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Article

Yield and Agronomic Performance of Sweet Corn in Response to Inoculation with *Azospirillum* sp. under Arid Land Conditions

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Abstract: Nitrogen is the most common limiting factor for crop productivity, and most maize cultivars require fertilizing. Here, we report on the possibility of partially replacing the nitrogenous fertilizer in sweet corn inoculated with a native strain of *Azospirillum* sp. in arid land on the coast of Peru. We performed an agronomic experiment in a crop field with arid soil under drip irrigation in Huacho (Peru) using a commercial variety of sweet corn. The treatments were two levels of nitrogen (90 and 180 kg N ha^{-1}), one or two applications of a native strain of *Azospirillum* sp. (1×10^8 CFU/mL) and a control treatment with only nitrogen fertilizer. Eleven agronomic variables related to productive aspects were evaluated by performing statistical analyses and the comparison of treatment means. Inoculation with *Azospirillum* sp. did not significantly ($p > 0.05$) affect the total weight of ears, the number of ears per plant and the number of male flowers, but it significantly ($p < 0.05$) influenced the grain yield per hectare, the survival of plants, grain weight per plant, and the diameter and length of the cob. In some productive characteristics of sweet corn cv "Pardo", a significant effect was found following inoculation with *Azospirillum* sp., which outperformed the control with only nitrogen fertilization in grain yield, suggesting that it is possible to complement the application of nitrogen to soil with the inoculation of this strain, replacing up to 50% of the levels of fertilizer application, since the benefit/cost ratio increases.

Keywords: arid soils; *Azospirillum*; nitrogen fixation; nitrogen levels; sweet corn

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1. Introduction

Corn (*Zea mays*) is one of the most important agricultural products in Latin America; in this region, local maize varieties are critical for food security, livelihoods and cultures [1]. In Peru, the area under cultivation exceeds half a million hectares [2], and the local variety "choclo" (sweet corn) is a highly demanded food on the market given its importance in regional gastronomy, as Andean maize is a basic food of the rural population [3]. Due to the need to obtain sustainable agricultural production, new ecological alternatives have been investigated worldwide, considering the use of biofertilizers as an option to partially or fully replace the use of chemical fertilizers [4]. Recent studies reinforce the concept that corn yields are controlled not only by the N supply in the soil but also by factors that modify the demand of the plants and the ability to capture N [5].

The arid soils of the Peruvian coast have a low amount of organic matter, normally less than 1% [6]; the balance between mineralization and decomposition of soil organic matter is due to functional differences in key soil microbial groups that influence the mineralization of carbon sources [7]. For this reason, it is necessary to explore those microbial groups that can be related to the biological fixation of atmospheric nitrogen and the mineralization of soil organic matter, which affect the agronomic performance of maize [4]. Likewise, corn producers need to improve crop management, especially in technological aspects of nitrogen fertilization that reduce high production costs [8].

Azospirillum bacteria develop in the roots supplying N to the plant and in various edaphic environments associated with cereals and other species; they are capable of synthesizing phytohormones that promote growth and morphological and physiological changes in the root and exert biocontrol [9], improving the use of water and nutrients and increasing yield and productivity [10]. *Azospirillum* was originally selected for its ability to fix atmospheric nitrogen (N_2). Since the 1970s, it has consistently shown a very promising PGPR, based on physiological, molecular, agricultural and environmental studies conducted in this bacterium [11]. In recent years, studies on *Azospirillum*–plant interactions have introduced a wide range of mechanisms to demonstrate the beneficial impacts of this bacterium on plant growth [12]; alternative strategies have been developed to replace chemical fertilizers through the use of beneficial microorganisms that maintain soil balance and support plant growth through various mechanisms, including phosphate solubilization and nitrogen fixation [13]. The use of plant growth-promoting bacteria (PGPR) for the formulation of biofertilizers has become one of the most promising clean technologies for the development of sustainable agriculture [14]. Among these bacteria, the ones that stand out the most are *Azospirillum*, which can fix nitrogen, produce cytokinins, gibberellins and indols and reduce nitrates, which allows it to be used as a biofertilizer without generating consequences for the environment [11,15]. Through direct and indirect mechanisms of action, these bacteria can significantly reduce the use of chemical fertilizers [16].

Nitrogen fixation is the main mechanism by which *Azospirillum* influence plant growth [17]. In recent years, some studies have focused on the nitrogen cycle within cells and the genes involved in this process. The ability of *Azospirillum* strains is often naturally maintained, enhancing their ability to express exceptional nitrogenase enzyme activity [18]. The efficiency for atmospheric nitrogen fixation and denitrification can be regulated through the concentration of oxygen, nitrates and molybdenum. In addition, *Azospirillum* can adapt to low temperatures and low oxygen concentrations, depending on the ability of the bacteria to efficiently use nitrites and nitrates [19], as well as to various temperature, pH and salinity conditions [20].

According to Walters [21], some indigenous varieties of maize grown under traditional agricultural practices with little or no fertilizer have developed strategies to improve plant performance under conditions of low nitrogen content in the soil. There is evidence that *Azospirillum* spp. have high affinity for cereals, particularly corn and sorghum [22] and that they can be inoculated even under nitrogen fertilizer application [23]. In traditional maize cultivars, high crop affinity has been found with native strains of *Azospirillum* [24]. Still, in yellow dent maize, no significant interactions have been found between *A. brasiliense* strains and commercial hybrids [25].

In Peru, some plant growth-promoting bacteria have been identified to induce significant increases in root and shoot dry weight in purple corn (*Z. mays* var. purple amylaceum) [26]. Also, *Azospirillum* sp. native strains have been isolated and applied in lowland rice [27] and in yellow dent corn hybrids [28,29] in a complementary way with nitrogen fertilization. The objective of this study was to determine the influence of inoculation with a native strain of *Azospirillum* sp., in addition to nitrogen fertilization in sweet corn, and to explore a reduction in the use of chemical fertilizers in this crop under the arid conditions of the coast of Peru.

2. Materials and Methods

2.1. Study Area

The work was carried out in aridisol (residual formation soil) during the spring of 2019 in “El Paraíso”, province of Huaura, Lima (coordinates: −11.188352, −77.492155).

The soil presented a sandy texture (90% sand), alkaline reaction (pH 8.1), very low organic matter content (0.36%) and moderate- to- high salinity (6.46 dS/m).

According to the Köppen climate classification, the study area is defined as BWh (dry, desert and hot climate); climatic records show relative humidity between 65 and 91%, a maximum temperature of 19 °C and a minimum of 13 °C, with no rainfall.

2.2. Crop Management

The maize crop had a vegetative period of 120 days from planting; during this time, all variable evaluations were carried out until harvest. Irrigation was carried out by pumping through drip irrigation tapes to guarantee adequate water supply to the plants, due to the aridity and soil salinity conditions. Fertilization was carried out locally with nitrogen, phosphorus and potassium 15 and 45 days after planting. For nitrogen fertilization, the application of the doses of the treatments (Table 1) in the experimental units was taken into account. A total of 30 and 45 days after planting, manual weed control was practiced, and some agrochemicals such as chlorpyrifos and diazinon were applied for pest control. No fungicides were applied to avoid possible interactions with the inoculant; harvesting took place 120 days after sowing.

Table 1. Description of treatments.

Treatment	Code	Description
100% N	Control	Conventional fertilization at 180 kg N ha^{-1}, uninoculated
100% N + A_1	T_1	180 kg N + *Azospirillum* inoculation at sowing
100% N + A_1 + A_2	T_2	180 kg N + *Azospirillum* inoculation at sowing and hilling
50% N + A_1	T_3	90 kg N + *Azospirillum* inoculation at sowing
50% N + A_1 + A_2	T_4	90 kg N + *Azospirillum* inoculation at sowing and hilling

A_1, one inoculation per treatment, A_2 two inoculations per treatment.

2.3. Materials

Characterization of *Azospirillum* sp.

The bacterial strain was isolated from the rhizosphere of maize in fields along the central coast of Peru and identified as *Azospirillum* sp. by the Biotechnology Production Laboratory (National University Jose Faustino Sanchez Carrion, Huacho, Peru). The strain was characterized by morphology on Petri dishes with nutrient agar, exhibiting a mucoid structure, transparent color and circular colony shape. Gram staining revealed it to be a Gram-negative bacterium. Biochemical profiling included a growth assay (Bashan et al., 2011) on nitrogen-free semi-solid malate (NFb), positive catalase reaction (+), positive urease reaction (+), motility test and positive oxidase assay (+). To formulate the *Azospirillum* sp. Treatments, biomass was increased in nutrient broth, and colony-forming units (CFU) were quantified, resulting in a final concentration of 1×10^8 CFU/mL.

2.4. Plant Material

The sweet corn variety "Pardo" was purchased from a local seed supplier. The "Pardo" variety is a starchy white maize biotype with a sweet endosperm adapted to the Peruvian coast [30].

2.5. Dosage and Frequency of Inoculant Application

A first dose of 30 mL of inoculum of *Azospirillum* sp. (1×10^8 CFU) as a seed treatment was applied. This dose was applied according to the following combinations of nitrogen fertilization and inoculants: T_0 control (without inoculation), 180 kg N ha^{-1}; T_1, one application of *Azospirillum* in the seed + 180 kg N ha^{-1}; T_2, two applications of *Azospirillum* (in the seed and at hilling) + 180 kg N ha^{-1}; T_3, one application of *Azospirillum* in the seed + 90 kg N ha^{-1}; T_4, two applications of *Azospirillum* (in the seed and at hilling) + 90 kg N ha^{-1} (Table 1).

Nitrogen fertilization treatments were formulated according to Table 1, using a urea source (46% N), and were applied 15 and 45 days after planting, according to each level considered. The control treatment (without inoculation) was applied at the full dose of 180 kg of nitrogen per hectare, dividing fertilizer application 15 and 45 days after sowing. Treatments T_2 and T_4 received the second dose of the inoculum sprayed on the foliage at the time of hilling (45 days after sowing). The plots without bacterial inoculants (T_0) were only sprayed with running water on the foliage of the plant. All experimental units (inoculated or non-inoculated) received a base application with 100 units of phosphorus and 120 units of potassium per hectare as standard to complete the fertilization formula.

2.6. Evaluated Variables

The variables related to maize production that were recorded for evaluation were yield per hectare (kg), grain weight per plot (kg), weight of 100 seeds (g), ear length (cm), number of ears per plant and ear diameter (cm). Likewise, days to germination, plant survival to harvest (%), days to harvest and the number of male flowers per plot were also considered. For this, random samples of 10 plants were taken for each variable, except for total yield per ha, grain weight per plot, days to germination and plant survival, for which the two central rows of each experimental unit were evaluated.

The yield per hectare was projected as follows:

Yield kg ha^{-1} = (Weight of ears $plot^{-1}$) × (sample area of the two central rows of the experimental plot) × plant density adjustment (% plants surviving to harvest).

Additionally, a benefit/cost ratio (BCR) projection was made for each combination of treatments to compare them with the control without inoculation. For this purpose, information was taken from the production cost structure of MIDAGRI [2] and a projection of income per hectare (gross value of production (GVP) in USD per ha) according to the total yield results of the treatments under study (kg per ha). The benefit/cost ratio was then projected for each fertilization inoculation alternative: $BCR = GVP - PCost/PCost$.

2.7. Data Analysis

A Randomized Complete Block Design with 4 repetitions was used. The size of the experimental unit was 160 maize plants for each treatment; the experimental units were randomly assigned for each inoculation treatment and had a dimension of 4 rows 4 m long; the distance between rows was 0.90 m, and between plants, it was 0.15 m.

For the hypothesis tests, the level of statistical significance of α = 5% was used. Analysis of variance (ANOVA) was performed for the agronomic characteristics evaluated. Previously, they were subjected to statistical tests to verify the normality of the data; then, we compared the means of treatments by the Scott–Knott test. The data were processed by using the R Studio program.

3. Results

Statistical significance was observed for total yield per hectare, diameter and length of the ear. Still, no differences were found for the characteristics of grain weight per plant, the weight of the ear and the number of ears per plot. Likewise, statistical differences were found in plant survival, days to harvest and weight of 1000 seeds. No significance was observed for days to emergence and the number of flowers per plot.

Regarding the total yield per hectare, the treatments with one or two inoculations and with 50 to 100% of the nitrogen level had mean values higher than the control without inoculation. On the other hand, for the ear diameter, the treatments with one or two doses of Azospirillum statistically outperformed the control without inoculation, while for the ear length, the treatments with one or two inoculations outperformed the control; there were no significant differences in grain weight and the number of ears per plant due to the effect of the inoculation treatments compared with the control (Table 2).

Table 2. Effects of inoculation + fertilization treatments on yield parameters.

Treatment	Yield kg ha^{-1}	Grain Weight, g	Ear Diameter, cm	Ear Length, cm	Ear Weight, kg	Ear Number
50% N + A_1	3633.26 ^{a}	601.25 ^{a}	5.06 ^{b}	21.73 ^{b}	0.28 ^{b}	0.92 ^{a}
50% N + A_1 + A_2	3269.33 ^{b}	586.25 ^{a}	5.12 ^{b}	20.82 ^{b}	0.27 ^{b}	0.96 ^{a}
Control (100% N)	2968.32 ^{b}	577.50 ^{a}	5.32 ^{a}	21.30 ^{b}	0.35 ^{a}	1.04 ^{a}
100% N + A_1	3709.28 ^{a}	561.25 ^{a}	5.39 ^{a}	23.82 ^{a}	0.36 ^{a}	0.95 ^{a}
100% N + A_1 + A_2	3434.23 ^{a}	496.25 ^{a}	5.37 ^{a}	22.65 ^{a}	0.34 ^{a}	0.88 ^{a}
SE	130.93	28.38	0.08	0.55	0.03	0.05

Means with equal letters do not show statistical differences ($p > 0.05$). SE, standard deviation.

The effects of inoculation treatments were significant in the case of plant survival at harvest, where it was observed that the inoculation with one or two applications of *Azospirillum* was superior to the control even with a 50% nitrogen contribution to the soil (Table 3). It was also possible to observe that days to harvest were statistically fewer (33.5 days) with the treatment of two inoculations and 50% of the applied nitrogen, for the control and the rest of the treatments. Likewise, a slight increase in the seed weight was found with the inoculation of *Azospirillum* plus 100% of the nitrogen applied to the soil, compared with the non-inoculated control. There were no statistical differences in days to germination and the number of flowers per experimental unit due to the effect of any of the treatments evaluated.

Table 3. Effects of inoculation + fertilization treatments on agronomic traits.

Treatments	Days to Emergence	Plant Survival %	Days to Harvest	Flower Number	1000 Seed Weight, kg
50% N + A_1	6.91 ^{a}	78 ^{a}	43.00 ^{a}	34.25 ^{a}	0.93 ^{b}
50% N + A_1 + A_2	6.65 ^{a}	70 ^{b}	33.50 ^{b}	25.75 ^{a}	0.94 ^{a}
Control (100% N)	7.50 ^{a}	64 ^{b}	47.75 ^{a}	33.50 ^{a}	0.93 ^{b}
100% N + A_1	6.03 ^{a}	79 ^{a}	48.50 ^{a}	30.75 ^{a}	0.94 ^{a}
100% N + A_1 + A_2	7.04 ^{a}	74 ^{a}	45.50 ^{a}	35.25 ^{a}	0.93 ^{b}
SE	0.31	0.03	3.08	3.07	0.01

A_1: first inoculation at sowing; A_2: second inoculation at hilling. SE, standard error. Means with equal letters do not show statistical differences ($p > 0.05$).

Table 4 shows that the effect of inoculation with *Azospirillum* sp. impacted the benefit/cost ratio (BCR) in the production of sweet corn "Pardo", increasing this ratio from 0.32 to 0.49 when nitrogen levels were reduced to 50% and from 0.23 to 0.35 when applying the full dose of nitrogen fertilizer (180 kg N ha^{-1}); in both cases, the effect of inoculation with *Azospirillum* sp. improved the BCR compared with the non-inoculated control.

Table 4. Total yields and benefit-/cost ratios (BCRs) per hectare (USD$).

Code	Treatment	Yield [1]	GVP [2]	PCost [3]	Benefit/ha [4]	BCR [5]
T_3	50% N + A_1	3633.26	1963.92	1321.08	642.84	0.49
T_4	50% N + A_1 + A_2	3269.33	1767.21	1334.59	432.61	0.32
T_0	Control	2968.32	1604.50	1469.73	134.77	0.09
T_1	100% N + A_1	3709.28	2005.02	1483.24	521.77	0.35
T_2	100% N + A_1 + A_2	3434.23	1856.34	1510.27	346.07	0.23

[1] Total yield, kg per ha, projected according to experimental results, [2] Gross value of production, USD$ per ha, [3] Production Costcost, USD$ per ha, [4] Benefit/ha: GVP − PCost (USD$). [5] Benefit-/Cost Ratio.

4. Discussion

In the present investigation, it was found that the total yield in the sweet corn variety "Pardo" was lower with the control dose (180 kg N ha^{-1}) without inoculation, compared with the treatments with 90–180 kg N ha^{-1} inoculated with the strain of *Azospirillum* sp. This result is consistent with the findings of various authors [31,32] (Galindo et al., 2020), which indicate an improvement in corn grain yield under the effect of inoculation with *Azospirillum* sp. The evidence shown by Ferrerira [33], who found that corn hybrids showed greater expressiveness in yield components in the presence of *A. brasilense* applied in seed treatment, is consistent with this experiment carried out on sweet corn, where the seed was also inoculated. Likewise, Alvarado [32] maintain that the joint use of synthetic fertilizers with microbial inoculants increases grain yield in various varieties of hybrid maize, which shows evidence similar to that obtained in this investigation.

Regarding the other characteristics that are considered yield components and were evaluated in the experiment, such as ear diameter, ear length and 1000 seed weight, these were significantly affected by inoculation with *Azospirillum* sp., although other variables, such as the number of ears, were not affected by inoculation. The significance of these findings suggests that the response in total yield in this variety of sweet corn can be balanced with nitrogen fertilization and inoculation with *Azospirillum* sp.; this aspect was studied by Wagner [34], who found that interactions with soil microorganisms could be important for the expression of yield heterosis in corn.

Inoculation treatments with *Azospirillum* sp. were significant in the case of plant survival at harvest; concerning this finding, Bashan and Levanony [35] indicated that the most important effects of inoculation with this microorganism refer to morphological changes in the root system of the plant, which may be in agreement with the results of the present investigation; it was also found by Nunes [36] that inoculation with *Azospirillum brasilense* led to an increment in root dry weight and nitrate reductase activity.

Mechanisms by which bacteria can promote plant growth include the mobilization of nutrients and the production of phytohormones [37]. In the hypothesis put forward in the existing research, *Azospirillum* may contribute to atmospheric nitrogen fixation in maize [33] and thus to the acquisition of this key element for plant growth [17]. It was also proposed [37] that free-living rhizobacteria such as *Azospirillum* have a clearer plant growth-promoting effect in soils with aridity or salinity problems, similar to the environmental conditions encountered in this research study.

Azospirillum spp. and other bacterial strains that can fix atmospheric nitrogen, solubilize phosphorus or produce growth regulators [31,38] can be used as biofertilizers in a complementary way to the application of chemical fertilizers [17], a fact that is corroborated by the present investigation under the conditions of the Peruvian coast, in which alluvial soils devoid of organic matter and scarce in water predominate [6].

The intensive use of fertilizers and phytosanitary agents can increase the toxic agents in rivers and soils, and it has been observed that modern intensive agriculture can strongly impact traditional agriculture in desert areas [39]; the environmental impacts are influenced by the high energy intensity linked to the production of inorganic fertilizers used and by phytosanitary agents [40]. In this sense, this research study is a contribution to evaluating different nitrogen fertilization alternatives in maize that include the use of soil microbiota such as *Azospirillum* sp., since maize is a product in high demand in agribusiness [29,41]. This aspect is especially important in the current worldwide situation of increased fertilizer prices [42]. It is also worth mentioning that Peru and several Latin American countries are importers of fertilizers, due to the absence or deficit in the national production of these inputs; there is an indirect trade effect of international conflicts that can be expected to be more relevant for the economies of Latin America and the Caribbean [43].

In conclusion, a significant effect was found for inoculation with *Azospirillum* sp. in some productive characteristics of sweet corn cv. "Pardo", outperforming the control (with only nitrogen fertilization) in total yield. The total yield with half the dose of nitrogen applied to the soil (90 kg N/ha) plus an inoculation dose at sowing in *Azospirillum* sp. was superior to the control treatment without inoculation and with the full dose of nitrogen fertilization (180 kg N/ha). The inoculation with *Azospirillum* sp. improved the survival rate of maize plants under conditions of arid soil devoid of organic matter and with salinity problems. It could be possible to partially replace nitrogenous fertilizer in the cultivation of sweet corn with *Azospirillum* inoculation, since the BCR increases substantially with the use of this bacterium in Peruvian arid soils.

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