BIOSCIENCE JOURNAL

OPTIMIZATION OF SOYBEAN YIELD IN ULTISOLS THROUGH ADAPTIVE VARIETIES SCREENING AND PLANT GROWTH PROMOTING RHIZOBACTER

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How to cite: AFRIDA, E., SYAHRIL, M. and TAMPUBOLON, K. Optimization of soybean yield in ultisols through adaptive varieties screening and plant growth promoting rhizobacter. *Bioscience Journal*. 2022, **38**, e38013. https://doi.org/10.14393/BJ-v38n0a2022-57214

Abstract

This research was aimed at obtaining varieties of soybean adaptive to acid soils and to obtain Plant Growth-Promoting Rhizobacteria (PGPR) isolates that can improve the agronomic characteristics of soybean and increase the ultisols fertility. This research was conducted in two-stages research on Sampali Village, Percut Sei Tuan sub-District, Deli Serdang District, North Sumatra Province, Indonesia from August 2019 until March 2020. The first stage (adaptive varieties screening) using the non-factorial Randomized Block Design (RBD) with the varieties of Argomulyo, Wilis, Kaba, Dena-1, Devon-1, Dega-1, Demas-1, Burangrang, Detam-1, and Kipas Merah. The second stage (application of PGPR isolates singly and in combinations) using the factorial RBD, the first factor of applicative single and the combination of PGPR isolates, the second factor of adaptive varieties including Detam-1 and Wilis. Data were analyzed with ANOVA and followed by DMRT at $P<0.05$. The results showed that the Detam-1 and Wilis varieties had significantly higher yield per plant of 14.73 g and 14.54 g, respectively, than other varieties. The applications of a single and combination of PGPR isolates significantly increased the number of branches, stem diameter, plant height, yield per plant, soil pH, organic-C, available-P, and total-N and decreased the soil C/N. The Detam-1 variety had the higher in yield per plant compared to Wilis variety. The isolates combination of *Rhizobium leguminosarum+Rhizobium sp2+Bacillus sp+Burkholderia sp* for Detam-1 and Wilis varieties can be recommended to support the growth and yield of soybean on ultisols.

Keywords: Acid Soils. Nitrogen-Fixing Bacteria. Phosphate Solubilizing Bacteria. Screening. Soybean Varieties.

1. Introduction

The ultisols cover an area of 41,919 million ha that is 40.77% of land in Indonesia as compared to other acid soils (entisols-3.70%, inceptisols-39.76%, oxisols-13.75%, and spodosols-2.02%). However, the area of the ultisols in North Sumatra on the 2nd place with 36.68% of land coverage after the Inceptisols that covers 58.10% of the land surface (Center for Land and Agro-Climate Research 2000; Mulyani et al. 2004). The land area used for dry field and shifting cultivation in North Sumatra Province on 2017 was 691,622 ha and 345,481 ha, respectively (Ministry of Agriculture 2019). The extent of the land is used for plant cultivation, one of these food plants is soybean. Based on the data from Statistics of Sumatera Utara (2019) reported that the soybean area was 5,563 ha with the productivity by 1.73 ton ha^{-1} in 2019 which was lower than the national average. The low productivity of soybeans could be caused by several factors, which include planting soybean on acid soil such as the ultisols.

Several issues with the ultisols have been reported to be impacting the growth and yield of soybean [*Glycine max* (L.) Merr]. Subagyo et al. (2004) reported that the primary issues with the use of ultisols for agricultural were aluminum (Al) and iron (Fe) toxicity and nutrient deficiency, especially phosphorus (P). Al-toxicity causes the plant to have a poor ability to absorb nutrients and water. The elements of Al and Fe are most soluble in acid soils and effortlessly bind to P. This reduces the efficiency of P-absorption and prevents the plants from benefiting by application of P fertilizer. Prasetyo and Suriadikarta (2006) stated that the high acidity of ultisols land was another issue. It is very acidic soil with a pH less than 4.50, low organic matter content, exchangeable base, availability-P, cation exchange capacity, but high Al saturation. Mossor-Pietraszewsk (2001) reported the interaction of Al^{3+} (the primary form of Al-toxic) with oxygen donor compounds such as proteins, nucleic acids, and polysaccharides resulted in the inhibition elongation and lengthening of the plant cell. Ezeh et al. (2007) and Duressa et al. (2011) stated that high soil acidity can inhibit the root growth, decreased the availability of nutrients, and produces plant of poor character. Uguru et al. (2012) stated that soil pH had a strong influence on the root growth, agronomic character, and yield of soybean.

Therefore, efforts such as the use of soybean varieties adapted to acidic soil, application of PGPR isolates such as nitrogen-fixing bacteria, and phosphate solubilizing in situ are needed to increase the growth and yield of soybean and increase the fertility of acid soil. According to Adie and Krisnawati (2016), soybean genotypes G115H/Kaba//Kaba///Kaba-8-6 among 15 soybean genotypes yielded an average of 2.23 ton ha^{-1} and was categorized as adaptive to acidic soil in three locations (pH 5.87; 5.04; and 4.73). Soybean genotypes that are adaptive to acid soils are characterized by the ability to maintain the plant height, larger number of nodes per plant, and pods per plant. Gangasuresh (2010) stated that the combination of *Rhizobium* and phosphate solubilizing bacteria is more synergistic compared to a single inoculation on the growth of soybean. Ahemad and Kibret (2014) and Kafrawi (2015) also reported that PGPR was able to induce systemic resistance and indole-3-acetic acid (IAA) hormone production to support the plant growth.

PGPR genera have been reported to increase the growth and yield of soybean through the production of amino acids, gibberellins, IAA, and other polyamines, increase root growth and increase absorption of water and nutrients (Schmidt et al. 2015; Yadav et al. 2017), such as the genera *Bacillus* (Mishra et al. 2009; Tonelli et al. 2017), *Burkholderia* can produce the ACC-deaminase, IAA, siderophore, solubilize of heavy metal and phosphate (Jiang et al. 2008), *Rhizobium sp* can produce the hormones IAA, HCN, ammonia, siderophores (Wani et al. 2007) and likewise, *Bacillus, Azotobacter, Beijerinckia, Enterobacter, Burkholderia, Erwinia, Microbacterium, Flavobacterium, Rhizobium, Pseudomonas,* and *Serratia* are reported significant phosphate solubilize (Bhattacharyya and Jha 2012). Zahir et al. (2010) reported that *Rhizobium phaseoli* significantly increased plant height, nodulation, biomass, grain yield, and nitrogen content in seeds of *Vigna radiata*. Wani and Khan (2010) also reported that *Bacillus* species PSB10 significantly increased growth, nodulation, chlorophyll, seed yield, and grain protein, reduced chromium uptake in the roots, shoots, and seeds of chickpeas (*Cicer arietinum*).

Screening of soybean varieties to find the ones that can produce a good yield under soil acidity stress and efforts to develop PGPR isolates that can support growth and yield of soybean and also increase the ultisols fertility are needed. This research was aimed at identifying varieties of soybean that can adapt to acid soils, and PGPR isolates that can improve agronomic characteristics of soybean and ultisols fertility.

2. Material and Methods

Research area

The research was conducted in Sampali Village, Percut Sei Tuan Sub-district, Deli Serdang District, North Sumatra Province, Indonesia. Ultisols is the predominant soil type in the region. Soil analysis was conducted in the Laboratory of Sentral, Faculty of Agriculture, Universitas Sumatera Utara, Medan. It was two-stages research. The first stage was the screening of soybean varieties to identify the adaptive varieties from August until November 2019. The second stage was application isolate of single and the combination of PGPR in varieties of adaptive soybean from January until March 2020.

The first stage (screening for adaptive varieties soybean): plot determination and soil analysis

This research was conducted by forming a plot with a size of 4.2 m × 2 m separated by 0.5 m and replicates were 0.75 m. Soybean plants were spaced 40 cm × 25 cm. A soil sample was taken from each plot at the depth of 25 cm then composited and analyzed for several chemical characteristics (Table 1).

Table 1. Analysis of the chemical characteristics of ultisols.

No	Soil chemical characteristics	Method	Value	Category*
1	Actual pH	H_2O	4.59	Acid
2	Potential pH	KCl	4.31	-
3	Organic-C (%)	Walkley and Black	1.80	Low
4	C/N	-	8.40	Low
5	Total-N (%)	Kjeldahl	0.20	Low
6	Available-P (ppm)	Bray-I	8.23	Very Low
7	CEC (me/100g)	AAS	9.24	Low
8	Exchangeable-K (me/100g)	AAS	0.36	Low
9	Exchangeable-Na (me/100g)	AAS	0.38	Low
10	Exchangeable-Ca (me/100g)	AAS	0.64	Very Low
11	Exchangeable-Mg (me/100g)	AAS	0.45	Low
12	Exchangeable-Al (me/100g)	AAS	2.48	Very Low

Note: *Indonesia Soil Research Institute (2009) with category in pH H_2O (acid= 4.5-5.5;); organic-C (low= 1-2%); C/N (low= 5-10); total-N (low= 0.1-0.2%); available-P (very low <15 ppm); CEC (low= 5-16 me/100 g); exchangeable-K (low= 0.1-0.3 me/100 g); exchangeable-Na (low= 0.1-0.3 me/100 g); exchangeable-Ca (very low <2 me/100 g); exchangeable-Mg (low= 0.4-1 me/100 g); exchangeable-Al (very low < 1 me/100 g).

The first stage (screening for adaptive varieties soybean): design methods and data analysis

This research was arranged using the non-factorial randomized block design with soybean varieties include Argomulyo, Wilis, Kaba, Dena-1, Devon-1, Dega-1, Demas-1, Burangrang, Detam 1, and Kipas Merah and was with three replications. The root length, plant height, stem diameter, number of branches, and yield per plant were measured at 108 days after planting and yield ha^{-1} was converted using the formula:

$$\text{Yield ha}^{-1} = \frac{\text{Land area ha}^{-1}}{\text{Plant spacing}} \times \text{yield per plant} \quad (1)$$

$$\Delta\ \text{Yield ha}^{-1} = \frac{\text{Yield ha}^{-1}\ \text{of varieties release} - \text{Yield ha}^{-1}\ \text{of treatment}}{\text{Yield ha}^{-1}\ \text{of varieties release}} \times 100\% \quad (2)$$

The second stage (application isolate PGPR on adaptive varieties): selection of adaptive varieties

The adaptive varieties of soybean were selected by the highest yield per plant in the first stage. Wilis and Detam-1 varieties showed significantly higher yield than others in the first stage and were selected for the second stage of the experiment.

The second stage (application isolate PGPR on adaptive varieties): isolation of plant growth promoting rhizobacter (PGPR)

Isolation PGPR was conducted by taking rhizosphere from all soybean varieties. PGPR of N-fixing bacteria were isolated and grown in the solid Yeast Peptone Agar (YPA) medium and incubated for 48 hours. Bacteria colonies were grown, then suspended in a liquid medium of Yeast Peptone Broth (YPB) until the suspension achieved the population density with an absorbance value of 106-108 OD using a spectrophotometer. PGPR of phosphate solubilizing bacteria were isolated using the Pikovskaya medium with the composition of 10 g glucose, 5 g Ca_3PO_4, 0.5 g $(NH_4)2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.01 g $MnSO_4.H_2O$, 0.5 g yeast extract, and 0.01 g $FeCl_3.6H_2O$ to make one liter of aqueous solution at pH 7.0 using the pour plate method (Pikovskaya 1948).

The second stage (application isolate PGPR on adaptive varieties): morphological identification of PGPR colonies

The isolate selection was based on the morphological identification of the colonies. Pure isolates were obtain with the streak-plate technique using the Pikovskaya medium. Index phosphate solubilizing pure isolates were identified using the Pikovskaya medium with the marked formation of a clear zone around the colony. The test medium was inserted in the petri dish and allowed to solidify. Furthermore, each isolate was grown by the spot inoculation technique and incubated for seven days. Colonies are grown and able to establish clear zones showed to phosphate solubilizing isolate (qualitative). Isolation of bacteria was found the characteristics in N-fixing and phosphate solubilizing, then matched with a guide book namely *Bergey's Manual of Determinative Bacteriology 9th Edition* (Holt et al. 2000). Based on the morphological identification of colonies, five types of PGPR isolates were obtained three N-fixing bacteria (*Rhizobium leguminosarum*, *Rhizobium sp1*, *Rhizobium sp2*) and two phosphates solubilizing bacteria (*Bacillus sp, Burkholderia sp*).

The second stage (application isolate PGPR on adaptive varieties): PGPR application and design method

This research was conducted by forming a plot with a size of 4.2 m × 2 m separated by 0.5 m and replicates were 0.75 m. The plant spacing was used of 40 cm × 25 cm. This research was arranged using randomized block design factorial. The first factor was the applicative combination of N-fixing and phosphate solubilizing bacteria (Table 2) and the second factor was the adaptive varieties of soybean including Detam-1 and Wilis.

Table 2. Applicative combination of N-fixing and phosphate solubilizing bacteria in selected varieties (Detam-1 and Wilis).

Isolates	Bacteria Combination
0	Untreated
1	*Rhizobium leguminosarum*
2	*Rhizobium sp1*
3	*Rhizobium sp2*
4	*Bacillus sp*
5	*Burkholderia sp*
6	*R. leguminosarum+Bacillus sp*
7	*R. leguminosarum+Burkholderia sp*
8	*Rhizobium sp1+Bacillus sp*
9	*Rhizobium sp1+Burkholderia sp*
10	*Rhizobium sp2+Bacillus sp*
11	*Rhizobium sp2+Burkholderia sp*
12	*R. leguminosarum+Rhizobium sp1*
13	*R. leguminosarum+Rhizobium sp2*
14	*R. leguminosarum+Rhizobium sp1+Rhizobium sp 2*
15	*R. leguminosarum+Rhizobium sp1+Bacillus sp*
16	*R. leguminosarum+Rhizobium sp1+Burkholderia sp*
17	*R. leguminosarum+Rhizobium sp2+Bacillus sp*
18	*R. leguminosarum+Rhizobium sp2+Burkholderia sp*
19	*R. leguminosarum+Rhizobium sp1+Bacillus sp+Burkholderia sp*
20	*R. leguminosarum+Rhizobium sp2+Basillus sp+Burkholderia sp*

The second stage (application isolate PGPR on adaptive varieties): parameters and data analysis

The agronomic characters including plant height, stem diameter, the number of branches, yield per plant and the soil fertility parameters including soil pH measured the H_2O method, C/N, organic-C measured the Walkley and Black method, available-P using the method by Bray-I and total-N using the Kjeldahl method were recorded at 108 days after planting. Determine the effect of isolates on yield per plant and ultisols fertility (pH, organic-C, available-P, and total-N) are calculated the rate of increase or decrease (Δ) using the equations 3 to 8:

$$\Delta \text{ Yield per plant } = \frac{\text{Yield plant}^{-1} \text{ of treated} - \text{Yield plant}^{-1} \text{ of untreated}}{\text{Yield plant}^{-1} \text{ of untreated}} \times 100\% \quad (3)$$

$$\Delta \text{ Soil pH} = \frac{\text{Soil pH of treated} - \text{Soil pH of untreated}}{\text{Soil pH of untreated}} \times 100\% \quad (4)$$

$$\Delta \text{ C/N} = \frac{\text{C/N of treated} - \text{C/N of untreated}}{\text{C/N of untreated}} \times 100\% \quad (5)$$

$$\Delta \text{ Organic-C} = \frac{\text{Organic}-\text{C of treated} - \text{Organic}-\text{C of untreated}}{\text{Organic}-\text{C of untreated}} \times 100\% \quad (6)$$

$$\Delta \text{ Available-P} = \frac{\text{Available}-\text{P of treated} - \text{Available}-\text{P of untreated}}{\text{Available}-\text{P of untreated}} \times 100\% \quad (7)$$

$$\Delta \text{ Total-N} = \frac{\text{Total}-\text{N of treated} - \text{Total}-\text{N of untreated}}{\text{Total}-\text{N of untreated}} \times 100\% \quad (8)$$

The data were analyzed by ANOVA and followed by DMRT at the 5% level using SAS 9.4 software. The relationship between the plant growth and ultisols fertility to yield per plant of soybean were analyzed using the Pearson correlation with IBM SPSS Statistics v.20 software. Correlation coefficient values were categorized based on Evans (1996) with very low (0.00 to 0.19), low (0.20 to 0.39), moderate (0.40 to 0.59), strong (0.60 to 0.79), and very strong (0.80 to 1.00).

3. Results and Discussion

Screening of adaptive varieties soybean

The ANOVA showed that the varieties significantly affected the root length, plant height, stem diameter, the number of branches, and yield per plant of soybean on ultisols (Table 3). The results showed that the Detam-1 variety had higher the root length, plant height, stem diameter, and the number of branches compared to other varieties. Detam-1 and Wilis varieties of soybean have higher yield per plant and were significantly different from 14.73 g and 14.54 g respectively compared to other soybean varieties. All varieties in this screening test had lower yield ha^{-1} as compared to varieties released by the Ministry of Agriculture. The high yield per plant and yield ha^{-1} of Detam-1 and Wilis varieties indicated that both varieties were tolerant to ultisols with acidic pH, total-N, organic-C, CEC, and cations exchange (Ca, Mg, K, Na) were classified as very low and low, which cause marked by the occurrence of elongation and lengthening of the roots. Root lengthening will affect the nutrient uptake needed by soybean plants and lead to an increase in plant height, stem diameter, the number of branches, and yield per plant. According to Ermolayev (2001) the acid-tolerant soybean had the root system that survived by forming a plasma membrane that prevented the penetration of aluminum (Al) in the zone elongation and lengthening of the root. Tolerant soybean can exudate or restrict the entry of aluminum into cells of apex radicis. The ability to restrict aluminum penetration in the cell could be the result of organic acids exudation, increased pH in the rhizosphere region, lignification, and aluminum transport in the vacuole. Wang et al. (2008) reported that the acid-tolerant soybean in the dryland was able to translate carbon from the shoots to the roots of the plant. Uguru et al. (2012) reported that the root length, dry weight of root, the number of leaves per plant and seeds weight per plant were decreased by 27.98%; 83.33%; 31.10% and 68.00% respectively for seven varieties of soybean grown in soils pH ranged from 6.0 to 4.50. Adie and Krisnawati (2016) reported that yield ha^{-1}, the number of branches per plant and the number of filled pods per plant decreased by 11.52%; 10.93% and 40.15% respectively for 15 varieties of soybean at potential soil pH ranged from 4.70 to 4.20.

Table 3. Effect of varieties on plant height, stem diameter, the number of branches, yield per plant of adaptive variety soybean in the ultisols.

Varieties	Root Length (cm)	Plant Height (cm)	Stem Diameter (mm)	Number of Branches	Yield per Plant (g)	Yield ha^{-1} (ton ha^{-1})	Yield ha^{-1} of variety release*	Δ of variety release
Argomulyo	32.47±0.27^{bc}	56.13±0.23^{bc}	5.62±0.07^{bc}	5.17±0.07^{b}	13.12±0.14^{b}	1.312	2.03	-35.37
Wilis	29.50±0.38^{bcd}	53.17±0.34^{bcd}	5.32±0.11^{bcd}	4.80±0.10^{bc}	14.54±0.10^{a}	1.321	1.60	-17.44
Kaba	23.57±0.44^{e}	48.57±0.44^{de}	4.87±0.14^{de}	4.47±0.15^{cd}	12.31±0.15^{c}	1.231	2.13	-42.21
Dena-1	24.17±0.29^{de}	49.17±0.29^{de}	4.92±0.09^{de}	4.43±0.08^{cd}	11.13±0.15^{ef}	1.113	1.70	-34.53
Devon-1	23.60±0.09^{e}	45.93±0.28^{de}	4.59±0.09^{e}	4.10±0.08^{d}	10.98±0.08^{f}	1.098	2.75	-60.07
Dega-1	26.87±0.37^{de}	50.87±0.44^{cde}	5.09±0.14^{cde}	4.57±0.13^{cd}	11.97±0.14^{cd}	1.197	2.78	-56.95
Demas-1	24.33±0.32^{de}	49.33±0.32^{de}	4.93±0.10^{de}	4.47±0.10^{cd}	11.42±0.15^{ef}	1.142	1.70	-32.82
Burangrang	27.47±0.42^{cde}	52.80±0.39^{bcd}	5.28±0.12^{bcd}	4.77±0.12^{bc}	11.87±0.21^{cde}	1.187	1.60	-25.81
Detam-1	38.93±0.25^{a}	63.93±0.25^{a}	6.39±0.08^{a}	5.97±0.04^{a}	14.73±0.13^{a}	1.473	2.51	-41.31
Kipas Merah	33.13±0.35^{b}	58.13±0.35^{b}	5.81±0.11^{b}	5.20±0.12^{b}	13.12±0.15^{b}	1.312	2.50	-47.52

Note: Mean values followed by the same letter within the column are not significantly different by DMRT at level of 5% ± standard error. *Ministry of Agriculture (2016).

Effect of PGPR isolates on the agronomic characters for adaptive varieties

The ANOVA showed that the PGPR isolates had significantly effect on the agronomic characters of soybean including the number of branches, plant height, stem diameter, and yield per plant. Adaptive varieties showed the significantly effect on the yield per plant of soybean. However, the effect was not significant on the number of branches, plant height, and stem diameter. Interaction between PGPR isolates and varieties was not significantly influenced the agronomic characters of soybean including the number of branches, plant height, stem diameter, and yield per plant in ultisols (Tables 4 and 5).

Application of single isolate and combination PGPR had a positive effect in increasing the number of branches and yield per plant of soybean, which ranged by 5.26 to 97.81% and 17.34 to 106.72% compared to untreated plants. An increase in the stem diameter of soybean caused by the application of single and combination PGPR ranged by 2.50 to 33.85% compared to untreated plants except for the isolates combination of *R. leguminosarum+Rhizobium sp1*. An increase in the plant height of soybean caused by the application of single and combination PGPR ranged by 3.75 to 27.87% compared to untreated except for single isolate of *R. leguminosarum* and the isolates combination of *R. leguminosarum+Rhizobium sp1*. It was showed that the N-fixing bacteria such as *R. leguminosarum+Rhizobium sp1* undeveloped under acid soil conditions and inhibited the infection process. It was observed that the soil pH in the untreated and the isolate single application of *R. leguminosarum* and the isolates combination of *R. leguminosarum+Rhizobium sp1* experienced the change in soil pH from 4.84 (acid) to 5.49 and 5.60 (Table 6). Soil pH conditions in the two isolates were classified as acid to slightly acid. According to Giordano and Hirsch (2004) the process of N-fixing bacteria infection occurs when rhizobia penetrates root hairs and forms threads of infection and then penetrates cortical cells and forms bacteroids to form nodules. Vacheron et al. (2013) also added the PGPR can modulate root development and growth through the production of phytohormones, secondary metabolites, and enzymes characterized by reduction in the primary root growth rate, and an increase in the number and length of lateral roots and root hairs. PGPR also influences plant nutrition through nitrogen fixation, phosphorus solubilization, or siderophore production, and modifies root physiology by changing gene transcription and metabolite biosynthesis in plant cells. However, it has been reported that several *Rhizobium* strains are unable to survive in acid soil. Wolff et al. (1993) several strains of *Rhizobium* still survive in soil pH of 5, but in soil pH of 4.4 most strains of *Rhizobium* undeveloped in the soil and their infection process was also inhibited. The optimal pH for *Rhizobium* development and infection ranged between under neutral to slightly alkaline. Several *Rhizobium* were classified as susceptible to the low pH and uninfected the root hairs in acid soil. Weisany et al. (2013) stated that soil acidity causes Ca-deficiency, Al- and Mn-toxicity that inhibited nodulation process and nitrogen-fixing. Naibaho et al. (2019) reported that the Al-stress at 1.5 g and 3 g decreased the fresh weight of tomato by 57.64 to 69.49%, dry weight by 47.33 to

62.21%, root epidermis size by 12.28 to 82.44%, cortex by 4.52 to 60.40%, and stele by 17.40 to 75.75% compared to the control.

Application of the *R. leguminosarum+Rhizobium sp2+Basillus sp+Burkholderia sp* isolates combination on the adaptive varieties of soybean caused the highest increase in the number of branches, plant height, stem diameter, and yield per plant by 7.52 branches; 61.42 cm; 6.52 mm; and 22.37 g respectively compared to other treatments. It was caused by a single isolate of phosphate solubilizing bacterial (*Bacillus sp*) could be increased the higher in soil pH compared to single isolate of nitrogen-fixing (*R. leguminosarum*) and single isolate *Burkholderia sp* could be increased the higher in soil pH compared to the three isolates of nitrogen-fixing (*R. leguminosarum, Rhizobium sp1,* and *Rhizobium sp2*) (Table 6). It was showed that phosphate solubilizing bacteria secreted several organic acids that can chelate Al and Fe ions causing the acidity of the soil and raise the soil pH. These changes in the soil pH could stimulate the growth and activity of *Rhizobium.* PGPR bacteria have also been reported as capable of producing hormones to stimulate the growth and yield of soybean in acid soil conditions. According to Rao (1994) the phosphate solubilizing bacteria secreted several of organic acids such as formic, acetic, propionic, lactonic, glycolic, and succinic, which can form chelates with Al and Fe cations, thereby affecting soil pH to support the growth and activity of *Rhizobium.* Rodríguez and Fraga (1999), Ahmed and Shahab (2011) and Walpola and Yoon (2012) reported that the organic acids such as carboxylic, glycolic, malonic, succinic, fumaric, and alpha-ketoglutaric could accelerate the maturity and increase the straw ratio and total yield of phosphate solubilizing bacteria. Vikram and Hamzehzarghani (2008), Mittal et al. (2008), Yousefi et al. (2011) and Santana et al. (2016) reported that phosphate solubilizing microorganisms to support the plant growth through the phytohormone formation such as auxin, gibberellins, cytokinins, and polyamides. Thakuria et al. (2004) reported that the endophytic bacteria isolated from plant roots could produce the IAA (*indole-3-acetic acid*). Shahab et al. (2009) reported that PGPR bacteria from the genus *Burkholderia* could solubilize phosphate and produce the IAA hormone.

Adaptive varieties were significantly affected on the yield per plant of soybean in ultisols. Detam-1 and Wilis varieties showed increased the yield per plant in isolated compared to un-isolated. However, Detam-1 variety had higher the yield per plant in both isolated and un-isolated of PGPR compared to Wilis variety. It was evident in the number of branches, plant height, soil pH, available-P, and total-N were significantly and positively correlated with the coefficient of 0.710 (strong); 0.502 (moderate); 0,476 (moderate); 0.547 (moderate); and 0.772 (strong) respectively with the yield per plant of Detam-1 variety, meanwhile the Wilis variety be found the number of branches, soil pH, available-P, and total-N had correlated positively and significantly with the coefficient of 0.772 (strong); 0.546 (moderate); 0.518 (moderate); and 0.736 (strong) respectively in the yield per plant (Table 7). According to Adie et al. (2009) Detam-1 variety had higher the yield ha^{-1} at 2.51 ton ha^{-1} and weight of 100 seeds at 14.84 g compared to Wilis variety of 2.36 ton ha^{-1} and 10.86 g respectively. Krisnawati and Adie (2016) stated that plant height was significantly and positively correlated with the coefficient by 0.315 in the yield ha^{-1} of soybean.

Effect of PGPR isolates on the ultisols fertility

The ANOVA showed that PGPR isolates had significantly effect, but the variety and their interactions insignificantly to ultisols fertility including soil pH, C/N, organic-C, available-P, and total-N (Table 6). Applications of a single and the combination of PGPR isolates significantly increased the soil pH in a range of 13.43% to 30.99% compared to untreated and the highest was found in the combination of *R. leguminosarum+Rhizobium sp2+Bacillus sp.* Applications of a single and the combination of PGPR isolates significantly decreased the soil C/N in the range of 5.90% to 20.54% compared to untreated and the highest was found in the combination of *R. leguminosarum+Rhizobium sp1+Bacillus sp+Burkholderia sp*. Applications of a single and the combination of PGPR isolates significantly increased the organic-C ranged by 41.87% to 89.02% compared to untreated and the highest was found in the combination of *R. leguminosarum+Bacillus sp.* Applications of a single and the combination of PGPR isolates significantly increased the available-P ranged by 41.83% to 98.77% compared to untreated and the highest was found in the combination of *R. leguminosarum+Rhizobium sp1+Bacillus sp+Burkholderia sp*. Applications of a single and the combination of PGPR isolates significantly increased the total-N ranged by 23.81% to 319.05% compared to untreated and

the highest was found in the combination of *Rhizobium sp2+Burkholderia sp* and *R. leguminosarum+Rhizobium sp2+Basillus sp+Burkholderia sp*.

It was caused by the phosphate solubilizing bacteria ability of *Bacillus sp+Burkholderia sp* combined with various nitrogen-fixing isolates could be secreted several organic acids which chelate the ions that cause acidity to the soil. This was evidenced by the changes in soil pH which increase from 4.84 to 6.24 and 6 10, which caused a decrease in the C/N ratio and an increased the organic-C, which made the nutrients available for the plants to absorb. The highest increases in available-P and total-N were 98.77% and 319.05%. According to Rao (1994) the phosphate solubilizing bacteria could secrete several organic acids which chelate with Al and Fe cations, and affect the soil pH to encourage growth and activity of *Rhizobium.* Horner (2008) stated that the availability of N nutrients helped plants to absorb P nutrients more effectively and lead to increased the plant growth. Afzal and Bano (2008) reported that combination inoculation of *Rhizobium* and phosphate solubilizing bacteria without the use of P fertilizer could increased the seed yield up to 20% of wheat compared to the application of a single P fertilizer. Rodríguez and Fraga (1999) and Satyaprakash et al. (2017) added that the inoculation of phosphate solubilizing bacteria such as *Pseudomonas, Bacillus, Rhizobium, Micrococcus, Flavobacterium, Achromobacter, Erwinia,* and *Agrobacterium* have been reported to increase P solubility and ensures higher yield. Bachtiar et al. (2019) also reported that the isolate combination of *Rhizobium* R1 and phosphate solubilizing microbial in FPF4 increased the dry weight of soybean by 41.67% and N-uptake by 196.47% compared to the control. A combination of *Rhizobium* and phosphate solubilizing microbes could decrease the need for the use of chemical fertilizers by 50%.

Table 4. Effect of PGPR isolates and adaptive varieties on the number of branches and plant height of soybean in the ultisols.

PGPR isolates	Number of Branches				Plant Height (cm)			
	Varieties		Average ± SE	% of untreated	Varieties		Average ± SE	% of untreated
	Detam-1	Wilis			Detam-1	Wilis		
0	3.70^{ns}	3.90^{ns}	3.80±0.034^{m}	-	48.33^{ns}	47.73^{ns}	48.03±0.058^{c}	-
1	3.97^{ns}	4.03^{ns}	4.00±0.019^{lm}	5.26	48.27^{ns}	47.13^{ns}	47.70±0.080^{c}	-0.69
2	6.60^{ns}	6.50^{ns}	6.55±0.024^{c}	72.37	48.77^{ns}	50.90^{ns}	49.83±0.109^{c}	3.75
3	5.80^{ns}	5.50^{ns}	5.65±0.041^{gh}	48.68	49.63^{ns}	51.80^{ns}	50.72±0.110^{c}	5.59
4	5.10^{ns}	5.30^{ns}	5.20±0.034^{ij}	36.84	52.20^{ns}	54.93^{ns}	53.57±0.124^{abc}	11.53
5	3.80^{ns}	4.10^{ns}	3.95±0.041^{lm}	3.95	54.27^{ns}	57.57^{ns}	55.92±0.136^{abc}	16.42
6	4.13^{ns}	4.30^{ns}	4.22±0.031^{k}	10.96	55.87^{ns}	54.37^{ns}	55.12±0.092^{abc}	14.75
7	6.30^{ns}	6.20^{ns}	6.25±0.024^{def}	64.47	54.50^{ns}	58.60^{ns}	56.55±0.152^{abc}	17.74
8	6.20^{ns}	6.50^{ns}	6.35±0.041^{de}	67.11	55.03^{ns}	56.53^{ns}	55.78±0.092^{abc}	16.14
9	5.17^{ns}	5.50^{ns}	5.33±0.043^{i}	40.35	52.47^{ns}	49.67^{ns}	51.07±0.125^{bc}	6.32
10	5.87^{ns}	5.60^{ns}	5.73±0.039^{g}	50.88	51.33^{ns}	51.60^{ns}	51.47±0.039^{bc}	7.16
11	5.60^{ns}	5.50^{ns}	5.55±0.024^{h}	46.05	53.87^{ns}	48.77^{ns}	51.32±0.169^{bc}	6.84
12	5.00^{ns}	5.10^{ns}	5.05±0.024^{h}	32.89	46.27^{ns}	49.63^{ns}	47.95±0.137^{c}	-0.17
13	5.10^{ns}	5.10^{ns}	5.10±0.000^{h}	34.21	52.30^{ns}	52.20^{ns}	52.25±0.024^{abc}	8.79
14	6.10^{ns}	6.23^{ns}	6.17±0.027^{f}	62.28	55.20^{ns}	54.27^{ns}	54.73±0.072^{abc}	13.96
15	6.20^{ns}	6.20^{ns}	6.20±0.000^{ef}	63.16	52.60^{ns}	55.87^{ns}	54.23±0.135^{abc}	12.92
16	6.30^{ns}	6.30^{ns}	6.30±0.000^{def}	65.79	55.97^{ns}	54.50^{ns}	55.23±0.091^{abc}	15.00
17	6.40^{ns}	6.20^{ns}	6.30±0.034^{def}	65.79	55.23^{ns}	55.03^{ns}	55.13±0.034^{abc}	14.79
18	6.50^{ns}	6.33^{ns}	6.42±0.031^{cd}	68.86	55.00^{ns}	52.47^{ns}	53.73±0.119^{abc}	11.87
19	7.20^{ns}	7.10^{ns}	7.15±0.024^{b}	88.16	59.80^{ns}	61.00^{ns}	60.40±0.082^{ab}	25.75
20	7.77^{ns}	7.27^{ns}	7.52±0.053^{a}	97.81	60.63^{ns}	62.20^{ns}	61.42±0.094^{a}	27.87
Average ± SE	5.66±0.094^{ns}	5.65±0.088^{ns}			53.22±0.170^{ns}	53.66±0.181^{ns}		

Mean values followed by the same letter within the column are not significantly different by DMRT at level of 5% ± standard error.

Table 5. Effect of PGPR isolates and adaptive varieties on the stem diameter and yield per plant of soybean in the ultisols.

PGPR isolates	Stem Diameter (mm)				Yield per Plant (g)			
	Varieties		Average ± SE	% of untreated	Varieties		Average ± SE	% of untreated
	Detam-1	Wilis			Detam-1	Wilis		
0	4.97^{ns}	4.77^{ns}	4.87±0.033^{fg}	-	10.58^{ns}	11.05^{ns}	10.82±0.051^{l}	-
1	5.16^{ns}	4.88^{ns}	5.02±0.040^{d-g}	3.08	13.04^{ns}	12.35^{ns}	12.70±0.062^{l}	17.34
2	4.89^{ns}	5.09^{ns}	4.99±0.033^{efg}	2.50	19.72^{ns}	19.85^{ns}	19.78±0.027^{efg}	82.84
3	4.96^{ns}	5.18^{ns}	5.07±0.035^{d-g}	4.14	14.38^{ns}	13.18^{ns}	13.78±0.082^{k}	27.31
4	5.21^{ns}	5.49^{ns}	5.35±0.040^{b-g}	9.92	19.93^{ns}	18.58^{ns}	19.25±0.087^{fgh}	77.95
5	5.43^{ns}	5.76^{ns}	5.59±0.043^{b-f}	14.85	16.15^{ns}	14.35^{ns}	15.25±0.101^{h}	40.94
6	5.59^{ns}	5.44^{ns}	5.51±0.029^{b-g}	13.18	16.70^{ns}	15.50^{ns}	16.10±0.082^{h}	48.80
7	5.45^{ns}	5.87^{ns}	5.66±0.048^{b-e}	16,19	20.65^{ns}	19.45^{ns}	20.05±0.082^{d-g}	85.30
8	5.52^{ns}	5.65^{ns}	5.59±0.028^{b-f}	14.68	22.21^{ns}	20.68^{ns}	21.44±0.093^{ab}	98.17
9	5.25^{ns}	4.97^{ns}	5.11±0.040^{c-g}	4.86	22.35^{ns}	20.15^{ns}	21.25±0.111^{bc}	96.40
10	5.13^{ns}	5.16^{ns}	5.15±0.012^{c-g}	5.68	21.50^{ns}	20.30^{ns}	20.90±0.082^{b-e}	93.16
11	5.39^{ns}	4.89^{ns}	5.14±0.053^{c-g}	5.54	21.54^{ns}	20.68^{ns}	21.11±0.070^{bcd}	95.09
12	4.63^{ns}	4.96^{ns}	4.80±0.043^{g}	-1.54	20.80^{ns}	19.60^{ns}	20.20±0.082^{c-f}	86.69
13	5.56^{ns}	5.21^{ns}	5.39±0.044^{b-g}	10,64	19.38^{ns}	16.18^{ns}	17.78±0.134^{i}	64.28
14	6.19^{ns}	5.76^{ns}	5.98±0.049^{ab}	22.72	18.49^{ns}	18.63^{ns}	18.56±0.027^{hi}	71.52
15	5.93^{ns}	5.59^{ns}	5.76±0.044^{cd}	18.21	20.30^{ns}	16.10^{ns}	18.20±0.154^{hi}	68.21
16	6.26^{ns}	5.45^{ns}	5.86±0.068^{abc}	20.26	19.60^{ns}	18.40^{ns}	19.00±0.082^{gh}	75.60
17	6.19^{ns}	5.85^{ns}	6.02±0.044^{ab}	23.61	20.98^{ns}	20.12^{ns}	20.55±0.070^{b-e}	89.93
18	6.17^{ns}	5.91^{ns}	6.04±0.038^{ab}	24.02	19.53^{ns}	19.00^{ns}	19.27±0.055^{fgh}	78.07
19	6.48^{ns}	6.47^{ns}	6.47±0.009^{a}	32.92	22.10^{ns}	21.23^{ns}	21.66±0.070^{ab}	100.22
20	6.65^{ns}	6.39^{ns}	6.52±0.038^{a}	33.85	23.30^{ns}	21.43^{ns}	22.37±0.102^{a}	106.72
Average ± SE	5.57±0.067 ^{ns}	5.46±0.062 ^{ns}			19.20±0.162^{a}	17.94±0.157^{b}		

Mean values followed by the same letter within the column are not significantly different by DMRT at level of 5% ± standard error.

Table 6. Effect of PGPR isolates in the soil pH, C/N, organic-C, available-P, and total-N in the ultisols.

PGPR isolates	pH H_2O			C/N		Organic-C (%)			Available-P (%)			Total-N (%)		
	Average and Category		% of untreated	Average	% of untreated	Average and Category		% of untreated	Average and Category		% of untreated	Average and Category		% of untreated
0	4.84^{f}	A	-	8.13^{e}	-	2.46^{f}	M	-	13.03^{h}	M	-	0.21^{e}	M	-
1	5.49^{e}	A	13.43	7.49^{d}	-7.87	3.49^{e}	H	41.87	18.48^{g}	H	41.83	0.38^{d}	M	80.95
2	5.79^{a-e}	SA	19.63	7.46^{cd}	-8.24	4.46^{ab}	H	81.30	23.63^{a-d}	H	81.35	0.84^{ab}	VH	300.00
3	5.87^{a-e}	SA	21.28	7.37^{cd}	-9.35	4.37^{abc}	H	77.64	23.17^{a-d}	H	77.82	0.83^{ab}	VH	295.24
4	5.65^{b-e}	SA	16.74	7.15^{bcd}	-12.05	4.15^{a-d}	H	68.70	22.01^{b-f}	H	68.92	0.79^{ab}	VH	276.19
5	5.89^{a-e}	SA	21.69	7.56^{de}	-7.01	4.56^{a}	H	85.37	24.17^{abc}	H	85.50	0.26^{e}	M	23.81
6	5.98^{a-e}	SA	23.55	7.65^{de}	-5.90	4.65^{a}	H	89.02	24.62^{ab}	H	88.95	0.26^{e}	M	23.81
7	6.13^{a-d}	SA	26.65	7.03^{a-d}	-13.53	4.03^{a-e}	H	63.82	21.38^{c-g}	H	64.08	0.76^{abc}	VH	261.90
8	6.05^{a-e}	SA	25.00	7.39^{cd}	-9.10	4.39^{abc}	H	78.46	23.24^{a-d}	H	78.36	0.83^{ab}	VH	295.24
9	5.57^{de}	SA	15.08	7.57^{de}	-6.89	4.57^{a}	H	85.77	24.24^{abc}	H	86.03	0.87^{a}	VH	314.29
10	5.61^{cde}	SA	15.91	7.28^{cd}	-10.46	4.28^{abc}	H	73.98	22.70^{a-d}	H	74.21	0.81^{ab}	VH	285.71
11	5.77^{a-e}	SA	19.21	7.64^{de}	-6.03	4.64^{a}	H	88.62	24.61^{abc}	H	88.87	0.88^{a}	VH	319.05
12	5.60^{de}	SA	15.70	7.34^{cd}	-9.72	4.34^{abc}	H	76.42	23.01^{a-d}	H	76.59	0.82^{ab}	VH	290.48
13	5.69^{b-e}	SA	17.56	7.16^{bcd}	-11.93	4.16^{a-d}	H	69.11	22.07^{b-e}	H	69.38	0.79^{ab}	VH	276.19
14	6.06^{a-e}	SA	25.21	6.84^{abc}	-15.87	3.84^{b-e}	H	56.10	20.37^{d-g}	H	56.33	0.73^{bc}	H	247.62
15	6.01^{a-e}	SA	24.17	7.17^{bcd}	-11.81	4.17^{a-d}	H	69.51	22.12^{b-e}	H	69.76	0.79^{ab}	VH	276.19
16	6.27^{ab}	SA	29.55	6.50^{a}	-20.05	3.50^{e}	H	42.28	18.54^{fg}	H	42.29	0.66^{c}	H	214.29
17	6.34^{a}	SA	30.99	6.53^{a}	-19.68	3.53^{e}	H	43.50	18.70^{efg}	H	43.51	0.67^{c}	H	219.05

18	6.12^{a-d}	SA	26.45	7.07^{a-d}	-13.04	4.07^{a-e}	H	65.45	21.54^{b-g}	H	65.31	0.77^{abc}	VH	266.67
19	6.24^{abc}	SA	28.93	6.46^{a}	-20.54	3.63^{de}	H	47.56	25.90^{a}	VH	98.77	0.87^{a}	VH	314.29
20	6.10^{a-e}	SA	26.03	6.64^{a}	-18.33	3.81^{cde}	H	54.88	25.18^{a}	VH	93.25	0.88^{a}	VH	319.05

Mean values followed by the same letter within the column are not significantly different by DMRT at level of 5%. Criteria for pH H_2O (acidic/A= 4.5-5.5; slightly acidic/SA= 5.5-6.5); organic-C (moderate/M= 2.01-3%; high/H= 3.01-5%); available-P (moderate/M= 8-10 ppm; high/H= 11-15 ppm; very high/VH >15 ppm); total-N (moderate/M= 0.21-0.5%; high/H= 0.51-0.75%; very high/VH >0.75%) (Indonesia Soil Research Institute 2009).

Table 7. Correlation analysis of the number of branches, plant height, stem diameter, pH, C/N, organic-C, available-P, and total-N on the yield per plant of soybean for Detam-1 and Wilis varieties.

Varieties	Parameter	NB	PH	SD	SP	CN	OC	AP	TN	YP
Detam-1	NB	1								
	PH	0.567 **	1							
	SD	0.615 **	0.889 **	1						
	SP	0.589 **	0.727 **	0.769 **	1					
	CN	-0.772 **	-0.584 **	-0.782 **	-0.625 **	1				
	OC	-0.011	0.052	-0.246	0.170	0.398	1			
	AP	0.235	0.336	0.027	0.273	0.165	0.896 **	1		
	TN	0.730 **	.0149	0.117	0.226	-0.369	0.343	0.440 *	1	
	YP	0.710 ** (S)	0.502 * (M)	0.393 (L)	0.476 * (M)	-0.469 * (M)	0.410 (M)	0.547 * (M)	0.772 ** (S)	1
Wilis	NB	1								
	PH	0.594 **	1							
	SD	0.653 **	0.929 **	1						
	SP	0.724 **	0.782 **	0.778 **	1					
	CN	-0.557 **	-0.580 **	-0.655 **	-0.758 **	1				
	OC	0.112	-0.014	-0.106	0.191	0.140	1			
	AP	0.458 *	0.417	0.360	0.410	-0.183	0.737 **	1		
	TN	0.798 **	0.225	0.236	0.439 *	-0.332	0.415	0.575 **	1	
	YP	0.772 ** (S)	0.404 (M)	0.433 (M)	0.546 * (M)	-0.509 * (M)	0.285 (L)	0.518 * (M)	0.736 ** (S)	1

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed). N= 21. NB= number of branches; PH= plant height (cm); SD= stem diameter (mm); SP= soil pH H_2O; CN= C/N; OC= organic-C (%); AP= available-P (%); TN= total-N (%); YP= yield per plant (g). The correlation coefficient was adopted from Evans (1996) with category as Very Low/VL= 0.00-0.19; Low/L= 0.20-0.39; Moderate/M= 0.40-0.59; Strong/S= 0.60-0.79; and Very Strong/VS= 0.80-1.00.

Correlation analysis of plant growth and ultisols fertility on the yield per plant of adaptive varieties soybean

The relationship between plant growth in adaptive varieties (Detam-1 and Wilis) and ultisols fertility was presented in Table 7. The relationship of the number of branches, plant height, soil pH, available-P, and total-N were significant and positively correlated to the yield per plant in Detam-1 variety of soybean. An increase in the number of branches, plant height, soil pH, available-P, and total-N lead the higher the yield per plant in Detam-1 variety. The relationship of the number of branches, soil pH, available-P, and total-N were significantly and positively correlated to the yield per plant for Wilis variety. It was shown that an increase in the number of branches, soil pH, available-P, and total-N resulted in increasing the yield per plant in Wilis variety. This relationship between parameters and the yield in the case of both varieties has been moderate until strong. The stem diameter and organic-C had a positive correlation, but it were insignificantly affect the yield per plant in Detam-1 variety. The plant height, stem diameter, and organic-C shown a positively correlated, but insignificant affect the yield per plant for Wilis variety. The relationship of soil C/N ratio was significantly and negatively correlation with the yield per plant for Detam-1 and Wilis varieties. It was shown that the lower C/N of ultisols resulted in increased the yield per plant in both varieties. According to Taufiq et al. (2007) an increase in the size of soybean seeds was correlated positively with the increases in the soil pH, available-Ca, -Mg, and decreasing the exchangeable-Al, -H, as well as available-Fe, -Mn.

4. Conclusions

The Detam-1 and Wilis varieties are classified as adaptive on acid soils which are characterized by the highest yield per plant. The isolates combination application of *R. leguminosarum+Rhizobium sp2+Bacillus sp+Burkholderia sp* significantly increased the agronomic characteristics of soybean compared to other PGPR isolates. The isolates combination application of N-fixing and phosphate solubilizing bacteria significantly increased the ultisols fertility include pH; organic-C; available-P; total-N and C/N compared to untreated. The isolates combination of PGPR for several varieties can be recommended to support the growth and yield of soybean and increasing the ultisols fertility.

Authors' Contributions: AFRIDA, E.: conception and design, acquisition of data, analysis and interpretation of data, critical review of important intellectual content; MUHAMMAD, S.: conception and design, acquisition of data, drafting the article; TAMPUBOLON, K.: conception and design, acquisition of data, drafting the article, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: Not applicable.

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Received: 10 September 2020 | **Accepted:** 17 December 2021 | **Published:** 16 February 2022