Received March 28th 2019// Accepted July 29th 2019 // Published online July 14th 2021

Yield response of yam (*Dioscorea rotundata* Poir.) to inoculation with *Azotobacter* and nitrogen chemical fertilization in the Caribbean region of Colombia

SÁNCHEZ, D.B.[1]; LUNA, L.L.[1]; ESPITIA, A.A.[1]; CADENA, J.[1]

ABSTRACT

In previous studies, we collected roots and soils associated with yam crops (*Dioscorea rotundata* Poir.) along the Caribbean region of Colombia from which several strains of *Azotobacter chroococcum* and *Azotobacter vinelandii* were identified, which in laboratory and nursery studies showed growth promotion activity in yam. In this research, we obtained from Agrosavia two of these strains (*A. chroococcum* DBC12, *A. vinelandii* DBC9) and evaluated their effect on yam yields and tuber quality under field conditions, in combination with four nitrogen levels (0, 50, 75 and 100% of the recommended N fertilization dose). In a first instance, *in vitro* tests confirmed the N fixation capacity and the NH_3 production of both bacterial strains, while under field conditions, the highest yields were obtained from treatments that combined the individual inoculation with strains *A. chroococcum* DBC12 or *A. vinelandii* DBC9, with 50% of the recommended N fertilization level. On the other hand, yam tubers were classified according to market quality, and the same treatments induced the production of higher yields of first category or export-type tubers, suggesting that these two bacterial strains were also able to improve tuber quality. From this study it was concluded that *A. chroococcum* DBC12, *A. vinelandii* DBC9 strains have the potential for replacing up to 50% of the recommended N fertilization dose and present potential as possible bio-inoculants, which could be an alternative for reducing levels of chemical nitrogen fertilization, thus contributing to a more sustainable and competitive yam culture.

Keywords: PGPR, Rhizobacteria, tuber yield, tuber quality.

RESUMEN

En estudios previos, realizamos una colecta de raíces y suelos asociados a cultivos de ñame (Dioscorea rotundata Poir) en la región Caribe de Colombia, de los cuales se lograron aislar e identificar varias cepas de Azotobacter chroococcum y Azotobacter vinelandii, que en estudios de laboratorio y casa de malla mostraron promoción del crecimiento en plantas de ñame. Para la presente investigación, se obtuvieron de AGROSAVIA, dos de estas cepas (A. chroococcum DBC12, A. vinelandii DBC9) y se evaluaron, bajo condiciones de campo, sus efectos sobre los rendimientos y la calidad de los tubérculos de ñame, en combinación con la aplicación de cuatro niveles de fertilización química con nitrógeno (0, 50, 75 and 100% de la dosis recomendada

[1]Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Centro de Investigación Turipaná, km 13, Vía Montería-Cereté, Colombia. Correo electrónico: dbsanchez@corpoica.org.co

para el cultivo). Por un lado, en una primera prueba realizada bajo condiciones in vitro, se confirmó la capacidad de fijación de N y la producción de NH_3 en ambas cepas de bacterias, mientras que, bajo condiciones de campo, los más altos rendimientos se obtuvieron con la combinación de la inoculación individual con las cepas A. chroococcum DBC12 o A. vinelandii DBC9, y el 50% de la dosis recomendada de fertilización con nitrógeno. Por otro lado, la clasificación de los tubérculos de ñame de acuerdo con la calidad para el mercado, indicaron que los mismos tratamientos indujeron la producción de una mayor cantidad de tubérculos de primera categoría o calidad tipo exportación, lo que sugiere que estas dos cepas de bacterias son efectivas para mejorar también los aspectos de calidad de los tubérculos. De estos resultados, se concluye que las cepas A. chroococcum DBC12 y A. vinelandii DBC9 tienen la potencialidad de reemplazar hasta el 50% de la dosis recomendada de fertilización nitrogenada y presentan potencial como posibles bioinoculantes, lo que las convierte en una alternativa para reducir los niveles de fertilización química, contribuyendo así a una agricultura más sostenible y competitiva en el cultivo del ñame.

Palabras claves: *PGPR, rizobacterias, rendimiento y calidad de tubérculos.*

INTRODUCTION

Yam comprises a group of climbing plant species, which produce tubers or roots, some of which are edible and important for the food supply of many rural populations in the world (Dumet and Ogunsola, 2008). Yam belongs to the *Dioscorea* genus, being a very common plant crop in the humid and sub-humid tropics, especially in Africa, West Indies, and in some parts of Asia, Central and South America. There are about 600 species of yams registered, being *D. alata, D. bulbifera, D. cayenensis, D. esculenta, D. opposita-japonica, D. nummularia, D. pentaphylla, D. rotundata and D. trifida* the main species grown worldwide as edible food (Siqueira, 2009). Some of the *Dioscorea* spp. species have also been reported to have antioxidant capacity and a high steroidal and sapogenin content, substances used in the manufacture of oral contraceptives, sex hormones and cortisone (Applezweig, 1977; Rodríguez, 2000).

One of the most cultivated species in the world is *D. rotundata* Poir, a highly appreciated species due to its importance as a food source, high carbohydrate and starch content (Bömer *et al*., 2018; Markson *et al*., 2010; Vashi *et al*., 2018). Most yam crops and cultivated areas are concentrated in African countries with approximately 96% of world production, while America contributes approximately 2.64% to the global world production. Colombia is second in production in the Americas, with 381,468 tons per year (FAOSTAT, 2017). In Colombia, yam is grown mainly in the Caribbean region by small-holder farmers, for whom it is the main source of food, income, and employment (Benítez *et al*., 2007).

At the present, there is an urgent need to intensify the production of food for an increasing world population, but at the same time, it is also necessary to promote a more sustainable agriculture that protects natural resources. Nitrogen fertilization has been established as the main tool to increase crop yields. However, the continuous increase in fertilizer prices has made it unreachable for many rural farmers, especially small-scale ones (Savci, 2012). One alternative that has recently drawn great attention is the production of biopreparations or bioproducts made up from microorganisms, which in part replace the inputs of chemical origin. Some of these bioproducts are based on the use of Plant Growth Promoting Rhizobacteria or PGPR. These PGPR are bacteria that colonize the soil and establish a relationship with the roots of plants, improving their ability to access nutrients (Chauhan *et al*., 2015). The mechanisms through which these bacteria stimulate growth of many plants include biological fixation of atmospheric nitrogen (N_2), production of phytohormones (auxin, i.e., Indole acetic acid (IAA), cytokinins, gibberellins, abscisic acid), solubilization of phosphate compounds in the soil, and the production of antimicrobial substances (production of lytic enzymes, siderophores), among others (Chauhan *et al*., 2015; Compant *et al.,* 2005).

Among the genera that have been identified associated with the roots of different plant species are *Acinetobacter, Azotobacter, Alcaligenes, Arthrobacter, Azospirillium, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Gluconacetobacter, Herbaspirillum, Rhizobium*, and *Serratia* (Ahmadi-Rad *et al*., 2016; Ramirez *et al*., 2018).

One of the main genus of bacteria that is part of the PGPR group is *Azotobacter*. The bacteria belonging to the genus *Azotobacter* are aerobic, free-living bacteria, very common and dominant in the rhizosphere of plants (Chennappa *et al.,* 2018). Several reports in the literature mention that the *Azotobacte*r genus provides a series of byproducts that can be used by the roots of plants (Vejan *et al*., 2016), especially the fixation of atmospheric nitrogen (N_2), the production of some hormones, and metabolites or secondary factors that stimulate the growth of plants. Within these, the role played by *Azotobacte*r in the fixation of atmospheric nitrogen stands out, due to its importance for agriculture, since its presence in the rhizosphere partially ensures the release of ammonium ions that would be available for growth of plants. In the soil, it has been calculated that this type of bacteria fixes about 20 kg N ha^{-1} $year^{-1}$ (Dilworth *et al*., 1988). As such, *Azotobacte*r has been reported in several studies as a promoter of growth in various crops such as

wheat, corn, rye, oats, and vegetables (Baba *et al*., 2018; Kizilkaya, 2008; Kushwaha *et al*., 2013).

In 2015, we collected roots and soils associated with yam production in the Caribbean region of Colombia from which we identified microorganism species associated with yam roots. Among the species isolated, we identified several strains of *Azotobacte*r, which in laboratory studies showed IAA production and solubilization of phosphorus. Subsequently, nursery trials showed their ability to establish relationships with the roots of yam, showing also growth promotion activity (Sánchez and Pérez, 2018). We hypothesize that these microorganisms have the potential to be used as biofertilizers in yam crops. The present investigation was carried out to evaluate, under field conditions, the capacity of *Azotobacte*r strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9 to reduce the use of chemical nitrogen fertilization and increase yield and quality in yam crops in the Caribbean region of Colombia.

MATERIALS AND METHODS

Location

The experiment was carried out at an experimental station of the Colombian Agricultural Research Corporation (Agrosavia), in Carmen de Bolívar, located in the Caribbean region of Colombia, at 09° 42' 56.0 "North and 75° 06' 15.5" West, at an altitude of 197 masl. According to Holdridge's agro-ecological classification (Holdrige, 2000), this location belongs to a Tropical Dry Forest (TDF), and presents an average annual rainfall of 1,179 mm, average relative humidity of 72%, and annual temperature of 26.9°C. The experiment was performed on clay loam soil, with pH of 7.93, organic matter 2.37%, available phosphorus 105.39 mg kg^{-1}, Calcium 6246 mg kg^{-1}, Magnesium 566.4 mg kg^{-1}, Sodium 59.8 mg kg-1, Potassium 351.0 mg kg^{-1}, and Sulfur 8,99 mg kg^{-1}.

Plant Material

Yam tubers (*Dioscorea rotundata*) cv Criollo were obtained and divided into 100-g sections, that were sown in polyethylene bags (0.15x0.15 m), filled with a substrate composed of a mixture of sand and clay soil at a 1:1 ratio: This substrate was previously sterilized by solarization and application of a fungicide containing a mixture of Carboxymethyl + Thiram, at a rate of 4 g L^{-1}. The resulting seedlings were maintained under nursery conditions for a period of 30 days and then transplanted to the experimental plots in the field when reached a height between 0.15 and 0.20 m.

Rhizobacteria

In this experiment, we evaluated bacterial strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9. These strains are part of a group of several *Azotobacter* bacteria that were collected by Agrosavia, associated with roots and soils of yam crops in the Caribbean region of Colombia. These two strains were selected for this experiment because in previous laboratory and nursery studies, had shown growth promotion activity in yam plants (Sánchez and Pérez, 2018). In particular, strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9 were collected at a yam crop in the municipality of Carmen de Bolívar (County of Caracolí Grande), located at 9° 44' 24.8 N and 75° 13' 28.2" W. For the purpose of this research, strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9 were reactivated in an Ashby-sucrose culture medium, ensuring their purity and viability. The inoculant to use in these experiments was produced from discontinuous fermentation, using in nutritive culture medium composed of (g L-1): glucose 0.5, yeast extract 0.5, peptone 0.5, casein 0.5, starch 0.5, K_2HP0_4 0.30, $MgSO_4$ 0.05 and pH: 7.0, which was maintained at 30°C and 120 rpm in an orbital shaker. The inoculum was prepared in a flask of 2000 mL of total volume with 1000 mL of working volume in 2/1 ratio to allow sufficient aeration starting from an adjusted pre-inoculum OD_{540} = 0.500.

The inoculant to use in these experiments was produced in nutritive culture medium composed of (g L^{-1}): glucose 0.5, yeast extract 0.5, peptone 0.5, casein 0.5, starch 0.5, K_2HP0_4 0.30, $MgSO_4$ 0.05 and pH: 7.0, which was maintained at 30 °C and 120 rpm, in a discontinuous fermentation media, starting from an adjusted pre-inoculum OD_{540}=0.500.

Experimental design

We used a randomized complete block design, with an unbalanced 4x4 factorial arrangement, with three replications, where the first factor corresponded to the bacterial

Azotobacter strain	Nitrogen fertilization level (%)			
	0	100	75	50
Without *Azotobacter*	T0	T1	T2	T3
A. chroococcum DBC12	-	-	T5	T4
A. vinelandii DBC9	-	-	T7	T6
A. chroococcum DBC12+ *A. vinelandii* DBC9	-	-	T9	T8

Table 1. Treatments for evaluating two strains of *Azotobacte*r and four levels of nitrogen fertilization on the productivity and quality of yam in the Caribbean region of Colombia.

strains (without bacteria, *A. chroococcum* DBC12, *A. vinelandii* DBC9, and a mixture of both), and the second factor corresponded to levels of nitrogen fertilization (0%, 50%, 75% and 100% of the recommended N fertilization dose). The experimental design used was unbalanced due to the elimination of treatments that combined the application of rhizobacterial strains, alone or as a mixture, with 0% and 100% N fertilization levels, since, in previous experiments, these treatments produced null yields in yam cultivation (data not shown). The experimental unit consisted of plots with five yam rows, 8.0 m long, 1.0 m apart and 0.5 m separation between plants, for a population density of 20,000 plants per hectare. The area of each plot was 32 m^2. The total experimental area was 1,350 m^2, including circulation areas and borders.

Treatments

Ten treatments were evaluated, corresponding to the combination of bacterial strains, alone or as a mixture, with the levels of N fertilization, in an unbalanced factorial design. The treatments evaluated are shown in table 1.

To calculate the adequate dose of N fertilization, the availability of nutrients in the soil were compared with nutritional requirements for yam reported by O'Sullivan (2010) and based on that, a dose of 183 kg of N ha^{-1} was calculated. Nitrogen was supplied as chemical fertilizer ammonium sulfate $(NH_4)_2SO_4$ with a concentration of 21% Nitrogen and 24% Sulfur. This source of N was selected due to the tendency of these soils to have an alkaline reaction and high pH. No other sources of fertilizer were applied since the soil had enough supply of P, K, and microelements. The recommended dose of ammonium sulfate in each treatment was split in two applications, 50% of which was applied 70 days after transplanting (DAT), and the remaining 50% at 130 DAT. The fertilizer was applied around each individual yam plant. Eight days after chemical fertilization, 10 mL of bacterial inoculum, corresponding to 10^8 Colony Forming Units (CFU) mL^{-1} bacteria was injected around each individual plant. A second inoculation of bacteria, with 10 mL of bacterial inoculum, was carried out eight days after the second N fertilization, i.e. at 138 DAT.

Response variables

PGPR activity

Three *in vitro* tests were done to corroborate the PGPR activity of the bacterial strains *A. chroococcum* DBC12, *A. vinelandii* DBC9. In the first instance, a qualitative test that marked positive or negative for the capacity of the biopreparations to fix atmospheric N was carried out, using a semi-solid N-free media (Wilson and Knight, 1952), in which the bacterial strains were inoculated. For this test, a non-inoculated medium was used as a negative control. A second test was carried out to detect the production of ammonia (NH_3), by inoculating the bacterial strains in a nutritive broth composed of (g L^{-1}): 0.5 yeast extract; 0.5 peptone; 0.5 casein; 0.5 glucose; 0.5 starch; 0.30 K_2HPO_4; 0.30 $MgSO_4$ and with pH 7.2. In this medium the bacterial strains were incubated for a period of 24 h at 30°C. Solutions of phenol/nitroprusside and sodium hydroxide/sodium hypochlorite were added to the supernatant, in a 1:1 ratio, and allowed to react for 30 minutes at 60°C. After this, a reading of absorbance was performed on a spectrophotometer at 630 nm (Spectronic 601, Milton Roy). Ammonia production by bacteria was calculated based on an absorbance curve calibrated previously (figure 1). A third test was carried out to verify the *in vitro* compatibility between both bacterial strains, by means of a dual confrontation, in a petri dish

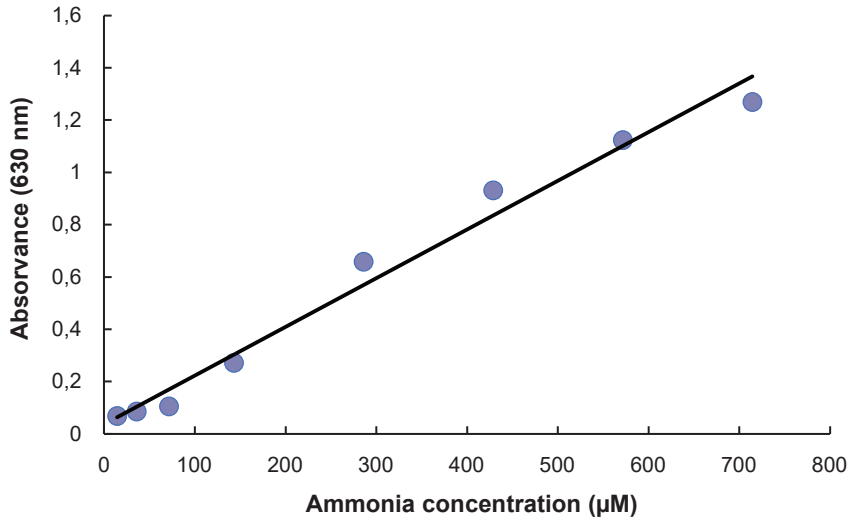

Figure 1. Absorbance curve used for determination of NH_3 production by bacteria.

with Luria-Bertani (LB)® culture medium, consisting in drawing a stria with one of the strains, while the other is planted perpendicularly. Next, the petri dishes were incubated at 30°C for 5 days. The results of bacterial growth under this test indicated that the strains were compatible, as they did not show visual growth inhibition to each other.

Tuber yam yields

Harvest was gathered 12 months after transplanting using the three central rows of each plot, eliminating plants at the ends of each row (1.50 m at each end), for an effective harvest area of 15 m^2. Fresh roots or tubers were extracted from the soil and classified according to the local market as: 1) first category tubers or export-type tubers, which correspond to rounded shape tubers, free of physical, insect or diseases damages, with individual weight between 1.5-3.0 kg; 2) second category tubers, that are destined to the local market, and correspond to irregular shape tubers, with size and weight higher or lower than the export type; 3) third-category tubers, which are not suitable for commercialization, which correspond to tubers that are rejected in the market, since they have irregular shape and have physical, insect or disease damages. These classification parameters were established in Colombia by Procaribe to classify yam tubers destined for the export and local market (Procaribe, 2012).

Statistical analysis

The data obtained were subjected to tests of normality and homogeneity of variances through Shapiro-Wilk and Levine method, respectively. Once normality and variance assumptions were confirmed, an analysis of variance was performed, considering the unbalanced factor. In cases where differences were detected at a level of significance of 0.05, a separation of means was performed using Tukey´s HSD test. Group treatments were also compared by orthogonal contrasts. Statistical package SAS v.9.04® was used for all statistical analyses.

RESULTS

PGPR activity

When cultured in a semi-solid N-free medium, each of the rhizobacteria *A. chroococcum* DBC12 and *A. vinelandii* DBC9, registered positive in the qualitative test for biological N fixation 72 hours after inoculation (table 2). Similarly, in the quantitative test, both strains were able to produce NH_3, thus, corroborating the ability of both bacterial strains to fix atmospheric N.

On the other hand, the compatibility assessment showed no inhibition of growth or incompatibility between rhizobacteria *A. chroococcum* DBC12 and *A. vinelandii* DBC9, thus, indicating that a mixture of both bacteria might be used in the production of bioinoculants. Therefore, in the field experiments, we considered the use of a 1:1 mixture (5 mL each) of *A. chroococcum* DBC12 + *A. vinelandii* DBC9 to inoculate yam plants.

Yam yields

Yam productivity was calculated by extracting yam tubers from the soil in each plot and classifying the tubers according to Procaribe (2012). With this data, we calculated the first, second and third category tuber yields, corresponding to tubers or roots that are destined to the export, local market, and rejected tubers, respectively. According to the results, total tuber yields were affected ($p<0.0001$) by the interaction between bacterial strains and the N fertilization levels, which indicates that the total tuber yields were dependent on the level of N fertilization applied to the crop, in conjunction with the effect of the strains of rhizobacteria used. Indeed, as shown in figure 2, the highest yields were obtained with a combined application of 50% N fertilization level with either rhizobacteria *A. chroococcum* DBC12 (21.6 t ha^{-1}) or *A. vinelandii* DBC9 (22.9 t ha^{-1}). As observed, when the N fertilization level was raised up to 75% of the recommended dose, this positive effect of bacterial inoculation is reduced. This in contrast with the yields of the chemical fertilization treatments, in which yam plants responded positively to mineral N fertilization, showing an increase in the yields, concomitant with the increase in the N fertilization level.

However, the yields obtained with the individual inoculation of rhizobacteria, at 50% of the N fertilization level, were higher than any of the yields obtained with the chemical fertilization treatments (0, 50, 75 and 100% of the N fertilization level). On average, yields obtained by the individual strains of rhizobacteria, at 50% of nitrogen fertilization, were 19.3 to 25.9% higher than with treatment with 100% of the N fertilization dose. These findings indicate that there

Bacterial Strains	Biological N fixation (Qualitative test) 72 h	NH_3 production (Quantitative test) (µM)
A. chroococcum DBC12	+	8.08±0.04
A. vinelandii DBC9	+	7.83±0.02
Control	+	6.17±0.05

Table 2. Tests for determination of biological N fixation and NH_3 production of rhizobacteria *A. chroococcum* DBC12 and *A. vinelandii* DBC9.

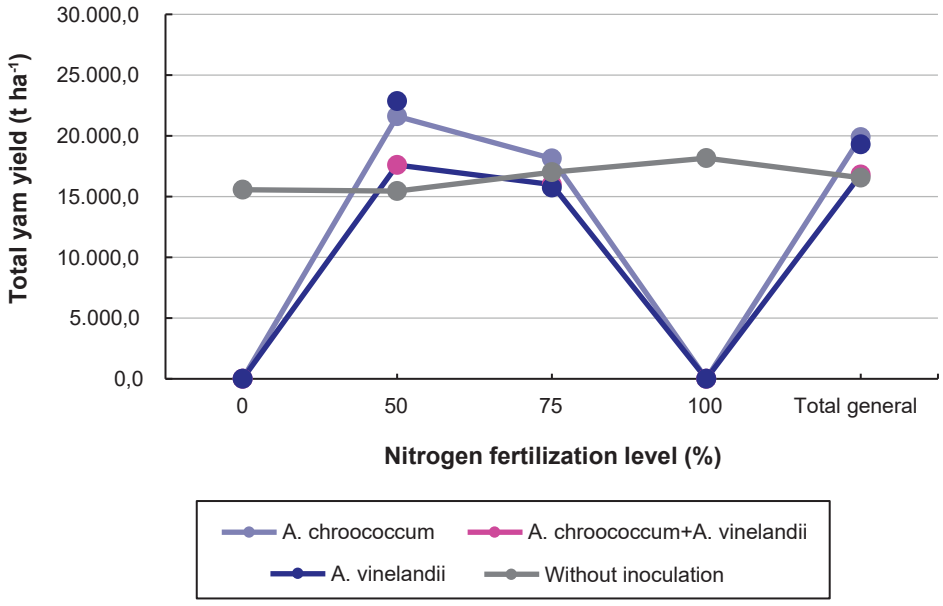

Figure 2. Total yam tuber yields affected by two rhizobacteria strains and four N fertilization levels.

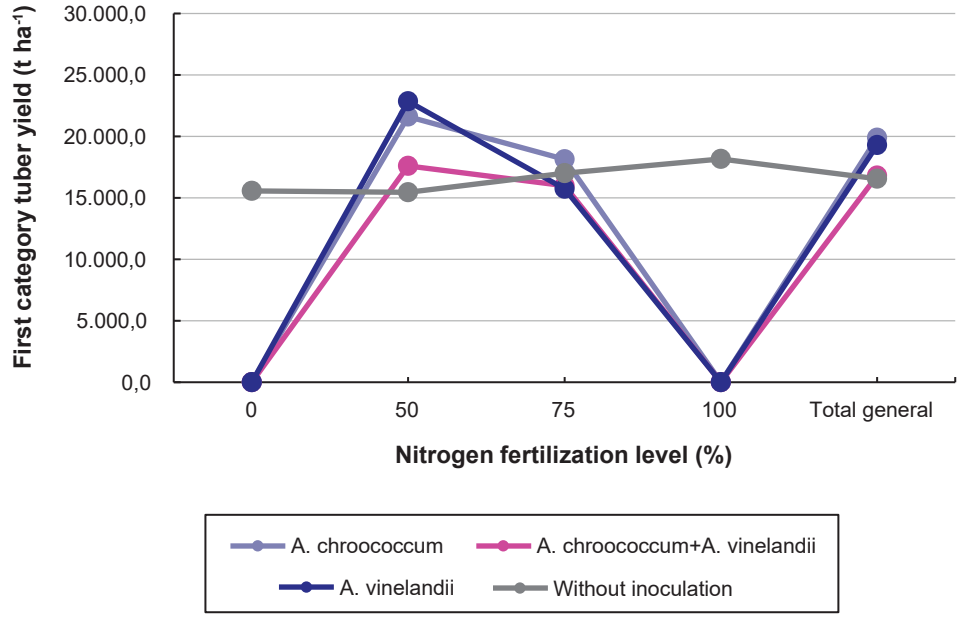

Figure 3. First category yam tuber yields affected by two rhizobacteria strains and four N fertilization levels.

is a positive interaction between *Azotobacter* and the chemical fertilization for increasing yam yields. However, the fact that individual inoculation with the *Azotobacter* strains exceeded the yield increases that could be obtained by any nitrogen fertilization dose shows that there are more mechanisms induced by bacteria involved, in addition to biological nitrogen fixation.

With respect to quality (figure 3), the production of first category roots or tubers is of the utmost importance due to their greater price and market value. Regarding yields of first category tubers, higher yields were also obtained with the combination of either one of the two bacterial strains (*A. chroococcum* DBC12 and *A. vinelandii* DBC9), at 50% N fertilization level ($p<0.0001$). As was the case with total

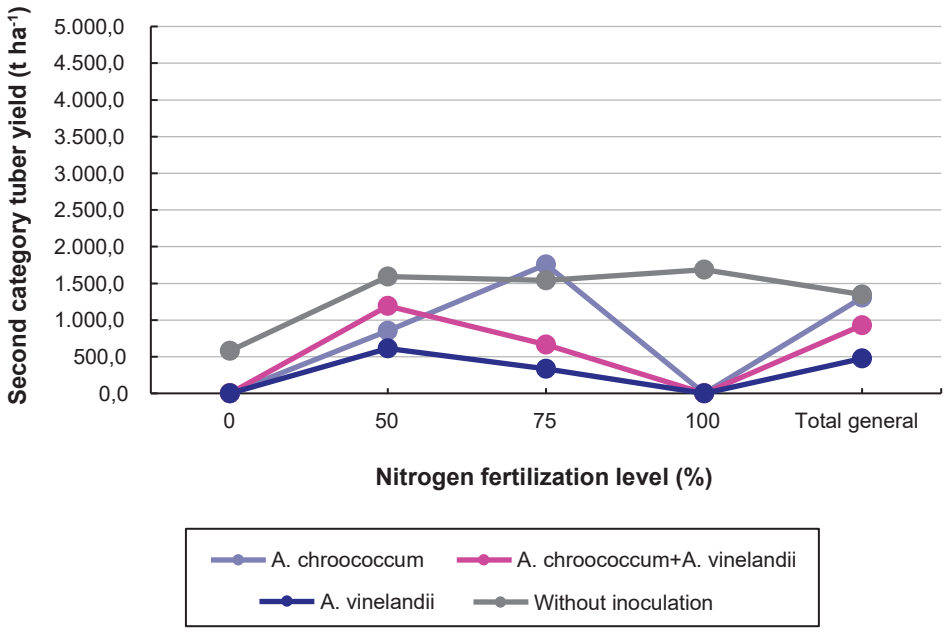

Figure 4. Second category yam tuber yields affected by two rhizobacteria strains and four nitrogen fertilization levels.

yields, bacterial inoculation effect was nearly lost at 75% N fertilization, showing yields decreases at higher N fertilization levels. Similarly, it was found that first quality yields obtained with the individual inoculation with bacteria strains *A. chroococcum* DBC12 or *A. vinelandii* DBC9, at a level of 50% N fertilization level, were superior to the chemical control (0, 50, 75 and 100% of the N fertilization level). On an average, the yield of these two treatments were 19.3 and 21.3 t ha^{-1}, respectively, which was 40.8% and 55.4% higher than the yield obtained with chemical fertilization at a full N dose (100% N). These results show the potential of these bacterial strains to increase tuber quality and to reduce N fertilization levels in yam crops in the Colombian Caribbean Coast.

In relation to second category tubers for local market, the results of these experiments indicated better yields ($p<0.0001$) with the use of higher N fertilization levels alone or with the inoculation of the bacterial strain *A. chroococcum* with 75% N fertilization level (figure 4).

On the other hand, the production of third-category or reject tubers did not depend ($p>0.05$) on the effects of bacterial strains, fertilization levels or their interaction (data not shown). This means that the yields of deformed tubers depend on factors other than those evaluated in these experiments, such as physical soil impediments, insects or disease attacks.

DISCUSSION

These results showed that both strains of *Azotobacter* evaluated in these experiments have the capacity to reduce atmospheric N to ammonium, which allows them to grow without external N sources. The group of microorganisms that possess this capacity are collectively known as diazotrophs and are considered very useful for N fertilizer substitution programs in important crop species (Norman and Friesen, 2017; Rodríguez *et al*., 2018; Russelle, 2008; Kushwaha *et al*., 2013; Kumar *et al*., 2001).

We found that rhizobacteria strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9, were beneficial to increase the total and the first quality yields in yam crops, for which the best results were obtained with either strain with 50% of the N fertilization level. The fact that the effectiveness of these rhizobacteria, was reduced at higher N fertilization levels, would indicate negative effects of high nitrogen levels on the fixation capacity of bacteria, which in turns seems to be affecting tuber growth and yields. The mixture of both rhizobacterial strains had no synergistic effect since yields obtained with the mixture were lower than those obtained with either one of the individual bacterial strains. Even though the *in vitro* compatibility tests indicated no incompatibility between the bacterial strains evaluated in these experiments, the field results suggest the existence of other factors that negatively influence the establishment of the coculture. In this respect, Brahmaprakash and Sahu (2012) reported that bacteria inoculated to the soil can be affected by the competition established with the native microflora in the soil, and by the environmental conditions in the experimental site.

Nitrogen fertilization, on the other hand, seems to exert a positive influence on yam yields, although its effect

seems to be superior on second and third category tubers. In this case a fertilization level of 75% of the recommended N dose seems to be the most convenient. The fact that yam yields obtained with both rhizobacteria strains, at 50% of the N fertilization level, were higher than the yields obtained with any of the chemical fertilization treatments (0, 50, 75 and 100 percent of the N fertilization level), indicates that some other factors, different to N supply from fixation, could also be contributing to improving yam yields. These other factors could be hormones or secondary metabolites that act as stimulants to yam growth and productivity. In this respect, some reports indicate that *Azotobacte*r might positively influence growth and yield of some plants, not only due to the contributions in nitrogen, but also due to the production of a series of hormones, in particular auxins, gibberellins, and cytokinins (Noumavo *et al*., 2013). This production of hormones and growth regulators promotes the growth of plants and enhances the uptake of nutrients, which leads to a higher yield in crops. In previous experiments, Sánchez and Pérez (2018) working with the same bacterial strains (*A. chroococcum* DBC12 and *A. vinelandii* DBC9), confirmed the production of IAA at a level of 27.70 µg L^{-1} and 24.29 µg L^{-1}, respectively, in pure liquid cultures. In this regard, several studies have shown that *in vitro* production of IAA and other physiologically active hormones derived from L-tryptophan are characteristic of PGPR strains (Teixeira *et al*., 2007).

Similarly, it has been reported that this type of bacteria produces different types of secondary metabolites that are released into the soil, such as vitamins, amino acids, antifungal substances, among others, which have a direct influence on the growth of outbreaks and roots in crops (Chennappa *et al*., 2018). These hormones have been associated in the literature with positive effects in plants, such as the promotion of elongation and cell division, apical dominance, tissue differentiation and development of a greater number of roots in plants (Scagliola *et al*., 2016). Sánchez and Pérez (2018) also reported that the strains of *A. chroococcum* DBC12 and *A. vinelandii* DBC9 had the ability to solubilize phosphates *in vitro*, which was measured as the production of orthophosphate from phosphate rock, with values of 15.90 mg L^{-1} for *A. chroococcum* DBC12 and 18.94 mg L^{-1} for *A. vinelandii* DBC9. The same authors reported that these rhizobacteria promoted the plant growth of yam seedlings (*D. rotundata*), at the greenhouse level.

Dixon and Kahn (2004) reported that the joint application of mycorrhizae with *Azotobacter* phosphate solubilizers, allows the fixed amounts of atmospheric nitrogen to be greater, because the fixing bacteria have a greater amount of available phosphorus (an essential element for the fixation of nitrogen), supplied by the activity of the solubilizing organisms. The phosphorus-solubilizing bacteria have become an important habitat of the soil and the inoculation with these has shown substantial increases in the growth of the plants. Such bacterial populations could be of importance in the development of diverse agricultural ecosystems (Wei *et al*., 2018). It has been reported that these solubilizing bacteria modify the nutrition of phosphorus and increase its solubilization in the soil through many processes such as the decrease in soil pH, through the production of organic acids, alkaline phosphatases, phytohormones, H^+ protonation, anion exchange, chelation and production of siderophores, which promote the solubilization of phosphorus in the soil (Adnan *et al*., 2017).

Results obtained with other species of bacteria in yam crops report similar results. For example, Swain *et al*. (2007) working with strains of *Bacillus subtilis* CM4-CM5, report the production of 2.0 and 2.5 mg L^{-1} of IAA, respectively, associated with positive effects on the stimulation of root growth (length and fresh weight), with an increase in the root-stem relation and the number of shoots, in comparison with the non-inoculated *Dioscorea rotundata* plants. On the other hand, Jimtha *et al*. (2017), in studies with *Dioscorea nipponica* with the strain *Proteus* sp. (R6), report this strain as positive for nitrogen fixation, HN_3 production, IAA, siderophore, ACC deaminase and solubilization of phosphates. In these studies, it was found that plants treated with the bacterial strain R6 showed improved size of tubers and a number of roots in comparison to plants without bacterial application.

Our results allow us to conclude that the bacterial strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9 collected in the Caribbean region of Colombia can become an effective alternative for reducing chemical N fertilization in production of yam tubers destined to the export market since they potentiate yield and the production of first category tubers. These bacteria are also an alternative for a more sustainable agriculture since they present the possibility of reducing the use of chemically synthesized N fertilizers. In these experiments, we obtained between 89.1 and 93.3% of yield of first category yam tubers, the yield being higher when N fertilization at a level of 50%, the recommended dose, was combined with either of the bacterial strains *A. vinelandii* DBC9 and *A. chroococcum* DBC12. This last observation might be of great importance, because as Pérez and Campo (2016) mentioned, the size, shape, and weight of yam tubers are very important for the export market and constitute the most limiting factors that confront yam farmers in Colombia. In practice, it is estimated that only 30% of the total yam tuber production received at the market centers in the Caribbean region of Colombia meets the specifications of the international market.

In relation to the yield of second category tubers for the local market, our results indicated that better yields were obtained ($p<0.01$) with the use of higher N fertilization levels alone or, with the inoculation of the bacterial strain *A. chroococcum* with 75% N fertilization level. These results suggest that, with respect to second category tubers, irregular growth and overweight are favored by higher N levels, while for first category tubers for the export market, it is more advisable he use of a 50% N fertilization level, accompanied by the inoculation of the bacterial strains *A. chroococcum* DBC12 and *A. vinelandii* DBC9.

On the other hand, the production of third-category or reject tubers was not affected by our treatments, which indi-

cates that the number of deformed tubers depends on factors other than those evaluated in these experiments, such as physical soil impediments or attacks of insects or diseases. Studies advanced by Cardona (2007), in this regard indicate that if planted at wide distances (0.23 and 0.30 m between plats), an obvious deformation occurs in yam tubers, with high individual weight (1.71 kg and 1.96 kg, respectively). This author indicates that reducing the distance to 0.15 m between plants reduces deformation and decreases individual tuber weight to 1.35 kg. Large tubers are difficult to extract from the soil, split, or suffer wounds at the time of harvest, increasing the number of cuts and the probability of losses due to greater decay resulting from pathogens attacks. Likewise, it is suggested that the variability in the shape of tubers could also be related to the leaf area of each yam plant. Costas *et al*. (1968), observed an increase of 50% in the size of tubers of *D. rotundata*, with irregular shape when they increased the leaf area using 1.83 m tutors versus non-tutored plants. Rodríguez (2000) determined that the use of low planting densities and yam cultivars with excessive growth of stems and leaves keeps the plant in a juvenile state, delays tuberization, reduces commercial production and increases the formation of deformed tubers. Further studies should concentrate on finding the best dose and formulation of these two *Azotobacter* strains as potential bioinoculants to substitute N fertilization and improve the productivity of yam crops in the Caribbean region of Colombia.

CONCLUSIONS

From the above results, it can be concluded that the bacterial strains evaluated in these experiments, *A. chroococcum* DBC12 and *A. vinelandii* DBC9, present a high potential for the preparation of bio-inoculums with the purpose of improving productivity and quality of yam at the Caribbean region of Colombia. These bacterial strains were positive in *in vitro* tests for nitrogen fixation and NH_3 production, and field tests showed the ability to increase the yields of total and first category tubers. The best effects were obtained when inoculation of strains was combined with a level of 50% of the N recommended dose. Therefore, it was concluded that these rhizobacteria present potential as possible bio-inoculants, which could be an alternative to reducing levels of nitrogen chemical fertilization, thus contributing to a more sustainable and competitive yam culture.

ACKNOWLEDGMENTS

This study was funded through the Cooperation Agreement N° 5144 between the Semana Foundation and the Colombian Agricultural Research Corporation (Agrosavia).

REFERENCES

ADNAN, M.; SHAH, Z.; FAHAD, S.; ARIF, M.; ALAM, M.; KHAN, I.A.; RAHMAN, I.U. 2017. Phosphate-solubilizing bacteria nullify the antagonistic effect of soil calcification on bioavailability of phosphorus in alkaline soils. Scientific Reports 7 (1): 16131. http://doi.10.1038/s41598-017-16537-5

AHMADI-RAD, S.; GHOLAMHOSEINI, M.; GHALAVAND, A.; ASGHARZADEH, A.; DOLATABADIAN, A. 2016. Foliar application of nitrogen fixing bacteria increases growth and yield of canola grown under different nitrogen regimes. Rhizosphere 2:34-37. https://doi.org/10.1016/j.rhisph.2016.08.006

APPLEZWEIG, N. 1977. *Dioscorea:* the pill crop. In: SIEGLER, D. (Ed.). Crop Resources. New York, Academic Press. 149-163 pp.

BABA, Z.A.; TAHIR, S.; WANI, F.S.; HAMID, B.; NAZIR, M.; HAMID, B. 2018. Impact of *Azotobacter* and inorganic fertilizers on yield attributes of tomato. Int. J. Curr. Microbiol. App. Sci. 7:3803-3809. https://d doi.org/10.20546/ijcmas.2018.702.450

BENÍTEZ, L.P.; TOVAR, C.T.; ORTIZ, A.V.; DUNOYER, A.T.; MINDIOLA, R.B.; BENÍTEZ, L.M. 2007. Aprovechamiento del ñame espino (*Dioscorea rotundata*) en la producción de bioplásticos. Prospectiva 5:68-72.

BÖMER, M.; RATHNAYAKE, A.I.; VISENDI, P.; SEWE, S.O.; SICAT, J.P.; SILVA, G.; SEAL, S.E. 2018. Tissue culture and next-generation sequencing: A combined approach for detecting yam (*Dioscorea* spp.) viruses. Physiol. and Mol. Plant. Path. https://doi.org/10.1016/j.pmpp.2018.06.003

BRAHMAPRAKASH, G.P.; SAHU, P.K. 2012. A review: Bio-fertilizers for sustainability. J Indian Institute Sci. 92:37-62.

CARDONA, J. 2007. Distancia de siembra en la producción y calidad de ñame Guinea negro (*D. rotundata*). J. Agr. U. Puerto Rico 91:61-65.

COSTAS, R.C.; BONETA, E.; SILVA, S. 1968. Effect of various cultural practices on yields of yams in Puerto Rico. J. Agr. U. Puerto Rico 52:356-61.

CHAUHAN, H.; BAGYARAJ, D.J.; SELVAKUMAR, G,; SUNDARAM, S.P. 2015. Novel plant growth promoting rhizobacteria-prospects and potential. Appl. Soil Ecol. 95:38-53. https://doi.org/10.1016/j.apsoil.2015.05.011

CHENNAPPA, G.; SREENIVASA, M.Y.; NAGARAJA, H. 2018. *Azotobacter salinestris*: A novel pesticide-degrading and prominent biocontrol PGPR bacteria. In*:* PANPATTE, D.; JHALA, Y.; SHELAT, H.; VYAS, R. (Eds.). Microorganisms for green revolution, microorganisms for sustainability. Springer, Singapore. 23-43 pp. https://doi.org/10.1007/978-981-10-7146-1_2

COMPANT, S.; DUFFY, B.; NOWAK, J.; CLÉMENT, C.; BARKA, E.A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and prospects. Appl. Environ. Microbiol. 71:4951-4959. https://doi:10.1128/AEM.71.9.4951-4959.2005

DILWORTH, M.J.; EADY, R.R.; ELDRIDGE, M.E. 1988. The vanadium nitrogenase of *Azotobacter chroococcum* reduction of acetylene and ethylene to ethane. Biochem. J. 249: 745-751.

DIXON, R; KAHN, D. 2004. Genetic regulation of biological nitrogen fixation. Nature Reviews Microbiology 2(8):621-631.

DUMET, D.; OGUNSOLA, D. 2008. Guías para la regeneración de germoplasma: ñame. In: DULLOO, M.E.; THORMANN, I.; JORGE, M.A.; HANSON, J. (Eds.). Crop specific regeneration guidelines [CD-ROM], CGIAR System-wide Genetic Resource Programme SGRP Rome, Italy 8 p.

FAOSTAT-ORGANIZACIÓN PARA LAS NACIONES UNIDAS PARA LA ALIMENTACIÓN Y LA AGRICULTURA FAO. 2017. Disponible vía DIALOG http://www.fao.org/faostat/es/#data/QC

HOLDRIDGE, L. 2000. Ecología basada en zonas de vida. Quinta reimpresión. Instituto Interamericano de Cooperación para la Agricultura IICA 1996, c1978, San José, Costa Rica, 225 p.

JIMTHA, J.C.; MATHEW, J.; RADHAKRISHNAN, E.K. 2017. Bioengineering of *Dioscorea nipponica* with rhizospheric Proteus spp. for enhanced tuber size and diosgenin content. Biotech 7:261. https://doi.org/10.1007/s13205-017-0886-3

KIZILKAYA, R. 2008. Yield response and nitrogen concentrations of spring wheat (*Triticum aestivum*) inoculated with *Azotobacter chroococcum* strains. Ecol. Eng. 33:150-156.

KUMAR, V.; BEHL, R.K.; NARULA, N. 2001. Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol. Res. 156:87-93. https://doi.org/10.1078/0944-5013-00081

KUSHWAHA, A.; BAILY, S.B.; MAXTON, A.; RAM, G.D. 2013. Isolation and characterization of PGPR associated with cauliflower roots and its effect on plant growth. The Bioscan 8:95-99.

MARKSON, A.A.; OMOSUN, G.; MADUNAGU, B.E.; AMADIOHA, A.C.; WOKOCHA, R. 2010. Physicochemical Alteration of Tissues of White Yam (*Dioscorea Rotundata* Poir) Tubers Incited by Botryoiplodia Theobromae Pat. Int J Curr Res. 4:055-061.

NORMAN, J.S.; FRIESEN, M.L. 2017. Complex N acquisition by soil diazotrophs: how the ability to release exoenzymes affects N fixation by terrestrial free-living diazotrophs. The ISME journal 11:315-326. https://doi.org/10.1038 / ismej.2016.127

NOUMAVO, P.A.; KOCHONI, E.; DIDAGBÉ, Y.O.; ADJANOHOUN, A.; ALLAGBÉ, M.; LAMINE, B. 2013. Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. Am. J. Plant Sci. 4:1013-1021. http://dx.doi.org/10.4236/ajps.2013.45125

O'SULLIVAN, J.N. 2010. Yam nutrition: nutrient disorders and soil fertility management. ACIAR Monograph No. 144. Australian Centre for International Agricultural Research, Canberra. 112 p.

PÉREZ, P.J.; CAMPO, A. 2016. Efecto de la densidad poblacional sobre el rendimiento de ñame espino (*Dioscorea rotundata* Poir.) tipo exportación. Rev. Col. Cienc. Hortic. 10:89-98. http://dx.doi.org/10.17584/rcch.2016v10i1.5072

PROCARIBE, F. 2012. Guía práctica para el manejo orgánico del cultivo de ñame tipo exportación. 49p. (Available at: https://www.swissaid.org.co/sites/default/files/Cartilla%2BÑame_Julio%2B2012.pdf).

RAMIREZ, A.; PACHECO, M.R.; MORENO, S.J.; XIQUI, M.L.; BACA, B.E. 2018. Versatile use of *Azospirillum* brasilense strains tagged with egfp and mCherry genes for the visualization of biofilms associated with wheat roots. Microbiol. Res. 215:155-163. https://doi.org/10.1016/j.micres.2018.07.007

RODRIGUEZ, M.Â.; LADEIRA, L.C.; ARROBAS, M. 2018. *Azotobacter*-enriched organic manures to increase nitrogen fixation and crop productivity. Europ. J. Agron. 93: 88-94. https://doi.org/10.1016/j.eja.2018.01.002

RODRÍGUEZ, W. 2000. Botánica, domesticación y fisiología del cultivo del ñame (*Dioscorea alata*). Agron. Mesoamericana 11:133-152. https://doi.org/10.15517/am.v11i2.17326

RUSSELLE, M.P. 2008. Biological dinitrogen fixation in agriculture. In: SCHEPERS, J.S.; RAUN, W.R. (Eds.). Nitrogen in Agricultural Systems. Agronomy Monograph n.° 49. ASA, CSSA, SSSA, Madison, WI, USA. 281-359 pp.

SÁNCHEZ, D.; PÉREZ , J.V. 2018. Caracterización y evaluación de PGPRs sobre el crecimiento de plántulas de *Dioscorea rotundata in vitro*. Agron. Costarricense 42:75-91 https://doi. org/10.15517/rac.v42i2.33780

SAVCI, S. 2012. An agricultural pollutant: chemical fertilizer. Int. J. Environ. Sci. Dev. (3): 73-80. http://dx.doi.org/10.7763/IJESD.2012.V3.191

SCAGLIOLA, M.; PII, Y.; MIMMO, T.; CESCO, S.; RICCIUTI, P.; CRECCHIO, C. 2016. Characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (*Hordeum vulgare L.*) and tomato (*Solanum lycopersicon* L.) grown under Fe sufficiency and deficiency. Plant Physiol. Biochem. 107:187-196. https://doi.org/10.1016/j.plaphy.2016.06.002

SIQUEIRA, M.V.B.M. 2009. Inhame (*Dioscorea* spp.): uma cultura ainda negligenciada. Hortic. Bras. 27: S4075-S4090.

SWAIN, M.R.; NASKAR, S.K.; RAY, R.C. 2007. Indole-3-acetic acid production and effect on sprouting of yam (D*ioscorea rotundata* L.) minisetts by *Bacillus subtilis* isolated from culturable cow dung microflora. Pol. J. Microbiol. 56:103-110.

TEIXEIRA, M.A.; DE MELO, I.S.; VIEIRA, R.F.; CARVALHO, F.E.; HARAKAVA, R. 2007. Microrganismos endofíticos de mandioca de áreas comerciais e etnovariedades em três estados brasileiros. Pesquisa Agropecuária Brasileira 42(1) :43-49.

VASHI, J.M.; SARAVAIYA, S.N.; DESAI, K.D.; PATEL, A.I.; PATEL, H.B.; SRAVANI, V. 2018. Effect of planting distance on growth and tuber yield of greater yam (*Dioscorea alata* L.) under different growing conditions IJCS 6 :1475-1481.

VEJAN, P.; ABDULLAH, R.; KHADIRAN, T.; ISMAIL, S.; NASRULHAQ BOYCE, A. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability: A review. Molecules 21: 1-17. http://dx.doi.org/10.3390/molecules21050573

WEI, Y.; ZHAO, Y. SHI, M.; CAO, Z.; LU, Q.; YANG, T.; WEI, Z. 2018. Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. Bioresource Tech. 247:190-199. https://doi.org/10.1016/j.biortech.2017.09.092

WILSON, P.W.; KNIGTH, S.G. 1952. Experiments in bacterial physiology. 3d. Burguess, Minneapolis, USA. 49 p.