Effect of Soil Physicochemical Properties on PGPR Density at A Coffee Plantation in Malang, Indonesia

Ervinda Yuliatin1*, Tri Ardyati1, and Suharjono Suharjono1

1Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia

*Corresponding author: ervindayuliatin02@gmail.com

Abstract. The objective of this research was to determine the effect of physicochemical properties of coffee plantation soil on density of plant growth-promoting rhizobacteria (PGPR). The PGPR bacteria were isolated from rhizosphere of Coffea arabica and Coffea canephora soil using serial dilution method. The number of IAA-producing, phosphate-solubilizing, and nitrogen-fixing bacteria were determined by Tryptic Soy Agar, Pikovskaya Agar and Nitrogen-free-Bromothymol Blue Agar medium, respectively. The density of IAA producing bacteria and nitrogen-fixing bacteria of Coffea arabica soil 1.5×10⁵ cfu/g and 2×10⁴ cfu/g, respectively were higher than Coffea canephora soil. The density of phosphate-solubilizing bacteria 1.7×10⁵ cfu/g of Coffea canephora was higher than Coffea arabica soil. Environmental parameters of both coffee species were significantly influenced by latitude, light intensity and tree height (p<0.05). The C/N ratio was significantly higher of Coffea arabica soil 11.33 % than Coffea canephora soil 8.66 %. The highest correlation (correlation > 0.86) was between nitrogen-fixing bacteria and IAA-producing bacteria. This analysis can be performed that the density of PGPR may vary due to some soil properties, environment factors, and plant characteristics.

Keywords: IAA, phosphate, nitrogen, PGPR, density, coffee

1. Introduction

Around 125 millions of world population countries in America, Africa, and Asia consume coffee [1,2]. The coffee commodity has developed more than 50 years to improve coffee production. Increasing quantity and quality of coffee is followed by high cost production due to solving the problem of pest, soil health, climate change, rainfall and plant diseases [3]. Researchers predicted that coffee demanding will increase double by 2050 while the land availability of coffee plantation will decrease by half. Therefore, it is necessary to improve the management of coffee production for gaining the best quality of coffee in the future [4].

Indonesia is one of Asian country that produces various coffee species and it significantly support the economic sector. Malang, a city in East Java, is in the third position as the main coffee producer. A central for coffee production is at Universitas Brawijaya Forest (UB Forest). Interestingly, the coffee plantation grows along with vegetables, pine tree, and Mahogany on the same area. The last two years, coffee production decreased due to high rainfall frequency while demand for coffee
increases. Despite of those soil physicochemical properties such as organic carbon content, nitrogen and phosphorus availability, and C/N ratio also influence coffee production.

The role of soil physicochemical properties is important due to its interaction between microbes and plant in the soil. The community structure of soil microbes depend on the soil. For instance, soil microbes on agriculture soil may different with other soil types. Soil microbe-associated with plant have the capability to overcome soil in fertility. Although the use of synthetic fertilizer improves the agricultural yield, but the impact is dangerous for human and environment. Hence, application of potential soil microbe as an alternative to synthetic fertilizer can be a solution for regaining soil fertility [5]. These soil microbes, especially rhizosphere bacteria, have the capability to improve plant growth and soil fertility. Many plant growth promoting rhizobacteria (PGPR) have been reported such as Alcaligenes, Azospirillum, Arthrobacter, Acinobacter, Bradyrhizobium, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium, Azorhizobium, Alorhizobium, Sinorhizobium, Frankia and Mesorhizobium [6-13]. In other studies, it is reported that rhizobacteria associated with Coffea arabica were screened biofertilizer [14] and biopesticide candidate [15,16].

The study of rhizosphere associated with Coffea arabica and Coffea canephora in UB Forest is unclearly understand. In order to support sustainable agriculture, this study was done to determine the density of rhizobacteria as indigenous PGPR from coffee plantation in UB Forest, Malang and investigate the relationship between physicochemical properties and environmental parameters to PGPR density.

2. Research Method

2.1. Soil sampling

Soil samples were collected from coffee plantation of UB Forest, Malang, East Java, Indonesia at 978 m a.s.l. (07°44’23.3”S and 112°32’01.4”E) for C. arabica and 1139 m a.s.l (07°44’23.4”S and 112°32’01.2”E). Each sample was randomly collected to a depth of 10 cm. Subsequently, the equivalent weight of the samples from each field was mixed in a vessel to obtain a composite sample. This procedure was repeated with the sample from every three different points. The soil was transported in sealed bags and stored at 4°C until further processing.

2.2. Measuring soil physicochemical properties and environmental parameters

Soil pH was measured using glass electrode (1:1) in soil distilled H2O suspension. Determination of water holding capacity (WHC) based on Blazka and Fisher (2014). Firstly the soils were sieved and dried at 105 °C then these were placed in the crucible. The crucible unsaturated soil was weighed again. The dry samples was placed in a soaking tray that was filled with distilled water which was evident by the shining film of water on the soil surface. The crucible was then removed from the soaking tray, the water allowed to drain and the crucible was again weighed (water-saturated soil). The organic C was determined by oxidation with K2Cr2O7 and titration with (NH4)2FeSO4. Available P in 0,5 M NaHCO3 extract was determined by the antimony-pottasium-tartrate method. Potassium was determined using Bray’s method. Characteristics of the environmental parameters were included temperature, altitude, tree diameter, tree distance, tree height, and light intensity.

2.3. Isolation and Quantification of PGPR

Soil samples (25 g) was dissolved in 225 mL of NaCl solution (0,85%). The PGPR were isolated using serial dilutions method (10^−1-10^−6). Each of aliquot (0,1 mL) was inoculated in triplicate onto (1) Triptyc Soy Agar plates with tryptophan 200 µg / mL for isolation of IAA-producing bacteria; (2) Pikovskaya agar plates for isolation of phosphate-solubilizing bacteria and (3) Nitrogen-free-bromothymol medium agar for isolation of nitrogen-fixing bacteria. All the isolations were incubated at 30°C for 48-72 hours. The number of colonies was enumerated for total plate count. Each isolated bacteria was purified by spread plate and incubated at 28°C for 24-48 hours.
2.4. Statistical Analysis

The results were analysed by independent T-Test with level significance was $p<0.05$ to determine the comparison of soil physicochemical properties and environmental parameters to PGPR’s density between two soil coffee. The statistical analysis were performed by SPSS 22.00. The multivariat analysis between soil physicochemical properties, environmental parameters and PGPR density was used Principal Component Analysis (PCA) that performed by PAST 3 program.

3. Result and Discussion

3.1. Soil physicochemical properties of coffee plantation

Soil samples were taken from two different species of coffee plantation (Robusta and Arabica) at altitude of 976-1137 m a.s.l. Two coffee soil types were dark brown which is a character of Andosol soil usually found at an altitude of 750-3000 m a.s.l and contains volcanic material [17].

| Parameter                  | Arabica       | Robusta       | $P$ value |
|----------------------------|---------------|---------------|-----------|
| pH                         | $4.0 \pm 0.1^a$ | $4.5 \pm 0.4^a$ | 0.11      |
| Organic carbon (%)         | $4.33 \pm 0.7^a$ | $5.11 \pm 0.82^a$ | 0.27      |
| Total N (%)                | $0.51 \pm 0.02^a$ | $0.45 \pm 0.06^a$ | 0.15      |
| C/N ratio                  | $11.33 \pm 0.57^b$ | $8.66 \pm 1.52^a$ | 0.047     |
| Phosphorous availability (ppm) | $3.07 \pm 0.95^a$ | $1.96 \pm 0.46^a$ | 0.27      |
| Organic matter             | $8.85 \pm 1.42^a$ | $7.49 \pm 1.21^a$ | 0.14      |
| Water holding capacity     | $99.55 \pm 0.05^a$ | $99.6 \pm 0.008^a$ | 0.19      |

$^a$ The same row followed by the different of letter in Arabica and Robusta coffee soil column is significantly different ($p<0.05$) using T-Test

Soil physicochemical properties (table 1) showed that the C/N ratio was significantly different between Arabica and Robusta soil. Arabica soil was 11.33 ratio that performed the highest ratio rather than Robusta soil. Arabica and Robusta soil had similarly value of pH, organic carbon, total N, P-availability and organic matter content. According to Balai Penelitian Tanah, soil physicochemical properties of Arabica and Robusta soil in UB Forest had similarity category such as the C/N ratio was medium (figure 1a), organic matter content was very high (figure 1b) and the p-availability was low (figure 1c). In contrast, the soil physicochemical properties of Arabica soil was very acid 4.0 in pH and total N was high compare to Robusta soil was acid 4.5 in pH (figure 1a) and total N was medium (figure 1b) respectively. Both of coffee species were planted on agroforestry mixture with Pine and Mahogany that produce plant litter as a provider of highly organic matter. This agroforestry have important role to maintain the ecosystem balance for litter decomposer bacteria. Therefore, the value of soil physicochemical properties such as organic carbon, total nitrogen and organic matter are high [18],[19].
Figure 1. The soil physicochemical properties comparison of Arabica and Robusta Coffee (a) soil acidity degree and C/N ratio, (b) percentage of organic carbon, total N and organic matter, (c) phosphorus available. The data was analysed using T-Test (α=0.05). The variation of letter show significantly differences.

| Parameter        | Coffee plantation soil | Arabica | Robusta | P value |
|------------------|------------------------|---------|---------|---------|
| Temperature      | 20.01 ± 1.6<sup>a</sup> | 22.11 ± 0.91<sup>a</sup> | 0.13    |
| Altitude         | 976 ± 1.4<sup>a</sup>  | 1137 ± 2.5<sup>b</sup>  | 0.00    |
| Tree diameter    | 7.21 ± 0.84<sup>a</sup> | 8.17 ± 1.5<sup>a</sup>  | 0.39    |
| Tree distance    | 162.44 ± 19.5<sup>a</sup> | 166.22 ± 6.8<sup>a</sup> | 0.76    |
| Tree height      | 2.26 ± 0.06<sup>b</sup> | 1.8 ± 0.07<sup>a</sup>  | 0.02    |
| Light intensity  | 66 ± 9<sup>a</sup>     | 412.38 ± 14.8<sup>b</sup> | 0.00    |

* The same row followed by the different of letter in Arabica and Robusta coffee soil column is significantly different (p<0.05) using T-Test.

Environmental parameters showed that altitude, tree height, and light intensity were different between Arabica and Robusta coffee soil (table 2). Another factor such as temperature, tree diameter, and tree distance was a relative similar number (p>0.05). The temperature is within the optimal range for plant growth while altitude influences coffee growth and production [20]. It was performed by tree diameter and tree height. The altitude above 1000 m a.s.l showed average of tree diameter which had a high of size number from Robusta coffee. In contrast, the number of tree height was lower than Arabica coffee. The light intensity between two coffee species was significantly different due to weather change and observation time in the field.

3.2. Isolation and density of PGPR bacteria

The density of IAA-producing, phosphorus-solubilizing and nitrogen-fixing bacteria were similar between soil samples from Arabica and Robusta (table 3). The density of IAA-producing bacteria was the highest (1.5 × 10⁵ cfu/g) in the Arabica soil while phosphorus-solubilizing bacteria of Robusta soil was the highest (1.7×10⁵ cfu/g) among all isolated bacteria (figure 2). The IAA-producing bacteria from rhizospheric soil in large quantities can provide IAA hormones for cell elongation and plant growth [21-22]. The role of phosphorus-solubilizing bacteria was important due to it can release phosphorus that is bound to soil minerals for plant growth and development [23]. Meanwhile, it was clear that the nitrogen-fixing bacteria had a similar density on both coffee species soil. It means that
the ability of nitrogen-fixing bacteria supported ammonium for assembling amino acid and plant proteins.

Table 3. Density of potential PGPR

| Parameter                    | Arabica | Robusta | P value |
|------------------------------|---------|---------|---------|
| IAA producing bacteria       | 1.5 \times 10^{5b} | 1.0 \times 10^{5a} | 0.05    |
| Phosphorus solubilizing bacteria | 1.4 \times 10^{5ab} | 1.7 \times 10^{5a} | 0.10    |
| Nitrogen fixing bacteria     | 2.0 \times 10^{4a} | 2.2 \times 10^{4a} | 0.36    |

a The same row followed by the different of letter in Arabica and Robusta coffee soil column is significantly different (p<0.05) using T-Test

Figure 2. The comparison of PGPR density of both coffee species soil was analysed by T-Test analysis (0<0.05)

3.3. The correlation of soil physicochemical properties and environmental factors to density of PGPR

Multivariate analysis of soil physicochemical properties, environmental factors and PGPR diversity was determined by biplot analysis using PCA (Principal component analysis). It was performed the general factor that characterize the coffee soil. There was two groups of coffee soil including Arabica coffee soil (KA) and Robusta coffee soil (KR) (figure 3). The KA group was characterized by IAA-producing bacteria density (IAA), nitrogen-fixing bacteria density (NFB), organic matter (OM), P-availability (PA) and C/N ratio (C/N). It showed that the higher of C/N ration, organic matter and P-availability of Arabica soil can increase the number of IAA-producing bacteria and nitrogen-fixing bacteria density.
The high quantity of P-availability decreased the density of phosphorus-solubilizing bacteria. Meanwhile, the KR group was determined by temperature (T), light intensity (LI), soil pH (SpH), organic carbon (OC), and water holding capacity (WHC) was high respectively. It means that factors contributed to the low-density number of IAA producing and nitrogen-fixing bacteria but those factors did not influence the density of phosphorus-solubilizing bacteria. It was reported that the altitude can affect the soil acidity while the high number of temperature and organic matter increases the density of bacteria [24].

4. Conclusion
Soil physicochemical properties and environmental parameters influence the PGPR density at Arabica and Robusta soil. It can be performed using statistical and biplot analysis to describe the characteristic of each coffee soil. The coffee soil can produce indigenous PGPR from rhizosphere coffee as biofertilizer to support plant growth. Further research is suggested to investigate the potency of PGPR isolate for IAA-producing, phosphate-solubilizing and nitrogen-fixing.

5. Acknowledgment
Authors are thankful to the Ministry of Research, Technology and Higher Education of Indonesia that provided a research grant for the master degree thesis (No. 055/SP2H/LT/DRPM/2019), the director of UB Forest for the opportunity and facility to conduct this research, and the Head of Microbiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University for laboratory support.

References
[1] Musoli P, Cubry P, Aluka P, Billiot C, Dufour M, De Bellis F, Pot D, Bieysse D, Charrier A and Leory T 2009 Genetic differentiation of wild and cultivated population: diversity of Coffea canephora Pierre in Uganda Genome 52 634-46. doi: 10.1139/G09-037.
[2] Osorio N 2002 The global coffee crisis: a threat to sustainable development. international coffee organization London (http://www.ico.org/documents/globalcrisise.pdf)
[3] International Coffee Organization 2014 World coffee trade (1963-2013). A Review of The Markets Challenges and Opportunities Facing The Sector.
[4] World Coffee Research 2017 World coffee research the first five years: 2012-2017. (www.worldcoffeeresearch.org)
[5] Putri O H , Utami S R, Kurniawan S 2018. Sifat kimia tanah pada berbagai penggunaan lahan di UB Forest. J.Tanah dan Sumberdaya Lahan 6:1-5
[6] Sharma P, Kumawat K C, Kaur S 2016 Plant growth promoting rhizobacteria in nutrient
enrichment: *Current perspectives. in biofortification of food crops* springer India 2016 263-289.

[7] Patel S, Sayyed R Z and Saraf M 2016 Bacterial determinants and plant defense induction: their role as biocontrol agents in sustainable agriculture. *In Plant, Soil and Microbes, Springer International Publishing* 2016 187-204.

[8] Bashan Y, de-Bashan L E and Prabhu S R 2016 Superior polymeric formulations and emerging innovative products of bacterial inoculants for sustainable agriculture and the environment. *In Agriculturally Important Microorganisms Springer Singapore* 15-46.

[9] Shaikh S S, Sayyed R Z and Reddy M S 2016 Plant growth promoting rhizobacteria: a sustainable approach to agro-ecosystem *In: Plant, Soil and Microbes - Interactions and Implications in Crop Science.* Switzerland: Springer international publishing AG p 181-201.

[10] Shaikh S S, Patel P R, Patel S S, Nikam S D, Rane T U and Sayyed R Z 2014 Production of biocontrol traits by banana field fluorescent pseudomonads and their comparison with chemical fungicides. *Ind J Exp Biol* 52 917-920.

[11] Shaikh S S and R Z Sayyed 2015 Role of plant growth promoting rhizobacteria and their formulation in biocontrol of plant diseases. *In: Plant Microbes Symbiosis: Applied Facets* Springer India p 337-351.

[12] Shaikh S S, Wani S J and Sayyed R Z 2016 Statistical based optimization of siderophore production and scale-up on bioreactor *2016 Biotech* p 6: 69.

[13] Lal S, Chiarini L and Tabacchioni S 2016 New insights in plant-associated paenibacillus species: biocontrol and plant growth-promoting activity *In Bacilli and Agro biotechnology Springer International Publishing* 2016 p 237-279.

[14] Muleta D, Assefa F, Borjesson E, Granhall U 2013 Phosphate-solubilising rhizobacteria associated with Coffea arabica L. in natural coffee forests of southwestern Ethiopia. *Journal of the Saudi Society of Agricultural Sciences* 12 73-84.

[15] Kumar A, Prakash A and Johri B N 2011 Bacillus as PGPR in crop ecosystem, in: *bacteria in agrobiology: crop ecosystems* Springer-Verlag Berlin Heidelberg.

[16] Amutha R, Karunakaran S, Dhanasekaran S, Hemalatha K, Monika R, Shammugapriya P and Sornalatha T 2014 Isolation and mass production of biofertilizer (Azotobacter and Phosphobacter). *International Journal of Latest Research in Science and Technology* 3 79-81.

[17] Soil Survey Staff 2010 Keys to soil taxonomy eleventh edition *Natural Resources Conservation Service-United States Department of Agricultural,* Washington DC.

[18] Suhartati 2007 Kajian karakteristik tanah pada tegakan jenis tanaman cepat tumbuh. *Info Hutan.* Pusat Penelitian dan Pengembangan Hutan dan Konservasi Alam (Bogor).

[19] Khalif U, Utami SR and Kusuma Z 2014 Pengaruh penanaman sengon (Paraserianthes falcatoria) terhadap kandungan C dan N tanah di Desa Slamparejo, Jabung, Malang *Jurnal Tanah dan Sumberdaya Lahan* 1 9-15.

[20] Amutha K and Priya K J 2011 Effect of pH, temperature and metal ions on amylase activity from Bacillus Subtilis Kcx 006. *Int. J. Pharma and Bio Sci* 2 407-413.

[21] Mohite B 2013 Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth *Journal of Soil Science and Plant Nutrition* 13 638-649.

[22] Premachandra D L, Hudek and Brau L 2016 Bacterial modes of action for enhancing of plant growth *Journal of Biotechnology & Biomaterials* 6 1-8.

[23] Li Y, Liu X, Hao T and Chen S 2017 Colonization and maize growth promotion induced by phosphate solubilizing bacterial isolates *International Journal of Molecular Sciences* 18 1-16.

[24] Sari N P, Santoso T I and Mawardi S 2013 Sebaran tingkat kesuburan tanah pada perkebunan rakyat kopra arabika di dataar tinggi Ijen-Raung menurut ketinggian tempat dan tanaman penaung. *Pelita Perkebunan* 29 93–107.