



Formulation of bacterial consortium for improvement growth and yield of maize (*Zea mays* L.)

Ali Ikhwan, Aniek Iriany*, Erny Ishartati, Faridlotul Hasanah

Department of Agrotechnology, Faculty of Agriculture, University of Muhammadiyah Malang, Indonesia

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* Corresponding Author

Email address:

aniek55@yahoo.co.id

ABSTRACT

Plant growth-promoting bacteria (PGPB) have become an important subject of research to increase maize production. The PGPB consortium should provide more benefits than single or dual inoculation. This study aimed to investigate the effect of a PGPB consortium on improving maize growth and yield. The field experiment used a split-plot design. The main plot consisted of three maize varieties (Talenta, Pertiwi-3, and Bisma), and the subplot consisted of three formulations of PGPB consortia [endophytic bacteria isolates, *Acetobacter* sp., cellulolytic, and ligninolytic (F1); endophytic bacteria isolates, *Azospirillum* sp., cellulolytic, and ligninolytic (F2); and endophytic bacteria isolates, cellulolytic, ligninolytic, *Acetobacter* sp., and *Azospirillum* sp. (F3)] and one control. PGPB consortia formulation did not influence maize growth significantly, but maize varieties did. Pertiwi-3 showed the highest value in all growth variables, followed by Bisma and Talenta, respectively. The effect of PGPB consortia formulation upon ear fresh and dry weight depends upon the maize variety, and Pertiwi-3 showed the highest value in yield variables. PGPB consortia formulation 2 was the most effective to apply for Pertiwi-3 cultivation, while PGPB consortia formulation 3 produced higher yields for Talenta and Bisma. These findings indicated that specific PGPB formula could improve the yield for specific maize varieties.

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1. Introduction

Maize (*Zea mays* L.) is an important crop not only consumed as a staple food (30%) but also as feed (65%) and raw materials (5%) (Panikkai et al., 2017). Approximately 12,500 million tons (Mt) of maize were consumed in Indonesia in 2017/2018. However, national maize production in that year only generated 11,900 Mt. Approximately 0.6 Mt were imported to make up for the shortfall (Mcdonald & Meylinah, 2019). Compared to top maize producers in the world e.g., USA and China, Indonesian maize productivity (tons ha⁻¹) lagged behind, with 5.3 compared to 11.1 of the US, 6.1 of China, and 5.7 of global maize production. Several efforts to increase maize production involved extensification, which increased 1.4% of the harvest area in 2018–2019 (Mcdonald & Meylinah, 2019). However, any extensification efforts must be accompanied by intensification because the total of arable land available for cultivation is limited; some of them are being rapidly converted to non-agricultural use (Li et al., 2019). Currently, agricultural intensification is taking an

interest in environmentally sustainable approaches. This perspective modifies the agroecosystem to minimize the negative impact of synthetic input (fertilizer, pesticide, etc.) and maintain economic profitability with on-farm resources, such as native beneficial microbiota, to promote plant growth and increase yield (Pérez-Montaño et al., 2014; Velten et al., 2015).

Plant growth-promoting bacteria (PGPB) has become an important subject of research to increase maize productivity (Di Salvo et al., 2018). PGPB can fertilize soils and promote plant growth when applied as a biofertilizer (Olanrewaju & Babalola, 2019) through several mechanisms, including phytohormone production, nutrient release, and biotic/abiotic stress alleviation (Santos et al., 2019). Those properties increased yield and boosted crop production, aligning with the intensification goal (Fukami et al., 2016). Particularly in maize, PGPB inoculation has been demonstrated promising, e.g., it improved agronomic

performance, increased yield, and reduced susceptibility to biotic/abiotic stress (Fitriatin et al., 2017; Olanrewaju & Babalola, 2019; Ullah et al., 2020).

In biofertilizer formulation, it is possible to mix and combine several PGPB strains in a consortium to apply in the field. This idea comes from the fact that many bacteria could live together and establish a mutualistic relationship (Olanrewaju & Babalola, 2019). Furthermore, not all PGPB are capable of exerting the same mechanism. Some excel in fixing nitrogen, while others may be proficient at producing phytohormone, etc. Hence, by combining PGPB in a consortium, more benefits can be provided (Malusà et al., 2016). The superiority of consortium compared to single or dual inoculation was reported in several papers. For example, Akhtar et al. (2018) reported better maize performance with bacterial consortium than single or dual inoculation in *Fusarium* infested soils. Irfan et al. (2019) found the same benefit in a saline environment. This may occur because the consortia convert multiple nutrients, enhance microbial activity in the soil, and convert nutrients through the symbiotic association of bacteria and plant roots. This, in turn, promotes nodulation, nitrogen and phosphorus cycling, promotion in plant growth, increased grain yield, and protein concentration (Moretti et al., 2020).

This study investigated the effect of inoculating a PGPB consortium on maize performance and yield. The consortium comprises isolates of endophytic, cellulolytic, ligninolytic bacteria, *Acetobacter* sp. and *Azospirillum* sp. Studies on PGPB consortia to apply in maize are still rare. Therefore, this paper may be a reference for future studies on using PGPB consortia as a biofertilizer to improve maize production in Indonesia. This study aimed to investigate the effect of a PGPB consortium on improving the growth and yield of several maize varieties.

2. Materials and Method

2.1. Preparation of PGPB consortia formulation

PGPB consortia used in this study were composed of unidentified endophytic isolates (JC2A, JC4D, JC5B, JC5D, JC5T, JH3A, JMH3B, and JMH3T), unidentified cellulolytic, ligninolytic, *Acetobacter* sp., and *Azospirillum* sp. These isolates were prepared and obtained by Kurniawan (2016).

Before use, all isolates were mixed, grown in M63 medium, and harvested in a large-scale fermentor as described by Kurniawan (2016).

2.2. Experimental design

Laboratory work was carried out in the Department of Agrotechnology's laboratory, University of Muhammadiyah Malang. Meanwhile, a field study was conducted in Karangploso, Malang, Indonesia (−7.9175924 °S, 112.6142577 °E) with an altitude of 550 m, daily temperature range of 25–32 °C, and rainfall 3600 mm year⁻¹. The field experiment was carried out using a split-plot design with twelve treatment combinations and three replications. The main plot was three maize varieties i.e. Talenta, Pertiwi-3, and Bisma. The subplot was PGPB consortia formulation, arranged in four combinations: F0 (control without inoculation), F1 (5 ml of each JC2A, JC4D, JMH3B, JMH3T, *Acetobacter* sp., cellulolytic, and ligninolytic), F2 (5 ml of each JMH3T, JC5B, JC5D, JC5T, *Azospirillum* sp., cellulolytic, and ligninolytic), and F3 (5 ml of each JH3A, JC5D, JMH3B, JC5T, cellulolytic, and ligninolytic; and 2.5 ml of each *Acetobacter* sp. and *Azospirillum* sp.). PGPB consortia formulation was applied through soil drenching three times: sowing day, 14, and 28 days after sowing, with a dose of 200 ml plant⁻¹ in each application.

2.3. Measured variables

Variables measured included plant growth and yield parameters. They are plant height, number of leaves, leaf area, fresh root weight, plant biomass, fresh ear weight, ear dry weight, cob weight, and 100 seed weight. Plant height, number of leaves, and leaf area were measured during weeks 2–7 (tasseling period). Fresh root weight and plant biomass were measured by weighing the lower and upper parts of plants (soil level as the reference) once at the end of the observation. Fresh and dry ear weights were collected before and after the water content was removed by sun-drying. Cob weight was determined by weighing the dry maize ear without husk, and 100 seeds weight were obtained by weighed on water content 14%–15% (w/w).

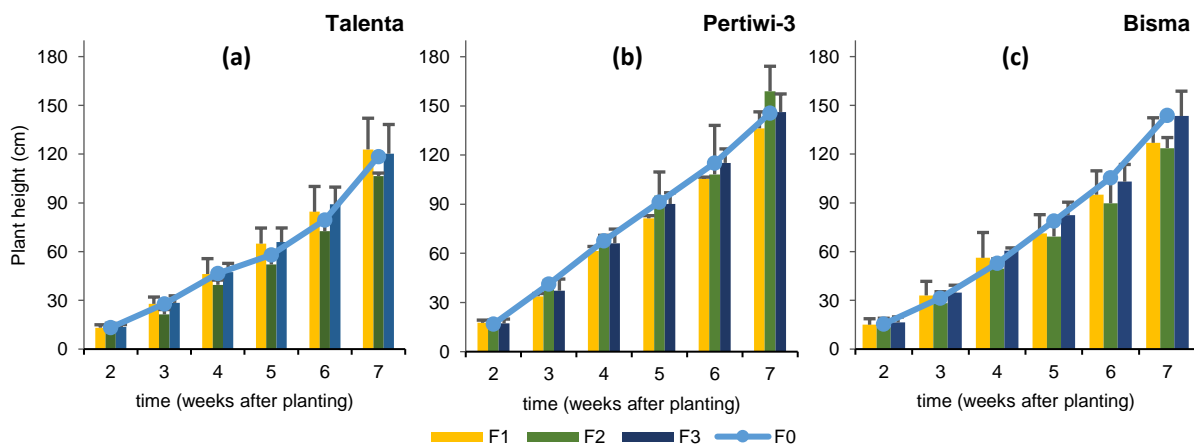


Figure 1. Plant height of three maize varieties under various endophytic bacteria formulations at 2 until 7 weeks after planting (Note: F0 = control, F1 = Plant growth-promoting bacteria (PGPB) consortia formulation 1, F2 = endophytic bacteria formulation 2, F3 = PGPB consortia formulation 3; bars represent standard deviation)

Table 1. Growth of maize under Plant Growth-Promoting Bacteria (PGPB) consortia formulations and three maize varieties at the end of the observation

Treatments	Plant height (cm)	Number of leaves	Leaf area (cm ²)	Root fresh weight (g)	Plant biomass (g)
<i>PGPB consortia formulations</i>					
F0	163.84 a	12.92 a	481.32 a	38.72 a	214.75 a
F1	152.35 a	12.67 a	470.42 a	46.22 a	207.06 a
F2	151.17 a	12.94 a	493.93 a	39.81 a	225.25 a
F3	169.01 a	13.42 a	507.86 a	43.14 a	232.31 a
<i>Maize varieties</i>					
Talenta	131.92 b	11.67 c	445.87 b	20.83 c	129.25 c
Pertiwi-3	177.67 a	14.19 a	525.77 a	60.71 a	319.69 a
Bisma	167.68 a	13.10 b	493.50 ab	44.38 b	210.58 b

Remarks: Mean followed by the same letter in the same column showed no significant difference based on Tukey’s HSD test ($\alpha=0.05$); F0 = control, F1 = PGPB consortia formulation 1, F2 = PGPB consortia formulation 2, F3 = PGPB consortia formulation 3.

2.4. Statistical and data analysis

The data were analyzed using analysis of variance to determine the effect of the treatments. The differences were assessed using Tukey’s HSD test (α 5%) to find out the best treatment for any significant values. An interaction plot was used to understand the behavior of one variable depending on the other variables, while the main plot clarified the behavior of each factor level of a certain factor.

3. Results

3.1. Effect of PGPB consortia formulation on growth parameters of three maize varieties

During the observation time, maize plant height was not significantly different under different PGPB consortia formulations ($P > 0.05$). However, F3 tends to show higher plant height than F1 and F2 on Talenta dan Bisma, while on Pertiwi-3, F2 tends to show better plant height with the order was F2, F3, and F1, from highest to lowest respectively. A significant difference occurred among maize varieties (Figure 1).

Pertiwi and Bisma varieties showed significantly higher plant height (21–26%) than Talenta during observation time ($P < 0.05$) (Table 1).

The number of maize leaves did not show a significant difference under the application of various PGPB consortia formulations ($P > 0.05$). However, F3 treatment tends to show more leaves than other formulations. A significant difference occurred among maize varieties (Figure 2). Pertiwi-3 and Bisma had significantly more leaves than Talenta during the observation period ($P < 0.05$), with the order from the most to least number of leaves was Pertiwi-3, Bisma, dan Talenta (Table 1).

The difference of leaf maize area under the application of various PGPB consortia formulations was not visible during the observation period. However, each formula tends to exhibit better leaf area in a specific variety e.g., F1 in Talenta, F2 in Pertiwi-3, and F3 in Bisma (Figure 3). Pertiwi-3 showed a wider leaf area (6–15%) than Bisma and Talenta. In addition, Pertiwi-3 produced a significantly wider leaf area (15%) than Talenta (Table 1).

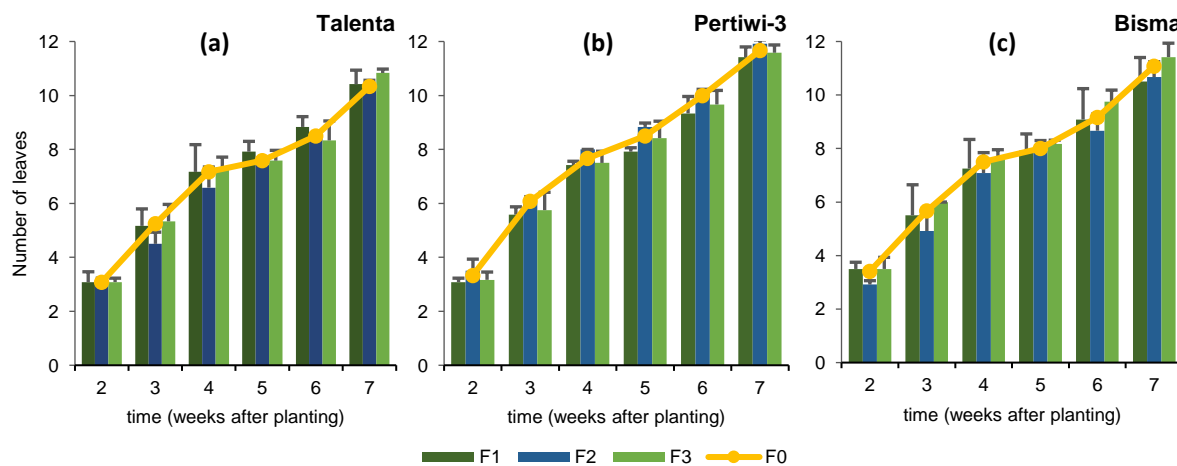


Figure 2. Number of maize leaves under various Plant growth-promoting bacteria (PGPB) consortia formulations on three maize varieties at 2 until 7 weeks after planting (Note: F0 = control, F1 = PGPB consortia formulation 1, F2 = PGPB consortia formulation 2, F3 = PGPB consortia formulation 3; bars represent standard deviation)

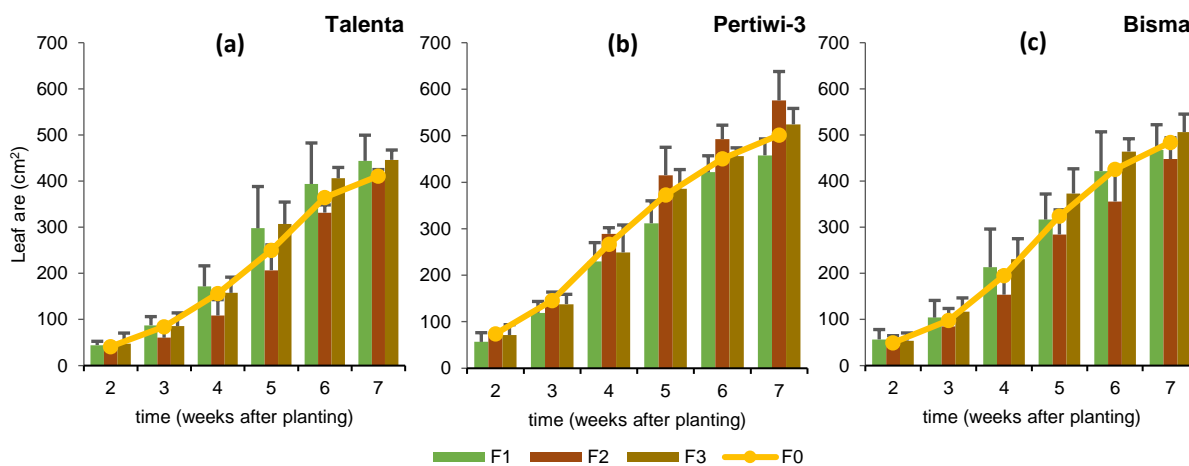


Figure 3. Leaf area of three maize varieties under various Plant growth-promoting bacteria (PGPB) consortia formulations at 2 until 7 weeks after planting (Note: F0 = control, F1 = PGPB consortia formulation 1, F2 = PGPB consortia formulation 2, F3 = PGPB consortia formulation 3; bars represent standard deviation)

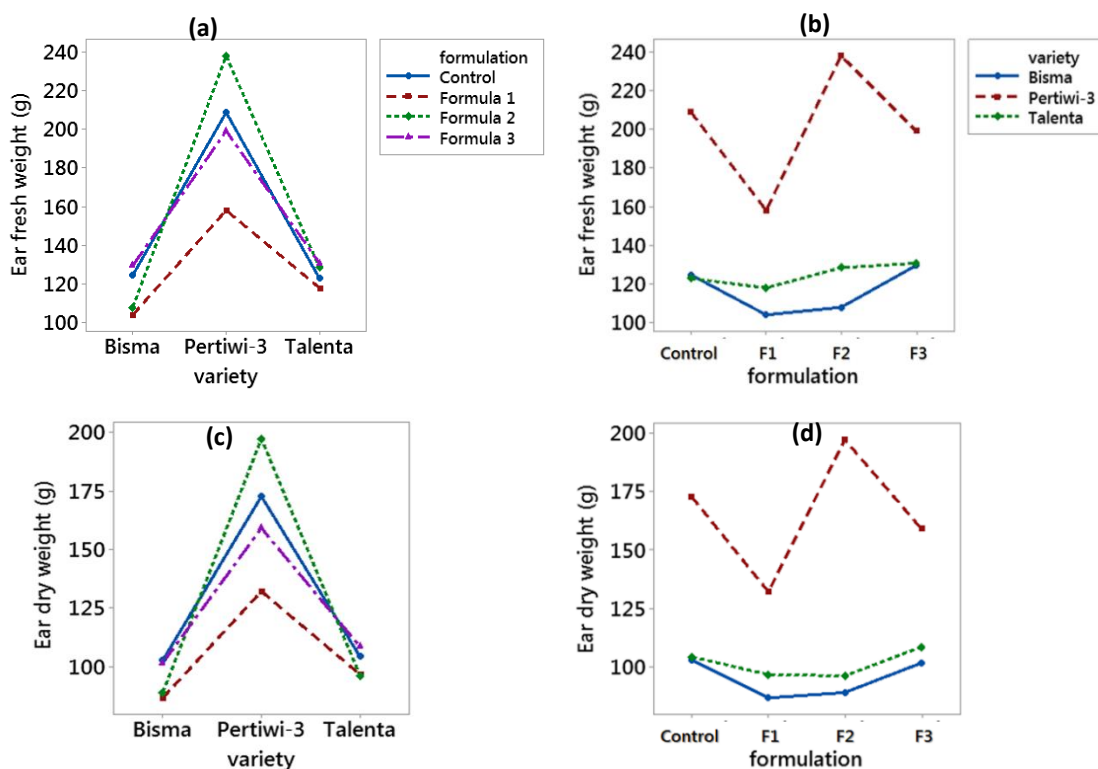


Figure 4. Interaction plot for fresh ear weight (a and b) and dry ear weight (c and d) of three maize varieties under various Plant growth-promoting bacteria (PGPB) consortia formulation

Fresh root weight and plant biomass did not show a significant difference in all PGPB consortia formulations at the end of observation, but F1 tends to show heavier fresh root weight than F3 and F2. F3 tends to exhibit higher plant biomass than F2 and F1. Pertiwi-3 demonstrated significantly heavier fresh root weight (27–66%) and plant biomass (34–60%) than Bisma and Talenta at the end of observation ($P < 0.05$) (Table 1).

3.2. Effect of PGPB consortia formulation on yield parameters of three maize varieties

Table 2 shows the influence of the PGPB consortium application on yield variables of three maize varieties. Pertiwi-3 showed the heaviest fresh ear weight and dry ear

weight of about 40% higher than other varieties in all PGPB consortia formulations. The order from the heaviest to the lightest was F2, F3, and F1 (Figure 4). Overall, the significant difference of fresh and dry ear weight between Talenta and Bisma did not appear in all those formulations (Table 2), but the highest mean value of ear fresh and dry weight of both varieties produced by F3 (Figure 4). The interaction plot of three maize varieties under various PGPB consortia formulation for ear fresh and dry weight variables (Figure 4) shows apparent interaction. Application of various PGPB consortia formulations did not show a significant difference in the cob weight or 100 seed weight variables ($P > 0.05$), but there was a significant difference due to maize varieties (Table 2).

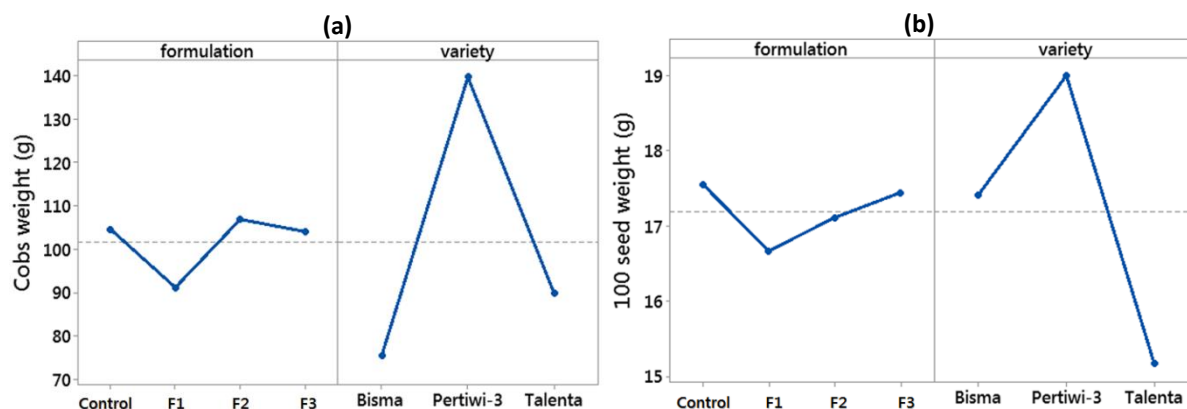


Figure 5. Main effects plot for cobs weight and 100 seed weight of three maize varieties under various Plant growth-promoting bacteria (PGPB) consortia formulation (Note: dash line represents the reference line of the response)

The highest cob weight appeared on the application of the F2 formula, followed by F3 and F1 treatments. In contrast, the highest 100 seed weight was seen on the application of the F3 formula, followed by F2 and F1, respectively. Furthermore, Pertiwi-3 produced significantly heavier cobs weight and 100 seed weight than Talenta and Bisma. The maize varieties' effects upon cob weight and 100 seed weight are larger than the PGPB consortia formulation's effects (Figure 5).

4. Discussion

The F2 and F3 treatments were the suitable PGPB consortia formulation for improving the growth of several maize varieties (Table 1), but the most effective PGPB consortia formulation to obtain better yield was depend on the maize variety (Table 2). The F2 treatment was the best PGPB consortia formulation to be applied for Pertiwi-3 cultivation, while the F3 treatment produced better yields for Talenta and Bisma (Figure 4). It can be explained by the composition of the PGPB consortia formula, either the interaction of bacteria strain or the presence of other non-symbiotic bacteria. The F3 treatment consists of four strains of endophytic bacteria isolates (JH3A, JC5D, JMH3B, JC5T), combined with cellulolytic bacteria, ligninolytic bacteria, *Azospirillum* sp, and *Acetobacter* sp. The F3 formulation consists of four strains of endophytic bacteria isolates (JMH3T, JC5B, JC5D, JC5T), without *Acetobacter* sp. The previous studies reported that bacteria isolated from maize rhizosphere in several countries, including Indonesia, were classified to genera *Bacillus* sp., *Pseudomonas* sp., *Serratia* sp, *Agrobacterium* sp., *Azospirillum* sp., *Acetobacter* sp, *Achromobacter* sp., and *Stenotrophomonas* sp. (Abiala et al., 2015; Agbodjato et al., 2015; Arsita et al., 2020; Sondang et al., 2019; Tchakounté et al., 2018; Wen et al., 2016; Youseif, 2018). Those bacteria directly promote plant growth by producing phytohormones (Jambhulkar et al., 2016) and enhance the stress tolerance of plants (Sirari et al., 2016). Various benefits of bacteria isolated from maize rhizosphere, e.g., genera *Stenotrophomonas* sp., *Agrobacterium* sp., and *Achromobacter* sp. as mentioned before, proved to contribute to nutrient availability. *Azospirillum* sp. is a non-symbiotic bacteria, characterized as a free-living N₂-fixer. It plays a role in phosphate solubilization and gibberellic acid production (Pérez-Montañó et al., 2014; Richard et al., 2018; Zayed, 2016). *Acetobacter* sp. produced indole acetic acetic

(IAA) in free environment conditions and increased root length, fresh root weight, and protein content in palm oil seedlings (Purwanto et al., 2019).

Based on this study, varieties strongly influence maize growth. Pertiwi-3 showed the highest value in all growth variables, followed by Bisma and Talenta, respectively (Table 1). The trend of these results in accordance with research reported by Pesireron & Senewe (2011) and Zufahmi et al. (2013). The PGPB consortia formulation did not significantly influence the growth of maize. A study by Lin et al. (2018) indicated that several PGPR formulations consisting of 4 strain bacteria classified as *Pseudomonas* sp. and *Bacillus* sp., did not significantly affect the growth of maize at V₄, V₆, and V_T growth stages. PGPR treatment on maize seeds also did not significantly affect the growth parameters of maize under field conditions (Nezarat & Gholami, 2009). Although there was no significant difference between formulations in this study, F1 tend to exhibit inferior maize growth, except for fresh root weight variable, compared to non-inoculated control, while F2 and F3 showed better growth than non-inoculated control (Table 1). It can be caused by the inter-relation between each isolate or bacteria inoculant. In general, various studies reported on better plant growth with bacteria inoculation, such as *Azospirillum* sp., *Acetobacter* sp., and other symbiotic or non-symbiotic bacteria, especially root growth variables (Jhala et al., 2016). Cassán et al. (2009) suggest photostimulation as a role of genera *Azospirillum*, *Achromobacter*, *Burkholderia*, and *Arthrobacter*. These strains have positive effects on root weight and nutrient uptake of maize plants, which might be attributable to phytohormone production and nutrient solubilization (Pérez-Montañó et al., 2014). Sirari et al. (2016) assert that hormones (IAA, gibberellin, and others) produced by PGPR increases root length, root tips, and root surface area. Youseif (2018) added that maize plants inoculated with *Agrobacterium* sp., one of the bacteria reported present in maize rhizosphere, showed significantly higher root length and fresh and dry weight of plant roots compared to uninoculated controls. It explained the heavier fresh root weight on inoculated (F1) than non-inoculated (control) one, although all growth variables showed inversely.

In summary, PGPB application, i.e., *Acetobacter* sp., *Azospirillum* sp. and other bacteria inoculated from the maize rhizosphere improved plant growth (Arsita et al., 2020; Curá

et al., 2017; Di Salvo et al., 2018; Olanrewaju & Babalola, 2019). A strong increase in total plant biomass of maize was obtained with inoculations of *Burkholderia cepacia*, *Azospirillum brasilense*, and *Herbaspirillum seropedicae* in individual experiments, in comparison with non N fertilize soil (Pérez-Montaño et al., 2014). In the broader range, Mehta et al. (2016) reported enhanced nitrogen availability after application of various strains of Azotobacter, Azospirillum, Phosphobacter, and Rhizobacter, increased plant height, the number of leaves, and stem diameters of *Helianthus annuus*. These results describe the role of PGPB to enhance nutrient use efficiency of fertilizer, solubilization, and mineralization of soil phosphorus (inorganic and organic), and its ability as free-living nitrogen-fixing PGPR so that nutrients became bioavailable. Nutrient absorption, especially N accumulation in plants, helps increase overall plant growth (Kumar et al., 2016; Mehta et al., 2016; Pérez-Montaño et al., 2014).

In terms of yield, Pertiwi-3 showed the highest value in yield variables (Table 2), which is consistent with studies reported by Pesireron & Senewe (2011), Maryamah et al. (2017), and Prasetyo et al. (2018). It is supported by the main

effects plot shown in Figure 5. The main effects plot displays the response means for each factor level in sorted order. The effects are the differences between the means and the reference line (Shah & Freedman, 2011). Pertiwi-3 showed a higher means value than the reference line in yield variables (cobs weight and 100 seed weight).

Interaction between maize varieties and PGPB consortia formulation is present when the response at maize varieties depends upon the PGPB consortia or vice versa. The greater the difference between the lines from the parallel state, the higher level of interaction (Shah & Freedman, 2011). Figure 4 shows a greater difference between varieties in the same PGPB consortia formulation than in the same varieties. It implies that PGPB consortium formulation's effect upon fresh and dry ear weight depends upon the maize variety. Previous work reported that the application of PGPR did not significantly affect growth parameters of maize under field conditions but did significantly affect several yield variables (Nezarat & Gholami, 2009). Bacteria inoculation, e.g., *Azospirillum* sp. and its consortia, affected maize yield (increased up to 14% dry weight) (Cassán et al., 2009; Pérez-Montaño et al., 2014).

Table 2. Yield observation of three maize varieties under application of Plant growth-promoting bacteria (PGPB) consortia formulation

Treatment	Ear fresh weight (g)	Ear dry weight (g)	Cobs weight (g)	100 seed weight (g)
<i>Control</i>				
Talenta	122.58 cd	104.17 c		
Pertiwi-3	208.75 ab	172.75 ab		
Bisma	124.33 cd	102.67 c		
<i>PGPB consortium formula 1</i>				
Talenta	117.67 cd	96.50 c		
Pertiwi-3	157.92 bc	132.08 bc		
Bisma	103.67 d	86.50 c		
<i>PGPB consortium formula 2</i>				
Talenta	128.17 cd	96.00 c		
Pertiwi-3	238.17 a	197.42 a		
Bisma	107.58 cd	88.83 c		
<i>PGPB consortium formula 3</i>				
Talenta	130.50 cd	108.42 c		
Pertiwi-3	199.17 ab	159.17 ab		
Bisma	129.25 cd	101.50 c		
<i>Formulation x Maize varieties</i>	*	*	ns	ns
<i>PGPB consortium formulation</i>				
F0			104.61 a	17.56 a
F1			91.17 a	16.67 a
F2			106.89 a	17.11 a
F3			104.06 a	17.44 a
<i>Formulation</i>			ns	ns
<i>Maize varieties</i>				
Talenta			89.90 b	15.17 b
Pertiwi-3			139.73 a	19.00 a
Bisma			75.42 b	17.42 ab
<i>Maize varieties</i>			**	*

Remarks: Mean followed by the same letter in the same column showed no significant difference based on Tukey's HSD test ($\alpha=0.05$); F0 = control, F1 = PGPB consortia formulation 1, F2 = PGPB consortia formulation 2, F3 = PGPB consortia formulation 3.

Microbial consortia increased yield variables of the crop compared to single inoculant treatments (Olanrewaju & Babalola, 2019). PGPB promotes it as phytostimulation, biofertilization (N), and biocontrol (several root pathogens) (Cassán et al., 2009). Bacteria inoculation also reported had a positive response in other crops, i.e., wheat and *Helianthus annuus*, which resulted in a significant increase N accumulation in wheat grain, seed filling, and seed dry weight (Gopalakrishnan et al., 2016; Kumar et al., 2016; Mehta et al., 2016).

In brief, the results confirmed an improvement aspect of growth and yield of three maize varieties by application of PGPB consortia formulation. It was also presented as information regarding the best PGPB consortia formulation for each maize variety. However, characterization and identification of PGPB consortia at a molecular level and their role should be considered to understand the enhancement of growth and yield mechanism clearly.

5. Conclusion

PGPB consortia formulation did not influence maize growth significantly, but maize varieties did. Pertiwi-3 showed the highest value in all growth variables, followed by Bisma and Talenta, respectively. The effect of PGPB consortia formulation upon ear fresh and dry weight depends upon the maize variety, and Pertiwi-3 showed the highest value in yield variables. PGPB consortia formulation 2 was the most effective formulation for Pertiwi-3 cultivation, while PGPB consortia formulation 3 produced higher yields for Talenta and Bisma.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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