Study on rhizobium interaction with osmoprotectant rhizobacteria for improving mung bean yield

Y Maryani\textsuperscript{1,2,3}, Sudadi\textsuperscript{1}, W S Dewi\textsuperscript{1} and A Yunus\textsuperscript{1}

\textsuperscript{1} Doctoral Program in Agricultural Science, Graduate School, Sebelas Maret University, Surakarta, Indonesia
\textsuperscript{2} Faculty of Agriculture, Universitas Sarjanawiyata Tamansiswa, Jl. Batikan no. 7 Yogyakarta, Indonesia
\textsuperscript{3} Corresponding author: ym_ust@yahoo.com

Abstract. Gunungkidul has calcareous soil with limitations including calcareous stone, mostly hilly terrain, and shallow cultivated layer. Furthermore, nowadays we face the disadvantages of long dry seasons, a short rainy season, and high temperatures caused by climate change. To overcome these problems, it is necessary to investigate the interactions between microbes in the soil and plants to support mung bean yield. Osmoprotectant rhizobacteria were isolated and screened from the calcareous soil in Gunungkidul with disadvantageous climates such as a long dry season, a short rainy season, and high temperature. This research was arranged in Completely Randomized Design. The result showed that osmoprotectant rhizobacteria isolate of strain A124-k and Ver5-k can produce 9.6306 mg g\textsuperscript{-1} cell of glycine betaine in a soil density 1.7667 x 10\textsuperscript{7} CFU g\textsuperscript{-1} and 11.4870 mg g\textsuperscript{-1} cell of glycine betaine in a soil density 1.9667 x 10\textsuperscript{7} CFU g\textsuperscript{-1}. Inoculation of the isolate into mung bean rhizosphere can support mung bean yield. Osmoprotectant rhizobacteria isolate did not affect rhizobium in the mung bean rhizosphere.

1. Introduction
Gunungkidul has limitations by having calcareous soil including calcareous stone, deep hardpan layer, mostly hilly terrain, and shallow cultivated layer. Furthermore, we face the climate drawbacks such as long dry seasons, short rainy season, and high temperatures which caused by climate change nowadays. It leads to irregular rainwater availability for microorganisms and crops. Therefore, it is necessary to investigate the interactions between microbes in the soil and plants to boost crop production. Rhizobium always lives in the rhizosphere. It always helps mung bean to synthesize nodules at mung bean root. This research aimed to study the ability of osmoprotectant rhizobacteria isolates and rhizobium to support mung bean yield. Calcareous rhizobacteria isolates were A124-k and Ver5-k strains. It can produce osmoprotectant glycine betaine.

Mung bean is the third most important food plant in Indonesia after soybean and peanut. Mung bean production is low and it is not capable of fulfilling the need and productivities of 1.44 ton h\textsuperscript{-1} which is low potential yield [1].

Mung bean always lives with rhizobium symbiotically by nature. Rhizobium helps mung bean to tie up Nitrogen in the air. There are many other bacteria in it. Some of the bacteria are free living. The
rhizobium is always living with many other rhizobacteria in rhizosphere’s mung bean. The rhizobacteria do not affect rhizobium growth and activity [2].

Shokley et al. [3] explained that inoculation rhizobium and *Arbuscular mycorrhiza* at legume could boost the agroforestry plant prosperity. It is at nonlegume did not influence plant growth. It can escalate legume and nonlegume plant development.

The rhizobacteria can help to maintain water and nutrient. Supporting nutrition for a plant such as, tie up nitrogen, siderophore, decomposed organic, antagonist activity to plant disease and pest, and plant growth which promoting rhizobacteria [4].

2. Material and methods
The research was employing 2 factorial experiments in *Completely Randomized Design*. The first-treatment factors were non inoculated rhizobium (R0) and inoculated rhizobium (R1). The second-factor treatment was inoculation of osmoprotectant rhizobacteria isolates which was consisting of non inoculation of rhizobacteria isolates (I0), inoculation osmoprotectant rhizobacteria isolate Al24-k (I1), and inoculation osmoprotectant rhizobacteria isolate Ver5-k (I2).

This experiment used a sterile insectisol soil media. Mung bean seeds were washed by using sterile water. Mung bean seeds were submerged in a solution which containing osmoprotectant rhizobacteria isolates with $10^6$ of density during 30 minutes. Seeds were plated in sterile media.

The observed variable in this research was **Rhizobacteria density in mung bean rhizosphere**. It was performed by plating method which according to [2]. Rhizobacteria density was observed by six weeks after planting age. Rhizobacteria density was observed by soil dilution method and measured in Colony Form Unit (CFU) [2]. **Glycine betaine production**, the procedure for determining glycine betaine adopted the periodide method which was developed by [4] [5]. **Mung bean yield**, mung bean yield include dry grain weight, 100-grain weight per mung bean plant. **Analysis of variance (ANOVA)**, using statistical analysis system (SAS) was performed on the data (glycine betaine) and the treatment means were compared using Duncan’s test at $p \leq 0.05$.

3. Results and Discussions

**Table 1.** The density of osmoprotectant rhizobacteria isolates in mung bean’s rhizosphere and glycine betaine production.

| Treatment                        | Osmoprotectant rhizobacteria isolates in mung bean’s rhizosphere | Density | Glycine betaine |
|----------------------------------|------------------------------------------------------------------|---------|----------------|
| Inoculation of Rhizobium         |                                                                  |         |                |
| Non inoculated of Rhizobium      | $1.4556 \times 10^7$ p                                           |         | 6.8503 p       |
| Inoculated of Rhizobium          | $1.6237 \times 10^7$ p                                           |         | 7.1402 p       |
| Inoculation of osmoprotectant rhizobacteria : |                                                                  |         |                |
| Non inoculated rhizobacteria     | 0                                                                | c       | 0.0000 c       |
| Inoculated rhizobacteria Al24-k  | $1.7559 \times 10^7$ a                                           |         | 9.7006 b       |
| Inoculated rhizobacteria Ver5-k  | $1.8279 \times 10^7$ a                                           |         | 10.8670 a      |

Information: Numbers followed by the same lower case letters show nonsignificant difference based on Duncan’s test $p \leq 0.05$.

Table 1 shows the glycine betaine density and production of osmoprotectant rhizobacteria isolates in mung bean’s rhizosphere of non inoculated rhizobium were same as inoculated rhizobium. This indicates that rhizobium did not affect the osmoprotectant rhizobacteria isolates activity.

Table 1, displays osmoprotectant rhizobacteria isolates Ver5-k density in mung bean’s rhizosphere has the same density of osmoprotectant rhizobacteria isolates Al24-k. Glycine betaine production of osmoprotectant rhizobacteria isolates Ver5-k was higher than glycine betaine production of
osmoprotectant rhizobacteria isolates Al24-k. This illustrates that osmoprotectant rhizobacteria isolate Ver5-k have more activity than osmoprotectant rhizobacteria isolates Al24-k.

Table 2. Dry weight plant, dry grain weight, 100-grain weight per mung bean plant in six weeks after planting.

| Treatment                      | Plant dry weight (g) | Dry grain weight (g) | 100-grain weight (g) |
|--------------------------------|----------------------|----------------------|----------------------|
| Inoculation of rhizobium:      |                      |                      |                      |
| Non inoculated rhizobium       | 1.6419 b             | 49.8701 b            | 4.70 b               |
| Inoculated rhizobium           | 1.7337 a             | 51.7899 a            | 5.25 a               |

Information: Numbers followed by the same lower case letters show nonsignificant difference based on Duncan’s test \( p \leq 0.05 \).

Table 2, Inoculation of Rhizobium in mung bean shows significant effects on dry plant weight, dry grain weight, 100-grain weight per plant. Inoculation of Rhizobium in mung bean displayed dry plant weight, dry grain weight, 100-grain weight per plant was higher than non inoculated rhizobium [6]. Inoculation rhizobium and Arbuscular mycorrhiza at legume can escalate agroforestry plant growth. Inoculation rhizobium at nonlegume cannot boost plant growth. Rao [2] demonstrated that rhizobium only helps legume to synthesis nodule root and gives hand to tie up nitrogen in the air. Perruod and LeRudulier [8] justified that nodule synthesis and activity of external and indigenous rhizobium illustrated significant effects. Inoculated rhizobium of legume improved disease and pest plant endurance, and also raised plant growth and yield.

Table 3. Plant dry weight, dry grain weight, 100-grain weight per plant mung bean six weeks after planting.

| Treatment                      | Plant dry weight (g) | Dry grain weight (g) | 100-grain weight (g) |
|--------------------------------|----------------------|----------------------|----------------------|
| Inoculation of osmoprotectant rhizobacteria: |                      |                      |                      |
| Non inoculated rhizobacteria   | 1.5661 b             | 43.2029 b            | 4.77 b               |
| Inoculated rhizobacteria Al24-k | 1.7544 a             | 54.9857 a            | 5.04 a               |
| Inoculated rhizobacteri Ver5-k | 1.7428 a             | 54.3014 a            | 5.11 a               |

Information: Numbers followed by the same lower case letters show nonsignificant difference based on Duncan’s test \( p \leq 0.05 \).

Table 3 inoculated osmoprotectant rhizobacteria isolates for mung bean demonstrates significant effects on plant growth and yield mung bean. Inoculated osmoprotectant rhizobacteria isolates of strain Al24-k and Ver5-k on mung bean gave higher plant growth and yield than non inoculated osmoprotectant rhizobacteria isolate. Rhizobacteria supported water for plant growth and yield through many activities synthesis glycine betaine [4]. Nicolaus et al. [6] explained that organic osmolyte was produced by a microorganism to accumulate in the cell. By employing this microorganism adaptation mechanism has wide range adaptation.

Salmonella typhimurium, Escherichia coli, Klebsiella pneumonia, Azospirillum amadaerahnse, A. lipoferum, A. brasilense and A. halopraeferens were bacteria that produce osmolyte under osmotic stress [8][9][10]. Osmolite can motivate water potential. It can cause water to flow into the cell, so cell turgidity can be maintained. According to [11] Rhizobium meliloti, ectothiorhodospira halochloris secretes glycine betaine. Glycine betaine was osmoprotectant.

4. Conclusion
Osmoprotectant rhizobacteria were isolating and screening from the calcareous soil of Gunungkidul with climates disadvantageous such as, long dry season, short rainy season, and high temperature
which are caused by the climate change. Inoculation of isolates osmoprotectant rhizobacteria can support mung bean yield. Osmoprotectant rhizobacteria isolate did not affect rhizobium in mung bean rhizosphere.

References
[1] Widyawati, Harjasa T and Taufik T T 2016 Organik fertilizer application on yield green bean (Vigna radiata L) varieties N ultisol Jurnal Kultivasi 15(3) pp 159-163
[2] Rao N S S 1994 Soil microorganism, and plant growth Indonesia University Press Jakarta.
[3] Shokley F W, R McGrow and H F Gareet 2004 Growth and nutrient concentration of two native forage legumes inoculated with rhizobium and mycorrhiza in Missouri USA Agroforestry System 60 pp 137 – 142
[4] Maryani Y 2010 Khemotaksis rhizobacteria osmotolerant pada rhizosphere mug bean (Vigna radiata L) Biota 15 (3) pp 486 - 493
[5] Barak A J and Tuna D J 1981 Determination of choline, phosphorylcholine and betain Methods of Enzymol 72 pp 287 – 292
[6] Nicolaus, Lanzotti B V, Trincone A, DeRosa M, Grant W D and Gambacarta A 1989 Glycine-betaine and polar lipid composition in Halophytic archaeabacteria in response to growth in different salt concentration FEMS Microbiol Leff 59 pp 157 – 160
[7] Suki K 2011 Rhizobiol measures to evade host defense strategy and indigenous threats to present symbiotic nitrogen fixation: a focus two legume-rhizobium model system Cellular and Molecular Life Sciences 68 pp 1327 – 1339
[8] Perruod B D and LeRudulier 1985 Glycine-betaine Transport in E. coli: Osmotic Modulation J Bacteriol 161 pp 391 – 401
[9] Smith L T, Pocard J A, Bernard T and LeRudulier D 1988 Osmotic control of glycine beta in biosynthesis and degradation in Rhizobium meliloti. J. Bacteriol 170 pp 3142 – 3149
[10] Hartmann A 1988 Ecophysiological aspects of growth and nitrogen fixation in Azospirillum sp Plant and Soil 110 pp 225 – 238
[11] Tschichholz I and Truper H G 1990 Fate of compatible solutes during dilution stress in Ectothiorhodospira halochloris FEMS Microbiol Ecol 73 pp 181 -186