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RESEARCH ARTICLE

IMPACT OF SELECTING PHOSPHATE SOLUBILIZING MICROORGANISMS ON THE GROWTH OF MAIZE AND SORGHUM USING VIVIANITE AS INORGANIC PHOSPHATE SUPPLY

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ABSTRACT

Phosphate Solubilizing Microorganisms (PSM) were isolated in NBRIP medium containing tricalcium phosphate (TCP) as the sole P source, from roots and rhizospheric soils of *Zea mays* collected in three localities (Bini, Mbang-boum, Borongo) of the Vina Division of Adamawa Region (Ngaoundéré, Cameroon). Ability of these PSM to solubilize phosphate was tested in modified nutrient agar medium, on 4 inorganic P forms: $Ca_3(PO_4)_2$, $AlPO_4.H_2O$, $FePO_4.2H_2O$ and vivianite (Phosphate Rock) as natural phosphate source. At least 117 isolates were obtained from roots and soils samples. To conduct test in Reyes basal liquid media, 20 preselected isolates showing excellent solubilizing results in solid medium were selected. Isolates BGL12 (from Borongo soil), BNL (from Bini soil) and RBNBL5 (from Mbang-boum root) successfully solubilize 3 types of phosphate out of 4 used. At the end of the solubilizing process; low pH (pH 4.5) instead of pH 7 in the initial media, revealed the release of organic acid in those media. The isolates RBNBL5, BGL12 and BNL boosted the phosphorus uptake of *Zea mays* and *Sorghum bicolor* L. Moench when growth in pot with phosphate rock (vivianite). Increase of the leaves number, the height, the dry biomass and the phosphorus content of these plants was noticed 42 days after planting.

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INTRODUCTION

More than 923 million people are chronically hungry while most of them leave in rural areas of poor countries (FAO, 2005). If world population continues to grow at the mean estimated annual rate of 1.1%, it will reach 9.3 billion people in 2050 compared to 7 billion today. Between 2005 and 2010, Cameroon had an annual population increase of 2.6%; it can then be projected that in 27 years (2037), its manpower will increase (MINEPAT, 2010). Following, FAO (2000, 2012) estimates that food production should satisfy 70% to 100% of the country's population. Therefore, the world urgently needs to increase its agricultural productivity, particularly in the less developed countries. The reduced agricultural productivity could be a result of increased demographic pressure coupled to bad practices of soil management. The low soil fertility is a fundamental problem with limits crop production.

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This problem becomes crucial in the tropical and subtropical countries where the soil exposed to erosions becomes poor in organic matter. This erosion leads to deficiency in soil nutrients, especially nitrogen and phosphorus. Elsewhere, in these tropical and subtropical soils, crop productivity is limited by acidity, aluminium (Al) toxicity, low cation exchange capacity and drought (Mohamed, 2012). As phosphorus is the second major nutritive element of the agricultural production after nitrogen, it is essential in crop production (Ehrlich, 1990; Bado, 2002) and need for making it available to improve the agricultural outputs is imperative. The use of chemical fertilizer's often results in immediate solution to the problem of biogenic soil deficiency. Indeed, these inputs can improve the production by 82% (Nyembo *et al.*, 2012). However, these fertilizers cannot easily be affordable and easily applied by farmers; it often leads to environmental hazards (Verdura, 2008). Beneficial microorganisms such as nitrogen fixers, mycorrhizal fungi and P solubilizing microbes are being selected and used by some authors for significantly increase diverse crop productivity in sub Saharan African soils (Nwaga *et al.*, 2010). Microorganisms are involved in a range of

processes that affect phosphorus transformation in the soil and are thus an integral component of P cycle (Deubel and Merbach, 2005). In particular, soil microorganisms are effective in releasing P from organic pools of total soil P by mineralization (Bishop *et al.,* 1994) and from inorganic complexes through solubilization (Narula *et al.,* 2000). Preliminary work carried out in South Cameroon on the bacteria isolated from root and rhizospheric soil of palm tree showed their beneficial effects on maize P supply and its production in greenhouse (Fankem, 2007; Fankem *et al.*, 2011). These results indicate that the selected bacteria could stimulate grain production of maize from 36 to 46% on Cameroon oxisols. Also these selected Phosphate Solubilizing Microorganisms (PSM) have shown their potential to improve by 50 to 160% P absorption by *Sorghum bicolor* and *Vignaunguiculata* under greenhouse conditions (Abba, 2006). Some of these beneficial micro-organisms can also protect crops against root diseases (Nwaga *et al.*, 2007a; Nwaga *et al.*, 2007b). Results of this work carried on in the humid forest zone of Cameroon could be useful for cereal production in the Sudano-sahelian and Guinea-savannah zones. Sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays* L.) are the main cereals produced and consumed by the majority of the population of Northern Cameroon (Mouna, 2007). Unfortunately, cereal production decreased in Sudano-sahelian and Guinea-savannah zones during this last decade, due to decrease of soil fertility. This soil fertility is linked to P availability and great need of cereal for P is well documented (Lott *et al.,* 2011). A rate of 30kg P/ha can substantially increase maize grain yield (Ojiem, 2004).

The main objective of this work is to assess ways of increasing P mobilization from insoluble phosphorus forms by the use of PSM, in order to improve the production of cereals in the Guinnea –Savannah zones of Cameroon. This may enhance food security and safety as well as alleviate poverty in Cameroon. Hypotheses of our study are:

Hypothesis 1: Microorganisms concentration in soils vary according to cultivation practice.

Hypothesis 2: All microorganisms available in soils are not PSM and PSM strains varies according to the substrate (Soils or Roots).

Hypothesis 3: Efficiency of each strain of PSM varies according to different forms of insoluble phosphorus that exist in soil.

Hypothesis 4: Each strains of PSM has its characteristics.

MATERIALS AND METHODS

Description of sites

Soils and roots samples were collected in three localities of Adamawa Region, at Bini, Borongo and Mbang-Mboum sites (Figure 1), while pot culture was conducted in Bini locality, inside the University campus. Adamawa Region is located in an area extending over about 72.000 km^2 with an average altitude of 1200m. It is located between 7°26'164" North latitude and 13°33'34" East longitude. Its climate is of the Sudano-Guinean moist trend, with two seasons: one rainy season from March to October, and one dry season from November to March. The annual average rainfall is 2381mm, the average annual temperature is 25.59 and the relative humidity is 66.47%. Bini site is about 15 km from the regional head quarters of Adamawa and is situated between 7.4° North latitude and 13.5° West longitude. Borongo site is about 30 km from the headquarter of Adamawa and is situated between 7.46° North latitude and 13.59° West longitude. Mbang-Mboum site is situated between 7.51° North latitude and at the 13.83° West longitude.

Cultural practices

Cultural practices are the concrete ways of acting farmers based on the objectives and in the context of constraints and opportunities (Papy, 1998). Regarding culture muskwari sorghum and maize, major cultural practices are plowing, clearing, burning, and herbicides treatments. These practices vary depending on the dominant vegetation.

Characteristics of vivianite

The vivianite rock phosphate was collected in Hangloa's locality situated between 7°20 and 7°30 North latitude and between 13°20 and 13°25 East longitude. It constitutions is as follows: Fe_2O_3(68.72 %), P_2O_5 (9.17%), Al_2O_3 (7.72 %) and SiO2 (9.67%) (Fodoué, 2012). This natural phosphate rock is reduced to fine powder before use.

Characteristics of maize and sorghum seeds

Maize and Sorghum were used to study the beneficial effect of selected PSM isolated from soil and roots samples. *Zea mays* seed Shaba variety (CMS 2019) with high productivity, large seeds and a three-month cycle phenotype was used. Seeds are obtained at the IRAD (Wakwa, Ngaoundéré). *Sorghum bicolor* L. Moenchseed local Safrarivariety grown in the FarNorth Cameroon for its productivity, resistance to drought, and short cycle (3 months), were chosen.

Collection of soils and roots samples

Three fields per site were chosen according to agricultural system, based on three different methods of weeding during the establishment of the field (labor, burnt and herbicide). Soils and roots fragment samples were collected on Z*ea mays* in the selected sites in Adamawa region (Bini, Mbang-Mboum and Borongo) according to Swift *et al.* (2001) method. Ten soils samples were air dried, crushed to pass through 2 mm sieve and thoroughly mixed to represent one composite sample. In the same way, roots fragments were collected in ten randomized *Zea mays* rhizospheres and thoroughly mixed to represent one composite sample. One part of samples was aseptically transferred to microbiology unit of the veterinary research laboratory of the IRAD Wakwa in the Adamawa Region (Ngaoundéré, Cameroon) for isolation of total microorganisms and Phosphate Solubilizing Microorganisms. Another part of samples was used for physico-chemical characterization according to standard methods.

Isolation of total microorganisms from soil and roots samples

Total micro-organisms population was isolated on Tryptone Soya Agar medium (TITAN BIOTECH L.T.D, 2008)). Ten gram (10 g) of soil sample was suspended in 90 ml of sterile distilled water to obtain the stock solution (10^{-1} dilution). Serial dilutions were prepared by adding 1ml of this stock solution into 9 ml sterile water until the 10^{-7} dilution was obtained. The 10^{-5}, 10^{-6} and 10^{-7} dilution were plated on Petri dishes at room temperature (28°C) for 48 to 72 hours and total micro-organisms were enumerated.

Isolation of PSM from soil and roots samples

The PSM were isolated and enumerated in National Botanical Research Institute's Phosphate growth medium (NBRIP) (Nautiyal, 1999), containing per liter of distilled water: 20g glucose, 5g $MgCl_2.6H_2O$, 0.25g $MgSO_4.7H_2O$, 0.25g KCl, 0.1g $(NH_4)_2SO_4$ and 5g $Ca_3(PO_4)_2$ with 0.5% Bromocresol green (BCG) as dye (Gadagiand Sa, 2002). Stock solution of 0.5% dye was prepared by dissolving a corresponding weight of BCG into 70% ethanol and the final pH adjusted to 6.5 with 1M KOH. The pH of the media was adjusted to 7.5 before autoclaving. A soil sample of 10g was suspended in 90ml of sterile distilled water and 10^{-1} dilution was obtained. Serial dilutions were prepared by adding 1 ml of the suspension made into 9ml sterile phosphate buffer, until the 10^{-7} dilution was obtained. Each dilution was plated in NBRIP medium plus 5g/l of $Ca_3(PO_4)_2$, and left to incubate for three days at 28-30°C according to the method described by Mehta and Nautiyal (2001). To isolate the bacteria from root, the adhering soil was gently shaken. Roots were washed with tap water for 2min to remove all adhering soil particles, followed by washing with sterile NaCl solution (0.85% (wt/vol)). Ten grams (10g) of roots were mortified in 90ml of 0.85% NaCl. After that, serial dilution samples were plated on the NBRIP medium as described above and left to incubate for five days at 28°-30°C. In both cases, colonies surrounded with halo/yellow zone, ascertain the solubilization of phosphorus. The index of solubilization (IS) (Qureshi *et al.,* 2012) was used as indicator for the isolate efficiency:

IS = (Colony diameter n + diameter of halo zone z) / Colony diameter n.

Colonies with great index of solubilization will be retained for the following experiments.

Estimation of PSM solubilization in solid media

To test the capability of the PSM to solubilize inorganic phosphates, the modified Nutrient Agar medium (Fankem, 2007) was used plus dye. It made as follows per liter of distilled water: 3.0g NaCl, 3.0g yeast extract, 5.0g peptone, 5.0ml of 0.5% BCG (pH indicators), 15.0g agar. Different inorganic phosphate types were added: 5g Ca_3 $(PO4)_2$, 5g $FePO_4.2H_2O$, 5g AlPO4, or 5g vivianite (PR) at pH 7.5. A 10µl of suspension of two days grown culture were used to inoculate each quarter part of the Petri-dish. Five days later, the diameter of the colonies (n) and that of the halo zone (z) were measured with a Vernier caliper and index of solubilization ((z+n)/n or IS) was evaluated.

Estimation of PSM solubilization in liquid media

To test the capability of the PSM to solubilize inorganic phosphates, the modified Nutrient Agar medium (Fankem, 2007) was used plus dye. It is made as follows per liter of distilled water: 3.0g NaCl, 3.0g yeast extract, 5.0g peptone, 5.0ml of 0.5% BCG (pH indicators), 15.0g agar. Different inorganic phosphate types were added: 5gCa_3 $(PO4)_2$, 5g $FePO_4.2H_2O$, 5g AlPO4, or 5g vivianite (RP) at pH 7.5. A 10µl of suspension of two days grown culture were used to inoculate each quarter part of the Petri-dish. Five days later, the diameter of the colonies (n) and that of the halo zone (z) were measured with a Vernier caliper and index of solubilization ((z+n)/n or IS) was evaluated.

Estimation of PSM solubilization in liquid media

Reyes basal medium (Reyes *et al.,* 1999) described as followed per liter distilled water: 0.1g NaCl, 0.5g $MgSO_4.7H_2O$, 0.1g $CaCl_2.2H_2O$, 0.5mg $FeSO_4.7H_2O$, 1.56mg $MnSO_4.H_2O$, 1.40mg $ZnSO_4.7H_2O$, 2.0µg vitamin B12 and 30.0g of sucrose was used to assess Phosphorus concentration. Inorganic phosphate sources were added at a concentration of 5g/l of individual inorganic phosphate types ($Ca_3(PO_4)_2$, $FePO_4.2H_2O$, $AlPO_4.H_2O$ or vivianite). After sterilization, each flask containing 50ml of Reyes basal medium received 200µl of a two days culture growth in medium described subsequently in above paragraph. Inoculated flasks (samples) and un-inoculated ones (control) in triplicate were incubated at 28°C on a rotary shaker at 150 $rev.min^{-1}$ in the dark. Seven days after, 3 ml of each flask were centrifuged at 10.000g for 10 minutes at 4°C and used for pH measurement and for colorimetric determination of dissolved phosphate (P) following the method described by Murphy and Riley (1962).

Green house trials with the Selected PSM

Five liter plastic pot was used in green house experiment with 5kg of a mixture of sterilized sand and soil in which 1g of vivianite was added before cultivation. Plastic pot was filled with Rorisonnutrient solution made of macro-nutrients ($MgS0_4.7H_2O$:120,02g/l; $Ca(NO_3)_2.4H_2O$: 238,04g/l; KH_2PO_4. $3H_2O$: 115,38g/l and micro-nutrients (FeDTA: 12,5g/l; $MnSO_4.4H_2O$: 1,121g/l; H_3BO_3: 1,142g/l; $(NH_4)_6$ $Mo_{24}.4H_2O$: 0,930g/l; $ZnSO_4.7H_2O$: 0,220g/l; $CuSO_4.5H_2O$: 0,198g/l). When necessary, 8ml of the nutrient solution was added to 1000 ml of distilled water and the final solution was used during the growth period of plant. *Zea mays* and *Sorghum bicolor* L. Moench seeds were sterilized with sodium hypochlorite (25g/l) for 5 min and then finely washed twice with sterile distilled water before sowing. All the sowed seeds received 1 ml of a 2 days microbial culture. Uninoculated (control) and inoculated pots were irrigated and randomly disposed in a greenhouse subjected to light. The experiment included 7 inoculated bacterial treatments (BG12, BN, RBNB5, BG12+BN, BG12+RBNB5, BN+RBNB5 and BG12+ BN+RBNB5) and a control without inoculation; all were performed in 12 replications. Plants height and leaf number were evaluated at 42 days after planting (DAP) as growth characteristics. All the plants were harvested and parameters such as shoot dry weigh and P content was evaluated using the method described by Murphy and Riley (1962).

Statistical Analysis

Means data of results were compared after statistical analysis using One-way ANOVA Turkey test performed by Graph Pad Prism version 5.00 for Windows, Graph Pad Software, San Diego California USA, and www.graphpad.com".

RESULTS

Physico-chemical characteristics of different sites and soil samples

Table 1 showed that all the soils were classified as acidic soil because the pH is less than 6. Furthermore, soils in the Adamawa Region are acidic, with the most acidic ones in Bini and Borongo sites (pH 4.8-5.0). The available phosphorus is very low and varied from 8.15mg/kg for Borongo to medium at 20.3mg/kg for Mbang-Mboum. Quantities of iron and

aluminum are high in Bini site (192.0meq/100g and 93.89meq/100g respectively) and low in Borongo site (54.85meq/100g and 41.40meq/100g respectively). In Mbang-Mboum site, these two elements accounted for 60.12 and 19.42meq/100g respectively. The organic matter is around 3% for Biniand Borongowhile 6.7% for Mbang-Mboum.

Total microorganisms and Phosphate Solubilizing Microorganisms

The total number of microorganisms and PSM in soils vary according to site and cultural practices (Table 2). In Mbang-Mboum site, the density of PSM from soils samples is high (16.7 to 40%) while it is low in Borongo site (16.7 to 28.6%) and in Bini site (10-14.3%) compared to the total microorganisms. In Bini (44.8% to 80%) and Borongo (42.2% to 81.3%) site, the density of PSM from roots samples is also high but, in Mbang-Mboum (13.8% to 21.4%) site, it is very low.The percentage of PSM is higher in root samples (42.8%) andvery low in soil samples (18.1%). In general, the percentage of PSM is high in the Burn and Herbicide practice than in the labor practice.

Phosphate-Solubilizing Microorganisms Efficiency

The selected isolates were able to show P-solubilizing halo zone (z) either on $Ca_3(PO4)_2$, $FePO_4 \cdot 2H_2O$, $AlPO_4 \cdot H_2O$ or on vivianite (Rock Phosphate) on solid Nutrient Agar medium (Figure 2). Many isolates showed significant results on agar plates with high Solubilization Index (SI) after 5 days of incubation at 28±2°C temperature. The SI varied from one phosphate type to another (Table 3). The SI of isolates on (Ca-P), varied from 1.68 (BGH1) to 3.82 (BGL1), from 1.00 (RBNBL5) to 4.36 (BGH1) on $FePO_4.2H_2O$, from 1.00 (BNBH26, BNBH, RBNBH7, RBNBL12, RBNL3, RBNBBr1, BNL) to 3.26 (RBGL7) on $AlPO_4.H_2O$ and from 1.00 (RBNBL5, RBNBH2, RBGBr7, RBGL7, BNBL17) to 3.27 (RBNBBr1) on vivianite (RP). The acidification of the medium showed the process of phosphate solubilization by changing the color in the zone surrounding the colony into yellow by some PSM. This yellow activity indicates a decrease of pH and an increase in acidity.

Quantitative determination of phosphorus solubilization in liquid media

Table 4 show the values of P (mg/l) solubilized in liquid media after seven days of incubation. In media with $Ca_3(PO_4)_2$, the concentration of P obtained with all the isolates was significantly different from those of control, showing that the tested isolates have converted the inorganic insoluble phosphate into soluble form. In that medium, the most efficient is represented by strain BGL12 (159.5mg P/l) followed by RBNBL5 (152.16 mg P/l) isolated from soil and root samples collected at Borongo and Mbang-Mboum sites respectively. According to the medium with $FePO_4 \cdot 2H_2O$, all the tested isolates have converted the insoluble phosphate into the soluble form. In these media, the most efficient is represented by strain RBNBL5 (92.35mg P/l) followed by BGL12 (86.82mg P/l) isolated from root and soil samples from Mbang-Mboum and Borongo sites respectively. The values of P obtained with all the isolates in the medium with $AlPO_4.H_2O$, were significantly different from that of control. In this medium, the most efficient is represented by strain BNBLl7 (56.97 mg P/l) followed by BNBH (48.55 mg P/l) from Mbang-mboum site. The values of P obtained with all the isolates in the medium with Phosphate Rock were significantly different from that of control. In this medium, the most efficient is represented by strain BNBH (36.58mg P/l) followed by BNBL (36.48 mg P/l) isolated from soil sample collected at Mbang-Mboum. In general, Ca-phosphate, Fe-phosphate, Al-phosphate and rock phosphate (vivianite) solubilization seems to be linked with acidity increase (pH decrease) of the medium but this pH decrease was not correlated to the amount of the phosphate solubilized.

Activity of PSM on the Growth and Phosphorus Uptake of Maize and Sorghum

To study the effect of PSM on plant growth and phosphorus uptake after 42 days, maize and sorghum were selected because they are the main cereal produced and consumed by most population of the Northern Cameroon. The inoculation of seeds by the PSM has significantly affected the two cereal plants.

Activity of Phosphate Solubilizing Microorganisms on the Growth of *Zea mays*

Plant height

In general, maize plants growth was stimulated by inoculation with the selected PSM (Figure 3a). The highest score was obtained with treatments BGL12+BNL+RBNBL5 (27.3 cm), BGL12+RBNBL5 (26.0 cm), BGL12+BNL (25.4cm), BGL12 (24.3cm) and BNL (24.0 cm). The results obtained by isolates RBNBL5 (22.6 cm) and BNL+ RBNB5 (20.2cm) are not significantly different from that of control (21.5cm) with P value < 0.05.

Plant leaves

Inoculation of PSM and vivianite (RP) improved maize number of leaves (Figure 3b). The best number of leaves is obtained by the treatments BGL12+BNL+RBNBL5 (5.92), BGL12 (5.75), follow by BGL12+RBNBL5 (5.50), BNL (5.17), BNL+RBNBL5 (5.08) and are significantly different from the Control (P<0.05). The treatments made of isolates RBNBL5 (4.67), BGL12+BNL (4.42) is not significantly different from that of control (4.58). Treatments can be classified in the following order: BGL12+BNL+RBNBL5> BGL12>BGL12+RBNBL5>BNL>BNL+RBNBL5>RBNBL5 >Control>BGL12+BNL.

Shoot dry weight

All PSM isolates inoculation significantly increased the shoot dry weight compared to the non-inoculated control(Figure 3c). The best performance was obtained with treatment made with the mixture of 3 PSM isolates BGL12+BNL+RBNBL5 (4.20g) followed by the mixture of 2 PSM isolates BGL12+BNL (3.12g), BNL+RBNBL5 (2.52g) and 1 PSM; BNL (2.51g), BGL12 (2.46g). The result obtained by BG12+RBNBL5 (1.54g) and RBNBL5 (1.46g) are not significantly different (P<0.05) from the Control (1.47g). The best result obtained by the association of 3 PSM, BGL12+BNL+RBNBL5 (4.2g) is significantly different from the Control (1.47 g) with P value<0.05.

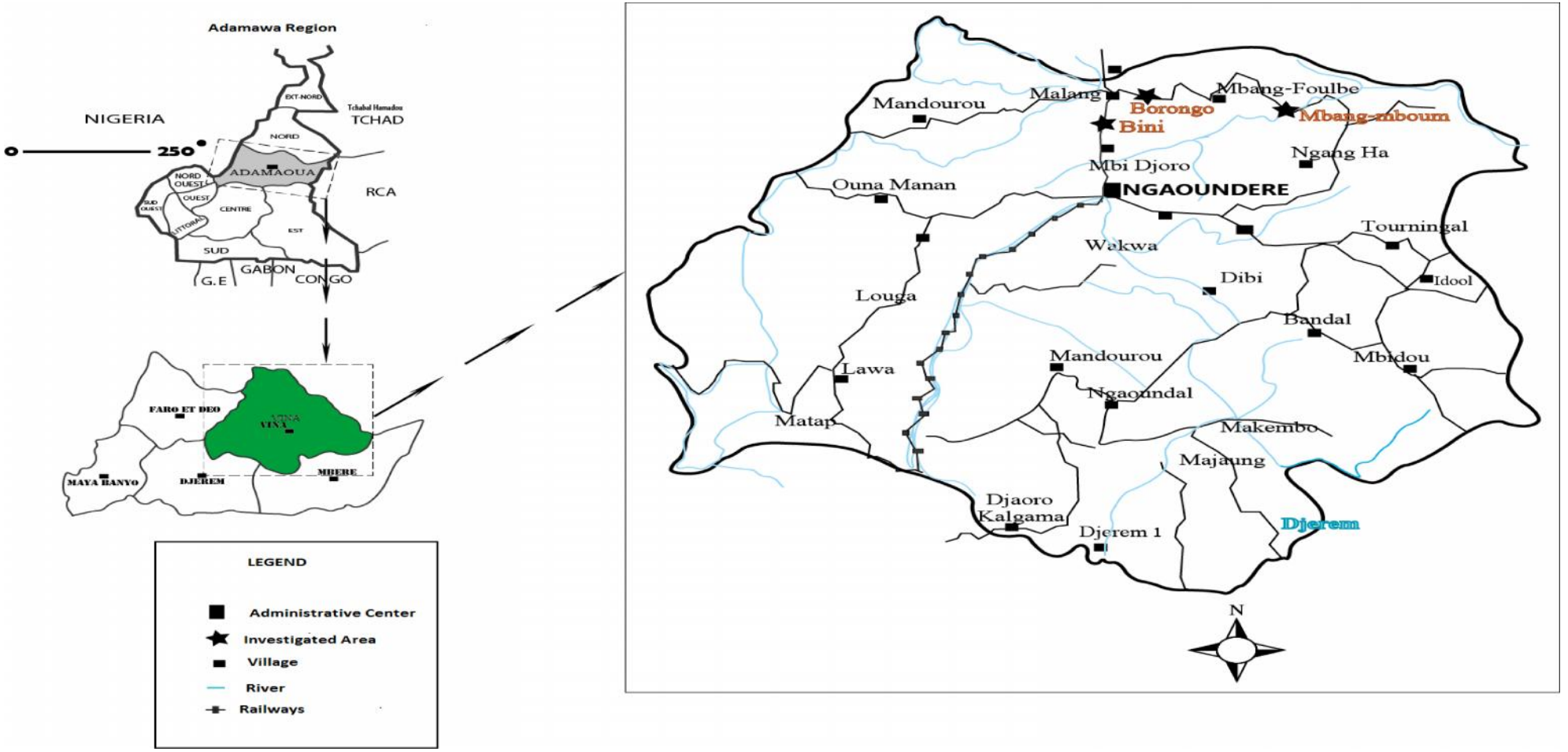

Figure 1. Localization of Adamawa in Cameroon and distribution of investigated area in Sahelian zone

(1) BGL12 **(2) BNL** **(3) BGL12** **(4) RBNBL5**

Figure 2. (1-4): Halo zone surrounding colonies of PSM on five days agar plates culture containing $FePO_4 \cdot 2H_2O$ (1), $AlPO_4 \cdot H_2O$ (2), $Ca_3(PO_4)_2$ (3) and Vivianite (4) as phosphates sources

Table 1. Physico-chemical characteristics of different soil samples

		Parameters											
Sites	Isolates codes	Sand (%)	Silt (%)	Clay (%°)	pH	MO (%)	CEC (méq/100g)	K (méq/100g)	Ca* (méq/100g)	Mg (méq/100g)	Available P* (mg/kg sol)	Fe* (méq/100g)	Al* (méq/100g)
	BNL	27	35	37.30	5.0	3.20	25.2	0.2	15.04	3.73	12.35	192.0	93.89
Bini	BNbr	25.70	34.27	38.20	5.07	4.03	25	0.25	15.02	3.96	12.89	188.16	90.25
	BNH	26	36	38	5.12	3.85	25.5	0.31	15.00	3.84	13.06	190.03	92.05
	BGL	40	29	31	4.8	6.40	11.7	0.21	0.4	0.16	8.15	54.85	41.40
Borongo	BGbr	42.61	27.95	30	4.82	6.35	11	0.26	0.6	0.15	9.10	56.10	40.21
	BGH	41	28.38	31.84	4.62	6.12	11.5	0.22	0.42	0.18	8.85	55.12	41.0
Mbam-mboung	BNBL	14	49	37	5.9	6.75	22.0	3.42	3.52	1.52	20.3	60.12	19.42
	BNBbr	13.56	49.5	37.25	5.72	6.80	22.0	3.35	3.58	1.65	20.48	62.13	18.95
	BNBH	14.25	47.58	36	5.55	6.52	21.0	3.40	3.61	1.50	21.26	60.15	20.42

All data are mean of two replications; * aqua regia extract

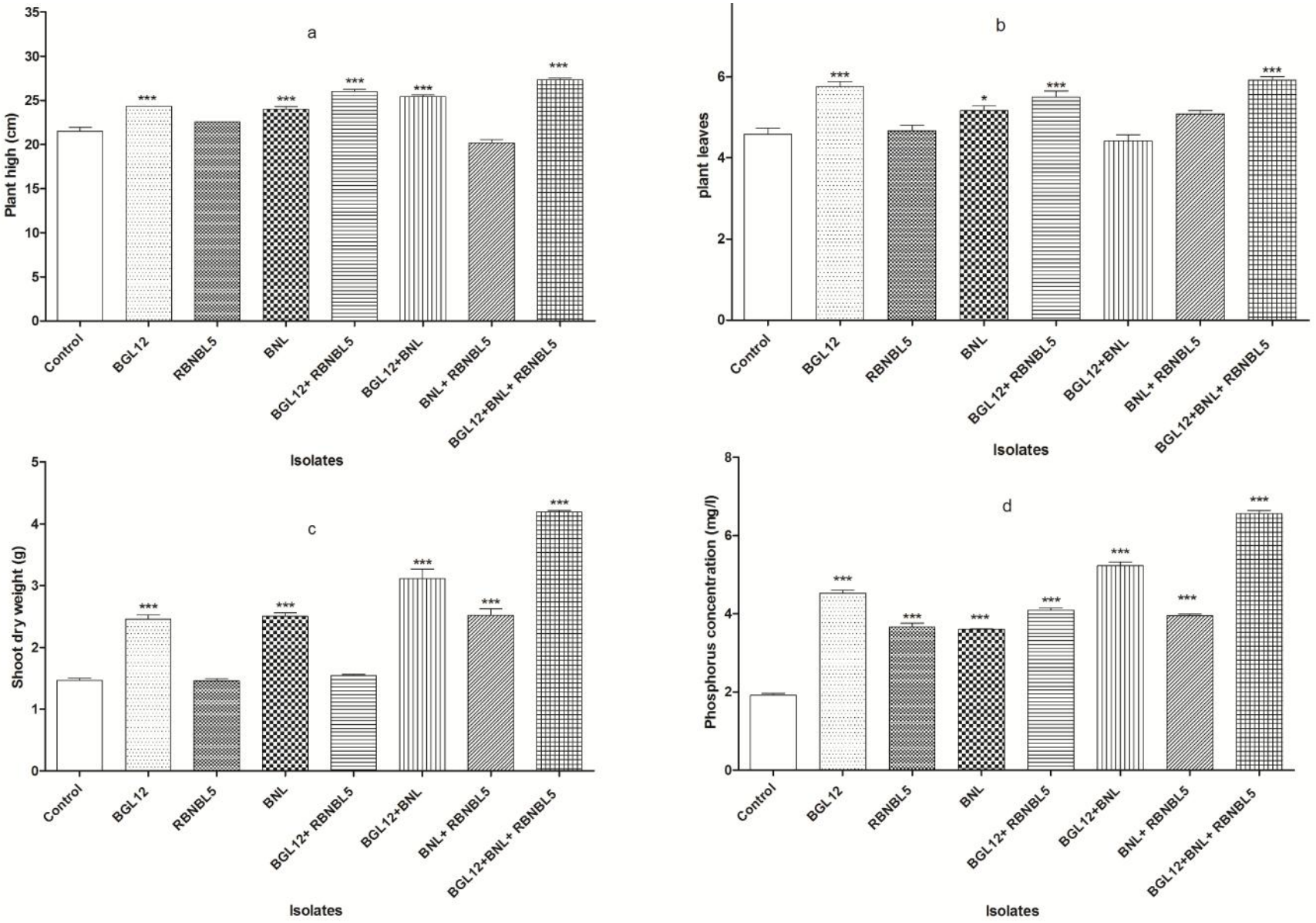

Figure 3. Effect of inoculation of selected PSM on the growth (a), plant leaves (b), shoot dry weigh (c) and P uptake (d) of maize (Zea mays)

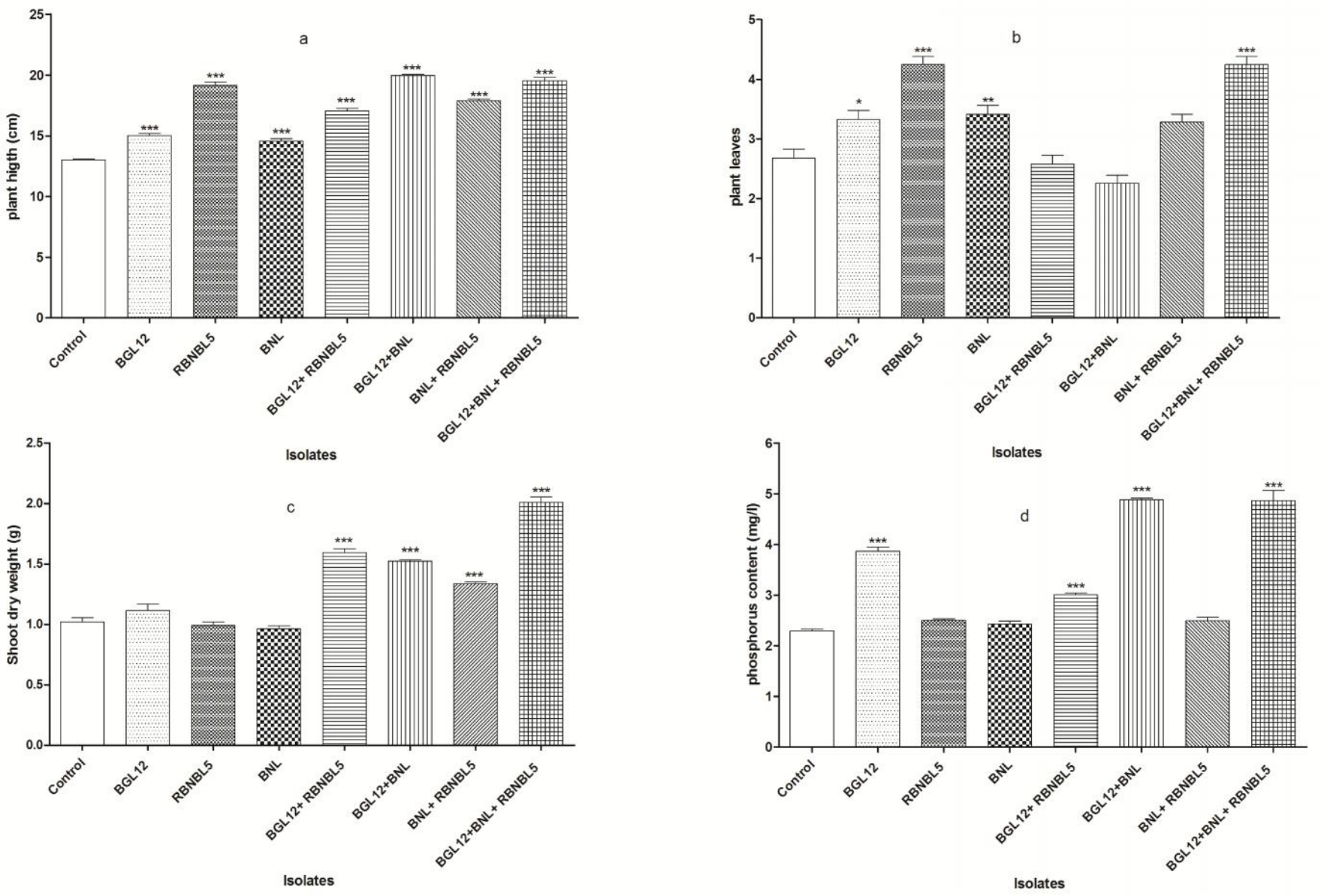

Figure 3. Effect of inoculation of selected PSM on the growth (a), plant leaves (b), shoot dry weigh (c) and P uptake (d) of maize (Zea mays)

Table 2. Total microorganisms and Phosphate Solubilizing Microorganisms from soils and roots samples

Region	Samples	Sites	Codes	Total Microorganisms (10^6 CFU/g)	PSM (10^6 CFU/g)	Percentage (%)
Adamawa	Soil	Bini	BNL	26	3	11.5
			BNbr	10	1	10.0
			BNH	7	1	14.3
		Borongo	BGL	10	2	20.0
			BGbr	7	2	28.6
			BGH	6	1	16.7
		Mbang-mboum	BNBL	6	2	33.3
			BNBbr	6	1	16.7
			BNBH	5	2	40.0
			Average	9.22	1.67	18.1
	Roots	Bini	RBNL	29	13	44.8
			RBNbr	10	8	80.0
			RBNH	5	3	60.0
		Borongo	RBGL	45	19	42.2
			RBGbr	23	18	78.3
			RBGH	16	13	81.3
		Mbang-mboum	RBNBL	42	9	21.4
			RBNBbr	29	4	13.8
			RBNBH	10	2	20.0
		Average		23.2	9.9	42.6

CFU = colony-forming unit

Legend of isolates codes

BNL : Bini Labor	BGL : Borongo Labor	BNBL : Mbang-mboum Labor
BNbr : Bini burnt	BGbr : Borongoburnt	BNBbr : Mbang-mboum burnt
BNH : Bini Herbicide	BGH : Bororngo Herbicide	BNBH : Mbang-mboum Herbicide
RBNL : Root Bini Labor	RBGL : Root Borongo Labor	RBNBL : Root Mbang-mboum Labor
RBNbr : Root Bini burnt	RBGbr : Root Borongo burnt	RBNBbr : Root Mbang-mboum burnt
RBNH : Root Bini Herbicide	RBGH : Root Borongo Herbicide	RBNBH : Root Mbang-mboum Herbicide

Table 3. Phosphate Solubilizing Microorganisms halo's zone on nutrient agar with diverse phosphate sources:$Ca_3(PO_4)_2$, AlPO4.H2O, Vivianite or $FePO_4$, $2H_2O$

	Medium with $Ca_3(PO_4)_2$			Medium with $FePO_4.2H_2O$			Medium with $AlPO_4 \cdot H_2O$			Medium with Vivianite			
Isolates	z	n	(z+n)/n	z	n	(z+n)/n	z	n	(z+n)/n	z	n	(z+n)/n	
BNBL22	5.68	2.8	3.03	7	4	2.75	1	1.6	1.63	8.42	4.5	2.87	abcd
BNBH26	7.44	5.22	2.43	4	2.5	2.6	0	5.5	1.00	10.38	5.79	2.79	abc
BGL12	10.28	3.65	3.82	8.5	3.85	3.21	8.5	4.5	2.89	8.42	4.16	3.02	abcd
BNBH	5.52	5.42	2.02	6.5	5	2.3	0	6	1.00	5.82	3.48	2.67	abc
BG9dBr	8.92	7.7	2.16	2.6	2.5	2.04	1.57	2.6	1.60	7.16	4.64	2.54	abcd
RBNBH7	4.37	3.5	2.25	6.5	5.98	2.09	0	4.2	1.00	3.13	2.48	2.26	abc
RBNBL12	4.68	3.1	2.51	4.2	2	3.1	0	4.6	1.00	8.44	5.62	2.50	abc
RBNL3	3.77	2.77	2.36	3.57	2.46	2.45	0	3.6	1.00	5.79	2.98	2.94	abc
RBNBBr1	0.18	0.12	2.5	6.22	2.43	3.56	3.35	4.9	1.68	6.71	2.95	3.27	abcd
RBNBL5	3.15	2.8	2.13	0	1.7	1.00	2.5	2.4	2	0	2.38	1.00	ab
RBNBH2	4.1	3.9	2.05	1.6	1.52	2.05	3.5	3	2.17	0	3.16	1.00	abc
RBNBBr8	4.77	3.67	2.30	3.18	2.8	2.14	3.5	2.43	244	1.35	0.6	3.25	abcd
RBNBH6	5.15	3.3	2.56	8.98	4.96	2.81	5.3	3.6	2.47	3.02	2.45	2.23	abcd
RBNBL3	3.7	2.6	2.42	5.44	4.2	2.30	5.36	3.98	2.35	4.02	3.12	2.29	abcd
RBNBH13	3.68	2.64	2.39	3.98	3.5	2.14	0	3.5	1.00	3.75	2.5	2.5	abc
BGH1	3.48	5.1	1.68	4,2	1.25	4.36	5.9	3	2.97	3.5	5	1.7	abcd
BGBr5	6.3	2.62	3.40	3.5	2.67	2.31	5.32	4	2.33	0	4.6	1.00	abc
RBGL7	5.38	2.64	3.04	5	2	3.5	6	2.65	3.26	0	5.4	1.00	abc
BNBL17	3.5	2.45	2.43	8.5	4.2	3.02	5	4	2.25	0	5.66	1.00	abc
BNL	5.55	4.6	2.21	7	2.5	3.8	0	6.3	1.00	8.3	6.32	2.31	abc

Data are mean of two replicationsa) Solubilization of $Ca_3(PO_4)_2$b) Solubilization of $FePO_4 \cdot 2H_2O$ c) Solubilization of $AlPO_4.H_2O$d) Solubilization of Vivianite (Rock Phosphate); z) Diameter of halo zone; n) Diameter of colony; (z+n)/n)) ratio between halo zone and colony diameter

Table 4. Phosphate Solubilizing Microorganisms (PSM) activities in liquid medium with diverse phosphate sources: $Ca_3(PO_4)_2$, $Al_3(PO_4)_2$, Vivianite and $FePO_4.2H_2O$

Isolates	Medium with $Ca_3(PO_4)_2$		Medium with $AlPO_4.H_2O$		Medium with $FePO_4.2H_2O$		Medium with Vivianite	
	P (mg/l)	pH	P (mg/l)	pH	P (mg/l)	pH	P (mg/l)	pH
BNBL22	61.48c	5	23.81b	4.52b	43.44d	4.27a	18.51a	4.37c
BNBH26	34.68a	5.11d	28.45b	4.76c	72.02g	4.37b	26.68b	4.47d
BGL12	159.5e	5.33d	46.72d	5.08d	86.82h	4.28a	19.57a	4.66e
RBNBL5	152.16e	4.67a	20.99b	4.72c	92.35i	4.1a	34.00c	4.08a
BG9dbr	62.38c	4.78b	31.70c	4.58b	25.33b	4.5c	25.58b	4.18b
BNBH7	57.88b	5.19d	15.47a	4.62c	63.35f	4.32b	34.07c	4.54d
RBNBL12	34.00a	4.82b	20.77b	4.39b	9.99a	4.5c	20.38a	4.21b
RBNL3	46.17b	4.8b	31.93c	5.1d	17.20b	4.43b	33.71c	4.2b
RBNBbr1	71.62c	5c	11.17a	4.55b	3.78a	4.6c	28.31b	4.37c
BNBH	162.38e	5.17d	48.55d	4.38b	53.74e	4.32b	36.58c	4.52d
RBNBH2	109.63d	5.09c	11.16a	4.07a	7.20a	4.35b	33.01c	4.45d
RBNBbr8	40.98b	4.74b	31.13c	4.43b	20.54b	4.5c	18.46a	4.14a
RBNBH6	62.16c	4.85b	20.51b	4.61b	24.57b	4.52c	20.60a	4.24b
RBNBL3	40.53b	4.96c	19.26a	5.15d	24.99b	4.25a	21.44a	4.34b
RBNBH13	29.27a	5.93e	15.36a	4.08a	34.48c	4.4b	24.39b	4.18b
BGH1	46.39b	4.87b	23.12b	4.85c	67.99f	4.42b	18.46a	4.26b
BGbr5	52.92b	4.55a	16.43a	4.16a	41.66d	4.31b	21.68a	3.98a
RBGL7	34.23a	4.81b	21.28b	5.4d	24.27b	4.5c	20.72a	4.2b
BNBL17	158.5e	4.7a	56.97e	4.2a	77.43g	4.28a	36.28c	4.11a
BNL	161.71e	4.72a	32.38c	4.4b	81.30h	4.4b	36.48c	4.13a
control	27.47a	6.70e	16.9a	6.92e	4.00a	6.43d	15.48a	6.25f

Data are means from experiments performed in triplicate. For each isolate, means in each column followed by different letters are significantly different ($P<0.05$)

Phosphorus content

Data observed show that maize plants have responded to inoculation with PSM isolates (Figure 3d). All treatments gave significant result (100%) and the best record was obtained with treatments BGL12+BNL+RBNBL5 (6.56mg/ plant), followed by BGL12+BNL (5.23 mg/plant), BGL12 (4.52mg/plant), BGL12+RBNBL5 (4.09mg/plant), BNL+RBNBL5 (3.95 mg/plant), BNL (3.66mg/plant), RBNBL5 (3.6mg/plant) and finally by Control (1.92 mg/plant) respectively. Treatments BGL12+BNL+RBNBL5 (6.56mg/plant) is significantly different from the control with P value<0.05.

Activityof Phosphate Solubilizing Micro-organisms on the Growth of Sorghum

Plant height

The Figure 4 showed that plants of sorghum were affected by inoculation with the selected PSM isolates. The highest score in plant height (Figure 4a) was obtained with treatments BGL12+BNL (20cm), BGL12+BNL+RBNBL5 (19.5cm) and RBNBL5 (19.1cm) while BNL+ RBNBL5 (17.9cm), BGL12+ RBNBL5 (17.1cm), BGL12 (15 cm) and BNL (14.6cm) represent the lowest score. These results are all significantly different (P<0.05) from that of the control.

Plant leaves

Inoculation of PSM isolates improved the number of leaves of Sorghum(Figure 4b). The best number of leaves is obtained by the treatments BGL12+BNL+RBNBL5 (4.25) and RBNBL5 (4.25). The inoculation of Sorghum with treatments BGL12 (3.33) and BNL (3.41) also affected the number of leaves and are significantly different (P<0.05) from the Control. But BNL+RBNBL5 (3.28), BGL12+RBNBL5 (2.58) and BNL+BGL12 (2.25) has not significantly affected the number of leaves and is not different from that of Control (2.68). According to these results, we can classify the treatments in the following order: BGL12+BNL+RBNBL5 = RBNBL5 >BGL12> BNL>BNL+RBNBL5>Control> BGL12+RBNBL5 > BNL+BGL12.

Shoot dry weight

PSM isolates are significantly increased the shoot dry weight of Sorghum compared to the control. Some treatments are not significantly different (P<0.05) from that of control. The best performance is obtained with treatment constituted with isolates BGL12+BNL+RBNBL5 (2.00g), BGL12+RBNBL5 (1.59g), BGL12+BNL (1.52g) and BNL+RBNBL5 (1.33g) compared to that of the control (0.91 g). The obtained result helps us to classify the treatments in the following order: BGL12+BNL+RBNBL5>BGL12+RBNBL5>BGL12+BNL>BNL+RBNBL5>BG12>BNL>RBNBL5 >Control.

Phosphorus content

Data observed show that plants of sorghum have responded to inoculation of PSM and Phosphate Rock. Treatments made by isolates RBNBL5 (2.50mg/plant), BNL+RBNBL5 (2.49mg/plant) and BNL (2.29mg/plant) are not different compared to that of the control (2.42mg/plant). Meanwhile, treatments made of BGL12+BNL+RBNBL5 (4.88mg/plant), BGL12+BNL (4.86mg/plant), BGL12 (3.87mg/plant) and BGL12+RBNBL5 (3.01mg/plant) significantly provide more phosphorus compared to that of the control with p<0.05. But treatments made of RBNBL5 (2.50mg/plant), BNL+RBNBL5 (2.49mg/plant) and BNL (2.29mg/plant) have a negative effect. According to the results of the absorption of phosphorus, the treatments will be classified as followed:

BGL12+BNL+RBNBL5>BGL12+BNL>BGL12> BGL12+RBNBL5>RBNBL5 > BNL+RBNBL5 >Control>BNL.

DISCUSSION

The main objective of our study was to isolate and evaluate the effect of PSM on the growth of *Zea mays* and *Sorghum bicolor* in pots culture. PSM was isolated in all our samples such as many authors found (Babana *et al.*, 2013; Fankem *et al.*, 2014). Their soil densities depended on the properties of theses soils (Khan *et al*., 2009) and some cultural practices. According to cultural practice, application of some type of herbicidecan stimulate the population of some PSM (Amal *et al.*, 2003). Alexander (1978), Gupta and Surat (1987) and Cork and Krueger (1991) report that those PSM utilized the herbicides and their degraded products for their energy, carbon and other nutrients. The characteristic of acidic soil and the small quantity of organic matter are factors that can affect bacteria density and diversity (Benaduzi *et al.*, 2013). In this case, NBRIP medium was used to isolate Phosphate Solubilizing Microorganisms and modified Nutrient Agar with Green Bromocresol dye as a pH indicator was used for a better observation of halo zone (Fankem, 2007). Many isolates showed halo zone on plates with insoluble inorganic phosphate ($Ca_3(PO4)_2$, $FePO_4.2H_2O$, $AlPO_4.H_2O$ and Rock Phosphate) according to other authors (Mehta and Nautiyal, 2001; Fankem *et al.*, 2006;N. Uma Maheswari and S. Sudha, 2013). The value of the ratio ((z+n)/n)) is an indicator for the phosphate solubilization. This technique of identification of PSM is not infallible. It is reported that some isolates which did not show any halo zone on Agar plates could solubilize insoluble inorganic phosphate in liquid media (Leyval and Barthelin, 1989; Deubel and Merbach, 2005 and Fankem *et al*., 2011) due to the low quantity of organic acid produced (Babana*et al.*, 2013). The inoculation of maize with PSM and Rock Phosphate positively increased plant height, number of leaves, shoot dry weight and phosphorus content. This result is similar to those of Abou-el-Seoud and Abdel-Megeed (2012) who reported the increase of dry weight of maize inoculated with bacteria and similar to Fankem *et al*. (2014) who showed that, in general, all the strains in single and in consortia significantly increased the number of leaves, stem base diameter, total dry weight, shoot dry mass and root dry weight of maize compared to non-inoculated control. In addition, there are many reports where researchers have found that PSM could increase maize growth and yield (Hussain*et al.*, 2013). This increase in growth may be attributed to auxin production, organic acids, synthesis of phytohormones, or phosphatases (Chabot *et al.*, 1996; Gyaneshwar *et al.*, 2002; Arcand and Schneider, 2006; Rodríguez *et al.*, 2006; Fankem *et al.*, 2008). These observations strongly confirmed the high P solubilization capacity of the isolates which might have released P from the Natural Phosphate and native inorganic phosphorus due to the action of organic acids and enzymes. Similar response was obtained with other authors who experimented the solubilization of Rock Phosphate and confirmed that P uptake

varied depending on the efficiency of isolates. Many observations on the increased P uptake in different crops due to inoculation with P solubilizers have been made by several researchers (Sandeep *et al.*, 2008; Tao *et al.*, 2008; Mamta *et al.*, 2010; Panhwar *et al.*, 2012; Muhammad *et al.*, 2013; Kaur and Reddy, 2014). The inoculation with phosphate solubilizing microorganisms having ability concurrently improved plant P uptake and crop growth.

Conclusion

Soils in the northern Cameroon constituted a good reservoir for phosphate-solubilizing bacteria, which could be useful in improving crop productivity in poor or medium fertile soils. It is necessary to confirm the good response of maize and sorghum improvements by PSM isolates and rock phosphate on greenhouse conditions. Our study demonstrates that Phosphate Solubilizing Microorganisms are able to significantly improve the crop growth, yield and phosphorus uptake.

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