DEVELOPMENT OF CORN HYBRIDS (*Zea mays*) UNDER INOCULATION OF DIAZOTROPHIC BACTERIA AND NITROGEN FERTILIZATION

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ABSTRACT: the aim of this work was to evaluate the development of corn genotypes under the effect of inoculation with *Azospirillum brasilense* in association with nitrogen fertilization. The experimental design was completely randomized in a (5x2x2) factorial design with three replicates. Five corn hybrids were used, combined with two nitrogen (N) doses (without N and with 100 kg ha^{-1} N) and two levels of *A.brasilense* inoculation (without and with inoculation). Plant height and stem diameter at stadiums V8 and R1, leaf chlorophyll content at stadium R1 and dry mass at stadium R2 were evaluated. It was observed that variations in the evaluated parameters are due to the different genetic materials and mainly due to the application of N. Inoculation with *A.brasilense* was not effective for changing corn development.

KEY WORDS: *Azospirillum brasilense*, biological nitrogen fixation, genotypes

DESENVOLVIMENTO DE HÍBRIDOS DE MILHO (*Zeamays*) SOB INOCULAÇÃO DE BACTÉRIAS DIAZOTRÓFICAS E ADUBAÇÃO NITROGENADA

RESUMO: o objetivo deste trabalho foi avaliar o desenvolvimento de genótipos de milho sob efeito da inoculação com *Azospirillum brasilense* (AZ) em associação com adubação nitrogenada. O delineamento experimental utilizado foi o inteiramente casualizado em esquema fatorial (5x2x2), com três repetições. Foram utilizados cinco híbridos de milho, combinados com duas doses de nitrogênio (N) (sem N e com 100 kg ha^{-1} de N) e com dois níveis de inoculação com *A.brasilense*(sem e com inoculação). Avaliou-se a estatura de planta e o diâmetro de colmo nos estádios V8 e R1, o teor de clorofila na folha em estádio R1 e a massa seca da parte aérea no estádio R2. Observou-se que quando houve variação nos parâmetros avaliados, esta foi devido aos diferentes materiais genéticos e principalmente devido à aplicação de N. A inoculação com *A.brasilense* não foi eficiente em alterar o desenvolvimento do milho.

PALAVRAS-CHAVE: *Azospirillum brasilense*, fixação biológica de nitrogênio, genótipos

INTRODUCTION

Corn is the world's largest volume-produced cereal, exceeding one billion tonnes (FAO, 2013a), and Brazil is the third world's largest producer (FAO, 2013b). Brazilian corn production in the 2016/2017 harvest reached 92.8 million tonnes and average productivity of 5,300 kg ha^{-1}, representing 40 % of total grain production (Conab, 2017). However, the average productivity is relatively low, since there are regions in the country with corn crops under no-tillage system, with average yields of 9,000 to 10,000 kg ha^{-1} (Fontoura, 2005; Nogara Neto et al., 2011).

Nitrogen is one of the nutrients with the greatest effects to increase corn production, being fundamental for plant metabolism, by participating in the synthesis of proteins and chlorophylls (Hawkesford et al., 2012). Adequate nitrogen supply is essential to achieve high yields, although it is the nutrient most deficient in practically all soil classes, and NO_3^- and NH_4^+ are the inorganic forms absorbed by plants (Okumura et al., 2011). The availability of this nutrient through nitrogen fertilizers represents one of the highest costs in the production of non-leguminous crops, as corn, wheat and rice crops consume approximately 60 % of the total world's nitrogen as fertilizer (Ladha et al., 2005) and less than 50 % applied in this form is used by plants (Halvorson et al., 2002).

Diazotrophic bacteria can be an alternative to reduce the use of nitrogen fertilizers in cereals (Hungria et al., 2010) and consequently reduce production costs. Among these bacteria, the most important are those of the genus *Azospirillum* in association with grasses (Radwan et al., 2004), which fix atmospheric N_2 in association with several non-leguminous species (Döbereiner and Pedrosa, 1987) and produce various growth hormones (Roesch et al., 2007; Tien et al., 1979). This genus presents contributions for several plant characteristics, among them plant height (Braccini et al., 2012), leaf N content (Nunes et al., 2015; Pereira et al., 2015), shoot dry matter yield (Braccini et al., 2012; Lana et al., 2012; Marini et al., 2015) and grain yield (Ferreira et al., 2013; Hungriaet al., 2010; Lana et al., 2012).

Plant genotype may influence N_2 fixation efficiency and/or the ability to promote plant growth, for example corn with *Azospirillum* (Pereira et al., 2015; Reis Junior et al., 2008). Dartora et al. (2013) observed different behaviors of corn genotypes submitted to the combined inoculation of *Azospirillum brasilens* and *Herbaspirillum seropedicae*, providing increments in stem basal diameter, shoot dry matter and grain yield. However, results on the inoculation of grasses have been discrepant, which do not always follow the same trend, being able to raise or reduce a certain variable (Lana et al., 2012; Nunes et al., 2015).

Based on the above, the aim of this work was to evaluate the development of corn genotypes under the effect of inoculation with *A. brasilense* in association with nitrogen fertilization.

MATERIAL AND METHODS

The experiment was conducted in open field vases at the Department of Agrarian Sciences - Federal University of Paraná (UFPR) from January to May 2013 in Curitiba, PR, located at geographic coordinates 25º24'47'' south latitude and 49º14'58'' west longitude with average altitude of 915 m. It is located in the first Paraná plateau, with Cfb type climate, according to the Köppen classification.

According Marques and Motta (2004), the chemical characteristics of soil collected in the 0 - 20 cm depth layer before experiment installation were: pH in $CaCl_2$ of 5.8; pH in SMP of 6.1; potential acidity (H + Al) of 4.6 $cmol_c$ dm^{-3}; 27.4 g dm^{-3} of carbon (C); 8.0 $cmol_c$ dm^{-3} of calcium (Ca^{2+}); 4.9 $cmol_c$ dm^{-3} of magnesium (Mg^{2+}); 2.1 $cmol_c$ dm^{-3} of potassium (K^+); 44.3 mg dm^{-3} of phosphorus (P) (Mehlich 1); 7.37 mg dm^{-3} of iron (Fe^{2+}); 0.11 mg dm^{-3} of copper (Cu^{2+}); 5.02 mg dm^{-3} of manganese (Mn^{2+}); 0.95 mg dm^{-3} of zinc (Zn^{2+}); 15 $cmol_c$ dm^{-3} of base sum; 19.6 $cmol_c$ dm^{-3} of cation exchange capacity (CEC) and 76.5 % of base saturation (V %).

The experimental design was completely randomized in a (5x2x2) factorial design with three replicates. The experimental unit consisted of a vase (8 L) containing one plant. Five commercial corn hybrids were used (DKB 566 PRO, $BG7060_{H,}$ PRE 32D10, P30F53, FÓRMULA TL) in combination with two N doses (without and with application of 100 kg ha^{-1} of N) and with two levels of *A. brasilense* inoculation (without and with inoculation).

Corn seeds were inoculated with *A. brasilense* through a commercial peaty solid inoculant containing AbV5 and AbV6 (bacteria) strains with $2x10^8 CFUg^{-1}$ in the ratio of 200 g of inoculant to 25 kg of seed. A 10 % sugar solution in the ratio of 300 ml to 50 kg of seeds was used to increase peat adhesion on the seed surface (Hungria et al., 2010). Inoculation was carried out by adding the sucrose solution through an automatic pipette on seeds, followed by the inoculant and, with subsequent mixing in seeds prior to sowing.

All treatments received of P (160 mg kg^{-1}) and K (150 mg kg^{-1}) applications before sowing, incorporated into the vase soil (6.650 kg), in the forms of single superphosphate (SSP) and potassium chloride (KCl), respectively. The N source was urea (46 % of N), incorporated into the soil in two stages, 75 mg kg^{-1} of N in V2 and 177 mg Kg^{-1} of N in V5, according to pre-established treatments.

Corn sowing was carried out on January, with three seeds per vase at 4 cm depth. After 27 days of emergence, thinning was performed, leaving one plant per vase. Weed control was manually performed, with no disease control. Parameters plant height, stem diameter at the smaller and larger diameter, leaf chlorophyll content, shoot dry matter yield (SDMY) and shoot nitrogen content were evaluated. Plant height and stem diameter were evaluated at stadiums V8 and R1 by using a measuring tape from the soil surface to the insertion of the flag leaf and a digital pachymeter was used to measure the stem diameter in the first internode visible above the soil, respectively.

The determination of the relative chlorophyll content in leaves was carried out with readings in stadium R1, through N-Tester® (Yara) manual chlorophyllometer. Readings were performed on the first leaf above and opposite to the main corn ear, at points located in the region of the middle third of the leaf (thirty per leaf), from the base and 2 cm from the two leaf margins. For greater accuracy, the N-Tester® automatically displays the average value of thirty readings.

The nitrogen content (N) in the index leaf was determined in the middle third of the opposite leaf and below the point of insertion of the main corn ear, collected at the flowering stadium (Argenta et al., 2001). Index leaf samples were placed in greenhouse with forced air circulation for drying at 65 ± 2 °C for 72 h and milled in a Willye knife mill, for N determination through Kjeldahl digestion followed by distillation and titration as described in Tedesco et al. (1995).

In order to determine the shoot dry matter content, plants were cut in the first visible internode above the soil in the R2 stadium, then they were sectioned and conditioned in paper bags, identified and placed in greenhouse with forced air circulation for drying at 65 °C ± 2 °C until constant weight for final matter quantification.

Data were submitted to analysis of variance (ANOVA), by SISVAR statistic software (Ferreira, 2014), and in case of significance for corn hybrids, means obtained were compared by the Tukey test at 5 % significance level.

RESULTS AND DISCUSSION

In the evaluated characteristics, no interaction between hybrids, nitrogen fertilization and *A. brasilense* inoculation was observed (Table 1), and these results are similar to those observed by Cavallet et al. (2000), when carrying out experiments with commercial product containing *Azospirillum* spp. Similarly, Dartora et al. (2013) observed no significant interaction between *A. brasilense* inoculation and *H. seropedicae* and nitrogen fertilization for variables plant height, stem basal diameter and shoot dry matter. Perhaps, this was due to competition among soil organisms, considering that the soil used for this experiment was not previously sterilized.

Table 1. Plant height variation (PHV) and stem basal diameter (SD) in the vegetative (V) and reproductive phases (R) and chlorophyll content (ClorF), leaf N content (LN) and shoot dry matter (SDM) in the reproductive phase of corn plants as a function of genotype, *Azospirillum brasilense* inoculation and nitrogen doses

Treatments	PHV (cm)		SD (mm)		ClorF	SDM (g plant^{-1})	LN (g kg^{-1})
	V	R	V	R	R	R	R
Genotype							
BG7060H	41.7 a*	125.4 ab	22.2 a	20.8 a	242.7 c	108.7 ab	16.9 a
DKB566 PRO	44.3 a	133.3 a	21.2 ab	19.7 ab	315.8 ab	115.0 ab	10.8 a
FÓRMULA TL	43.8 a	135.06 a	21.6 ab	20.0 ab	334.2 a	125.8 a	10.1 a
P30F53	40.2 a	114.9 b	20.0 b	18.54 b	259.0 bc	97.56 b	12.5 a
PRE32D10	40.0 a	112.0 b	21.0 ab	19.4 ab	316.5 ab	101.22 b	14.6 a
N in topdressing (Kg ha^{-1})							
0	36.2 b	117.5 b	18.7 b	17.3 b	209.8 b	73.1 b	9.0 b
100	47.7 a	130.7 a	23.6 a	22.1 a	377.6 a	146.2 a	17.0 a
Inoculation							
without	41.7 a	123.3 a	21.3 a	19.9 a	302.0 a	113.3 a	12.0 a
with	42.3 a	125.0 a	20.9 a	19.5 a	385.3 a	106.0 a	13.9 a
CV %	15.40	11.5	8.4	8.8	11.6	17.7	25.1

*Means followed by the same letter in the column do not differ at 5 % probability level by the Tukey test.

In general, the highest values for the evaluated variables were observed in Formula TL hybrid, while the smallest ones were observed in P30F53 (Table 1). This difference can be attributed to the genetic potential of each genotype. Dotto et al. (2010) also found differences in the performance of corn hybrids in an experiment with *H. seropedicae* inoculation and N doses.

The leaf nitrogen content did not vary among analyzed hybrids, and all presented lower values compared to reference values (20-24 g kg^{-1}) (NEPAR, 2017), unlike that verified by Marini et al. (2015), which obtained differences in nitrogen content using two corn hybrids (30F53; CD383).

Nitrogen application provided increases in plant height, stem diameter, chlorophyll content and foliar nitrogen as observed by Morais et al. (2015) and shoot dry matter yield, similar to that found by Lana et al. (2012). Okumura et al. (2011) verified a positive relationship between nitrogen application and plant height. The positive effect as a function of N is due to the performance of this nutrient in plant metabolism, used in the synthesis of proteins and chlorophylls (Hawkesford et al., 2012), in addition to the influence in processes such as leaf expansion and photosynthetic rate (Okumura et al., 2011). According to Gomes et al. (2007), leaf nitrogen level has positive correlation with grain production.

Inoculation of corn genotypes with *A. brasilense* AbV5 and AbV6 strains did not modify plant height, stem diameter, dry matter production, as well as the chlorophyll and nitrogen content in leaves of plants in both vegetative and reproductive phase (Table 1). Marini et al. (1999), also observed no variation in plant height, stem diameter and nitrogen content with the inoculation of two corn hybrids with *A. brasilense*. Pereira et al. (2015) and Morais et al. (2015) observed no effect of this inoculation on chlorophyll and leaf nitrogen contents. In general, the increase in chlorophyll content corresponds to an increase of photosynthesis, which is related to the increase of plant vigor and production (Bashan et al., 2006; Okumura et al., 2011).

The effects on stem basal diameter, chlorophyll content and dry matter production are contrasting, since Dartora et al. (2013) verified increases in stem basal diameter, Lana et al. (2012) and Quadros et al. (2014) observed increase in shoot dry matter yield, chlorophyll content and plant height, when corn was inoculated with *A. brasilense*. These variations in results obtained with *A. brasilense* inoculation corroborate Lana et al. (2012), who reported that the same trend of effects does not always occur.

The greatest variations observed were due to nitrogen fertilization and to the hybrid used, pointing that the plant response was not due to the fixation of atmospheric N_2 by *A. brasilense* or root growth provided by hormones produced by this bacterium (Pedrinho et al., 2010; Roesch et al., 2007; Tien et al., 1979), but to the higher supply of nitrogen and variation in genotype efficiency in soil nitrogen absorption (Dobbelaere et al., 2002).

It could be concluded that there is variation among corn genotypes regarding development, with Formula TL hybrid presenting the highest chlorophyll content and shoot dry matter values, while BG7060H, stem basal diameter and DKB566 PRO, plant height in the reproductive phase and nitrogen application provided significant increase in corn development and their content on leaves, shoot dry matter and in all other parameters evaluated, while the same was not observed with *A. brasilense*, showing no effect with inoculation.

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