> | 103.8<sup>a</sup> | 100.6<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 α = 0.05

The results of the analysis of the effect of treatment on the dry weight of the upland rice plants showed that bacterial inoculation markedly increased the dry weight of the upland rice plants. Inoculated plants have higher dry weight than control plants. Of the three types of bacteria used *K. pneumoniae* gives the lowest dry weight of the upper plants, but statistically not significantly different from the results of *A. brasilense* SP27 inoculation.

The dry weight of upland rice roots inoculated with no bacteria was the lowest compared to other bacteria, and significantly different from the dry weight of plants inoculated with *A. brasilense* and *K. pneumonia*. The highest root dry weight was obtained from plants which were inoculated with *A. brasilense* SP7 but statistically not different from other bacterial treatments. The use of fertilizers with various doses, also the interaction between fertilization and inoculation did not show a significant effect on root dry weight. The absence of influence of the use of N fertilizer on root dry weight, also caused root dry weight and N fertilization dose were not correlated for all bacterial treatments.
Table 3. Inoculation effect of *A. brasilense* and *K. pneumonia*, and N fertilizer on upland rice upper plant dry weight

| Fertilizer dose (Kg N/Ha) | upper plant dry weight (g)a | Average |
|--------------------------|-----------------------------|---------|
|                          | C  | SP7 | SP27  | Kp  |
| 0                        | 6.73 | 8.6 | 10.6  | 10.5 | 9.10a |
| 25                       | 4.75 | 10.2| 7.6   | 6.9  | 7.35a |
| 50                       | 6.33 | 12.1| 9.0   | 8.8  | 9.10a |
| 75                       | 6.53 | 9.2 | 10.6  | 7.5  | 8.44a |
| Average                  | 6.10a | 10.0b | 9.5b  | 8.4b |

*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 α = 0.05

Table 4. Inoculation effect of *A. brasilense* and *K. pneumonia*, and N fertilizer on upland rice root dry weight

| Fertilizer dose (Kg N/Ha) | root dry weight (g)a | Average |
|--------------------------|----------------------|---------|
|                          | C  | SP7 | SP27  | Kp  |
| 0                        | 0.45 | 0.8 | 0.7   | 0.9  | 0.70a |
| 25                       | 0.33 | 1.1 | 0.1   | 0.5  | 0.64a |
| 50                       | 0.53 | 0.8 | 0.7   | 0.6  | 0.66a |
| 75                       | 0.53 | 0.8 | 0.9   | 0.6  | 0.72a |
| Average                  | 0.46a | 0.85b | 0.75b | 0.64b |

*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 α = 0.05

The results of measurements of the effect of N2-fixing bacteria inoculation on upland rice biomass, it is known that different types of bacteria from the same species or from different species will give different results. *A. brasilense* SP7 and SP27 give the best response of upland rice root dry weight. However, *K. pneumonia* does not seem to provide a good response when combined with the use of fertilizers. It seems that N fertilizer application inhibit the upland rice root dry weight.

3.2. Total N content and Total Plant N production

The N content of upland rice plants is not affected by inoculation with N2-fixing bacteria, but by N fertilization. Inoculated plants and control plants show almost the same total N rate. The average N (%) rate of plants fertilized by 25 kg N/Ha was highest among all doses, but these results did not show any significant difference with doses of 0 kg N/Ha and 50 kg N/Ha.

Table 5. Inoculation effect of *A. brasilense* and *K. pneumonia*, and N fertilizer on upland rice total plant N content

| Fertilizer dose (Kg N/Ha) | Total plant N content (%)a | Average |
|--------------------------|-----------------------------|---------|
|                          | C  | SP7 | SP27  | Kp  |
| 0                        | 2.19 | 2.16 | 2.04  | 2.16 | 2.14ab |
| 25                       | 2.25 | 2.14 | 2.20  | 2.19 | 2.19b  |
| 50                       | 2.11 | 2.29 | 2.10  | 2.07 | 2.14ab |
| 75                       | 2.16 | 2.04 | 2.08  | 2.13 | 2.10b |
| Average                  | 2.18b | 2.16b | 2.10b | 2.14ab |

*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 α = 0.05

The effect of inoculation on total N production of upland rice is very clear. Inoculated plants have a very high total N production compared to plants without inoculation. This is due to these bacteria are
microaerophilic, able to fix nitrogen under free-living conditions, motile, and able to navigate in gradients of various chemicals, including oxygen [13]. Of the three types of bacteria used, the SP7 strain gave the lowest yield total N production of plants, but it was not significantly different from *Klebsiella pneumonia*. The total N production of plants of SP27 statistically different from other bacterial treatments. Different fertilizing doses produced different total N production of plants. Fertilizer dose of 25 Kg N/Ha gave the highest yield total N production of plants but it was not significantly different from the dose of 0 and 50 Kg N/Ha, and significantly different from the dose of 75 Kg N/Ha. These result suitable with the research that showed that C/N, C and N were important factors influencing the abundance and community structure of nitrogen-fixing bacterial [10]. From the results of the regression analysis of the correlation of dry weight of upland rice roots with plant dry tops showed that the root dry weight correlated linearly with the dry weight of the top of the plant for all inoculation treatments.

**Table 6.** Inoculation effect of *A. brasilense* and *K. pneumonia*, and N fertilizer on upland rice total plant N production.

| Fertilizer dose (Kg N/Ha) | Total plant N production (g)a | Average |
|--------------------------|-------------------------------|---------|
|                          | C | SP7 | SP27 | Kp     |         |
| 0                        | 0.15 | 0.18 | 0.22 | 0.23 | 0.20b  |
| 25                       | 0.10 | 0.22 | 0.17 | 0.16 | 0.16b  |
| 50                       | 0.13 | 0.28 | 0.19 | 0.18 | 0.20b  |
| 75                       | 0.13 | 0.19 | 0.23 | 0.16 | 0.18b  |
| Average                  | 0.13a | 0.22b | 0.20b | 0.18b |         |

*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 α = 0.05

4. Conclusion

Inoculation of upland rice plants with N2-fixing bacteria *A. brasilense* and *K. pneumonia* can increase tiller formation from 6.8 to 8.6, can increase plant dry biomass 2 times fold and also can increase total plant N production from 0.13g to 0.22g. 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.

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