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Effect of the inoculation of nitrogen-fixing rhizobacteria in the sweet potato crop (*Ipomoea batatas* Lam.)

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Abstract

The excessive use of nitrogen fertilizers in the nutrition of plants Generates environmental problems and Increases the production costs of crop. Plant Growth Promotingrhizobacteria(PGPR) are an alternative to the use of chemical fertilizers, Favoring the yield of crops. The objective of esta investigation was to determine the effect of inoculation of rhizobacteria With fixed nitrogen sweet potato crop in the Montes de Maria microregion. Sweet potato cutting of the variety Tainung 66, provenance was established under a design of complete blocks at random With an Increased factorial arrangement 3x2+2, with three replications, Where the was evaluated effect of native states of *Azotobacter sp*. (IBCB10) and *Azotobacter vinelandii* was EVALUATED (IBCB15) mixed With fertilization levels (50% and 75%). The results Obtained Indicate That the inoculation of the IBCB10+IBCB15 bacteria in a mixture With 50% of the nitrogen fertilizer dose, Increased yields the crop by 57% Significantly With respect to the chemical and 93% Control T_2 to T_1 With relation (Without inoculation). Likewise, the application of the bacterium IBCB10 stimulated the production of dry matter greater tuberous roots of sweet potato, by optimizing the absorption of nitrogen fertilizer reduced to 50%. These results allow to rhizobacteria be included in management alternatives for the mineral nutrition of the sweet potato crop.

Keywords: *azotobacter vinelandii, escribir separado*, biofertilizer, growth promoting, production, sustainable agriculture

Introduction

The sweet potato (*Ipomoea batatas Lam*), ranked fifth in importance of roots and tubers in developing countries, it is a food with high content of total carbohydrates (25-30%) considered easily digestible presents outstanding nutritional characteristics, culinary and can be used as feed supplement for livestock.[1]The sweet potato tubers are used as industrial raw material for the production of starch, alcohol, pectin, etc. In addition to providing energy, it is a good source of minerals and vitamins.[2,3]

The productivity of the crop, the same as most plants of agricultural interest, shows a strong dependence on nitrogen and water during their growth cycle; therefore, this element is a determining factor in the fertilizer plan synthesis in sweetpotato.[4,5] However, excessive use of fertilizers resulting in higher production costs, soil and water pollution, and decreased microbial activity engaged in plant nutrition; consequently they are causing significant losses in terms of performance.[6,7]

The use of microorganisms with potential biofertilizer is one of the main strategies to implement sustainable farming practices that help improve rhizosphere soil dynamics.[8–10] Organic farming can provide comprehensive solutions for sustainable food system that contributes to improving food security in the world.[11,12]

The PGPR correspond to a heterogeneous set of rhizosphere microorganisms of the soil, they remain in association on the surface of the roots and have beneficial effects on plants.[13–15] Rhizosphere free-living bacteria stimulate plant growth by various processes such as nitrogen fixation, solubilization of nutrients, siderophore production, synthesis regulators plant growth and control of phytopathogenic of the floor.[16,17] The most studied genera belong to *Azotobacter, Azospirillum,Herbaspirillum, Beijerinckia, Burkholderia,Pseudomonas, Bacillus and Enterobacter*.[18–20] these rizobacteriasphytohormones synthesized which induce changes in plant physiology, enabling improved processes flowering, germination and establishment of plants.[21–23] Likewise, PGPR indirectly promote systemic resistance against phytopathogenic[24–26]and one of the mechanisms used is the synthesis of volatile compounds such as hydrogen cyanide (HCN) and metalitos bioactive secondary (antibiotics).[18] Bacteria of the genus *Azotobacter* possess an enzymatic complex (nitrogenase) able to reduce the atmospheric nitrogen to ammonia which can be assimilated by plants,[27] generating significant effects on the crop yields,[28] I save of mineral fertilizers and reduction of environmental pollution.[29,30] These bacteria and other microorganisms used in fertilization of agricultural soils are complementary in formulating bioestimulantes in sustainable agriculture. In this context, the objective of this investigation was to determine the effect of inoculation with rhizobacteria fixing nitrogen in the sweet potato crop in the microregion of Montes de Maria.

Materials and methods

Location

This study was cabor the Colombian Corporation for Agricultural Research (AGROSAVIA) in the The Carmen based Bolivar, geographically located at 9°42' 50.29" N y 75°06' 27.2" W in the town of Carmen Bolivar, Bolivar, Colombia. The area belongs to the warm climate zone Y dry, formation of dry tropical woods (BS-T), average temperature of 27.7°C, relative humidity average of 76%, altitude of 148 meters, bimodal rainfall with rainfall of 1,100 mm annually. The experiment was conducted under field conditions during the months of June to October 2017. The floor where the trial was installedpresents a texture loam, pH=7,96; M.O=2,32%; P= 79,71mg/kg; Ca_2+=28,71 Cmol/kg; Mg_2+=3,07 mg/kg; K = 0,63 mg/kg; CIC=34,36 Cmol/kg.

Vegetal material

In planting apical cuttings were used potato variety Tainung 66, obtained from healthy, free plants the pests and diseases spread in seedbeds. With an average length of 20-25 cm, with 5-6 buds, stem thickness and good vigorous appearance, cuttings were planted with a planting distance of 1.0 m between rows and 0.4 m between plants, which it corresponded to a seeding density of 25,000 plants/ha.

Rizobacterias

Native bacterial strains of the micro-region of Montes de Maria*,* *Azotobacter sp.* IBCB10 and *Azotobacter vinelandii* IBCB15 and were isolated and supplied by the laboratory of Agricultural Microbiology Research Center Turipaná.[31]

Qualitative test of nitrogen fixation *in vitro*

The binding capacity *in vitro* nitrogen strains IBCB10 and IBCB15 qualitative test was determined by using the culture medium free nitrogen NFB;[32]strains were seeded in triplicate in semisolid culture medium puncture and incubated for 72 hours at a temperature of 30±2°C. As negative control uninoculated medium was used. The ability of isolates to grow was observed in medium without nitrogen, which was indicated as positive or negative according to the presence or absence of growth.

Preparation of inocula rhizobacteria

Bacterial strainsIBCB10 and IBCB15 were seeded in brothLuria Bertani[33]in a volume corresponding to 10% of the final volume required and left on an orbital shaker for 24 hours at a temperature of 30±2°C and 150 rpm. The bacterial suspension was measured with an absorbance at 540 nm, corresponding to a bacterial population $1x10^8$UFC.ml.Plants were inoculated with 10 ml of the bacterial suspension at the base of the stem, in different phenological times of cultivation: the first inoculation was performed eight days after sowing (DAS), a second bacterial inoculation 30 DDS and the third at 40 DDS, these inoculations were performed in conjunction with the application of nitrogen fertilizer.

Fertilization

Nitrogen fertilization in yam cultivation was performed as ammonium sulfate $(NH_4)_2SO_4$, with fertilization of 25.80g/plant. The application was made in installments:30% at 20 DDS and the second fraction of 70% to 40 DDS. Doses of the fertilizer were calculated taking into account the requirements of the cultivation and soil analysis.

Experimental design and treatments

The structure of the treatment consisted of a 3x2 factorial arrangement; where qualitative factors were bacterial strains (three levels), and quantitative factor corresponded to two levels of fertilization. In addition two control treatments (absolute and chemical) were established. Combinations (Table 1) were assigned a complete block design with three replications randomized, for a total of 24 experimental units (EU) 36.0m2 (7.2 m longx5.0 m wide).

Table 1 Treatments evaluated in growing potato Tainung 66

Treatment	Description	Dose $(NH4)_2SO_4$ (Kg. Ha-1)
T1	witness all	0
T2	Chemical witness 100% Nitrogen fertilization (FN)	645
T3	*Azotobacter sp.* (IBCB10)+50% FN	322,50
T4	*Azotobacter sp.* (IBCB10)+75% FN	483,75
T5	*A. vinelandii* (IBCB15)+50% FN	322,50
T6	*A. vinelandii* (IBCB15)+75% FN	483,75
T7	*Azotobacter sp.* (IBCB10)+A. vinelandii (IBCB15)+50% FN	322,50
T8	*Azotobacter sp.* (IBCB10)+A. vinelandii (IBCB15)+75% FN	483,75

To quantify the effect of foliar treatments analysis was performed at 60 (DDS): Nitrogen (EPA 3513 Modified) Phosphorus (Digestion open nitric: perchloric (5:2)/spectrophotometry) Potassium, Calcium and Magnesium (open Digestion nitric: perchloric (5:2)/emission spectrophotometry inductively coupled plasma). Production performance and root dry matter: A the time of harvest (DDS 120), the following variables were evaluated.

Statistic analysis

The obtained data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene respectively. Checked assumptions and analysis of variance test was made orthogonal contrasts 5% significance for the performance variables and root dry matter. Comparisons between means were analyzed as follows: A: vertical comparisons between treatments inoculated mixed with different levels of fertilizer; B: horizontal comparisons between treatments vs inoculated T_1 and T_2 controls all chemical control. Tukey test ($p \leq 0.05$) was made for the components of the foliar analysis. SAS statistical package (version 9.2) was used.

Results

In vitro nitrogen fixation by rhizobacteria

Strains IBCB10 and IBCB15 incubated for 72 hours, showed the ability to grow in medium free of semisolid nitrogen, such a fact evidenced by the formation of a white, thick wavy film below the surface of the medium, there were also color change of the culture medium from green to blue,which is a presumptive qualitative test of the ability of nitrogen fixing.

Effect of nitrogen-fixing rhizobacteria sweet potato yield

Inoculating rhizobacteria IBCB10+IBCB15 mixed with 50% of the dose of the nitrogen fertilizer, increased yields 4.24 cultivation $t.ha^{-1}$ with respect to $T_2$and 5.64 $t.ha^{-1}$ treatment T_1 (No inoculation). It should be noted that the yields obtained between treatments T_1 and T_2yielded no statistical difference(Table 2).

The results indicate that the T_3, T_4, T_5, T_6 and T_8 treatments are

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not statistically different (p≥0.05) between them or with respect to the control treatments. a differential behavior was evident employing strains individually reduced IBCB15 2.58 t.ha^{-1} to the applied with 50% of the recommended fertilizer dosage in relation to T6. In contrast, IBCB10+50% fertilizer did not return statistical difference T_7.

Table 2 Effect of rhizobacteria on performance yam tuberous roots (t ha^{-1})

Treatment	Bacterial strain	Fertilization	Yield (t. Ha-1) Inoculation	Fertilization 100% (T2)	Without inoculation (T1)
T7	IBCB10+IBCB15	50%	11.71±0.59 a	7.47±0.28 b	6.07±1.14 b
T3	IBCB10	50%	8.20±0.48 ab	7.47±0.28 b	6.07±1.14 b
T4	IBCB10	75%	7.16±1.38 b	7.47±0.28 b	6.07±1.14 b
T6	IBCB15	75%	6.78±0.81 b	7.47±0.28 b	6.07±1.14 b
T8	IBCB10+IBCB15	75%	5.06±0.54 b	7.47±0.28 b	6.07±1.14 b
T5	IBCB15	50%	4.20±0.74 b	7.47±0.28 b	6.07±1.14 b

The values show the mean and standard error, the same letters have no significant statistical difference (p ≥0.05) for horizontal and vertical comparisons

Effect of nitrogen fixing rhizobacteria on dry matter accumulation in potato

The application of bacteria *Azotobacter* sp. IBCB10 plus 50% of the nitrogen fertilizer, had the highest amount of dry matter accumulated in the roots of sweet potato, achieving increases of 105% with respect to $T_1$and $T_2$with 16.19%. Averages minor presented with T_2, T_4 and T_7 respectively.

The results obtained in this research suggest that *Azotobacter* sp. IBCB10 stimulated increased formation of secondary roots and therefore optimized absorption reduced nitrogen fertilizer to 50%. Applying fertilizer to 100% does not favor the accumulation of root dry matter in potato cultivar Tainung 66, because reduced by 1.47 kg ha^{-1} compared with the T_3.

Response inoculation rhizobacteria in nutrient accumulation in potato

In Figure 1 the behavior is observed in the absorption of nutrients by the sweet potato crop 60 DDS. Tukey's test indicated no significant differences (p≥0.05) between treatments. However, there were increases in the average values of nutrients (nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca)) with the application of microorganisms as compared to the T_1controls and T_2(Figure 1).

The results indicate that the greater amount of nutrient extracted at 60 DDS by sweetpotato plant Tainung 66 corresponds to the element nitrogen, which may be related to the maximum absorption of N for this phenological stage of the crop corresponds to the period of active growth. In Figure 1 it is evident that the treatments T_4 and T_5 increased nitrogen uptake, although no significant differences with respect to T_1.

Discussion

Bacterial strains and IBCB15 IBCB10 study were cultured in semisolid medium free nitrogen and growth was obtained, which is presumptive qualitative test of nitrogen fixing ability (Table 3). Studies by Perez and Sanchez[31]show that the strains under study have the ability to form acetylene reduce *in vitro*, *Azotobacter* sp. 0412 IBCB10 reduced mmol.mol^{-1}.h^{-1} and *Azotobacter* vinelandii IBCB15 0366 mmol.mol^{-1}.h^{-1} respectively, and these strains have the ability to solubilize and produce phosphorus AIA. Among biofertilizer, *Azotobacter* strains play a key role in the cycle of nitrogen in nature because they become inaccessible atmospheric nitrogen to plants and release it in the form of ammonium available ions to plants in soil that fix an average 20 kg of N/ ha per year.[34]

Table 3 Test qualitative of nitrogen fixation by rhizobacteria

Bacterial strains	Biological fixation of N 72 h
Azotobacter sp. IBCB10	+
Azotobacter vinelandii IBCB15	+
Control (no inoculation)	-

Inoculation Azotobacter sp. IBCB10 and Azotobacter vinelandii IBCB15 mixed with 50% of the dose of chemical fertilizer, substantially increased the yield of tubers of potato. Bacteria of the genus *Azotobacter* favor vegetative growth of different crops, stimulating root elongation, allowing better absorption of water and nutrients from the soil.[35,36] Furthermore, participated by various mechanisms in addition to the traditional fertilization on many crops of agricultural importance, because the capacity of this microorganism to fix nitrogen and supply to the plant through the biological fixation of nitrogen.[37,38]Significant increases in performance potato, sweet potato and wheat have resulted from the application of a consortium of PGPR, especially those with complementary skills as nitrogen fixation, solubilization of phosphorus, auxin production and siderophores among others.[39–41] The results are consistent with reports of Ruisánchez et al.[42] where it was determined that the combined inoculation of two bioproducts: Dimabac® (*A. chroococcum+Bacillus subtilis*) and Fitomas E® (bionutrient based minerals) increased productivity of the cultivation of tomato variety INIFAT-28 with decreased of 30% of the recommended nitrogen fertilizer. The greater effectiveness of the consortium is explained by interactions as cooperation and symbiosis exists in microorganisms and can be beneficial to improving the development and growth or allow survival of microorganisms.[43,44] Inoculation with *Azotobacter* has been used to induce seed germination, stimulate plant growth and increasing yield of tubers *Ipomoea batatas*L. var. Rancing up to 32.3%.[45,46] Recent

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studies by Singh et al.[47]to determine the answer to eleven sources of organic fertilizer in growing sweet potato cv. NDSP-65 in the 2015-2016 season it was found that treatment with the combination of NPK 50:25:50 kg dose. ha^{-1}+bacteria genus Azospirillum sp. (2.5 kg ha^{-1}) solubilizing bacteria phosphorus (5 kg. Ha^{-1}) poultry manure (2.5 t. Ha^{-1}) significantly increased yields of tubers of *Ipomoea batatas L* 20% with respect to the absolute control.

Regarding the production of root dry matter significant ($p\leq0.05$) occurred, the highest values were obtained with T3 (Table 4). Similarly, the Roman et al.[48]concluded that *Azotobacter* sp. It is a nitrogen fixing promotes free-living root growth, leading to an increase in the concentration of dry matter. Kader et al.[49]found that wheat plants var. Kanchani dry matter increased by 76% relative to control plants treated with *Azotobacter* sp. in mixture with 50% of the nitrogen fertilizer. For his part, The application of the nitrogenous fertilize.[50]mentioned that among the beneficial effects of *Azotobacter* sp. in plants it is considered an increase in height, root mass and performance.

Table 4 Effect of rhizobacteria on dry matter production of sweet potato root (kg ha^{-1})

			Root dry matter (kg. ha-1)		
Treatment	**Bacterial strain**	**Fertilization**	**Inoculation**	**Fertilization 100% (T2)**	**Without inoculation (T1)**
T3	IBCB10	50%	2.87±0.13 a	1.40±0.06 e	2.47±0.09 ab
T8	IBCB10+IBCB15	75%	2.23±0.14 bc	1.40±0.06 e	2.47±0.09 ab
T5	IBCB15	50%	2.18±0.12 bc	1.40±0.06 e	2.47±0.09 ab
T6	IBCB15	75%	2.13±0.14 bcd	1.40±0.06 e	2.47±0.09 ab
T7	IBCB10+IBCB15	50%	1.80±0.19 ecd	1.40±0.06 e	2.47±0.09 ab
T4	IBCB10	75%	1.55±0.11 ed	1.40±0.06 e	2.47±0.09 ab

The values show the mean and standard error. Letters not common differ statistically ($p\leq0.05$) for the horizontal and vertical comparisons.

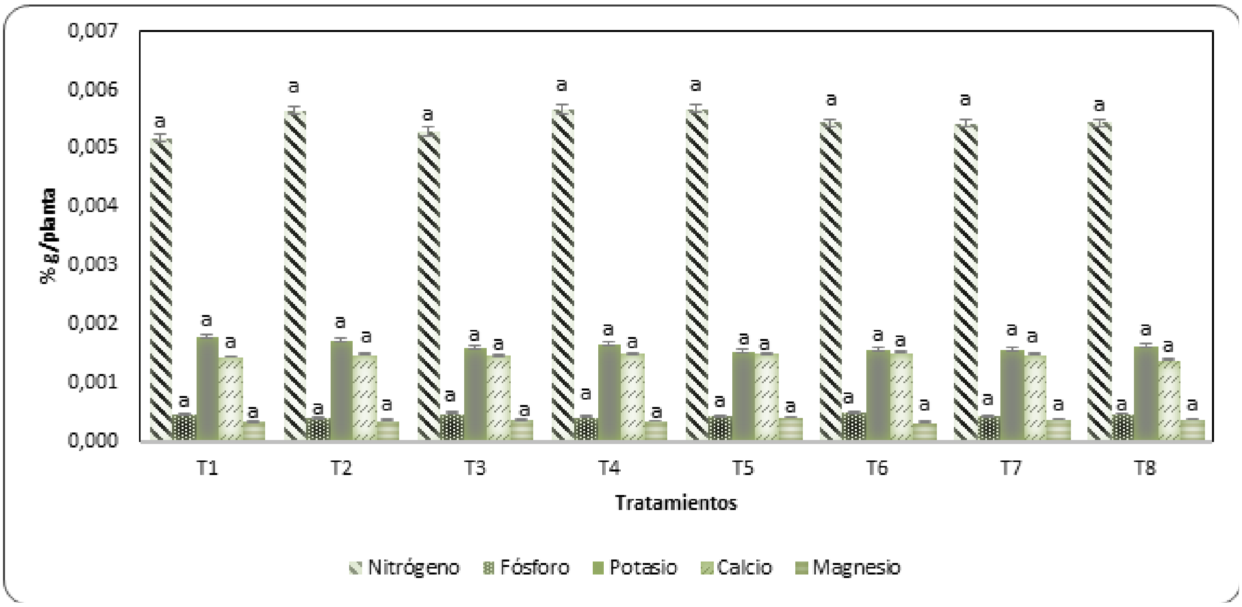

Figure 1 Response to inoculation with rhizobacteria on nutrient absorption in the sweet potato crop.

The application of the nitrogenous fertilizer T_2 as $(NH_4)_2SO_4$ adversely affects the dry matter of sweet potato tubers (Table 4). According to the points made by Luna et al.[51] the high availability of nitrogen can prolong vegetative growth, delaying the start of tuberization reducing yield and the percentage of dry biomass of roots. A increasing the amount of nitrogen is a decrease in the quality of tubers. Because, to the lower the percentage of dry matter, the concentration of nitrate stored in the vacuole, increasing the concentration of proteinaceous compounds that decrease the properties of the tubers.[52]

Regarding nutrient absorption at 60 DDSTainung66 batata, no significant differences were found between treatments, however, there was a trend to increased absorption of nitrogen in the $T_4$and T_5. Coraspe et al.[53] found that potato plants cv Atlantic as nitrogen removal 4.08 g/plant at 66 DDS under greenhouse conditions was recorded. Nitrogen plays an essential role in plant growth and stimulating growth favoring cell division. In addition, it is involved in a large number of processes; one of the most important is the production of chlorophyll, which is necessary for their synthesis and constitutes molecules such as essential amino acids, proteins, enzymes, nucleoproteins, hormones,

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adenosine triphosphate (ATP).[1]Nitrogen is required in large quantities for forming nitrogenous substances, which move with water and stored in the tissues of stems and root, in most species, the juvenile phase requires N to form green matter in the growth process.[54] Promoting bacteria growth plants can facilitate growth and development, either indirectly or directly. Indirect plant growth occurs when these bacteria reduce or prevent some of the harmful effects of a plant pathogen. The direct effect is to promote the growth of plants using plant growth-promoting bacteria, these facilitate the acquisition of nutrients from the environment, including nitrogen, iron and phosphate fixed.[55,56]

Conclusion

The development of this investigation established that inoculation with native strains *Azotobacter* sp. IBCB 10 and *Azotobacter vinelandii* IBCB15 allowed to reduce by 50% nitrogen fertilization recommended for cultivation in soils microregion Montes de Maria, which is an alternative to the traditional management of the sweet potato crop. The combined use of these microorganisms with biofertilizer potential increased yields growing significantly by 57% compared to the T_2chemical control and 93% relative to the T_1 No inoculation, I have indicated that these bacteria help plants improve absorption nutrient, stimulating growth and thus improve the productivity of the crop with friendly technologies environment.

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Conflicts of interest

Authors declare no conflict of interest exists.

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