Seed treatment using rhizobacteria as plant growth promotion of two chili variety (*Capsicum annuum* L.)

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Abstract. The research aims to find out the effect of pre-germination treatment seeds using rhizobacteria as plant growth promotion of two varieties of red chili peppers in the field. The experiment used a randomized design of factorial groups. Factors studied were varieties (V) and rhizobacteria types (R). The variety factor consists of 2 varieties namely PM999 (V₁) and Kiyo F1 (V₂). While the type of rhizobacteria factor tried consists of 8 treatment, namely, control (R₀), Azotobacter sp. (R₁), *B. megaterium* (R₂), *P. atmuta* (R₃), *B. alvei* (R₄), Flavobacterium sp. (R₅), *B. coagulans* (R₆), *B. firmus* (R₇) and *B. pilymixa* (R₈). Each treatment was repeated 3 times, so there were 48 experimental units. Each unit of experiment is represented by 5 sample plants. The data was analyzed using ANOVA and continued with DMRT test at real level α = 0.05. The results showed that vegetative growth and production of chili plants until the age of 45 days after planting in each variety is not dependent on the pre-germination treatment of seeds with rhizobacteria. But the varieties of chili plants used affect vegetative growth and production. PM999 varieties are superior to the Kiyo F1 variety. Pre-germination treatment of seeds using rhizobacteria is relatively effective in improving vegetative growth and yield of chili plants. Among the 8 isolates rizobacteria isolate *Azotobacter* sp., *B. megaterium*, *B. coagulans*, *Flavobacterium* sp., and *P. atmuta* relatively effective to provide an increased effect on the growth and production of chili plants.

1. Introduction

Chili peppers are one of the significant vegetable commodities in Indonesia. Statistical data on the area of chili cultivation in 2017 reached 120,275 ha, ranked first widest compared to other vegetable crops [1]. However, the productivity level still in the third ranks after potatoes and shallots. The average productivity of chili peppers has only reached 7.93 tons ha⁻¹, this figure showed the lowest when compared to its production potential that can reach 20 tons ha⁻¹ [1].

Rhizobacteria symbiotic with plant roots, it also serves as plant growth promoting rhizobacteria (PGPR). The plant growth increased by PGPR able to occur through one or more mechanisms associated with the physiological character of rhizobacteria and the conditions in rhizosphere environment. Indol acetic acid (IAA) is a functioning auxin chemical found in plants and act as pgpr
to increase the growth and yield. The function of IAA for plants among others increases cell development, stimulates the formation of new roots, spurs the growth, stimulates the flower, and increases the enzyme activity [2]. Generally, plants are not able to produce enough IAA for their growth and development. Some strains of rhizobacteria able to synthesize IAA from precursors (base materials) contained in the root exudates as well as from organic matter. Depending on the concentration, this active compound can increase or inhibit the plant growth [3].

Several rhizobacteria species have been investigated as PGPR belonging in genus *Azotobacter* spp., *Azospirillum* spp., *Acetobacter* spp., *Bacillus* spp., *Burkholderia* spp., *Enterobacteriaceae* spp., *Pseudomonas* spp., and *Serratia* spp., [4]–[6]. As PGPR, rhizobacteria competitively colonizes the roots and utilizes exudate and lysate issued by plant roots [7]. In connected with the role of the rhizobacteria as PGPR, the purpose of this research to find out the effect of pre-germination treatment seeds using rhizobacteria as PGPR of two varieties of red chili peppers in the field.

2. Materials and methods
Rhizobacteria isolates were isolated from the healthy rhizosphere of chili plants in Cikanyawar Village, Sirna Galih I and Sirna Galih II, Mega Mendung District, Bogor Regency, West Java Province, Indonesia. Total of 10 g of soil around the roots were taken from several plants, then mixed and stirred until homogeneous. Soil sample composite as much as 10−5, 10−6, and 10−7 dilution levels. 0.1 ml of soil suspension was poured and stirred into King's B medium which was added with cyclohexamide 50 mg L−1, ampicillin 50 mg L−1, and chloramphenicol 12.5 mg L−1 for the isolation of rhizobacteria *P. fluorescens* [8]. To isolate rhizobacteria from the group *Serratia* spp. 0.1 ml of soil suspension was poured into King's B media with 100 mg L−1 tetracycline antibiotics and 500 mg L−1 ampicillin [9]. Isolation of *Bacillus* spp. This is done by heating the soil suspension to a temperature of 80 °C for 30 minutes in a water bath and then pouring it into tryptic soy agar (TSA) media with a concentration of 0.1. The rhizobacteria culture obtained was incubated at 27 °C for 48 hours. Each growing colony was isolated and pure culture made. Rhizobacteria that potential as plant growth promoters were identified using a standardized test procedure by Schaad et al. (2001) method [8] and Holt et al. (1994) [10].

The experiment used a randomized design of factorial groups. Research factors were varieties (V) and rhizobacteria species (R). The variety factor consists of 2 varieties namely PM999 (V1) and Kiyo F1 (V2). While the species of rhizobacteria factor tried consists of 8 levels, namely, control (R0), *Azotobacter* sp.(R1), *B. megaterium* (R2), *P. amenta* (R3), *B. alvei* (R4), *Flavobacterium* sp. (R5), *B. coagulans* (R6), *B. firmus* (R7) and *B. pilimixa* (R8). Each treatment was repeated 3 times so that in total there were 48 experimental units. Each unit of experiment is represented by 5 sample plants. The data was analyzed using ANOVA, and continued with DNMRT test at a real level α = 0.05.

Before seedling the chilli seed are treated with rizobacteria. Rhizobacteria isolates were previously grown in TSA medium (*Azotobacter* sp., and *Bacillus* sp.) or King's B (*Flavobacterium* sp. and *Pseudomonas* sp.), then incubated until 48 hours. Colonies of rhizobacteria are suspended in sterile water until the population density of 10⁶ cfu ml⁻¹ [11] or absorbant values OD₆₀₀=0.164 (*Azotobacter* sp., and *Bacillus* sp.), and OD₆₀₀=0.192 (*Flavobacterium* sp. and *Pseudomonas* sp.) using spectrophotometer. The chili seeds are then soaked in rhizobacteria suspenze for 24 hours, then germinated.

Germination of seeds is carried out in a seeding pot with measure 10 x 10 cm. Seedlings are moved to the field after 4 weeks after planting. The planting media used is a mixture of soil and manure with a ratio of 1:1 (v:v). Before planting in the field, the soil is first processed three times. Along with soil processing, manure is given 15 tons ha⁻¹. The experimental unit is an planting shed with a width of 1 m and a length of 3.5 m. The shed planting used mulch black silver plastic. The planting distance was 50 x 60 cm. Maintenance includes fertilization and control of pests and weeds. Fertilizer used is a complete fertilizer NPK (15:15:15). Fertilizer dissolved in water at a dose of 2 kg 100 L⁻¹ water. Fertilization is done once every 2 weeks by watering at the base of the stem. The
parameters observed include the height and stem diameter at 30 and 45 days after planting (DAP), the number of branches and productive branches observed at 30 and 45 DAP, the number of flowers and the number of fruits observed at 60 and 90 DAP. The data was analyzed using ANOVA, which followed by a different test between the treatment and DMRT (Duncan Multiple Range Test) at \( \alpha = 0.05 \).

3. Results and discussion

3.1. Vegetative plant growth
The results showed that the vegetative growth of red chili plants from each varieties gave the same response to the treatment of seeds using various isolates of rhizobacteria in pre-germination of seeds. All the observed growth parameters indicated the significant differences in value of the two varieties tried due to differences in rhizobacteria isolates used in the pre-germination treatment of seeds. However, the results of Test F tested a single factor, the varieties of chili plants are significant different on the observed vegetation growth parameters based on the height change of plants at 30 and 45 DAP, as well as the stem diameter at 45 DAP. The F test results also showed that a single factor of pre-germination treatment of seeds using rhizobacteria had a noticeable effect on vegetative growth parameters observed based on the high change of plant at 30 and 45 DAP, stem diameter ages 30 and 45 DAP. The average height of plants at 30 and 45 DAP on two varieties and for each pre-germination treatment of seeds with different types of rhizobacteria presented in Table 1 until 4.

Table 1. The plant height average two varieties of chili plants and pre-germination treatment of seeds using rhizobacteria.

| Rhizobacteria treatment | Plant Height (30 DAP) | Plant Height (45 DAP) |
|-------------------------|------------------------|------------------------|
|                         | PM999 (\( V_1 \)) | Kiyo F1 (\( V_2 \)) | Average | PM999 (\( V_1 \)) | Kiyo F1 (\( V_2 \)) | Average |
| Control (R\(_0\))      | 31.79                  | 25.13                  | 28.46 ab | 33.09                  | 25.07                  | 29.08 ab |
| Azotobacter sp. (R\(_1\)) | 29.63                  | 29.17                  | 29.40 ab | 30.40                  | 28.97                  | 29.69 ab |
| B. megaterium (R\(_2\)) | 26.25                  | 26.99                  | 26.62 ab | 26.53                  | 27.53                  | 27.03 b  |
| P. atmuta (R\(_3\))    | 29.87                  | 28.97                  | 29.42 ab | 29.73                  | 29.60                  | 29.65 ab |
| B. alvei (R\(_4\))     | 30.95                  | 26.29                  | 28.62 ab | 32.30                  | 26.60                  | 29.45 b  |
| Flavobacterium sp. (R\(_5\)) | 33.77                  | 26.17                  | 29.97 a  | 34.53                  | 27.68                  | 31.11 a  |
| B. coagulans (R\(_6\)) | 31.71                  | 26.81                  | 29.26 ab | 32.73                  | 26.62                  | 29.68 ab |
| B. firmus (R\(_7\))    | 27.12                  | 24.71                  | 25.91 b  | 28.05                  | 26.40                  | 27.23 b  |
| B. pilimica (R\(_8\))  | 30.41                  | 27.07                  | 28.74 ab | 31.19                  | 27.53                  | 29.36 b  |
| DMRT 0.05               | 30.17 A                 | 26.81 B                 | 30.95 A  | 27.33 B                 |                        |         |

Description: The numbers followed by the same letter in the same column (lowercase) and the same row (capital letter) are no different than those in test level 0.05 (DMNRT).

Table 1 shows that the chili plants height at 30 DAP and 45 DAP significantly varieties of PM999 produces higher growth of seedlings than the Kiyo F1 variety. PM999 variety was superior than Kiyo F1 variety. The difference in rhizobacteria isolates used in the pre-germination treatment of seeds is followed by differences in the value of vegetative growth parameters of different chili plants. Seed treatment using various rhizobacteria isolates from the results of this study has not shown a significant increase in plant vegetation growth when compared to control. However, it can be seen that there is an increase in the height growth of chili plants at 30 and 45 DAP in seed treatment using rhizobacteria isolate Flavobacterium sp., P. muta, B. coagulans, and Azotobacter sp. is higher than control.

The increase of plant height was thought to be closely related to the role of these rhizobacteria as PGPR. The ability of rhizobacteria to the production of IAA and the ability to dissolve phosphate previously. Treatment of seeds with PGPR rhizobacteria plays an important role, especially beneficial in the process of seed germination [12]. Application of PGPR enhances directly phosphates
solubilization, atmospheric nitrogen fixation and secretion of plant hormones (indole acetic acid, gibberellins, cytokinins and ethylene) needed for growth [13]. Admittedly, PGPR have been reported to improve the growth of a variety of crop species, such as tomato [14], red pepper [15], maize [16], mung bean [17-18], and lettuce [19]. Three PGPR strains (Microbacterium oleivorans, Brevibacterium iodinium, and Rhizobium massiliae) exhibited significantly greater plant height, fresh weight, dry weight, and total chlorophyll content than control treatment on chili plant [20].

Table 2. The stem diameter average two varieties of chili plants and pre-germination treatment of seeds using rhizobacteria.

| Rhizobacteria treatment | Stem diameter (30 DAP) | Stem diameter (45 DAP) |
|-------------------------|------------------------|------------------------|
|                         | PM999 (V₁)  | Kiyo F₁ (V₂)  | Average     | PM999 (V₁)  | Kiyo F₁ (V₂)  | Average     |
| Control (R₀)            | 6.39        | 5.92        | 6.16 ab     | 9.86        | 8.49        | 9.18 ab     |
| Azotobacter sp. (R₁)    | 5.78        | 6.66        | 6.22 ab     | 9.33        | 8.87        | 9.10 ab     |
| B. megaterium (R₂)      | 5.12        | 5.65        | 5.39 b      | 8.37        | 7.60        | 7.99 b      |
| P. atmuta (R₃)          | 5.51        | 6.10        | 5.81 a      | 9.30        | 8.46        | 8.88 ab     |
| B. alvei (R₄)           | 6.33        | 6.04        | 6.19 ab     | 8.89        | 8.67        | 8.78 ab     |
| Flavobacterium sp. (R₅) | 7.01        | 6.17        | 6.59 a      | 11.06       | 8.51        | 9.79 a      |
| B. coagulants (R₆)      | 6.10        | 5.58        | 5.84 a      | 9.38        | 7.72        | 8.55 ab     |
| B. firmus (R₇)          | 6.64        | 6.06        | 6.35 ab     | 9.18        | 8.42        | 8.80 ab     |
| B. pilymixa (R₈)        | 6.37        | 6.15        | 6.26 a      | 9.12        | 7.48        | 8.30 ab     |
| DMRT 0.05               | -           | -           | 9.39 A      | 8.25 B      |             |             |

Description: The numbers followed by the same letter in the same column (lowercase) and the same line (capital letter) are no different than those in test level 0.05 (DMRT).

The results of the diameter stems of chili plants at 30 and 45 DAP (Table 2) showed that the pattern is almost the same as the results of measurement of plant height. In stem diameter observation, rhizobacteria isolate Flavobacterium sp., Azotobacter sp. B. firmus and B. pilymixa had a positive impact on the increase the diameter stem. Auxin triggers a number of cellular function ranging from differentiation of vascular tissues, initiation of lateral and adventitious roots, stimulation of cell division, elongation of stems and roots, and orientation of root and shoot growth in response to light and gravity [21,22]. Six rhizobacteria strains, were produce IAA that able to increased shoot, stem and root length of chili pepper [23].

3.2. Generative plant growth

The results showed the reproductive parameters of the red chili plants of each variety tried to provide the same response to the treatment of rhizobacteria isolates used in the pre-germination treatment of seeds. The entire of the the production parameters did not show significant differences in value from the two varieties tried due to differences in rhizobacteria isolates used in the pre-germination treatment of seeds. However, the single factor varieties significantly affect the production of plants based on the components of reproductive parameters that is the number of fruits at 8-90 DAP. The F test results also showed that a single factor of seed pre-germination treatment using rhizobacteria species had not significant effect on all observed plant reproduction parameters based on the change in the number of branches per plant, the number of productive branches, flowers and fruits per plant at 30-90 DAP. Average number of branches per plant, productive branches, flowers and number of fruits on each red chili variety seed treatment with various species of rhizobacteria presented in Tables 3 and 4.

Table 3 shows that the treatment of pre-planted seeds using 8 isolates of rizobacteria has not provided an increase in the value of red chili plant reproduction parameters observed based on the change in the number of branches and the number of productive branches at 30-45 DAP, the number of flowers and the number of fruits per plant at 60-90 DAP. However, relatively the treatment of seeds using rizobacteria provides an increase in the number of flowers per plant more in
number when compared to control, except in the treatment of seeds using isolate rizobacteria species \textit{B. pilymixa}, the value of the number of flowers per plant is lower than the control.

**Table 3.** The average number of branches and number of productive branches two varieties of chili plants and pre-germination treatment of seeds using rhizobacteria.

| Rhizobacteria treatment | Number of Branches (30 DAP) | Number of Productive Branches (45 DAP) |
|-------------------------|-----------------------------|----------------------------------------|
|                         | PM999 (V₁)                  | Kiyo F1 (V₂)                           | Average | PM999 (V₁) | Kiyo F1 (V₂) | Average |
| Control (R₀)            | 58.00                       | 41.80                                  | 49.90   | 54.87       | 57.00        | 55.94   |
| \textit{Azotobacter} sp. (R₁) | 61.47                       | 74.40                                  | 67.94   | 59.00       | 72.33        | 65.67   |
| \textit{B. megaterium} (R₂) | 48.60                       | 53.40                                  | 51.00   | 45.60       | 51.40        | 48.50   |
| \textit{P. atmuta} (R₃)  | 63.27                       | 60.60                                  | 61.94   | 57.87       | 58.40        | 58.14   |
| \textit{B. alvei} (R₄)   | 54.47                       | 80.47                                  | 67.47   | 53.27       | 73.27        | 63.27   |
| \textit{Flavobacterium} sp. (R₅) | 69.47                       | 64.87                                  | 67.17   | 67.27       | 62.20        | 64.74   |
| \textit{B. coagulants} (R₆) | 47.47                       | 37.20                                  | 42.34   | 46.67       | 33.20        | 39.94   |
| \textit{B. firmus} (R₇)   | 65.47                       | 68.53                                  | 67.00   | 64.40       | 66.13        | 65.27   |
| \textit{B. pilymixa} (R₈) | 44.20                       | 52.27                                  | 48.24   | 42.93       | 49.93        | 46.43   |

Description: The numbers followed by the same letter in the same column (lowercase) and the same line (capital letter) are no different than those in test level 0.05 (DMRT).

**Table 4.** The number of flowers and fruits average two varieties of chili plants and pre-germination treatment of seeds using rhizobacteria.

| Rhizobacteria treatment | Number of Flowers | Number of Fruits |
|-------------------------|-------------------|-----------------|
|                         | PM999 (V₁)        | Kiyo F1 (V₂) |
|                         | (70 DAP)          | (60 DAP)       | Average | (90 DAP) | (80 DAP) | Average |
| Control (R₀)            | 56.13             | 42.13          | 49.13   | 52.67    | 38.13    | 45.40   |
| \textit{Azotobacter} sp. (R₁) | 60.33             | 73.53          | 66.93   | 58.87    | 53.67    | 56.27   |
| \textit{B. megaterium} (R₂) | 46.47             | 52.13          | 49.30   | 41.20    | 41.07    | 41.14   |
| \textit{P. atmuta} (R₃)  | 58.60             | 59.73          | 59.17   | 55.87    | 46.73    | 51.30   |
| \textit{B. alvei} (R₄)   | 55.40             | 73.87          | 64.64   | 43.60    | 55.47    | 49.54   |
| \textit{Flavobacterium} sp. (R₅) | 68.93             | 63.13          | 66.03   | 68.73    | 33.60    | 51.17   |
| \textit{B. coagulants} (R₆) | 48.20             | 34.73          | 41.47   | 46.13    | 31.07    | 38.60   |
| \textit{B. firmus} (R₇)   | 66.33             | 67.33          | 66.83   | 51.73    | 50.87    | 51.30   |
| \textit{B. pilymixa} (R₈) | 48.13             | 51.33          | 49.73   | 49.00    | 33.00    | 41.00   |

DMRT 0.05

Description: The numbers followed by the same letter in the same column (lowercase) and the same line (capital letter) are no different than those in test level 0.05 (DMRT).

PGPR can invigorate plant development through an assortment of instruments, including the fixation of atmospheric nitrogen, solubilization of phosphate, and production of phytohormones (i.e., indole-3-acetic acid (IAA), gibberellin, cytokinin, and abscisic acid), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and exopolysaccharide (EPS) [24]. Some PGPR strains of \textit{Agrobacterium}, \textit{Azospirillum}, \textit{Bacillus}, \textit{Pseudomonas}, and \textit{Rhizobium}, able to increase plant height [25]. Rhizobacteria strains (\textit{Microbacterium oleivorans}, \textit{Brevibacterium iodinum}, and \textit{Rhizobium massiliae}) exhibited significantly greater plant height, fresh weight, dry weight, number of productive branches, flowers, fruits and total chlorophyll content than control treatment on chili plant [20]. Two rhizobacterial strains \textit{Streptomyces} sp. and \textit{Bacillus} sp. were advance the chili plant height, wider the plant canopy, number of branches and number of fruit [26].
In the Table 4, chili plants derived from seeds that get pre-germination treatment using rizobacteria produce more fruit per plant than control treatment. Although the statistic is not increase significantly, in the number of fruits per plant, the number of rizobacteria that give a positive effect to the increase in the number of fruits per plant there are only 5 isolates, ie. *B. megaterium, Azotobacter sp.*, *P. dimuta, B. alvei*, and *B. firmus*. Depend on Table 4, two varieties were tried to see the response of chili plants to the treatment of seeds using rizobacteria, it turns out that both varieties give different responses to seed treatment based on the change in the number of fruits per plant. PM999 varieties provide an excellent response to seed treatment using rizobacteria compared to Kiyo F1 varieties. As an evidenced in the measurement of the number of fruit changes per plant, that the variety of PM999 is significantly higher than the Kiyo F1. The PM999 variety produces an average number of fruits per plant (51.98), while the Kiyo F1 variety produces only an average number of fruits per plant (42.62).

The positive effects of rizobacteria in particular that belong to the group of plant growth booster agents (PGPR) can take place through the production of IAA growth regulators, dissolving phosphates and induction of systemic resistance of plants. Colonization of the root system by rizobacteria increases the growth and development of root systems, recientence to abiotic insecurity, absorption and utilization of nutrients more efficiently [12, 27]. Two rhizobacterial strains *Streptomyces* sp. and. *Bacillus* sp. were advance the chili plant height, wider the plant canopy, and promoted the number of fruit [26]. Two strains of rhizobacterial increased plant height, number of leaves, earlier flower’s phase, and fruit weight of chilli [28]. Two rhizobacteria isolates were able to increase shoot length, root length, number of fruit, and weight of chilli fruit [29].

Based on the influence of single factors of varieties tried, from the results of this study it can be concluded that different varieties produce different growth and production. PM999 varieties were superior to the Kiyo F1 variety, in vegetative and generative phase. The differences in nature of excellence are thought to be related to the genetic character of both varieties. Each variety has different genetic characters that can be seen by phenotype characters.

The results of observations in the vegetative growth phase of plants, it was shown that not all of the 8 rizobacteria isolates tested had the potential as plant growth-promoting rhizobacteria, as evidenced by the various benchmarks observed. Although statistically no rizobacteria species have shown maximum results. The same thing has also been proven previously in the germination phase and seedling growth phase, which was observed based on seedling height, number of leaves, and dry weight biomass of seedlings. In addition, stand on the results of the physiological characterization of the rhizobacterial species, has the ability to produce IAA growth regulators and the ability to dissolve phosphate. These two abilities are rhizobacterial characteristics that must be possessed by rhizobacteria that act as PGPR [12].

The study concluded that rhizobacteria used in the pre-germination treatment of seeds showed the role as PGPR, in the vegetative phase and reproductive phase. Theoretically, the level of production of a plant is highly dependent on the conditions of its vegetative growth. The role of rhizobacteria that promote plant growth in the vegetative growth phase in this study turned out to also play a role in the plant reproduction phase. The group of rhizobacteria that are effective in the reproductive phase is related to the ability of these rhizobacteria as PGPR. One of the characteristics of rhizobacteria that act as PGPR is their ability to produce growth regulators (auxins, gibberellins, and cytokinins), nitrogen fixation and the ability to dissolve phosphate and induce systemic resistance to disease. As previously stated, these rhizobacteria have played a role as PGPR since the process of seed germination, seedling growth, vegetative growth, thus it is suspected that the positive impact will also affect the reproductive phase. The ability to fix nitrogen, solubilize phosphate, and produce growth hormones (gibberellins, auxins, and cytokinins) has been widely reported as a mechanism for rhizobacteria in their role as PGPR [30–32].
4. Conclusions
In summary, the PM999 variety has higher effect on vegetative and production than Kiyo F1 variety. Pre-germination treatment using rhizobacteria (Azotobacter sp., B. megaterium, B. coagulans Flavobacterium sp., and P. atmuta) was relatively effective in increasing vegetative growth and production of chili plants.

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