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# The Effect of *Bacillus altitudinis* P-10 Combination Treatments on the Plant Growth and Seed Quality of Corn (*Zea mays* L)

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## Abstract

Increasing production of Corn in recent years must be supported with the supply of good quality of seeds. *Bacillus altitudinis* P -10 is a plant growth promoting rhizobacteria that has biofetilizer and biopesticide activities. The aim of this research was to find out the effect of combination treatments of *B. altitudinis* P -10 on the growth pf the plant and seed quality of Corn (*Zea mays* L). The research design used was randomized group design with monofactor pattern of 4 combination treatments with 6 groups. The corn seed used was Lamuru varieties. The treatments consisted of D1 (manure), D2 (manure and *B. altitudinis* P-10), D3 (manure and chemical fertilizer), and D4 (manure, chemical fertilizer, and *B. altitudinis* P-10). The parameters observed included the height of the plant, the length of the root, the root dry matter, the crown dry matter, the total dry matter, the emergence time of male flower, the emergence time of female flower, the time of physiological ripening, the length of the cob, the diameter of the cob, the number of seeds per cob, the number of seeds per row per cob, the weight of 100 seeds, the production of the seed, the moisture content, the germination percentage, the purity and the impurity of seed. The results showed that the combination treatments of D4 gave the best parameter for the emergence time of flowers, the time of physiological ripening, the length of the cob, the diameter of the cob, the number of seeds per cob, the number of cobs per row, the weight of 100 seeds, and the production of the seed.

***Keywords*****:** *Bacillus altitudinis* P-10, PGPR, corn plant growth, corn seed quality
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## INTRODUCTION

Corn is a strategic commodity that can be developed as the other foodstuff product. Corn becomes the most consumed second foodstuff by Indonesian. According to the data of *Badan Pusat Statiska* in 2011-2015 projected in 2018, the national corn production in Indonesia has reached 30 million tons or increase 12,49% per year with 20,2 million tons of national needs demand (Bulletin Supply and Food Price, 2018). The compliance of national needs must be supported by the increasing of the plant production. One of factors that affects the plant production is the availability of seeds.

The increasing of seeds availability must be accompanied by the increasing of seed quality. Seed quality covers everything related to physical, physiological, and genetic that determine the plant production. The seeds that satisfy the standard quality produce profitable growth and production. The good corn seed can be produced with the good seed production process.

To rise seed production, it can be done by giving the combined nutrient that satisfies the plant's need, such as combination of manure, chemical fertilizer and PGPR (*Plant Growth Promoting Rhizobacteria*) to the plant. The use of manure can improve the soil fertility, the soil permeability, the soil porosity, the soil structure, the water resistance, and the soil cation content, so that these can accelerate the growth of the plant.

The weakness of the manure is it takes long time to be absorbed by the plant. Therefore, to maximize the use of manure, the fast release chemical fertilization can be added. In addition**,** chemical fertilizer contains the essential nutrients needed such as nitrogen, phosphor, and potassium. These nutrients must be available through the life cycle of the plant both in vegetative and generative phases. During the vegetative phase, photosynthate will be allocated for the forming of plant vegetative organs. Meanwhile, during the generative phase, photosynthate will be translocated in the part that will be harvested. Therefore, it is necessary to know the right combination to increase the corn seed production.

The enhancement of the corn production can be supported with the use of Plant Growth Promoting Rhizobacteria (PGPR) that acts as the provider of nutrients for plant such as nitrogen, phosphate, potassium, and sulfur, so that it can increase the growth and the yield of plant. One of genus that has the ability of PGPR is *Bacillus.* Genus *Bacillus* is one of PGPR that is widely used as biofertilizer and biopesticide. Molecular and Applied Microbiology Laboratory, UPT Integrated Laboratory, Dipenogoro University had succeeded isolating the Rhizobacteria *Bacillus altitudinis* P-10 from the root of organic rice plant that is potential as biopesticide and biofertilizer (Budiharjo et all, 2017).

The application of PGPR to plant can be done with various methods. One of them is through the coating of the seed. The coating of *Bacillus* to plant seed needs to be formulated to ease the application in the field and to increase the effectiveness of the bacteria used. Kaolin is one of substance that can be used as the carrier media. Based on the previous statement, it is necessary to conduct research to determine the best combination of manure, chemical fertilizer, and *Bacillus altitudinis* P-10 on the growth and the quality of corn seed.

## MATERIALS AND METHODS

This study was conducted on November 2019 –February 2020 in the agricultural farm in Bulusan, Tembalang, in the Physiology and Crop Breeding Laboratory, and in the Molecular and Applied Microbiology Laboratory UPT Integrated Laboratory, Dopenogoro University, Semarang. This study was arranged in randomized monofactor group design with 6 groups. The treatments consisted of D1 (manure), D2 (manure and *B. altitudinis* P-10), D3 (manure and chemical fertilizer), and D4 (manure, chemical fertilizer, and *B. altitudinis* P-10).

The PGPR used in this study is *B. altitudinis* P-10. The preparation of the PGPR was started with the cultivation of *B. altitudinis* P-10 on Nutrient Agar (NA). The isolate was incubated for 24 hours then the Gram staining was conducted to determine the purity of isolate. The inoculum was made by taking 1-2 ose of isolate and inoculated it into 250 ml Nutrient Broth (NB) medium. The culture was incubated at 37$^{o}$C 170 rpm for 24 hours or until the cell density reach $\geq 10^{7}$ sel/ml (Budiharjo et all, 2017). The number of population calculation was done with total plate count method.

The formulation of PGPR was conducted in accordance with the procedure(Kumar T (1), 2012) that was, packed with 100 grams per package. The packed Kaolin was sterilized with autoclave. The inoculation of *B. altitudinis* P-10 was done by mixing bacteria into sterile kaolin. The mixing of bacteria into kaolin followed the procedure [4] that was 40 ml/ 100 gram kaolin incubated at 37$^{o}$C 170 rpm for 24 hours (Kumar T (2), 2012).

The cultivation of corn began with the land preparation by making 2 x 7.5 m of beds. The cow manure was given at 7 HST as much as 30 kg/bed or 20 ton/ha. The planting was done with spacing of 70 x 25 cm, the depth of the hole was 5 cm, and every hole was filled with 5 seeds. The seeds used for D2 and D4 treatment were combined with formulation of *B. altitudinis* P-10 and kaolin. The maintenance was done by watering, thinning, weeding, and replanting. The application of chemical fertilizer in D3 and D4 treatments used 120 kg N/ha, 80 kg/ha $P_2O_5$/ha dan 80 kg $K_2O$/ha. The giving of Urea was done 3 times at 10, 30, and 45 HST. SP-36 was given to 10 HST. While KCI was given at 10 and 30 HST. The harvesting was done when it was physiologically ripe that was marked with dried and brown cornhusk also had black layer on it. The post-harvest process covered drying the cobs, shelling the seeds, sorting the seeds, and drying the seeds. Then the seed quality testing will be conducted.

The observation parameter consists of the height of plant observed every week by measuring from the base of the stem to the highest leaf. The length of the root was observed at 5 MST by measuring the longest root from the base of root to the tip of the root. The root dry matter was observed at 5 MST by heating the root in the oven at 105$^{o}$C for 24 hours. The crown dry matter was observed by heating the crown in the oven at 105$^{o}$C for 24 hours at 5 MST. The total dry matter was obtained from the sum of root dry matter and crown dry matter. The emergence time of the male and female flower was calculated based on the number of days needed from the planting until the male and female flower appear minimal 50% from the entire plants in every experiment plot. The time of physiological ripening was calculated based on the number of days needed by plant from the beginning until showing the harvest criteria. The length of the cob, without the cornhusk, was measured from the base until the tip of the cob. The diameter of the cob was obtained from the measurement of the base, the middle and the tip of every cob. The number of seeds per cob was calculated based on the number of seeds in every cob. The number of seed per row per cob is calculated from the entire seeds in row per cob. The weight of 100 seeds was observed by

weighting 100 grains of dry seeds. The seed production was observed by weighting the seeds that have been dried and shelled. The moisture content was done by heating 10 g seed in the oven at 105$^{o}$C for 17 hours. The germination percentage was done with rolled paper in plastic test method with germinating 50 seeds with 3 repetition for 7 days. The purity of seed was done by weighting pure seed that had been separated from the seed impurity. The impurity of seed was done by weighting the impurity seed that had been separated from the purity seed.

The data obtained from the research were analyzed statistically by using the Analysis of Variance (ANOVA). The data were analyzed using the model of Yij = μ + Kj + Di + εij. The differences among means were tested using *Duncan Multiple Range Test* (DMRT) with $p<0.05$ accepted as representing a statistically significant difference.

## RESULTS AND DISCUSSIONS

*The Height of the Plant*

The result of variance analysis showed that the treatment of manure, chemical fertilizer, and *B. altitudinis* P-10 gave not significant effect toward the height of the corn plant. The corn plant height observation result due to the giving of manure, chemical fertilizer, and *B. altitudinis* P-10 combination is showed in the Table 1.

Table 1. The Height of Corn Plant

| Treatment | Plant Height Average (cm) Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| D1 | 8,10 | 26,64 | 51,34 | 79,06 | 123,78 | 159,89 | 190,80 |
| D2 | 7,51 | 25,42 | 50,60 | 79,10 | 142,23 | 179,90 | 205,21 |
| D3 | 5,12 | 21,24 | 48,51 | 81,32 | 148,12 | 190,80 | 225,01 |
| D4 | 8,00 | 27,24 | 57,95 | 87,47 | 161,56 | 205,45 | 241,05 |

Explanation : D1 = manure, D2 = manure and *Bacillus altitudinis* P-10, D3 = manure and chemical fertilizer, D4 = manure, chemical fertilizer and *Bacillus altitudinis* P-10.

The nutrient consisted in the manure, chemical fertilizer, and *B. altitudinis* P-10 accelerate the growth of corn plant. The plant can absorb the available nutrient well, so that it resulted in good height of the plant. The absorbed nutrient will be used for the metabolism process so that it will enhance the plant height. The manure can improve the biological and physical soil properties such as improving soil structure and increasing microorganism activities in helping the process of organic matter decomposition (Wijayanti, 2013).

The content of nitrogen and phosphor in chemical fertilizer take an important role in the plant height growth. The nitrogen functioned as the constituent of chlorophyll, protein, and protoplasm needed in the forming of plant vegetative organs such as root, stem, and leaf (Fahmi, 2101). The available phosphor acts in the forming of amino acid so that the forming of new plant cell can occur. Phosphor acts in plant respiration and metabolism process, especially in the forming of new cell that can improve plant (Faizin, 2015).

*B. altitudinis* P-10 has the ability to produce hormone that can improve the growth. *B. altitudinis* P-10 in plant root produce plant growth regulator or phytohormone such as indole acetic acid (IAA), cytokinins, gibberellin, that can support the growth of the plant (Majeed, 2015).

*The Length of the Root*

The result of the variance analysis show that all treatments give unreal effect toward the length of the corn plant root. The result of the observation of root length as the result of giving manure, chemical fertilizer, and *B. altitudinis* P-10 combination is shown in the Table 2.

Table 2. The Length of Corn Plant Root

| Treatment | Average |
|---|---|
| | .....cm..... |
| D1 | 25,36 |
| D2 | 23,07 |
| D3 | 22,47 |
| D4 | 24,57 |

Explanation : D1 = manure, D2 = manure and *Bacillus altitudinis* P-10, D3 = manure and chemical fertilizer, D4 = manure, chemical fertilizer and *Bacillus altitudinis* P-10.

The length of the plant shows the ability of plant to obtain water supply and nutrient in the deeper soil layer. The treatments given to corn plant give unreal effect toward the length of the root. It is caused of the nutrient needs of corn plant have been satisfied by the treatments given. Therefore, the growth of root has stopped. The root will grow longer as long as it still absorb the nutrient, so that when the absorption of nutrient has satisfied, the root will stop growing (Fitri, 2017). The length of corn plant can be seen in Fig 1.

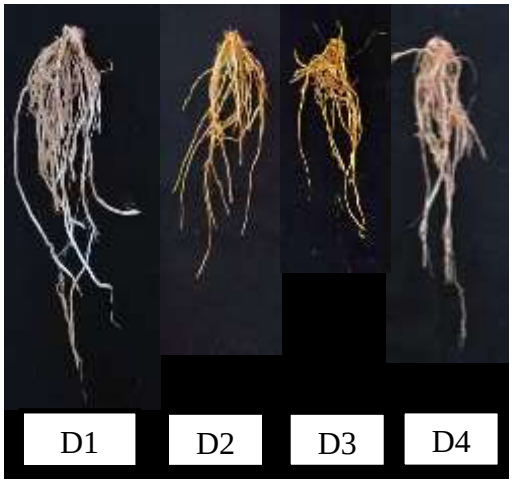



Fig. 1. The length of corn

The root morphology is influenced by the availability of nutrient in the soil. The root morphology will also be determined by the content of nutrient. When the nutrient is provided in the sufficient number, the plant will form a shallow root system. Otherwise, the soil that is consisted of less nutrient will lengthen the root to obtain nutrient. The root will have an increasing of lengthening and the number of root as the form of adaptation to the minimum of available nutrient in the soil (Moelyohadi, 2015).

*The Emergence Time of Male and Female Flower*

The emergence time of male and female flower as the treatment result of giving manure, chemical fertilizer, and coating the seed with *B. altitudinis* P-10 based on the test result of Duncan's multiple range test ($p<0,05$) can be seen in the table 3

Table 3. The Emergence Time of Male Flower

| Treatment | Tassel | Anther |
|---|---|---|
| | .....hari..... | |
| D1 | $57,33^{a}$ | $59,33^{a}$ |
| D2 | $56,83^{a}$ | $59,50^{a}$ |
| D3 | $55,33^{ab}$ | $58,00^{ab}$ |
| D4 | $53,17^{b}$ | $55,67^{b}$ |

The different superscript in the same column show the real difference ($P < 0,05$).

The D1, D2, and D3 treatments tend to show the longer flowering age of male and female flower rather than the D4 treatment. It is caused when the forming of male flower in the corn plant needs nutrient. The giving of complex nutrient in the form of manure, chemical fertilizer and *B. altitudinis* P-10 is able to satisfy the plant needs of nutrient. The metabolism process requires nutrient such as N, P and K in sufficient number in the vegetative and generative growth phase (Seipin, 2016). The P element is an indispensable element in the flowering process. Phosphor acts in the division of cell, flowering process also fruit and seed ripening process. (Suntoro, 2014).

The female flower appears 1-3 days after the emergence of male flower. The seed coating using the formulation of *B. altitudinis* P-10 and kaolin can stimulate the growth of corn plant. The growth that increases will accelerate the emergence of female corn flower. This is because *B. altitudinis* P-10 has the ability to stimulate the growth of the plant due to its ability to produce IAA hormone. The seed treatment using rhizobacteria can stimulate the growth of the plant because it has the ability to produce IAA and to dissolve the previous phosphate (Mardiah, 2016). Cytokinin produced by *B. altitudinis* P-10 is able to stimulate the induction of corn plant flowering. *B. altitudinis* in plant root helps to produce plant growth regulator or phytohormone such as indole acetic acid (IAA), cytokinin, and gibberellin that are able to support the growth of the plant (Majeed, 2015).

*The Length of the Cob, the Diameter of the Cob*

The length and the diameter of corn cob as the result of the giving of manure, chemical fertilizer, and *B. altitudinis* P-10 based on the test result of Duncan's multiple range test ($p<0,05$) are shown in the Table 4.

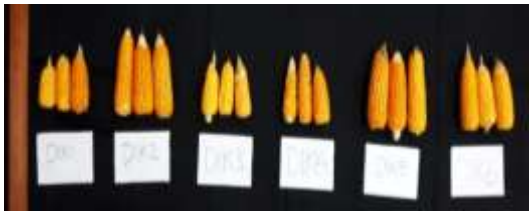
Table 4. The Length and the Diameter of Corn Cob

| Treatment | Cob Length | Cob Diameter |
|---|---|---|
| | .....cm..... | |
| D1 | 14,93[c] | 4,47[b] |
| D2 | 15,58[bc] | 4,50[b] |
| D3 | 18,09[ab] | 4,82[ab] |
| D4 | 19,54[a] | 5,09[a] |

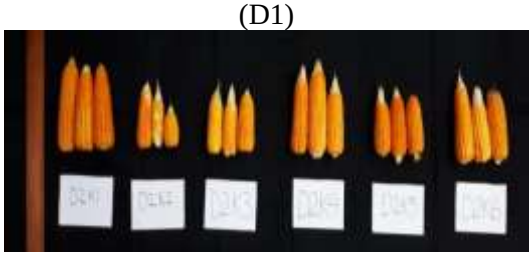
The different superscript in the same column show the real difference (P < 0,05).

From the result of Duncan's multiple range test (p<0,05) showed in the Table 4, it can be seen that the length and the diameter of the corn cob as the result of the treatment given show the different result. The length and the diameter of cob are one of corn plant generative parameters that is the result of photosynthate accumulation. The plant production is determined by the ability of distribution and photosynthate accumulation to the harvested part (Sarawa, 2014).

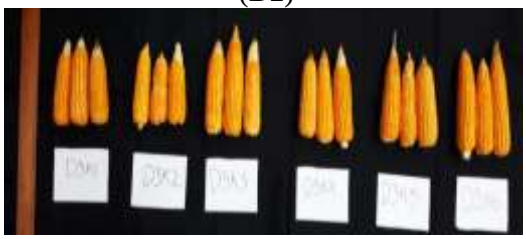
The corn cob filling is influenced by the nutrient received by the plant. The received nutrient will be used to form assimilate. The need of assimilate will continue to increase during the filling period of cob. The sufficient assimilate supply will enhance the growth of cob and the filling of seed so that it can obtain the maximum result (Aprilyanto, 2016).



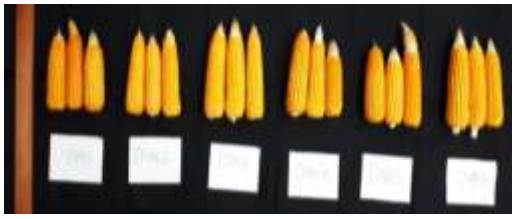
(D1)



(D2)



(D3)



(D4)

Fig 2. The Corn Cobs

The giving of manure, chemical fertilizer, and the coating of seed with *B. altitudinis* P-10 and kaolin formulation (D4) gives the best result (Fig 2.). Phosphor is one of the environment factors that influences the filling of cob. This statement is in accordance to the statement of Wahyudin *et al.* (2017) that stated the availability of P element causes the more photosynthate that is allocated to the cob. Therefore the fruit size becomes bigger. Nitrogen is an important element that is always needed by corn plant during the growing period to the ripening of the seed, so nitrogen must be available continuously. The giving of nitrogen must be done periodically because it is volatile and washable. The character of N fertilizer is volatile and in the rainy season, the abstersion can happen. Therefore, it must be given periodically (Lestari, 2018).

*The Number of Seeds per Cob and the Number of Seeds per Row*

The number of corn seeds per cob and the number of seeds per row as the result of giving the manure, chemical manure, and the coating of seed with *B. altitudinis* P-10 based on the test result of Duncan's multiple range test (p<0,05) are shown in the Table 5.

Table 5. The Number of Seeds per Cob and the Number of Seeds per Row

| Treat-ment | The Number of Seeds per Cob | The Number of Seeds per Row |
|---|---|---|
| D1 | 266,80[c] | 21,92[b] |
| D2 | 248,81[bc] | 21,36[b] |
| D3 | 336,13[ab] | 27,09[ab] |
| D4 | 427,17[a] | 32,38[a] |

The different superscript in the same column show the real difference (P < 0,05).

The giving of manure, chemical fertilizer, and the coating of seed using the *B. altitudinis* P-10 and kaolin formulation gives the best result (D4). The forming of the seeds start with the pollination process. The number of seeds per cob is affected by the fertilization and pollination process. The fertilization process happens if the pollen sticks to the

pistil or corn hair. Then, it is distributed through the pistil duct until it meets the egg cell (Maintang, 2013). The growth of the cob begins since the fertilization happen, so that all photosynthates are allocated to the cob part to form the seeds. The large number of seeds shows the great photosynthate distribution. Plant has the ability to produce and to distribute photosynthate to the sink and convert it to economic product that is an important element for the enhancement of the yield. (Mastur, 2013).

The number of seeds per row relate with the length and diameter of the cob. The longer cob produced, the larger the number of the seeds per row produced. The larger number of seeds per row indicate a perfect fertilization process. The fertilization process happens if the pollen sticks to the pistil or corn hair. Then, it is distributed through the pistil duct until it meets the egg cell (Maintang, 2013). The giving of manure, chemical fertilizer, and the coating of seeds using *B. altitudinis* P-10 is one of methods to improve the photosynthate. The photosynthate produced will be allocated to the corn seed. The deficiency of nutrition in 10-14 HST before the hair emergence can reduce the ovule formed. (Syaifuddin, 2019).

*100 Corn Seeds Weight*

The weight of 100 seeds as the result of giving manure, chemical fertilizer, and the coating of seeds with *B. altitudinis* P-10 based on the test result of Duncan's multiple range test ($p<0,05$) are shown in the Table 6.

Table 6. 100 Corn Seeds Weight

| Treatment | Average |
|---|---|
| | .....g..... |
| D1 | $35,39^{c}$ |
| D2 | $37,31^{bc}$ |
| D3 | $39,34^{ab}$ |
| D4 | $42,14^{a}$ |

The different superscript in the same column show the real difference ($P < 0,05$).

The giving of manure, chemical fertilizer, and the coating of seeds using *B. altitudinis* P-10 and kaolin formulation (D4) give the best result. The weight of 100 seeds are the carbohydrate accumulation from photosynthesis process. The better absorption of nutrient, water, and sun will make a good photosynthesis process. The photosynthate produced will be more and will be distributed to the seeds so it affects the weight of seeds produced. The more photosynthate produced, the seeds produced will also increase (Zainal, 2014).

The giving of nutrient through fertilizing can improve the rate of plant photosynthesis. The better vegetative growth of plant, the photosynthesis process will run well. Nitrogen and phosphor are continuously absorbed until the ripening of seeds. The most of N and P are distributed to the growth point, stem, leaf, and male flower then transferred to the seeds (Nugroho, 2015).

*The Production of Seeds per Plot*

The production of seeds per plot as the result of giving the manure, chemical fertilizer, and the coating of seeds with *Bacillus altitudinis* P-10 based on the test result of Duncan's multiple range test ($p<0,05$) are shown in the Table 7.

Tabel 7. The Production of Seeds per Plot

| Treatment | Average |
|---|---|
| | .....kg/plot..... |
| D1 | $3,35^{c}$ |
| D2 | $3,00^{bc}$ |
| D3 | $4,63^{b}$ |
| D4 | $6,32^{a}$ |

The different superscript in the same column show the real difference ($P < 0,05$).

The treatment of giving manure, chemical fertilizer, and *B. altitudinis* P-10 (D4) results the high corn seed production per plot. The corn seed production is determined by the availability of nutrient during the growth period. The obstructed plant growth causes the obstruction of photosynthate translocation to the seed so it causes the reduction of the seed weight and low production. The decreased photosynthesis activity causes the reduction of photosynthate allocation to the reproduction organ so that the size of the seeds become smaller (Afandi, 2013).

The nutrient in corn plant is available from the first growth until the seed ripening period. The seed coating using the formulation *B. altitudinis* P-10 and kaolin and the giving of inorganic fertilizer affect the growth and the production of corn plant. The absorbed nutrient will be used for photosynthesis process. The photosynthate produced will be translocated to the seed so that it can improve the production. The result of photosynthate is translocated to generative organ in purpose to improve the production of seeds per hectare (Polnaya, 2012)

## CONCLUSION

Combination of manure, chemical fertilizer and *B. altitudinis* P-10 did not affect

the height of the plant and the length of the root. The application of manure, chemical fertilizer, and *B. altitudinis* P-10 resulted in the best emergence time of flower, cob length, cob diameter, the number of seeds per cob, the number of seeds per row, the weight of 100 seeds and the seed production per plot..

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