Scientific Paper

Effect of the application of three *Bradyrhizobium* strains on the morphoagronomic development of *Glycine max* L.

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Abstract

Objective: To evaluate the effectiveness of three *Bradyrhizobium* strains in the morphoagronomic development of *Glicine max* L, cultivar Incasoy 27, in the Puerto Padre municipality.

Materials and Methods: A study was conducted under field conditions, on a reddish Brown Fersialitic Soil of the Puerto Padre municipality, Las Tunas, Cuba. A randomized block design was applied with five treatments (absolute control, fertilization with NPK and the *Bradyrhizobium* strains, ICA 8001, USDA 110 and GIE 109) and four replicas. The distance between replicas was 1 m and the seed had 98 % germination. During the cultivation cycle, the number of pods per plant, number of grains per pod, weight of 100 grains, yield in t ha^{-1} were evaluated and an economic assessment was made.

Results: The lowest number of pods per plant was obtained in the absolute control, without differing from the treatment with NPK. In turn, both differed statistically ($p > 0,05$) from the treatments with *Bradyrhizobium*. The number of grains per pods was also higher in the inoculated treatments, which differed significantly from the control. The highest grain yield was obtained in the inoculated plants, with values between 1,06 and 1,23 t ha^{-1}.

Conclusions: The application of Bradyrhizobium strains positively influenced the morphoagronomic indicators of the crop, which allows to decrease the use of mineral fertilizers.

Keywords: organic fertilizers, inoculation, yield

Introduction

Biofertilizers constitute a sustainable economic and ecological alternative in integrated crop management. They allow to reduce external inputs, improving the quantity and quality of internal resources, as well as to ensure higher efficiency of mineral fertilizers (León and Mesa, 2016). They are, along with biopesticides, key inputs in sustainable agricultural production (Pathak and Kumar, 2016). Among them, arbuscular mycorrhizal fungi (AMF) and *Bradyrhizobium* are widely used worldwide in the fertilization of economically important crops.

The use of biofertilizers in agricultural production systems has reached a high peak today, especially to achieve higher quantity and availability of nutrients assimilable by the plant and to accelerate all the microbial processes of decomposition and synthesis that occur in the soil. Biopreparations contribute thus to soil fertility and sustainable crop yield, with the consequent conservation of the environment (Dibut-Álvarez *et al.*, 2010). For such reason, the use of microorganisms in substitution of inorganic fertilizers is greatly important, guaranteeing the natural and safe production of crops.

In 2016, Cuba exported 2,77 billion dollars in food and imported $ 13,6. Of the latter, $ 13,3 million was invested in soybean (*Glycine max* L. Merrill) meal, with a negative trade balance of $ 10,9 billion, according to Conformity Assessment Bodies (OEC, 2017). This reality forces us to look for less expensive alternatives to obtain this product.

The high protein content of a grain of flour makes it the crop with the highest nitrogen demand. This requirement can be fulfilled from the contribution of the soil (by the mineralization of organic nitrogen) and fertilization. As a legume, by means of biological nitrogen fixation, it can satisfy up to 90 % of the necessary nitrogen (Pastorino, 2016).

In the Puerto Padre municipality, in Las Tunas province, Cuba, agricultural crop yields are low and vary between 0,8 and 1,0 t ha^{-1}. To counteract the negative effect of chemical fertilization, in recent times the use of biofertilizers has been increased,

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which allows plants to overcome the stress caused by adverse environmental conditions.

Due to the above-explained facts, this study was developed to evaluate the effectiveness of three *Bradyrhizobium* strains in the morphoagronomic development of *Glycine max* L, cultivar Incasoy 27, in the Puerto Padre municipality.

Materials and Methods

Location. The research was carried out under field conditions, on a Reddish Brown, Fersialitic soil (Hernández-Jiménez *et al*., 2015), between November 8, 2018, and February 8, 2019, in the La Cana farm, belonging to the Cooperative of Credits and Services (CCS) Paco Cabrera, in the Vázquez town, Puerto Padre municipality. The farm is located on km 40 of the Las Tunas-Puerto Padre road, Nueve Palmas. It has a total area of 1,28 ha and is geographically located at coordinates 21°07'21.17" N and 76°40'25.0" W.

Characteristics of the soil in the experimental area. To know the soil indicators, samples were taken at a depth of 20 cm by means of the experimental technique of sampling in grid form (Almendros *et al*., 2010). Drying and sieving was carried out through a 2 mm mesh. The pH was determined by the potentiometric method and organic matter, according to Walkley and Black (1934). The available phosphorus was calculated by the method proposed by Olsen *et al*. (1954) with molecular spectrophotometry (EDULST01-13). Cation exchange capacity (CEC), exchange cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and base exchange capacity (BEC) were found by the Mehlich (1984) method, modified (NC-65: 2000) (table 1).

Table 1. Some components of soil fertility (0-20 cm).

Indicator	Value
OM, %	2
pH, KCL	8,26
EC, dS m^{-1}	0,4
Ca^{2+},cmol(+) kg^{-1}	20
Mg^{2+}, cmol(+) kg^{-1}	10
K^+, cmol(+) kg^{-1}	0,17
Na^+, cmol(+) kg^{-1}	0,06
P_2O_5 ppm	12,5

Experimental design and treatments. A randomized block design was applied, with five treatments and four replicas, in plots with an area of 11,2 m^2 (2,80 x 4 m) and four rows. The two central rows (5,6 m^2) were taken as calculation area. The distance between replicas was 1 m and the cultivar Incasoy-27 from INCA was used, with 98 % germination. The treatments were the following: Absolute control, Fertilization with NPK, *Bradyrhizobium* strain E 109; *Bradyrhizobium* strain USDA 110 and *Bradyrhizobium elkanii* strain ICA 8001.

Experimental procedure

Crop management was carried out as established by the technical instructions (Hernández and Bello, 2010).

The sowing was done on November 8, 2018, manually and two seeds were placed per nest, at a depth of 4 cm, with a distance between rows of 0,70 and 0,10 m between plants.

During the crop cycle, irrigation was applied seven times by sprinkler technology in the critical periods of water demand, framed in the pre-flowering, flowering, pod formation and grain filling stages, with an interval of 7 to 8 days, depending on the rainy season.

To meet the nutritional demands of the crop, a dose of 770 kg ha^{-1} of NPK was applied, with a complete 10-8-6 formula. This guaranteed 100 kg ha^{-1} of nitrogen, 80 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O at the time of planting in the row bottom. The seeds were inoculated when they were to be sown with 1 mL per seed of bacterial suspension (10^8 CFU mL^{-1}) of the inoculants.

Measurements. After harvest, the number of pods per plant and the number of grains per pod in 20 plants per plot were evaluated. One hundred grains (g) were weighed with an analytical balance (SARTORIUS, BS 2202S model). In addition, the yield obtained in each plot was estimated. The data were transformed into tons per hectare. Also, an economic evaluation of the different treatments was carried out.

Statistical analysis. The test of variance homogeneity and normality was performed. When the two premises were fulfilled, a double classification variance analysis was used. For the comparison of means, Tukey's test was used, for a 5 % error probability. The information was processed with the statistical software InfoStat®, version 2017 (Di Rienzo *et al*., 2017).

Results and Discussion

Table 2 shows the morphoagronomic variables of *G. max* per treatment. The lowest number of pods per plant was obtained in the absolute control, without differing from the NPK treatment. In turn, both differed statistically ($p > 0,05$) from the other *Bradyrhizobium* treatments, which showed no differences among them.

Nitrogen fixation in legumes such as soybeans is of great agronomic interest because it constitutes an economic means to maintain or increase the nitrogen content in the soil. In addition, it produces quality protein, in a context in which the world faces the explosive increase of the population and the shortage of feed and food, among other problems.

With inoculation, the number of grains increased, for which efficiency similar to that achieved with mineral fertilization was observed (Costales-Menéndez *et al.*, 2017). In this sense, the symbiosis between the legume and the *Rhizobium* bacteria enhances the growth and development of the soybean crop.

The isolation of rhizobia strains and cultivars tolerant to stress conditions is an important objective for many researchers, especially as it is an alternative for reforestation and recovery of productive areas with legumes. However, the search for genetic diversity in rhizobia species is a relevant complement to achieve these objectives and to substitute mineral fertilization and imports (Martínez *et al.*, 2018).

The colonization capacity is a key factor in the prevention and treatment of fungal diseases, because host plants are closely related to biofilm formation (Zhou *et al.*, 2016).

The best results in the cultures depend on an adequate bacterial colonization in the rhizosphere. The application of the correct inoculation technique on the seeds will be manifested in a higher percentage of germination, as well as in the crop productivity. It will also be shown in the increase of resistance to stress (Mahmood *et al.*, 2016).

The number of grains per pod was higher in the inoculated treatments, which differed significantly from the control (table 2).

The weight of 100 grains and the yield showed significant differences with regards to the control, which showed the lowest values; while the highest ones were obtained in the treatments inoculated with the three *Bradyrhizobium* strains (table 2).

The inoculation of *Bradyrhizobium* strains in the soybean crop cultivar Incasoy 27, under the soil conditions of Las Tunas province, achieved a higher productive response than that obtained with mineral fertilization.

In a research conducted by Cairo-Cairo y Álvarez-Hernández (2017), it was reported that the grain weight for the cultivar Incasoy-27 was 9,78 g, which coincides with the result obtained in this study.

From the scientific and practical point of view, it is important to consider the variables described above, as they offer criteria that can be used for the selection of rhizobium species with better adaptation to stress, which positively influences the improvement of the symbiotic fixation process and the crop yield (Bruno *et al.*, 2017).

The economic calculation of the crop showed that inoculation is an alternative to substitute imports. With this technique, profits of $ 5 720,00 (absolute control) and $ 12 373,00 CUP (strain ICA 8001) per hectare were obtained (table 3).

The planting of the soybean crop with biofertilizers is a viable alternative for crop production, in order to increase the agricultural yield and sustainability of agroecosystems. In this sense, Aguado-Santacruz (2012) argues that biofertilizers

Table 2. Morphoagronomic variables of *G. max* L.

Treatment	Number of pods/plant	Number of grains/pod	Weight of 100 grains, g	Yield, t ha^{-1}
Absolute control	11^{c}	2^{b}	$8,6^{c}$	$0,6^{c}$
Fertilization with NPK	15^{bc}	3^{a}	$9,4^{b}$	$0,9^{b}$
Bradyrhizobium strain E 109	19^{ab}	3^{a}	$11,6^{a}$	$1,0^{ab}$
Bradyrhizobium strain USDA 110	23^{a}	3^{a}	$12,0^{a}$	$1,2^{a}$
Bradyrhizobium elkanii strain ICA 8001	24^{a}	3^{a}	$12,3^{a}$	$1,2^{a}$
SE ±	0,13	0,01	0,01	0,05
VC %	6,22	1,67	1,42	9,19

Means with different letters in the same column differ for $p > 0,05$

Table 3. Economic appraisal of the soybean cultivar.

Indicator	Absolute control	Fertilization with NPK	Bradyrhizobium		
			strain E 109	strain USDA 110	strain ICA 8001
Yield, t ha^{-1}	0,60	0,93	1,06	1,17	1,23
Value of production, \$ ha^{-1}	6 360,00	9 858,00	11 236,00	12 402,00	13 038,00
Cost of production, \$ ha^{-1}	640,00	940,00	665,00	665,00	665,00
Profit	5 720,00	8 918,00	10 571,00	11 737,00	12 373,00
Cost per peso, \$ ha^{-1}	0,10	0,09	0,05	0,05	0,05

offer good yields in crops and provide facilities for their application, in addition to reducing the use of chemical fertilizers in agriculture. These criteria coincide with the report by Armenta-Bojórquez *et al*. (2010), who stated that the inoculation of biofertilizers containing rhizospheric bacteria causes significant increases in the productivity of agricultural crops and improves profits.

The new technologies should focus on maintaining and preserving the sustainability of the production system through the rational exploitation of natural resources and the application of relevant measures to preserve the environment (Grageda-Cabrera *et al*., 2012). Inoculation and agronomic management of microorganisms with biofertilizing properties constitute rational technologies, which emerged as innovative and promising practices for the agricultural activity (Moreno-Reséndez *et al*., 2018).

Conclusions

The application of *Bradyrhizobium* strains positively influenced the indicators evaluated in the crop, which allows to decrease the use of mineral fertilizers.

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Authors' contribution

- Aracelis Romero-Arias. Worked on the research conception and design; participated in the data acquisition and interpretation; wrote and reviewed the article.
- Raquel María Ruz-Reyes. Worked on the research conception and design; participated in the data acquisition and interpretation; wrote and reviewed the article.
- María Caridad Nápoles-García. Worked on the research conception and design; participated in the data acquisition and interpretation; wrote and reviewed the article.
- Ernesto Javier Gómez-Padilla. He worked on the conception and design of the research; participated in the data acquisition and interpretation; wrote and reviewed the article.
- Sergio Rodríguez-Rodríguez. He worked on the research conception and design; participated in the data acquisition and interpretation; wrote and reviewed the article.

Conflicts of interest

The authors declare that there are no conflicts of interests.

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