The effect of different species of cyanobacteria on the rice yield and nitrogen use efficiency under different levels of nitrogen fertilizer on Alluvial West Java

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Abstract. Cyanobacteria or blue-green algae play an important role in rice cultivation. Nitrogen-fixing bacteria Cyanobacteria are found in rice fields, some of which can fix N₂ up to 30%, produce some phytohormones, vitamins, amino acids, and organic acids increase soil fertility and rice productivity. The study was conducted in Alluvials wetland rice in Jatitujuh, Majalengka Regency, West Java. This research aimed to analyze the influence of cyanobacteria in increasing rice yield and the efficiency of inorganic N fertilizers. The study was arranged in a randomized block design with two factors. The first factor was the cyanobacteria formula, i.e.: (1) without cyanobacteria, (2) Pseudanabaena sp.+Chlorogloea sp., (3) Pseudanabaena sp.+Nostoc sp., (4) Chlorogloea sp.+Nostoc sp. The second factor was the dose of N fertilizer (0%, 50%, 75% and 100%). The highest rice yield obtained by Chlorogloea sp.+Nostoc sp with 100% N, increased by 14.75%. Application of Pseudanabaena sp. + Nostoc sp. was increased rice grain yield and straw biomass by 11.47% and 37.49%, reduced N fertilizer by 25 to 50%, and increased nutrient uptake of N, P, K by 43.73%, 34.80 %, 34.40%. Using cyanobacteria is a promising strategy to increase rice yield and reduce chemical fertilizers.

1. Introduction

Current agricultural practices rely heavily on the application of synthetic fertilizers and pesticides, intensive tillage, and the use of excess water. These activities have helped many people in developing countries meet their food needs. However, these activities have caused environmental problems as including deterioration of soil fertility. So that in increasing agricultural production, it is necessary to find a technology that can increase output by utilizing limited resources without destroying environmental quality [1, 2]. Biofertilizers are better known as microbial inoculants, including bacteria (Azotobacter), cyanobacteria (blue-green algae), and mycorrhizal fungi, which provide nutrients for plants, contribute to soil fertility and maintain soil structure [3]. Cyanobacteria as potential candidates for their application to develop environment-friendly and sustainable agricultural practices [4, 5]

Cyanobacteria inhabit the earth since 3.8 billion years ago, and its oxygenic photosynthetic nature can change the earth's atmosphere from anaerobic conditions to aerobes. They spread in freshwater, marine, and terrestrial ecosystems but also found in extreme habitats such as hot springs, regions hypersaline, frozen environment, and desert [6]. Cyanobacteria fix nitrogen up to 30% and this will increase soil fertility and increase crop production by 34% [7]. They are environmentally friendly...
biofertilizers in increasing crop productivity, especially in lowland rice ecosystems. Nitrogen-fixing of cyanobacteria significantly complements on soil nitrogen [8].

Cyanobacteria are photosynthetic, N-fixation, autotrophic capacities, produce growth-promoting substances such as hormones, vitamins, amino acids, and organic acids [9]. Cyanobacteria contribute significantly to the biogeochemical cycles of carbon, nitrogen, and oxygen [10,11]. Nitrogen fixation by algal in helping to maintain the fertility of rice fields was recognized by De in 1938 [12]. The utilization of cyanobacteria was reported to have a beneficial effect on barley, oats, tomatoes, radishes, cotton, sugar cane, maize, chilies and lettuce [13]. The use of cyanobacteria to increase lowland rice yield in Indonesia is not widely used, so information on its use is required.

Modern green technologies by using biofertilizers such as cyanobacteria, fungi, and bacteria that produce plant growth-promoting rhizobacteria (PGPR) could improve and restore soil fertility and ensure sustainable agricultural production. Prasanna et al. [14] reported that inoculation cyanobacteria to the soil increased the rice yield by 19% and an increase in nitrogen fixation. In Nepal, Prasad and Prasad [15] recommended some suitable cyanobacteria strains as a source of biofertilizer for the use to increase rice productivity. Moreover, these microorganisms can reduce the use of inorganic fertilizer, mitigate stressed agroecosystems, and wastelands [16]. Some research results indicate a positive effect of cyanobacteria inoculation on rice cultivation. Saadatnia and Riahi [17] reported that inoculation cyanobacteria in the pot experiment increase rice plant growth and improved soil properties. Biofertilizer technology based on cyanobacteria can be a powerful tool to enrich soil fertility and increase rice yield.

The use of cyanobacteria to increase rice production is a promising strategy to reduce the dependence on the use of chemical fertilizers. On the other hand, the N content of most rice fields is low, so it requires a very high N fertilizer [18]. Cyanobacteria in paddy fields are important microbes to be used as bio inoculants to increase soil fertility, improve soil structure, and increase yield [14]. Excessive use of chemical fertilizers causes environmental problems such as greenhouse effects, ozone layer depletion, and water acidification are prevented by using biofertilizers [19]. To determine the effect of cyanobacteria on lowland rice yield, it has been carried out in Majalengka Regency, West Java, Indonesia. In this study, cyanobacteria were formulated using kaolinite powder as a carrier material and inoculated to the seeds with a dose of 500 g ha⁻¹. For most of the researches that have been done, cyanobacteria were inoculated to the soil. This research aimed to analyze the influence of cyanobacteria in increasing rice yield and the efficiency of inorganic N fertilizer used.

2. Materials and methods

The topography in Jatitujuh District Majalengka Regency is relatively flat, and sloping is at an altitude of 30 to 53 meters above sea level, with Alluvial soil type site in 6°37’48” S - 108°14’5” E. The research was carried out from April to August 2018. The materials used in this study were: rice seeds (Inpari 32 variety), cyanobacteria (Pseudanabaena sp., Chlorogloea sp., and Nostoc sp.) with kaolinite powder carrier material, compost, urea, SP-36, KCl. The experiments were laid out with a randomized complete block design (RCBD) by two factors with three replications. The first factor was the cyanobacteria formula, which consists of four types i.e., (1) Without cyanobacteria (S0), (2) Pseudanabaena sp.+Chlorogloea sp. (S1), (3). Pseudanabaena sp.+ Nostoc sp. (S2), (4) Chlorogloea sp.+Nostoc sp.(S3). The second factor was the level dose of urea fertilizer which consists of four levels, namely (1) 0% N (N0), (2) 50% N (N1), (3) 75% N (N2), (4) 100% N (N3).

Each unit plot size area was 5 m x 4 m. Urea was applied at three equal splits, the first given when the rice plants are one week after planting (WAP), while the remaining at two equal splits at maximum tillering and panicle initiation stage of crop growth. Urea, SP-36, and KCl fertilizers were applied by spreading in experimental plots. SP-36 and KCl were given before transplanting. The fertilizer rate of recommendation doses (100% dose) is 300 kg ha⁻¹ Urea, 100 kg ha⁻¹ SP36, and 100 kg ha⁻¹ KCl. Cyanobacteria biofertilizer applied at two splits, 500 g ha⁻¹ to seed treatment and 1 kg ha⁻¹ to 7 days after transplanting. Parameters were observed on plant height, number of tillers, number of panicles, panicle length, the weight of straw biomass, rice yield, and N, P and K uptake. The data obtained were analyzed using Analysis of Variance (ANOVA) following the standard procedure given by [20] using DSTAAT
computer software, and the differences of the mean among the treatments were analyzed by Duncan’s Multiple Range Test (DMRT) at the level of p = 0.05.

3. Results and Discussion

3.1. Soil chemical characteristics of the site

The soil characteristics of Alluvial Majalengka, West Java has neutral soil reaction (pH 6.6), low organic-C content (1.47%), low total-N (0.17%), and low C/N ratio (8.65). The potential-P (extracted with 25% HCl) is classified as very high, while the K₂O (extracted with 25% HCl) is classified moderate, but the available-P was moderate. The exchangeable-Ca and Mg are classified as very high, while the exchangeable-K and Na are low. Cation exchange capacity (CEC) is classified as moderate, but the level of base saturation is very high. The main problems of alluvial Majalengka, West Java are low C-organic and N (table 1). Application of biofertilizer containing inoculants of N-fixing microbes (i.e. cyanobacteria) are well-known potential to increase available N to wetland rice.

| Soil characteristics | Value     | Method         | Criteria          |
|----------------------|-----------|----------------|-------------------|
| pH H₂O               | 6.6       | pH meter       | Neutral           |
| N (%)                | 0.17      | Kjeldahl       | Low               |
| C-Org (%)            | 1.47      | Walkley & Black| Low               |
| C/N                  | 8.65      |                | Low               |
| P₂O₅ (mg 100g⁻¹)     | 139.00    | HCl 25 %       | Very high         |
| K₂O (mg 100g⁻¹)      | 31.75     | HCl 25 %       | Moderate          |
| CEC (emol (+) kg⁻¹)  | 23.34     | N NH₄OAc pH 7  | Moderate          |
| Ex. Ca (cmol (+) kg⁻¹) | 27.25   | N NH₄OAc pH 7  | Very high         |
| Ex. Mg (cmol (+) kg⁻¹) | 8.56    | N NH₄OAc pH 7  | Very high         |
| Ex. K (cmol (+) kg⁻¹) | 0.27     | N NH₄OAc pH 7  | Low               |
| Ex. Na (cmol (+) kg⁻¹) | 0.31    | N NH₄OAc pH 7  | Low               |
| Base saturation (%)  | >100      |                | Very high         |

The application of BGA in the rice fields promotes rice growth and yield [21]. Matrix analysis of variance (ANOVA) showed that the effect of cyanobacteria significantly affected to straw biomass, rice yield and nutrient uptake of N, P and K. Application of N-level fertilizer gave significantly affected to plant height at 8 WAP, the number of tillers at 6 and 8 WAP, straw biomass and rice grain yield. The interaction of both of the treatments gave significantly affected the rice yield observed (table 2).

3.2. Effect of cyanobacteria and nitrogen level to the growth of rice

The statistical analysis results showed that the combination of cyanobacteria and N fertilization did not significantly interact with the growth of plant height at 2 to 8 WAP. However, it appears that 50% to 100% N fertilization has a significant effect on plant height compared to the 0% N. Fertilization of 100% N showed the highest plant height and was significantly different from the treatment of 0% N, 50% N and 75% N. This indicated that rice plants still require high N elements until the plant growth period of 8 WAP. The highest plant height was 88.39 cm, an increase of 8.84% compared to the 0% N (table 3).

3.3. Effect of cyanobacteria and N fertilizer dose to the yield variable component

Cyanobacteria and N fertilizer did not significantly interact with straw biomass, number of tillers per hill, number of panicles per hill, and panicle length (table 5). However, there appears to be an increase in both cyanobacteria and N fertilizer treatments significantly affected the straw biomass compared to the control. The highest straw biomass in cyanobacteria treatment was achieved of Pseudanabaena sp. + Nostoc sp. (S2), straw biomass was 11.16 t ha⁻¹, increased by 37.49%. Meanwhile, the N fertilization treatment that had the highest effect on biomass was 75% N (N2), straw biomass was 10.91 t ha⁻¹, on the other research reported that cyanobacteria affected the number of panicles per hill and length of panicles [22].
Table 2. Matrix analysis of variance (ANOVA) to the variables were observed

| No | Variables                                      | S   | N   | S X N |
|----|-----------------------------------------------|-----|-----|-------|
| 1  | Plant height at 2, 4 and 6 WAP (cm)            | ns  | ns  | ns    |
| 2  | Plant height at 8 WAP (cm)                    | ns  | *   | ns    |
| 3  | Number of tillers 2 and 4 WAP                | ns  | ns  | ns    |
| 4  | Number of tillers 6 and 8 WAP              | ns  | *   | ns    |
| 5  | Straw biomass (t ha⁻¹)                       | *   | *   | *     |
| 6  | Number of tillers (hill⁻¹)                  | ns  | *   | ns    |
| 7  | Number of panicles (hill⁻¹)                 | ns  | ns  | ns    |
| 8  | Panicles length (cm)                         | ns  | ns  | ns    |
| 9  | Grain yield (t ha⁻¹)                         | *   | *   | *     |
| 10 | N-uptake                                      | *   | *   | ns    |
| 11 | P-uptake                                      | *   | *   | ns    |
| 12 | K-uptake                                      | *   | *   | ns    |

Remarks: S=Cyanobacteria, N=Urea level, S X N=Interaction of cyanobacteria and Nitrogen level, ns = not significant, * = significant

Table 3. The average plant height at the observation of 2 – 8 WAP

| Treatment                        | 2 WAP | 4 WAP | 6 WAP | 8 WAP |
|---------------------------------|-------|-------|-------|-------|
| **Cyanobacteria formula**       |       |       |       |       |
| Control (S0)                    | 32.43 | 48.28 | 65.78 | 84.65 |
| *Pseudanabaena* sp.+*Chlorogloea* sp. (S1) | 32.80 | 48.48 | 72.20 | 85.25 |
| *Pseudanabaena* sp+*Nostoc* sp (S2) | 32.28 | 48.28 | 72.03 | 86.63 |
| *Chlorogloea* sp + *Nostoc* sp (S3) | 33.70 | 49.83 | 75.13 | 85.04 |
| **The dose of N (Urea)**        |       |       |       |       |
| 0% N (N0)                       | 31.88 | 47.74 | 71.03 | 81.21 |
| 50% N (N1)                      | 32.73 | 49.26 | 70.75 | 86.18 |
| 75% N (N2)                      | 33.08 | 48.45 | 72.71 | 85.79 |
| 100% N (N3)                     | 33.53 | 49.42 | 70.65 | 88.39 |
| CV (%)                          | 6.67  | 5.69  | 5.90  | 5.90  |

Remarks: WAP=Weeks After Planting. The numbers followed by the different letters in the same column are significantly different at 5% DMRT

Table 4. The average the number of tillers at the observations of 2 to 8 WAP

| Treatment                        | 2 WAP | 4 WAP | 6 WAP | 8 WAP |
|---------------------------------|-------|-------|-------|-------|
| **Cyanobacteria formula**       |       |       |       |       |
| Control (S0)                    | 6.88  | 18.72 | 23.43 | 18.09 |
| *Pseudanabaena* sp.+*Chlorogloea* sp. (S1) | 6.67  | 18.61 | 23.08 | 17.93 |
| *Pseudanabaena* sp+*Nostoc* sp (S2) | 6.45  | 18.84 | 24.51 | 18.30 |
| *Chlorogloea* sp + *Nostoc* sp (S3) | 6.89  | 20.22 | 23.87 | 17.82 |
| **The dose of N (Urea)**        |       |       |       |       |
| 0% N (N0)                       | 6.48  | 17.37 | 20.52 | 16.43 |
| 50% N (N1)                      | 6.39  | 18.75 | 23.81 | 17.93 |
| 75% N (N2)                      | 6.76  | 19.55 | 24.47 | 18.07 |
| 100% N (N3)                     | 7.25  | 20.72 | 26.09 | 19.28 |
| CV (%)                          | 12.75 | 13.27 | 13.43 | 12.31 |

Remarks: WAP=Weeks After Planting. The numbers followed by the different letters in the same column are significantly different at 5% DMRT
Table 5. The average the number of tillers per hill, number of panicles per hill, and panicle height of rice Inpari 32 variety at harvest.

| Treatment                  | Straw biomass (t ha\(^{-1}\)) | Number of tillers (tillers hill\(^{-1}\)) | Number of panicles (sheets hill\(^{-1}\)) | Panicle length (cm) |
|----------------------------|--------------------------------|-------------------------------------------|-------------------------------------------|---------------------|
| **Cyanobacteria formula**  |                                 |                                           |                                           |                     |
| Control (S0)               | 8.12 a                         | 18.09                                    | 15.09                                     | 22.77               |
| *Pseudanabaena* sp.+*Chlorogloea* sp. (S1) | 10.21 bc                     | 17.49                                    | 15.13                                     | 23.67               |
| *Pseudanabaena* sp.+*Nostoc* sp. (S2) | 11.16 c                      | 18.30                                    | 16.42                                     | 22.92               |
| *Chlorogloea* sp. + *Nostoc* sp. (S3) | 9.75 b                        | 17.82                                    | 15.27                                     | 23.45               |
| **Dose of N (Urea)**       |                                 |                                           |                                           |                     |
| Control (N0)               | 8.41 a                         | 16.42 a                                  | 15.08                                     | 22.84               |
| 50% N (N1)                 | 9.57 b                         | 17.92 a                                  | 15.40                                     | 23.63               |
| 75% N (N2)                 | 10.91 c                        | 18.01 ab                                 | 14.58                                     | 23.05               |
| 100% N (N3)                | 10.36 bc                       | 19.28 b                                  | 16.85                                     | 23.29               |
| C V (%)                    | 22.45                          | 12.31                                    | 13.11                                     | 12.16               |

Remarks: The numbers followed by the same letters in the same column show no significant difference at 5% DMRT.

The combination of cyanobacteria and N fertilizer gave a significant interaction to rice yield (table 6). The highest grain yield was achieved by S3N3 (*Chlorogloea* sp. + *Nostoc* sp., 100% N) was 8.40 t ha\(^{-1}\). The treatment of S0N3 (without cyanobacteria, 100% N) showed the grain yield was 7.32 t ha\(^{-1}\). This result showed that inoculation cyanobacteria were able to maximize rice yield at the 100% N fertilization, the rice yield increase of 1.08 t ha\(^{-1}\) or 14.75%. Whereas in the S1N1 (*Pseudanabaena* sp. + *Chlorogloea* sp., 50% N), S1N2 (*Pseudanabaena* sp. + *Chlorogloea* sp., 100% N), S2N1 (*Pseudanabaena* sp. + *Nostoc* sp., 50% N), and S2N2 (*Pseudanabaena* sp. + *Nostoc* sp., 75% N) showed that the grain yield of rice was not significantly different to the S3N3 (*Chlorogloea* sp. + *Nostoc* sp., 100% N) and significantly of S0N3 (without cyanobacteria, 100% N). This illustrates that cyanobacteria biofertilizer is an alternative to reducing the dose of N fertilizer to lowland rice. At the same time, it can increase the higher yield than the yield is obtained by urea fertilizer follow the recommended dose (300 kg Urea ha\(^{-1}\)). The increased grain yield of rice in the application of cyanobacteria (*Pseudanabaena* sp. + *Nostoc* sp.) increased rice yields up to 14.75%. It saved N fertilizer (urea) by 25 to 50% compared to the recommended N dose (100% N). The results of the research by Setyawati and Pu jawati [23] stated that the yield of rice grain at 120 kg N ha\(^{-1}\) fertilization was 67.343 g pot\(^{-1}\). Meanwhile the treatment of 90 kg N ha\(^{-1}\) + cyanobacteria (10 kg ha\(^{-1}\)) gave a higher grain yield, namely 88.573 g pot\(^{-1}\). This showed that cyanobacteria able to increase grain yield, besides that it can save N fertilizer, increase grain yield by 21,23% and save N fertilizer by 25% N. The research results by Mishra and Pabbi [24] the rice yield by cyanobacteria increased rice yield about 12.3 to 19.5%, while the research result by Prasad [25] reported cyanobacteria able to increase rice yield 7.53 to 21.2%.

The relationship between N fertilizer level and cyanobacteria ((*Pseudanabaena* sp. + *Nostoc* sp.) with to the rice yield shown in Figure 1, this figure shows the relationship between the cyanobacteria of (*Pseudanabaena* sp. + *Nostoc* sp.) and the level of N fertilizer level, indicating that above 75% recommended N (urea) combined with cyanobacteria (*Pseudanabaena* sp. + *Nostoc* sp.) tends to reduce rice grain yields, represented by the regression equation \(Y = -0.3125x^2 + 2.6825x + 2.4675\) by the \(R^2\) value of 0.998. Based on this equation the optimal dose of N fertilizer with a combination of cyanobacteria (*Pseudanabaena* sp. + *Nostoc* sp.) is 79.31% N.
Table 6. Interaction effect of cyanobacteria and N fertilizer dose to the grain yield of rice Inpari 32 variety

| The dose of N (Urea) | Cyanobacteria | Grain yield (t ha\(^{-1}\)) | Average |
|----------------------|---------------|----------------------------|---------|
|                      | S0            | S1                        | S2      | S3      |       |
| N0- PK (N0)          | 6.86 a        | 6.39 a                    | 6.57 a  | 6.71 a  | 6.63 A |
| 50% N (N1)           | 6.82 a        | 7.81 b                    | 7.74 b  | 7.09 a  | 7.37 B |
| 75% N (N2)           | 7.39 a        | 7.89 b                    | 8.16 b  | 7.30 a  | 7.68 BC|
| 100% N (N3)          | 7.32 a        | 7.98 b                    | 8.08 b  | 8.40 b  | 7.94 BC|
| Average              | 7.09 A        | 7.37 B                    | 7.64 B  | 7.94 C  | 7.41   |

Remarks: The numbers followed by the lowercase in the same row and column or the numbers followed by the uppercase in the row or column show no significant difference at 5% DMRT

3.4 Nutrient Uptake

The effects of cyanobacteria and N fertilizer dose gave no significant interaction effect on nutrient uptake is shown in table 7. The research conducted in Majalengka rice fields with alluvial soil types showed that cyanobacteria affected increasing uptake of N, P and K nutrients compared without cyanobacteria. It is suspected that the increased availability due to the N fixation activity by cyanobacteria causes better plant growth, thereby increasing the growth of straw biomass (table 5), so that the increase in biomass will increase the amount of plant nutrient uptake. Cyanobacteria have the ability to fix nitrogen in rice fields through the fixing of N\(_2\) atmosphere to converted into ammonium, besides that it can dissolve insoluble phosphorus in the soil into a form available for plants [26], with the ability of cyanobacteria to dissolve P nutrients will increase P nutrient uptake by plants.

Wilson [9] and Sharma et al. [27] reported some species of cyanobacteria can dissolve insoluble phosphate through the mechanism of releasing organic acids, produce biomass and exopolysaccharides and increase the activity of soil enzymes which participate in the release of nutrients needed by plants. De Caire et al. [28] and Chakdar et al. [29] stated that cyanobacteria also are known to dissolve and mobilize phosphorus (P) and make it available to plants, producing extracellular phosphatase, organic
acids, polysaccharides [30]. Polysaccharides secreted by cyanobacteria contribute to the stability of the soil structure, increase soil C and N levels, thereby promoting plant growth.

The uptake of N, P and K increased significantly with the increasing N application dose, but at 100% N uptake of N and K began to decline. While the highest absorption of N and K was found in urea application of 75% N, while the highest absorption of P was in the implementation of urea as much as 100% N.

**Table 7.** The average of N, P, K uptake of rice straw Inpari 32 variety

| Treatment                          | N            | P            | K            |
|------------------------------------|--------------|--------------|--------------|
| Control (S0)                       | 143.89 a     | 12.79 a      | 131.83 a     |
| *Pseudanabaena* + *Chlorogloea* (S1)| 190.63 bc    | 15.73 b      | 163.21 b     |
| *Pseudanabaena* + *Nostoc* (S2)    | 206.82 c     | 17.24 c      | 177.17 c     |
| *Chlorogloea* + *Nostoc* (S3)      | 181.36 b     | 15.21 b      | 152.72 b     |

*The dose of N (Urea)*

| Treatment (N0)                     | N            | P            | K            |
|------------------------------------|--------------|--------------|--------------|
| Control (S0)                       | 106.57 a     | 13.78 a      | 146.64 a     |
| 50% N (N1)                         | 214.96 bc    | 15.12 a      | 154.65 ab    |
| 75% N (N2)                         | 242.34 c     | 15.78 b      | 179.17 c     |
| 100% N (N3)                        | 168.39 b     | 18.19 c      | 166.99 b     |
| CV (%)                             | 14.16        | 13.11        | 12.16        |

Remarks: The numbers followed by the same letters in the same column show no significant difference at 5% DMRT

4. **Conclusion**

With the results of the above research, it can be concluded that giving cyanobacteria as a biofertilizer using kaolinite as a carrier material and inoculating to the rice seeds is a promising strategy with an easier application, can increase rice yield and reduce the dose of N fertilizer application. A combination of *Chlorogloea* sp. + *Nostoc* sp. and 100% urea can maximize rice production, increase rice yield by 14.75%. Formula cyanobacteria *Pseudanabaena* sp. + *Nostoc* sp. increase the rice yield and straw biomass by 11.47% and 37.49%, reduce the dose of N fertilizer application by 25 to 50%, and increase nutrient uptake of N, P, K by 43.73%, 34.80%, 34.40% respectively. The use of cyanobacteria as a biofertilizer to increase rice production is a promising strategy and reduce chemical fertilizers.

4. **Acknowledgments**

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