Proceedings of the Pakistan Academy of Sciences: B
Life and Environmental Sciences 61(2): 159-169 (2024)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(61-2)925

Pakistan Academy of Sciences

Research Article

Improving Soil Quality and Yield of Intercropping-System Crops in a Dry Land Area through Plant Growth Promoting Rhizobacteria Application Frequency

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Abstract: The future of agriculture is prone to choose technology that can enhance the quality of the resources to support the sustainability of food production. Plant Growth Promoting Rhizobacteria (PGPR) is a reliable technology for future agriculture as it is environment-friendly, and able to optimize resource utilization and decrease external input. This research aimed to analyze the effect of PGPR (*Pseudomonas fluorescens* + *Bacillus polymyxa*) application frequency on chemical soil properties, a yield of an intercropping system in dry land, the in-between correlation of the parameters, and to determine the best PGPR application frequency. Randomized Complete Block Design (RCBD) was used in this research to put the treatment in the experimental unit properly. The treatments consisted of i) one-time application of PGPR at the planting time, ii) twice application of PGPR at the planting time and 15 Days After Planting (DAP), iii) three times application of PGPR at the planting time, 15 DAP and 30 DAP, iv) without application of PGPR as control. The results showed that PGPR application frequency improved chemical soil properties, yield, and total by-products as livestock feed. The activity of soil enzymes, nitrogenase, and phosphatase, was enhanced compared to the control. The application of PGPR in dryland areas is recommended to maintain soil fertility and support sustainable intercropping crop production. Further studies are needed to conduct mixed farming between agriculture, animal husbandry, clean energy (biogas), and organic fertilizer (residue from the biogas digester).

Keywords: Arid Land, Bio Fertilizer, Environment-Friendly Technology, Mixed Farming, Multiple Cropping Practice, Sustainability Food Production**.**

1. INTRODUCTION

Agriculture in the future will face a significant challenge: providing proper food for the increasing world population. In 2050, the world population is projected to reach 9.7×10^9 [1]. Consequently, food production must be improved to cover the future population. Dryland can be an alternative source of food production as it has 40 % of the total land in the world [2]. The ecosystem in dry land has been proven to contribute to world development and promise for the future. However, converting dry land to agriculture has many obstacles, such as limited water availability, easily eroded soil, low organic matter content, and low nutrient content [3]. This condition implies that cultivating crops on this land reasonably needs a strategy to maintain sustainable resources and production. Sustainability is a demand for agriculture in the future [4], and conservation-based agriculture is more suitable to be developed [5]. In addition, apart from the land resource, other constraints of cultivation in dry land are narrow land tenure and a low number of educated farmers. This situation will further suppress sustainable food production. From the mentioned constraints, soil fertility and water availability are the main limiting factors for dryland food production. The limited water availability in dryland causes low

Received: June 2023; Revised: April 2024; Accepted: June 2024
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soil moisture, affecting soil microorganisms' life [6, 7]. An insufficient amount of soil moisture also affects both the low population and low activity of microorganisms [8]. In contrast, the population of microorganisms in dry land is an important factor as a bio-indicator for soil fertility [9, 10]. A high population of microorganisms indicates better soil fertility. To support the growth and production of crops in dry land, the population of beneficial microorganisms in the soil needs to be increased, especially plant growth promoting rhizobacteria (PGPR) [11]. These bacteria can provide available nutrients for crops [12, 13]. In addition, PGPR is also capable of producing phytohormone [14–17] and acting as a biocontrol [18–20]. Under environmental stress, PGPR helps plants overcome these unfavorable conditions to survive [6, 21, 22]. The role of PGPR can increase the growth potential of crops cultivated in unfavorable lands, such as dry land.

Another study reported that rhizobacteria *Pseudomonas flourescens* (Trevisan) Migula 1895; and *Bacillus* sp. could dissolve phosphate [23]. Moreover, these bacteria can synthesize plant growth hormone, namely indole acetic acid (IAA) [24]. *P. flourescens* and *Paenibacillus polymyxa* (Prazmowski 1880) can reduce salt stress's effect on the shoot and root growth of barley (*Hordeum vulgare* L.) [25]. Applying *P. fluorescence* as a biofertilizer in banana [*Musa* (*genus*)] nurseries resulted in colonized roots and encouraged the growth of banana seedlings [26].

There are several studies of PGPR applications in intercropping systems; however, they only compare PGPR inoculation and without PGPR [27, 28]. However, the effectiveness of PGPR application frequency in improving soil quality and yield in the intercropping system has not been widely reported. PGPR application in the intercropping system is an attempt to increase the availability of nutrients in the soil for crops. The availability of sufficient nutrients can reduce competition between plants, affecting the production of intercropping crops per unit of land area. The PGPR population in the rooting area needs to be maintained to still provide nutrients for plants in sufficient quantities, especially during the growth period of the plants. These reasons are the underlying reasons why PGPR application frequency should be taken into account. This study aimed to analyze the effect of PGPR application frequency on improving soil chemical properties, yield, and a total by-product as livestock feed in an intercropping system on dry land.

2. MATERIALS AND METHODS

2.1. Location

The experiment was conducted on dry land on Poteran island, Sumenep regency, Indonesia with a dry climate (Table 1) and an 8 m above sea level, from January to April 2019. This location is located at S 7°03'57.2832", E 113°56'31.7076". The soil type on this island is categorized as the Mediterranean, with soil temperature regimes as hot (is hyperthermic). Chemical soil properties on the site (soil analysis before conducting research) were reported that the soil contains 1.5% organic matter, 6.5 for pH level, 21.99 me100 g^{-1} soil of Cation Exchange Capacity (CEC), and 0.12%, 3.16 mg kg^{-1}, 0.18 me 100 g^{-1} soil for nitrogen, phosphate, and potassium, respectively. Data on organic matter, nitrogen, phosphate, and potassium are categorized as low to very low, as Prasetyo showed in Probolinggo [29]. This condition of low soil fertility will be discussed further in "future research" at the end of this manuscript.

2.2. Materials

The research used planting materials for local

Table 1. Climate condition on research location.

Average	Year					
	2015	**2016**	**2017**	**2018**	**2019**	**2020**
Number of rainy days (d)	9.50	15.08	13.25	10.83	8.00	14.00
Number of precipitation (mm)	2.44	170.89	152.33	119.66	99.25	162.87
Humidity (%)	79.25	81.88	80.75	76.00	73.79	76.29
Temperature (0C)	28.19	27.96	27.96	28.12	28.37	28.68
Atmospheric pressure (mb)*	1 012.35	1 011.80	1 011.12	1 011.17	1 011.59	1 008.95

*1 mb = 0.001 bar = 100 Pa, Source [30].

maize (*Zea mays* L.) var. Guluk-Guluk, of which groundnut (*Arachis hypogaea* L.) seeds were obtained from the direct harvest of local farmers and stem cuttings of Adira cultivar cassava (*Manihot esculenta* Crantz). The fertilizer consisted of cattle manure, burned rice husk, phonska, urea, trisodium phosphate (TSP), and potassium chloride (KCl). PGPR used in the research was a consortium of *P. fluorescens* and *B. polymyxa* from the Laboratory of Plant Disease and Pest Management of Pamekasan, Madura, East Java, Indonesia.

2.3. Experimental Design

Randomized Complete Block Design (RCBD) was used to arrange treatment for each experiment unit. This research consisted of four treatments, namely i) without PGPR application, ii) one-time application of PGPR at the planting time, iii) twice application of PGPR at the planting time, and 15 Days After Planting (DAP), iv) three times PGPR application at the planting time, 15 DAP, and 30 DAP. Each treatment had four replications. The placement of treatment in each group was done randomly.

2.4. Overview of the Experiment

Before planting, land preparation is done by clearing the land of weeds. Furthermore, 16 experimental plots were created, divided into four groups. The plot size was 8.30 m × 8.30 m. A 30 cm wide water channel with a depth of 30 cm was made between the plots. Each plot carried out minimum tillage, only tilling the soil in the row where corn, groundnut, and cassava will be planted. Basic fertilization used Bokhasi cattle manure fertilizer at 2 t ha^{-1}, burned rice husks 500 kg ha^{-1}, Phonska 200 kg ha^{-1}. Follow-up fertilization was carried out 20 days after planting using urea 150 kg ha^{-1}, and TSP 100 kg ha^{-1}. Planting in the experimental plot was arranged in three rows of groundnut, three rows of corn, three rows of groundnut, one row of cassava, three rows of groundnut, three rows of corn, and three rows of groundnut. The spacing of groundnut was 20 cm × 20 cm, corn 60 cm × 20 cm, and cassava with a distance of 90 cm in rows. Planting was carried out in the rainy season in 2019. The PGPR solution sprayed onto the soil was made by mixing 10 mL of PGPR in 1 L of water. Each plant was sprayed with 10 mL of PGPR solution. Each plot's need for a PGPR solution was calculated based on the total plant population. As the population of all plants per plot was 729, the need for PGPR solution was 7 290 mL. This solution was given evenly on the experimental plots. The time of PGPR application was adjusted to the treatment. In terms of determining changes in soil quality, soil chemical properties were observed, such as soil C-organic content, total N nutrient content, available P, exchangeable K, and cation exchange capacity. In addition, soil enzyme activities, namely nitrogenase and phosphatase enzymes, were observed. Crop production variables, namely corn, groundnut, and cassava, were observed per plot (kg ha^{-1}). The plant buds as forage for animal feed were also observed in fresh form (kg ha^{-1}).

2.5. Procedures of Soil Chemical Analysis

Soil samples were air-dried and ground for the preparation of chemical analysis. Nitrogen content was determined by the micro-Kjeldahl digestion method, available P was analyzed by the Bray-1 method, and K was extracted from soil using NH_4OAC 1N pH 7 and measured by a flame photometer. The wet combustion method was used to determine soil organic carbon, and cation exchange capacity was determined using the extractor NH_4OAC 1N pH 7 method. Soil nitrogenase activity was analyzed by Acetylene Reduction Assay (ARA) [31] and phosphatase activity was determined according to Zechmeister-Boltenstern [32].

2.6. Statistical Analysis

The obtained data were analyzed by analysis of variance (ANOVA). If the treatment effect is significant, the analysis is continued with multiple comparison analyses of the Least Significant Difference (LSD) with an error of 5%. Correlation between variables was analyzed using Pearson Product Moment correlation analysis, and all statistical analysis using SPSS-25 software [33, 34].

3. RESULTS AND DISCUSSION

The effects of PGPR application frequency on the intercropping system (groundnut-corn-cassava) showed an improvement in the crops' soil chemical properties, yield, and by-products. The detailed results are provided as follows.

3.1. Soil Chemical Properties

The PGPR application frequency significantly affected available phosphate levels and exchangeable potassium levels compared to those without PGPR treatment. However, there was no significant effect among the frequency of PGPR 1, 2, and 3 times of applications. The application of PGPR in dryland effectively increased the availability of these two nutrients, and the increase reached 96.97% to 129.11% and 108.57% to 128.57% for available phosphate and exchangeable potassium, respectively. This result shows the role of phosphate-solubilizing bacteria *P. flourescens* and *B. polymyxa* in the PGPR solution. *B. polymyxa* is a bacterium that can dissolve phosphate and is effective in helping to overcome water stress [35]. This statement is reinforced by the results of [36], which showed that *Bacillus* sp. could dissolve phosphate fertilizers. *P. fluorescens* inoculation also increased soil P availability and phosphatase activity and positively affected soil improvement [37]. Phosphate solubilizing bacteria could dissolve unavailable phosphate into available by producing phosphatase enzymes and organic acids [38]. In addition, phosphate dissolution can occur through the production of inorganic acids, a decrease in pH with the release of protons, and the production of exopolysaccharides [39].

The application of PGPR to the treatment two times and three times showed no significant effect when compared to PGPR treatment one time. PGPR in the one-time treatment has succeeded in changing phosphate to be available to plants, so the application of PGPR in treatment two times and three times is no longer practical. The reason is because the microbes also use the available phosphate to reproduce themselves. Thus, the enzyme activity increased significantly (Table 2), but the number of P available did not experience a significant increase [40]. The research conducted by [41] also showed similar results to this study. The reason behind this finding is the fact that microorganisms also need P to carry out their breeding. The improvement in exchangeable potassium found in this experiment is in line with the previous research conducted by [42] where several groups of bacteria (*Bacillus* and *Pseudomonas*) were reported to dissolve potassium. Insoluble potassium is converted into potassium available to plants which in general is through the mechanism of producing organic acids [43]. Thus, the increase of P and K in this study indicates that PGPR can be an environmental-friendly solution to overcome the limited availability of nutrients in dry land. This rhizosphere engineering strategy has become an important way to achieve sustainable crop production.

The application of PGPR frequency had no significant effect on N levels. However, *B. polymyxa* was reported to be able to fix N in the air in addition to dissolving phosphate [44]. Likewise, C-organic content and soil CEC were not significantly affected by the frequency of PGPR application. However, there is a tendency for these three variables to increase. PGPR microbial growth is influenced by organic compounds produced by plant roots [45, 46]. Increasing microbial biomass will increase soil organic matter because the main constituents of microbial bodies are protein, homo, and heteropolysaccharides [47]. In addition, microbes also produce polysaccharide compounds that add soil organic carbon and soil aggregation [48]. The application of PGPR improved the chemical properties of the dry land soil used in this study (Table 2). This result will support the sustainability of agricultural cultivation in dry land, as stated by Ojuederie *et al.* [13].

3.2. Enzyme Activity

The observed soil enzymes were phosphatase and nitrogenase. These enzymes were related to

Table 2. Soil chemical properties affected by PGPR application frequency on intercropping system.

PGPR Application	N total (%)	Available P (mg kg^{-1})		Exchangeable K (cmol kg^{-1})		C-Organic (%)	CEC (cmol kg^{-1})
Without PGPR	0.21	58.28	a	0.70	a	1.56	32.03
1 × PGPR	0.20	110.70	b	1.46	b	1.73	40.57
2 × PGPR	0.22	98.37	b	1.60	b	1.61	41.02
3 × PGPR	0.24	114.42	b	1.29	b	1.68	37.14

Note: Numbers with different letters in the same column are significantly different at the 95% probability level.

the PGPR given to the soil. The average activity of the two types of enzymes differed between treatments with the frequency of PGPR application. The more frequently given PGPR, the activity of the two enzymes increased (Table 3). The observed relationship between soil phosphatase enzyme activity and available phosphate showed a significant positive correlation with an r of 0.87. This result indicates the availability of nutrients for plants. Costa *et al.* [49] also reported that enzyme acid phosphatase was positively and significantly correlated with PGPR application. The phosphatase enzymes in soil are primarily derived from bacteria, fungi, and plants [50]. Its activity is influenced by temperature, soil pH [51, 52], soil organic carbon, and soil water content [53]. However, this study showed a weak correlation between phosphatase activity and C-organic levels. The correlation coefficient (r) was only 0.346. However, the phosphatase activity capable of providing relatively high available P (Table 2). The highest activity of nitrogenase enzymes was only 0.65 μg g^{-1} soil and showed a weak correlation with the total N content of the soil. The total N content of the soil in this study was in the low category [29]. The activity of microorganisms in the soil, including releasing enzymes and dissolving minerals such as phosphorus [54], also produces enzymes to degrade organic matter to produce nutrients available to plants [55]. The presence of these beneficial microorganisms helps the plant to produce biomass. In addition, Almeida *et al*. [56] had proven that soil enzyme activity could be used to indicate quality changes in degraded soils.

3.3. Crop Yield

Applying PGPR in groundnut-maize-cassava intercropping can increase groundnut production compared to treatment without PGPR (Table 4). The increase in production reached 30.08%. The twice application of PGPR showed the highest harvest dry production for all intercropped plants. Compared with no PGPR or control, dry-shelled corn production increased with PGPR application. The twice application of PGPR, namely at the time of planting and 15 DAP, corn production increased by 41.62%, and cassava production increased by 28.12%. This result indicates that rhizobacteria in the roots encouraged better plant growth so that crop production increased. Its role is to dissolve phosphate as well as increase exchangeable potassium (Table 2). This finding is in line with recent research which stated that the application of PGPR was able to enhance results and nutrition content on some crops such as horticulture and oil palm (*Elaeis guineensis* Jacq) [16, 57–59]. In addition, bacteria produce growth hormones such as indole acetic acid (IAA) that can stimulate growth and increase the yield of crops [24, 60, 61].

Table 3. The average of soil enzyme activity affected by PGPR application frequency on intercropping system.

PGPR Application	Phosphatase enzyme (μg g^{-1})		Nitrogenase enzyme (μg g^{-1})	
Without PGPR	0.47	a	0.38	a
1 × PGPR	0.63	b	0.45	b
2 × PGPR	0.78	c	0.58	b
3 × PGPR	0.88	c	0.65	b

Note: Numbers with different letters in the same column are significantly different at the 95% probability.

Table 4. Production of groundnut, corn, and cassava affected by PGPR application frequency on intercropping system.

PGPR Application	Groundnut (kg ha^{-1})		Maize (kg ha^{-1})		Cassava (kg ha^{-1})	
Without PGPR	323.40	a	1 255.96	a	224.25	a
1x PGPR	401.15	b	1 718.78	b	220.75	a
2x PGPR	428.87	b	1 778.28	b	287.32	b
3x PGPR	381.42	b	1 769.52	b	241.77	b

Note: Numbers with different letters in the same column are significantly different at the 95%.

3.4. Production of the Shoot as Animal Forage

The by-product of crops was utilized as livestock's forage to suffice the needs of feed. In Table 5, the statistical analysis result shows that the PGPR application did not show any significant difference which means it did not lead to an increase in shoot production. The observed forage production was a by-product of groundnut, maize, and cassava shoot. However, there was a tendency for growth in shoots compared to control or without PGPR. The increase can reach 20.38 %. Actually – with data that showed low soil fertility before the study – there is a conflict of interest between using agricultural waste in the form of shoots for animal feed and composting as a material to improve soil quality. Hence, it is interesting to study the benefits of these two things in mixed farming.

Some of the harvest waste should be incorporated into the soil to maintain the survival of PGPR and increase its population on dry land. Not all harvest waste is used for animal feed. In addition, livestock manure needs to be returned to the soil to increase organic matter input. Thus, it is expected that the soil organic matter content will increase and can be used as an energy source for the growth, maintenance of microorganism cells and the production of extracellular enzymes [62]. Components of cellulose and lignocellulosic organic matter are broken down into simple carbohydrates such as glucose for growth. Increased glucose levels cause increased bacterial growth [63]. Therefore, in the future, it is still necessary to research the application of PGPR combined with the return of organic matter from crop waste and livestock manure to improve soil quality so that plant performance is better and healthier and productivity enhancement. This recommendation is supported by research results [64–70] which reported that organic matter significantly improves soil quality. Goenadi *et al*. [68] explained on oil palm (*E. guineensis*), Sumatran *et al*. [69] reported the positive effect of the midrib decomposition of salak [*Salacca zalacca* (Gaertn.) Voss], while Pramulya *et al*. [70] wrote the addition of organic matter from the leaves of the shade plant of the lamtoro species [*Leucaena leucocephala* (Lam.) De Wit] in coffee plantations.

However, in mixed farming, the cost of transporting livestock manure from pens to farmland is a major consideration. It should be studied, for example, only 50 % of the remaining harvest is transported to the cattle shed as feed. The remaining 50 % was decomposed at the edge of agricultural land by adding local decomposing microbia [71, 72].

Biogas digesters (individual or communal) build near the cattle sheds [73, 74]. Daily, the digester is filled with manure and urine from cattle, which act as anaerobic fermentation. Likewise, all household organic waste (leftovers, kitchen wastes, tree leaves in the yard) is put into the digester with specific rules [75, 76]. This biogas digester is also connected to the latrines or septic tanks in people's homes [73, 77, 78] with this action, there are several advantages, namely renewable energy— clean energy for household kitchens and solid and liquid organic fertilizers [73,79, 80], which are helpful for plant productivity and soil amendments [81, 82] The amount of by-product as fertilizer from this digester is smaller, so it reduces transportation costs, but the quality is excellent [83, 84].

The results of other studies showed that the use of the PGPR bacterial consortium, which can provide N, P, and K was proven to increase the growth and physiological parameters of cereal plants. Several studies related to PGPR have also reported that Mycorrhiza has a positive effect on various plants and improves soil properties [85–88]. Therefore, besides the bacteria applied in

Table 5. The production of the shoot as animal forage after PGPR application per hectare.

Treatment	Groundnut (kg ha^{-1})	Maize (kg ha^{-1})	Cassava (kg ha^{-1})	Total (kg ha^{-1})
Without PGPR	323.40	1 225.96	224.25	1 682.61
1 × PGPR	401.15	1 718.78	220.75	2 340.68
2 × PGPR	428.87	1 778.28	287.32	2 494.47
3 × PGPR	381.42	1 769.52	241.77	2 392.71

Note: Numbers with different letters in the same column are significantly different at the 95% probability level.

this study, it is better to add other PGPR bacteria, namely N-fixing bacteria and mycorrhiza fungi, so that they become a consortium that is expected to increase the availability of nutrients and increase plant growth and production.

4. CONCLUSIONS

Applying PGPR significantly improved soil chemical properties and production in an intercropping system on dry land. The output of the shoot as forage for animal feed increased by 20.38 %. Enzyme activity increased when compared with no application of PGPR. The twice application of PGPR showed the best effect on soil quality, plant production, and fresh shoots. Using PGPR in intercropping plant cultivation in dry land is an alternative to maintaining soil quality. Thus, the sustainability of production can support the provision of food in the future.

5. ACKNOWLEDGEMENTS

The authors would like to thank to the Directorate of Research and Community Service under The Ministry of Research, Technology and Higher Education of Indonesia for funding through Applied Research Program (07/SP2H/PEN/MONO/ LPPM/UNIJA/2019).

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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