Study on osmoprotectant rhizobacteria to improve mung bean growth under drought stress

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Abstract. Climate change leads to irregular rainwater availability for crops and thus enhances drought stress. Furthermore, nowadays we face climate disadvantages such as long dry season, short rainy season and high air temperature caused by climate change. This research aimed at studying the ability of osmoprotectant rhizobacteria isolates to support mung bean growth under drought stress. The rhizobacteria were isolated from mung bean’s rhizosphere. The results showed that isolates of strain A124-k and Ver5-k produced glycine betaine 9.6306 mg g⁻¹ cell, 1.7667 x 10⁷ CFU g⁻¹ soil and 11.4870 mg g⁻¹ cell, 1.9667 x 10⁷ CFU g⁻¹ soil. The isolated rhizobacteria from mung bean’s rhizosphere under field capacity of soil moisture produced glycine betaine 6.8000 mg g⁻¹ cell, 1.2556 x 10⁷ CFU g⁻¹ soil. Under 75% field capacity of soil moisture, isolates produced glycine betaine of 6.4059 mg g⁻¹ cell, 1.3111 x 10⁷ CFU g⁻¹ soil, while under 50% from field capacity, the isolates produced glycine betaine of 7.4108 mg g⁻¹ cell, 1.6667 x 10⁷ CFU g⁻¹ soil. The osmoprotectant rhizobacteria improved the resilience of mung bean to drought stress.

1. Introduction
Gunungkidul regency is occupied by calcareous soils. The calcareous soil has the limitations which majorly dominated by hilly terrain, deep hardpan layer, calcareous stone and shallow cultivation layer. Furthermore, nowadays we are facing the climate disadvantages such as long dry seasons, short rainy season and high air temperatures due to climate change. Climate change leads to irregular rainfall and thus enhances drought stress on crops because the water availability is insecure. This research aimed to study the ability of osmoprotectant rhizobacteria isolates to support mung bean growth under drought stress. Strains A124-k and Ver5-k are calcareous rhizobacteria isolates, which can produce osmoprotectant as glycine betaine and tolerant to osmotic stress.

The rhizobacteria isolates are capable to produce the osmoprotectant compound as glycine betaine, can help other organisms secreting glycine betaine into the environment. Glycine betaine is an osmolyte synthesized by rhizobacteria to protect it from osmotic stress such as dry soil, which also indicator of E. coli osmotic stress during rhizobacteria colony forming [1].

Mung bean is the third most important commodity in Indonesia after soybean and peanut, but the production is low and not sufficient to meet the needs. Productivity is very low, only of 1.162 ton ha⁻¹ [2]. The efforts to mung bean production can be done by field extension, including to marginal land such as dry land. Mung bean can be cultivated on dry land with technology support called inoculation of osmoprotectant rhizobacteria, the plant is tolerant to drought stress.
2. Material and methods
The research was designed with 2 factor in the Completely Randomized Design. The first factor was the inoculation of osmoprotectant rhizobacteria consisted of three levels, i.e., (1) inoculation of osmoprotectant rhizobacteria isolates consisting of non inoculation of rhizobacteria isolates (I0), (2) inoculation osmoprotectant rhizobacteria isolate Al24-k (I1), and (1) inoculation osmoprotectant rhizobacteria isolate Ver5-k (I2). The second factor was drought stress consisting of three levels, i.e. (1) field capacity (C1), (2) 75% field capacity (C2), and (3) 50% field capacity (C3).

This experiment used to sterile insectisol soil media. Mung bean seeds were washed using sterile water. And then submerged in a solution containing osmoprotectant rhizobacteria isolates with $10^6$ density during 30 minutes. And then planted in sterile media.

Variable observed was Rhizobacteria density in mung bean rhizosphere, using serial dilution plating technique according to [3]. Rhizobacteria density was observed at six weeks after planting, by soil dilution method and in Colon Form Unit (CFU). Glycine betaine content was investigated by adopting the periodide method developed by Barak and Tuma [4] and Nicolaus [5]. Mung bean growth parameter, including plant height, leaf number, leaf width, and biomass were measured using the volumetric method. Analysis of variance (ANOVA) and Duncan’s advanced test (DMRT) test at $p \leq 0.05$ was performed to the analyzed data.

3. Results and Discussions

Table 1. Osmoprotectant rhizobacteria isolate density mung bean’s rhizosphere and glycine betain production.

| Treatment | Osmoprotectant rhizobacteria isolates in mung bean’s rhizosphere | Density | Glycine betain |
|-----------|-------------------------------------------------------------------|---------|---------------|
|           |                                                                   | c       | c             |
| Inoculation of osmoprotectant rhizobacteria : |                                                                   |         |               |
| Non inoculated rhizobacteria | 0 $\times 10^7$ | b | 9.6306 |
| Inoculated rhizobacteria Al24-k | 1.7667 $\times 10^7$ | a | 11.4870 |
| Inoculated rhizobacteria Ver5-k | 1.9667 $\times 10^7$ | a | 11.4870 |

Soil moisture :
Field capacity | 1.2556 $\times 10^7$ | p | 6.8000 |
75 % field capacity | 1.3111 $\times 10^7$ | p | 6.9059 |
50 % field capacity | 1.6667 $\times 10^7$ | p | 7.4108 |

*Mean followed by the same letters is non significantly different at $\alpha = 5\%$.

Table 1, shows the density of osmoprotectant rhizobacteria isolates ver5-k in mung bean rhizosphere was higher than that at osmoprotectant rhizobacteria isolates Al24-k. It also can be seen in table 1 that glycine betain production of osmoprotectant rhizobacteria isolates Ver5-k is higher than that at osmoprotectant rhizobacteria isolates Al24-k. This indicates that osmoprotectant rhizobacteria isolate Ver5-k had more activity and ability than that of osmoprotectant rhizobacteria isolates Al24-k.

It performed in table 1 that, soil moisture treatment showed no significant effects on osmoprotectant rhizobacteria isolates density. but significantly effected the glycine betain production. Soil moisture 75% field capacity and 50% field capacity increased glycine betaine production compared in that of field capacity. It indicates that drought stress can increase the activity of osmoprotectant rhizobacteria isolates to produce glycine betain.

Table 2 shows that inoculation of osmoprotectant rhizobacteria isolates in mung bean rhizosphere showed that strain Al24-k and Ver5-k higher plant height, leaf number and leaf width than non inoculated osmoprotectant rhizobacteria isolates.

Table 2 also displays that moisture influenced plant height, leaf number, and leaf width, where 75% and 50% of field capacity decreased plant height, leaf number, and leaf width.
Table 2. Plant height, leaf number and leaf width per plant six weeks after planting.

| Treatment                          | Plant height | Leaf number | Leaf width (cm²) |
|-----------------------------------|--------------|-------------|------------------|
| Inoculation of osmoprotectant rhizobacteria: |              |             |                  |
| Non inoculated rhizobacteria      | 16.37 b      | 16.7 b      | 188.679 b        |
| Inoculated rhizobacteria Al24-k   | 16.78 ab     | 17.7 a      | 215.479 a        |
| Inoculated rhizobacteria Ver5-k   | 17.00 a      | 17.6 a      | 221.169 a        |
| Soil moisture:                    |              |             |                  |
| Field capacity                    | 17.24 a      | 17.9 a      | 226.376 a        |
| 75 % field capacity               | 16.69 ab     | 17.2 b      | 205.931 b        |
| 50 % field capacity               | 16.23 b      | 16.9 b      | 193.020 c        |

*Mean followed by the same letters is non significantly different at α = 5%.

Table 3. Dry weight per plant six weeks after planting.

| Treatment                          | Dry weight (g) |
|-----------------------------------|---------------|
| Non-in_field capacity (control)   | 1.613 cd      |
| Non-in_75% field capacity         | 1.520 d       |
| Non-in_50% field capacity         | 1.180 e       |
| Al24-k_field capacity             | 1.883 a       |
| Al24-k_75% field capacity         | 1.743 bc      |
| Al24-k_50% field capacity         | 1.630 cd      |
| Ver5-k_field capacity             | 1.786 ab      |
| Ver5-k_75% field capacity         | 1.776 ab      |
| Ver5-k_50% field capacity         | 1.620 cd      |

*Mean followed by the same letters is non significantly different at α = 5%.

Table 3 performs that, the inoculation between osmoprotectant rhizobacteria isolates and moisture soil affected the biomass. The interaction of inoculation osmoprotectant rhizobacteria isolates Al24-k with field capacity soil moisture; Ver5-k at field capacity soil moisture; Al24-k at 75% field capacity soil moisture; Ver5-k at 75% field capacity soil moisture showed higher plant growth than at non inoculation in field capacity (control). This indicates that osmoprotectant rhizobacteria isolate Ver5-k and Al24-k increased glycine betaine production along with the decrease of soil moisture.

The results also show that inoculation of osmoprotectant rhizobacteria isolates Al 24-k and Ver5-k improved mung bean tolerance on drought stress. According to Truper and Galinski [6], the microorganism under osmotic stress have two adaptation mechanism i.e. KCl type and organic osmolyte type, respectively. According to Smith [7], organic osmolyte was produced by a microorganism and accumulated in cell and blocked the cellular process, so resulting in the compatible solute. Microorganism with this adaptation mechanism has wide range adaptation, as isolate A82 was able to help upland to growth well under 40 % field capacity soil moisture [8]. Isolate A82 in osmotic stress condition produced glycine betain. The isolation and screening of soil in Rajasthan desert, India obtained 24 bacteria isolates which resistant to osmotic stress [9], namely Bacillus sp 45.8 %, Corynebacterium sp 41.7 % and Acinetobacter sp, Aeromonas sp, Staphylococcus sp group 2.5 %. Bacillus megaterium BOFC15 rhizobacteria increased plant growth and help the plant to be tolerated for drought stress [10] because Bacillus megaterium produced spermidine (Spd) which is polyamine type. Spermidine can increase plant dry weight and improve the architecture of plant root, as well as increase plant adaptation to drought stress.

4. Conclusion

Inoculation of osmoprotectant rhizobacteria which isolated and screened from the calcareous soil of Gunungkidul supported mung bean growth under field capacity soil moisture and improved the
resilience of mung bean to drought stress. This is important for face the recent climate disadvantages affected by climate change.

Acknowledgement
The authors are indebted to the ministry of research DIKTI Department, Indonesia for the Doctor Grant research funding of Research DIKTI.

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