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Inoculation effect of *Azospirillum* sp. and two levels of nitrogen on the performance of the hybrid corn 'Insignia 800'

Efecto de la inoculación de *Azospirillum* sp. y de dos niveles de nitrógeno sobre el rendimiento de maíz híbrido 'Insignia 800'

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Abstract

The objective of this investigation was to evaluate the effect of inoculating *Azospirillum* sp. and two levels of nitrogen on the productive characteristics of hard yellow corn variety 'Insignia 800' under the conditions of Nuevo Imperial, Cañete (central coast of Peru). A randomized complete block design with five treatments and four replications was used in a field experiment using the commercial corn hybrid 'Insignia 800'. The treatments were two levels of nitrogen (90 and 180 kg N ha^{-1}), plus one or two applications to the foliage of *Azospirillum* sp. (1.08 x 10^7 CFU per plant), and a control treatment (without inoculation). Ten agronomic variables related to productive traits were evaluated. Treatment comparison was executed with the Scott Knott test at the 5% significance level using the Infostat program. Inoculation with *Azospirillum* sp. did not significantly affect the grain yield per hectare, grain weight per plot, shelling percentage, grain depth, number of ears per plant and diameter of the cob, but it influenced significantly some productive traits such as cob weight per plot, grain weight per plant, cob diameter and ear length, as well as a high benefit-cost ratio due to inoculation. Inoculating the plant foliage with *Azospirillum* sp. had a significant effect and greater economic efficiency for some productive characteristics of the hard yellow corn cv. ´Insignia 800´, and did not significantly affect grain yield.

Keywords: *Yellow dent corn, Azospirillum, agronomic characters, nitrogen dose*

Resumen

El objetivo de esta investigación fue evaluar el efecto de inocular *Azospirillum* sp. y dos niveles de nitrógeno sobre las características productivas del maíz amarillo duro variedad 'Insignia 800' en las condiciones de Nuevo Imperial, Cañete (costa central del Perú). Se utilizó un diseño de bloques completos al azar con cinco tratamientos y cuatro repeticiones en un experimento de campo utilizando el híbrido de maíz comercial 'Insignia 800'. Los tratamientos fueron dos niveles de nitrógeno (90 y 180 kg N ha^{-1}), más una o dos aplicaciones al follaje de *Azospirillum* sp. (1.08 x 10^7 UFC por planta), y un tratamiento control (sin inoculación). Se evaluaron diez variables agronómicas relacionadas con rasgos productivos. La comparación de tratamientos se realizó con la prueba de Scott Knott al nivel de significancia del 5% utilizando el programa Infostat. La inoculación con *Azospirillum* sp. no afectó significativamente el rendimiento de grano por hectárea, peso de grano por parcela, porcentaje de desgrane, profundidad del grano, número de mazorcas por planta y diámetro de la mazorca, pero influyó significativamente en algunos rasgos productivos como el peso de mazorca por parcela, peso de grano por planta, diámetro de la mazorca y longitud de la mazorca, así como también se obtuvo una alta relación beneficio-costo por la inoculación. La inoculación al follaje de la planta con *Azospirillum* sp. tuvo efectos significativos en algunas características productivas del maíz amarillo duro cv. ´Insignia 800´ y una mayor eficiencia económica, pero no afectó significativamente el rendimiento de grano.

Palabras Clave: *Maíz amarillo duro, Azospirillum, caracteres agronómicos, dosis de nitrógeno*

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Introduction

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. Among these bacteria, the ones that stand out the most, are those of the *Azospirillum* genus. These bacteria have the capacity to fix nitrogen, solubilize phosphorus, produce cytokinins, gibberellins and auxins, and reduce nitrates, making them useful as bio fertilizers that improve agricultural product quality without generating consequences to the environment (Bashan et al., 2013; Fibach-Paldi et al., 2012).

Maize (*Zea mays*) is one of the most important agricultural products in Peru, with the area under cultivation exceeding half a million hectares nationwide, of which 265,000 are planted with the hard yellow corn (Ministerio de Agricultura y Riego [MINAGRI], 2017) and this is a product in high demand in the agricultural and industrial market, mostly on the coast of Peru. Hard yellow corn is sustained, thanks to the high demand of the poultry industry that require twice the volume produced in the country (Eguren, 2003).

According to Walters et al. (2018), some corn landraces grown under traditional agricultural practices with little or no fertilizer could have developed strategies to improve yield in conditions of low nitrogen content in the soil, and in these landraces, from 29 % -82% of assimilated nitrogen was derived from atmospheric nitrogen (N_2).

Rangel-Lucio et al. (2011), found some degree of affinity or effect of a homologous strain, of *Azospirillum* sp., obtained from the traditional varieties H-28 and Chalqueño maize, re-inoculated in these same varieties and the subsequent recognition of the strain in modern varieties. Likewise, Rangel-Lucio et al., (2014) found that the bio fertilization of *Azospirillum* sp. has a potential benefit in the production of sorghum, and in particular, the strains of *A. brasilense* VS-7 and VS-9, presenting an effect on yield of 55% and 49% higher than the control mean fertilized with nitrogen; their results also demonstrated the existing affinity between strain and plant genotype. Sangoquiza et al. (2018) investigated the biological response of *Azospirillum* sp. against different types of stress, for which they performed the characterization of the isolates as well as their biological response to stress conditions due to temperature, salinity, pH. Their results demonstrated that lyophilized *Azospirillum* isolates grow best at temperatures of 28° C to 38° C and at a pH of 7 to 8.

Piscoya & Ugaz (2016) demonstrated that an application of diazotrophic bacteria (*Azotobacter* sp., 22%, *Azospirillum* sp., 49% and *Enterobacter* sp., 16%) and 50% of chemical fertilizer (240 kg of nitrogen per hectare in the form of urea) increases plant height, number of leaves and stem diameter, as well as grain yield in hard yellow corn. According to the research by Zambonin et al. (2019), no interaction between hybrids and inoculation was found for any phenotypic variable studied, and the specificity between hybrids and inoculation was not verified. Likewise, they found that inoculation with *A. brasilense* did not interfere with grain yield and corn yield components. Alvarado et al. (2018), found that the combined use of synthetic fertilizer and inoculants, maintains and increases grain size and maintains yield similar to that obtained with just synthetic fertilization in 18 varieties of corn. According to Munaretto et al. (2019), the foliar application of *A. brasilense*, whether alone or combined with seed treatment, increased grain yield and yield components of wheat cultivars. Martínez et al. (2018) concluded that the use of the biofertilizer *Azospirillum brasilense* represented an increase in grain yield in corn of 28%.

The objective of the work was to evaluate the effect of *Azospirillum* sp. and two doses of nitrogen on yield components of hard yellow corn ´Insignia 800´ under the conditions of Nuevo Imperial, Cañete (central coast of Peru).

Materials and Methods

The research was carried out in Nuevo Imperial, Cañete (central coast of Peru), located at the geographical coordinates of 13°4′34.39 ″ S, 76°19′4.04 ″ W, altitude 205 masl. The research was carried out from July to December. The average environmental temperature fluctuated between 17 and 23° C.

A soil characterization analysis was carried out and the results showed low in salts, very low organic matter, medium available phosphorus, low available potassium and sandy loam texture, according to standards (Prialé, 2016; Sono, 2018) (Table 1).

Table 1. Soil characterization analysis at Nuevo Imperial, Cañete

Chemical characterization						Physical characterization			Textural Class
pH (1:1)	*E.C.(1:1)*[1] dS/m	*CaCO3* %	*O.M.*[2] %	*P* ppm	*K* ppm	*Sand* %	*Lime* %	*Clay* %	*Texture*
7,80	0,28	2,90	0,83	9,1	86	75	15	10	Fr.A.

[1] CE= Electric conductivity
[2] O.M. = soil organic matter content, %

According to the soil analysis, a general fertilizer formula of 180-100-150 kg ha^{-1} of N-P_2O_5-K_2O was determining, which was applied according to the treatments proposed in the experiment, considering the levels of phosphorus and potassium as fixed factors. Nitrogen levels were dosed according to Table 2, in each of the experimental units.

Table 2. Nitrogen levels and inoculation treatments in the experiment

Code	Treatments (kg N ha^{-1})[1]
Control	180 N (no inoculation)
T_1	180 N + A_1
T_2	180 N + A_1+ A_2
T_3	90 N + A_1
T_4	90 N + A_1 +A_2

[1]A_1, the first application of *Azospirillum* sp. to the foliage of the plants 15 days after sowing, A_2, the second application of *Azospirillum* sp. to the foliage of the plants 45 days after planting

The combinations of nitrogen (N) doses were in the form of urea (46% N) and one or two of foliar applications of *Azospirillum* sp. The treatments were: 180 N without inoculating (control), 180 N + A_1 (T_1), 180 N + A_1 + A_2 (T_2), 90 N + A_1 (T_3) and 90 N + A_1 + A_2 (T_4) (Table 2). The nitrogen doses were applied in two equal parts, 50% at the first hilling two weeks after sowing, together with all the phosphorus and potassium doses, which were constant for all treatments in amounts of 100 and 150 kg ha^{-1} of P_2O_5 and K_2O respectively, in the form of triple calcium superphosphate (46% P_2O_5) and potassium sulfate (50% K_2O). The remaining 50% nitrogen was incorporated into the second hilling, about seven weeks after sowing.

´Insignia 800´ is a simple hybrid of corn, tolerant to black spot (*Phyllachora maydis*), according to information from the supplying company (Interoc SA) and was used in the field experiment, at a planting density of 65 000 plants per hectare (0.90 m x 0.40 m). Furrow irrigation was supplied to field experiments with a weekly frequency, from emergence to harvest maturity.

The bacterial strain was isolated from the rhizosphere in maize fields of the central coast of Peru and was identified as *Azospirillum* sp. in the Laboratorio de Biotecnología de la Producción -Universidad Nacional José Faustino Sánchez Carrión (Huacho), by morphological characterization and biochemical profile (catalase test, urease reaction, motility test and oxidase test).

Catalase test: it was carried out on a slide adding 2 drops of 30% hydrogen peroxide and it was put in contact with the biomass of the bacterium, observing the presence of bubbles indicating the release of oxygen.

Urease test: the microorganism was cultured in Christensen's Urea Agar, incubating at 37ºC for 24 hours, and the colour variation of the culture medium from yellow to red was observed, due to the variation of pH by the phenol red indicator.

Motility test: the semi-solid mobility mannitol medium was used in a tube in a straight position and it was seeded by sting, incubating at 37 ºC for 24 hours, producing cloudiness and elongation in the line of the sting.

Oxidase test: It was carried out with a filter paper and 3 drops of Kovacs's reagent were added to the centre of the paper, spreading the seeding loop with bacterial biomass, resulting in a positive reaction with a colour change to purple-black.

The increment of the *Azospirillum* strain was carried out in nutrient broth culture medium and diluted at a concentration of 1.08 x 10^7 colony-forming unit (CFU) per plant. Then foliage and surrounding soil were sprayed after the emergence of the plants (15 days after planting) and hilling (45 days after planting), according to the indicated treatments. Herbicides were not applied to avoid interactions in the soil with the inoculating agent.

The variables related to maize production that were submitted to evaluation were: yield per hectare (kg), grain weight per plot (kg), shelling percentage (%), grain depth (cm), ear weight per plot (kg), grain weight per plant (kg), ear diameter (cm), ear length (cm), number of ears per plant and cob diameter (cm). Random samples of 10 plants were evaluated for each variable, except for grain yield, in which two central rows of each experimental unit were harvested.

A benefit-cost (B/C) analysis was performed on the treatments under evaluation. For this, the information based on Escalante (2018) was obtained to calculate production costs, the gross value of production (GVP) and profitability rate (B/C) for each treatment, in order to compare the level of economic efficiency.

The design used was randomized complete blocks with four replications per treatment; the experimental units were randomized for each inoculation treatment and had a dimension of 6 rows of 4 meters in length; for the evaluations, only the two central rows were taken into account.

The data were processed in the Infostat program (2017 version). The analysis of variance (ANOVA) was done to test for the effects of the variables. Also, the Scott-Knott test (Bhering et al., 2008) was executed because of its unambiguous mean grouping to compare the treatment efficacy, at a significance level of 5%.

Results

It is shown that the inoculation treatments did not significantly affect the yield per hectare, grain weight per plot, shelling percentage, grain depth, number of ears per plant and ear diameter (Table 3); the yield of inoculated plots, were not statistically different from the control plots, but it can be noted that the inoculation treatments with

Table 3. Grain yield components and *Azospirillum* sp. inoculation in hybrid corn 'Insignia 800'

Treatment	Grain yield	Grain weight per plant	Grain depth	Shelling percentage	Ear number per plant	Ear diameter	Cob weight per plot	Cob Diameter	Ear length
	kg ha-1	*kg1*	*cm.1*	*%*	*N plant1*	*cm.1*	*kg plot-1*	*cm.1*	*cm.1*
Control	6 250,50 a	1,08 b	1,77 a	82 a	0,86 a	4,08 a	0,22 b	2,30 b	14,53 b
T1	6 756,50 a	1,08 b	1,79 a	83 a	0,88 a	4,16 a	0,22 b	2,31 b	14,59 b
T2	7 786,75 a	1,28 a	1,92 a	82 a	1,00 a	4,32 a	0,27 a	2,47 a	15,74 a
T3	7 301,50 a	1,09 b	1,85 a	82 a	0,89 a	4,22 a	0,23 b	2,37 b	14,86 b
T4	7 351,75 a	1,10 b	1,91 a	83 a	0,94 a	4,29 a	0,24 b	2,38 b	14,76 b
%CV	21,55	7,68	6,52	0.98	17,82	3,16	7,62	2,89	4,11

[1]Means with the same letter do not differ significantly *P*>0,05; % CV is the coefficient of variability expressed as a percentage. The yield was projected as a density of 65 000 plants per ha

Table 4. Grain yield and benefit-cost ratio per hectare (soles)

Code	Treatment	Yield [1]	GVP [2]	Cost [3]	B/C[4]
T2	180 N + A_1+ A_2	7 788	7 632,24	6 100	0,2512
T4	90 N + A_1+ A_2	7 352	7 204,96	5 785	0,2455
T3	90 N + A_1	7 302	7 155,96	5 735	0,2478
T1	180 N + A_1	6 757	6 621,86	6 050	0,0945
Control	180 N (no inoculated)	6 251	6 125,98	6 000	0,0210

[1] Grain yield, kg per ha.,
[2] Gross value of production, soles per ha^{-1},
[3] Production cost, soles per ha^{-1},
[4] Benefit/Cost ratio

Azospirillum sp. influenced some product characteristics such as ear weight, grain weight per plant, cob diameter and ear length (Table 3).

In the variables in which statistical significance was found, the best inoculation treatment found was T_2 (180 N ha^{-1} + two applications of *Azospirillum* sp., about 15 and 45 days after sowing), affecting weight and number of ears per plot, as well as cob diameter and ear length, both characters correlated to grain yield (Hallauer et al., 2010); however, in the present experiment the inoculation with *Azospirillum* sp., did not affect these grain yield components significantly (Table 3).

The number of ears per plant and ear diameter (table 3) were characters that were not significant due to the effect of inoculation treatments in relation to the control, in which a commercial dose of nitrogen (180 kg N per hectare) was applied.

Likewise, respect to cob weight and cob diameter (Table 3), with the application of two doses of inoculation with *Azospirillum* sp. in addition to the full dose of nitrogen (T_2), both characters improved significantly; there are not many studies related to these variables and their relationship to grain yield per hectare It can be distinguished that ear length (Table 3) was significantly greater with the application of the T_2 treatment, this trait is considered to be correlated to grain yield in hard yellow corn (Hallauer et al., 2010; Chura & Tejada, 2014).

It is also important that in the characters affected by *Azospirillum* inoculation, the number of applications seems to influence significantly, this could be observed in the treatments with two applications (A_2), differing from the inoculation with a single application (A_1) at 15 days after sowing (Table 3).

In table 4 shows the economic analysis of the treatments evaluated in the research and it is noted that in the treatments with the complete dosage of nitrogen (180 N) or with half the dose of nitrogen (90 N), and received one or two doses of *Azospirillum* sp., the highest economic profit rate per hectare was obtained with the highest benefit-cost rate (B/C). The control treatment (180 N, without inoculating) showed a low B/C rate.

Discussion

The results obtained by some authors show that when inoculants are used, there is an increase in corn yield due to the application of beneficial microorganisms (Martínez et al., 2018; Alvarado et al., 2018), which coincides in part with the findings in this study, although not specifically for grain yield. As proposed by Zambonin et al. (2019), in the case of inoculation with *A. brasilense*, the inoculation treatment did not interfere with grain yield and corn yield components; in the present investigation a growth-promoting effect was shown by the inoculation of *Azospirillum* sp. in the hard yellow corn hybrid but without significantly affecting the grain yield under field conditions in Cañete. These results are also similar to those found by Rockenbach et al. (2017), regarding ear diameter but they differ in relation to grain yield since this author found no significant differences in hybrid Pioneer 30K73 for inoculating *A. brasiliense*.

The present results coincide with the references of various authors, regarding the use of *Azospirillum* spp. and nitrogen fertilization in corn. The inoculation with *A. brasilense* increased Nitrogen Use Efficiency (NUE), maize grain yield and agronomic characteristic in the AS1572 hybrid (Skonieski et al., 2019) but there was no response in another hybrid; the use of this diazotrophic bacterium is viable even when high rates of nitrogen in the form of urea applied to the soil (Galindo et al., 2016). However, Schaefer et al. (2019) consider that *Azospirillum brasilense* helps plant growth and yield but does not replace the effect of N fertilization. Interestingly, we found no differences in grain yield for control (180 kg N, no inoculation) or the half N dose per hectare (90 kg N plus *Azospirillum* inoculation) in hybrid corn cv. ´Insignia 800´.

Some characters are yield components in maize hybrids (Chura & Tejada, 2014), so there is consistency with the findings of the present experiment and are similar to Piscoya & Ugaz (2016) and Alvarado et al. (2018), regarding the promotion of growth by *Azospirillum* sp. inoculation for traits that are components of maize yield.

The varietal factor could be a cause to which this difference or lack of effects of the inoculation on the yield is attributed, as suggested by Rangel et al. (2014). This is an aspect that has not been developed in the present investigation where a simple hybrid (a single genotype) of hard yellow corn was used.

Regarding the number of applications of *Azospirillum*, we found that two applications are the best treatment for maize inoculation; for De Souza et al. (2019), the application of 30 kg ha^{-1} of N at sowing and a single application of 150 kg ha^{-1}, or two applications of 75 kg ha^{-1} in topdressing, inoculated with *Azospirillum brasilense* provided better nutrition and development, with a positive effect for the mass of 100 grains which reflects an increase in grain yield, similarly as we found in the research. Also, the application dose of *Azospirillum* was important as found by Numoto et al. (2019) who suggested that the dose of inoculant that provided the best agronomic result was 100 mL ha^{-1} in conjunction with the application of N at sowing or top dressing and as we found, in relation to *Azospirillum* application, that best performed when it considered two inoculations to maize foliage, for some characteristics such as ear weight, grain weight and ear length per plant.

Conclusions

A significant effect of *Azospirillum* sp. was found in some characteristics in hybrid corn cv. ´Insignia 800´, but no significant effect was detected for grain yield. The recommended number of applications in this cultivar was two foliar applications at 15 and 45 days after planting plus a commercial dose of nitrogen in order to obtain a greater economic efficiency at Nuevo Imperial, Cañete.

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