Phosphate Solubilizing Microorganisms for Cereal Production in Adamawa (Cameroon)

A. Maimouna, T. L. Tchuenteu, D. Nwaga, J. P. Nguetnkam, and C. Megueni

ABSTRACT

Phosphorus is usually a limiting factor of acidic tropical soil and limited by the low availability and high cost. The present study aimed to evaluate the interaction of Hangloa vivianite (as phosphorus supply), the selected Mycorrhizal fungi strains (M) and Phosphate Solubilizing Microorganisms (PSM) on maize production in the Adamawa Region of Cameroon. An experimental design with 15 combinations of bacteria strains (B), (BGL12 as B1, SSL9 as B2, BNBL17 as B3, RBNBL5 as B4, and the combinations of these singles strains, namely B1B2, B1B3, B1B4, B2B3, B2B4, B3B4, B1B2B3, B1B2B4, B1B3B4, B2B3B4, B1B2B3B4) and 05 inputs (mycorrhiza (M), Rock Phosphate (RP) and mixture of M with RP (RP+M)), negative control (C-) and positive control (C+) was used. After four months of growth, nutrients (Phosphorus and Nitrogen) content and grain yield were assessed. Results indicated that total phosphorus content of M, RP and RP+M plants were 2.42, 2.11 and 4.00 fold higher than that of unfertilized plants. M, RP and RP+M increased maize seeds yield by 26.20%, 26.89% and 165.51% relative to negative control. The benefit of the selected PSM with Mycorrhizal fungi strains (246.76% for N; 216.34% for P) and with the Rock Phosphate (314.23% for N; 167.26% for P) was more pronounced with the combination of RP+M (1030.45% for N and 967.31% for P). These results showed that PSM associated with Mycorrhiza can be used as inoculants to improve the efficiency of vivianite as phosphate fertilizer for sustainable maize production under Sudano-Guinean Climate of Adamawa Cameroon region.

Keywords: Grain yield, Hangloa vivianite, Adamawa Cameroon, Phosphates Solubilizing Microorganisms, Phosphorus uptake.

I. INTRODUCTION

Cereals are species generally sown for their grains, whose starchy albumen, reduced in flour, is eaten by human or by domestic animals. Most cereals belong to the Poaceae family. These are: wheat, rice, millet, sorghum, barley, oats, rye, corn. The main cereals produced in the world, is Maize (*Zea mays* L.) with approximately 900 million tons produced per year [32]. *Zea mays* L. is an important food crop in sub-Saharan Africa (SSA) that contributes significantly to food security [3]. In Northern Cameroon, where Maize is the most important cereal food, the quantities produced are not enough for this Region. Soil fertility is among the major factors affecting maize production in this Region. Arbuscular Mycorrhizal Fungi (AMF) present in soils, forming associations with roots provide phosphate (P) and other nutrients to their plant hosts by producing hyphae that grow out from roots [31]. However, AMF can only exploit soluble P sources and much P in the soil is in an insoluble form. To increase the production of some cereals, chemical fertilizers such as phosphorus were used, but their high cost limited their availability for farmers. Phosphorus (P) is an essential nutrient for plant such as maize and plays many roles in nutrient uptake, in respiration, biological oxidation, photosynthesis and this P is a structural component of

nucleic acid [9]. It is also important in the process of the flowering and grain filling stages [33]. To ensure the plant production, millions of tons of P fertilizer were added to soils each year [14]. The use of these P chemicals fertilizers has increased crop yields, but their excessive and abusive use has resulted in human health pollution and environment pollution [28]. In this respect, the use of many natural fertilizers (Rock Phosphate Powder or Rock Potassium Powder) and biofertilizers is an alternative. Rock Phosphate are sources of P but not readily available to a plant because this mineral is released slowly and their use as fertilizer often causes insignificant yield increases of crop production [36]. Microorganisms have the ability to solubilize the insoluble phosphates and maintain the nutrient status of soil [27]. The use of Plant Growth Promoting Rhizobacteria (PGPR) and Phosphorus Solubilizing Microorganisms (PSM) as biofertilizers, contribute to improve plant growth, plant nutrition and agricultural production [7], [22], [24], [29], [19]. PSM are known for their ability to solubilize inorganic phosphates, making phosphorus available to plants [10], [8], [16]. Unfortunately, cereal production decreased in Sudano-Sahelian and Guinea-savannah zones during this last decade, due to the decrease of soil fertility. In the region of Adamawa (Cameroon) where soils are ferrallitic, this loss of fertility is mainly due to high soil acidity and nutrient leaching by rainfall [20]. In this region,

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there are several geological materials rich in P and other mineral element that can be used to amend soils and improve crop production. Hangloa vivianite is one of the local geological materials rich in phosphate and accessible to peasants [35]. Our study focused on improvement of cereal production in the Sudano-Guinean zones of Cameroon. In this respect Phosphate Solubilizing Microorganism (PSM) was isolated from Sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays* L.) rhizosphere. Results indicate that the selected bacteria stimulated maize growth in pots and in fields. Also, PSM have shown their potential to improve by 50 to 160% Phosphorus absorption by *Sorghum bicolor* and *Vigna unguiculata* under greenhouse conditions [15]. The author [7] Reported that inoculation of Phosphate Solubilizing Bacteria (*P. fluorescens*), isolated from acid soils in Cameroon and able to mobilize P from Al- and Fe-phosphates, increased grain yield and P uptake of maize when tested in the greenhouse. The main objective of this study was to improve durably cereal production in the Northern Cameroon. Therefore, PSM isolates, Rock Phosphate and Mycorrhizae were used to improve grain yield, nitrogen uptake and phosphorus-solubilization. The interest and usefulness of this work was that the combination of PSM, vivianite powder and mycorrhizae which better improve maize productivity will be popularized.

II. MATERIAL

A. Maize Seeds

Shaba variety of maize obtained from the Regional Delegation of MINADER was used (Fig. 1). It is an improved variety with a four-month life cycle. This variety of maize is cultivated by Adamawa's farmers because of its good quality of flour used as food.

Fig. 1. Seeds of *Zea mays* Var Shaba.

B. Biofertilizers

Phosphates Solubilizing Microorganisms (PSM) and selected Mycorrhizal fungi strains (M) were used as biofertilizers. Four single selected strains of PSM (**BGL1** as B1, **SSL9** as B2, **BNBL17** as B3 and **RBNBL5** as B4) isolated from maize and sorghum rhizosphere and 11 combinations of these strains were used in these experiments.

The Mycorrhizal biofertilizers (Fig. 2) used were supply by the Laboratory of Microbiology and Soils of the Biotechnology Center of the University of Yaounde I. Selected isolates were multiplied on sorghum and peanut

plants (hosts plants) and tested on maize and sorghum [23]. It contains infective spores and propagules of three genera: *Glomus*, *Scutellospora* and *Gigaspora* species with a concentration of 20 spores/g of substrate.

Fig. 2. Inoculum of mycorrhizal fungi.

C. Rock Phosphate

A Rock Phosphate used was obtained from vivianite (Fig. 3). It was sampled from the Hangloa Basin located at about 25 km in the Northwest of Ngaoundere city. Chemical analyses of this powder were done by [35] and were given the following composition: Fe_2O_3 (68.72%), P_2O_5 (9.17%), Al_2O_3 (7.72%) and SiO_2 (9.67%), total Phosphorus (671.50 mg/kg) and available phosphorus (81.13 mg/kg).

Fig. 3. a) Vivianite and b) Rock powder of vivianite.

III. METHODS

A. Effects of Phosphate Solubilizing Microorganisms (PSM), Mycorrhizal fungi and Rock Phosphate on Maize Growth in Field.

A 15×5 experimental design (Table I) with 15 combinations of bacteria (B), (BGL12 as B1, SSL9 as B2, BNBL17 as B3, RBNBL5 as B4, and the combinations of these singles strains, namely B1B2, B1B3, B1B4, B2B3, B2B4, B3B4, B1B2B3, B1B2B4, B1B3B4, B2B3B4, B1B2B3B4) and 05 inputs [combinations of mixture of Rock Phosphate with the selected Mycorrhizal fungi strains (mycorrhizae (M), Rock Phosphate (RP) and mixture of M with RP (RP+M)) negative control (C-) and positive control (C+)] was used. The elementary plots were installed in 3 randomized blocks. Each block had the following measure: 32.25 m x 30.5 m. The dimension of one elementary plot was 3.75 m x 3.5 m (area = 13.125 m²) separated from another by 1 meter. There were 145 elementary plots (15 isolates and combination of isolates plus positive and negative control) replicated three times. The space between the lines was 75 cm and 50 cm in line. The quantity of

inputs was 15 g of M, 50 g of RP and mixture of 15 g of M with 50 g of RP were applied per hole.

Maize crops were grown during the rainy cropping season in the Adamawa Region. Maize seeds were disinfected alternately in 70% ethanol for 1 min and in sodium hypochlorite 5% during 5 min then rinsed several times with sterile water. These sterilized seeds were pregerminated in a Petri dish with sterile distilled water. Two pregerminated seeds were introduced in each hole. Inoculation of PSM was carried out before sowing by coating of pregerminated seeds maize. Mycorrhizal fungi were inoculated at a rate of 15 g per hole. Likewise, the Rock Phosphate was applied in the front hole planted at 50 g per hole before sowing.

TABLE I: CODES OF PSM ISOLATES AND THEIR COMBINATION

Codes	Isolates
B1	BGL12
B2	SSL9
B3	BNBL17
B4	RBNBL5
B1B2	BGL12+SSL9
B1B3	BGL12+BNBL17
B1B4	BGL12+RBNBL5
B2B3	SSL9+BNBL17
B2B4	SSL9+RBNBL5
B3B4	BNBL17+RBNBL5
B1B2B3	BGL12+SSL9+BNBL17
B1B2B4	BGL12+SSL9+RBNBL5
B1B3B4	BGL12+BNBL17+RBNBL5
B2B3B4	SSL9+BNBL17+RBNBL5
B1B2B3B4	BGL12+SSL9+BNBL17+RBNBL5

B. Nitrogen and Phosphorus contents

Nitrogen and Phosphorus analysis was undertaken at the Laboratory of Microbiology and Soils at the University of Yaoundé I. The colorimetric methods described by [21] for phosphorus and [5] for nitrogen content determination were used. Dried plants were crushed for those analyses and yield parameters were undertaken.

C. Statistical Analysis

The data were statistically analyzed with “XLSTAT 2017 and EXCEL 2013” software. Analysis of variance (ANOVA) allowed the variability of dependent data to be assessed. Means were separated by Duncan's test.

IV. RESULTS

A. Weight of Maize Cobs

After four months of growth, a highly significant difference ($P < 0.001$) was observed between the effects of PSM isolates on the average weight of maize cobs for the RP+M treatment in general (Fig. 4). The Rock Phosphate associated with PSM improved the weight of the maize cobs for B3, B4, B1B2, B1B3, B1B4, B3B4, B2B3B4 and B1B2B3B4 isolates. Elsewhere, with mycorrhizae, all PSM isolates alone or in combination, significantly increased highly the weight of maize cobs ($P < 0.01$). The highest values were obtained with the combination of isolates B2B3B4 (267.06 ± 0.76 g), B2B3B4 (308.76 ± 4.05 g) and B1B2 (512.33 ± 1.79 g) in treatments M, RP and RP+M respectively; In the RP+M treatment, analysis of variance

showed that all isolates were significantly different to the negative control (C-).

B. Plant Nitrogen Content

Fig. 5 showed nitrogen content in maize plant. It ranges from 1.97 ± 0.60 mg/100 g (C-) to 5.13 ± 0.14 mg/100 g (B1) for M treatment, from 2.44 ± 0.26 mg/100 g (B1B3B4) to 5.57 ± 0.308 mg/100 g (B2B3B4), for RP treatment and from 1.97 ± 0.60 mg/100 g (C-) to 6.85 ± 0.15 mg/100 g of dry matter (B2B3B4) for the RP+M treatment. All PSM isolates have significantly improved ($P < 0.05$) the plants nitrogen content in the three treatments when compared with positive control. It appears that there was a significant difference between treatments M and RP+M and between treatments RP and RP+M. In the RP+M treatment, PSM improve nitrogen absorption. The interaction between treatments and isolates was highly significant ($P < 0.001$).

C. Plant Phosphorus Content

The highest value was obtained with isolates B1B3 (0.30 ± 0.02 mg/100 g), B1B3B4 (0.42 ± 0.01 mg/100 g) and B2B3B4 (0.60 ± 0.03 mg/100 g) in treatments M, RP and RP+M respectively (Fig. 6). The lowest values were obtained by the C+ (0.05 ± 0.01 mg/100 g) and the C- (0.09 ± 0.02 mg/100 g). As with nitrogen, mycorrhizae alone or associated with RP improved the plant phosphorus content.

D. Maize Field Yield

The positive effect of PSM inoculations with mycorrhizae and Rock Phosphate were clearly observed in Table II and III. The highest straw yield is observed with the associated isolate B1B2 (5.71 ± 0.00 t/h) and the minimum is observed with B1B3B4 (2.63 ± 0.01 t/h) in the treatment RP+M. The maximum in the isolated RP and M treatments were observed with association of isolates B2B3B4 (2.83 ± 0.00 t/h), B1B3B4 (2.57 ± 0.01 t/h) while the minimums were observed with association of isolates B1B3B54 (1.18 ± 0.01 t / h) and B1B3 (1.40 ± 0.01 t/h), respectively.

All isolates significantly ($P < 0.001$) improved the yield of maize in the three treatments with the exception of isolates B4 and B1B2 which did not show any significance compared to the negative control in the RP treatment.

E. Total Nitrogen Yield and Response to Total Nitrogen Uptake by Maize Plants

The highest total nitrogen yield was obtained with isolates B1B3B4 (202.89 ± 8.10 Kg/ha), B2B3B4 (321.30 ± 3.20 Kg/ha), and B1B2 (570.7 ± 1.98 Kg/ha) in treatments M, RP and RP+M respectively (Table II) and the smallest percentage was rated with the negative control ($0.00 \pm 0.00\%$). Analysis of variance showed that a significant difference was observed between the effects of isolates and the mean yield content and nitrogen absorption for the RP+M treatment ($P < 0.001$).

F. Total Phosphorus Yield and Response to Total Phosphorus Uptake by Maize Plants

The results reported in Table III showed that the highest values were obtained with isolates B1B3B4 (15.70 ± 0.01 Kg/ha), B2B3B4 (9.60 ± 1.62 Kg/ha), and

treatments M, RP and RP+M, respectively. The analysis of variance showed that a significant difference is observed between the effects of isolates and the mean yield content and absorption of phosphorus for RP+M treatment ($P<0.001$).

Legend: B3: SSL9; B1B2: BGL12+SSL9; RP: Rocks Phosphates; RP+M: Rocks Phosphates and Mycorrhiza.

Legend: M: Mycorrhizal fungi, RP: Rock Powder (vivianite), RP+M: Mixing Rock Powder and Mycorrhizal fungi, C+: Positive control, C-: Negative Control with B1: BGL12, B2: SSL9, B3: BNBL17, B4: RBNBL5 as different PSM isolates.
The values with different letters are significantly different.

Legend: M: Mycorrhizal fungi, RP: Rock Powder (vivianite), RP+M: Mixing Rock Powder and Mycorrhizal fungi, C+: Positive control; C-: Negative Control with B1: BGL12, B2: SSL9, B3: BNBL17, B4: RBNBL5 as different PSM isolates.

TABLE II: EFFECTS OF DIFFERENT TREATMENTS ON TOTAL NITROGEN UPTAKE OF MAIZE PLANTS

Treatments	M				RP				RP+M			
	GY	TNU	TNY*	(%)**	GY	TNU	TNY*	(%)**	GY	TNU	TNY*	(%)**
B1	1.49±0.05c	7.70±0.51de	107.41±5.66e	190.53±4.33e	1.58 ± 0.08e	5.62 ± 0.047c	88.86±7.51c	140.36±1.80d	3.75 ± 0.09d	9.97 ± 0.44d	325.10±3.10d	779.36±1.96e
B2	1.64±0.04e	8.45±1.13e	145.16±7.91g	292.61±3.96g	1.72 ± 0.04g	11.90 ± 0.56m	204.70±9.63g	453.70±3.60h	3.33 ± 0.00g	11.45 ± 0.55h	426.90±6.07e	1054.72±1.96f
B3	2.10±0.03j	7.15±1.30de	160.76±9.69h	334.83±2.84h	2.09 ± 0.09k	10.76 ± 0.67l	224.70±14.00h	507.80±3.60i	4.46 ± 0.03l	11.68 ± 0.46hi	489.30±1.90h	1223.50±1.00gh
B4	1.44±0.05b	6.39±0.97de	83.45±1.16c	125.72±8.80c	1.55 ± 0.09e	7.11 ± 0.42fg	110.33±6.63d	198.43±1.05e	3.74 ± 0.02i	11.14 ± 0.36fg	525.72±2.90ij	1327.42±1.00h
B1B2	1.60±0.01d	7.74±0.042de	119.83±0.98f	224.13±1.60f	1.44 ± 0.05c	7.81 ± 0.34hi	112.80±4.99d	205.11±1.66e	5.71 ± 0.01e	13.36 ± 0.55j	570.75±1.98j	1443.81±2.80i
B1B3	1.40±0.03a	7.52±1.33de	115.84±3.20ef	231.33±1.96f	2.21 ± 0.03m	7.66± 0.56ghi	169.40±12.40f	358.21±2.20g	3.2 ± 0.00f	11.64 ± 0.65hi	411.66±4.90e	1013.50±1.50f
B1B4	1.97 ± 0.06gh	5.93 ± 0.67cd	122.33±3.80f	230.88±1.08f	2.35 ± 0.07n	8.63 ± 0.46i	202.60±9.94g	448.12±1.95h	3.95 ± 0.02k	12.16 ± 0.33i	421.60±3.70e	1040.38±1.03f
B2B3	2.07 ± 0.04i	6.50 ± 0.20de	108.43±1.50e	193.29±1.35e	1.87 ± 0.05j	9.62 ± 0.51k	179.60±9.66f	385.80±2.32g	3.10 ± 0.02e	9.64± 0.31d	290.96±2.41d	687.01±1.70d
B2B4	1.76 ± 0.07f	8.34 ± 1.90e	163.90±5.40h	343.33±9.32hi	1.80 ± 0.02i	7.53 ± 0.46gh	135.40±8.36e	266.24±3.50f	3.31 ± 0.07g	8.50 ± 0.47c	336.98±5.40d	811.43±9.05e
B3B4	1.51 ± 0.09c	6.52 ± 0.04de	96.00±3.21d	159.67±7.12d	2.14 ± 0.05l	6.34 ± 0.29de	135.80±6.14e	267.32±1.75f	3.89 ± 0.01j	11.21 ± 0.11fg	460.77±3.86fg	1146.33±2.55g
B1B2B3	2.30 ± 0.03l	5.91 ± 1.47cd	154.24±2.56gh	317.20±1.63gh	1.64 ± 0.04f	8.31 ± 0.44ij	136.10±7.31e	268.14±1.52f	5.25 ± 0.07n	10.16 ± 0.47de	480.56±3.42gh	1199.86±3.83g
B1B2B4	1.99 ± 0.05h	5.99 ± 1.37cd	96.98±1.16d	162.32±2.08d	1.76 ± 0.04h	6.34 ± 0.44e	111.50±7.77d	201.60±2.98e	3.60 ± 0.04h	10.12 ± 0.21de	336.39±5.70d	809.907±1.83e
B1B3B4	2.50 ± 0.07m	7.80 ± 0.44de	202.89±8.10j	448.80±8.73k	1.18 ± 0.04a	5.89 ± 0.37cd	69.28±4.32b	87.40±2.83bc	2.63 ± 0.05b	10.73 ± 0.37ef	433.70±6.70f	1073.11±1.60f
B2B3B4	2.27 ± 0.06k	6.63 ± 0.40de	176.75±9.35i	378.10±7.93j	2.83 ± 0.00o	11.33 ± 0.01m	321.30±3.20i	769.08±2.80j	5.17 ± 0.01m	14.37 ± 0.30k	518.97±2.50i	1303.76±2.67h
B1B2B3B4	1.43 ± 0.02ab	4.20 ± 0.80bc	62.38±7.10b	68.73±9.90b	1.40 ± 0.05b	6.74 ± 0.25ef	94.71±3.63c	156.18±1.14d	2.64 ± 0.06b	10.11 ± 0.44de	237.61±2.41c	542.71±3.85c
C+	1.95 ± 0.04g	3.17 ± 0.56ab	66.82±3.24b	80.74±3.27b	1.50 ± 0.02d	4.28 ± 0.09b	64.31±1.50b	73.95±1.22b	2.80 ± 0.08c	6.33 ± 0.40b	134.81±5.17b	264.64±35.95b
C-	1.45 ± 0.02b	2.55 ± 0.16a	36.97±2.13a	0.00±0.00a	1.45 ± 0.02c	2.55 ± 0.16a	36.97±2.13a	0.00±0.00a	1.45 ± 0.02a	2.55 ± 0.16a	36.97±2.13a	0.00±0.00a
Average	1.83±0.36	6.85±1.13	127.76±38.41	246.76±103.61	1.84±0.43	8.11±1.99	153.14±65.54	314.23±177.30	3.85±0.93	11.08±1.48	417.80±95.20	1030.45±257.94

Legend : * N (%) x 10 x Yield (t/ha), ** (Nitrogen uptake – Control- Nitrogen Uptake)/ Control- Nitrogen Uptake x 100 , M: Mycorrhizal fungi, RP: Rock Powder (vivianite), RP+M: Mixing Rock Powder and Mycorrhizal fungi, GY: Grain Yield (t/ha), TNU: Total Nitrogen Uptake (g/100g), TNY: Total Nitrogen Yield (Kg/ha), C+: Positive control, C-: Negative Control, B1: BGL12 ; B2: SSL9, B3: BNBL17; B4: RBNBL5. The values with different letters are significantly different.

TABLE III: EFFECTS OF DIFFERENT TREATMENTS ON TOTAL PHOSPHORUS UPTAKE OF MAIZE PLANTS

Treatments	M				RP				RP+M			
	GY	TPU	TPY*	(%)**	GY	TPU	TPY*	(%)**	GY	TPU	TPY*	(%)**
B1	1.49±0.05c	0.57±0.02gh	8.57±0.06i	248.37±1.20h	1.58 ± 0.08e	0.38±0.08defg	6.11±1.36e	148.37±8.4de	3.75 ± 0.09d	0.51±0.14c	16.5±0.23b	570.73±1.90d
B2	1.64±0.04e	0.42±0.02e	7.04±0.04f	186.17±1.90e	1.72 ± 0.04g	0.34±0.06cdef	5.95±1.15d	141.86±1.62de	3.33 ± 0.00g	0.60±0.03cd	20.15±0.75cd	719.10±1.03f
B3	2.10±0.03j	0.33±0.01cd	6.97±0.03f	183.33±2.24de	2.09 ± 0.09k	0.32±0.04cde	6.74±0.94ef	173.98±5.52f	4.46 ± 0.03l	0.64±0.00d	30.61±0.46gh	1144.30±1.30l
B4	1.44±0.05b	0.46±0.01ef	6.70±0.03e	172.35±1.70d	1.55 ± 0.09e	0.46±0.08g	7.16±1.34f	191.05±1.86g	3.74 ± 0.02i	0.78±0.02de	28.6±1.38g	1062.60±4.5k
B1B2	1.6±0.01d	0.37±0.01d	5.48±0.06d	122.76±4.2c	1.44 ± 0.05c	0.42±0.08efg	6.11±1.14e	148.37±1.01de	5.71 ± 0.01e	0.75±0.05de	42.13±1.75j	1612.60±2.10o
B1B3	1.4±0.03a	0.48±0.06f	7.2±0.06f	192.68±1.95e	2.21 ± 0.03m	0.44±0.06fg	9.87±1.27h	301.21±1.22i	3.2 ± 0.00f	0.71±0.17de	21.94±0.31d	791.86±3.70g
B1B4	1.97 ± 0.06gh	0.25±0.06b	5.04±0.06c	104.87±1.6b	2.35 ± 0.07n	0.34±0.08cde	7.94±1.83g	222.76±4.50h	3.95 ± 0.02k	0.58±0.03c	23.78±2.01ef	866.66±2.90i
B2B3	2.07 ± 0.04i	0.37±0.03cd	7.74±0.20g	214.63±1.70f	1.87 ± 0.05j	0.33±0.06cde	6.20±1.17e	152.03±1.82e	3.1 ± 0.02e	0.66±0.01d	19.88±0.92c	708.13±3.80ef
B2B4	1.76 ± 0.07f	0.54±0.02g	9.26±0.36j	276.42±2.20i	1.8 ± 0.02i	0.41±0.06efg	7.37±1.16f	199.59±1.56g	3.31 ± 0.07g	0.60±0.03cd	22.85±2.40e	828.86±6.30h
B3B4	1.51 ± 0.09c	0.37±0.03d	5.63±0.23d	128.86±2.12c	2.14 ± 0.05l	0.33±0.05cde	7.16±1.14f	191.05±1.40g	3.89 ± 0.01j	0.85±0.10f	31.02±1.09h	1160.97±2.50l
B1B2B3	2.3 ± 0.03l	0.35±0.00cd	8.20±0.15h	233.33±2.31g	1.64 ± 0.04f	0.26±0.05bc	4.32±0.80c	75.60±2.08c	5.25 ± 0.07n	0.68±0.04d	35.48±1.04i	1342.27±1.90m
B1B2B4	1.99 ± 0.05h	0.43±0.03ef	8.64±0.40i	251.22±1.02h	1.76 ± 0.04h	0.33±0.06cde	5.82±1.12d	136.58±1.72d	3.60 ± 0.04h	0.69±0.09d	24.46±1.80f	894.30±2.70j
B1B3B4	2.57 ± 0.07m	0.60±0.01h	15.7±0.01l	538.21±1.80k	1.18 ± 0.04a	0.35±0.07cdef	4.11±0.82c	67.07±1.61c	2.63 ± 0.05b	0.66±0.03d	19.56±1.17c	695.12±1.40e
B2B3B4	2.27 ± 0.06k	0.43±0.00ef	9.93±0.06k	303.66±1.08j	2.83 ± 0.00o	0.34±0.05cde	9.60±1.62h	290.24±2.40g	5.17 ± 0.01m	0.90±0.20f	41.04±1.23j	1568.29±1.74n
B1B2B3B4	1.43 ± 0.02ab	0.32±0.00c	4.63±0.11b	88.21±1.89b	1.4 ± 0.05b	0.36±0.05cd	4.16±0.71c	69.10±1.33c	2.64 ± 0.06b	0.55±0.00c	15.84±0.42b	543.90±2.87c
C+	1.95 ± 0.04g	0.23±0.01b	4.50±0.18b	82.93±2.14b	1.5 ± 0.02d	0.17±0.03ab	2.60±0.50b	5.69±1.20b	2.8 ± 0.08c	0.35±0.09b	15.48±0.80b	529.26±2.40b
C-	1.45 ± 0.02b	0.17±0.00a	1.30±0.00a	0.00±0.00a	1.45 ± 0.02c	0.17±0.00a	1.30±0.00a	0.00±0.00a	1.45 ± 0.02a	0.17±0.06a	1.30±0.00a	0.00±0.00a
Average	1.83 ± 0.37	0.42 ± 0.10	7.78 ± 2.69	216.34 ± 109.26	1.84 ± 0.43	0.36 ± 0.05	6.57 ± 1.73	167.26 ± 70.19	3.85 ± 0.93	0.68 ± 0.11	26.26 ± 8.32	967.31 ± 338.16

Legend : * N (%) x 10 x Yield (t/ha), ** (Nitrogen uptake – Control- Nitrogen Uptake)/ Control- Nitrogen Uptake x 100 , M: Mycorrhizal fungi, RP: Rock Powder (vivianite), RP+M: Mixing Rock Powder and Mycorrhizal fungi, GY: Grain Yield (t/ha), TNU: Total Nitrogen Uptake (g/100g), TNY: Total Nitrogen Yield (Kg/ha), C+: Positive control, C-: Negative Control, B1: BGL12; B2: SSL9, B3: BNBL17; B4: RBNBL5. The values with different letters are significantly different.

V. DISCUSSION

In our field experiment, PSM isolates, Rocks Phosphates and Mycorrhiza associated or not were used to evaluate some parameters as maize cobs, nitrogen uptake, phosphorus uptake and grain yield. The performance of the four isolates in association or alone (B1: BGL12, B2: SSL9, B3: BNBL17 and B4: RBNBL17) in increasing these parameters showed that they might have mineralized the rocks phosphate (RP) by releasing phosphatase enzyme, organic or inorganics acids. The results are similar to that reported by [4], [27], [6], [7], [18]. A highly significant difference ($P < 0.001$) was observed between the effects of PSM isolates and the average weight of maize cobs for the RP+M treatment. The effect of RP+M treatment associated with isolates promoted nitrogen content than with the RP and M treatments alone. The average nitrogen content of plants (B2B3B4: 6.85 ± 0.15 mg/100 g) in the RP+M treatment improved nitrogen yield (B1B2: 570.75 ± 1.98 Kg/ha) and the percentage of nitrogen absorption (B1B2: $1443.42 \pm 2.80\%$) of this element compared to the negative control (C-). However, the RP treatment without PSM isolates has no effect on these two parameters, this indicate the importance of PSM and Mycorrhiza. The analysis of variance showed that a significant difference was observed between the effects of PSM isolates and the phosphorus mean yield content (B2B3B4: 41.04 ± 1.23 Kg/ha) and the percentage of phosphorus absorption (B2B3B4: $1568.29 \pm 1.74\%$) for RP+M treatment. Many authors showed the role of Phosphate Solubilizing Microorganisms (PSM) in releasing P from rock phosphates [11], [25], [17], [1], [8], [34], [26] which may lead to increased P uptake and plant growth [12]. Co-inoculation of PSM, Arbuscular Mycorrhizal fungi (AMF) with Rocks Phosphates resulted in significantly higher of nitrogen and phosphorus uptake in plant than where these microorganisms were used alone. For all the inoculation effect, co-inoculation with the AMF and PSM resulted in the highest plant growth response. Some reports of field trials have showed the beneficial effects of co-inoculations with Phosphate Solubilizing Bacteria (PSB) and AMF [13], [25] and PSM survive longer when associated to Mycorrhizal roots rather than to non-Mycorrhizal roots [30], [2], thus increasing the possibility of delivering P into the soil solution.

VI. CONCLUSION

The objective of this work was to evaluate the effect of PSM associated with RP and Mycorrhizae on *Zea mays* growth. Results indicate that these isolates can effectively improve the solubility of P content in rock phosphate applied as fertilizer, thus increase the amount of available nitrogen and phosphorus in the soil and maize productivity. Also results showed that solubilization or RP was better with double inoculation of PSM and Mycorrhiza. PSM and RP can be used as natural fertilizers to improve plants productivity in the Northern Cameroon.

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