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## INOCULATION AND CO-INOCULATION OF BACTERIA OF THE GENERA *BACILLUS*, *PSEUDOMONAS* AND *AZOSPIRILLUM* IN CORN CULTIVATION IN NORTHWESTERN SÃO PAULO\*

### ABSTRACT

Corn is a species of plant of the Gramineae family that is widely used by farmers for animal feed, for its grains, and for straw. Microorganisms play an important role in the soil, such as in the decomposition of organic materials, soil structuring and availability of nutrients, improving the physical, chemical and biological characteristics of the soil. The aim of this work was to evaluate the agronomic characteristics and yield of corn in the 2021/22 harvest when treated with different bacteria inoculations and co-inoculation. The experiment was carried out at NEPE/UNIFUNEC in a randomized block design, with eight treatments consisting of isolated bacteria and combinations of bacteria of the genera *Bacillus*, *Pseudomonas* and *Azospirillum*, in 5 blocks. The following evaluations were carried out: plant stand, plant height, stem diameter, first ear insertion height, ear diameter, ear length, number of grains per row and number of rows, weight of 100 grains and yield. The software used for the statistical analyses was SISVAR. Significant results were found for the following variables: plant height, stem diameter and number of grains per ear. The best treatments for these variables were: T3 – culture inoculated with *Pseudomonas fluorescens*; T4 – culture inoculated with *Bacillus subtilis*; T5 – culture inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis* and T8 – culture inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasilense*. The inoculation and co-inoculation of bacteria promoted satisfactory growth, which may be reflected in production with an increase in the number of grains per ear, especially the association of *Pseudomonas fluorescens* with *Bacillus subtilis*.

**Keywords:** Phosphorus in the soil. Microorganisms. Sustainable agriculture. *Zea mays*.

## INOCULAÇÃO E CO-INOCULAÇÃO DE BACTÉRIAS DO GÊNERO *BACILLUS*, *PSEUDOMONAS* E *AZOSPIRILLUM* NO CULTIVO DE MILHO NO NOROESTE PAULISTA

### RESUMO

O milho é uma espécie de planta da família das gramíneas, bastante utilizada por agricultores para alimentação animal, aproveitamento dos grãos e palhada. Já os microrganismos desempenham importante papel no solo, como decomposição de materiais orgânicos, estruturação do solo e disponibilização de nutrientes, melhorando as características físicas, químicas e biológicas deste. O objetivo do presente trabalho foi avaliar as características agrônômicas e a produtividade do milho na safra 2021/22 a diferentes inoculações e co-inoculação de bactérias. O experimento foi realizado no NEPE/UNIFUNEC em um delineamento de blocos casualizados, com 8 tratamentos sendo bactérias isoladas e a combinação de bactérias do gênero *Bacillus*, *Pseudomonas* e *Azospirillum*, em 5 blocos. As avaliações realizadas foram: estande de plantas, altura de plantas, diâmetro de caule, altura de inserção da primeira espiga, diâmetro de espiga, comprimento de espiga, número de grãos por fileiras e número de fileiras, massa de 100 grãos e produtividade. O software usado para as análises estatísticas foi o SISVAR. Os resultados significativos encontrados foram para as variáveis: altura de plantas, diâmetro de caule e número de grãos por espiga. Os tratamentos superiores para essas variáveis foram: T3 – cultura inoculada com *Pseudomonas fluorescens*; T4 – cultura inoculada com *Bacillus subtilis*; T5 – cultura inoculada com *Pseudomonas fluorescens* + *Bacillus subtilis* e T8 - cultura inoculada com *Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasilense*. A inoculação e co-inoculação de bactérias promoveram um crescimento satisfatório, podendo refletir em produção com aumento de número de grãos por espiga, em especial a associação de *Pseudomonas fluorescens* com *Bacillus subtilis*.

**Palavras-chave:** Fósforo no solo. Microrganismos. Agricultura sustentável. *Zea mays*.

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## 1 INTRODUCTION

Corn (*Zea mays*) is a plant belonging to the gramineae family, and is a crop of national and global importance due to its characteristics as a source of both human and animal food and as a raw material for various industrialized products, generating jobs and driving industries, with direct social and economic repercussions for the areas where it is cultivated (MORAIS et al., 2017).

According to data from the National Supply Company (*Companhia Nacional de Abastecimento* – Conab) (2021), the estimated total national production area of corn, for November 2021, is 20,888.7 thousand hectares with productivity of 5587 kg per hectare. It should be noted that cultivation can often be done in up to 3 crops during the year, being classified as corn of the 1st, 2nd and 3rd crops.

According to Harger et al. (2007), corn is cultivated in Brazil under different climate and soil conditions. The productivity of the crop depends, among other things, on the adequate supply of nutrients and the supply of phosphorus (p) through mineral fertilization of the soil, an essential and common practice in Brazil.

According to Awasthi *et al.* (2011), phosphorus is one of the most vital nutrients for crops. However it is also the most deficient, resulting in serious repercussions for plant performance and productivity levels. Other factors that hinder the chemical supply of phosphorus are its high energy and economic cost, low efficiency (5-30%), chronic accumulation of phosphate in the environment, and global scarcity of phosphoric rock (PATIÑO-TORRES and REYES, 2014). In Brazil, phosphate rock reserves are represented by igneous rocks, with low phosphorus content and the presence of contaminants. As a consequence, due to high demand and low production, the country has become a major importer of phosphate fertilizers and phosphate rocks. This dependence on the international market exposes it to greater vulnerability in relation to external prices (BENITES, 2015).

Brazilian soils are deficient in phosphorus, as there is a strong interaction of P with soil colloids, providing a high fixation capacity and low availability of P, competing with the response to phosphate fertilization in a large portion of Brazilian soils (OLIVEIRA et al., 1982; LEE; GUILHERME, 2007). According to Santos et al. (2015), P is associated with important functions in plants; for example, it is strongly linked to metabolic processes, and plays a role in the constitution of ATP, DNA and enzymes such as phosphorylase, being essential for plant life and a key factor in promoting better production.

The rhizosphere (the zone of soil that is influenced by the plant roots) of some plants has microorganisms capable of solubilizing P; therefore, studying the behavior of these microorganisms for better acquisition and use of P by the plant is of paramount importance (INUI, 2009). According to Silveira and Freitas (2007), microorganisms play a role in biochemical reactions, in the availability of nutrients for plant development, being a beneficial relationship for agricultural crops and with the use of these microorganisms in agriculture in regional contexts for the improvement of global agricultural sustainability. According to the president of Embrapa (2018), Mauricio Antônio Lopes, Brazil has the potential for growth in the agricultural microorganisms market, due its vast biological diversity, the largest on the planet.

Bacteria of the genus *Pseudomonas* are common in soils. According to Naz and Bano (2010), who studied phosphate solubilization, there are three species of the genus capable of solubilizing inorganic phosphate through the production of phytohormones.

Another genus of bacteria that also plays the role of solubilizing phosphorus in the soil is *Bacillus*, which acts by improving both the development of the root system and the aerial part of the plant. Oliveira *et al.* (2017), evaluating the effect of different *Bacillus* strains on promoting corn seedling growth, found that the products used were efficient in promoting seedling growth, when inoculants were used individually or combined.

The use of microorganisms together with different functionalities (co-inoculation) has been highlighted, thanks to the synergistic effect, as a means of increasing production in some crops (FERREIRA *et al.*, 2016). The same authors evaluated the effect of co-inoculation of phosphorus-solubilizing microorganisms (*Bacillus* sp.), mycorrhizal fungi and *Azospirillum* in the cultivation of pearl millet fertilized with phosphorus sources with different solubilities. The authors found that co-inoculation with *Azospirillum*, phosphate-solubilizing *Bacillus* and mycorrhizal fungi, provided greater plant height of corn fertilized with Araxá phosphate.

According to Guimarães *et al.* (2021), research aimed at promoting plant growth and plant production with inoculation containing bacteria based on phosphate solubilization in the soil is more recent and promising, according to the importance and difficult dynamics of this nutrient in the soil. The use of multifunctional microorganisms is fundamental for sustainable agriculture, promoting plant growth and improving crop yield and quality.

Santos *et al.* (2021) report that the successful performance of phosphate-solubilizing bacteria is influenced by factors such as temperature, pH, and soil salinity. Therefore, it is

important to evaluate the efficiency of the use of treatments containing phosphate-solubilizing bacteria in different environments.

The aim of the present study is to evaluate the agronomic characteristics and yield of corn crops (2021/2022) subjected to different inoculations and co-inoculation of phosphorus-solubilizing bacteria of the genus *Bacillus*, *Pseudomonas* and plant growth-promoting biostimulant bacteria of the genus *Azospirillum* in the municipality of Santa Fé do Sul, in the Northwest of São Paulo, seeking to enhance plant development and increase the efficiency of land use by the crop.

## 2 METHODOLOGY

### 2.1 Experiment location, preparation of the area and corn sowing

This study was carried out in a rainfed area at the Teaching, Research and Extension Center of the Centro Universitário de Santa Fé do Sul, São Paulo, Brazil, under a eutrophic, abruptic, TO moderate, sandy/medium RED-YELLOW CLAYEY soil, according to Oliveira et al. (1999), located between 20°22'17" latitude south and 50°90'52" longitude west, at an altitude of 384 m. The average annual rainfall is 1,221 mm and the average annual temperature is 24.4°C. The climate of the region, according to the Köppen classification, is of the Aw type, classified as humid tropical, with a dry, mild winter and a hot, rainy summer.

The soil in which the research was established has been cultivated with annual crops for two years, the previous crop being off-season corn. Before installing the experiments, chemical characterization of the soil for fertility purposes was carried out, using the methods proposed by Raij et al. (2001). Twenty soil samples with deformed structure were collected using a screw auger in the 0.00-0.20 m layer. Then, after interpretation of the analysis, the necessary soil fertility corrections were made.

The data obtained from the soil analysis were: pH (CaCl<sub>2</sub>) = 5.3; 12.0 mg dm<sup>-3</sup> of P; 1,7; 19,0; 13,0; 18,0; 33,7 and 51,7mmolc dm<sup>-3</sup> of K, Ca, Mg, H+Al, SB and CTC, respectively; 15.0 g dm<sup>-3</sup> of organic material and base saturation (V%) = 65.0.

Next, the liming requirement was calculated using the following equation:

$$Nc = \frac{(V2 - V1) \times CEC}{RTNP \times 10}$$

Where:

V2 = desired saturation per base.

V1 = current saturation per base (soil analysis).

CEC = cation exchange capacity [ $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^{+} + (\text{H} + \text{Al})$ ], in  $\text{cmolc.dm}^{-3}$ .

RTNP = relative total neutralization power of the limestone to be applied.

Through the calculations, a liming requirement for the area of  $300\text{kg.ha}^{-1}$  of limestone with RTNP of 86% was obtained.

For the statistical analysis, a randomized block design (RBD) was used, with 5 replications, and the experiment consisted of 8 treatments:

T1 – Control;

T2 – culture inoculated with *Azospirillum brasilense*

T3 – culture inoculated with *Pseudomonas fluorescens*

T4 – culture inoculated with *Bacillus subtilis*

T5 – culture inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis*

T6 - culture inoculated with *Azospirillum brasilense* + *Bacillus subtilis*

T7 - culture inoculated with *Azospirillum brasilense* + *Pseudomonas fluorescens*

T8 – culture inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasilense*

The treatments were applied by planting furrow, following the manufacturers' recommended doses for each inoculant (*Pseudomonas fluorescens*: 400 ml/ha ( $1 \times 10^9$  cfu/ml); *Bacillus subtilis*: 500 ml/ha ( $1 \times 10^9$  cfu/ml) and *Azospirillum brasilense*: 400 ml/ha ( $1 \times 10^8$  cfu/ml)).

Each experimental plot consisted of 6 rows of 10 m with 0.5 m spacing between each crop, making a plot area of 3 x 10 m (30 m<sup>2</sup>).

Before sowing the crops, the planting area was prepared, and the area was desiccated with the herbicides glyphosate (1.56 kg a.i. ha<sup>-1</sup>), carfentzone-ethyl (20 g a.i. ha<sup>-1</sup>) and haloxyfop-p-methyl (60 g a.i. ha<sup>-1</sup>) to control any remaining weeds in the area.

Simple corn hybrid seeds were used, with Viptera 3 technology submitted to seed treatment with fungicides (Carbendazim + tiram) and insecticides (Bifenthrin

The corn was sown on December 1, 2021, in a no-tillage system, with row spacing of 0.5 m and 3 plants per linear meter, totaling a population of 60 thousand plants ha<sup>-1</sup>, with sowing fertilization of 300 kg ha<sup>-1</sup> of the formulation 8-28-16 (N-P-K).

Figure 1 – Corn sowing, 2021.



Source: Prepared by the authors.

After sowing, the seeds were left to germinate so that the experimental plots could be staked, in order to identify the treatments.

Figure 2 – Staking the area, 2021.



Source: Prepared by the authors.

Nitrogen fertilization was performed 23 days after planting, when the crop was between vegetative stages the V4 and V5. A dosage of 100 kg of N per hectare was used.

During the crop development, two herbicide applications were carried out; the first was glyphosate (2.5 Kg b.c. h-1) and the second was chlorimuron, which was applied by directed jet. Three insecticide applications were also carried out: two to control fall armyworm and leafhopper (Methomyl and Imidacloprid) and one specifically to control leafhopper, and a preventive application of fungicide.

## 2.1 Evaluations carried out

When the corn crop had reached physiological maturity (stage R6), the following evaluations were performed:

- Plant stand: a tape measure was used to count the number of viable plants in each treatment plot. The tape measure was extended 3 meters between the rows of each plot and the total number of plants in each row was counted. The total number was divided by the total distance of 6 linear meters to obtain the population density of each plot.

- Plant height: defined as the distance (m) from soil level to the apex of the corn tassel; Ear insertion height: defined as the distance (m) from soil level to the insertion of the main ear;

- Basal diameter of the stalk: using a manual caliper for the measurement, based on the second internode of the plant;

- Mass of 100 grains: determined on a precision balance (0.01g) at 13% moisture (wet basis – b.u.);

- Grain yield: determined by collecting the ears of the plants contained in 5 meters of the 4 useful rows of each plot. After threshing, the grains are quantified and the data transformed into  $\text{kg ha}^{-1}$  at 13% (wet basis).

- Subsequently, 10 ears of corn were collected at harvest for the following evaluations: Length of the ear (peeled): determined from the apex to the base of the ear; Ear diameter (peeled); Number of rows per ear: obtained as a function of the ratio of the number of rows of grains in each ear; Number of grains per row of the ear: determined as a function of the ratio between the number of grains in each row of the ear; Number of grains per ear: obtained by counting the number of grains in each ear of corn, from each experimental unit.

All the results were tabulated and submitted to analysis of variance using the F test ( $p < 0.05$ ). When there were significant differences, the Snott-Knott test was performed at 5% probability level. The statistical software used to carry out the analyzes was SISVAR® (FERREIRA, 2014).

## 3 RESULTS AND DISCUSSION

The data on the biometric and production characteristics analyzed are presented in Table 1.

**Table 1.** Analysis of variance for: Height (HT), plant diameter (DIA); spike insertion height (HT\_E); ear diameter (DIA\_E); ear length (LT\_E); plant stand (STAND); grains per ear (GR\_E); mass of 100 seeds (M\_100) and yield (Y). Santa Fe do Sul, 2022.

TREATMT	HT (m)	DIA (mm)	HT_E (m)	DIA_E (mm)	LT_E (cm)	STAND (un)	GR_E (un)	M_100 (g)	Y (kg/ha)
T1	1.36 B	15.28 B	0,67	28,28	11,79	2,4	407.52 B	8,92	1486,67
T2	1.52 B	17.44 A	0,73	28,08	12,22	2,93	425,60 B	9,95	1658,00
T3	1.60 A	18.84 A	0,84	28,8	12,62	2,57	467.92 A	10,16	1692,67
T4	1.64 A	17.64 A	0,79	29,24	12,66	2,53	462.96 A	10,14	1690,67
T5	1.69 A	17.08 A	0,81	29,6	12,6	2,3	466.64 A	10,2	1699,33
T6	1.50 B	15.40 B	0,7	27,76	11,34	3,03	395.36 B	9,28	1546,00
T7	1.58 A	17.36 A	0,82	27,8	11,71	2,76	420.40 B	9,14	1523,67
T8	1.72 A	19.44 A	0,83	28,16	12,02	2,93	439.72 A	9,31	1551,33
<i>F-test</i>	3,56**	2,97*	1.90ns	1.34ns	1.38ns	1.07ns	2,52*	0.87ns	0.87ns
<i>Average</i>	1,58	17,31	0,77	28,47	12,12	2,68	435,77	9,64	1606,04
<i>CV</i>	8,62	10,91	13,57	4,6	7,68	21,85	9,07	13,09	13,09

Averages followed by the same letter do not differ from each other by the Snott-Knott test at a 5% probability level. \*\*:significant ( $p < 0,01$ ); \*: significant ( $p < 0,05$ ); ns: Not significant; CV (%): Coefficient of variation.

The data obtained in the analysis were significant for the following requirements: height, diameter and grains per ear. For height, treatment 8 (culture inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasilense*) was highlighted, as it had higher growth rate than the other treatments. The same occurred in relation to stem diameter, where there was greater stem thickening, this difference was 2.13 mm higher than the analyzed measurement, providing better support for the plant.

Microorganisms such as *Azospirillum*, *Pseudomonas sp* and *Bacillus* can stimulate growth and reduce stress in plants through the production of auxin (IAA), gibberellin, cytokinin and other hormones (KHOSHURU et al., 2020; LOPES et al., 2021).

Cândido et al. (2022), studying the residual co-inoculation of growth-promoting bacteria for corn crops, found that co-inoculation with *Azospirillum brasilense* in soybean promoted improvements in the physiology of corn grown in succession, providing an alternative to give the plants better stress tolerance potential and generate plants with higher yield potential.

In relation to grain generation (grains per ear), treatments 3, 5, 4 and 8 were significant, especially treatments 3, 4 and 5 (T3 – culture inoculated with *Pseudomonas fluorescens*; T4 –

culture inoculated with *Bacillus subtilis*; T5 – culture inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis*) with a grain yield per ear around 7.4% above than the average.

Araújo (2008) evaluated the inoculation of *B. subtilis* in seeds of corn, soybean and cotton crops. The author noted that in relation to plant development, represented by the production of dry matter, it was observed that corn was the only crop that responded to *B. subtilis* inoculation.

The other results were non-significant, which showed that, statistically, they are the same.

Dalcin (2021) evaluated the inoculation of *B. Megaterium* and *B. subtilis* with doses of P in the corn crop. The author reported that it was economically viable with the 0% P dose; however, the 100% P dose was not viable. Seed inoculation with *P. fluorescens*, without the addition of P, produced 87% relative agronomic efficiency (CHAVES, 2013).

Martins (2020) evaluated the efficiency of *Bacillus megaterium* and *Pseudomonas fluorescens* applied in the planting furrow and as a seed treatment in corn plants. The author observed that when applied directly to the seed, these treatments generated significant results in relation to stem diameter, plant height, leaf width, ear weight, number of ear grains, and fresh plant matter. The author reports that inoculation of bacteria of the genus *Pseudomonas* and *Bacillus* aids the solubilization of P present in the rhizosphere, promoting greater availability of nutrients to the plants, greater vigor and initial start-up. This results in an increase in productivity of corn plants, through the sum of factors related to absorption, nutrition, accumulation and use of water, leaf chlorophyll index and productive components, shoots, leaf area, root mass and grain mass.

Co-inoculation results in better gains in productivity compared to the inoculation technique alone.

#### 4 CONCLUSION

In conclusion, the inoculation and co-inoculation of bacteria promoted satisfactory growth, which may be reflected in production, with an increase in the number of grains per ear. The treatment that proved particularly beneficial was the association of *Pseudomonas fluorescens* with *Bacillus subtilis*.

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