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BASIC AREA - Article

Plant growth-promoting bacteria associated with nitrogen fertilization at topdressing in popcorn agronomic performance

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ABSTRACT: The use of plant growth-promoting bacteria is a promising alternative with low environmental impact to increase the efficiency of use of chemical fertilizers, ensuring high yield with better cost-effective ratio. In maize crops, several studies have demonstrated an increased yield when Azospirillum-based inoculants are used. In the case of popcorn, there are no available studies related to use of inoculation and its response on yield parameters. Thus, the aim of this study was to evaluate the field performance of popcorn when inoculated with the commercial product Masterfix L (A. brasilense Ab-V5 and A. brasilense Ab-V6) and the non-commercial inoculant UEL (A. brasilense Ab-V5 + Rhizobium sp. 53GRM1) associated with nitrogen fertilization. The trials were conducted in Londrina and Maringá, Paraná State, Brazil, in a randomized block design with four replications, in a split plot design with the inoculation treatments located in the plots (uninoculated, Masterfix L, and UEL) and the different N rates located in the subplots where ammonium sulphate was applied in the topdressing at the V6 stage (0, 50, 100, and 150 kg·ha^{-1}). The variance analysis showed significant effects ($p < 0.05$) of inoculation (Londrina environment) and N rates (both environments) only for grain yield. There was no inoculation effect in the grain yield when inoculants were applied together with N-fertilization at topdressing. In the absence of N-fertilization at topdressing, the inoculants Masterfix L. and UEL promoted higher grain yield as compared to the uninoculated plants, with resulting increases of 13.21 and 26.61% in yield, respectively.

Key words: *Azospirillum brasilense*, *Rhizobium* sp., inoculants, grain yield.

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INTRODUCTION

The use of chemical fertilizers, along with breeding and crop management, has provided a significant increase in maize yield (Galvão et al. 2014). However, the dependence of the current agriculture on chemical fertilizers raises great concern, due to the risks associated with the indiscriminate use of these inputs, such as eutrophication of soil and groundwater and emissions of greenhouse gases (Chien et al. 2011; Dungait et al. 2012; Marks et al. 2013). In addition to environmental problems, chemical fertilizers participate in more than 28% of the maize crop production costs, according to CONAB (2015).

Among the alternatives to increase the use efficiency of chemical fertilizers, which may decrease the amount applied to crops, is the use of inoculants containing plant growth-promoting bacteria (PGPB), which comprise a group of microorganisms that stimulate the growth and development of plants through direct and/or indirect mechanisms, coexisting associatively on root surfaces, the rhizosphere and phyllosphere, and internal tissues from different plant species (Hungria et al. 2010). These bacteria act directly by supplying the plant demand for nutrients (P, K, Fe) and water, through direct growth-promoting mechanisms, or by increasing resistance and tolerance of plants to biotic and abiotic stresses through the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC); they act also as biocontrol agents and induce systemic resistance (Hungria et al. 2010; Brattacharyya and Jha 2012; Singh et al. 2013; Bashan et al. 2014).

PGPB have been found in association with a large number of species of cereals and forage grasses, with a wide range of described genera including *Pseudomonas*, *Burkholderia*, *Bacillus*, *Bradyrhizobium*, *Rhizobium*, *Gluconacetobacter*, *Herbaspirillum* and *Azozpirillum* (Hungria et al. 2010; Videira et al. 2012). Among these genera, *Azospirillum* is one of the best studied groups, with several ones related to the production of phytohormones that induce root growth and improve the absorption of water and nutrients by plants (Doornbos et al. 2012). Furthermore, as well as rhizobia, *Azospirillum* bacteria are able to fix atmospheric nitrogen and thus can directly contribute to the nitrogen nutrition of various non-legume species, promoting their growth and decreasing the use of nitrogen fertilizers without decreases in yield (Hungria et al. 2010; Ferreira et al. 2013).

Based on several studies, the contribution of *Azospirillum* to maize yield has shown contrasting results (Hungria et al. 2010; Ferreira et al. 2013; Repke et al. 2013; Quadros et al. 2014). Repke et al. (2013) found that the application of *A. brasilense* (AZ) solution to seeds, with or without synthetic nitrogen levels, showed no effect on plant development and yield of maize. On the other hand, Hungria et al. (2010) evaluated the use of AZ on maize and observed an increase of 27% in yield compared to the control without inoculation. Ferreira et al. (2013) analyzed the response of corn to AZ inoculation in soils of the Brazilian Cerrado and recorded yield increases of 14.9, 26.7 and 29.4% in the treatments P-K + AZ, P-K + AZ + 100 kg N and P-K + AZ + 200 kg N, respectively, when compared with the control (P-K). Quadros et al. (2014) investigated the yield in three maize hybrids inoculated with a mixture of three *Azospirillum* species (*A. brasilense*, *A. lipoferum* and *A. oryzae*) and detected an interaction between hybrids and inoculation, obtaining a significantly increased yield only for hybrid P32R48, with increase of 750 kg·ha^{-1}.

The inoculation response may vary according to the genotype of the plant, bacterial strain, environmental conditions, agricultural practices, as well as the number and quality of PGPB cells used as inoculant (Matsumura et al. 2015). In the case of popcorn maize, to the best knowledge of the authors, there is no literature related to the use of inoculants containing PGPB and responses on the development and yield of the crop. Thus, this study aimed to evaluate the effect of inoculation of the commercial product Masterfix L, formulated with two strains of *Azospirillum brasilense* (*A. brasilense* Ab-V5 and Ab-V6), and a non-commercial inoculant developed at the State University of Londrina (inoculant UEL) formulated with a strain of *Azospirillum brasilense* and a strain of *Rhizobium* sp. (*A. brasilense* Ab-V5 and *Rhizobium* sp. 53GRM1) — under different levels of nitrogen fertilizer as top dressing — on the agronomic performance of popcorn maize.

MATERIAL AND METHODS

The experiments were conducted in the farms of the State University of Londrina (UEL) and State University of Maringá (UEM), in the 2014/2015 growing season.

The farm of UEL is located in Londrina, Northern Paraná State (geographic coordinates: 23°20’32”S and 51°12’32”W; altitude: 550 m). The soil is characterized as eutrophic Red Latosol (ERL). The farm of UEM is located at the Iguatemi District, Maringá, Northern Paraná State (geographic coordinates: 23°11’S and 52°03’W; altitude: 550 m). The soil is characterized as dystrophic Red Latosol (DRL). The climate of the regions of Londrina and Iguatemi is Cfa, according to the Köppen classification. Data on average rainfall as well as average maximum and minimum temperatures, observed in the study period, were obtained from a weather station located at the headquarters of the Agronomic Institute of Paraná (IAPAR) in Londrina, Paraná State (Figure 1).

The chemical properties of the soil (0 – 20 cm) before installing the experiments, Londrina and Maringá, were: pH ($CaCl_2$): 5.50 and 4.60; P: 8.55 and 12.89 mg·dm^{-3}; K: 1.60 and 0.28 cmol$_c$·dm^{-3}; Ca: 5.30 and 2.45 cmol$_c$·dm^{-3}; Mg: 2.20 and 0.81 cmol$_c$·dm^{-3}; and CTC: 13.38 and 7.51 cmol$_c$·dm^{-3}, respectively.

In each experiment, soil was prepared by plowing and light harrowing and fertilized with 300 kg·ha^{-1} of the commercial mix 8:28:16, at sowing. The experiments were arranged in a split plot randomized block design with four replications, with inoculants assigned to the plots (without inoculant; commercial inoculant: Masterfix - *A. brasilense* Ab-V5 and Ab-V6; inoculant UEL - *A. brasilense* Ab-V5 and *Rhizobium* sp. 53GRM1), and the nitrogen levels at the V_6 stage assigned to subplots

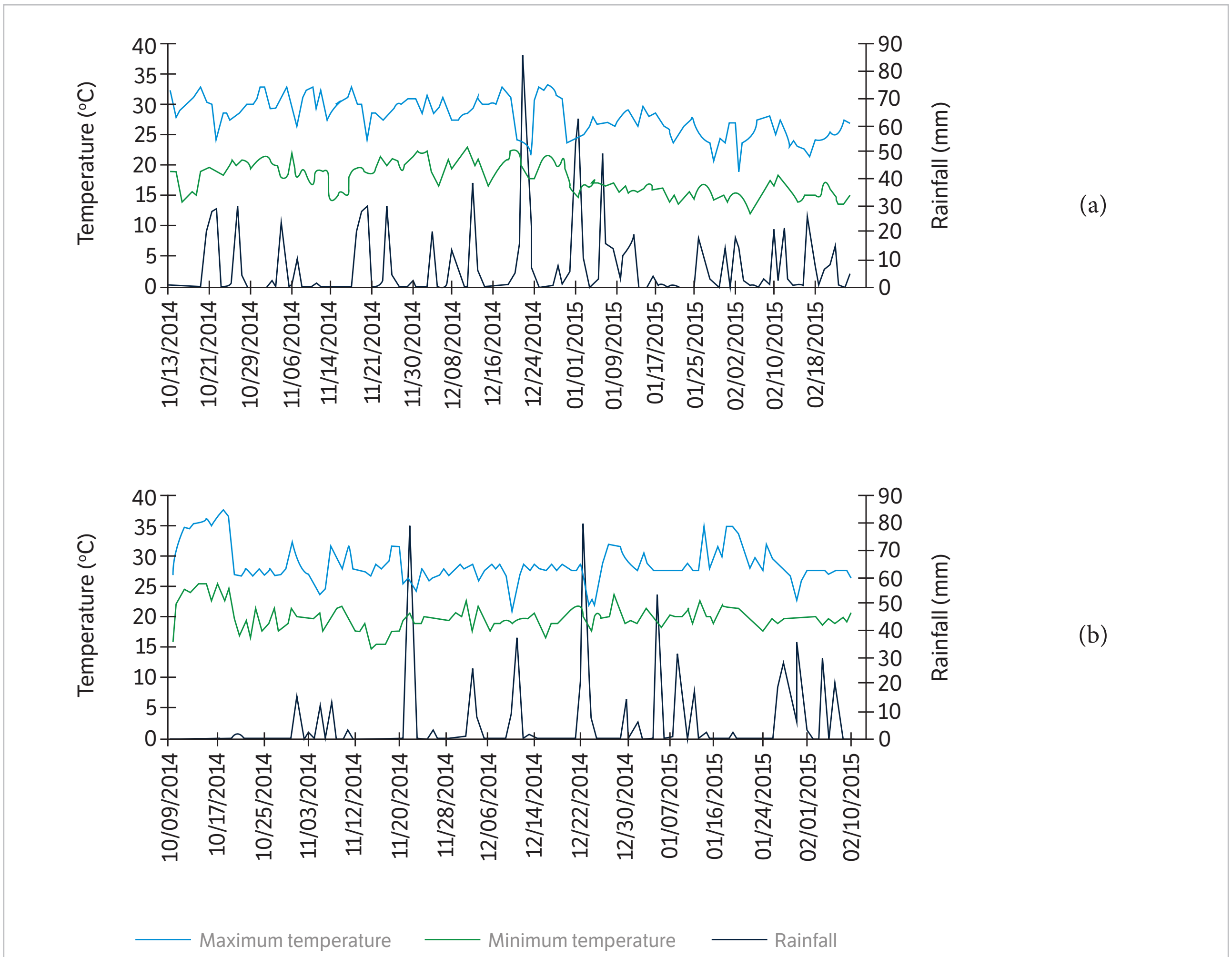


Figure 1. Daily data of maximum and minimum temperatures and rainfall during the experimental period. (a) Londrina and (b) Maringá (PR) (2014/2015).

using ammonium sulphate as a source of nitrogen (0, 50, 100 and 150 kg·ha^{-1}).

The different inoculants were applied to provide a bacterial concentration of 1 × 10^6 cells per seed. Bacteria used in the inoculant UEL are deposited in the collection of bacterial cultures of Molecular Biochemistry Laboratory, State University of Londrina (LBM-UEL). Isolated colonies of Ab-V5 and 53GRM1 were grown as a pre-inoculum in Dygs liquid medium (2 g glucose, 1.5 g peptone, 2 g yeast extract, 0.5 g K_2HPO_4; 0.5 $MgSO_4$, and distilled water q.s.p. 1 L, pH 6.0) for 24 h; then they were grown in 250 mL M15 liquid medium — composition formulated by LBM-UEL, under patenting process of the Brazilian National Institute of Industrial Property (INPI; BR deposit 1020140171746) — and cultured for 48 h under stirring in an orbital incubator (180 rpm at 28 °C). After this period, the cultures were quenched and the cell concentration was determined by counting in a Neubauer chamber, and then cell suspension was normalized to 1 × 10^9 cells mL^{-1} with dilution in liquid inoculant vehicle UEL.

The subplots were 3.2 × 4.0 m in size and consisted of four rows of 4.0 m length, with the working area represented by the two central rows. The spacing between rows was 0.8 m. Within the row, the spacing was 0.2 m between holes, which resulted in 20 holes per row, where two seeds of the hybrid popcorn maize IAC 125 were sown in each hole. The working area was formed by the two central rows, eliminating 0.5 m at the ends. Sowing was performed manually in the first half of October of each year. Thinning was held at the V_2 stage leaving one plant per hole.

The following variables were evaluated: plant height (PH, in cm), ear height (EH, in cm), number of ears per plant (NEP) and grain yield (GY, in kg·ha^{-1}). Individual analysis of variance was run in order to estimate the residuals associated with plots and subplots. Significant interactions between factors were unfolded. The qualitative factor (inoculation) was subjected to the comparison of means by Tukey's test ($p < 0.05$) and the quantitative one (N fertilizer levels as top dressing), to linear regression analysis. The best fit equation was chosen according to the regression coefficient, tested by the t-test corrected on the basis of residues from the analysis of variance. Data were analyzed with the aid of the R software (http://www.r-project.org) using the ExpDes package.

RESULTS AND DISCUSSION

The individual analysis of variance evidenced significant effects for inoculation and N levels only for grain yield in the Londrina environment. In Maringá, this difference was also detected for grain yield, but only for N levels. Repke et al. (2013) evaluated two levels of *A. brasilense* (with and without the application of the bacterial solution to the seeds) at six levels of N as top dressing (0, 80, 105, 130, 155 and 180 kg·ha^{-1}) and observed no effects from the application of inoculant on maize seeds and interaction between inoculant *versus* nitrogen levels for all variables. Dartora et al. (2013) investigated the inoculation of seeds (without *A. brasilense* — Ab-V5, *Herbaspirillum seropedicae* — SmR1 and combination Ab-V5 and SMR1) with five levels of N (0, 40, 80, 120 and 160 kg·ha^{-1}) and verified effects of inoculation only for stem diameter (vegetative and reproductive phase) and dry weight of shoots. Moreover, significant results for grain yield when inoculated with *A. brasilense* have been reported in several studies (Hungria et al. 2010; Ferreira et al. 2013; Quadros et al. 2014).

The unfolding of the interaction inoculation *versus* N levels was carried out despite the lack of significant interaction for both experiments. According to Barbin (2013), although the interaction was not significant, it is often advisable to unfold the degrees of freedom, because it can detect any significant effect diluted in the mean value (interaction). By the unfolding of the interaction inoculant *versus* N levels (0, 50, 100 and 150 kg·ha^{-1}), only the absence of N was significant for both experiments.

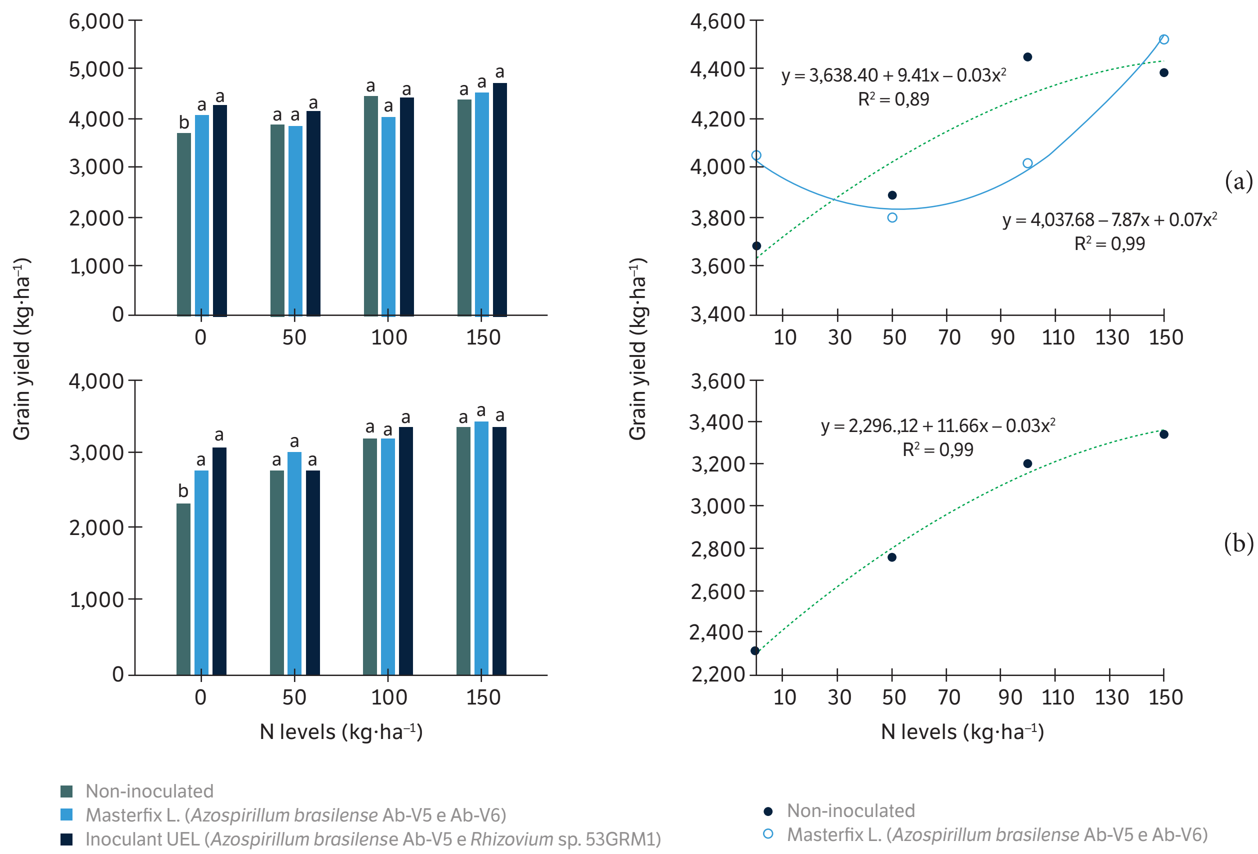
In this experiment, we found that the application of N-fertilizer as top dressing did not provide any yield increase to inoculation treatments, suggesting that these technologies are competitive and not additive. According to Carvalho et al. (2014), biological fixation efficiency in diazotrophic bacteria is rapidly reduced or even inhibited in the presence of high levels of nitrogen compounds in the soil, especially ammonia, which can cause the rapid inhibition of nitrogenase activity, responsible for the conversion of atmospheric nitrogen (N_2), the most accepted form of nitrogen by the plant. Beyond the control of nitrogenase activity, there is evidence that the N content in the soil can also regulate bacterial colonization.

Moreover, Oliveira et al. (2003) found that the number of diazotrophic bacteria isolated from sugar cane tissue decreases in plants fertilized with high levels of N compared with the number of bacteria in plants treated with low doses of N-fertilizer. The determination of the nitrogen use efficiency and the balance of this element under inoculation conditions *versus* levels of N-fertilizer are required to determine how inoculation with diazotrophic bacteria contributes to nutrition of inoculated plants.

Matsumura et al. (2015) assessed the effects of nitrogen fertilization on the diversity of the total community and active metabolites of endophytic bacteria in maize plants inoculated with *A. brasilense* Ab-V5. The authors verified that the fertilization management had a strong effect on the dominant populations of the metabolically active bacterial community, and 16S rRNA gene libraries of plants with regular N level suggest a lower diversity of such populations in comparison with the libraries of plants with low N. Additionally, the combination of treatments with regular N levels and plant inoculation showed no additive effect, but a downward trend in yield.

In the absence of top dressing nitrogen, there was a significant effect of commercial and UEL inoculants on the non-inoculated for experiments Londrina and Maringá (Figure 2). The grain yield increase was 9.98 and 16.44% (corresponding to 367.03 and 604.93 kg·ha^{-1}) for commercial and UEL inoculants, respectively, for the Londrina environment while, in the Maringá environment, the increase was 20.14 and 32.81% (corresponding to 465.25 and 757.98 kg·ha^{-1}), respectively.

The commercial formulation contains two strains of *Azospirillum brasilense* registered in the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) for use in commercial formulations (Hungria et al. 2010), while the inoculant of UEL contains the strain Ab-V5 of *A. brasilense*



Means followed by the same letter are not statistically different by Tukey's test ($p < 0.05$).

Figure 2. Grain yield (kg·ha^{-1}), in popcorn maize IAC 125, according to inoculants in different N levels, in two locations(a) Londrina and (b) Maringá (PR) (2015).

(registered in MAPA) and the strain 53GRM1 of *Rhizobium* sp., a strain isolated from maize rhizosphere and without registration in MAPA. In addition, differences in chemical composition are difficult to analyze, since the commercial formulation composition is taken as an industrial secret as well as the inoculant formulation of UEL, which has application for intellectual property protection in INPI (deposit in BR 1020140171746). According to Oliveira et al. (2014), many factors influence the efficient use of inoculant technology, being the formulation a major factor. In order to develop and commercially produce inoculants, it is necessary an integration of physical, chemical and biological parameters in these products, thus allowing the maintenance of high populations of the target organism and a longer survival in shelf and in the field.

The unfolding of N levels *versus* inoculants (non-inoculated, Masterfix L and inoculant UEL) indicated significant effects for non-inoculated in both experiments and inoculant Masterfix L in the Londrina environment. For non-inoculated at different levels of N, it was obtained a quadratic response with significance level of 1% (Figure 2), in which, according to the obtained production function, the maximum grain yield (4,366.30 and 3,429.08 kg·ha^{-1}) would be theoretically achieved with the application of 156.83 and 194.33 kg·ha^{-1} N for Londrina and Maringá environments, respectively.

For the inoculant Masterfix L at different N levels in Londrina, it was found that the lowest yield (3,816.47 kg·ha^{-1}) would theoretically be achieved with the application of 56.21 kg·ha^{-1} N. In contrast, with inoculants Masterfix L (in Maringá) and UEL (in both environments), at different N levels, there was no significance, that is, increasing N levels did not influence the increase in yield.

Inoculation of grasses with PGPB has been applied in addition to the use of mineral fertilizers — disregarding the increase in nutrient use efficiency from interactions between *Azospirillum* and inoculated plants —, which are primarily observed in studies conducted in the absence or with reduced doses of fertilizers, especially nitrogen (Veresoglou and Menexes 2010). The practice of inoculation with PGPB alters the environment where the plant grows as a result of biochemical interactions between associative pairs and leads to changes in gene expression of the plant inoculated (Amaral et al. 2014).

Furthermore, Partida-Martínez and Heil (2011) introduced the cost-effective concept in associative interactions, in which production increases resulting from these interactions may occur only if the microorganisms provide benefits surpassing the metabolic cost of the plant to maintain large microbial populations associated. In this context, the inoculation of plants with PGPB, with activity on plant nutrition (facilitating nitrogen nutrition, for example), may serve as an energy sink if the cultivation environment presents full nutrition conditions to the plant. Once PGPB consist of heterotrophic organisms and require energy from the plant to remain active and at large populations, it is most likely that an efficient inoculation results in a neutral or even negative effect on plant yield (Hartmann et al. 2009)

CONCLUSION

In the absence of top dressing, Masterfix L and UEL inoculants were superior to non-inoculated with average increases in yield of 13.21 and 26.61%, respectively. N levels (50, 100 and 150 kg·ha^{-1}) as top dressing did not influence the increase in yield of popcorn maize IAC 125 when inoculated with Masterfix L (in the Maringá environment) and UEL (in both environments).

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REFERENCES

Amaral, F. P., Bueno, J. C. F., Hermes, V. S. and Arisi, A. C. M. (2014). Gene expression analysis of maize seedlings (DKB240 variety) inocuated with plant growth promoting bacterium *Herbaspirillum seropedicae*. Symbiosis, 62, 41-50. http://dx.doi.org/10.1007/s13199-014-0270-6.

Barbin, D. (2013). Planejamento e análise estatística de experimentos agronômicos. Londrina: Mecenas.

Bashan, Y., Bashan, L. E., Prabhu, S. R. and Hernandez, J. B. (2014). Advances in plant growth-promoting bacterial inoculant technology: formulations and pratical perspectives (1998-2013). Plant and Soil, 378, 1-33. http://dx.doi.org/10.1007/s11104-013-1956-x.

Brattacharyya, P. N. and Jha, D. H. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology, 28, 1327-1350. http://dx.doi.org/10.1007/s11274-011-0979-9.

Carvalho, T. L. G., Balsemão-Pires, E., Saraiva, R. M., Ferreira, P. C. G. and Hemerly, A. S. (2014). Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. Journal of Experimenal Botany, First published online: August 11, 2014, 1-12. http://dx.doi.org/10.1093/jxb/eru319.

Chien, S. H., Prochnow, L. I., Tu, S. and Snyder, C. S. (2011). Agronomic and environmental aspects of phosphate fertilizers varying in source and solubility: an update review. Nutrient Cycling in Agroecosystems, 89, 229-255. http://dx.doi.org/10.1007/s10705-010-9390-4.

Companhia Nacional de Abastecimento (2015); [accessed 2015 Nov 15]. http://www.conab.gov.br

Dartora, J., Guimarães, V. F., Marini, D. and Sander, G. (2013). Adubação nitrogenada associada à inoculação com *Azospirillum brasilense* e *Herbaspirillum seropedicae* na cultura do milho. Revista Brasileira de Engenharia Agrícola e Ambiental, 17, 1023-1029. http://dx.doi.org/10.1590/S1415-43662013001000001.

Doornbos, R. F., Van Loon, L. C. and Bakker, P. A. H. M. (2012). Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agronomy for Sustainable Development, 32, 227-243. http://dx.doi.org/10.1007/s13593-011-0028-y.

Dungait, J. A., Cardenas, L. M., Blackwell, M. S. A., Wu, L., Withers, P. J. A., Chadwick, D. R., Bol, R., Murray, P. J., Macdonald, A. J., Whitmore, A. P. and Goulding, K. W. T. (2012). Advances in the understanding of nutrient dynamics and management in UK agriculture. Science of the Total Environment, 434, 39-50. http://dx.doi.org/10.1016/j.scitotenv.2012.04.029.

Ferreira, A. S., Pires, R. R., Rabelo, P. G., Oliveira, R. C., Luz, J. M. Q. and Brito, C. H. (2013). Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. Applied Soil Ecology, 72, 103-108. http://dx.doi.org/10.1016/j.apsoil.2013.05.020.

Galvão, J. C. C., Miranda, G. V., Trogello, E. and Fritsche-Neto, R. (2014). Sete décadas de evolução do sistema produtivo da cultura do milho. Revista Ceres, 61, 819-828. http://dx.doi.org/10.1590/0034-737x201461000007.

Hartmann, A., Schmid, M., van Tuinen, D. and Berg, G. (2009). Plant-driven selection of microbes. Plant and Soil, 321, 235-257. http://dx.doi.org/10.1007/s11104-008-9814-y.

Hungria, M., Campo, R. J., Souza, E. M. and Pedrosa, F. O. (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant and Soil, 331, 413-425. http://dx.doi.org/10.1007/s11104-009-0262-0.

Marks, B. B., Megías, M., Nogueira, M. A. and Hungria, M. (2013). Biotechnological potential of rhizobial metabolites to enhance the performance of *Bradyrhizobium* spp. and *Azospirillum brasilense* inoculants with soybean and maize. AMB Express, 3, 21. http://dx.doi.org/10.1186/2191-0855-3-21.

Matsumura, E. E., Secco, V. A., Moreira, R. S., Santos, O. J. P., Hungria, M. and Oliveira, A. L. M. (2015). Composition and activity of endophytic bacterial communities in field-grown maize plants inoculated with *Azospirillum brasilense*. Annals of Microbiology, First published online: April 01, 2015, 1-14. http://dx.doi.org/10.1007/s13213-015-1059-4.

Oliveira, A. L. M., Canuto, E. L., Reis, V. M. and Baldani, J. I. (2003). Response of micropropagated sugarcane varieties to inoculation with endophytic diazotrophic bacteria. Brazilian Journal of Microbiology, 34, 59-61. http://dx.doi.org/10.1590/S1517-83822003000500020.

Oliveira, A. L. M., Costa, K. R., Ferreira, D. C., Milani, K. M. L., Santos, O. J. P., Silva, M. B. and Zuluaga, M. Y. A. (2014). Aplicações da biodiversidade bacteriana do solo para a sustentabilidade da agricultura. Biochemistry and Biotechnology Reports, 3, 56-77. http://dx.doi.org/10.5433/2316-5200.2014v3n1p56.

Partida-Martínez, L. P. and Heil, M. (2011). The microbe-free planta: fact or artifact? Frontiers in Plant Science, 2, 100. http://dx.doi.org/10.3389/fpls.2011.00100.

Quadros, P. D., Roesch, L. F. W., Silva, P. R. F., Vieira, V. M., Roehrs, D. D. and Camargo, F. A. O. (2014). Desempenho agronômico a campo de híbridos de milho inoculados com *Azospirillum*. Revista Ceres, 61, 209-218. http://dx.doi.org/10.1590/S0034-737X2014000200008.

Repke, R. A., Cruz, S. J. S., Silva, C. J., Figueiredo, P. G. and Bicudo, S. J. (2013). Eficiência da *Azospirillum brasilense* combinada com doses de nitrogênio no desenvolvimento de plantas de milho. Revista Brasileira de Milho e Sorgo, 12, 214-226.

Singh, R. K., Malik, N. and Singh, S. (2013). Impact of rhizobial inoculation and nitrogen utilization in plant growth promotion of maize (*Zea mays* L.). Bioscience, 5, 8-14. http://dx.doi.org/10.13057/nusbiosci/n050102.

Veresoglou, S. D. and Menexes, G. (2010). Impact of inoculation with *Azospirillum* spp. on growth properties and seed yield of wheat: a meta-analysis of studies in the ISI Web of Science from 1981 to 2008. Plant and Soil, 337, 469-480. http://dx.doi.org/10.1007/s11104-010-0543-7.

Videira, S. S., Oliveira, D. M., Morais, R. F., Borges, W. L., Baldani, V. L. D. and Baldani, J. I. (2012). Genetic diversity and plant growth promoting traits of diazotrophic bacteria isolated from two *Pennisetum purpureum* Schum. genotypes grown in the field. Plant and Soil, 356, 51-66. http://dx.doi.org/10.1007/s11104-011-1082-6.