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Original article

Study of the association *Gluconacetobacter diazotrophicus*-tropical tubers and roots. Effect on performance under extension conditions

Estudio de la asociación G*luconacetobacter diazotrophicus*-viandas tropicales. Efecto sobre el rendimiento en condiciones de extensión

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ABSTRACT: In order to evaluate the effect of *G. diazotrophicus* application on the yield of tropical tubers and roots, under extension conditions, the INIFAT Abn-1 strain of the microorganism was inoculated in sweet potato, cassava and taro, during two production campaigns. At the end of the crop cycle in the inoculated sweet potato plants, the growth indicators increased between 30 and 60 %, with a yield of 13 t ha-1 more in the plots applied in relation to controls. In the case of cassava, the growth indicators increased with the application of microorganism between 36-48 % and the yield by 40 %, with the obtaining of 17 t ha^{-1} more in applied plots; while for taro, the growth indicators increased between 58 and 94 % in the inoculated plots and the yield increased by 40 and 58 % in each campaign, respectively. The concentration of *G. diazotrophicus* in leaves of the three cultures ranged between 10^3 and 10^7 CFU per gram of fresh tissue and it was between two and four orders lower in the control plants than in the inoculated ones. The work demonstrated the growth stimulating effect of *G. diazotrophicus* in sweet potato, cassava and taro under production conditions, the need to inoculate the microorganism to increase its autochthonous population in these crops, as well as the possibilities of using the endophytic bacteria in the agronomic management of these plant species.

Key words: sweet potato, biofertilization, taro, cassava.

RESUMEN: Con el objetivo de evaluar el efecto de la aplicación de *G. diazotrophicus* en el rendimiento de viandas tropicales, en condiciones de extensión, se inoculó la cepa INIFAT Abn-1 del microorganismo en boniato, yuca y malanga, durante dos campañas de producción. Al finalizar el ciclo de los cultivos en las plantas de boniato inoculadas se incrementaron los indicadores de crecimiento entre 30 y 60 %, con un rendimiento de 13 t ha^{-1} más en las parcelas aplicadas en relación a los controles. En el caso de la yuca los indicadores del crecimiento aumentaron con la aplicación del microorganismo entre 36-48 % y el rendimiento en un 40 %, con la obtención de 17 t ha^{-1} más en las parcelas aplicadas; mientras que para la malanga, los indicadores del crecimiento aumentaron entre 58 y 94 % en las parcelas inoculadas y el rendimiento se incrementó un 40 y 58 % en cada campaña, respectivamente. La concentración de *G. diazotrophicus* en las hojas de los tres cultivos osciló entre 10^3 y 10^7 UFC por gramo de tejido fresco y fue entre dos y cuatro órdenes menor en las plantas controles que en las inoculadas. El trabajo demostró el efecto estimulador del crecimiento de *G. diazotrophicus* en boniato, yuca y malanga en condiciones de producción, la necesidad de inocular el microorganismo para incrementar su población autóctona en estos cultivos, así como las posibilidades de uso de la bacteria endófita en el manejo agronómico de estas especies vegetales.

Palabras clave: boniato, biofertilización, malanga, yuca.

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INTRODUCTION

In the current biorevolution, more and more attention is paid to soil biology and plant biofertilization, linked to different nutrition management schemes (1,2). In this sense, much progress has been made in the design of biopreparations for various crops, with an emphasis on beneficial associative or symbiotic relationships (3,4). Endophytic microorganisms are also used to obtain bioproducts for agricultural use, although most of the research on the plant-endophyte ratio focuses on polyphasic taxonomy and genetic engineering, ecology and studies of genetic transformation and gene identification, in regarding the biological fixation capacity of atmospheric nitrogen (5,6). An example of endophytic bacteria is *G. diazotrophicus*, which has demonstrated its potential as a promoter of plant growth, mainly associated with grasses (7,8). In the case of Cuba, different investigations carried out with tropical tubers and roots, fruit trees and vegetables, demonstrate the close interaction that these crops establish with the bacteria (9,10), although most of these studies were carried out under controlled conditions or in small plots.

In order to know the potential of the endophytic species *G. diazotrophicus* as a growth stimulator, the study was extended in the present investigation with the aim of evaluating the bacteria application effect on of sweet potato (*Ipomoea batatas*, L.) yield, cassava (*Manihot esculenta* Crantz) and taro (*Xanthosomas* spp.), in production, under the edaphoclimatic conditions of Cuba.

MATERIALES AND METHODS

The trials to determine the inoculation effect of *G. diazotrophicus* bacterium, on indicators of the growth and agricultural yield of crops of cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas* L.) and taro (*Xanthosomas* spp.) were carried out in agricultural areas of "Alejandro de Humboldt" Institute for Fundamental Research in Tropical Agriculture, INIFAT, in Santiago de Las Vegas. A Compacted leached red ferrallitic, Gleyic and Ferruginous Nodular soil (11) was used, with neutral pH, medium content of organic matter, with a predominance of calcium and medium contents of phosphorus and potassium (Table 1).

The trials for the three plant species were carried out in two production campaigns, the first during 2009-2010 and the second, between 2010 and 2011. In both, the clones CENSA 78-354 of sweet potato, CMC-40 were used cassava and Mexico 1, taro. An experimental design in Random Blocks with a plot size of 50 m^2 was used in all trials. Two treatments were included: inoculated and control (without inoculation), with four replications in each case, in each of the two trials that were carried out by plant species.

For the application of *G. diazotrophicus*, the INIFAT Abn-1 (9) strain, conserved in the INIFAT, was multiplied in a submerged fermentation process under orbital shaking, in the SG (12) culture medium, at 32 °C temperature and 180 rpm of stirring, for 72 hours. Under these conditions, the microorganism reached a concentration between 3.0-3.3 x 10^{11} CFU mL^{-1}. Bacteria inoculation was always carried out in the late afternoon with the help of a phytosanitary backpack, by foliar spraying and on the ground a step of a man. The final product of strain fermentation was used, diluted in common water, at a dose equivalent to 2 L ha^{-1}, according to previously established criteria for the bacterial species (9).

Once the crop cycle was finished, the following indicators were evaluated: plant height (cm), stem diameter (cm) and tuber diameter (cm), for the sweet potato. Plant height (cm), number of primary branches, stem diameter (cm) and root diameter (cm), for cassava and tuber diameter and length (cm), for taro. For the three crops, the yield in t ha^{-1} was also quantified. To measure plant height, a wooden rod graduated in cm was used and a vernier's foot (0.05 mm error) was used for the diameter, both of the stem and of roots and tubers.

The concentration of *G. diazotrophicus* in leaves of sweet potato, cassava and taro, was determined as CFU g fresh tissue^{-1}. For this, at the end of the crop cycle, five plants were used for each of the plant species under study. 1 g of fresh plant tissue was taken, which was processed by the serial dilution method (13) with subsequent sowing in Petri dishes with the LGI culture medium. The plates were incubated at 28-30 °C for five days. Only colonies with intense yellow-orange coloration were considered.

The experimental data were statistically evaluated using the Newman Keuls test at 5 % significance, using analysis of variance for parametric data, after checking the normality of the variables. The processing of all the information was carried out using the Statgraphics 6.0 program.

RESULTS AND DISCUSSION

The application of *G. diazotrophicus* stimulated the growth and increased the yield of the sweet potato under production conditions, in the two sowing campaigns. Plants inoculated with bacteria showed an increase of between 33-34 % in plant length; between 31-62 % for the number of leaves; between 38-40 % in stem diameter and between 34-45 % in the tuber diameter, with respect to plants without bacterial application. For its part, agricultural yield increased by 51 and 48 % for the years 2009 and 2010, respectively, equivalent to 13 t ha^{-1} of additional tubers (Table 2).

Table 1. Chemical characteristics of the soil used in trials.

OM (%)	pH (H_2O)	P_2O_5	K_2O	Ca	Mg
		($mg100g^{-1}$)		$cmol_c\ kg^{-1}$	
3.12	7.2	47	60	8.7	3.2

Analysis methods: pH (potentiometry); organic matter (Walkley and Black); P2O5 (Oniani, by extraction with H2SO4); K2O (by calculation from exchangeable potassium); Ca and Mg (atomic absorption spectrophotometry)

Table 2. Response of the culture of the sweet potato clone CENSA 78-354 to inoculation with *G. diazotrophicus*, INIFAT Abn1 strain. Campaign 2009 and Campaign 2010.

Variants	Plant height (cm)		Stem diameter (cm)		Tuber diameter (cm)		Yield (t ha^{-1})	
	2009	2010	2009	2010	2009	2010	2009	2010
Control	21.35 b	23.48 b	3.59 b	3.51 b	5.10 b	6.01 b	27.81 b	29.32 b
Inoculated	27.59 a	31.10 a	4.83 a	4.90 a	7.46 a	8.16 a	41.75 a	43.96 a
Se x	0.34	0.10	0.21	0.30	0.11	0.17	0.72	0.60

Means with unusual letters differ significantly from each other by Anova test and Newman Keuls test with p <5 %

Studies carried out in Brazil with the *G. diazotrophicus-Ipomoea batatas* Lam interaction, although in smaller extensions, coincide in the positive effect caused by bacteria on this plant species (10).

One aspect to be highlighted is the increase in number of leaves in plants inoculated with the microorganism, with respect to control plants, with an average of 5-7 more leaves per plant at harvest time. This increase is explained by the production of physiologically active substances, mainly indole acetic acid and cytokinins, which can release *G. diazotrophicus* (5), substances that stimulate cell division and the elongation of cells and tissues, an effect that translates into greater organ development (14). The fact that a plant has more than two active leaves gives it advantages, from the point of view of reserve substance production and other biomolecules, through photosynthetic processes, which are favored at the expense of a larger photosynthetically active surface and nutrient translocation. In other studies, an increase in the leaf surface of grass plants inoculated with *G. diazotrophicus* (15) has been reported, which suggests that the bacterium, in interaction with different crops, induces physiological modifications that lead to an increase in growth indicators and, finally, the performance of plant species. It constitutes a promising microorganism to be used as an active ingredient in bioproducts for agricultural use.

Leaves of the sweet potato plants inoculated with *G. diazotrophicus* showed in both campaigns an increase in two orders of endophytic bacteria concentration, with respect to non-inoculated plants. In the first campaign (2009), the concentration of the microorganism averaged 5.1x10^5 CFU gram of fresh tissue^{-1}, in the control plants and 2.4x10^7 CFU gram of fresh tissue^{-1}, in the inoculated plants. In the second trial (2010 campaign), the concentration of the microorganism was 4.3x10^5 CFU gram of fresh tissue^{-1}, in the leaves of control plants and 3.5x10^7 CFU gram of fresh tissue^{-1}, in inoculated plants.

The presence of *G. diazotrophicus* in control plants shows that sweet potato is a natural host for bacteria, an aspect previously discussed by other authors (16). However, the increase in the value of this indicator in leaves of the inoculated plants indicates that the microorganism colonizes plant species once it is inoculated into it, an important aspect for its use in practice as a plant growth stimulator, such as part of the agronomic management of plant species such as the sweet potato itself.

For cassava cultivation, positive results were also obtained with *G. diazotrophicus* application. In this case, the height of the plant increased between 22-31 %, the number of primary branches by 96 %, the diameter of the stem between 50 and 87 % and the average diameter of roots harvested per plant by 50 % in inoculated plants, in relation to the untreated control plantations. In these trials, the yield per amount of surface evaluated increased by 40 % with microorganism application, obtaining an additional 17 t ha^{-1} compared to control areas (Table 3).

The stimulation in the number of branches and the stem diameter, of microorganism application product, enhances the translocation of nutrients and water for the formation of roots in plants. This effect produced by plant growth promoting bacteria is closely related to the potential for synthesis of active substances, of which, particularly for *G. diazotrophicus*, there are references to the release of auxins, gibberellins and cytokinins (5). However, the possibility that, from new physiological-biochemical studies, new compounds or strains that produce high amounts of the aforementioned phytohormones and that, due to this, induce a greater stimulatory effect on growth indicators, are not ruled out and crop yield. The increase in plant productivity that was achieved in the present study was higher than that obtained for cassava in previous investigations carried out in the conditions of Cuba, but in small plots (9) and demonstrates the potential of the endophyte bacterium to be used as a growth stimulator in this plant species, in different production modalities.

G. diazotrophicus concentration in leaves of cassava plants was three orders higher when the microorganism was applied. In the first campaign (2009-2010) a value of 4.2x10^4 CFU gram of fresh tissue^{-1} was obtained in leaves

Table 3. Response of the cassava clone CMC-40 culture to inoculation with *G. diazotrophicus*, INIFAT Abn1 strain.

Variants	Plant height (cm)		No. of primary branches		Stem diameter (cm)		Root diameter (cm)		Yield (t ha^{-1})	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Control	2.10 b	2.35 b	2.65 b	2.70 b	2.71 b	2.81 b	3.84 b	4.06 b	31.19 b	42.71 b
Inoculated	2.76 a	2.80 a	5.15 a	5.35 a	5.10 a	6.05 a	5.85 a	7.15 a	52.64 a	59.47 a
Se x	0.12	0.20	0.90	0.83	0.19	0.18	0.12	0.21	1.06	0.87

Means with unusual letters differ significantly from each other by Anova test and Newman Keuls test with p <5 %

from control plants and $3.1x10^7$ CFU gram of fresh tissue^{-1}, in leaves sampled from plants biofertilized. In the second trial (2010-2011 campaign), the concentration of the bacteria in leaves of control plants was $2.1x10^4$ CFU gram of fresh tissue^{-1} and $5.3x10^7$ CFU in leaves of inoculated plants gram of fresh tissue^{-1}. The presence of 1000 times more CFU in inoculated plants indicates that the endophytic bacteria colonize plant species after its application in this culture, which leads to obtaining a growth and yield stimulating effect. It can also be deduced that cassava is more sensitive to *G. diazotrophicus* colonization than sweet potato, taking into account that in the first case a lower concentration of bacteria was obtained.

G. diazotrophicus application in taro plants also had a positive effect on the development and performance of plant species. In this crop, the agricultural yield increased between 40 and 58 % in each campaign, respectively, obtaining an average of 13 additional t ha^{-1} after endophytic bacteria application. The length and diameter of tubers increased by 94 and 58 %, respectively, in the first season; while in the second it increased by more than double and 48 %, in relation to tubers from untreated control plants (Table 4).

It should be noted that in the case of this study, the yield values obtained are higher than those achieved in trials carried out with the plant species and *G. diazotrophicus* in small plots (9), an aspect that demonstrates prospects for microorganism use, such as part of the agronomic management of taro.

Inoculation with *G. diazotrophicus* can contribute significantly to nitrogen gains in plants, due to its condition as a dinitrogen fixer (5). As an advantage of this plant-bacteria association, the continued effect of the microorganism during the entire development of the crop stands out, which, unlike chemical fertilizers, allows the availability of the nutrient (nitrogen) to be increased for the plant, according to its needs and depending on its physiological stage. However, due to the few studies carried out on *G. diazotrophicus* interaction and tropical root vegetables, it would be convenient to demonstrate the percentage of nitrogen that the microorganism makes available to the plant.

G. diazotrophicus concentration in young taro leaves increased by four orders (10 000) in inoculated plants with respect to control plants. In the first campaign (2009-2010), the values reached for this indicator were $4.1x10^3$ CFU per gram of fresh tissue^{-1} in control plants and $2.4x10^7$ CFU per gram of fresh tissue^{-1} for plants that were artificially inoculated with the INIFAT Abn1 strain of *Gluconacetobacter diazotrophicus*. In the second campaign (2010-2011) a similar behavior was obtained. Thus, control plants averaged a population value of $5.2x10^3$ CFU gram of fresh tissue^{-1}, while those inoculated showed an endophyte population of $3.1x10^7$ CFU g of fresh tissue^{-1}.

From the increase in bacteria concentration in inoculated plants, it can be deduced, as in the case of cassava and sweet potatoes, that it is necessary to increase the number of cells of the microorganism inside plants through inoculation. Natural populations of bacteria do not allow the growth stimulating *G. diazotrophicus* effect to be manifested on these cultures, as can be seen in differences discussed in growth indicators between the control and inoculated plants. However, unlike other tubers, in taro, the inoculation of vitroplants in Agamic Seed Reproduction Center (CRAS, according its acronyms in Spanish) can be suggested, in such a way that the seedling, once released by this unit, presents a *G. diazotrophicus* concentration suitable for its subsequent establishment and development in the field. In this sense, inoculations with different growth- promoting species, including *G. diazotrophicus*, have been carried out in cassava during the acclimatization phase with positive results (17), but it is a research topic that should be addressed in other research works.

The study demonstrated the beneficial effect on growth and yield of sweet potato, cassava and taro that is achieved by applying the endophyte *G. diazotrophicus*.

CONCLUSION

The application of INIFAT Abn-1 strain of *Gluconacetobacter diazotrophicus* allows improving the growth, development and yield of sweet potato, cassava and taro crops, under production conditions.

RECOMMENDATIONS

- To implement the use of *G. diazotrophicus* within agronomic practices for the agricultural management of sweet potato, cassava and taro crops.
- To demonstrate the possible potential as a nitrofixer of *Gluconacetobacter diazotrophicus*, in interaction with sweet potato, cassava and taro, using isotopic techniques.

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Table 4. Response of taro clone México 1 culture, to inoculation with *G. diazotrophicus*, INIFAT Abn1 strain.

Variant	Diameter of tuber (cm)		Tuber length (cm)		Yield (t ha^{-1})	
	2009-2010	2010-2011	2009-2010	2010-2011	2009-2010	2010-2011
Control	3.89 b	3.66 b	6.75 b	6.40 b	28.95 b	25.06 b
Inoculated	6.15 a	5.47 a	13.10 a	12.90 a	40.70 a	39.84 a
Se x	0.96	0.40	0.617	0.31	1.04	0.82

Means with unusual letters differ significantly from each other by Anova test and Newman Keuls test with p <5 %

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