LA GRANJA:
REVISTA DE
CIENCIAS DE LA VIDA
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n39.2024.09

Scientific paper/ Artículo científico

BIOTECHNOLOGY

RESPONSE OF FLOUR CORN (*Zea mays L. var. Amylacea*) TO THE INOCULATION OF *Azospirillum* AND *Pseudomonas*

RESPUESTA DEL MAÍZ HARINOSO (*Zea mays L. var. Amylacea*) A LA INOCULACIÓN DE *Azospirillum* Y *Pseudomonas*

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Article received on June 06th, 2020. Accepted, after review, on September 07th, 2021. Published on March 1st, 2024.

Abstract

Plant growth-promoting bacteria (PGPB) that inhabit root rhizosphere of plants are of great agricultural importance due to their ability to produce phytohormones during root colonization. These phytohormones produce physiological changes in the plant that favor a greater absorption of nutrients, promote growth and increase production. This study analyzed the effect of inoculation of two of these bacteria, *Azospirillum* sp. and *Pseudomonas fluorescens*, under field conditions representative of the Andean Highlands. The experiment was carried out using flour corn seed of the INIAP-101 variety in a randomized complete block design with six repetitions. The treatments corresponded to: T1 (*Azospirillum* sp.), T2 (*P. fluorescens*), T3 (*Azospirillum* sp. and *P. fluorescens*), T4 (control, conventional chemical fertilization) and T5 (absolute control). The application of these bacteria significantly increased ($p<0.05$) the root length, the diameter of the cob, and the yield of fresh corn ("elotes" or "choclos"). The combined inoculation of these bacteria (T3) obtained the highest yield, with 19.70 t ha^{-1} of fresh corn; while the chemical control and the absolute control got 17.12 and 13.58 t ha^{-1} of fresh corn, respectively. The economic analysis of T3 reported a benefit/cost of 1.35, which indicates that the synergism of these two bacteria could be a sustainable strategy to improve yields and reduce the use of chemical fertilizers for corn production in the Andean region.

Keywords: biofertilizers, bioinoculants, fresh corn, sustainable production.

Resumen

Las bacterias benéficas que habitan la rizósfera de las plantas son de gran interés agrícola debido a su capacidad para producir fitohormonas durante la colonización radicular. Estas fitohormonas producen cambios fisiológicos en la planta que favorecen una mayor absorción de nutrientes que provocan un rápido crecimiento vegetativo y una mayor producción. Esta investigación tuvo como objetivo evaluar el efecto de la inoculación de dos de estas bacterias, *Azospirillum* sp. y *Pseudomonas fluorescens* en el cultivo de maíz harinoso bajo condiciones de campo típicas de la región alto andina. El experimento se realizó utilizando semilla de maíz harinoso de la variedad INIAP-101 con un diseño de bloques completos al azar y seis repeticiones. Los tratamientos correspondieron a: T1 (*Azospirillum* sp.), T2 (*P. fluorescens*), T3 (*Azospirillum* sp. y *P. fluorescens*), T4 (testigo con fertilización química convencional) y T5 (testigo absoluto). La aplicación de estas bacterias incrementó de manera significativa (p<0,05) la longitud de raíz, el diámetro de la mazorca y el rendimiento del maíz tierno (elotes o choclos). La inoculación combinada de estas bacterias (T3) obtuvo el mayor rendimiento con 19,70 t ha^{-1} de choclos, mientras que el testigo químico y el testigo absoluto obtuvieron 17,12 y 13,58 t ha^{-1} de choclos, respectivamente. El análisis económico de T3 reportó un coste/beneficio de 1,35, lo cual indicó que el sinergismo de estos dos géneros podría ser una estrategia sostenible para mejorar los rendimientos y reducir el uso de fertilizantes químicos en el cultivo de maíz de la región Andina.

Palabras clave: biofertilizantes, bioinoculantes, choclo, producción sustentable.

Suggested citation: Sangoquiza-Caiza, C., Zambrano-Mendoza, J., Borgues-García, M. and Choi, K. (2024). Response of flour corn (Zea mays L. var. Amylacea) to the inoculation of Azospirillum and Pseudomonas. La Granja: Revista de Ciencias de la Vida. Vol. 39(1):152-161. http://doi.org/10.17163/lgr.n39.2024.09.

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1 Introduction

Flour corn (*Zea mays* L. var. *Amylacea*) is one of the most important crops in the Ecuadorian Highlands, due to the large amount of land used for its production and the role it plays as a basic component of the population's diet (Yánez et al., 2013). Around 62 581 ha of this cereal are grown annually, doubling the area planted with other important crops in the highlands such as potato, bean, wheat, and barley (Continuous Agricultural Production and Area Survey (ESPAC; by its acronym in Spanish) (ESPAC, 2018). Unfortunately, the yield of this type of corn reaches up to 0.93 t ha^{-1} for dry grain, and 3.75 t ha^{-1} for fresh corn, placing Ecuador among the lowest flour corn productivity levels in South America (Boada and Espinosa, 2016).

Flour corn is usually planted in small lots up to one hectare, and a large proportion of these lots are located on marginal soils that include hillside areas, with soils exposed to erosion and no irrigation facilities. In addition, most of the farmers use uncertified seeds and unimproved native varieties. All these, and other socioeconomic factors make corn yield very low, which limits farmer´s purchasing power and restricts the use of inputs, such as fertilizers.

The yield of corn is closely related to the availability of soil nutrients. For corn grown in the highlands, it is recommended to apply 80 kg ha^{-1} of nitrogen and 40 kg ha^{-1} of phosphorous in soils of intermediate fertility (Yánez et al., 2013). After nitrogen, phosphorus is one of the most important elements in the early stages of normal plant development; a deficiency of these elements in the crop can cause slow growth, little development of the root system and therefore reduces the yield of the harvest (Guzmán, 2012). Plant growth-promoting bacteria (PGPB) facilitates plant growth by providing either fixed nitrogen or phosphorus and induces the production of phytohormones during root colonization. These phytohormones produce physiological changes in the plant that favor a greater absorption of nutrients, promote growth, and increase production (Glick, 2012; Santoyo et al., 2016).

Currently, it is pivotal to investigate the soil microbiota to obtain strategies that improve agricultural productivity. One of these strategies is the application of bioinoculants with beneficial microorganisms which, applied to the soil or the seed, generate a positive impact on plant nutrition and yield (Glick, 2012; Díaz-Blanco and Márquez-Reina, 2011). The use of bioinoculants made from beneficial microorganisms, such as *Azospirillum* sp. and *Pseudomonas fluorescens*, that live associated or in symbiosis with the roots of corn, can efficiently contribute to nitrogen fixation, phosphorus solubilization, and natural soil fertility, providing a positive agrobiological effect that constitutes an important alternative for the reduction or partial substitution of mineral fertilizers (Grageda et al., 2012; Pereira et al., 2020).

The study and management of beneficial microorganisms has increased to such a degree that nowadays a whole commercial movement has been generated. The production and commercialization of bioinoculants are aimed to strength a sustainable production system with an ecological balance of the soil (Ruiz, 2015; Urrutia, 2019). Several studies have shown the benefits of the use of bioinoculants or biofertilizers in dent and flint corn in lowlands, increasing yield and cost effectiveness for farmers, but reports on flour corn in the highlands are scarce. In Mexico, García et al. (2012) showed that the inoculation with *A. brasilense* increased grain yield compared with the non-fertilized and non-inoculated control, increasing the benefit/cost by 56% on average. Later,Martínez-Reyes et al. (2018) reported that the use of a biofertilizer with *A. brasilense* increased the yield of grain up to 28.0% (1.67 t ha^{-1}) with respect to the absolute control, achieving a greater net benefit than the use of conventional chemical fertilization. In Brazil, Pereira et al. (2020) reported that the use of bioinoculants increased grain yield up to 39.5 and 34.7% when corn seed was inoculated with *Bacillus subtillis* and *A. brasilense*, respectively.

The National Institute for Agricultural Research-INIAP has a collection of *Azospirillum* sp. and *P. fluorescens* strains isolated from the rhizosphere of corn plants, collected throughout the Ecuadorian Highlands. These strains have been characterized and evaluated, and as results the C2 strain of *Azospirillum* sp. and the *nI5* strain of *P. fluorescens* with a concentration of $1{\times}10^9$ ufc mL^{-1} have shown the best results in preliminary studies (Rivadeneira, 2012; Pincay, 2014; Sangoquiza et al., 2019). The objectives of this research are to evaluate the effect of these native Andean strains in the production of fresh flour corn ("choclos" or "elotes") and estimate the benefit/cost of the inoculation.

2 Material and Methods

2.1 Study area

The experiment was established in a commercial corn production field located in the Province of Pichincha, Ecuador, in the parish of Amaguaña (0°23'9.87" S and 78°30'3.4" W), at an altitude of 2 675 masl, during the 2017-2018 cycle. The average temperature of Amaguaña is 17°C with an average rainfall of 960 mm (Bastidas, 2016).

2.2 Bacterial strains and bioinoculant

The inoculum was developed from the lyophilized strain *C*2 of *Azospirillum* sp., isolated from 'Laguacoto', parish Veintimilla, cantón Guaranda, province of Bolívar, and the strain *nl*5 of *P. fluorescens* isolated from 'Tunibamba' of the parish 'El Sagrario' cantón Cotacachi, province of Imbabura. The strains were collected from the rhizosphere of corn plants cultivated in the highlands of Ecuador (Carrera, 2012; Pincay, 2014). The strains are conserved at the laboratory of the Maize Program at the Experimental Station Santa Catalina of INIAP. The inoculum and the inoculation of the seeds were made as reported by Sangoquiza et al. (2019). Briefly, 1000 μL of 1% peptone were placed in the Eppendorf tubes, by shaking until the mixture was homogenized using a vortex. Subsequently, 50 μL of the inoculum were taken and placed in Petri dishes with the solid malic-acid Congo Red Agar culture medium for the case of *Azospirillum* sp., and *King B* for *P. fluorescens*. Subsequently, the inoculum was placed in an incubator at 30°C for 7 days. After this time, pure sections of the bacteria were taken and placed in Petri dishes with the specific growth media.

The bioinoculant was made in a liquid support with 2% molasses with sterile distilled water, pH 7, at a bacterial concentration of 1×10^9 ufc mL^{-1}. In 300 mL of the bioinoculant solution, 280 mL corresponded to the liquid support, and 20 mL to the bacteria (10 mL of *Azospirillum* sp. and 10 mL of *P. fluorescens*). The bacteria concentration was measured with a spectrophotometer (Thermo Scientific, GENESYS). The bacteria concentration was obtained by reaching the value of 1 in absorbance, which was confirmed by growth tests by dispersion on a plate. The ufc counting was performed using serial dilutions. The bioinoculant was applied directly to the seed at the time of sowing, as indicated by Yánez et al. (2010). Briefly, 420 seeds of maize were placed in a plastic bowl with a capacity of 5 L. Then, 200 mL of the bioinoculant was applied to the seed, mixing it uniformly to ensure contact with the seed surface. After inoculation, the seed was left to rest in the shade for an hour, before being used for sowing.

Bacteria viability test was performed with the remaining 100 mL of the bioinoculant. The viability evaluation was carried out by taking a sample of the bioinoculant in which serial dilutions were made, taking 0.1 mL of each dilution and sowing in Petri dishes with the specific culture medium for each bacterium. These were incubated at 30°C for 7 days. The presence of bacteria was determined by plate count.

2.3 Experimental design

The sowing was carried out in a sandy loam soil at the beginning of the rainy season in November, with the flour maize variety INIAP-101 (Caviedes, 2013). The treatments corresponded to: T1 (*Azospirillum* sp; 1×10^9 ufc mL^{-1}), T2 (P. fluorescens; 1×10^9 ufc mL^{-1}), T3 (Azospirillum sp. and P. fluorescens; 1×10^9 ufc mL^{-1}), T4 (control, conventional chemical fertilization) and T5 (absolute control). Chemical fertilization was applied only in the plots corresponding to the treatment T4. The fertilization, based on the results of the soil analysis (Table 1), was 57 kg of N ha^{-1}, 57 kg of P_2O_5 ha^{-1}, 10 kg K_2O ha^{-1}, 25 kg S ha^{-1}, and 10 kg of Mg ha^{-1}, distributing equally the nitrogen between sowing and hilling, which was carried out 45 days after sowing. The other nutrients were applied at sowing. The size of each plot was four furrows of 5.00 m long and 3.20 m wide, with 80 plants in each plot. The planting distance was 0.80 m between rows and 0.25 m between plants, with a density of 50 000 plants ha^{-1}. The nutritional content of the soil where the experiment was planted, analyzed in the Department of Soils of the Experimental Station Santa Catalina, is shown in Table 1.

Table 1. Nutritional values of the Amaguaña soil where the effect of inoculation of *Azospirillum* and *Pseudomonas* in flour maize was evaluated.

Nutrient	Value	Unit
N	57.0	ppm
P	11.0	ppm
S	4.70	ppm
K	0.59	meq 100 mL^{-1}
Ca	5.90	meq 100 mL^{-1}
Mg	1.90	meq 100 mL^{-1}
Zn	0.90	ppm
Cu	7.60	ppm
Fe	350	ppm
Mn	3.40	ppm
B	0.30	ppm
MO	4.40	%
pH	6.36	

The agronomic practices of the crop were carried out following the recommendations given to small farmers of the highlands of Ecuador (Yánez et al., 2013). In short, a plowing pass and a harrow pass were carried out to incorporate the remains of the previous crop (corn). During the development of the crop, the weeds were removed manually, and the insect pests were controlled with two applications of insecticides (Cypermethrin and Chlorpyrifos) at 60 and 120 days, with a dose of 1 L ha^{-1}. The crop was harvested and evaluated in a fresh state (fresh corn), at the growing stage R3 (milky stage), 150 days after planting (Yánez et al., 2013).

2.4 Variables evaluated

Agronomic evaluation was conducted at harvest, according to the International Maize and Wheat Improvement Center (CIMMYT) procedures for international trials (CIMMYT, 1999). The evaluation of the variables is detailed:

- **Plant height**: the length from the base of the plant to the point where the panicle begins to divide was measured on ten randomly selected plants in the central furrows of each plot. This variable was recorded in m using a height meter at harvest.
- **Root length**: at harvest, 10 plants were taken randomly form the central furrows of the plot. The aerial part of the plants was separated and the length of the root was measured from the base of the plant to the tip of the longest root with the help of a meter. This was measured in cm.
- **Ear length**: ten ears with bracts taken randomly from the central furrows of each plot were measured from the base to the tip of the ear with the help of a tape measure. This was measured in cm.
- **Ear diameter**: the central point of 10 randomly chosen ears from the central furrows with bracts of each treatment was measured with a caliper, the data were expressed in cm.
- **Yield**: ears with bracts, suitable for fresh consumption at R2-3 stage ("elote", "choclo"), from the two central furrows (40 plants) of each experimental plot were harvested. Fresh corn yield was recorded in kg, but data is shown as t ha^{-1}.
- **Inoculation effectiveness index (IEI)**: it was calculated as a percentage using the Equation 1 (Escobar et al., 2011).
- **Cost/benefit**: it was estimated based on the income from the sale of the fresh corn harvested and the production cost (inputs, labor, soil preparation, and indirect costs) of each treatment. The gross income (USD ha^{-1}) was estimated by multiplying the fresh corn yield (t ha^{-1}) by its commercial value (USD t^{-1}). The net profit per hectare was estimated by the difference between the gross income in dollars and the cost of production. The cost for each dollar obtained (CU) was calculated by dividing the cost of production (USD ha^{-1}) by the gross income (USD ha^{-1}), and the benefit cost ratio (B/C) was estimated by dividing the net profit (USD ha^{-1}) by the production cost (USD ha^{-1}).

$$IEI\,(\%) = \frac{(\text{yield of the inoculated treatment} - \text{yield of absolute control})}{(\text{yield of absolute control})} \times 100 \qquad (1)$$

2.5 Statistical analysis

A completely randomized block design with six replications was used to evaluate the effect of three bioinoculants (T1, T2, and T3) and two controls (T5 and T6). The results were subjected to a normality Shapiro-Wilks modified test, and then, analysis of variance and Tukey's multiple comparison test ($p < 0{,}05$) were performed for all agronomic traits. The statistical program INFOSTAT was used for the analysis.

3 Results

Table 2 shows the results of the agronomical traits evaluated in the different treatments. Significant statistical differences ($p < 0{,}05$) were observed among the treatments for all traits. The absolute control, as expected, showed the lowest values, and lowest Tukey-test ranking, meaning the poorest agronomic performance. The use of conventional chemical fertilization (T4) showed the highest height of the plant with 2.44 m; although it did not differ statistically from the inoculated treatments (T1, T2 and T3), it did differ from the absolute control (T5), which obtained the shortest plants with 2.20 m.

The fresh corn yield obtained with the use of bioinoculants (T1, T2, and T3) did not differ statistically from the yield obtained using chemical fertilization (T4), according to Tukey test ($p < 0{,}05$). This was consistent with the observed performance of plant height, ear length, and root length. The IEI of the inoculation with *Azospirillum* sp. and *P. fluorescens* (T3) was 46.58 %, almost twice than when it was done with the single bacteria (T1 or T2) (Table 2). The use of the bioinoculant with both isolates (T3, Azospirillum sp. and P. fluorescens) showed the best agronomical performance for ear diameter, yield, and IEI (Table 2). There were no significant differences ($p > 0{,}05$) between T3 and the chemical conventional fertilization control (T4) for any of the evaluated traits, except for ear diameter, where T3 obtained thicker ears than T4, with 21.47 cm and 20.13 cm, respectively.

The economic assessment showed the benefit/cost of the application of these bioinoculants on flour maize (Table 3). The inoculation of the seeds made with the combined biofertilizer T3 (*Azospirillum* sp. and *P. fluorescens*) got the highest net profit 3546.00 USD ha^{-1}, and the highest B/C ratio 1.35. Although the production cost of the combined bioinoculant (T3) was higher than the bioinoculants with a single isolate (T1 and T2), the gross income of T3 was higher due to the yield increase obtained when inoculating with both isolates (Table 2).

4 Discussion

4.1 Effect of the application of bioinoculants on agronomical traits of corn

The use of bioinoculants with *Azospirillum* sp. and *P. fluorescens* on fresh flour corn showed the same agronomic performance than using conventional chemical fertilization (Table 2). The results obtained in this study could be because these bacteria have the ability to fix nitrogen, solubilize phosphorus and produce plant growth promoting compounds (Aguirre and Espinosa Moreno, 2016; Rueda et al., 2016). These bacteria stimulated the growth and development of the flour corn plants that showed longer roots, taller plants, and thicker ears than the absolute control (T5). Cereals inoculated with these bacteria have shown a greater capacity to efficiently absorb water and nutrients from the soil (Sala et al., 2005; Oliveira et al., 2018; Pereira et al., 2020).

Bioinoculants based on *Azospirillum* and *Pseudomonas* strains have a high capacity to produce indole-acetic acid (IAA) causing root development and cell elongation. There are reports that assure the influence of *Azospirillum* on the formation of lateral roots due to nitrite secretion (Camelo et al., 2011; Bécquer et al., 2012). The production of IAA and the high sensitivity of the roots to this hormone are fundamental for the response to the inoculation of *Azospirillum* and *Pseudomonas*, where a greater radical development is frequently observed, which translates into a greater surface area for nutrient absorption, and thus, a greater development of the aerial part of the plant (García et al., 2007).

Table 2. Effect of the application of bioinoculants on agronomical traits in the cultivation of flour corn INIAP 101. Treatments: T1 (*Azospirillum* sp.), T2 (*P. fluorescens*), T3 (*Azospirillum* sp. and *P. fluorescens*), T4 (control, conventional chemical fertilization) and T5 (absolute control).

Treatment	Plant height (m)	Ear length (cm)	Ear diameter (cm)	Root length (cm)	Fresh corn yield (t ha^{-1})	IEI (%)
T1	2.37 ab*	38.33 a	21.20 a	38.67 a	16.72 b	22.68 b
T2	2.42ab	35.83 ab	21.00 a	32.67 bc	17.05 b	23.68 b
T3	2.33 ab	37.50 ab	21.47 a	37.00 ab	19.70 a	46.58 a
T4	2.44 a	36.33 ab	20.13 b	34.17 abc	17.12 ab	-
T5	2.20 b	32.33 b	18.47 c	31.00 c	13.58 c	0.00 c

IEI = Inoculation effectiveness index. *Different lowercase letters mean significant statistical difference according to Tukey test ($p < 0,05$).

The flour corn maize INIAP 101 showed an increase in the plant height, ear length and diameter of the inoculated plants, compared with the absolute control (Table 2). These results agree with Piscoya and Ugaz (2016), who pointed out that the Azospirillum genera showed an increase in the height, number of leaves and diameter of stems in the cultivation of hard corn; meanwhile Piromyou et al. (2011) demonstrate the beneficial action of individual and mixed inoculation of beneficial microorganisms in the cultivation of dent corn by improving their radical functioning in the absorption of the nitrogen by externally invading the corn root, favoring the synthesis of plant growth promoting substances.

The C2 strain of *Azospirillum* sp. and the nl5 strain of *P. fluorescens* used in this study have shown their capacity to promote nitrogen and phosphorous absorption before. Sangoquiza et al. (2019) reported a higher accumulation percentage of nitrogen and phosphorous in leave tissue of INIAP-101 plants inoculated with these isolates. Similar studies of the effect of *Azospirillum* sp. showed a higher percentage of nitrogen in corn leaf tissue (Ortiz, 2010), while the inoculation of *A. brasilense* and *P. fluorescens* increased the total phosphorus content by 187 kg ha^{-1} (Faggioli et al., 2003).

4.2 Inoculation effectiveness index (IEI) of the bioinoculants

For IEI, the treatment T3 showed statistically significant differences with treatments T1 and T2, according to Tukey-test ($p < 0,05$) (Table 2). These results indicated that the combination of Azospirillum and P. fluorescens favored corn performance, which suggests an associative symbiosis that can improve root morphology and physiology, achieving better use of water and nutrients such as nitrogen and phosphorus, generating better quality and development of the grain (Gálvez et al., 2014). Martins et al. (2018) pointed out that the effects of these microorganisms on plant development have favored the performance of various crops, when applied alone or in combination, achieving greater colonization and increasing the production. A more detailed review of the benefits of biofertilizers was recently published by Zambrano-Mendoza et al. (2021).

4.3 Economic assessment of the use of the bioinoculants

The analysis of the economic parameters showed how advantageous the application of these bioinoculants were by promoting a good development of the inoculated plants and greater economic performance (Table 3). The highest cost of production was obtained with T4 (conventional chemical fertilization control), while the lowest cost of production was obtained with T5 (absolute control), where no fertilization nor bioinoculant was applied. The main difference among the production costs of each treatment was given by the value of the chemical fertilizer and the cost of its application. In relation to the conventional chemical recommendation (T4), the use of the bioinoculant T3 (*Azospirillum* and *P. fluorescens*) allowed a saving of USD 281.12 ha^{-1}, which represents a decrease of approximately 16% in the production cost, without significantly affecting yield (Table 2). This shows that it is possible to substitute the use of chemical fertilizers by bioinoculants without affecting the production of corn, opening the opportunity to more sustainable and environmentally friendly production systems.

Table 3. Economic assessment of the application of bioinoculants in the flour corn INIAP 101 in the highlands of Ecuador.

	Treatment	Production cost (USD ha^{-1})	Raw Income (USD ha^{-1})	Net profit (USD ha^{-1})	CU* (USD)	Benefit/Cost (USD)
T1	*Azospirillum* sp.	1505.74	3009.60	1503.86	0.50	1.00
T2	*P. fluorescens*	1505.74	3069.00	1563.26	0.49	1.04
T3	*Azospirillum* sp. + *P. fluorescens*	1511.74	3546.00	2034.26	0.43	1.35
T4	Control, chemical fertilization química	1792.86	3081.60	1288.74	0.58	0.72
T5	Absolute control	1491.86	2444.40	952.54	0.61	0.64

*CU = Cost for each dollar obtained.

Many corn farmers in the highlands do not use chemical fertilizers at all, resembling treatment T5 (absolute control). Using *Azospirillum* and *P. fluorescens*, farmers may have the possibility to duplicate their net profit from 952.54 USD ha^{-1} to 2034.26 USD ha^{-1} with an additional investment of approximately 20.0 USD ha^{-1} (Table 3). Further studies in several environmental conditions could clarify this possibility.

The B/C of the fresh corn production using the bioinoculant T3 (*Azospirillum* and *P. fluorescens*) increased by 87.5% in relation to the conventional chemical fertilization (T4) (Table 3). This value is higher than the increase of 56.0% reported by García et al. (2012), and the increase of 36.0% reported by García et al. (2007), when evaluated the effect of the application of A. brasilense on dent corn in northern Mexico. In economic terms, these results showed that the inoculation with plant growth promoting bacteria (*Azospirillum* sp. and *P. fluorescens*) increased the net profit and the B/C ratio of the cultivation of corn; therefore, the use of these bioinoculants could generate an economically viable technology for the production of flour corn in the Andean Region.

5 Conclusions

The inoculation with *Azospirillum* sp. improved the yield of fresh corn ("choclo"), increased the development of the root, and the diameter and the length of the cob compared to the control. The inoculation with *P. fluorescens* showed higher yield and ear diameter than the control. The combination between *Azospirillum* sp. and *P. fluorescens* significantly increased fresh corn yield by 46.58% in relation to the control without fertilization. The yield of the plants treated with chemical fertilizer was similar to the yield obtained with the plants inoculated with these microorganisms. The combination of Azospirillum sp. and P. fluorescens produced the highest net gain and B/C ratio, increasing by 87.5% the B/C of conventional chemical fertilization.

Azospirillum sp. and *P. fluorescens* reduced the use of synthetic fertilizers by approximately 50%, lowering the cost of production. Hence, it is feasible to substitute the use of synthetic fertilizers for biofertilizers, opening the opportunity for a sustainable and environmentally friendly maize production system in the Ecuadorian highlands.

Acknowledgements

The authors thank the Korean Program on International Agriculture -KOPIA- and the project: "Development of cultivation technologies for corn and maize using biofertilizers in Ecuador Highlands", for financing the evaluation of the bioinoculant.

Author Contributions

CASC; Research, Writing. JLZM; Writing, review and editing. MBG; Conceptualization. KJC; Validation.

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