Effect of beneficial soil microbes and inorganic fertilizers on soil nitrogen, chlorophyll and yield of upland rice on Ultisols

B N Fitriatin 1), M E.Nabila2), E T.Sofyan1), AYuniarti1) and T Turmuktini3)

1Soil Science Department, Agriculture Faculty Universitas Padjadjaran
2Agrotechnology, Agriculture Faculty, Universitas Padjadjaran
3Agriculture Faculty, Winayamukti University

Email : betty.natalie@unpad.ac.id

Abstract. The beneficial soil microbes influence plant growth and soil quality. The soil microbes include phosphate solubilizing microbes and nitrogen-fixing bacteria. The experiments had been conducted to investigate the application of phosphate-solubilizing microbes and nitrogen-fixing bacteria and N,P,K fertilizers on soil nitrogen (N), chlorophyll and yield of upland rice on Ultisols. This experiment arranged in a factorial Randomized Block Design, consisted of two factors with three replications. The first factor consisted of beneficial soil microbes, which were without soil microbes; with microbial consortia 1 (Pseudomonas mallei, P. cepaceae, Aspergillus niger and Penicillium sp., Azotobacter chroococum, Azospirillum sp.); with microbial consortia 2 (Azotobacter chroococum, A. villandii, Azospirillum, Pseudomonas cepaceae, Penicillium and Actinobacteria); and mixed of microbial consortia 1+2. The second factor were NPK fertilizers with four levels (100%, 75%, 50% and 25% dosage of recommendation). The results showed that the application of the beneficial soil microbes and N,P,K fertilizers didn’t improve soil N, chlorophyll content and yield upland rice on Ultisols.

1. Introduction
The increasing of population caused more conversion of farmland to non agricultural use. Decreasing number of farmland causing local farmers to utilize suboptimal soil to fulfils Indonesia's food demand. Suboptimal land is a land with various soil problems such as low soil pH, also low soil nutrient and organic matters.

Ultisol is a dominant soil order in largest part of dry land in Indonesia and has not been used for agriculture. Acid reaction and high Al content are the main problems of Ultisol that prevent P solubilization, this is why Ultisol needs soil treatment such as calcification or fertilization [1]. Ultisol is beneficial enough for agriculture if land management pay attention to increase organic matters content and solve nutrient availability problem [2].

One of effective ways to increase nutrient availability is biofertilizer use Biofertilizers are products containing living cells of different types of microbes which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally
important elements (nitrogen, phosphorus) from unavailable to available form through biological process [3]. Regularly, biofertilizer is used nitrogen-fixing bacteria, phosphate-solubilizing microbes, potassium-solubilizing microbes, plant growth promoting rhizobacteria that produces phytohormones, decomposer, and biological agent [4]. Biofertilizer that suits for sub optimal land are phosphate-solubilizing microbes and nitrogen-fixing bacteria.

Microbe that has a role to facilitate available phosphate is phosphate-solubilizing microbes that enable to affects plant growth by releasing fixed phosphate[5], phosphatase enzyme excretion [6] and producing the phytohormone [7]. Phosphatase enzyme from microbes could catalyze mineralization of organic phosphate and transform it into inorganic phosphate [8].

*Azotobacter* sp. dan *Azospirillum* sp. are non-symbiotic and free-living bacteria that play a role in N fixation [9]. *Azospirillum* is the best genus from Plant Growth-Promoting Rhizobacteria (PGPR) genera group because of its interactions with several plant roots, enable to fixing nitrogen and solubilize phosphate also produce plant growth hormones [10].

Biofertilizer take an important role to sustainably increasing land productivity also affecting plant growth and yield positively [11]. Biofertilizer application that contains consortium of phosphate-solubilizing microbes and nitrogen-fixing bacteria and adding humic acid could increasing plant phosphate content and rice yield [12]. More research needed to know more about microbes with beneficial abilities such as phosphate-solubilizing microbes and nitrogen-fixing bacteria in increasing N soil also chlorophylls content and yield of upland rice grown on Ultisol.

2. Materials and Methods

Experiment was conducted at Green House in Faculty of Agriculture Universitas Padjadjaran, Jatinangor Sumedang District, Jawa Barat Indonesia. Upland rice var Situ Bagendit was grown in Ultisols (the soil is used 15 kg per pot). Soil order classified as acidic soil (pH H2O 4.8). Chemical properties of soil is N total 0,069% classified as very low, P-available (P2O5) 9.87 mg kg−1 classified as very low, P-potential (P2O5 HCl 25%) 18.29 mg 100 g−1 classified as low criteria). Biofertilizer that used in this experiment was a consortia of phosphate-solubilizing microbes and nitrogen-fixing bacteria from Soil Biology Laboratory Faculty of Agriculture, Universitas Padjadjaran.

The pot experiment was carried out in factorial Randomized Block Design consisted of two factors with three replications. The first factor consisted of beneficial soil microbes, which were without soil microbes; with microbial consortia 1 (*Pseudomonas mallei*, *P. cepaceae*, *Aspergillus niger* and *Penicillium* sp., *Azotobacter chroococum*, *Azospirillum sp.*); with microbial consortia 2 (*Azotobacter chroococum*, *A. viilandii*, *Azospirillum*, *Pseudomonas cepaceae*, *Penicillium* and *Acitenobacter*); and mixed of microbial consortia 1+2. The second factor were NPK fertilizers with four levels (100%, 75%, 50% and 25% dosage of recomendation). Biofertilizer dosage was 50 kg ha−1 meanwhile recommended dosage of inorganic fertilizer was Urea 250 kg ha−1, SP-36 100 kg ha−1, KCl 100 kg ha−1. Urea fertilizer was given periodically at 2, 4, and 6 weeks after planting, while SP-36 and KCl fertilizers were applied at the beginning of planting. Observed responds variable were N soil content (measured by the Kjeldahl method), leaves chlorophyll using a chlorophyll meter.and yield of upland rice (1,000 grain weight).

2.1. Application and Production of Biofertilizer

Multiplication of phosphate-solubilizing microbes and nitrogen-fixing bacteria isolates used nutrient broth (NB), while for phosphate-solubilizing fungi used potato dextrose broth (PDB). A total of 10% liquid culture of each isolates was added into scale up media then mixed with shaker 112 rpm for 3 days at room temperature. Before mixed, phosphate-solubilizing microbes, nitrogen-fixing bacteria, and phosphate-
solubilizing fungi population was counted by Total Plate Count Method; then 10% liquid inoculant was inoculated to a carrier consisted of peat and compost (1:1; w:w).

Biofertilizers that used in this research are solid biofertilizer with bacterial density $10^8$ CFU g$^{-1}$. Each pot received 0.375 g biofertilizer which is equal to 50 kg ha$^{-1}$. Biofertilizer application done with once time with mixing biofertilizer and 10,000 kg ha$^{-1}$ or 75 g pot$^{-1}$ of organic fertilizer (straw compost) then buried into the pot one week before planting.

3. Results and Discussion

Nitrogen content was analyzed at the end of vegetative period. The results showed that there was no interaction between the consortium and N, P, K fertilizers on all parameters (Table 1). The total N content of the soil was low based on the Soil Research Center. The application of 100% N, P, and K fertilizers gives a fairly high chlorophyll content when compared to other treatments (Table 1).

| Treatment | Total N (%) | Chlorophyll | 1000 grain weight (g/plant) |
|-----------|-------------|-------------|-----------------------------|
| Biofertilizers: |  |  |  |
| Without | 0.11 | 50.01 | 21.50 |
| Consortia 1 | 0.12 | 48.54 | 22.03 |
| Consortia 2 | 0.12 | 50.65 | 22.39 |
| Consortia 1+2 | 0.12 | 48.53 | 22.03 |
| Dosage of N, P, K: |  |  |  |
| 100 % | 0.11 | 51.06 | 22.49 |
| 75 % | 0.12 | 49.17 | 22.33 |
| 50 % | 0.12 | 49.57 | 21.60 |
| 25 % | 0.12 | 50.01 | 21.50 |

Note: The data that are not given letter notation, Duncan's multiple distance test is not carried out because it is not significantly different based on the analysis of variance at the 5% level.

Table 1 showed that both treatments has no significant effect on total N because the macronutrient N was used for microbial metabolism. Soil total N is very low before experiment; it cause N content in the soil after experiment became low. The total N content generally ranges from 2,000-4,000 kg ha$^{-1}$ in the 0-20 cm layer but only 3% is available to the plant. In addition, it is assumed that the N element is absorbed more by plants [1]. The pH of the experimental soil was acid so that the plants absorb more nitrogen [7].

Chlorophyll is the main pigment in the photosynthesis; the role of chlorophyll in photosynthesis are triggering CO$_2$ fixation to produce carbohydrates and provide energy for the ecosystem as a whole. Photosynthates produced from the photosynthesis process will be removed and spread to all parts of the plant by phloem vessels. Observation of chlorophyll content was carried out at the end of vegetative period. the application of 100% N, P, and K fertilizers gave a fairly high chlorophyll content when compared to other treatments [13].

This is presumably because the inorganic fertilizers are more available. The high or low content of chlorophyll is directly proportional to the availability of the N and plays an important role in the process...
of photosynthesis [14]. The chlorophyll meter and leaf color chart are simple, portable diagnostic tools that can measure the crop N status in situ in rice fields to determine the timing of N topdressing [15]. The increasing of nitrogen content and leaf area which correlated with an increase chlorophyll a and b by adding inorganic [16].

The combination treatment of the biofertilizer consortium and the 50% N, P, K fertilizer dosage in Table 1 showed that the microbes present in the biofertilizer play a role to increase the chlorophyll content. The availability of P and N due to phosphate solubilizing bacteria and N-fixing bacteria, can increase the content of chlorophyll and chloroplasts in leaves; and the process of photosynthesis to give a better plant growth [17], that the application of biofertilizer Azospirillum and Azotobacter resulted in highest chlorophyll levels [18].

The application of biofertilizers and N, P, and K fertilizers did not change the 1,000-grain weight of upland rice (Table 1). However, irrespective of statistical analysis application of microbial consortia 2 (P. cepaceae, A. chroococum, A. vilandii, Azospirillum, Penicillium, Acinetobacter) had the potency to increase the 1,000-grain weight of upland rice up to 4%.

Some factors that cause the application of biofertilizers and N, P, and K fertilizers did not have a significant effect on the rice yield, was caused by plant genetic. Rice var. Situ Bagendit have a small seed shape and size so that the weight of 1,000 grain was not significantly different. High and low weight of seeds depends on dry ingredients in the seeds. Dry matter in the seeds is obtained indirectly from photosynthesis which can then be used to fill the seeds [19].

It is supposed that insufficient plant nutrient in soil caused the formation of rice seeds become unoptimal [20]. Stated that the growth and yield of plants was strongly influenced by two factors; internal and external factors. Phosphate solubilizing bacteria was able to reduce inorganic fertilizers and increased growth of crops [21]. The combination of phosphate solubilizing bacteria and NPK fertilizer increased the height of paddy rice at 30 days after planting [22].

Internal factors such as plant age, plant morphology, yield, capacity to store food reserves, resistance to disease are influenced by genetic traits or inherited traits. Environmental factors, such as climate, soil and biotic factors were also dictate the yield. The difference in growth and the yield of upland rice were influenced by one or more of these factors. Differences in genetic composition is one of the factors causing diversity in plant appearance and differences in genetic composition will always occur even if the plant material used is from the same plant type.

4. Conclusion

Inoculation of phosphate solubilizing microbes and N-fixing bacteria as biofertilizers did not significantly affect the total N in soil, chlorophyll content in the leaves and 1,000 grain weight of upland rice. However, biofertilizer consortia 2 (Pseudomonas cepaceae, Azotobacter chroococum, Azotobacter vilandii, Azospirillum, Penicillium, Acinetobacter) enable to increase chlorophyll content and 1,000-grain weight of upland rice up to 4%.

Acknowledgements

This research was supported by grants received (applied research :16/UN6.E/LT/2018) from the Directorate General of Higher Education Ministry of Research and Technology Indonesia. We thank to our students for their assistance.
References

[1] Hardjowigeno, S. 2010. Soil Science. Akademika Presindo, Jakarta.

[2] Munir, M. 1996. Main Soils of Indonesia. Dunia Pustaka Jaya, Jakarta.

[3] Rokhzadi A., Asgharzadeh A., Darvish F., Nourmohammadi, G. and Majidi E. 2008. Influence of plant growth promoting rhizobacteria on dry matter accumulation and yield of chickpea (Cicer arietinum L.) under field condition. Am-Euras. J. Agric. Environ. Sci. 3(2): 253-257.

[4] Fitriatin, B.N., P. Tamara, O. Mulyani, E.T. Sofyan, A. Yuniarti and T. Turmuktini. 2018. Influence of biofertilizer and humic acid on NPK content and yield of rice (Oryza sativa L.). International Journal of Agriculture, Environment and Bioresearch. 3: 20-27

[5] Jayakumara, N., P. Paulraja, P. Sajeesha, K. Sajnaa, and A. Zineera. 2019. Application of native phosphate solubilizing bacteria for the use of cheap organic and inorganic phosphate source in agriculture practice of Capsicum annum (Chili) - A pilot scale field study. Proceedings 16 (2019) 1630-1639 Available online at www.sciencedirect.com

[6] Yadaf, by R. S. and J.C. Taradar. 2003. Phytase and phosphatase producing fungi in arid and semi-arid soils and their fouling in hydrolyzing different organic P compounds. Conducting Soil Biology and Biochemistry 35 : 1-7.

[7] Gentili, R., R. Ambrosini, C. Montagnani, S. Caronni, and S. Citterio. 2018. Effect of Soil pH on the Growth, Reproductive Investment and Pollen Allergenicity of Ambrosia artemisiifolia L. Plant Science 9 (1336): 1-12

[8] George., T.S., P. J. Gregory, M. Wood, D. Read, J. Buresh. 2002. Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. Conducting Soil Biology and Biochemistry 34 : 1487-1494.

[9] Fitriatin, B.N., A. Yuniarti, and T. Turmuktini. 2014. The effect of phosphate solubilizing microbe producing growth regulators on conducting soil phosphate, growth and yield of maize and fertilizer efficiency in Ultisols. Eurasian Journal of Soil Science Vol 3 pp. 104 -107

[10] Steenhoudt, O. and J. Vanderleyden. 2006. Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects.FEMS Microbiol. Rev. 24: 487–506.

[11] Itelima JU, WJ. Bang, MD. Sila, IA. Onyimba, and OJ. Egbere. 2019. A review: Biofertilizer - A key player in enhancing

[12] Sampson, P.H., T.P. Zarco, G.H. Mohammed, J.R. Miller, and T. Noland. 2003. Hyperspectral remote sensing of forest condition: estimating chlorophyll content in tolerant hardwoods. Forest Science, 49 (3): 381 – 391.

[13] Lakitan B. 2001. Fundamentals of Plant Physiology. Raja Grafindo Persada. Jakarta.

[14] Singh, T and S.S. Purohit. 2011. Biofertilizers Technology. Agrobios (India). ISBN. 13:978-81-7754-382-7.

[15] Balasubramanian, V., A.C. Morales, R.T. Cruz, and S. Abdurrahman. 1999. On-farm adaptation of knowledge-intensive nitrogenmanagement technologies for rice systems. Nutrient Cycle Agroecosyst. 53: 93–101

[16] Larimi, S. B., M. Shakiba, A.D. Mohamadinasab and M. M. Vaheid. 2014. Changes in nitrogen and chlorophyll density and leaf area of sweet basil (Ocimum basilicum L.) affected by biofertilizer and nitrogen application. International Journal of Biosciences, 5(9): 256 – 265

[17] Wu, F., J. Li, Y. Chen, L. Zhang, Y. Zhang, S. Wang, X. Shi, L. Li and J. Liang. 2019. Effects of phosphate solubilizing bacteria on the growth, photosynthesis, and nutrient uptake of Camellia oleifera Abel. Forests (10) : 348-357.

[18] Indah, R.Z. 2010. Effect of arbuscular mycorhiza fungi and Rhizobium on leaf anatomy characteristic and chlorophyll content of pea. Jurnal Biologi. 2(1) : 1-7.
soil fertility and crop productivity. Microbiol Biotechnol Rep Vol 2 No 1:22-28

[19] Masdar. 2007. Interaction of planting distance and number of seedlings per point of plant in the rice intensification system on vegetative growth of plants. Jurnal Akta Agrosia, (1): 92-98.

[20] Salamone, I. E.G.D., J.M. Funes, L.P.D. Salvo, J.S.E. Ortega, F. D’auria, L. Ferrando and A.F. Scavino. 2012. Inoculation of Paddy Rice with *Azospirillum brasilense* and *Pseudomonas flurescens*: Impact of Plant Genotypes on Rhizosphere Microbial Communities and Field Crop Production. Applied Soil Ecology 196-204.

[21] Prakash, J. and · NK. Arora. 2019. Phosphate-solubilizing *Bacillus* sp. enhances growth, phosphorus uptake and oil yield of *Mentha arvensis* L. Biotech. 9:126. https://doi.org/10.1007/s13205-019-1660-5

[22] Biswakarma, B., H. Verma and N.C. Sarkar. 2018. Effect of Phosphate Solubilizing Bacteria on Yield of Transplanted Rice under Lateritic Belt of West Bengal, India. *International Journal of Current Microbiology and Applied Sciences* 7(2): 3192-3204