

Grain Yield of Maize Hybrids in Response to Inoculation with *Azospirillum* sp. under Nitrogen Limiting Conditions in Huaura, Peru

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Abstract

This experiment was conducted to evaluate the effect of *Azospirillum* sp. inoculated with different nitrogen levels (0, 60, 90, 180 kg ha⁻¹) on grain yield, yield components and agronomic traits of hybrid maize at the arid conditions of the central coast of Peru. The experimental design used was randomized complete block design with four replications per treatment, under the arrangement of subdivided plots, in which three corn hybrids were assigned to complete plots and four inoculant-N fertilization treatments, to the subplots. The results showed that for dose fertilization of 180 kg ha⁻¹ of nitrogen (control), the grain yield of hybrid corn was similar as compared to 60-120 kg ha⁻¹ N fertilization inoculated at 15 and 45 days with a native strain of *Azospirillum* sp., suggesting that the response in grain yield for hybrid corn can be balanced with nitrogen fertilization and inoculation with *Azospirillum* sp. 1x10⁸ CFU mL⁻¹ in conditions of arid soils, with possible impact on the use of this microorganism in the maize production system

Keywords: nitrogen fertilization, *Azospirillum*, grain yield, maize

1. Introduction

The coast of Peru is a low-elevation plain adjacent to the Pacific Ocean and influenced by the cold Humboldt Current. It is a hyper-arid biome mostly devoid of vegetation, with the exception of the fog-dependent plant communities ('Lomas') and groundwater (Polk et al. 2020). In Peru, the area of corn under cultivation exceeds half a million hectares, of which 265,000 are planted with hard yellow corn (Ministerio de Agricultura y Riego, 2017), being a product of high demand in the agricultural and industrial market, mainly in the poultry and pig industry. The average grain yield in corn is 10 t ha⁻¹ in the department of Lima (Sistema Integrado de Estadística Agraria, 2020); however, production does not manage to supply national demand (Cabrera-Chavez, 2020). Corn remains in Peru because the agribusiness demands twice the volume produced in the country, through the operation of an agri-food chain (Eguren, 2003).

Maize is one of the world's most important agricultural products (Ngoone & Mutengwa, 2020), is generally planted in monoculture and can be held responsible for shaping the agricultural environment (Peiffer et al., 2013). Nitrogen recommendations for corn planting are less precise than desired and must be improved for economic and environmental reasons (Morris et al., 2018). With the exception of water resources, nitrogen is the most important limiting factor in the productivity of this crop (Franca et al., 2011). Globally, fertilizers support approximately 40% of the current world population and consume about 2% of the world's available energy supply (Reis et al., 2020).

Biofertilizers are defined as formulations of live microbial strains that are applied to seeds, plants or soil to colonize the rhizosphere, improving the supply and availability of nutrients and that can totally or partially replace fertilizers (Schmidt and Gaudin, 2018). Among plant-associated microorganisms, the mutualistic symbionts of fungi and bacteria have evolved successfully with their hosts since plants adapted to terrestrial ecosystems; they promote plant growth by facilitating the acquisition of scarce nutrients (Martin et al., 2017). The best-known example of a symbiotic association that can reduce the use of nitrogen fertilizers is that of bacteria and legumes that fix N₂. Bacterial taxa that are not rhizobia species can also develop associative symbiotic interactions with plants and fix atmospheric nitrogen. These include bacteria of the genera

Azospirillum, Azotobacter, and Bacillus, some of which are marketed as bio-inoculants (Dellagi et al., 2020).

The use of plant growth-promoting bacteria for the formulation of biofertilizers has become one of the most promising clean technologies for the development of sustainable agriculture (Gouda et al., 2017). Inoculants containing plant growth-promoting bacteria represent a new strategy to increase the efficiency of nitrogen use (NUE), providing better plant nutrition and grain yield in corn (Galindo et al. 2020). The genus *Azospirillum* has the ability to fix atmospheric nitrogen, solubilize phosphorus, produce cytokinins, gibberellins and auxins, which allows it to be used as a biofertilizer to obtain agricultural products, without generating consequences in the environment (Perez-Montano et al., 2013). Nitrogen-fixing microbial associations are efficient and well-characterized in legumes but are limited in cereals, including corn (Van Deynze et al., 2018).

Plant growth-promoting bacteria are considered a green technology that reduces the use of chemical fertilizers, thus improving soil health (Ramakrishna et al., 2019). Since the levels of nitrogen fixation achieved with nitrogen-fixing bacteria in cereals are not high enough to satisfy the needs of the plant and not as effective as those obtained with chemical fertilizers, it is a current research objective to increase nitrogen fixation in cereals, including corn (Rosenblueth et al., 2018). Schmidt & Gaudin (2018) found greater efficacy for biological nitrogen fixation in corn with strains of *Azospirillum* sp. than with *Bacillus* sp. and *Enterobacter* sp., under field conditions.

According to Walters et al. (2018), some local varieties of corn grown in Mexico under traditional agricultural practices with little or no fertilizer could have developed strategies to improve plant performance under conditions of low nitrogen content in the soil. In these local varieties of the 29% to 82% assimilated nitrogen, is derived from the atmospheric form N_2 . Bennett et al. (2020) proposed a model for the association of nitrogen-fixing microbes with the mucilage of the rhizosphere of corn and identified the main functionalities of a productive diazotrophic association.

Piscoya & Ugaz (2016) demonstrated that the application of diazotrophic bacteria and 50% of chemical fertilizer increases the height of the plant, the number of leaves and the diameter of the stem as well as the grain yield in hard yellow corn. According to Zambonin et al. (2019), no interaction was found between the hybrids and inoculation for the variables studied and the specificity between the hybrids and the inoculation was not verified. Likewise, they found that inoculation with *A. brasilense* did not interfere with grain yield and maize yield components. Alvarado et al. (2018), mention that the joint use of synthetic fertilizers with microbial inoculants maintained or increased grain yields compared to those obtained with only synthetic fertilization in 18 varieties of corn.

Munaretto et al. (2019) found that foliar application of *A. brasilense*, either alone or combined with seed treatment, increased grain yield and yield components of wheat cultivars. Reyes et al (2018) concluded that the use of the biofertilizer *A. brasilense* represented a 28% increase in corn grain yield. Silva et al. (2020) observed physiological responses when inoculants of corn root exudates were used, although these effects varied according to the genotype of the plant and the source of the exudates, finding that soluble compounds exudated by corn seedlings can increase the colonization by *A. brasilense*. Inoculation with *A. brasilense* increased Nitrogen Use Efficiency (NUE), maize grain yield and agronomic characteristic in the AS 1572 hybrid (Skonieski et al., 2019) also, there was no response in another hybrid; the use of this diazotrophic bacterium is viable even when high rates of N were applied (Galindo et al., 2016). However, Schaefer et al. (2019) considered that *A. brasilense* helps plant growth and yield but does not replace the effect of N fertilization.

Correa-Galeote et al. (2016) suggested that the genotype of Andean starchy maize could be the main factor in controlling bacterial diversity in its rhizosphere, either in monoculture or in association with other plants. The results of Wagner et al. (2020) show that interactions with soil microorganisms are important for the expression of heterosis in corn. Recently, SCAR-type molecular markers have been developed for the identification of *A. brasilense* (Coniglio et al. 2020).

There is a need for a biofertilizer development framework that integrates information from the soil microbial community and prioritizes validation of results in the field. To elucidate the roles of microbiomes in host performance, selection can be applied via contrasting treatments, in addition to controls (Mueller & Sachs, 2015). The objective of the work was to evaluate the effect of a native strain of *Azospirillum* sp. under nitrogen limiting conditions on the yield of hybrid corn, in the central coast of Peru.

2. Materials and Method

2.1 Description of Experimental Location

The research was carried out in the experimental field of the National University Jose Faustino Sanchez Carrion

located in Huacho (Lima), geographic coordinates 11 ° 07' 26" S, 77 ° 36' 32" W. During the development of the experiment (spring 2018) the climatic conditions in the coast of Peru were average temperature of 20.1 °C, minimum temperature of 13.1 °C, maximum temperature of 22.7 °C and relative humidity of 73-79%. No rainfall is recorded in this agroecosystem, with relatively cold and arid conditions prevailing along the Pacific coast that extends to the western slopes of the Andes (Garreud, 2009).

Two soil samples at 0 and 30 cm depth from the soil surface were collected prior to soil preparation and various physicochemical properties were analyzed during the growing season, as presented in Table 1. The characterization analysis shows that the experimental place presented a soil without salinity problems, moderately alkaline, with very low content of soil organic matter (OM), low in phosphorus (P), medium in potassium (K).

Table 1. Soil characterization analysis in the experimental site

| No. | EC dS/m | Clay % | Lime % | Sand % | pH | CaCO ₃ % | OM % | P ppm | K ppm | CIC | B ppm | NO ₃ | Ca+ | Mg+ | PSI % | Textural Class |
|-----|------------|-----------|-----------|-----------|------|------------------------|---------|----------|----------|------|----------|-----------------|-------|------|----------|-------------------|
| 1 | 1.4 | 82 | 14 | 4,0 | 8.37 | 4.4 | 0.4 | 10.6 | 165 | 6.88 | 1.3 | 0.45 | 6.87 | 2.58 | 5.66 | Sandy loam |
| 2 | 2.15 | 83 | 13 | 4,0 | 8.23 | 4.1 | 0.5 | 7.8 | 177 | 5.92 | 2.1 | 0.68 | 17.95 | 4.33 | 7.77 | Sandy loam |
| | 1.78 | 82 | 14 | 4,0 | 8.3 | 4.25 | 0.45 | 9.2 | 171 | 6.4 | 1.7 | 0.57 | 12.41 | 3.46 | 6.72 | |

2.2 Plant Materials

The bacterial strain used in the research was isolated from the maize rhizosphere in cultivated fields of the central coast of Peru and identified as *Azospirillum* sp. by the Laboratorio de Biotecnología de la Producción (National University Jose Faustino Sanchez Carrion), by morphological characterization and biochemical profile (catalase⁺ test, urease⁺ reaction, motility test and oxidase⁺ test). The biomass of the bacterial strain was increased in a nutrient broth and it was quantified in Colony Forming Units (CFU) obtaining an inoculum concentration of 1x10⁸ CFU mL⁻¹. The inoculation was carried out by spraying the foliage after the emergence of maize 15 days after sowing) and at the time of hilling (45 days after sowing), according to the indicated treatments; herbicides were not applied to avoid interactions in the soil with the inoculating agent.

The inoculant treatments were applied to three commercial cultivars of yellow corn; these were the hybrids 'Agricol XB8010', 'Dekalb 7508' and 'INIA 619', which were randomly distributed for sowing, in each replication. These corn cultivars are popular and often used by farmers under the growing conditions of the central coast of Peru.

2.3 Experimental Design, Layout and Treatments

The experimental design used was that of random complete blocks with four replications per treatment, under the arrangement of subdivided plots, in which the corn hybrids were assigned to complete plots and the inoculant treatments, to the subplots. The experimental units were randomly assigned for each treatment within each variety and had a dimension of 4 rows of 10 meters long. For the evaluations, only the two central rows were taken into account, to avoid the effect of edges.

Nitrogen (N) treatments were formulated in decreasing form for fertilization, being coded according to Table 2. The urea source (46% N) was used, applied 15 and 45 days after sowing, according to each level of N considered. These nitrogen treatments coincided with the applications of the inoculant *Azospirillum* sp. when it corresponded to the indicated combinations (T₁, T₂, T₃). The control treatment (T₀, without inoculation) was applied with the complete dose of 180 units of nitrogen per hectare, also fractionating the fertilizers at 15 and 45 days after sowing. P and K levels were constant for all treatments.

Table 2 Inoculant and N-fertilization treatments used in the field experiment

| Code | Treatments (N-P-K) |
|------------------------|--------------------------------------|
| T ₀ control | 180-100-150 no inoculated |
| T ₁ | 120-100-150 + <i>Azospirillum</i> sp |
| T ₂ | 60-100-150 + <i>Azospirillum</i> sp. |
| T ₃ | 00-100-150 + <i>Azospirillum</i> sp. |

2.4 Cultural Practices

A planting density of 65 000 plants per hectare (0.90 x 0.40 m) was used in the field experiment. Irrigation to the

field was by furrows with a weekly frequency, from emergence to harvest maturity, which occurred 150 days after sowing. The control treatment (without inoculation) was applied with the complete dose of 180 kilos of nitrogen per hectare, fractionating the fertilizer 15 and 45 days after sowing. Herbicides were not applied to avoid interactions in the soil with the inoculating agent.

2.5 Data Collection

The agronomic characters evaluated were: percentage of emergence in the field 10 days after sowing (% E), the number of leaves per plant (NLP), length of leaves (LL), average width of the leaf (LW), stem diameter (SD), fresh weight of foliage at 30 days (FW30), fresh weight of foliage at 60 days (FW60), fresh weight of foliage at 90 days (FW90), dry weight of foliage at 30 days (DW30), foliage dry weight at 60 days (DW60).

The yield components evaluated were: number of ears per sample (NM), average fresh weight of ear (FWE, transformed data), the diameter of the ear (ED), the diameter of the stem (SD), length of the ear (EL), the weight of 100 seeds (WS), average dry weight of cob (DWC), dry weight of grain per plant (DWG, transformed data), percentage of survival to harvest (% S), yield grain per hectare (YG).

To evaluate each variable in the experiment, random samples were taken from each treatment. With the exception of the grain yield per hectare in which the ears were weighed and shelled in the two central furrows of each experimental unit, in the rest of the variables 10 plants were randomly taken for each sample per treatment.

2.6 Statistical Analysis

The data were processed using the Infostat program (2017 version), performing the analysis of variance and the Scott-Knott test to compare the treatment means, at a significance level of 5%.

3. Results

3.1 Grain Yield and its Components

In the analysis of variance, it can be observed that regarding the grain yield per hectare, statistical differences were found for the inoculant treatments, but not for the corn hybrids (Table 3). Differences were found also between inoculant treatments for the following performance components: ear and cob diameter, ear length, the weight of 100 seeds and dry weight of the cob. Regarding the maize hybrids evaluated, the significant effects corresponded to the number of ears, fresh weight of the ear per plant, length of ear, the weight of 100 seeds and dry weight of the crown. In the case of ear length, a significant interaction was also found between corn hybrids and inoculant treatments, showing specific effects for this trait.

Table 3. Analysis of Variance for yield components

| MS (Yield components) | | | | | | | | | | | |
|------------------------------|-----------|-----------|------------------------|-----------|-----------|-----------|-----------|------------|------------------------|-----------|---------------------|
| Sources of Variation | dF | NE | FWE⁺ | DE | DC | LE | WS | DWC | DWG⁺ | %S | GY |
| | | n | g plant | cm | cm | cm | g | g | kg | % | kg ha ⁻¹ |
| Hybrids (H) | 2 | * | * | NS | NS | * | * | * | NS | NS | NS |
| Treatments(T) | 3 | NS | NS | * | * | * | * | * | NS | NS | * |
| H/T | 6 | NS | NS | NS | NS | * | NS | NS | NS | NS | NS |
| R ² | | 0.69 | 0.56 | 0.61 | 0.65 | 0.88 | 0.83 | 0.65 | 0.82 | 0.46 | 0.78 |
| CV % | | 23.4 | 21.7 | 9.7 | 5.9 | 7.6 | 5.7 | 23.3 | 26.3 | 18.1 | 26.3 |

⁺ Transformed data. Values of the mean squares with (*) **in bold**, are statistically significant ($p < 0.05$), R² coefficient of determination, CV% coefficient of variability in percentage. **MS** estimate of mean squares, **dF** degrees of freedom. **NE** number of ears per sample, **FWE⁺** fresh weight of the ear per plant (transformed data), **DE** diameter of the ear, **DC** diameter of the cob, **LE** length of the ear, **WS** weight of 100 seeds, **DWC** dry weight of the cob, **DWG⁺** dry weight of the grain per plant (transformed data), **% S** percentage of plant survival at harvest, **GY** grain yield per hectare.

In the comparison between the maize hybrids shown in Table 4, the cultivars Dekalb7508 and INIA619 outperformed XB8010 in grain yield. INIA619 also statistically outperformed the other two hybrids in dry weight of the crown and in crown length. For its part, XB8010 exceeded the other cultivars in fresh weight of the ear and in weight of 100 seeds. Dekalb7508 and XB8010 had a higher relative number of ears in relation to INIA619.

Table 4. Grain yield and its components comparison for corn hybrids

| Yield components | | | | | | | | | | |
|------------------|---------|------------------|--------|--------|---------|---------|----------|------------------|---------|---------------------|
| Hybrids | NE | FWE ⁺ | DE | DC | LE | WS | DWC | DWG ⁺ | %S | GY |
| | n | g plant | cm | cm | cm | g | g | kg | % | kg ha ⁻¹ |
| Dekalb7508 | 32.88 a | 21.96 b | 4.13 a | 2.33 a | 12.76 b | 27.63 c | 142.50 b | 1.97 a | 91.63 a | 7 885.00 a |
| XB8010 | 32.00 a | 27.67 a | 3.89 a | 2.27 a | 12.65 b | 32.25 a | 120.13 b | 1.70 a | 87.24 a | 6 269.44 b |
| INIA619 | 22.20 b | 21.31 b | 3.99 a | 2.34 a | 14.56 a | 30.69 b | 183.50 a | 1.66 a | 81.51 a | 7 592.19 a |

⁺ Transformed data. ¹ Means followed by the same letters in a column do not differ significantly by the SK test ($p < 0.05$)

In the comparison between inoculant treatments (Table 5), it is shown that the doses of nitrogen fertilization of 60-120 kg N ha⁻¹ to which *Azospirillum* sp. was inoculated were similar in grain yield to the control without inoculation (180 kg N ha⁻¹) and these treatments outperformed the plots that received the only inoculation with *Azospirillum* sp., without additional nitrogen fertilization (0 kg N ha⁻¹). Similar effects occurred for the cob dry weight, cob diameter and ear diameter. Only in the case of ear length, it was found that the dose of 120 kg N ha⁻¹ + *Azospirillum* statistically exceeded the control (180 kg N ha⁻¹) and the dose of 60 kg N ha⁻¹ + *Azospirillum*, existing interaction between maize hybrids and inoculant treatments.

Table 5. Grain yield and its components comparison for inoculant-N fertilization treatments

| Yield components | | | | | | | | | | |
|------------------|---------|------------------|--------|--------|---------|---------|----------|------------------|---------|---------------------|
| Treatments | NE | FWE ⁺ | DE | DC | LE | WS | DWC | DWG ⁺ | %S | GY |
| | n | g plant | cm | cm | cm | g | g | kg | % | kg ha ⁻¹ |
| 60 N + A | 31,50 a | 24,04 a | 3,99 a | 2,25 b | 13,05 b | 29,00 a | 147,83 a | 1,82 a | 85,01 a | 7 124,92 a |
| 120 N + A | 31,25 a | 23,82 a | 4,22 a | 2,37 a | 14,54 a | 30,00 a | 164,17 a | 1,84 a | 92,96 a | 8 478,08 a |
| 0 N + A | 27,45 a | 23,36 a | 3,65 b | 2,23 b | 11,41 c | 31,33 a | 112,67 b | 1,62 a | 80,38 a | 5 232,75 b |
| 180 N | 26,33 a | 23,36 a | 4,14 a | 2,39 a | 14,29 a | 30,42 a | 170,17 a | 1,81 a | 88,83 a | 8 159,75 a |

⁺ Transformed data. ¹ Means with different letters are statistically significant ($p < 0.05$)

3.2 Agronomic Characters

Among the agronomic traits evaluated (Table 6), the effect of inoculant treatments was significant for leaf width (LW), stem diameter (SD), fresh weight of foliage at 30 (FE30), 60 (FE60) and 90 (FE90) days, as well as for foliage dry weight at 30 (DW30) and 60 (DW60) days; In this last variable, a significant interaction was also found between corn hybrids and inoculant treatments. Regarding the maize hybrids evaluated, the significant differences corresponded to the width of the leaf, dry weight of the foliage at 90 days and dry weight of the foliage at 30 and 60 days. No differences were found between the hybrids regarding leaf length, fresh weight at 30 and 60 days, as well as in the percentage of the emergence of the plants.

Table 6. Analysis of Variance for agronomic traits

| Sources of Variation | dF | MS (Agronomic traits) | | | | | | | | | |
|----------------------|----|-----------------------|------|------|------|------|------|------|------|------|------|
| | | %E | NLP | LL | LW | SD | FW30 | FW60 | FW90 | DW30 | DW60 |
| | | % | n | cm. | cm. | cm. | kg | kg | kg | kg | kg |
| Hybrids (H) | 2 | NS | * | NS | * | * | NS | NS | * | * | * |
| Treatments(T) | 3 | NS | NS | NS | * | * | * | * | * | * | * |
| H/T | 6 | NS | NS | NS | NS | NS | NS | NS | NS | NS | * |
| R ² | | 0.39 | 0.75 | 0.87 | 0.86 | 0.65 | 0.8 | 0.72 | 0.83 | 0.73 | 0.79 |
| CV % | | 4.6 | 10.9 | 8.9 | 10.2 | 13.6 | 23.2 | 24.3 | 23.9 | 32.2 | 22.9 |

Values of the mean squares with (*) in bold are statistically significant ($p < 0.05$), R² coefficient of determination, CV% coefficient of variability in percentage. MS estimate of mean squares, dF degrees of freedom. % E field emergence percentage 7 days after planting, NLP number of leaves per plant, LL leaf length, LW leaf width, SD stem diameter, FW30 fresh weight of foliage at 30 days, FW60 fresh weight of foliage at 60 days, FW90 fresh weight of foliage at 90 days, DW30 fresh weight of foliage at 30 days, DW60 fresh weight of foliage at 60 days.

In the comparison between the maize hybrids shown in Table 7, Dekalb7508 surpassed the other two cultivars evaluated in leaf length, but was similar to the cultivar XB 8010 in terms of stem diameter, number of leaves per plant and in dry weight of the foliage at 30 days. Likewise, Dekalb 7506 was statistically similar to INIA 619

with respect to the dry weight of the foliage at 60 days; INIA 619 outperformed the other two cultivars in the case of fresh weight of the foliage at 90 days.

Table 7. Comparison of agronomic traits for corn hybrids

| Agronomic traits | | | | | | | | | | |
|------------------|---------|--------|---------|--------|--------|----------|----------|-----------|---------|----------|
| Hybrids | %E | NLP | LL | LW | SD | FW30 | FW60 | FW90 | DW30 | DW60 |
| | % | n | cm. | cm. | cm. | kg | kg | kg | kg | kg |
| Dekalb7508 | 94,44 a | 9,32 a | 49,13 a | 6,77 a | 1,77 a | 345,56 a | 442,19 a | 1925,13 b | 90,56 a | 210,31 a |
| XB8010 | 95,75 a | 9,71 a | 44,91 b | 5,18 b | 1,65 a | 329,50 a | 486,88 a | 1263,13 c | 88,00 a | 162,81 b |
| INIA619 | 94,75 a | 7,99 b | 35,09 c | 5,60 b | 1,48 b | 301,75 a | 440,94 a | 2458,13 a | 59,97 b | 215,81 a |

¹Means with different letters are statistically significant ($p < 0.05$)

In the comparison between inoculant treatments for agronomic characters (Table 8), it is shown that the doses of nitrogen fertilization of 60-120 kg N ha⁻¹ inoculated with *Azospirillum* sp., were similar to the control without inoculation (180 kg N ha⁻¹) and these treatments surpassed the plots that received the only inoculation with *Azospirillum* sp. without additional nitrogen fertilization (0 kg N ha⁻¹), with respect to the characters of length and width of the leaf, stem diameter, fresh weight of the foliage at 90 days as well as for the dry weight of the foliage at 30 and 60 days.

Table 8. Comparison of agronomic traits for inoculant-N fertilization treatments

| Agronomic traits | | | | | | | | | | |
|------------------|---------|--------|---------|--------|--------|----------|----------|-----------|---------|----------|
| Treatments | %E | NLP | LL | LW | SD | FW 30 | FW 60 | FW 90 | DW 30 | DW 60 |
| | % | n | cm. | cm. | cm. | kg | kg | kg | kg | kg |
| 60 N + A | 97,33 a | 8,87 a | 44,42 a | 6,13 a | 1,75 a | 245,33 b | 389,17 b | 1846,83 a | 95,17 a | 221,42 a |
| 120 N + A | 93,17 a | 9,35 a | 43,94 a | 5,90 a | 1,64 a | 344,75 a | 398,75 b | 2056,67 a | 86,46 a | 208,33 a |
| 0 N + A | 95,00 a | 8,63 a | 38,77 b | 5,13 b | 1,45 b | 408,33 a | 555,42 a | 1364,17 b | 51,58 b | 127,17 b |
| 180 N | 94,42 a | 9,18 a | 45,05 a | 6,25 a | 1,69 a | 304,00 b | 483,33 a | 2260,83 a | 84,83 a | 228,33 a |

¹Means with different letters are statistically significant ($p < 0.05$)

4. Discussion

In the present research, it was found that the grain yield in corn with the control dose (180 kg N ha⁻¹) without inoculation, was similar to that of the doses of 60-120 kg N ha⁻¹ inoculated with the strain of *Azospirillum* sp. 15 and 45 days after sowing, for the hybrid cultivars evaluated. This result is consistent with the findings of various authors (Galindo et al. 2020; Schmidt & Gaudin, 2018; Alvarado et al. 2018; Piscocoya & Ugaz, 2016), which indicate an improvement in corn grain yield under the effect of inoculation with *Azospirillum* sp. The results obtained also seem to agree with other researchers (Zamboni et al. 2019) regarding the little interaction of the inoculant effect of *Azospirillum* sp. with maize genotypes or hybrids, although there could be certain interaction effects between both factors for some physiological attributes (Silva et al. 2020), evidence also shown by Ferrerira et al (2020), who found that maize hybrids showed greater expressivity in yield components in the presence of *A. brasilense* applied in seed treatment. Likewise, Alvarado et al. (2018), argue that the joint use of synthetic fertilizers with microbial inoculants increases grain yield in various varieties of hybrid corn, which shows evidence similar to that obtained in the research.

Regarding other characteristics that are considered grain yield components evaluated in the experiment, such as ear diameter and length and seed weight, these were significantly affected by inoculation with *Azospirillum* sp., although other variables such as the number of ears and fresh weight of the cob, had no effect by inoculation. The significance of the variance components show in this case that the effect of treatments was similar to or greater than the effect of the corn hybrids *per se*, which suggests that the response in grain yield in the corn cultivars may be balanced with nitrogen fertilization and inoculation with *Azospirillum* sp.; this aspect has been studied by Wagner et al. (2020) who demonstrated that interactions with soil microorganisms are important for the expression of heterosis in corn.

Azospirillum spp. and other bacterial strains have the ability to fix atmospheric nitrogen, solubilize phosphorus or produce growth regulators (Perez-Montano et al. 2013; Schmidt & Gaudin, 2018), and there is evidence that they can be used as biofertilizers in a complementary way to the application of chemical fertilizers (Alvarado et al. 2018), a fact that is corroborated in this research under the conditions of the Peruvian coast, in which alluvial

soils devoid of organic matter and scarce in water predominate (Yang, 2020).

The intensive use of fertilizers and phytosanitary agents can increase the rate of nutrients and/or toxic agents in rivers and soils, observing that modern intensive agriculture can strongly impact traditional agriculture in desert areas (Lacroix et al. 2020); environmental impacts are influenced by the high energy intensity linked to the production of inorganic fertilizers used and by phytosanitary agents (Vazquez-Rowe et al. 2016), in that sense, this research can be considered a contribution to evaluate different alternatives of nitrogen fertilization in corn that includes the use of the soil microbiota such as *Azospirillum* sp. since hard yellow corn is a product in high demand and in deficit in Peruvian agribusiness (Eguren, 2003).

5. Conclusion

It was found that for the dose of 180 kg ha⁻¹ of nitrogen, the grain yield in maize hybrid was similar to that of the plots applied with 60-120 kg ha⁻¹ of nitrogen fertilization inoculated with *Azospirillum* sp. Similar responses were found in other components of grain yield and no differences were found in grain yield attributed to interactions between maize hybrids and the application of *Azospirillum* sp. It is suggested to explore the study of the characterization of the *Azospirillum* sp. and the impact of the use of this microorganism in the production system of maize.

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