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Combination of inoculation methods of *Azospirilum brasilense* with broadcasting of nitrogen fertilizer increases corn yield

Combinação de métodos de inoculação de *Azospirillum brasiliense* com adubação nitrogenada de cobertura aumenta produtividade de milho

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ABSTRACT

*Nitrogen (N) is the most limiting nutrient for corn production. Thereby, the goal of the paper was to evaluate inoculation methods of **Azospirillum brasilense** in order to partially supply N required by the crop. The experiment was carried out in Guarapuava, PR, Brasil, in 2011/2012 growing season. Randomized blocks with factorial 3 inoculation methods (seed treatment, planting furrow and non-inoculated control) x 5 doses of nitrogen (0, 75, 150, 225 and 300kg ha^{-1}) x 8 replications was used as the experimental design. Leaf are index, foliar nitrogen content, total chlorophyll, grains per ear and yield were evaluated. There was significant interaction between inoculation methods and nitrogen fertilization to leaf area index, but not for yield. Inoculation with the diazotrophic bacteria provided yield increase of 702kg ha^{-1} for inoculation in seeding furrow and 432kg ha^{-1} for inoculation in seed treatment compared to the control, but both treatments did not differ between each other. Furthermore, total chlorophyll, grains per ear and yield were positively affected, with quadratic response, by the nitrogen fertilization in broadcasting.*

Key words: *nitrogen fertilization, diazotrophic bacteria, seed treatment, planting furrow.*

RESUMO

*O nitrogênio (N) é o nutriente que mais limita a produtividade da cultura do milho. Dessa forma, o objetivo deste trabalho foi avaliar métodos de inoculação de **Azospirillum brasilense** para suprir em parte o nitrogênio requerido pela cultura. O experimento foi conduzido em Guarapuava, PR, Brasil, no ano agrícola de 2011/2012. Os tratamentos foram dispostos em delineamento de blocos ao acaso, em esquema fatorial 3 métodos de inoculação (tratamento de sementes, sulco de plantio e controle não inoculado) x 5 doses de nitrogênio (0, 75, 150, 225 e 300kg ha^{-1}), com oito repetições. As variáveis analisadas foram: índice de área foliar (IAF), teor de N foliar, clorofila total, grãos por espiga e produtividade. Houve interação significativa entre os métodos de inoculação e a adubação nitrogenada para a variável IAF, mas não para a produtividade. Concluiu-se que a inoculação com a bactéria proporcionou incremento na produtividade de 702kg ha^{-1} para inoculação no sulco de semeadura e de 432kg ha^{-1} no tratamento de sementes, comparado com o controle, mas não houve diferença significativa entre esses dois tratamentos. Além disso, o teor de clorofila total, número de grãos por espiga e produtividade foram afetados positivamente, obtendo-se resposta quadrática, com aplicação da adubação nitrogenada.*

Palavras-chave: *adubação nitrogenada, bactéria diazotrófica, tratamento de sementes, sulco de semeadura.*

INTRODUCTION

Nitrogen (N) is the most abundant element in the atmosphere and it is required in large amounts by plants. Since plants are not able to obtain N directly from the atmosphere, other sources such as biological fixation, organic matter and synthetic fertilizers could be used to supply the plant needs. The assimilation of N is a vital process for any plant because it is involved in growth and development and has remarkable effects on biomass production and final yield (AMADO et al., 2002).

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Biological nitrogen fixation is widely explored in legume crops, because it is a very effective method to supply N to the plants. One of the most successful examples is the use of ***Bradyrhizobium*** sp. in soybeans. However, differently from legume crops, biological nitrogen fixation for grass crops is in development, currently there is still no bacterial specie that could supply completely the N required by the plants.

Azospirillum is a genus of free-living bacteria that has been studied over the last years due to its ability to fix atmospheric N (BODDEY et al., 1995), and it has a huge potential to be used in inoculants as a N source for grass plants (BÁRBARO et al., 2008). There is evidence that the inoculation of corn seeds with ***A. brasilense*** is responsible for increasing the dry matter accumulation rate especially in the presence of high N levels, which seems to be related to the increased activity of photosynthetic enzymes and N assimilation (DIDONET et al., 1996).

High concentrations of N in the root zone are beneficial for promoting rapid initial plant growth and increasing grain yield (YAMADA, 1996). As noted by QUADROS (2014), inoculation of ***Azospirillum*** in corn associated with 50kg ha^{-1} N at the base had grain yield equivalent to using 130kg ha^{-1} N without inoculation, showing that somehow there is economy of N fertilizer when the inoculation is performed. This is very interesting and would be very helpful to reduce the need for N fertilizers and consequently the costs of production.

The traditional way of performing the inoculation of ***Azospirillum*** is through seed treatment. However, seed treatment is also a way to apply fungicides, insecticides and micronutrients, which could be toxic to the bacteria and consequently affect the effectiveness of the inoculation (VARGAS & SUHET, 1980). Therefore, other ways of inoculation such as in planting furrow should be considered. Planting furrow inoculation has the advantage over seed treatment by avoiding direct contact of the inoculant with toxic products applied on the seeds. In addition, planting furrow inoculation requires dilution of the inoculum, which improves the distribution of the bacteria in the soil by placing them where there are better conditions of temperature, humidity and it is near to the roots (VOSS, 2002), which has an advantage compared to application over the whole field.

The use of diazotrophic bacteria to supply N for grass crops would be very beneficial to production systems. However, there is lack of information about what the best inoculation method is and if there is any interaction of the inoculation with the normal practice of applying nitrogen fertilizer in broadcasting. Based on that, the objective of the study was to evaluate the effect of different inoculation methods of ***A. brasilense*** combined with N levels applied in broadcast fertilization on corn yield and its components. In addition, potential economy of N fertilizer and the reduction of production costs were assessed.

MATERIAL AND METHODS

The experiment was carried out during the 2011/2012 growing season in Guarapuava, Paraná, Brasil. The soil of the experimental site is a Brown Latosol (Oxisol) with medium to high fertility. According to Köppen's classification, the climate of the region is Cfb, being temperate, with no defined drought season and mild summer. Soil sampling was performed before planting at 0-20cm depth. Results indicated: pH in $CaCl_2$: 4.76; organic matter: 39.60g dm^{-3}; phosphorous (P): 7.98mg dm^{-3}; potassium (K^+): 0.36$cmol_c$ dm^{-3}; calcium (Ca^{2+}): 4.27$cmol_c$ dm^{-3}; magnesium (Mg^{2+}): 3.60$cmol_c$ dm^{-3}; aluminum (Al^{3+}): 0.04$cmol_c$ dm^{-3}; H+Al: 6,49cmolc dm^{-3}; cation exchange capacity (CEC): 14,72$cmol_c$ dm^{-3}.

The experimental field has been conducted in a crop-livestock integration system for five years. Soybean was grown during the previous summer season (October-March), whereas black oat (***Avena strigosa*** L.) and ryegrass (***Lolium multiflorum*** Lam.) were grown during the previous winter (April-October). Lambs occupy the field in a continuous grazing system during the winter season. Corn was manual planted on 10/03/2011, using the hybrid P30F53 with a population of 67.500 plants ha^{-1}. The experiment was in a randomized complete block design with factorial three (inoculation method) x five (N levels) with eight replications. The inoculation treatments were: 1) inoculation in seed treatment, 2) inoculation in planting furrow, and 3) non-inoculated control. The N levels were: 0, 75, 150, 225 and 300kg ha^{-1}. The experimental units were 2.4m width by 7m length, being composed of four rows. Urea (46% N) was used as a source of nitrogen, and the application was split in 50% at V2 and 50% at V5 growth stages (RITCHIE et al. 1993). Masterfix L Gramíneas® was the inoculant used for the treatments and it is composed of ***A. brasilense*** strains Abv5 e Abv6. Inoculation was performed with 100mL ha^{-1} of the commercial product in seed treatment before planting and 300ml ha^{-1} in planting furrow after planting. The application was done with backpack sprayer, with flat jet nozzles, 0.60m spacing apart, pressure of 30lb pol^{-2}, with spray volume of 200L ha^{-1}.

Chlorophyll content was determined by SPAD method at R1 growth stage (FALKER, 2008). Five plants from the central rows of the plot were selected randomly for sampling, and the chlorophyll content was measured in the index leaf. Five index leaves were also collected for determination of nitrogen levels using blue idophenol method (EMBRAPA, 2009). Leaf area index (LAI) was determined by measuring the length and width of all leaves with more than 50% green area of three plants.

Corn ears were harvested manually when grain moisture was 16%. Twenty-four ears were harvested from the third row of the plot, avoiding 0.5m from each edge. Six ears were used for counting the number of rows and grains per row. By the end, all ears were threshed, the grains were weighted, grain moisture was measured and the weight was corrected considering 13% moisture and finally the yield was transformed to kg ha^{-1}.

Data were analyzed by ANOVA, and means were compared by Tukey's test ($P<0.05$) for the qualitative factor (inoculation) and by regression analysis for the quantitative factor (nitrogen levels) using SISVAR software. For the significant regressions, the mathematical model was selected based on the best representation of the variable, using Microsoft Excel. For the quadratic regressions, it was estimated the nitrogen level for the maximum technical efficiency (MTE) and maximum economic efficiency (MEE) based on the current price of corn R$ 0.37 kg^{-1} of corn and R$ 3.07 kg^{-1} of N (CONAB, 2015).

RESULTS AND DISCUSSION

Corn yield was significantly ($P<0.05$) affected by inoculation of ***A. brasilense*** and by increased levels of N in broadcast application, however, the interaction of these two factors was not significant (Table 1). Both forms of inoculation, seed treatment and planting furrow, provided significant yield increase compared to the non-inoculated control, but there was not difference between each other (Figure 1a). Inoculation of ***A. brasilense*** in seed treatment or planting furrow had an average increase in yield of 567kg ha^{-1} compared to the non-inoculated control. BARROS NETO (2008) also observed positive effect of inoculation with diazotrophic bacteria in seed treatment with increase of 357kg ha^{-1} and 793kg ha^{-1} in corn yield. Nevertheless, other authors mentioned no effect of inoculation (REPKE et al., 2013).

It is considered that response to inoculation may be influenced by genetic characteristics of the plant, bacterial strain and environmental conditions (HUNGRIA, 2011), and that in recent years many efforts have been directed to the selection and study of nitrogen-fixing bacteria in grasses. Therefore, the results of this study contradict the lack of response on corn yield observed by CAMPOS et al. (2000), which may be related to enhanced compatibility of ***A. brasilense*** strains currently available in the market.

Inoculation of ***A. brasilense***, N levels and the interaction of these factors significantly affected the LAI (Table 1 and Figure 1b). Nitrogen levels provided quadratic response of corn yield regardless of the inoculation method. The maximum LAI in R1 growth stage was 5.6m² with planting furrow inoculation of ***A. brasilense*** and 244kg of N ha^{-1} N; 5.4m² with seed treatment inoculation and 197kg of N ha^{-1}; and 5.4m² for the non-inoculated control at the dose of 211kg of N ha^{-1}. LAI depends on plant spacing, number and size of leaves, plant growth stage, soil fertility, environmental conditions and genetic material. The LAI is the ability of the plant to explore the space available for its development (PEREIRA & MACHADO, 1987). Crop productivity increases significantly with increments of LAI, which ranges from 4 to 6 (FANCELLI, 2010). LAI allows estimating the degree of development of the

Table 1 - P values for chlorophyll content (CL), yield (Y), grains per ear (GE), leaf area index (LAI) and leaf nitrogen content (LNC) influenced by broadcasting of doses of nitrogen fertilizer and inoculation methods of ***A. brasilense***. Guarapuava, PR, Brasil, 2015.

Source of variance	CL	Y (kg ha^{-1})	GE	IAF (m^{-2})	NF (g ka^{-1} de MS)
Blocks	0.00^{**}	0.00^{**}	0.30^{ns}	0.37^{ns}	0.64^{ns}
A. brasilense (A)	0.19^{ns}	0.00^{**}	0.35^{ns}	0.01^{**}	0.15^{ns}
Nitrogen levels (N)	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}
A x N	0.98^{ns}	0.98^{ns}	0.70^{ns}	0.03^{*}	0.74^{ns}
Coefficient of variation (%)	3.76	6.32	6.19	5.7	15.49
Mean	53.29	10.897,97	530.24	5.11	28.43

**Significant to 1% of probability; *significant to 5% of probability, ^{ns}no-significant at 5% of probability by F test.

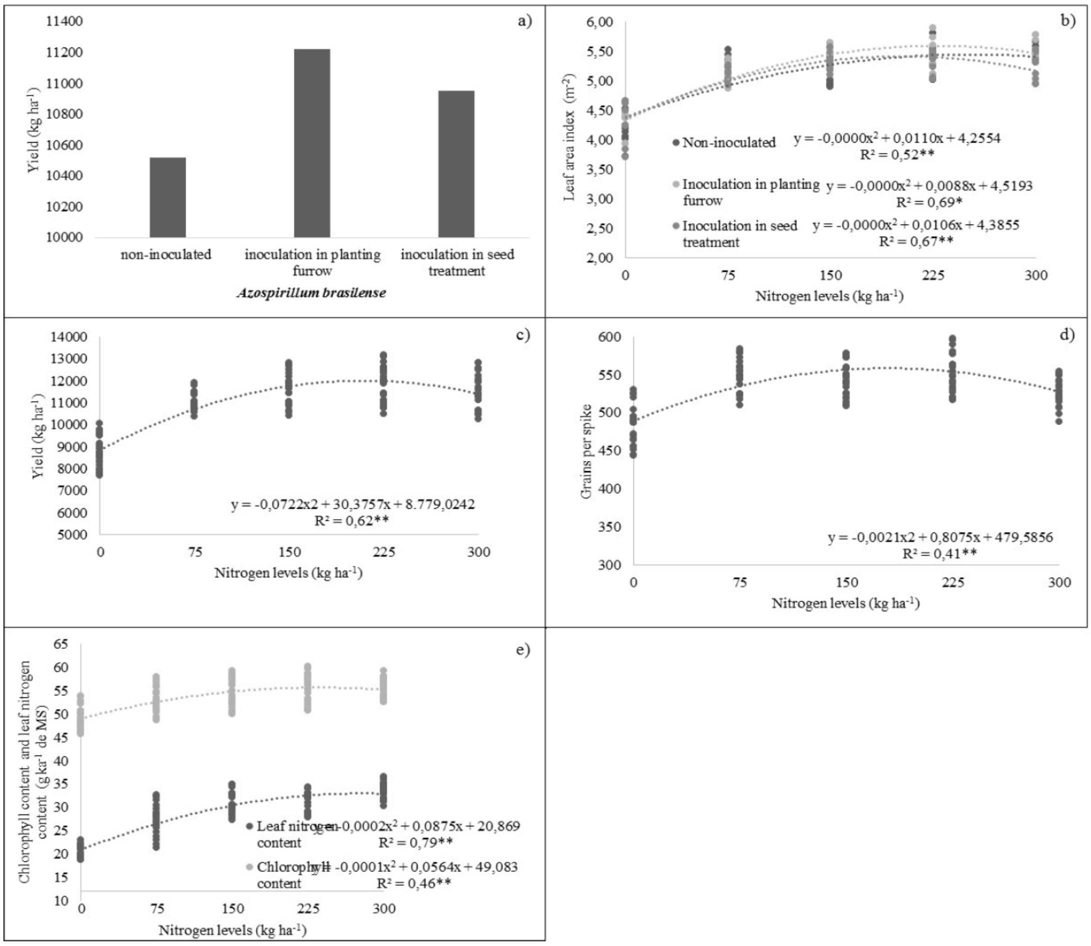

Figure 1 - a) Corn yield with inoculation of ***Azospirillum brasilense***; b) leaf area index; c) yield; d) grains per ear; e) chlorophyll relative content and leaf N content in this index leaf at the time of the R1 growth stage, influenced by nitrogen fertilization and inoculation of ***Azospirillum brasilense***. Guarapuava, PR, 2015. $^{**},^{*}$Significant at 1 and 5% probability by F test.

plant and the potential radiant energy interception (FANCELLI, 2000). Thus, we observed that the N dose that provided the maximum LAI was obtained with seed treatment inoculation of ***A. brasilense.***

The effect of inoculation of ***A. brasilense*** in corn with increase in yield and LAI could be due to two mechanisms. The first one is related to capacity of the bacterium to fix nitrogen from atmosphere (DOBBELAERE et al., 2003), which could supply partially the amount of the nutrient required by corn plants for their growth and development. The second mechanism relates to the production of plant hormones such as auxins, gibberellins and cytokinins (OKON & VANDERLEYDEN, 1997) that act on root morphology and physiology enhancing absorption of surface water and nutrients (PERIN et al., 2003). Nitrogen fixation is a process with high energy cost for the plant (FAGAN et al., 2007), so when large quantities of the nutrient are available in the soil, there is a reduction of the biological fixation (MENDES et al., 2003). Based on the previous evidences and the results obtained in this study, the effect on the plant hormones seems to be the main factor responsible for the increases in corn yield.

Corn yield also showed a quadratic response to the N levels in broadcast application (Figure 1c), which is related to the increased availability of nutrients in the soil, root uptake, better distribution of the nutrient in the plant and consequently greater transfer to the grain, ensuring superior productivity (FANCELLI & DOURADO NETO, 2004). Based on the regression models, the MTE of 11,97kg ha^{-1} would be obtained with 210kg of N ha^{-1}, whereas the MEE would be obtained with 92kg of ha^{-1}. ARAUJO et al. (2004) observed 28% increase in corn yield with 240kg of N ha^{-1}. There was no significant interaction in corn yield between N levels and inoculation methods of ***A. brasilense***. This result may be related to the factors that influence the process of interaction between plant and bacteria such as strain type, nutritional status of the plant and the bacteria, plant genotype, environmental conditions and competition with other microorganisms (QUADROS, 2014).

Nitrogen rates were significant for total chlorophyll content, grains per ear and leaf N content (Table 1). In experiment conducted by MUCHOW (1988) showed that N fertilization increased the number of grains per ear and crop yield, and the supply of N promoted increase in leaf chlorophyll content and N in corn, corroborating with the results obtained in this experiment. Similar to what was found for productivity, the number of grains per ear had quadratic response to the application of N rate (Figure 1d). According to the data obtained by regression, the MTE would be achieved with 193kg of N ha^{-1}, with 557 grains per ear.

BORTOLINI et al. (2001), evaluating different N doses and application timing, found that the number of grains per ear was mostly associated component with grain yield. The number of grains per ear is a result of the number of rows per ear and kernels per row, which are defined in the early stages of plant development between the emission of 7^{th} and 9^{th} leaf (FANCELLI, 2000). Therefore, the N availability in these stages is crucial to ensure ears with high number of grains. SANDINI et al. (2011) found increase in number of rows, kernels per row and grains per ear, with increasing N rate, which is consistent with the results of this study.

Nitrogen is a key component of the chlorophyll molecule, playing an important role in photosynthesis. Increase in chlorophyll levels were observed up to 223kg of N ha^{-1}, resulting in 56g kg^{-1} DM (Figure 1e). The importance of measurement of chlorophyll content in the leaf is because it predicts the nutritional level of N in plants, correlating positively with N content present in the plant (BOOIJ et al., 2000).

The N content in the index leaf showed a higher concentration with 29.64g of N kg^{-1} at a dose of 200kg of N ha^{-1} (Figure 1e), ranging from 27.84 and 32.21g kg^{-1} for the implementation of hedging doses. Suitable levels of N in the leaves, according to MALAVOLTA et al. (1997) ranged from 27.5 to 32.5g of N kg^{-1}, indicating that the values obtained in this study are in accordance with what is considered appropriate for corn plants. The higher leaf N content provides greater growth and plant development, contributing to a greater LAI and accumulation of carbohydrates, depending on photosynthesis (SILVA et al., 2006). However, when comparing the behavior of the averages obtained by leaf N with the other variables assessed, we noted that high doses of N may have negative trends for revenue.

Information about the effect of planting furrow inoculation of ***A. brasilense*** in corn is scarce in the literature, which shows the relevance of this research to investigate the effect of inoculation methods. In addition, studies with planting furrow inoculation of nitrogen fixing bacteria in soybean have considered it as a viable practice (HUNGRIA et al., 2013), especially in order to minimize or prevent toxic effects on bacteria by insecticides, fungicides and micronutrients applied as a seed treatment (CAMPO et al., 2010).

CONCLUSION

Corn yield was increased by inoculation with ***A. brasilense*** regardless of the inoculation method in planting furrow or seed treatment, presenting itself as a viable technology for the corn crop. Broadcast application of nitrogen fertilizer was efficient for the variables analyzed for leaf area index, total chlorophyll content, leaf nitrogen, and number of grains per ear, increasing grain yield with the use of the dose of 225kg of N ha^{-1}.

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