Colegio de Postgraduados

Effect of agronomic characteristics of hybrid and Creole corn using native plant growth-promoting bacteria to reduce the production cost

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ABSTRACT

Objective: To design and to evaluate an environmentally-friendly biofertilizer based on plant growth-promoting bacteria (PGPB), assessing the agronomic characteristics of two genotypes of C/Acceleron A7573 hybrid and Creole corn.

Design/methodology/approach: A biofertilizer based on PGPB was designed and assessed in a completely random experimental block design with nine treatments and four repetitions in *C/Acceleron A7573* hybrid and Creole corn in a plot at El Pericón, municipality of Tecoanapa, Guerrero, Mexico. The microorganisms *Rhizobium* sp., *A. brasilense* and *A. vinelandii* were used.

Results: The use of PGPB has greater effectiveness in all the agronomic variables and better yields because they are adapted to the environmental and soil conditions, with it being an excellent alternative to the use of fertilizers.

Limitations on study/implications: The demonstrative experimental plot had 5000 m^2 and it was the main limitation.

Findings/conclusions: Bacteria of the genus *A. brasilense* YOM9 and *A. vinelandii* YOC4 contributed to the higher yield of the *C/Acceleron A7573* hybrid corn seed, and *Rhizobium* sp. R01 and *A. vinelandii* YOC4 in the Creole grain of the olotillo race compared to the T9 fertilizer and the absolute control. A biofertilizer for corn is obtained based on results from this study, as an ecotechnology based on PGPB.

Keywords: Corn, plant growth-promoting bacteria, biofertilizer.

INTRODUCTION

Corn (*Zea mays* L.) is the most important agricultural crop in the Mexican economy and the most consumed cereal for the diet; it is produced for subsistence by farmers in rural communities. Corn occupies more productive surface than any other crop; although

it is cultivated in more than 160 countries, Mexico stands out among the eight countries that concentrate 75% of the total global consumption (Haytowitz *et al.*, 2018). In Mexico, corn is cultivated annually in nearly 8,500,000 ha, which produce around 24 million tons, while the annual demand is around 28 million tons (FIRA, 2020; SIAP, 2020). The Agrifood and Fishing Information Service (Servicio de Información Agroalimentaria y Pesca, SIAP) informed that Guerrero destines a cultivated surface annually of around 481,523.05 ha, 96% under rainfed conditions with 85% of native seeds, with annual state production of around 1,271,850.89 t (SAGARPA, 2018).

Presently, Mexico faces serious production problems due to low fertility and loss of soils that are becoming exhausted and making the production of crops of economic interest more expensive, derived from the excessive use of fertilizers and other agrichemicals (Reyes *et al.*, 2018), which affect the environment negatively.

These problems entail the need to seek efficient alternatives that ensure the sustainable production of the grain, through the application of biofertilizers or bioinnoculants that contribute in a high percentage to the development, nutrition, improvement and fertility of the soils, reducing the use of synthetic fertilizers in up to 50% (FAO, 2019). With great environmental and social impact, thanks to the reduction of costs and the balance in agroecosystems (Reyes *et al.*, 2018), the advantages attributed to the PGPB whose ability to fix atmospheric nitrogen, solubilize phosphate and produce phytohormones undoubtedly favor good results in growth and development. The main objective of this study was to design and to evaluate a biofertilizer that is environmentally friendly with PGPB from the genera *Rhizobium* sp., *A. brasilense* and *A. vinelandii*.

MATERIALS AND METHODS

The demonstrative plot is located in the community of El Pericón, municipality of Tecoanapa in the region of Costa Chica, Guerrero, Mexico (Figure 1) (INEGI, 2019).

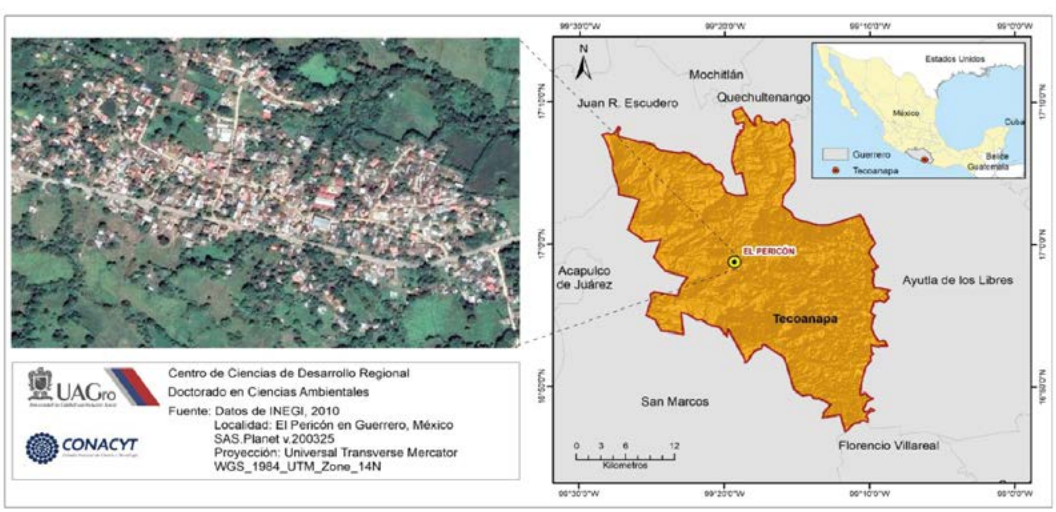

Figure 1. Geographic location of El Pericón, Municipality of Tecoanapa, Guerrero (INEGI, 2019).

Analysis of soil, genetic material, experimental design and economic analysis

The soil was analyzed physicochemically according to number 7 of the regulation NOM-21-RECNAT-2000, which establishes the specifications of fertility, salinity and classification of soils.

The corn genotypes used were the Creole race Olotillo and the *C/Acceleron A7573* hybrid. The experiment was established based on the completely random blocks with four repetitions with the following dimensions: 5 m of length by 0.81 m of width with nine furrows per block.

Experiment estabishment. The experiment was conducted in the period of February to July, 2021. Sowing was done with three seeds per sowing site at 0.40 m, and from these three, it was adjusted to two plants per shrub during the stage of vegetative growth (VG) that included 25 plants per treatment and 225 plants per block, equivalent to 900 plants per genotype of corn in four repetitions.

Farming activities in the experiment. Irrigation was conducted by gravity every 48 h, according to the hydric requirement of the plant. During the phenological stage of vegetative growth (VG), 1200 mL were added; in stage V1-V3 the amount was increased to 2400 mL of water; and finally 8400 mL of irrigation was applied in stage V7 to R1. Weed control was done manually.

Description of the bacteria strains. The strains that were used were donated by the biobank of the Laboratory of Molecular Microbiology and Environmental Biotechnology of the School of Chemical-Biological Sciences at the Universidad Autónoma de Guerrero, reactivated in NYDA agar and incubated at 37 °C during 24 h. The previously isolated strains of corn root, reed, bean nodules and rhizosphere, were confirmed *in vitro* as PGPB and labeled as: *Rhizobium* sp., R01, *A. brasilense* YOM9 and *A. vinelandii* YOC4.

Treatments

Combinations of the following treatments were made: T1 (Absolute control), T2 (*Rhizobium* sp. R01), T3 (*A. brasilense* YOM9), T4 (*A. vinelandii* YOC4), T5 (*Rhizobium* sp. R01 + *A. brasilense* YOM9), T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4), T7 (*A. brasilense* YOM9 + *A. vinelandii* YOC4), T8 (*Rhizobium* sp. + *A. brasilense* + *A. vinelandii*), T9 (chemical fertilizer).

Table 1. Identification and qualitative characterization of 6 strains from corn root, bean, reed and rhizosphere.

Strain No.	Identification	FBN	Solubilization $\left(PO_4^{-2}\right)$	Production (AIA)	Production (Gibberellins)	Production (enzymes)
R01		+++	+++	+++	+++	+
AYM9		+++		+++	+++	+
YOC4		+++	+++	++	++	++

+++: High concentrations of Auxins, Gibberellins, IS, FBN and PE.
++: Moderate concentrations of Auxins. Gibberellins, IS, FBN and PE.
+: Minimal concentrations of Auxins, Gibberellins, IS, FBN and PE.
-: Without production of Auxins, Gibberellins, IS, FBN and PE.

Preparation of inoculates

The inoculates were prepared in solid based on molasses at 2% at a concentration of 1-2$\times 10^8$ UFC/mL of each bacterial strain in nutritional agar (MCD LAB). In addition, the following inputs were used: two kilos of rice at a volume of 30 L (2 kg of sterile rice, 40 mL of molasses, 10 mL of saline solution at 0.9% and 100 mL of each concentrate). The final inoculates were left resting during three days for the strains to activate metabolically (Orbe-Díaz *et al.*, 2020).

Inoculation of plants and variables evaluated

The first inoculation was made 20 days after sowing of the Creole (olotillo race) and *C/ Acceleron A7573* hybrid corn genotypes, and for that purpose deposits of 20 L were used per treatment; with the support of a graduated cylinder of 250 mL, the stem base of each corn plant was inoculated, following the same procedure, and inoculations were carried out at 20, 34, 48 and 62 d with the same conditions and concentrations of PGPB.

The chemical fertilizer N80-P23-K15 and N10-P46-K30 was added to the side of the stem base of the corn plant (Martínez-Reyes *et al.*, 2018). Per treatment, 25 plants were selected to measure plant height (PH), stem diameter (SD), and leaf area (LA). The harvest of cobs was done at 135 days, and then data of the number of rows per cob (NRC) and cob length (CL) were taken, and the grain yield (GY) was estimated.

The data recorded and ordered were processed through analysis of variance and Tukey's test at 0.01% of probability through the support of the statistical software (SAS).

Economic analysis

The data recorded in the irrigation experiment logbook were subjected to analysis with the CEZACA software (Carpio *et al.*, 2022).

RESULTS AND DISCUSSION

The physicochemical properties of the soil in the study region are loam-clay-sand with evident deterioration that affects the grain yields due to bad practices. The soil analysis of the sample PF2 is loamy sandy texture, with a moderately acid pH of 5.64; according to López-Báez *et al.* (2019), corn adapts to all types of soils, where the optimal value of pH is 6.0 to 7, the soil presents Electric Conductivity 1:2 (H_2O) (mS/cm) of 0.08 free of salts, Electric Conductivity in Saturation Extract (mS/cm) 0.31 free of salts and is moderately rich in organic matter with 1.68; based on the NOM-021-RECNAT-2000 (Table 2).

In this sense, López-Báez *et al.* (2018) reported 64% of the soils with evident deterioration, among them generalized exchangeable acidity and a pH of 5.2 that evidences the need to design strategies that are environmentally friendly with the aim of increasing the productivity and the profitability in corn crops under sustainable agriculture. One year later, López-Báez *et al.* (2019) related the amendments in organic matter characteristic of the region as an alternative for degraded soils in response to the bad practices of burn, tilling and grazing and the non-incorporation of other sources of microorganisms to nourish the soils, as seen in the results from the soil analysis of the El Pericón sector (Table 2).

Table 2. Results from the physicochemical analysis of El Pericón sector (PF2).

Sector	Sample	Texture			Textural clss	pH	C.E ds/m	M.O. %
		Ao	Li	Ar				
Pericón	PF2	19.64	15.64	64.72	Sandy loam	5.64	0.08	1.68
Phosphorus (Bray) (mg/kg)		ppm. 72.17		Sample level: Medium				
Cations (+)								
Elements		**μg/ml**		**Interpretation**				
Sodium (Na^{+})		0.07		Under				
Potassium (K^{+})		0.14		Medium				
Calcium (Ca^{2+})		6.98		Medium				
Magnesium (Mg^{2+})		1.63		Moderately low				
Catión Exchange Capacity		8.82						
Microelements								
Elements		**μg/ml**		**Interpretation**				
Iron		136.41		Moderadately high				
Zinc		2.12		Medium				
Copper		0.57		Under				
Manganese		2.82		Very low				
Boron		<0.02		Very lowo				

Figure 2. Demonstrative plot. A) Start of cultivation, B) Irrigation, C) Fertilization and D) Biofertilization.

The basic elements (Na, K, Ca and Mg) present low, medium and moderately low viability in the soil analysis with 0.07, 0.14, 6.98 and 1.63 for Na, K, Ca and Mg, respectively, as shown in Table 2, based on the NOM-021-RECNAT-2000. In terms of micronutrients (Fe, Zn, Cu, Mn and B), iron and zinc presented adequate levels compared to copper, manganese, and boron which show deficient and marginal levels (Table 2).

Physical-chemical analysis according to numeral 7 of NOM-021- RECNAT-2000, which establishes the specifications of fertility, salinity and soil classification, El Pericón sector (PF2).

Throughout the experiment, a minimum temperature of 17 to 21 °C was recorded, and a maximum of 36 to 38 °C.

Agronomic variables of both corn genotypes

The analysis of variance detected statistically significant differences for the agronomic variables stem height and diameter between treatments ($p \leq 0.01$) (Tabla 3). It was seen that treatments T4 (*A. vinelandii* YOC4), T2 (*Rhizobium* sp. R01), and T9 (chemical fertilization) showed greater consistency in terms of PH, while the lowest height was obtained from the absolute control.

After analyzing the statistically significant data ($p \leq 0.01$) through Tukey's test (Table 3), it was found that treatments T3 (*A. brasilense* YOM9), T2 (*Rhizobium* sp. R01), and T8 (*Rhizobium* sp. R01 + *A. brasilense* YOM9 + *A. vinelandii* YOC4) showed greater consistency in terms of SD and the smallest stem diameter was obtained in T1 (absolute control); in terms of LA, the treatments T2 (*Rhizobium* sp. R01), T6 (*Rhizobium* sp. R01 + *Azotobacter vinelandii* YOC4), and T8 (*Rhizobium* sp R01 + *A. brasilense* YOM9 + *A. vinelandii* YOC4) showed better results compared to T1 (control); in the case of the CL, the treatments T2 (*Rhizobium* sp. R01), T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4), and T8 (*Rhizobium* sp. R01 + *A. brasilense* YOM9 + *A. vinelandii* YOC4); and lastly, in NRC, treatments T2 (*Rhizobium* sp. R01), T4 (*A. vinelandii* YOC4), and T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4).

Treatments T2, 3, 4, 6 and 8 showed greater biological effectiveness in corn growing when they were inoculated based on native PGPB, since they produce adhesive substances, excretion of substances that promote the growth and development of the plant, and indirect mechanisms, which agrees with Caiza *et al.* (2019). Creole corn (olotillo race) exceeded the plant height in comparison to the *C/Acceleron A7573* hybrid genotype. Authors like Zulueta-Rodríguez *et al.* (2020) showed statistical significance in plant height after confirming notable differences in their treatments with *A. brasilense*, catalogued as PGPB with great impact within sustainable agriculture due to the multiple promotion mechanisms attributed to them. However, Yousef *et al.* (2019) reported that *Rhizobium* sp. is associated to grasses as endophytes, and therefore, when the cereals are inoculated with PGPB they accumulate the nutrients P, K, Ca, Mg and even Fe.

Peréz-Peréz *et al.* (2021) reported that the inoculation of *Rhizobium* sp., in its 20 treatments, evidence that the height of the corn plants was statistically higher than the control in four out of the five variables, which is related to the mechanisms of plant growth promotion that they show. Therefore, a sustainable biofertilizer based on these bacteria genera is undoubtedly one efficient alternative for the producers. Caiza *et al.* (2019) reported statistically significant differences in the leaf area when inoculating PGPB of the genera *Azotobacter* and *Azospirillum* and, therefore, they conclude that the advantages from PGPB play an important role in the crops.

Pérez-Pérez *et al.* (2021) evaluated PGPB in corn and reported a significant increase in the stem diameter and root length so they highlight the importance of applying biofertilizer.

The analysis of variance and Tukey's test on the number of lines per cob showed significant differences between the *C/Acceleron A7573* hybrid and the Creole-olotillo, according to what is reported by Martínez-Reyes *et al.* (2018), who point out that the treatments based on *Azotobacter* sp., *A. brasilense* + *C. violaceum* + TQ 160-46-30, A. *brasilense* + FQ 80-23-15 increase the number of lines per cobs, evidencing the biotechnological potential of PGPB.

In the case of the results of cob length, the effectiveness of the PGPB is confirmed in this study compared to what was reported by Martínez-Reyes *et al.* (2018), where they quantified the best averages and determined that *Azospirillum* exerts a better effect since they attribute the capacity to fix nitrogen and the production of gibberellins, while the control shows a shorter length (Table 3). For their part, the purpose of Jaraba *et al.* (2020) of using *Rhizobium* sp., *A. brasilense* and *A. vinelandii* as biofertilizers was to substitute up to 60% the nitrogenous fertilizers, increasing the production, yields and cost-benefit rate, showing that their application replaces 20 to 60 kg N ha^{-1}. The results of this study give an advantage to future research to adopt sustainable agriculture with the aim of reducing the dependency of fertilizers in up to 50% using biofertilizers based on native PGPB.

Grain yield

The results point to a significant increase among corn genotypes, where the *C/Acceleron A-7573* hybrid presented higher grain yield of 7.69 t ha^{-1} with treatment T7 (*A. brasilense* YOM9 + *A. vinelandii* YOC4), and of 7.21 t ha^{-1} with T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4); concerning the Creole corn, the yield of 4.48 t ha^{-1} with treatment T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4) and of 4.16 t ha^{-1} with treatment T2 (*Rhizobium* sp. R01); and the lowest production for both genotypes was obtained in T1 (Table 4).

According to Beltrán-Pineda *et al.* (2022), the problems faced in farming regarding the excessive use of chemical fertilizers has given place to researchers seeking alternatives that are environmentally friendly with the aim of decreasing the dependency of producers on these toxic inputs, and this agrees with López-Báez *et al.* (2018), who report the effect of *Azotobacter* sp. on corn and the excellent results in the grain yield of 5.88 and 5.65 k ha^{-1} respectively.

Results show that *Rhizobium* sp., *A. brasilense* and *A. vinelandii* increase the yield, attributable to a greater absorption of nutrients.

Yousef *et al.* (2019) obtained similar results with a yield of 33.78 t ha^{-1} using *Rhizobium* sp., *A. brasilense* and *A. vinelandii*, attributing them as a sustainable alternative of biofertilizer for corn. In the same year, Hernández-Reyes *et al.* (2019) obtained grain yields of 1.79 and 2.07 t ha^{-1} attributable to *Azospirillum* sp., while Ayvar-Serna *et al.* (2020) obtained yields

Table 4. Corn grain yield expressed in t ha^{-1}.

Grain yield of hybrid genotype A7573 C/Acceleron and irrigated landrace-Olotillo.		
Treatments	**A7573 C/Acceleron Hybrid**	**landrace-Olotillo**
T1 Absolute witness	2.2 b	1.6 c
T2 *Rhizobium* sp. R01	**6.9 a**	**4.1 a**
T3 *Azospirillum* M9	5.4 a	3.4 b
T4 *Azotobacter* C4	5.7 a	3.5 b
T5 *Rhizobium* sp. R01 + *Azospirillum* M9	6.1 a	3.5 b
T6 *Rhizobium* sp. R01 + *Azotobacter* C4	**7.2 a**	**4.4 a**
T7 *Azospirillum* M9 + *Azotobacter* C4	**7.6 a**	**3.4 b**
T8 *Rhizobium* sp. + *Azospirillum* M9 + *Azotobacter* C4	6.6 a	3.8 a
T9 FQ 21-00-24 y 46-00-00	5.0 b	2.4 b
Media (Tukey = p≤0.01)	**5.9147 a**	**3.4034**

MH=maize hybrid A7573 C/Acceleron irrigation; MC=maize criollo-olotillo irrigation. Values are expressed as means ±SD for each treatment of the independent experiment. One-way ANOVA, Tukey's multiple comparison test; different letters in the bars indicate significant differences between experiments (P≤0.01).

of 14.13 Mg t ha^{-1} in DK357 hybrid corn, based on biofertilizers with *G. intraradices* + *A. brasilense*, and therefore, they conclude that using biofertilizers based on microbial consortiums increase the grain yields from 5 to 30%; thus, it is a field of opportunities focused on improving the soils with similar or better yields than those obtained with the use of fertilizers.

González-Mateos *et al*. (2018) relate the positive effect of microorganisms in corn grain yield with the capacity for atmospheric nitrogen fixation, solubilization of phosphates and production of phytohormones. Reyes *et al*. (2018) showed that *A. brasilense* and *A. vinelandii* strengthen the grain yield and, therefore, the results from this study agree slightly with Pérez-Luna *et al*. (2021) and Beltrán-Pineda *et al*. (2022).

This corroborates the effectiveness and the impact that PGPB have on the development of biofertilizers and the fundamental role they play within sustainable agriculture.

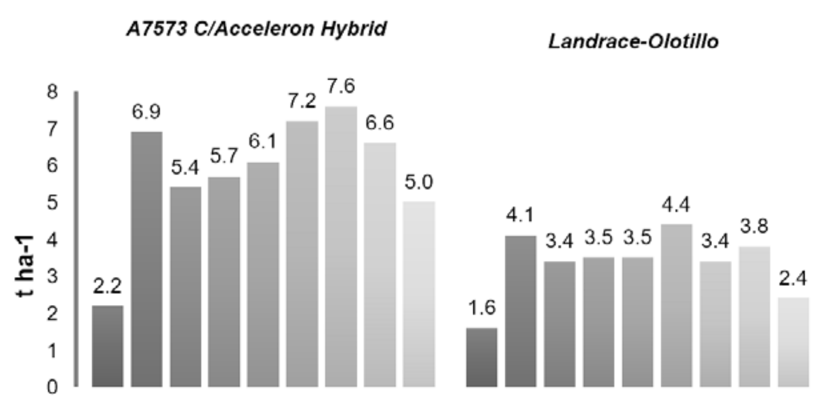

Figure 1. Corn grain yield assessed in nine treatments.

It is fundamental to specify that irrigation systems for rural development increase 60% of the territory destined to irrigation farming, since the superiority in yields of t ha^{-1} in comparison to rainfed farming is attributable. Therefore, the SHCP (2019) stated that the national average yield with irrigation is 7.5 t ha^{-1} and with natural precipitation it is 2.2 t ha^{-1}, which agrees with what was reported in this study, where yields of 7.6 t ha^{-1} were obtained using PGPB. Instead, Zepeda *et al.* (2021) state that irrigation farming generates more surfaces cultivated since a decisive factor in production is controlled and, therefore, the profits for small-scale and large-scale farmers increase.

Economic analysis for hybrid and Creole corn production

The results from the profitability analysis indicate that the treatments that contributed most to the grain yield are profitable for decision making, in the case of the *C/Acceleron A-7573* hybrid genotype with treatments T7 (*A. brasilense* YOM9 + *A. vinelandii* YOC4) and T6 (*Rhizobium* sp. R01 + *A. vinelandii* YOC4), with 7.69 t ha^{-1} and 7.21 t ha^{-1} of corn, respectively.

In this sense, the analysis indicates the total production cost of irrigation corn as $14,559.00, where the net income was $24,678.00, which is higher than the total cost, giving positive results of profitability by reflecting significant profits in grain production and yield. It should be highlighted that the sale price in the production cycle was stable and it remained at $18.00 L^{-1} with a production reached of 75. 341 (Table 5).

An important piece of data reflected in the analysis is the free net profit of $10,219.00, which is positive for the producers since it reflects their salary as worker within the production costs. The sum of profits is analyzed based on the production cycle in the six months, giving as a result a monthly profit of $1,703.00. This indicator of profitability is of utmost importance for the farmers in decision-making for their crops (Table 5).

Meanwhile, Ayvar-Serna *et al.* (2020) conducted a profitability analysis between corn genotypes, chemical NPK fertilization and biofertilizers, where they observed an increase per peso invested in the IT, IN and GPI obtained which fluctuated from $2.00 with NPK fertilization to $2.91 with biofertilizers (*G. intraradices* + *A. brasilense*). In comparison, Zepeda *et al.* (2021) conducted a study in irrigation corn and reported that their profits in irrigation exceed the profits from rainfed farming, from the production costs that have lower social and environmental cost, but the importance lies in that income with irrigation is a trigger that favors the region's economy due to the demand for goods and services.

However, Carpio *et al.* (2022) reported through an economic analysis the results from their experiment based on three corn genotypes, using a biofertilizer and a nitrogenated fertilizer, where they highlighted that for each investment they recovered what they invested despite the excessive costs of fertilizer and inputs. It is important to mention that corn farming is risky because of the excessive prices in the market and the social, environmental and economic problems that are attributed to the grain. Therefore, it is important to carry out a profitability analysis with the goal of generating important income and adopting new environmentally-friendly ecotechnologies.

Table 5. Cost structure and profitability indicators.

Concept	Unit	Quantity	Cost structure (%)
Surface area	(m^2)	5,000	
Production cycle	month	6	
Cycles per year	cycle	1.0	
Variable costs		7,738.00	
Manual tillage	($)	1,800.00	12.45%
Mechanized tillage	($)	300.00	2.07%
Seeds or seedlings	($)	568.00	3.93%
Biofertilizer	($)	200.00	1.38%
Chemical fertilizer	($)	240.00	1.66%
Energetics	($)	3,030.00	20.96%
Fees	($)	600.00	4.15%
Taxes	($)	1,000.00	6.92%
Fixed costs		6,721.00	
Land	($)	1,000.00	6.92%
Machinery and equipment	($)	4,209.00	29.11%
Tools and materials	($)	648.00	4.48%
Maintenance	($)	864.00	5.98%
Total, costs		14,459.00	
Production		1,371.00	
Average price	($)	18.00	
Revenues		24,678.00	
First	($)	24,678.00	
Profit	($)	10,219.00	
Cycle rate of return	(%)	7.6%	
Annual rate of return	(%)	7.7%	
Total, unit costs	($)	10.55	
Unit variable costs	($)	5.64	
Unit fixed costs	($)	4.90	

CONCLUSIONS

Bacteria from the genera *A. brasilense* YOM9 and *A. vinelandii* YOC4 contributed to the higher grain yield of *C/Acceleron A-7573* hybrid corn, and *Rhizobium* sp. R01 and *A. vinelandii* YOC4 in the Creole grain of the olotillo race, regarding the T9 fertilizer and the absolute control. Therefore, based on the results from this study to obtain a biofertilizer for corn as ecotechnology that plays an important role in the absorption and stabilization of the necessary essential elements, they are a good environmentally-friendly alternative to reduce the excessive use of fertilizers within farming. The use of biofertilizers by farmers with limited capital is recommended, because they are more economic, contribute nutrients in a natural way to the soil, and are highly profitable within sustainable agriculture.

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