ISSN: 1984-5529

Científica

Jaboticabal

v.45, n.3, p.321–324, 2017

http://dx.doi.org/10.15361/1984-5529.2017v45n3p321-324

Inoculation of *Azospirillum brasilense* and nitrogen doses in maize for grain production

Inoculação de *Azospirillum brasilense* e doses de nitrogênio em milho para produção de grãos

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Recebido em: 21-12-2014; Aceito em: 04-04-2017

Abstract

Nitrogen is the most required element and the one that most frequently limits productivity in maize crop. In recent years, the interest in technologies that contribute to increase the production indices, without entailing a greater consumption of inputs, is notable. In this context, the use of bacteria of the genus *Azospirillum brasilense* appears as a promising alternative in order to reduce the use of nitrogen fertilizers in maize crop, having as a consequence reduced production costs. This work involved two maize genotypes (AS1572 VTPRO and 30B39H) inoculated with *A. brasilense*, associated with five nitrogen doses in coverage (0, 60, 120, 240 and 480 kg ha^{-1}). No significant interaction was observed between the genotypes used and the inoculation by *Azospirillum brasilense*. There was interaction between the nitrogen doses and the tested genotypes, indicating that the genetic factors of the plant should also be considered at the moment of recommendation of the nitrogen fertilization for the maize crop. The cultivar AS1572 obtained a higher grain yield when compared to the cultivar 30B39H. A total of 9,327 kg ha^{-1} was achieved using 240 kg ha^{-1} nitrogen, and 7,896.29 with 480 kg ha^{-1} nitrogen, for the cultivars AS1572 and 30B39H, respectively.

Additional keywords: biological fixation; diazotrophic bacteria; nitrogen fertilization.

Resumo

O nitrogênio é o elemento mais requerido e o que mais frequentemente limita a produtividade na cultura do milho. Nos últimos anos é notável o interesse por tecnologias que contribuam para o aumento dos índices produtivos, sem acarretar em maior consumo de insumos. Neste contexto, a utilização de bactérias do gênero *Azospirillum brasilense* surge como alternativa promissora visando diminuir o uso de fertilizantes nitrogenados na cultura do milho, com consequência na redução dos custos de produção. Foi realizado um trabalho envolvendo dois genótipos de milho (AS 1572 VTPRO e 30B39H), inoculados com *A. brasilense,* associado a cinco doses de nitrogênio em cobertura (0, 60, 120, 240 e 480 kg ha^{-1}). Não foi observada interação significativa entre os genótipos utilizados e a inoculação por *Azospirillum brasilense.* Houve interação entre as doses de nitrogênio e os genótipos testados, indicando que, os fatores genéticos da planta também devem ser considerados no momento da recomendação da adubação nitrogenada para a cultura do milho. A cultivar AS1572 obteve maior rendimento de grãos, em comparação com a cultivar 30B39H. Foram alcançados 9.327 kg ha^{-1} utilizando 240 kg ha^{-1} de nitrogênio e 7.896,29 com 480 kg ha^{-1} de nitrogênio para a cultivar AS1572 e 30B39H, respectivamente.

Palavras-chave adicionais: adubação nitrogenada; bactérias diazotróficas; fixação biológica.

Introduction

The maize crop represents one of the main inputs of the production segment in Brazil, because, in addition to being present in human food in the form of by-products such as pasta and flour, this cereal represents a significant portion in the feed chain of cattle, poultry and swine. According to CONAB (2017), the estimates for the 2016/2017 crop project 215 million tons of grains, with maize grains accounting for 28 million tons. In this scenario, the southern region represents an important production pole, accounting for just over 29.8 million tons, with an average productivity of 6,113 kg ha^{-1}, above the national average of 5,057 kg ha^{-1}.

Although in the last 10 years there have been significant gains in maize productivity, mainly due to genetic improvement and improvement in crop management conditions, the average yield of Brazilian crops is still modest in view of its productive potential. One of the bottlenecks in the sector refers to nitrogen fertilization, since nitrogen is the most demanded nutrient by maize, and the one that most frequently limits grain yield (Lemaire & Gastal, 1997).

According to Fancelli & Dourado-Neto (2000), maize productivity is the result of several integrated factors, such as number of rows per ear, number of grains per ear and grain mass, which are the main components of crop yield. Grain formation in maize is closely related to the translocation of sugars and nitrogen from vegetative organs, especially from leaves to grains (Vorpagel, 2010).

Although N is the most abundant element in the atmosphere, plants of the family Poaceae, such as maize, cannot assimilate atmospheric N_2 due to the triple bonding between N_2 atoms, as opposed to Fabaceae species, which, given their symbiotic relationship with bacteria of the genus *Rhyzobium*, can extract the element from the atmosphere. This way, in order to reach high yield indices in maize crop, the addition of nitrogen fertilizers is necessary.

Maize is a crop that removes large amounts of nitrogen from the soil, therefore requiring high doses of the nutrient, which leads to an increase in the production cost. The N applied via mineral fertilizer, besides being a product obtained with the burning of fossil fuels, is susceptible to high losses by volatilization and leaching when applied to the crop. The latter loss can represent contamination of surface and underground waters. In recent decades, studies in search of new technologies that may contribute to the reduction of the use of nitrogen fertilizers in maize crop have been intensified.

In this sense, several bacteria present in the soil have the capacity to exert beneficial effects on crop growth, including the ability to fix atmospheric N. Bacteria of the genus *Azospirillum* are characterized by being free-living, and for many years have been known as plant growth promoters, being isolated in the rhizosphere of several grasses and cereals around the world, both in tropical and temperate species, presenting beneficial effects in experiments under greenhouse conditions and also under field conditions (Steenhoudt & Vanderleyden, 2000). For Hungria et al. (2010), inoculation with bacteria to promote plant growth has become interesting in view of the high costs of industrially produced fertilizers, the growing concern about environmental pollution and the search for sustainable agricultural systems.

Therefore, this work aimed to evaluate the possibility of reducing nitrogen fertilization in maize crop and the maize grain production in response to inoculation with *Azospirillum brasilense* and N doses.

Material and methods

The experiment was carried out at the experimental farm of the Universidade Tecnológica Federal do Paraná, Dois Vizinhos Campus, from November 2012 to March 2013. The soil is classified as Red Latosol, and the region is situated at an altitude of 520 m, latitude of 25°44” S and longitude of 53°04” W, with humid mesothermal subtropical climate (Cfa), according to the Köppen classification (Alvarez et al., 2013).

During the winter, the area where the work was installed was under black oat (*Avena strigosa* L.) cultivation; after flowering, the area was desiccated with the herbicide glyphosate (2.0 liters ha^{-1} Roundup Transorb).

The experiment was arranged in a randomized block design with 20 treatments and three replicates each. The treatments were distributed in a factorial arrangement composed of two maize hybrids (AS1572 VTPRO and 30B39H) and different levels of N (0, 60, 120, 240 and 480 kg ha^{-1}), inoculated or not with *A. brasilense*. Each plot had nine rows, with dimensions of 5.0 x 5.28 m, and the borders were disregarded from the evaluations.

The maize was established with a no-tillage seeder set with a spacing of 0.66 m between rows and 0.25 m between plants in the line, aiming to obtain a population of approximately 60,000 plants ha^{-1}. Seeding occurred on November 8, 2012, using the cultivars AS1572 and 30B39H, both simple hybrids, of early cycle, recommended for silage and grain, with resistance to fall armyworm, lesser cornstalk borer and corn earworm. Weed control was performed by applying the herbicide atrazine + simazine (2.0 L ha^{-1} Extrasin SC) at growth stage V2 to V3.

Prior to seeding, the seeds were inoculated with *A. brasilense* bacteria, strains AbV5 and AbV6, using 200 mL ha^{-1} of the commercial inoculant Azototal, containing at least $2x10^8$ cfu mL^{-1}. The plots without inoculation were sown first and, subsequently, the inoculated plots were sown, thus avoiding seed contamination. 50 kg ha^{-1} P_2O_5 and 75 kg K_2O ha^{-1} were used as base fertilizer; nitrogen fertilization was applied in coverage during the V6 stage using commercial fertilizer urea with 46% nitrogen.

During flowering, three plants per plot were randomly selected to determine the total height and the first ear insertion height. After determining the height, the leaves were removed to determine the leaf area index, using the formula (width x length x 0.75). After the measurements, the plants were conditioned in paper bags and taken to an oven with forced air circulation at 55 ºC for approximately 72 hours to determine the dry matter.

Harvesting was done manually when the grains presented approximately 16% moisture. The maize yield was determined in a useful area of $8m^2$ (2 lines x 0.8 m x 5 m), corrected to 13% moisture. Ten ears were randomly selected to determine the number of rows and the number of grains per row.

The results were submitted to F test at 5% probability, being submitted to Tukey test at 5% when significant, using the statistical software Assistat®.

Results and discussions

The interaction between cultivar AS1572 and cultivar 30B39H and the inoculation by *A. brasilense* was not significant, showing that the factors behaved independently in this work, according to Table 1. Campos et al. (2000) found similar results, not obtaining increased productivity for maize crop with the application of inoculant containing *A. brasilense*. The benefit of the inoculation of maize with diazotrophic bacteria depends on factors such as plant genotype, selected strains, interrelation between the two and the environment (Sala et al., 2007).

Table 1 - Yield of corn grains, cultivars AS1572 and 30B39H, submitted to inoculation by *Azospirillum brasilense*.

Cultivar	Grain yield (kg ha^{-1})		
	With *Azospirillum brasilense*	Without *Azospirillum brasilense*	Average $^{(*)}$
AS1572	8,371	8,074	8,222 a
30B39H	7,560	7,527	7,543 b
Average $^{(ns)}$	7,965	7,800	
CV (%)	8.01		

$^{(ns)}$: Non significant, F test ($p > 0.05$); $^{(*)}$ Significant, by F test ($p < 0.05$); Average values of the last column followed by different lowercase letters differ by the Tukey test ($p > 0,05$); CV: Coefficient of variation;

Although there was no interaction between the factors mentioned above, Didonet et al. (1996) report that there is a great deal of evidence that the inoculation of maize with *Azospirillum brasilense* is responsible for the increase in maize grain yield, mainly in high levels of nitrogen, which seems to be related to increased activity of photosynthetic enzymes and nitrogen assimilation. Okon & Itzigsohn (1995) reported that in 60 to 70% of the works with *Azospirillum* inoculation there was success with significant difference and an increase of productivity in the order of 5 to 30%. According to Vorpagel (2010), the contribution of bacteria of the genus *A. brasilense* can promote an increase in the surface of the roots and, consequently, in the volume of soil explored. In general, increased absorption of minerals and water in *A. brasilense*-infected cereals has been credited to the hormonal effect of the bacterium on promoting root growth (Bashand & Levanony, 1990). In addition, Saubidet et al. (2002) suggest that inoculation, therefore, does not replace nitrogen fertilizer, but promotes better absorption and utilization of the available N.

In this work, there was an interaction between hybrids and nitrogen doses for grain yield, according to Table 2.

Although the recommendation of nitrogen fertilization for maize crop is based on factors such as previous crop, level of organic matter in the soil and yield expectancy, this work demonstrates that the plant genotype is another important factor for the recommendations of nitrogen fertilization. The cultivar AS1572 presented better performance in relation to the cultivar 30B39H, with the highest yield being 9,326.98 kg ha^{-1}, when using 240 kg ha^{-1} N. In turn, the cultivar 30B39H obtained as best yield 7,896.29 kg ha^{-1}, when using 480 kg ha^{-1} nitrogen.

Table 2 - Yield of corn grains AS1572 and 30B39H, as function of rates of nitrogen.

Cultivar	Nitrogen (kg ha^{-1})				
	0	60	120	240	480
	Grain yield (kg ha^{-1})				
AS1572	7,546.12 aC	7,174.06 aC	8,051.26 aBC	9,326.98 aA	9,013.94 aAB
30B39H	6,654.79 bB	7,798.08 aAB	7,550.66 aAB	7,817.78 bAB	7,896.29 bA

Averages followed by the same letter, lowercase in the column and upper case in the row, do not differ by the Tukey test ($p > 0.05$).

Vorpagel (2010) reports that nitrogen plays a fundamental role in the plant life, acting as a constituent of chlorophyll, nucleic acids, phytochromes, coenzymes, enzymes and proteins. The nutrient availability may affect the leaf area development, impairing light interception and, consequently, the efficiency of the process of photosynthesis and grain filling (Campos et al., 2000). Hence, there is a positive correlation between grain yield and nitrogen fertilization levels. Ulger et al. (1995) reports the importance of applying N with the correct source and dose in order to promote better plant utilization. Mello (2012), in studies on maize hybrids and nitrogen doses in the state of Rio Grande do Sul, points to the interaction between the genotypes and the nitrogen rates used.

Conclusions

No significant interaction was observed between the tested hybrids and the inoculation by *A. brasilense*. The cultivar AS1572 obtained better grain yield when compared to the cultivar 30B39H, with less need for nitrogen.

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