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Growth and Yield of Corn (*Zea mays*) after treatment with Biofertilizer and an organic P sources cultivated on calcareous soil

Cahyo Prayogo[1], Mohammad Hamdani Rusydi[1], Nurul Istiqomah[2] and Al Gamal Pratomo[2]

[1]Soil Science Department , Faculty of Agriculture, University of Brawijaya,Jl.Veteran No.1 Malang-East Java-**Indonesia**

[2]East Java-Assessment Institute for Agricultural Technology (AIAT), Jl. Raya Karangploso KM 4, Malang-East Java-**Indonesia**.

***Correspondence:** c.prayogo@ub.ac.id, cprayogo73@gmail.com **Accepted: 09**April. 2019**Published online:** 06 May. 2019

Corn (*Zea mays*) is major in the world, but had a serious problem on their growth and yield when cultivated under calcareous soil. The availability of P elements on calcareous soil is relatively low since most of P on calcareous soil is bound by Magnesium (Mg) and calcium (Ca). With problems in calcareous soils, it is expected to find solution using a treatment under the combination of the utilization of an organic P fertilizer along with the application of biofertilizer. The experimental design used in this study was Randomized Block Design (RBD) with 2 factors in which each factor has 3 different levels of fertilizer application resulted in 9 different treatments. Each treatment was repeated 3 times. The treatments of biofertilizer involved 3 levels (40 g kg^{-1} of seed, 60 g kg^{-1} of seed, and 80 g kg^{-1} of seed), whilst an organic P fertilizer adopted 3 levels(100%, 125%, 150% of the application rate). The data obtained was analyzed using ANOVA and CVA to determine the significance of the treatment. The results showed that interaction between biological fertilizer and phosphorus fertilizer had significant effect on plant height of 45 HST and 60 HST, diameter of cob, soil pH, soil Organic-C, Total soil P, available soil P and P uptake by plant. Provisioning of biofertilizers and an organic phosphorus fertilizers has significant effect on available soil P and total P soil. The best treatment is generally indicated by the treatment of combination of 80 gr kg^{-1} of biological fertilizer treatment and 125% of an organic phosphorus fertilizer (H3P2). CVA multivariate analyses distingushed and clustered across different treatments based on available variables.

Keywords: Fertilizer, Phosporous, Multivariate analysis, Limestone, Nutrient uptake

INTRODUCTION

Corn (Zea mays) is one of the plants that are widely planted and utilized in the world (Asadu and Igboka, 2014). Corn is a relatively short-lived plant that is able to utilize fertilizer inputs more efficiently and has the potential to be able to produce large quantities of food grains per unit area. Increased attention to the development of corn plants was also followed by an increase in national production and harvest year after year. According to the Central Bureau of Statistics, Indonesia's corn production in 2015 was 19.61 million tons. (BPS, 2016).Meanwhile, according to the Directorate General of Food Crops, the total need to meet the needs of corn in 2016 is estimated at 21.4 million tons (BPS, 2016). This shows that Indonesia must increase corn production so that the need for corn consumption can be fulfilled. Expansion of the planting area is one of the efforts to increase corn production. One of the land that can be used to increase corn production is dry and calcareous soils.

Calcareous soil is frequently found in East Java, one of which is in the northern part of Gresik which consists of Bungah sub-district, Ujung Pangkah, Panceng and surrounding areas because the area is a limestone mountain area. Gresik Regency is in the mountains of the northern limestone folds that have undergone rapture. Limestone mountains come from sedimentation in the sea produced by coral animals so that they are called organic sediments (Ningsih and Hariyanto, 2015). Calcareous soil is generally characterized by low P and high soil pH, he difficulty of the P (phosphorus) available to plants is one of the problems in this condition. (Jagadeeswaran, et al., 2007). Availability of P elements in calcareous soils is relatively low because most P in calcareous soils are bound by Magnesium (Mg) and calcium (Ca) (Liu et al., 2015). In calcareous soils, phosphate ions are very reactive, and can move through absorption and / or precipitation with cations such as Ca^{2+} and Mg^{2+} With problems in such calcareous soils, it is expected to be overcome by input P fertilizer and also the use of biofertilizers. Giving SP-36 with a dose of 40 kg P ha^{-1} increased the dry weight of dry corn shell 1.5 times compared to without P fertilizer (Kasno, 2009).

Biofertilizers keep the soil environment rich in all types of macro and micro nutrients through nitrogen fixation, phosphate and potassium mineralization, release of plant growth regulators, antibiotic production and biodegradation of organic matter in the soil (Bhardwaj et al., 2014). When biological fertilizers are applied as seed treatments or soil inoculants, they multiply and participate in the nutrient cycle (Singh et al., 2011). Biofertilizers have emerged as an important component of integrated plant nutrition management programs that have the potential to increase crop yields through better nutrient supply. Biofertilizer application which contains various kinds of bacteria such as N binding bacteria, P solvent bacteria, and K solvent bacteria can increase corn plant growth (Wu et al., 2005). However those application on calcareous soils are very limited and the information of those effect are rarely found. This study intends to examine the utilization of biofertilizers and P applied to corn on calcareous soils are needed. P sources is important nutrient to improve crop yield (Anggraini et al., 2017;Rivaliati et al., 2017).

MATERIALS AND METHODS

Studi area and Experimental setup

The research was conducted in March 2017 until June 2017, located at TTP (Agricultural Technology Park) which is managed by AIAT East Java and located in Sukodono Village, Panceng District, Gresik Regency. The location is positioned at 6º55'48.02" S and 112º28'19.90" E, with the elevation at 50 m above sea level. For initial and final soil analysis were carried out in the Soil Chemical Laboratory, Department of Soil Science, Faculty of Agriculture, University of Brawijaya Malang.

Materials

The materials used were corn varieties BISI-222, organic fertilizer in the form of cow manure obtained from cattle farmers around the study site, AGRIMETH biofertilizers formulated with microbial active ingredients in the form of Azospirillum sp. (non symbiotic N fixers and fitohormone producers), *Bradhyzobium japonicum* (non symbiotic N fixers), *Azotobacter vinelandii* (non symbiotic N fixers and P solvents), *Methylobacterium sp.* (phytohormone producer), *Bacillus cereus* (P solvent and anti-pathogenic compound producer) and subsequently Urea fertilizer, Phonska fertilizer, SP-36 fertilizer and supporting chemicals for soil analysis.

Experimental Design

The experimental design used in this study was a Randomized Block Design (RBD) with 2 treatment factors, each of which had 3 levels to obtain 9 treatments. Each treatment was repeated 3 times. The treatments used were biological fertilizers with 3 treatment levels (40 g kg^{-1} of corn seed, 60 g kg^{-1} of corn seed, and 80 g kg^{-1} of corn seed), P fertilizer with 3 treatment levels (100%, 125%, 150%) , and manure as much as 20 tons ha^{-1} for each treatment as a basic fertilizer. Thus, there are 27 experimental plots in total. Details of treatment combination were presented in (Table 1) below.

Step of research and variables

Land clearing were performed to remove weeds and wild plants followed by setting up the plot trial. A trial plot of 27 experimental plots within the size of 4 m x 4 m was made then land were ploughed and the seed were germinated under field condition. The planting size was 80 cm x 20 cm. The seed number for each planting hole was in double (Figure 1)

Tabel 1. Detail information of treatment

code	Treatment
H1P1	Biofertiliser: 40 g $kg^{-1}$of corn seed + an organic P fertilizer : 100%
H1P2	Biofertilizer : 40 g $kg^{-1}$of corn seed + an organic P fertilizer 125%
H1P2	Biofertlizer : 40 g $kg^{-1}$of corn seed + an organic P fertilizer 150%
H2P1	Biofertilizer : 60 g $kg^{-1}$of corn seed + an organic P ferlilizer100%
H2P2	Biofertilizer: 60 g $kg^{-1}$of corn seed + an organic P fertilizer 125%
H2P3	Biofertilizer : 60 g $kg^{-1}$of corn seed + an organic P fertilizer 150%
H3P1	Biofertilizer : 80 g $kg^{-1}$of corn seed + an organic P fertilizer 100%
H3P2	Biofertilizer : 80 g $kg^{-1}$of corn seed + an organic P fertilizer 125%
H3P3	Biofertilizer : 80 g $kg^{-1}$of corn seed + an organicP fertilizer 150%

Description: The dose of 100% P fertilizer is equivalent to 52.5 Kg P_2O_5 contained in 350 kg of Phonska fertilizer 15:15:15

Figure 1. Germinating and cultivating corn seed in the calcareous soil

Soil was irrigated using manual system. Soil sampling of the sub-plot was taken by the surface using a shovel from 5 different sampling points to perform a composite sample. The soil sample were then put into a plastic bag and labeled. For chemical analysis, air dried soil is then grinded, and sieved using 2 mm and 0.5 mm of sieving equipments. Observations of variable were made to record plant growth (i.e plant height using handheld ruler 1.5 m), crop production (i.e. length and diameter of corn cob using handheld ruler 50 cm, total 1000 grain using a scale, yields using extrapolation from plots yield to ha (ton ha^{-1}), and soil fertility status including P available (P-Olsen) and total soil P (HCl-25%).

Data analysis

The data obtained during the research are tabulated using the Microsoft Excel program. Data analysis using analysis of variance (ANOVA) and CVA multivariate analysis were employed using Genstat software 18th editions. If there are significant differences in the experimental data, further testing will be carried out using the DMRT Advanced Test with a confident level of 5%.

RESULTSAND DISCUSSION

Initial Soil Analysis

The results of the initial soil analysis are shown in table 2 below.The pH value from initial soil analysis were obtained at 7.6 and it classified as nearly alkaline. In term of total organic C was about 0.09% and categorized into very low status.

The available P parameters at the level of 3.03 mg kg^{-1} which are in the low status and the total P was about 164.96 mg kg^{-1}which are in very high ccategory. Availability of P elements in calcareous soils is relatively low because most P in calcareous soils are bound by Magnesium (Mg) and calcium (Ca) (Liu et al., 2015).

Soil Fertility status

Soil pH

Generally, most of pH status were range between and it was lower than those initial value (7.6). There was a significant impact of treatment to soil pH (P<0.05). The treatment of H3P3 (biofertilizer :80 g kg^{-1} + an organic P fertilizer 150%) shown the highet soil pH which was not significantly different to H2P2 (biofertilizer : 60 g kg^{-1} + an organic P fertilizer 125%) (Table 4). This indicates that tere was a decreasing in the value of soil acidity after being given biological fertilizer treatment. Elfiati (2005) suggested that in P soluble microbial activity would produce organic acids such as oxalate, succinate, citrate, lactate, acetate, formate and others. The increase an organic acids is also followed by a decrease in pH. The decrease in pH is also due to the limited sulfuric acid, nitrate in the chemoautotrophic oxidation of sulfur and ammonium by bacteria.

Organic Soil C

Based on analysis of variance showed that treatments a significant effect (P <0.05) to soil organic C content. The lowest value of organic C content in the H1P1 treatment (biofertilizer 40 g kg-1 of corn seed + an organic P fertilizer 100%) with organic C content of 0.97% which was not significantly different to H1P2, H1P3, H2P1, similarly there was not significantly different in term of organic soil C at H3P3 (biofertilizer :80 g kg^{-1} of corn seed + an organic P fertlizer 150%) as the highest compare to H2P2. The raising soil organic C was due to the rapid mineralization or organic manure by microorganism contained inside biofertilizer. This was also affected the increasing availability of soil P. The soil P available at H3 treatment were 3 to 4 times higher compare to H1 treatment. In addition, similar trend were found on total soil P (mg kg^{-1}) (Table 2).

The highest treatment (H3P3) increased by 55.67% in term of total soil P from the lowest treatment (H1P1). The amount of total P of the soil is higher as the additional dose of treatment (biofertilizer or an organic P fertlizer addition) was increased. Siregar et al., (2015) also confirmed that regarding the application of SP-36 fertilizer (P sources fertlizer) and manure as well as the interaction of both factors have a significant effect on increasing available soil P.

Crop performance

The results of variance analysis showed that the combination of biofertilizer and phosphorus fertilizer had a significant effect (P <0.05) on the height of corn plants at 45 and 60 HST but not at 75 HST. Data on the effect of treatment on plant height are presented in Table 3.

There was a significant effect of the treatment to plant heigh (P<0.05). It can be seen that the plant height at 45 (day after planting) (DAP), treatment of H3P3 (biofertlizer : 80 g kg^{-1} + an organic P fertilizer : 150%) was the highest (118.5 cm) which was not significant to H1P1, H2P1 and H2P2. The lowest plant height in H1P2 treatment (Biofertilizer : 40 g kg^{-1} of corn seed + an organic P fertilizer 125%) which was at 100.7 cm, whereas the plant height was reduce to about 20% compare to H3P3. In contrary, the highest plant height at 60 DAP observed at H2P2 treatment (biofertilizer : 60 g kg^{-1} of corn seed + an organic P fertilizer : 125%) which was at about 130.8 cm, and this not significantly different to H1P1, H1P3, H2P1, H3P1 and H3P3, while the lowest plant height was detected at H3P2 (115.5 cm).

Other studies also showed that the application of biofertilizers and various doses of NPK fertilizer also had a significant effect on plant height and other corn plant growth variables on calcareous soils in the Egyptian region (Azab, 2016). In this study there was no significantly different on the plant height at 75 DAP. The different soil management can improved soil C along with soil microbe (Istiqomah et al, 2017;

Corn cob length and diameter

The treatment had a significantly effect to corn cob length and diameter (Table 4). Increasing on biofertizer and P application followed by the greater corn cob length and diameter. The changes in corn cob length were almost 50% when we compared the lowest value (H1P1) and the highest (H3P2) there was an increasing in length at about 50%. H3P2(biofertilizer :80 g kg^{-1} of corn seed + an organic P fertlizer 125%) treatment did not significantly different interm of corn cob and diameter to H3P3 (biofertilizer :80 g kg^{-1} of corn seed + an organic P fertlizer 150%). Similar trend were found at corn cob diameter.

Hadiyanto et al., (2016) confirmed that SP36 fertilizer had a significant effect on the corn cob length of each variety planted. Hasanudin and Bambang (2004) assumed that organic acids produced by microbes of phosphate solvents are able to increase the solubility of P not available to P available in the soil, so that the absorption of P by plants will also increase.

Table 2. Soil fertility status across different treatment

No	Perlakuan	pH	Total organic soil C	Available soil P (mg kg^{-1})	Total soil P (mg kg^{-1})
1	H1P1	7.23 a	0.09 a	5.00 a	186.9 a
2	H1P2	7.20 a	0.15 ab	5.52 a	221.5 ab
3	H1P3	7.38 b	0.10 a	5.98 a	206.6 ab
4	H2P1	7.18 a	0.16 ab	6.02 a	193.2 ab
5	H2P2	7.42 bc	0.20 b	6.48 a	198.6 ab
6	H2P3	7.38 b	0.19 b	7.02 a	206.6 ab
7	H3P1	7.39 b	0.20 b	19.79 c	227.9 b
8	H3P2	7.35 b	0.30 c	13.40 b	225.3 b
9	H3P3	7.49 c	0.39 d	14.43 b	287.2 c

Remarks: The same letters accompanying the average number in the same column show that the treatment is not significantly different from Duncan's 5% test. H1: Biofertilizer 40 g kg^{-1} of corn seed, H2: Biofertilizer 60 g kg^{-1} of corn seed , H3: Biofertilizer 80 g kg^{-1} of corn seed. P1: an organik P fertilizer 100% , P2: an organic P fertilizer 125%, P3: an organic P fertilizer 150%. * = significant at the P <0.05, DAP = Day After Planting, NS = Not significant.

Table 3. Plant height of corn across different treatment

No	Treatment	Plant height (cm)		
		45 DAP	60 DAP	75 DAP
1	H1P1	115.5 c	126.3 bc	133.6
2	H1P2	100.7 a	119.4 ab	127.7
3	H1P3	102.6 ab	123.2 abc	134.6
4	H2P1	115.6 c	127.1 bc	136.1
5	H2P2	115.5 c	130.8 c	138.4
6	H2P3	109.7 abc	119.3 ab	126.5
7	H3P1	112.8 bc	124.2 abc	131.9
8	H3P2	102.9 ab	115.5 a	122.7
9	H3P3	118.5 c	125.8 bc	139.8

Remarks: The same letters accompanying the average number in the same column show that the treatment is not significantly different from Duncan's 5% test. H1: Biofertilizer 40 g kg^{-1} of corn seed, H2: Biofertilizer 60 g kg^{-1} of corn seed , H3: Biofertilizer 80 g kg^{-1} of corn seed. P1: an organik P fertilizer 100% , P2: an organic P fertilizer 125%, P3: an organic P fertilizer 150%. * = significant at the P <0.05, DAP = Day After Planting, NS = Not significant.

Table 4. Corn cob length and diameter across different treatment

No	Treatment	Corn cob lenght (cm)	Corn cob diameter (cm)
1	H1P1	10.60 a	4.05 ab
2	H1P2	11.77 ab	4.20 bcd
3	H1P3	12.68 bc	4.24 cd
4	H2P1	12.84 bc	4.23 cd
5	H2P2	12.51 bc	4.24 cd
6	H2P3	12.35 abc	4.01 a
7	H3P1	12.33 abc	4.17 abc
8	H3P2	14.83 d	4.38 d
9	H3P3	14.15 cd	4.30 cd

Remarks: The same letters accompanying the average number in the same column show that the treatment is not significantly different from Duncan's 5% test. H1: Biofertilizer 40 g kg^{-1} of corn seed, H2: Biofertilizer 60 g kg^{-1} of corn seed , H3: Biofertilizer 80 g kg^{-1} of corn seed. P1: an organik P fertilizer

100% , P2: an organic P fertilizer 125%, P3: an organic P fertilizer 150%. * = significant at the P <0.05, DAP = Day After Planting, NS = Not significant.

Table 5. Weight of 1000 grain (g) and Yield (ton ha^{-1}) across different treatment

Treatment	Weight of 1000 grain (g)	Yield (ton ha^{-1})
H1P1	263.1 a	4.72 a
H1P2	274.7 a	6.22 a
H1P3	273.3 a	5.95ab
H2P1	272.0 a	4.63 a
H2P2	282.2 ab	4.81 a
H2P3	278.0 a	4.80 a
H3P1	277.7 a	6.47 ab
H3P2	298.6 bc	7.26 b
H3P3	302.0 c	6.54 ab

Remarks: The same letters accompanying the average number in the same column show that the treatment is not significantly different from Duncan's 5% test. H1: Biofertilizer 40 g kg^{-1} of corn seed, H2: Biofertilizer 60 g kg^{-1} of corn seed , H3: Biofertilizer 80 g kg^{-1} of corn seed. P1: an organic P fertilizer 100% , P2: an organic P fertilizer 125%, P3: an organic P fertilizer 150%. * = significant at the P <0.05, DAP = Day After Planting, NS = Not significant**.**

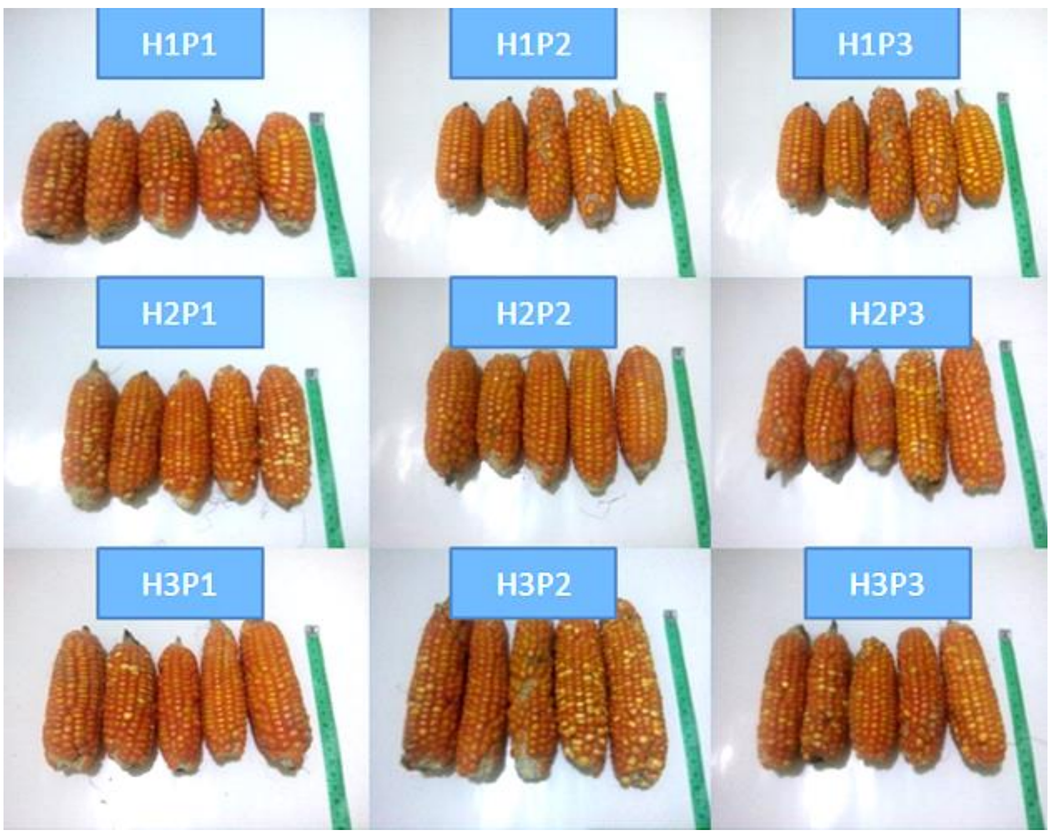

Figure 3. The performance of corn cultivated on calcareous soil under different biofertilizer an an organic P fertilizer.

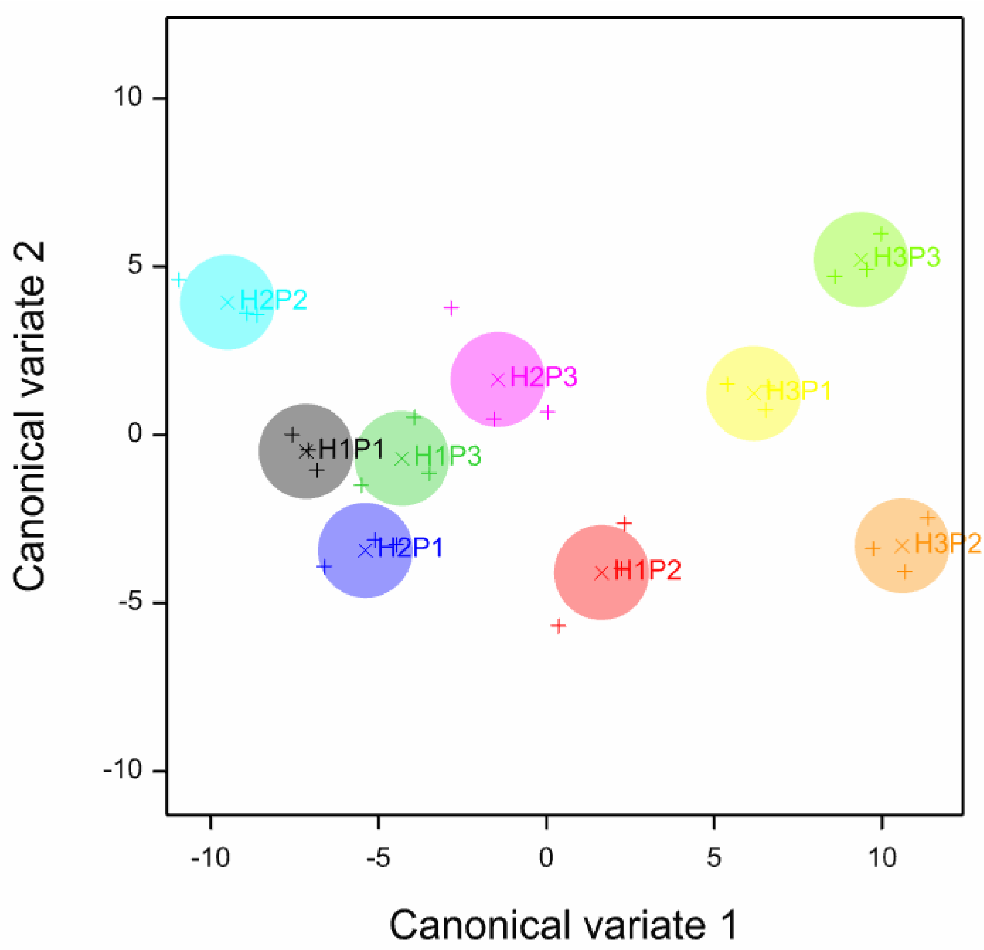

Figure 4. Multivariate analysis to cluster performance of corn cultivated on calcareous soil under different biofertilizer and an organic P fertilizer.

The availability and absorption of the P element causes more photosynthate to be allocated to the cob so that the size becomes larger.

Yield

Corn productivity is calculated in the form of weight of 1000 grains and yields based on total dry shelled grain per unit area. The result was presented in Table 5. It can be seen from those table that there was a significant different (P<0.05) in term of weight of 1000 grains and yields per unit area (ha). According to Table 5, it can be seen that H3P3 is the treatment with the highest weight value of 1000 grains at about 302,0 g followed by H3P2 which is not significantly different ono to another. Along with the addition of doses on each treatment followed by increasing the yield of 1000 grains. However, Wahyudin et al., (2017) could not show evidence that the weight of 100 dry seeds in experiments with phosphate fertilizer and the application biofertilizers could significantly affected the available treatment.

The table above shows that the yield across treatment were ranged between 4.72 to 7.26 tons ha^{-1} (dry shelled grain). Along with the addition of a given dose of biological fertilizer, it shows an increase in those productivity. Productivity in H3P2 treatment were increased by 40 % compare H1P1.

Multivariate analysis

Canonical variate analyses were employed to distinghuise the relationships between groups of variables (P-uptake, yield, weight of 1000 grain, plant height, corn con length, corn cob diameter, pH, C-org, available soil P, total soil P) in a two dimensional data set. The data set is split into two groups X (CV1) and Y (CV2), based on the multiple parameter above. The purpose of canonical analysis is then to find the relationship between X (CV1) and Y (CV2). CV1 represented 72 % whlist CV2 is 14,50%. Figure 3 showed that most of the plots separately along X(CV1) than following Y (CV2) axis. H1P1 is situated in the left side, and H3P2 is on the other side, whlist H3P3 is positioned in the top right side in the opposite of H2P2 treatment (Figure 4).

CONCLUSION

1. Provisioning of biofertilizers and an organic phosphorus fertilizers has significant effect on available soil P and total P soil.
2. The best treatment is generally indicated by the treatment of combination of 80 gr kg^{-1} of biological fertilizer treatment and 125% of an organic phosphorus fertilizer (H3P2).
3. CVA multivariate analyses could distingushed and clustered across different treatments based on available variables selected.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

CP designed and leading the experiments design and also wrote the manuscript. MHR, NI, and AGP performed setting up field treatments experiment, data collection, field support and facilitating the research equipment, financial along with data analysis. All authors read and approved the final version.

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