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RESEARCH

# Influence of plant growth promoting bacteria in seed yields of super-elite sweet potato (*Ipomoea batatas* Lam) in the field

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**ABSTRACT**

Incorporating microorganisms into plant culture systems has been a strategy for increased yields and to decrease the use and environmental impact of chemical fertilization. One of the main crops of the Colombian Caribbean is the sweet potato, due to its high fiber, mineral, vitamin and antioxidant contents, making it highly demanded worldwide, and leading to the search for better cultivating conditions. The purpose of this study was to determine the response in field performance of super-elite sweet potato vegetative seeds to the inoculation of microorganisms mixed with nitrogen fertilization (NF) in doses of 50 and 75 %. Field experiments were carried out in two areas of the Colombian Caribbean using the 3×2 factorial design, where each treatment consisted of 3 plots of 36 $m^2$. Results show that for the area of Sabanas Colinadas the inoculation of the combination of *Azotobacter chroococcum*-IBCR19, *Azospirillum lipoferum*-IBSC7 and 75 % NF, produced higher yields (14.5 ton/ha), with a significant increase of 32 %, compared to the treatment with no inoculation and 100% NF. For the Montes de María area the highest yield (15.3 ton/ha) was obtained with the inoculation of *Azotobacter vinelandii*-IBCB10 and 75 % NF, showing an increase of 21 % compared to the treatment having no inoculation and 100 % NF. In both areas, field yields increased with inoculation and there was a 25 % reduction of NF. These results demonstrate the increased potential of using microorganisms in sweet potato production systems of the Colombian Caribbean.

*Keywords:* PGPR, nitrogen fertilization, *Azotobacter vinelandii*, *Azospirillum lipoferum*, sweet potato

**RESUMEN**

**Influencia de bacterias promotoras de crecimiento vegetal en rendimiento de semilla super-elite de batata (*Ipomoea batatas* Lam.) cultivada en campo.** La incorporación de microorganismos a sistemas de cultivo se ha convertido en estrategia para incrementar los rendimientos de cultivo y disminuir los volúmenes de fertilización química. Uno de los cultivos de importancia en el Caribe Colombiano es la Batata que, por su alto contenido de fibra, minerales, vitaminas y antioxidantes, se ha convertido en un producto altamente apetecido internacionalmente, demandando la búsqueda de mejores condiciones para su cultivo. El objetivo de esta investigación fue determinar la respuesta de rendimiento en campo de semilla super-elite de batata a la inoculación de microorganismos en mezcla con fertilización nitrogenada (NF) en dosis de 50 y 75 %. En dos zonas del Caribe Colombiano se establecieron experimentos en campo con un arreglo factorial 3×2, cada tratamiento consta de 3 parcelas de 36 $m^2$. Como resultados se obtuvo que para la zona de Sabanas Colinadas con la inoculación de la mezcla de *Azotobacter chroococcum*-IBCR19, *Azospirillum lipoferum*-IBSC7 y 75 %NF se obtienen el mayor valor de rendimiento (14.5 ton/ha) y presenta un incremento significativo de 32 % respecto a tratamiento sin inocular con 100 % NF. Para la zona de Montes de María el mayor valor de rendimiento (15.3 ton/ha) se obtiene con la inoculación de *Azotobacter vinelandii*-IBCB10 y 75 % NF y se presenta un incremento del 21 % respecto al tratamiento sin inoculación al 100 % NF. En las dos zonas se logró el incremento del rendimiento en campo con la inoculación y una reducción del 25 % de NF, resultados que demuestran el potencial de los microorganismos en el sistema de cultivo de batata en el Caribe Colombiano.

*Palabras clave:* PGPR, fertilización nitrogenada, *Azotobacter vinelandii*, *Azospirillum lipoferum*, camote

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## Introduction

Sweet potatoes hold third place of preference in root and tuber crops worldwide, only surpassed by potatoes and cassava. The importance of this crop lies in that its tuberous root is considered to be a nutritional alternative when used as a fresh produce for human feeding [1] and forage for animal feeding, it is also a source of starch, alcohols, and compounds of added value, such as vitamins and antioxidants [2-4]. Because of its adaptability, sweet potatoes tolerate extreme conditions, and when it reaches its maximum coverage, the use of fungicides and herbicides is relatively low. Sweet potatoes are currently considered a promising crop for increasing agricultural productivity and contributing to the economic development of the country.

The Colombian Caribbean area is appropriate for growing sweet potatoes, and the Turipaná Research Center of the Colombian Agricultural Research Corporation (AGROSAVIA) works in the validation of technologies for the agronomical management of this crop. Seed production, as well as the establishment, nutrition and maintenance of the crop, are some of the factors assessed for improving yields. In nutrition, within the biotechnological field, research is aimed towards the search for plant growth promoting microorganisms, as an alternative to the use of chemical fertilizers [5].

1. Bovell-Benjamin, AC. Sweet potato: a review of its past, present, and future role in human nutrition. Adv Food Nutr Res. 2007;52:1-59.

2. Mohanraj R, Sivasankar S. Sweet Potato (*Ipomoea batatas* [L.] Lam)-A valuable medicinal food: A review. J Med Food. 2014;17(7):733-41.

3. Pradhan DMP, Archana M, James G, Chakrabarti SK, Vimala B, Naskar SK, *et al*. High starch, beta carotene and anthocyanin rich sweet potato: ascent to future food and nutrition security in coastal and backward areas. Int J Trop Agric. 2015;33(2):397-400.

The benefits of plant growth promoters (PGPR) are well supported [6-11]; however, there are limitations in the use of biofertilizers since this technology is unable to compete with chemical fertilizers [10]. This new approach is to evaluate the use of these microorganisms to reduce chemical fertilizers, and they are then scaled-up in the field to obtain results that can be extrapolated to a commercial level.

Vegetative seeds that are mainly formed by apical and pre-apical buds, are used to establish sweet potatoes in the field, but some plant health problems related to this type of propagation have been reported [12]. The use of super-elite vegetative seeds obtained from plants grown in vitro, can guarantee the health of the crops, as well as increasing viability, uniformity and the consistency of field yields [13]. Greenhouse reports of the increase of parameters such as height and radicular weight have been described in super-elite vegetative seeds of sweet potatoes inoculated with microorganisms [5], but they have not yet been evaluated in the field.

With all this in mind, this study was aimed to determine the resulting crop yields under field conditions of the super-elite vegetative seed of sweet potato, in response to the inoculation of PGPR microorganisms combined with reduced nitrogen fertilization (NF) at 50 and 75 % of that used in two Colombian Caribbean agricultural areas.

## Materials and methods

### Location of experiments

Two locations in two areas of the Colombian Caribbean were chosen for this study. These were the area of Sabanas Colinadas and that of Montes de María; details of each area are shown in table 1.

### Microorganisms

Strains were provided by the Microorganism Germplasm Bank-AGROSAVIA of the Turipaná Research Center, obtained from the research work reported by Pérez-Pazos [5]. Two strains were chosen for each area: Sabanas Colinadas Strain 1 was *Azotobacter chroococcum*-IBCR19 and Strain 2 *Azospirillum lipoferum*-IBSC7, and for Montes de María area Strain 1 was *Azotobacter vinelandii*-IBCB10 and Strain 2 *Azotobacter vinelandii*-IBCB15. These bacterial strains were chosen based on their previously demonstrated PGPR properties *in vitro*, specifically for the production of total indoles, phosphorus solubilization and growth stimulation parameters on *Ipomoea batatas* seedlings under greenhouse conditions [5].

### Fertilization

Urea, di-ammonium phosphate (DAP), and potassium chloride were used for fertilization. Fertilizer dosages are shown in table 2; they were calculated according to the fertilization requirements of sweet potatoes plants [14] and the chemical conditions of the soil in each area (Table 2).

### Plant material

Super-elite vegetative seeds of the accession number 15020063 from the germplasm bank of AGROSAVIA, were chosen as the plant material (Figure 1A).

Table 1. Locations where sweet potato (*Ipomoea batatas* Lam) cultures were established for studying the effect of plant growth-promoting (PGRP) bacteria plus fertilization in seed yields

| Area | Property | District | Municipality/State | Coordinates N | Coordinates W |
|---|---|---|---|---|---|
| Sabanas Colinadas | Villa Valeria | Las Tinas | Corozal/Sucre | 09°19'3" | 75°17'29" |
| Montes de María | U.L. Carmen de Bolívar | Carmen de Bolívar | Carmen de Bolívar/ Bolívar | 09°42'50.8" | 75°06'26.9" |

Table 2. Chemical conditions of the soil and fertilization requirements for the production of sweet potato in two areas of the Colombian Caribbean: Sabanas Colinadas and Montes de María

| Area | Chemical composition of the soil | | | | | | Fertilizer dose (g/plant) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Organic material (%) | P (mg/kg) | K (mg/kg) | Mg (mg/kg) | Ca (cmol/kg) | pH | Urea | DAP | KCl |
| Sabanas Colinadas | 1.49 | 36.09 | 0.24 | 2.76 | 23.37 | 7.45 | 18.8 | 2.2 | 8 |
| Montes de María | 2.01 | 55.28 | 1.00 | 5.66 | 26.67 | 7.57 | 17.0 | 0 | 0 |

DAP: Diammonium phosphate.

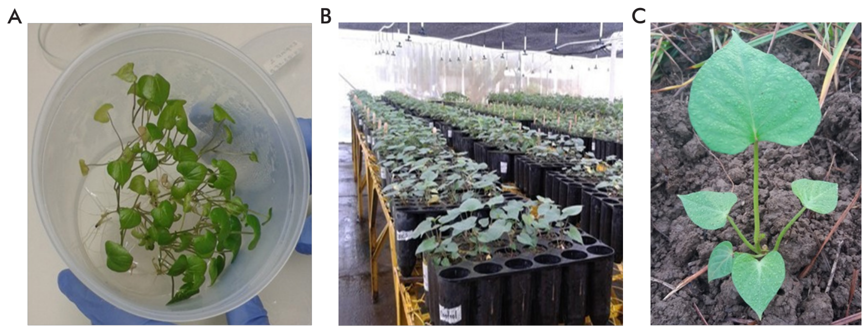


Figure 1. Super-elite seeds of sweet potato (*Ipomoea batatas* Lam), accession number 15020063. A) Seedlings produced *in vitro*. B) Hardening conditions of the super-elite sweet potato seeds in greenhouses. C) Super-elite sweet potato seeds suitable for field sowing.

The seeds were produced in vitro according to the micropropagation protocol established in the Biotechnology Laboratory of the Turipaná Research Center. The hardening of the super-elite seed was performed through two phases in a greenhouse (Figure 1B). The first phase started at sowing time until the third week. The plants remained under the sunlight at a luminosity of 24-27 klumen/$m^2$, with photoperiods of light/dark, of 12/12 hours, at 28-34 °C and a relative humidity of 80-95 %. Foggers blue base sprayers were used for irrigation, which worked at 40 psi with a discharge flow of 32 L/h, at intervals of 3 to 12 min and a frequency of 3 to 12 s. The second phase was carried out as of the third to the sixth week. The plants remained at a luminosity of 63-72 klumen/$m^2$, the temperature was at 34-38 °C and relative humidity of 60-80 %. Irrigation took place at intervals of 5 to 30 min and the frequency was of 5 to 20 s. Starting on the second week of hardening, the applications of fungicides and foliar fertilizations were carried out once a week. When the material reached the sixth week, it was considered to be suitable for sowing in field (Figure 1C). Constant irrigation in the afternoon was required, starting at sowing and for a period of 8 days.

### Inoculation of microorganisms and fertilization

The inoculation of microorganisms was made while using nitrogen fertilization (NF). The NF was applied in two fractions, the first one was of 30 % at 20 days

4. Tanaka M, Ishiguro K, Oki T, Okuno S. Functional components in sweetpotato and their genetic improvement. Breed Sci. 2017;67(1):52-61.

5. Pérez-Pazos JV, Sánchez-López DB. Caracterización y efecto de *Azotobacter*, *Azospirillum* y *Pseudomonas* asociadas a Ipomoea batatas del Caribe Colombiano. Rev Colomb Biotecnol. 2017;19(2):39-50.

6. Yasmin F, Othman R, Sijam K, Saad, MS. Effect of PGPR inoculation on growth and yield of sweetpotato. J Biol Sci. 2007;(2):421-4.

7. Dawwam G E, Elbeltagy A, Emara, H M, Abbas I H, Hassan M M. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Ann Agr Sci. 2013;58(2):195-201.

8. Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil. 2014;378(1-2):1-33.

9. Glick BR. Introduction to plant growth-promoting bacteria. In: Beneficial plant-bacterial interactions. Dordrecht: Springer International Publishing; 2015. p. 1-28.

10. Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq Boyce A. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules. 2016;21(5):573.

after sowing, and the second of 70 % at 35-40 days after sowing. At fertilization and inoculation, the plants had well developed leaves and longitudinal growth, represented in the coverage of 40-60 %. The inoculation of microorganisms was carried out directly in the soil at the foot of the plant with 10 ml of the bacterial suspension having a cellular concentration of $1 \times 10^8$ cfu/mL in the Luria Bertani medium (peptone 10.0 g/L, yeast extract 5.0 g/L, sodium chloride 5.0 g/L; pH 7.0 ± 2). Chemical fertilization was applied to the soil at each side of the plant at a distance of 5 cm from the stem. At the Sabanas Colinadas location where phosphorus and potassium were required, they were added at the time of sowing.


**Figure 2. Field cultures of super-elite seeds of sweet potato (*Ipomoea batatas* Lam), accession number 15020063 at Sabanas Colinadas area, Corozal municipality, Sucre State, Colombia. A) Sixty-days cultures. B) Tuberous roots of sweet potatoes obtained from super-elite seeds.**

### Experimental design

In order to evaluate the effect of strains of microorganisms and NF, a complete random block design with a 3×2 factorial design was established. This corresponded to three strains per zone (strain 1, strain 2 and a combination of the strains) and two levels of nitrogen fertilization (50 and 75 %), for a total of six treatments.

Three replicates were made for each one of the treatments. The field was distributed in experimental plots of 36 $m^2$ (5 rows that were 1-m wide and 7.2-m long). The sowing distance between plants was of 0.4 m for a total of 90 plants per plot. The response variable established was the total yield of tuberous roots after the development of the crop for 210 days. The best treatments resulting from the factorial analysis were compared to a treatment without the inoculation of microorganisms, having 100 % NF, in order to observe the presence of any significant differences.

### Statistical analysis

The yields were analyzed using a multifactorial analysis of variance using the 3×2 factorial arrangement, to determine the effect of the inoculation of microorganisms and NF in the field. The best treatments resulting from the factorial analysis were compared with the treatment without the inoculation having 100 % NF, using a one-way factorial analysis. Means were compared using the Tukey test with the 95 % confidence level. The analyses were made using the statistical software SAS v. 9.2.

## Results and discussion

### Sweet potato yields under the influence of PGPR bacteria and nitrogen fertilization in the area of Sabanas Colinadas

The percentage of survival of the super-elite seed was of 98 %, at eight days after sowing, reaching 100 % coverage at 60 days after sowing (Figure 2A). This indicates the low proportion of mortality of the seed, making this super-elite seed a promising material for sweet potato cultivation. Using the super-elite seed we obtained the tuberous roots under the normal tillage conditions used for sweet potatoes from the conventional seed (Figure 2B).

The multifactorial analysis of variance of yields, obtained 210 days after sowing, indicates that of the independent factors, only nitrogen fertilization (NF) had a statistically significant effect ($p = 0.003$), on the response variable. The interaction of the bacterial strain and NF showed a significant effect ($p = 0.036$) on the yield of the tuberous sweet potato roots with a 95 % confidence level.

It must be highlighted that the soils of Sabanas Colinadas are sandy and have low organic matter (1.49 %) and nutrients (Table 2), and therefore, the independent effect of fertilization on yields is probably related to changes of the nutritional conditions for the plant in this type of soil. The application of bacterial strains was only significant in the interaction with NF, indicating that in soils with nutritional deficiencies the bacterial strains depend on the availability of nutrients in the soil, which is a condition that possibly affects its effect as a growth stimulator. Similar results were reported by Singh [11], who evaluated sweet potato yields with treatments submitted to fertilization combined with *Azotobacter*, where it was found that with the combined application of organic and inorganic (NPK) fertilization and biofertilizers there was a significant improvement in plant growth, yield and quality parameters, demonstrating the interactive advantage of the use of inorganic NPK sources combined with biofertilizers, compared to the independent action of each factor.

Mean comparisons were used to determine the effect of the interactions of all factors (Table 3). Results indicated that the effect of the inoculation of microorganisms, both individually and combined, generated greater yields when they were applied with 75 % NF.

**Table 3. Mean comparison of the interaction of strains and fertilization for the total yield of the tuberous roots of sweet potato (*Ipomoea batatas* Lam) in the area of Sabanas Colinadas, Corozal municipality, Sucre State, Colombia**

| Bacterial treatment | Nitrogen fertilization (%) | Yield (Ton/ha)* |
|---|---|---|
| IBCR19 + IBSC7 | 75 | 14.538 ± 0.809 a |
| IBCR19 | 75 | 13.306 ± 1.393 ab |
| IBSC7 | 75 | 11.828 ± 1.189 ab |
| IBSC7 | 50 | 11.724 ± 0.927 ab |
| IBCR19 | 50 | 11.549 ± 1.366 ab |
| IBCR19 + IBSC7 | 50 | 10.559 ± 0.950 b |

* Means with the same letter not differ statistically according to the Tukey test ($\alpha \leq 0.05$). Values are presented in decreasing order. IBCR19: *Azotobacter chroococcum* strain IBCR19; IBSC7: *Azospirillum lipoferum* strain IBSC7.

11. Singh J. Effect of organic and inorganic sources of NPK and bio-fertilizer on growth, yield and quality of sweet potato (*Ipomoea batatas* Lam.). [Master Thesis] Master of Science in Agriculture (Horticulture). Department of Horticulture S.K.N. College of Agriculture, Jobner Sri Karan Narendra Agriculture University. 2017.

12. Barba M, Czosnek H, Hadidi A. Historical perspective, development and applications of next-generation sequencing in plant virology. Viruses. 2014;6(1):106-36.

13. Roy PK, Kabir MH. *In vitro* mass propagation of sugarcane (*Saccharum officinarum* L.) var. Isd 32 through shoot tips and folded leaves culture. Biotechnology.2007;6(4):588-92.

14. Folquer F. La batata (camote): estudio de la planta y su producción comercial Buenos Aires: Editorial Hemisferio Sur; 1978.

Although there were no significant differences between the strains inoculated at that fertilization level, the highest yields were obtained with the inoculation of the mixture, surpassing the treatment with each of the strains IBCR19 and IBSC7 at nearly 10 and 22 % respectively, and showing a statistically significant increase of 38 % compared to the treatment with 50 % NF that combined the inoculation of the mixture of bacterial strains (Table 3).

Results show that in soils such as those of Sabanas Colinadas, the increase in the level of fertilization from 50 to 75 %, favors the effect of the *A.chroococcum* and *A. lipoferum* bacterial strains in yields. Based on these results it can be inferred that the nutritional quality of the soil affects the establishment of the strains and the biological activity they offer under optimum concentrations. Similar results were found by Sharma [15], who evaluated the application of *Azospirillum* and *Azotobacter* in four levels of nitrogen and found that the application of *Azospirillum* with the maximum nitrogen level gave the maximum yield of *Brassica oleracea*. Also, Aswani *et al*. [16], who studied the effect of four levels of nitrogen and two sources of biofertilizers *Azotobacter* and *Azosprillium* on the yield and quality of onion bulbs (*Allium cepa* L.) observed that the combination of the high nitrogen fertilizer concentrations potentiated the beneficial effect of the microorganisms evaluated.

In order to validate the effect of the inoculation of microorganisms on the yield of sweet potato root tubers we compared, through a factorial design, the treatments showing the best results, with a treatment of the complete level of NF (100 %) without inoculating the microorganisms. The results obtained are shown in figure 3.

The inoculation of the IBCR19 strain using 75 % NF did not differ significantly from the treatment without inoculation having 100 % NF, although the average yield of the tuberous roots of sweet potatoes increased in 21 % (Figure 3). In relation to the inoculation of the combination of strains IBCR19 + IBSC7 + 75 % NF there was a significant increase ($\alpha \leq 0.05$) of 32 % in the average yield of the tuberous roots of sweet potatoes, of about 3.5 ton/ha compared to the 100 % NF treatment without the inoculation of microorganisms. In general terms, these results suggest that the use of microorganisms not only makes it possible to increase sweet potato yields, but also to substitute 25 % of the NF in the case of tuberous roots of sweet potatoes in the area of Sabanas Colinadas of the Columbian Caribbean, reaching significant yields with the combination of the *A. chrococcum*-IBCR19 and *A. lipoferum*-IBSC7 microorganisms.

Regarding the use of these two microorganisms, some reports on sweet potato cultivation in the field showed increases in yields when inoculating a combination of microorganisms with fertilization. Yasmin *et al*. [6], reached yields of 16.72 ton/ha with the inoculation of *Azospirillum* and 33 kg of N/ha. Also, Singh [11] reported that the combination of inorganic fertilization with the inoculation of Azotobacter significantly increased the weight of sweet potato tubercles compared to the treatment without the inoculation. These two bacterial strains show growth promotion characteristics, evidenced in the production of indoles and the solubilization of phosphorus *in vitro* (Table 4) [5]. These characteristics enable the interaction of these bacteria with the plant. The bacteria benefit from many compounds exudated by the plant, and the plant also benefits from the phytohormones, as well as from the transformation processes of compounds that are made available to them [7, 17]. Therefore, the incorporation of the bacterial strains *A. chrococcum*-IBCR19 and *A. lipoferum*-IBSC7 combined with 75 % NF in potato production systems, favor the growth of the plant that is finally reflected in the increase in yields of the tuberous roots.

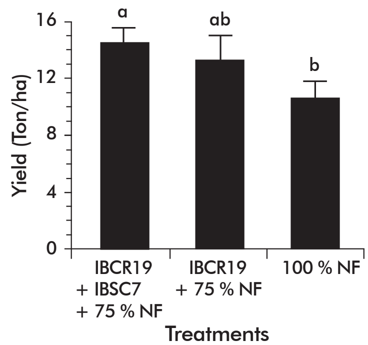


Figure 3. Yields of tuberous roots of sweet potato (*Ipomoea batatas* Lam) grown in the field under treatments with and without the inoculation of microorganisms at the Sabanas Colinadas area, Corozal municipality, Sucre State, Colombia. There were statistically significant differences (p = 0.0167) according to the Tukey test with a 95 % confidence level. BCR19: *Azotobacter chroococcum* strain IBCR19; IBSC7: *Azospirillum lipoferum* strain IBSC7. NF: nitrogen fertilization.

## Sweet potato yields under the influence of bacteria and nitrogen fertilization in the area of Montes de María

Mortality in the field of the super-elite seed was of 8 %, at eight days after sowing and reached 100 % coverage at 60 days after sowing (Figure 4A). This indicates that the seed had a high percentage of survival, thereby confirming the possibility of the use of this super-elite seed for sowing in the field. The tuberous roots obtained from the super-elite seed were equal to the normal ones obtained from the conventional seed (Figure 4B).

The multifactorial analysis of variance of yields indicate that in contrast to the Sabanas Colinadas area, in Montes de María the individual strains showed significant differences (p = 0.0352) in the response variable. The interaction of the strain and NF was statistically significant with a confidence level of 95 %.

Table 4. *In vitro* growth promotion activity of bacterial strains selected for studying their plant growth-promoting (PGRP) bacteria plus fertilization on sweet potato (*Ipomoea batatas* Lam) plants*

| Bacterial strain | Total indole production ($\mu$g/mL) | Solubilization of Phosphorus (ppm) |
|---|---|---|
| *Azotobacter chroococcum*-IBCR19 | 49.72 | 9.36 |
| *Azospirillum lipoferum*-IBSC7 | 7.41 | 7.14 |
| *Azotobacter vinelandii*-IBCB10 | 17.51 | 4.38 |
| *Azotobacter vinelandii*-IBCB15 | 40.56 | 4.34 |

* Data summarized from reference [5].

15. Sharma SK. Effect of *Azospirillum*, *Azotobacter* and nitrogen on growth and yield of cabbage (*Brassica oleracea* var capitata). Indian J Agr Sci. 2002.72(9):555-7.

16. Aswani G, Paliwal R, Sarolia DK. Effect of nitrogen and bio-fertilizer on yield and quality of rabi onion (*Allium cepa* L.) Cv. Puna Red Agric Sci Digest. 2005;25(2):124-6.

17. Nihorimbere V, Ongena M, Smargiassi M, Thonart P. Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol Agron Soc Envir. 2011;15(2):327-37.

Results suggest that the yields of sweet potatoes in the area of Carmen de Bolívar depend both on the bacterial inoculation and on the level of fertilization. Similar results were obtained for the Colinadas area in this study and the reports of other authors [15, 16]. This confirms that the incorporation of efficient microorganisms with NF is a possible alternative for improving the nutritional conditions of the crop and increasing the yields of sweet potatoes in the Columbian Caribbean. Means were compared to determine the effect of the interaction of the factors (Table 5).

The highest average yield in the field was reached when the sweet potato crop was inoculated with the IBCB10 strain using 75 % NF, which significantly surpassed ($\alpha \leq 0.05$) the rest of the combinations with 75 % NF (Table 5). Considering that the strains used for this area belong to the same species, the effect of the IBCB10 strain is significantly potentiated with the inclusion of 75 % NF, compared to 50 % NF in contrast to the IBCB15 strain. These results are probably related to the production capacity of the phytohormones of the *A. vinelandii*-IBCB10 strain, as shown in table 4. The IAA has an important impact on root development by elongating the primary roots, stimulating density and the length of the root hairs, which increase the total adsorption surface of the roots [18, 19]. All these factors are probably favoring the absorption capacity of the nutrients of plants inoculated with this microorganism [20-22]. Therefore, the 75 % NF level may be considered in this case the optimum nutrient concentration, enabling the establishment of the bacterial strain and the growth of the crop, shown by the increase in sweet potato yield.

It was shown that using 50 % NF with the inoculation of the combination of strains, yields did not differ significantly from the treatment with the highest average value (Table 5). This suggests that for the reduction of up to 50 % NF in the Montes de María soils, the IBCB10 and IBCB15 can offer good yields. The area of Montes de María has clay soils. This type of soil is characterized by an intermediate natural fertility, shown by its content in organic matter and nutrients (Table 2). In this type of soil 50 % NF would probably be appropriate for establishing the strains and for their biological activity, in contrast with 75 % NF, where the yields were lower. These results contrast with those obtained by other studies [15, 16], where the increase in fertilization favors the effect of the inoculation of microorganisms. These facts indicate that in the Montes de María soils, the activity of the bacterial strains are probably influenced by other factors, in addition to the mineral composition of the soil, the organic matter and even the microbial flora, which has not yet been characterized.

To validate the results obtained in the treatments with the inoculation of microorganisms where the highest average yields are obtained, a comparison was made with the treatment having 100 % NF without inoculation. The results are shown in Figure 5.

No statistically significant differences were observed between the treatments inoculated with microorganisms, IBCB10 + 75 % NF and the combination at 50 % NF compared to the treatment without any inoculation at 100 % NF. This demonstrates that the inoculation and reduction of the NF does not negatively affect the yields of tuberous roots. On the contrary, with the inoculation of microorganisms we obtained increases of up to 21 % in the average yields of tuberous roots with the inoculation

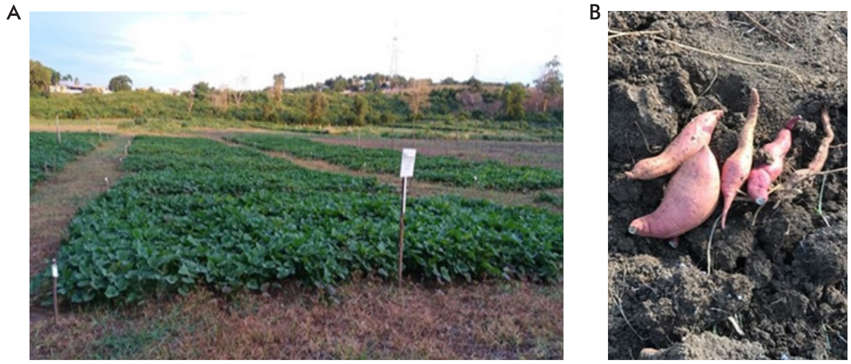


Figure 4. Field cultures of super-elite seeds of sweet potato (*Ipomoea batatas* Lam), accession number 15020063 at Montes de María area, Carmen de Bolívar municipality, Bolívar State, Colombia. A) Sixty-days cultures. B) Tuberous roots of sweet potatoes obtained from super-elite seeds.

Table 5. Mean comparison of the interaction of strains and fertilization for the total yield of the tuberous roots of sweet potato (*Ipomoea batatas* Lam) in the area of Sabanas Colinadas, Carmen de Bolívar municipality, Bolívar State, Colombia

| Bacterial treatment | Nitrogen fertilization (%) | Yield (Ton/ha)* |
|---|---|---|
| IBCB10 | 75 | 15.29 ± 1.29 a |
| IBCB10 + IBCB15 | 50 | 13.63 ± 1.44 ab |
| IBCB15 | 50 | 13.10 ± 0.52 ab |
| IBCB10 | 50 | 12.51 ± 0.28 b |
| IBCB15 | 75 | 12.05 ± 1.37 b |
| IBCB10 + IBCB15 | 75 | 11.02 ± 0.38 b |

* Means with the same letter not differ statistically according to the Tukey test ($\alpha \leq 0.05$). Values are presented in decreasing order. IBCB10: *Azotobacter vinelandii* strain IBCB10; IBCB15: *Azotobacter vinelandii* strain IBCB15.

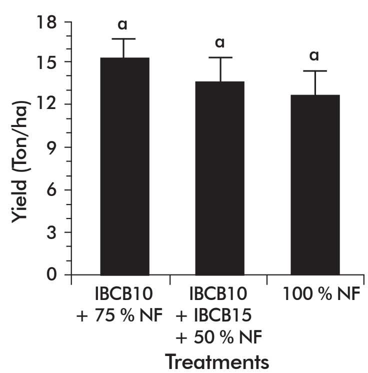


Figure 5. Yields of tuberous roots of sweet potato (*Ipomoea batatas* Lam) grown in the field under treatments with and without the inoculation of microorganisms at the area of Montes de María area, Carmen de Bolívar municipality, Bolívar State, Colombia. There were no statistically significant differences (p = 0.169) according to the Tukey test with a 95 % confidence level. IBCB10: *Azotobacter vinelandii* strain IBCB10; IBCB15: *Azotobacter vinelandii* strain IBCB15. NF: nitrogen fertilization.

18. Bucio J, Cueva J, Cantero, Becerra C, Rodríguez R., Rodríguez L. *Bacillus megaterium* modifica la arquitectura de la raíz de *Arabidopsis* independientemente de auxinas y etileno. Biológicas. 2013;11(1):1-8.

of the IBCB10 strain and 75 % NF observed in 2.7 ton/ha and 8 % with the treatment of the combination at 50 % NF. This confirms the potential use of microorganisms to favor yields of the tuberous roots of sweet potato and the decrease of NF in the cultivation of sweet potatoes in the area of Montes de María of the Colombian Caribbean (Figure 5).

Concerning the yield of sweet potatoes and as reported by FAOSTAT [23], in the year 2016 there were 8.6 million hectares cultivated in the world, with a total production of 105.2 million tons. The contribution to these statistics by South America corresponds to an area of 107 149 hectares (1.2 %) with a total production of 1.4 million tons (11.5 %), having an average yield of 13.58 tons/ha. In Colombia, sweet potato is an emerging crop. In 2008 the area sowed was of 5 hectares, with a production of 25 tons (5 tons/ha) in the Colombian Caribbean (Córdoba) [24]. In this study the area of Sabanas Colinadas (Sucre) produced a maximum average yield of 14.5 tons/ha, and for the area of Montes de María (Bolívar) it was of 15.3 tons/ha. These values are higher than those generally reported for the Colombian Caribbean, and within the range reported for South America, showing a positive balance for this area of the Colombian Caribbean.

## Conclusions

The use of super-elite vegetative seeds, defined as cuttings obtained from plants that have been produced by *in vitro* propagation, showed over 90 % viability in the field, generating high quality plants free from phytopathogens and with viable tuberous roots. The yields in the field were uniform and consistent. These facts show that the super-elite seed is of a good potential for the field cultivation of sweet potatoes.

In the soils of Sabanas Colinadas, 75 % NF and the inoculation of the combination of *A. chroococcum*-IBCR19 and *A. lipoferum*-IBSC7 bacteria, provides the highest yields (14.5 ton/ha). In the area of Montes de María, the highest yields (15.3 ton/ha) were obtained with the inoculation of the *A. vinelandii*-IBCB10 bacterium and 75 % nitrogen fertilization, but for a reduction in nitrogen fertilization of 50 %, the combination of strains can reach yields of 13.63 tons/ha, which does not differ statistically from the best treatment.

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## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

19. Lara C. Bacterias nativas con potencial en la producción de ácido indo acético para mejorar los pastos. Zootec Trop. 2014;29(2):187-94.

20. Ahmad F, Ahmad I, & Khan M S. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res. 2008;163(2):173-81.

21. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 2010;60(4):579-98.

22. Saharan BS, Nehra V. Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res. 2007;21(1):1-30.

23. FAOSTAT. Statistics Division. Food and Agriculture Organization of the United Nations. 1. Viale delle Terme di Caracalla, Rome, Italy. 2017 [cited 2017 Dec 18]. Available from: http://www.fao.org/faostat/en/#data/QC

24. AGRONET. Red de Información y Comunicación del Sector Agropecuario Colombiano. Bogotá: Ministerio de Agricultura y desarrollo rural. 2017 [cited 2017 Dec 18]. Available from: http://www.agronet.gov.co/estadistica/Paginas/default.aspx

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