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Improved Maize Growth in Condition Controlled by PGPR Inoculation on Ferruginous Soil in Central Benin

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Abstract

The use of microbial technologies in agriculture is rapidly expanding with the discovery of new bacterial strains effective in improving plant growth. In this study, we tested and highlighted the efficacy of PGPR (Plant Growth Promoting Rhizobacteria) alone or in a consortium on maize growth. For this purpose, a greenhouse experiment was carried out in pots containing sterilized ferruginous soil for 30 days. The corn seeds of the EVDT 97 SRT C1 variety were inoculated with bacterial suspensions of concentration 10^8 UFC/ml. The experimental device was a random block of 16 three-repeat treatments. The incidence of PGPR inoculated strains is assessed on the biomass growth and yield parameters of maize. At the end of the trial, the results showed that inoculation stimulated plant growth and development and resulted in a significant increase in the height, diameter at the collar, leaf surface and dry weight of aerial biomass of 20.15%, 21%, 32.77% and 37.73% respectively compared to controls, especially in corn plants inoculated with *B. thurengiensis* + *B. panthéthonicus* + *S. marcescens* and *Pseudomonas cichorii* + *Pseudomonas putida* + *Pseudomonas syringae*. These results show the potential of using these rhizobacteria as biological inoculants to improve maize productivity in Benin.

1. Introduction

Some bacteria that live in the rhizosphere define how the volume of soil influenced by the roots has the ability to promote plant growth [1] [2]. These microorganisms referred as PGPR drew attention to the need to reduce chemicals particularly in the context of sustainable agriculture and environmental protection [3]. PGPRs influence plant health and productivity through a variety of mechanisms that involve mineral solubilization, nitrogen fixation, phytohormone synthesis, hydrolytic enzyme synthesis and balance modulation plant hormone by deamination of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) [4] [5]. These rhizobacteria can improve the elongation and branching of the root system which promotes the absorption of water and minerals from the soil to the host plant including wheat, barley, corn and rice that are necessary for plant survival [6]. The benefits of PGPR inoculation on plant growth and productivity are well documented and have been correlated with phytohormone production and higher nutrient intake [7] [8] [9]. Various species of PGPR have been considered PGPR to stimulate plant growth and some of them marketed in recent years belong to the genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Mycobacterium*, *Enterobacter*, *Caulobacter*, *Serratia*, *Flavobacterium*, *Actinobacteria sp*. [10] [11].

Maize is a staple crop in Benin but soils are poor and inappropriate use of mineral fertilizers prevents producers from achieving the potential yield of this crop. Several research studies have reported that the excessive use of chemical fertilizers in agronomic systems aiming at improving yield, is less efficient in terms of stabilizing chemical elements that are quickly leached just after their application at ground level [12] [13]. This represents a deficit of absorption and nutritional assimilation in plants.

In Benin, the phenomenon of soil degradation is increasing especially in central regions dominated by ferruginous soils. This contributes to the depletion of organic matter in the soil as well as the nutrients essential for plant development [14]. The role of PGPRs in resolving several constraints such as soil degradation, water stress, and declining soil fertility that limit agricultural production has been widely proven [15]. The use of plant growth-promoting bacteria (PGPR) can be a promising alternative that can reduce the application of chemicals and improve crop yields and plant health [16] [17] [18]. For example, [19] has shown that PGPRs can increase nutrient bioavailability in the rhizosphere. They fix nutrients and prevent them from washing. In the work undertaken [20], the improvement in wheat yield was attributed to the co-inoculation of *Bacillus thuringiensis* and *Serratia sp*. Similarly, [21] [22] noted that inoculation of corn

seeds with strains of *Bacillus polymyxa*, *Pseudomonas alcaligenes* and *Actinomycetes sp.* (O19-AHB12) significantly improved the dry weight of maize plants by 19% to 52%, total maize biomass by 38% and the weight of 1000 corn seeds by 74.72% compared to controls. To date, little work has been done in Benin on the use of PGPRs to improve maize growth and yield. The objective of this study is to investigate the effects of nine (09) isolated rhizobacteria (PGPR) identified in central and northern Benin on the growth of greenhouse maize on ferruginous soil in central Benin.

2. Material and Methods

2.1. Material

- Corn seeds

The maize seeds used are those of the variety EVDT 97 STR C1 from the South Agricultural Research Centre (CRA-South) of the National Institute for Agricultural Research in Benin (INRAB). This variety is resistant to American rust, streak, helminthosporiosis, curvulariosis and drought [23].

- Provenance and characteristics of PGPR strains

Nine (9) strains of PGPR namely *Bacillus polymyxa*, *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus circulans*, *Bacillus panthothenicus*, *Pseudomona cichorii*, *Pseudomona putida*, *Pseudomona syringae* and *Serratiamarcescens* were used. These strains were isolated and characterized from the corn rhizosphere of the various agroecological zones of Central and Northern Benin by [24] and stored at −20˚C in Muller Hinton broth with glycerol (10%) laboratory of Biology and Molecular Typing in Microbiology at the University of Abomey-Calavi. The majority of strains show major fertilization and phytostimulation activities such as phosphate solubilization and acetic indole acid (IAA) production [25].

2.2. Methods

2.2.1. Preparing the PGPR Inoculum

The method described by Guiraud and Galzy, 1994 was used to rejuvenate by transplanting the three (3) strains of *Pseudomonas* on King B medium. The *Bacillus* and *Serratia* strains were revived on Nutritive Agar [26] [27]. The inoculum of each PGPR was obtained by culture in a nutrient medium (liquid HD) for 24 hours at 30˚C. The method described by [28] allowed us to adjust bacterial cultures to a concentration of approximately 1×10^8 UFC/ml (OD 0.45 to 610 nm) with a spectrophotometer 24 hours after incubation.

2.2.2. Experimental Device

The device adopted was a complete random block comprising sixteen (16) treatments with three (03) repetitions. Treatments have been defined as T1: *Bacteria-Free Control*, T2: *Bacillus polymysa*, T3: *Bacillus anthracis*, T4: *Bacillus circulans*, T5: *Bacillus thuringiensis*, T6: *Bacillus panthothenicus*, T7: *Pseudomonas cichorii*, T8: *Pseudomonas putida*, T9: *Pseudomonas syringae*, T10: *Serratiamarcescens*, T11: *Bacillus polymys*, *Bacillus anthracis*, *Bacillus. circulans*, *Ba-*

cillus thuringiensis, Bacillus panthothenicus, T12: *Bacillus thuringiensis, Bacillus panthothenicus, Serratia marcescens*, T13: *Pseudomonas cichorii, Pseudomonas putida, Pseudomonas syringae*, T14: *Pseudomonas putida, Serratiamarcescens*, T15: *Pseudomonas putidai, Bacillus thuringiensis*, T16: *Pseudomonas putida, Bacillus thuringiensis, Serratia marcescens*.

2.2.3. Processing the Substrate and Filling the Jars

The soil used consisted of depleted tropical ferruginous composite soil from the experimental site of the Agricultural Research Centre (CRA-Centre) in Gobé. This substrate is removed at a depth of 0 to 20 cm using a graduated shovel spade and is dried in the sun. It is then sifted to 2 mm and then doubly sterilized at 120˚C for 20 minutes at a 24-hour interval [29]. Then, 3 kg of the sterilized soil was weighed and distributed in each pot.

2.2.4. Description of the Chemical Characteristics of the Soil Used

The soil was analyzed in a previous study published in 2017 [30]. The data is in Table 1. This soil was moderately acidic, with a pH (water) of 6.5. The analysis also showed that the nitrogen (0.076%), phosphorus (5 ppm), potassium (0.16 meq)/100g) levels are very low. The sum of the exchangeable bases (3.66 meq/100g) is very small. On the other hand, the cationic exchange capacity (8 meq/100g) was average. These chemical properties reflect limited fertility of the study soil due to its low reserves of major nutrients (N, P and K) recorded.

2.2.5. Semi, Inoculation and Pot Maintenance

The pots were arranged inside the greenhouse and then watered at 2/9th of their maximum water retention capacity (CMR) 24 hours before sowing [31]. After opening the hole, two (02) seeds were deposited per pot and then inoculated with 10 ml of suspension of each bacterial strain of concentration 10^8 UFC/ml. The hole was immediately closed. A quantity of 500 ml water, or 1/9th of the soil's maximum water retention capacity (CRM), was brought to the plants every 48 hours after seed germination. Thinning to one plant per pot was performed one week after germination. The experiment was conducted in May 2019 under a greenhouse located within the grounds of the University of Abomeycalavi (West Africa, Republic of Benin) at an altitude of 22 m between latitude 6-258'N and longitude 2-20'E. The average temperature inside the greenhouse is 25.1˚C and 27.23˚C during the trial period.

2.2.6. Data Collection

Morphometric measurements were made 7 days after sowing. These morphometric

Table 1. Chemical characteristics of the soil.

pH (water)	Pa (ppm)	Nitrog (%)	MO (%)	K-ech (meq/100g)	Exchangeable bases (meq/100g)	CEC (meq/100g)
6.5	5	0.076	1.16	0.16	3.66	8

MO: organic matter; Pa (ppm): assimilable phosphorus; K-ech: exchangeable potassium; CEC: ability to exchange cationique, ppm: percentage per thousand; meq: milliequivalent.

characteristics were the height and diameter at the collar of the plants estimated respectively using a ruler tape and the caliper at the slide sliding every 96 hours for 30 days. At that time, the leaf surface of the plants was calculated according to the method described by [32] using the measurement of the length and width of the last two leaves ligulate of the plants. In addition, the young corn plants were gently dug up and the roots were immersed in a large bowl of tap water to rid them of soil particles. The freshly weighed biomass was sun-dried at 100°C for 72 hours to determine the dry weight of aerial and underground biomass.

2.2.7. Statistical Analysis

The Hierarchic Classification on Main Components (HCPC) preceded by a Main Component Analysis (ACP) was conducted to identify the pattern of discrimination caused by the effect of the PGPR and their combination on the growth and productivity parameters. The combination of these two consecutive multivariate analyzes is useful for showing the pattern across the data set [33]. The different classes obtained were described using descriptive statistics (mean, standard deviation). These multivariate analyzes were performed in the R 3.6.0 software [34] and required the use of the FctoMine R and factoextra packages. Within each group, the effect of PGPR and their combination on neck height and diameter was assessed for each parameter by fitting a linear mixed effects model to longitudinal data. In each model, the groups obtained were considered as fixed factors and time as a random factor. Adjusted averages were also calculated to represent the evolutionary trends of each growth parameter in each group. These analyzes were done with the R software using the packages nlme (for the fit of the model), lsmeans (for the computation of the adjusted averages), and ggplot2 (for the representation of the curves). The impact of PGPR and their combination on leaf area and plant yield parameters were assessed using an analysis of variance after verification of normality and homoscedasticity of the data respectively by Ryan-Joiner and Levene tests [35]. Post hoc or multiple comparison tests (SNK test) were performed to assess the statistical differences in averages when anova results are significant. Adjusted averages were also calculated to represent the averages of each parameter for each group. The car and lsmeans packages were respectively used to perform the anova, and the calculation of the adjusted averages.

3. Results

3.1. Identifying Homogeneous Groups of Treatments

The ACP carried out on the different parameters of plant growth and yield shows that the first two axes retain 78.67% of the total variance. All growth parameters and yield parameters are strongly correlated with axis 1 and only the BSS variable (dry underground biomass) which is strongly correlated with axis 2 (Figure 1(a)).

The hierarchical classification of main components (HCPC) applied to the averages of the measured variables allowed the definition of four treatment
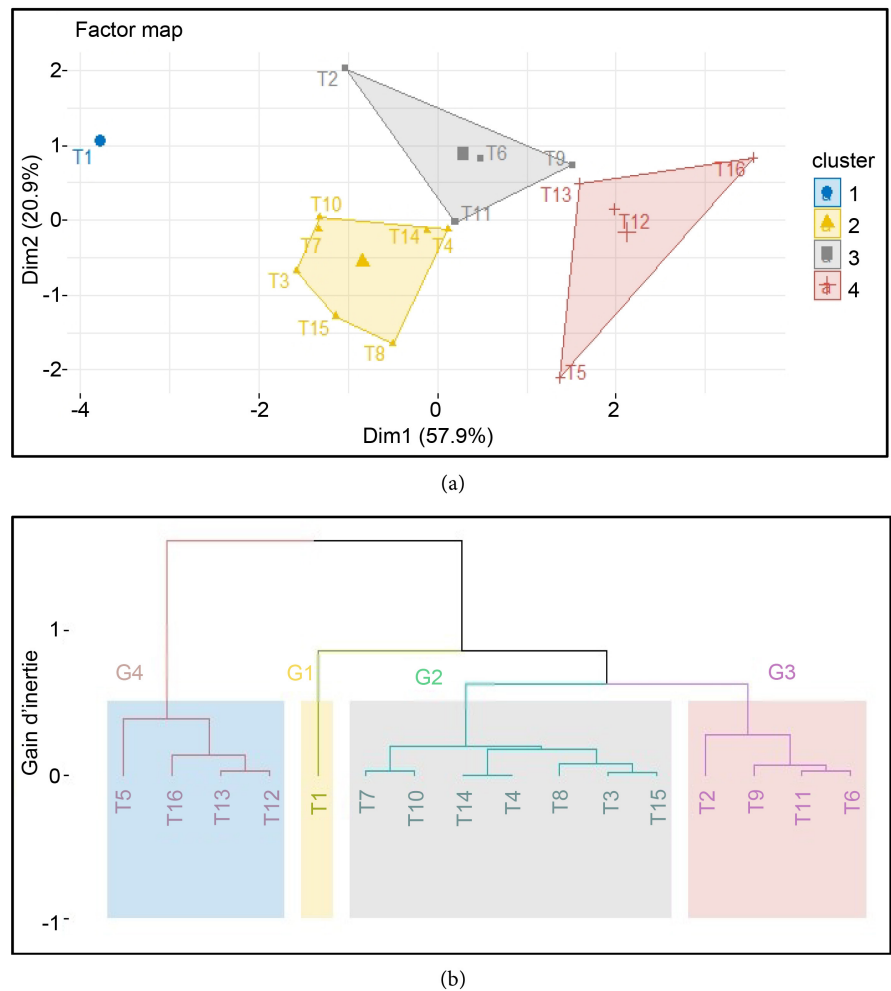
Figure 1. (a) ACP result showing different clusters based on the links between the measured variables; (b) Dendrogram of classes of different treatments (PGPR and combination) applied to plants.

groups (**Figure 1(b)**) discriminated against by the height, neck diameter and underground dry biomass variables. These homogeneous groups (G1, G2, G3 and G4) obtained were used to analyze the effect of PGPR and their combination on the growth and yield parameters of maize plants.

The first group (G1) consists of a single treatment, the control (T1). The second group (G2) consists of *B. anthracis* (T3), *B. circulens* (T4), *P. putida* (T8), *P. cichorii* (T7), *S. marcescens* (T10), *P. Putida, S. marcescens* (T14) and *P. Putida, B. Thuringiensis* (T15). Four treatments including a combination of PGPR are classified as Group 3 (G3). These are *B. polymyxa* (T2), *P. syringae* (T9), *B. panthothenicus* (T6) and *B. polymyxa, B. anthracis, B. circulatens, B. thuringiensis, B. panthethonicus* (T11). Group 4 (G4) consists of *B. thuringiensis* (T5), *B. thuringiensis, B. panthethonicus, S. marcescens* (T12), *P. cichorii, P. putida, P. syringae* (T13) and *P. putida, B. thuringiensis, S. marcesens* (T16). It should be noted that the treatments constituting group 4 are mostly combinations of several PGPRs.

3.2. Effect of PGPRs and Their Combination on the Growth Parameters of Corn Plants

3.2.1. Effect of PGPRs and Their Combination on the Height of Corn Plants

Based on the results of the mixed-effect linear model (Table 2), it appears that time has a significant effect on plant height growth (p-value < 0.0001). However, the treatment (groups) (p-value = 0.62) and the interaction between time and treatment (p-value = 0.45) are not significant, indicating that the variations observed over time do not at first depend on treatment.

The curve (Figure 2) shows the impact of treatment groups on plant height. Plants subjected to the effect of the G1 group (control treatment) are characterized by an average height of 26.4 cm. This is the lowest height performance recorded during the study. On the other hand, plants that benefited from G4 treatments had the best average heights (31.72 ± 0.28 cm) an improvement of

Table 2. Mixed-effect linear model results applied to plant height averages.

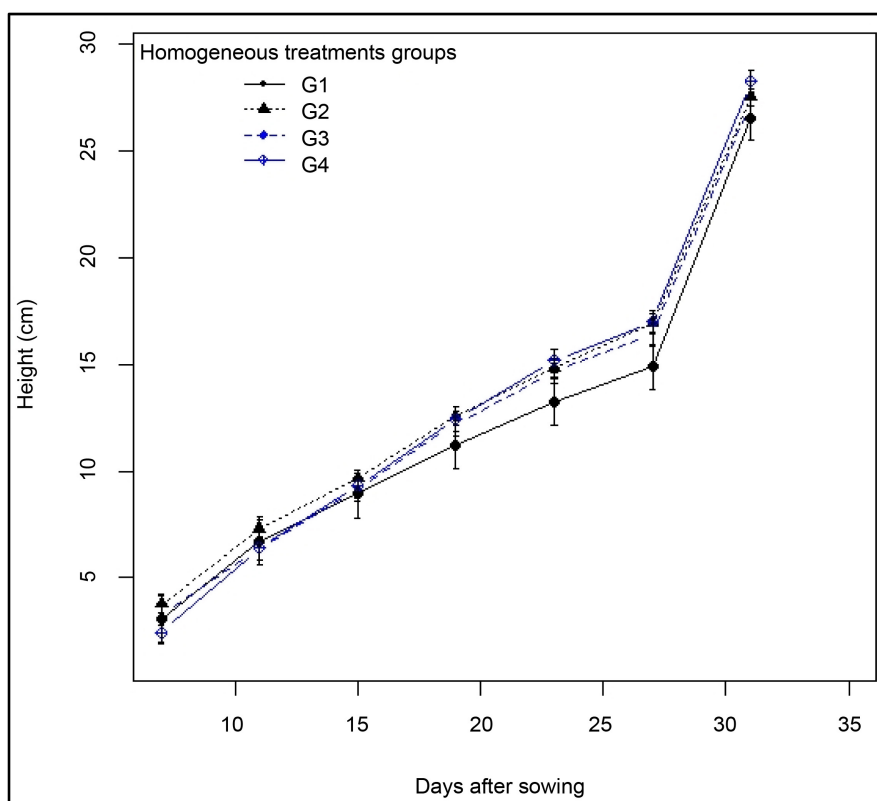|	numDF	denDF	F-value	p-value
(Intercept)	1	326	3824.441	<0.0001
Time	**1**	**326**	**2209.732**	**<0.0001**
Groups	3	326	0.598	0.6166
Time: Groups	3	326	0.870	0.4567

Figure 2. Evolutionary trend in the height of corn plants by groups of rhizobacteria.

20.15% compared to the G1 group (control). The minimum and maximum heights of this group (G4) were 31.33 cm and 31.93 cm respectively. At the end of the trial, the group 2 (G2) plants had a minimum height of 26.83 cm with a peak of 30.03 cm. For group 3, the minimum plant size was 27.83 cm while the highest recorded height was 29.16 cm. It should be noted that group 2 and group 3 (G3) treatments had similar effects on plant height after 30 days of sowing. The average values obtained were 28.48 ± 1.34 cm and 28.44 ± 0.58 cm respectively. This means that the height of the group 4 plants increased by about 12.11% compared to that of groups 2 and 3.

3.2.2. Effect of PGPR and Their Combination on the Diameter at the Collar of Corn Plants

Analysis of mixed-effect variance (Table 3) applied to plant collar diameter averages shows that time has a significant effect on the growth of plant collar diameter (p-value < 0.0001). The treatment (groups) (p-value = 0.02) and the interaction between time and treatment (p-value < 0.01) are also significant, indicating that the variations observed over time depend on treatment (group). The evolution curves of the diameter at the plant collar vary significantly from one collection period to another than from one treatment to another (Figure 3). In

Table 3. Mixed-effect linear model results applied to plant collar diameter averages.

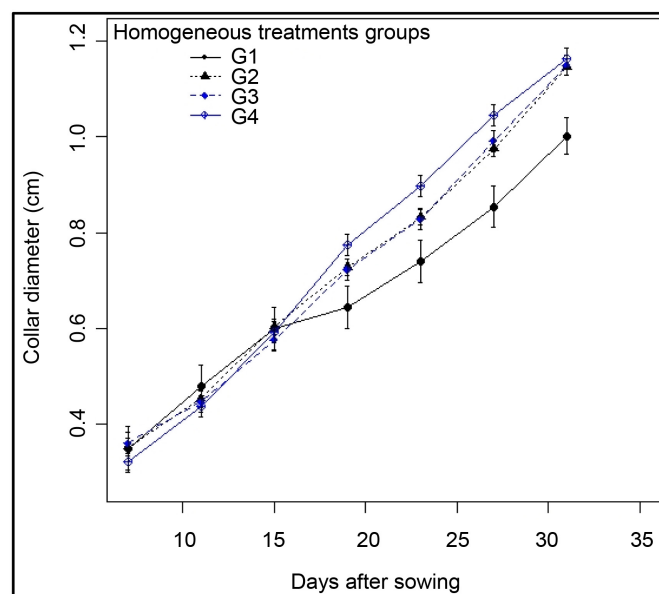|	numDF	denDF	F-value	p-value
(Intercept)	1	326	10320.943	<0.0001
Time	**1**	**326**	**1602.311**	**<0.0001**
Groups	3	326	3.103	0.0268
Time: Groups	3	326	4.476	0.0043

Figure 3. Evolutionary diameter trends at corn plant collars by treatment groups.

fact, non-inoculated plants representing group 1 (G1) members had the best diameters at the collar from the 7th to the 14th days after sowing. From this period, plants inoculated with group 4 (G4) rhizobacteria took over until the end of the test with an average diameter value at the collar of 1.21 ± 0.04 cm versus 1 cm for group 1 (G1) plants. This indicates that group 4 (G4) rhizobacteria induced a 21% increase over the control. Plants subject to the effect of the G3 group and group 2 give your intermediate tendencies between group 1 (G1) and group 4 (G4). The average collar diameter of 1.18 ± 0.03 cm was recorded in plants in these two groups (G2 and G3).

3.2.3. Effect of PGPR and Their Combination on Leaf Surface of Corn Plants

Figure 4 illustrates the effect of rhizobacteria inoculation by group of treatments on the leaf surface of plants. Measuring the leaf surface of plants shows a visible positive effect (**Table 4**) of inoculation by treatments (*B. thuringiensis*, *B. thuringiensis*, *B. panthethonicus*, *S. marcescens*, *P. cichorii*, *P. putida*, *P. syringae* and *P. putida*, *B. thuringiensis* *S. marcesens*) of group 4 followed by group 3 rhizobacteria with an average leaf area of 190.18 ± 19.90 m^2 and 173.74 ± 15.09 m^2 respectively. The performance of group 4 rhizobacteria improved the leaf surface of corn plants by more than 32.77% and 9.46%, respectively, compared
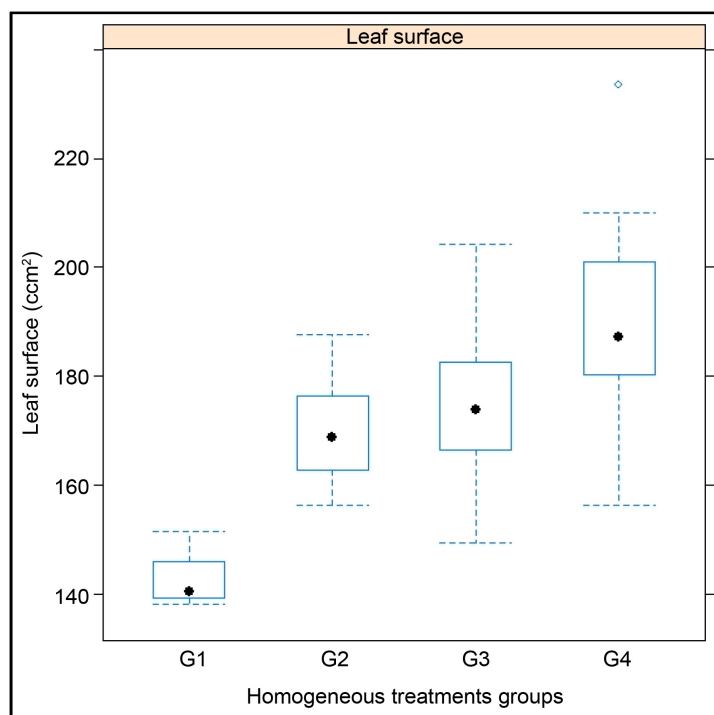

Figure 4. Effect of treatments by group on plant leaf surface.

Table 4. Results analysis of variance applied to averages of plant leaf surface.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)	
Groups	3	6522.1	2174.02	11.255	1.32e−05	***

to the control and group 3 (G3) plants.

3.2.4. Effect of PMPRs and Their Combination on the Biomass Yield Parameters of Corn Plants

In Figure 1, group 4 (G4) rhizobacteria inoculated plants produced the largest dry aerial biomass, an increase of 30.73% over the control. Based on the results of the variance analysis (Table 5), there was a significant difference (p < 0.001) between the different treatment groups. In addition, a highly significant improvement (p < 0.001) was observed between the treatment groups on dry underground biomass of the plants (Table 6). The best productions (3.42 ± 0.42 g) were recorded at the level of the plants of pin 3 (G3) followed by those of group 4 (G4) (3.06 ± 0.45 g). Non-inoculated plants of group 1 (G1) were the least productive.

4. Discussion

Various research has focused on the assessment of rhizobacteria for their ability to induce improvements in growth and yield parameters on controlled cereals (Qaisrani *et al.*, 2014) [36] (Karnwal, 2017) [37]. Based on the analysis of the data presented at the level of Figure 2, it is noted that the plants that benefited from the treatments *B. thuringiensis*, *B. thurenthonicus*, *B. panthothenicus*, *S. marcescenss*, *P. cichorii*, *P. putida*, *P. syringae* and *P. putida*, *B. thuringiensis S. marcesens* all in group 4 showed the best growth in height with an increase of 20.15% compared to control plants in the group 1. The promoter effect of these treatments exceeds by 12.11% that induced by *B. anthracis*, *B. circulens*, *P. putida*, *P. cichorii*, *S. marcescens*, *P. putida*, *S. marcescens* and *P. putida*, *B. thuringiensis* du gropue 2. It is the same for *B. polymyxa*, *P. syringae*, *B. panthothenicus* and *B. polymyxa* "*B. anthracis*" *B. circulatens*, *B. thuringiensis B. panthethonicus* classified in group 3. The same trend was observed on the diameter at the collar of the plants. Indeed, the highest neck diameter values were obtained by group 4 rhizobacteria. These treatments resulted in a 21% improvement over the diameter at the collar of non-inoculated group 1 plants. Our experimental results are consistent with those of Noumavo *et al.* (2013) [38], which reported increases of 26.77% and 30.25% respectively on the diameter at the collar as well as on the height of the corn plants inoculated with the combination of *P. fluorescens*, *P. putida* versus the witness. In addition, the work of Emami *et al.* (2019)

Table 5. Results analysis of variance applied to dry aerial biomass produced by plants.

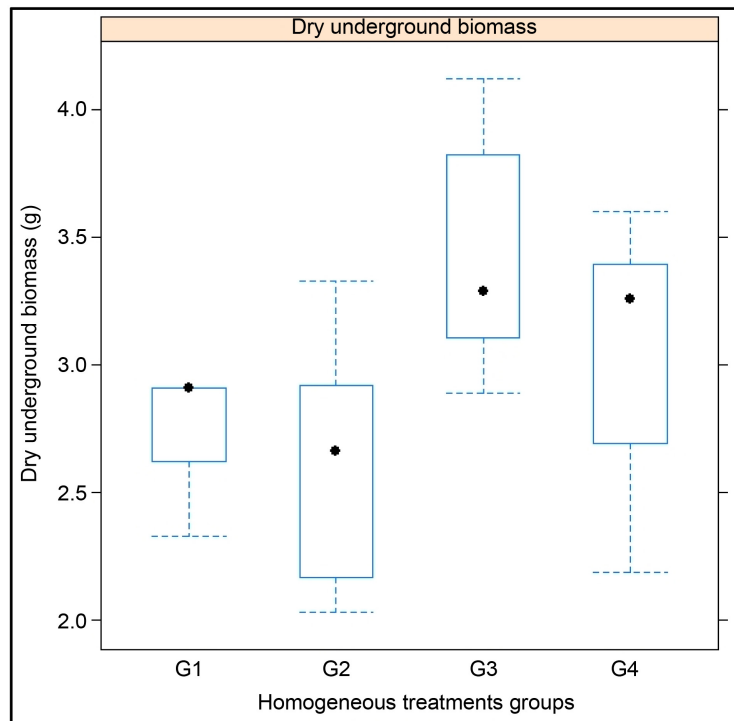|	Df	Sum Sq	Mean Sq	F value	Pr (>F)	
Groups	3	9.740	3.2468	4.4621	0.008041	**

Table 6. Results of variance analysis applied to dry underground biomass produced by plants.

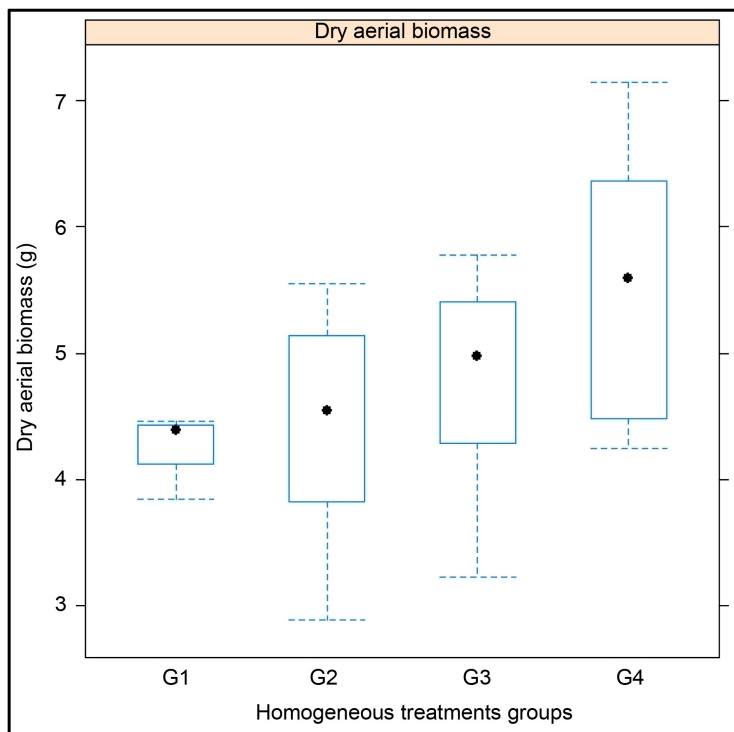|	Df	Sum Sq	Mean Sq	F value	Pr (>F)	
Groups	3	5.2194	1.73981	9.7649	4.675e−05	***

[39] have recently demonstrated that co-inoculation of eight bacterial strains of different taxa (*Pseudomonas*, *Bacillus*, *Stenotrophomonas*, *Serratia*, *Nocardia* and *Microbacterium*) with several PGP traits allows for increased growth of plants than mono-inoculation. From an agronomic point of view, the diameter at the collar appears as a very important parameter for the plants. The more robust they are, the more they will be able to withstand the environmental conditions that have become increasingly difficult in recent years as a result of climate change (Duponnois *et al.*, 2005) [40] (Biaou et Gnimadi, 2012) [41].

Among the parameters of agronomic interest is also the leaf surface used by researchers to measure the photosynthetic capacity of plants. In this study, inoculation of plants by rhizobacteria of different taxa had a highly beneficial effect on the leaf surface of the plants 30 days after sowing (Figure 4). The best leaf surfaces were recorded at the plant level both by mono-inoculation (*B. thuringiensis*) and by co-inoculation of group 4 rhizobacteria strains with an improvement of more than 32.77% over the control. Group 2 and 3 plants had leaves with an improvement of 21.29% and 17.92% respectively compared to non-inoculated plants. This means that the inoculation of group 4 rhizobacteria resulted in a leaf area gain of 11.48% and 14.85% respectively compared to the group 3 and group 2 plants. Such results were reported by Noumavo *et al.* (2013) [38] with inoculation of corn seeds on ferralitic soil in southern Benin. Other scientists have also observed increased plant height and leaf surface through inoculation with bacterial combinations (Govindappa *et al.*, 2011) [28]. Increased growth was also recorded on other crops such as wheat (Salantur *et al.*, 2006) [42], rice (Nandakumar *et al.*, 2001) [43] and on tomatoes (Oluwambeet Kofoworola, 2016) [44] due to co-inoculation of rhizobacteria. Akhtar *et al.* (2018) [45] justify the broadest leaves carried by corn plants under the influence of rhizobacteria combinations by better development of the root system of the plants; this is conducive to better absorption not only of nutrients but also of water and therefore of better productivity.

In terms of biomass yield, the dry weight of the aerial and underground biomass of the plants measured during our harvest study was significantly improved with inoculation compared to control plants (Figure 5). The results of the variance analysis applied to the average weights at the level of each group indicated a significant effect (Table 5) and a highly significant effect (Table 6) respectively for dry air biomass and dry underground biomass. Indeed, plants treated with *B. thuringiensis* and other combinations of group 4 rhizobacteria induced the best weight of aerial biomass (5.53 - 1.02 g) with an increase of 30.73% compared to the control. In contrast, the highest subterranean biomass dry weight (3.42 - 0.42 g) was obtained with *B. polymyxa*, *P. syringae*, *B. panthetonicus*, and *B. anthracis*, *B. circulans*, *B. thuringiensis*, *B. panthetonicus*, *B. polymyxa*) in group 3 followed by group 3 treatments 4. They induced an improvement rate of 26.19% and 12.91% respectively over the group 1 control plants. Similarly, Akhtar *et al.* (2018) [45] reported that the combination of 4 rhizobacteria (*Bacillus megaterium*, *Pseudomonas aeruginosa*, *Serratia spp.*, and

(a)

(b)

Figure 5. Effect of group treatments on production of dry aerial biomass (a) and dry underground biomass (b) of corn plants.

Pseudomonas fluorescens) significantly stimulated the dry weight of the aerial part of the 33.85% increase over controls, while the lowest dry aerial biomass

was recorded at the control corn feet. Significant increases in the biomass of wheat plants and improved absorption of nutrients have also been reported by Emami *et al.* (2018) [46] with greenhouse inoculation of *Pseudomonas* strains characterized by multiple PGP activities. The results of the dry weight of plant biomass obtained in our study show that dry matter production has been improved by inoculation. El Fakhri *et al.* (2010) [47] reported that dry matter is used for the production of new roots, their proliferation (root volume), their lengthening (length growth) and their maintenance.

The classification obtained on the basis of the dendrogram ([Figure 1(b)](#)) shows that most individually tested strains, for example *P. putida*, *P. cichorii*, *P. syringae*, *B. circulans*, *B. anthracis*and *S. marcescens*, did not express a high capacity of stimulation for all the parameters assessed compared to the performance of the combinations of the so-called strains. On the other hand, the inoculation of *B. thuringiensis* induced very interesting effects comparable to those produced by *B. thuringiensis*, *B. panthethonicus*, *S. marcescens*, *P. cichorii*, *P. putida*, *P. syringae* and *P. putida*, *B. thuringiensis*, *S. marcesens*. Several studies have reported the ability to promote the growth of plants in greenhouses or in the field conditions of *Bacillus strains*, alone or in combination with other strains (Lim and Kim, 2009) [48] (Win *et al.*, 2018) [49] Akinrinlola *et al.*, 2018 [50]. Our results are consistent with those of Ishizawa *et al.* (2017) [51] who stated that the effect of combining different strains of PGPR was similar to the effect of individual strains on Lemna minor growth stimulation. On the other hand, the results obtained in our study are at odds with those reported by Yamakawa *et al.* (2018) [52]. The authors of this work noted that the effect of combining two strains of PGPR promoting lentucle growth was less than their mono-inoculation. Previous studies have previously reported the stimulation capacity of *B. thuringiensis* on various crop species (Goes, 2012) [53] (Raddadi etal., 2008) [54] because of its ability to produce metabolites of agricultural interest (Raddadi *et al.*, 2008) [54]. This corroborates the results of work done in Canada by Emad *et al.* (2019) [55] which showed that R10 (*B. thuringiensis*) produced auxine (AIA), significantly improves the growth of *Lemna Minor*. Many authors have suggested that the effects of co-inoculation on plants depend on the particularity of the combinations used (Barea *et al.*, 1998) [56] Molina-Romero *et al.*, (2017) [57]. The work done by Agbodjato *et al.* (2018) [25] has demonstrated the ability of the majority of strains tested in our study to solubilize inorganic phosphate and produce metabolites of agricultural interest including acetic indole acid (AIA). The best results recorded on the parameters measured during our work would be related to the ability of PGPR strains to produce AIA, the siderophore, to solubilize phosphorus as it has been the case in many scientific works (Backer *et al.*, 2018) [58] (Amogou *et al.*, 2018) [59] (Won *et al.*, 2019) [60].

5. Conclusion

PGPRs are used in agriculture as soil biofertilizers. These microorganisms have

been shown to mediate interactions between plants and allow them to exploit soil resources. The results of this work confirm the importance of PGPR strains on height growth, neck diameter and dry weights of the aerial and underground portion of corn plants on depleted ferruginous soil in central Benin. Depending on whether combined or not, the best performance on the above parameters was recorded with *B. thuringiensis*; *B. thuringiensis B. panthethonicus S. marcescens* and *Pseudomonas cichorii, Pseudomonas putida*, and *Pseudomonas syringae* inoculation. Thus, the combined application of several strains of rhizobacteria to promote plant growth may have an effect comparable to that induced by mono-inoculation. These rhizobacteria presented desirable traits which might suggest promise for future field application to promote the growth of maize, thus contributing to sustainable agricultural practices in Benin.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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