ISSN: 1984-5529 Científica Jaboticabal v.46, n.1, p.95–100, 2018

http://dx.doi.org/10.15361/1984-5529.2018v46n1p95-100

Wheat cultivars submitted to seed inoculation with *Azospirillum brasilense* and nitrogen application in different environments

Cultivares de trigo submetidas à inoculação das sementes *com Azospirillum brasilense* e aplicação de nitrogênio em diferentes ambientes

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Recebido em: 04-01-2017; Aceito em: 02-10-2017

Abstract

The response of wheat to seed inoculation with *Azospirillum brasilense* is variable, depending on factors related to the interaction of the bacterium with the genotype, growing environment and nitrogen availability. The objective of this work was to evaluate the response of wheat cultivars to seed inoculation with two strains of *A. brasilense* (Ab-V5 and Ab-V6) and to application of nitrogen (N) doses in two environments. The experiments were conducted under field conditions in Eldorado do Sul (RS) and Itapiranga (SC), in the 2012 and 2013 growing seasons, under a randomized block design, in a 3 x 2 x 3 factorial arrangement, with three N doses (0, 50 and 100 kg ha^{-1}), with and without seed inoculation with *A. brasilense*, and three wheat cultivars (Mirante, Quartzo and TBIO Pioneiro). Nitrogen application increased wheat growth and grain yield, mainly in Eldorado do Sul (RS), where the soil of the experimental site shows lower fertility levels. Wheat seeds inoculation with two strains of *A. brasilense* showed variable responses in the growth and grain yield according to the cultivar, growing season and experimental site. Positive responses to seed inoculation were more consistent for the cultivar Mirante, in Eldorado do Sul (RS).

Additional keywords: diazotrophic bacteria; plant growth-promoting bacteria; *Triticum aestivum.*

Resumo

A resposta à inoculação de sementes de trigo com *Azospirillum brasilense* é variável, dependendo de fatores relacionados à interação da bactéria com o genótipo, o ambiente de cultivo e a disponibilidade de nitrogênio. O objetivo deste trabalho foi avaliar a resposta de cultivares de trigo à inoculação das sementes com duas cepas de *A. brasilense* (Ab-V5 e Ab-V6) e à aplicação de doses de nitrogênio (N) em dois ambientes de cultivo. Os experimentos foram conduzidos a campo, nos municípios de Eldorado do Sul (RS) e Itapiranga (SC), nas safras de 2012 e 2013, sob o delineamento em blocos casualizados, em arranjo fatorial 3 x 2 x 3, sendo três doses de N (0; 50 e 100 kg ha^{-1}), com e sem uso da inoculação das sementes com *A. brasilense*, e três cultivares de trigo (Mirante, Quartzo e TBIO Pioneiro). A aplicação de nitrogênio aumentou o crescimento e no rendimento de grãos do trigo, principalmente em Eldorado do Sul (RS), onde o solo da área experimental apresenta menores níveis de fertilidade. A inoculação das sementes de trigo com as duas cepas de *A. brasilense* apresentou respostas variáveis no crescimento e rendimento de grãos de acordo com a cultivar, o ano e o local de condução dos trabalhos. Respostas positivas à inoculação foram mais consistentes, na cultivar Mirante, em Eldorado do Sul (RS).

Palavras-chave adicionais: bactéria diazotrófica; bactérias promotoras de crescimento vegetal; *Triticum aestivum.*

Introduction

In wheat (*Triticum aestivum*), nitrogen (N) is the nutrient absorbed in greater quantity and the one that promotes greater effects on grain yield. Notwithstanding, less than 50% of the N applied via mineral or organic fertilizers is absorbed by plants (Halvorson et al., 2002). Low N use efficiency results in economic losses, since the application of nitrogen fertilizers represents a significant portion of the total production costs.

In contrast to the increase in wheat grain yield by using higher N rates, biological nitrogen fixation (BNF), or the promotion of increased plant N uptake by associative bacteria, becomes an alternative of lower cost and less environmental impact. Bacteria of the genus *Azospirillum* establish associative relations with wheat and have been increasingly highlighted by research. In Brazil, the isolation and selection of *A. brasilense* strains efficient in promoting wheat growth allowed the registration and commercialization of inoculants for this crop (MAPA, 2011). Results reported by

Hungria et al. (2010) showed that inoculation of wheat seeds with *A. brasilense* strains Ab-V5 and Ab-V6 promoted an increase in grain yield of up to 31%. This effect was attributed to the increase in macro- and micronutrient uptake and not specifically to nitrogen supply by BNF.

In addition to the BNF ability, other mechanisms of growth promotion by *Azospirillum* are reported in the literature, such as the production of nitric oxide and increased activity of the enzyme ACC deaminase, besides the production of plant growth regulators such as auxins (Bhattacharyya & Jha, 2012). These regulators interfere with plant growth by increasing root system growth, allowing the exploration of a larger soil volume and increasing nutrient and water uptake (Evseeva et al., 2011).

The wheat growth promotion through seed inoculation with *Azospirillum* is reported by several studies (Hungria et al., 2010; Veresoglou & Menezes, 2010; Silveira, 2012). Despite the positive results, there is a variation in the response as a function of genotype and growing environment (Sala et al., 2007). Moreover, competition with microorganisms, soil chemical and physical properties, environmental conditions and crop management practices (Sala et al., 2007; Días-Zorita & Canigia, 2008) may affect plant response to *Azospirillum* inoculation.

In view of the great variability of wheat response to seed inoculation with *A. brasilense* and the environmental concern in minimizing the use and losses of industrial N, a study was conducted to evaluate the effect of seed inoculation of three wheat cultivars with two strains of *A. brasilense*, in two growing environments and using different nitrogen rates.

Material and methods

The experiments were conducted under field conditions, in the 2012 and 2013 growing seasons, in two environments, Eldorado do Sul (RS) and Itapiranga (SC), both presenting subtropical climate with hot humid summer, type Cfa, according to Köppen classification (Pandolfo et al., 2002). In Eldorado do Sul (RS), the experiments were conducted at the Agriculture Experimental Station of the Federal University of Rio Grande do Sul (EEA/UFRGS), with an altitude of 46 m, in a soil classified as sandy clay loam Ultisol. In Itapiranga (SC), the experiments were conducted in the experimental area of FAI - Faculdades de Itapiranga, with an altitude of 350 m, in a typical Typic Haplustept. The results of the soil chemical analysis before the implementation of the experiment, in the 0-20 cm layer, were: pH (H_2O) = 5.8 and 5.8; P (Mehlich-1) = 55.5 and 33.1 mg dm^{-3}; K = 196 and 504 mg dm^{-3}; Organic matter = 34 and 36 mg dm^{-3}; Base saturation = 88 and 84%, respectively, in 2012 and 2013, for Itapiranga (SC), and pH (H_2O) = 5.5 and 5.6; P (Mehlich-1) = 32.0 and 34.0 mg dm^{-3}; K = 185 and 195 mg dm^{-3}; Organic matter = 19 and 16 mg dm^{-3}; Base saturation = 47 and 60%, respectively, in 2012 and 2013, for Eldorado do Sul (RS). The two experimental sites are located in Region 2, according to the wheat homogeneous adaptation regions, characterized as moderately warm, humid and with low altitude (Reunião..., 2015). Notwithstanding, the climate normals recorded from 1986 to 2013 in Itapiranga (SC) (Pandolfo et al., 2002) show higher average air temperature in July, August, September, October and November (14.2; 16.3; 17.8; 20.9 and 23 ºC, respectively), in comparison with the climate normals recorded from 1970 to 2009 in Eldorado do Sul (RS) (13.0; 14.5; 15.9; 18.7 and 20.8 ºC, respectively) (Bergamaschi et al., 2013). Furthermore, Itapiranga shows higher rainfall, mainly in the final three months of wheat cultivation, i.e., September, October and November (152.8; 243.8 and 142 mm, respectively), in comparison to Eldorado do Sul (142; 129 and 109 mm, respectively).

The treatments consisted of a 3 x 2 x 3 factorial, combining three nitrogen rates (0, 50 and 100 kg N ha^{-1} as urea), with 20 kg ha^{-1} applied at sowing and the rest applied as topdressing at the stage of six fully expanded leaves, two levels of seed inoculation with *A. brasiliense* (with and without inoculant application), and three wheat cultivars (Quartzo, Mirante and TBIO Pioneiro). In 2013, the cultivar TBIO Pioneiro was not used in Eldorado do Sul. The experimental design was a randomized block with split plots, and five (Eldorado do Sul, RS) and four replicates (Itapiranga, SC). The factors inoculation and N rates were located in the main plot and in the subplot, respectively.

For inoculation, a liquid commercial inoculant called Masterfix Gramíneas® containing the *Azopirillum brasilense* strains Ab-V5 and Ab-V6 was used, with 2.0 x 10^8 cells mL^{-1}, according to the manufacturer (Stoller). The inoculant was used in a dose of 300 mL ha^{-1} and homogenously mixed to the seeds immediately before sowing.

In Eldorado do Sul (RS), each plot was composed of 13 rows of 3 m in length and row spacing of 0.18 m, with a total area of 7.02 m^2. In Itapiranga (SC), each plot was composed of 7 rows of 3.5 m in length and row spacing of 0.17 m, with a total area of 4.17 m^2. At sowing, fertilization with P and K was performed, according to soil analysis, for a grain yield expectation of 5 Mg ha^{-1}.

Shoot dry biomass was determined at the stage of six fully expanded leaves (V6 stage) and at flowering by the sampling of plants in an area of 0.25 m^2. The plants sampled were oven-dried at 65 °C until constant weight and weighed in a semi-analytical balance. Subsequently, the N content in plant tissue was determined (Tedesco et al., 1995) and the amount of N accumulated in the shoots was calculated by multiplying the dry shoot biomass by its N content. Grain yield and its components (number of ears m^{-2}, number of grains ear^{-1} and grain weight) were determined by harvesting an area of 4.32 m^2 (Eldorado do Sul, RS) and 2.98 m^2

(Itapiranga, SC). After grain cleaning and weighing, grain yield data were corrected to 130 g kg^{-1} moisture. Data were submitted to analysis of variance by F-test using the software ASSISTAT version 7.7. When the F-test was statistically significant ($p \leq 0.05$), means were compared by Tukey's test at a 5% significance level.

Results and discussion

There was significant interaction for N rates and seed inoculation for the variable dry shoot biomass at the V6 stage, in Eldorado do Sul (RS), in the 2012 growing season, for cv. TBIO Pioneiro; for the amount of N accumulated at flowering, in this same site, in the 2013 growing season, for cv. Quartzo and cv. Mirante; as well as for the variable grain yield, in Itapiranga (SC), in 2012, for cv. TBIO Pioneiro. Regarding grain yield components, there was a significant interaction for the number of ears m^{-2}, in Eldorado do Sul, in 2013, for cv. Quartzo, and in Itapiranga, in 2012, for cv. TBIO Pioneiro; thousand grain weight, in Itapiranga, in 2013, for cv. TBIO Pioneiro; and number of grains per ear, in both sites, in 2012 for cv. Mirante (data not shown). For the other variables, the results for the main effect of N rate application and seed inoculation will be discussed.

In Eldorado do Sul (RS), there was effect of N rates for all variables evaluated in the two years, for the three wheat cultivars tested. In contrast, the effect of N rates in the experiments conducted in Itapiranga (SC) was variable according to the cultivar, growing season and variable considered. In 2012, the response to N application in this environment was significant in relation to the N accumulated in the shoots at the V6 stage only for cv. Mirante (Figure 1a), while at the flowering stage, the effect of N application occurred for all cultivars (Figure 1b). In 2013, a response of the N applied was observed for the amount of N accumulated at the V6 stage for cv. Quartzo (Figure 1c), in addition to shoot biomass (Figure 1d); N accumulated at flowering (Figure 1e) and grain yield for all cultivars (Figure 1f).

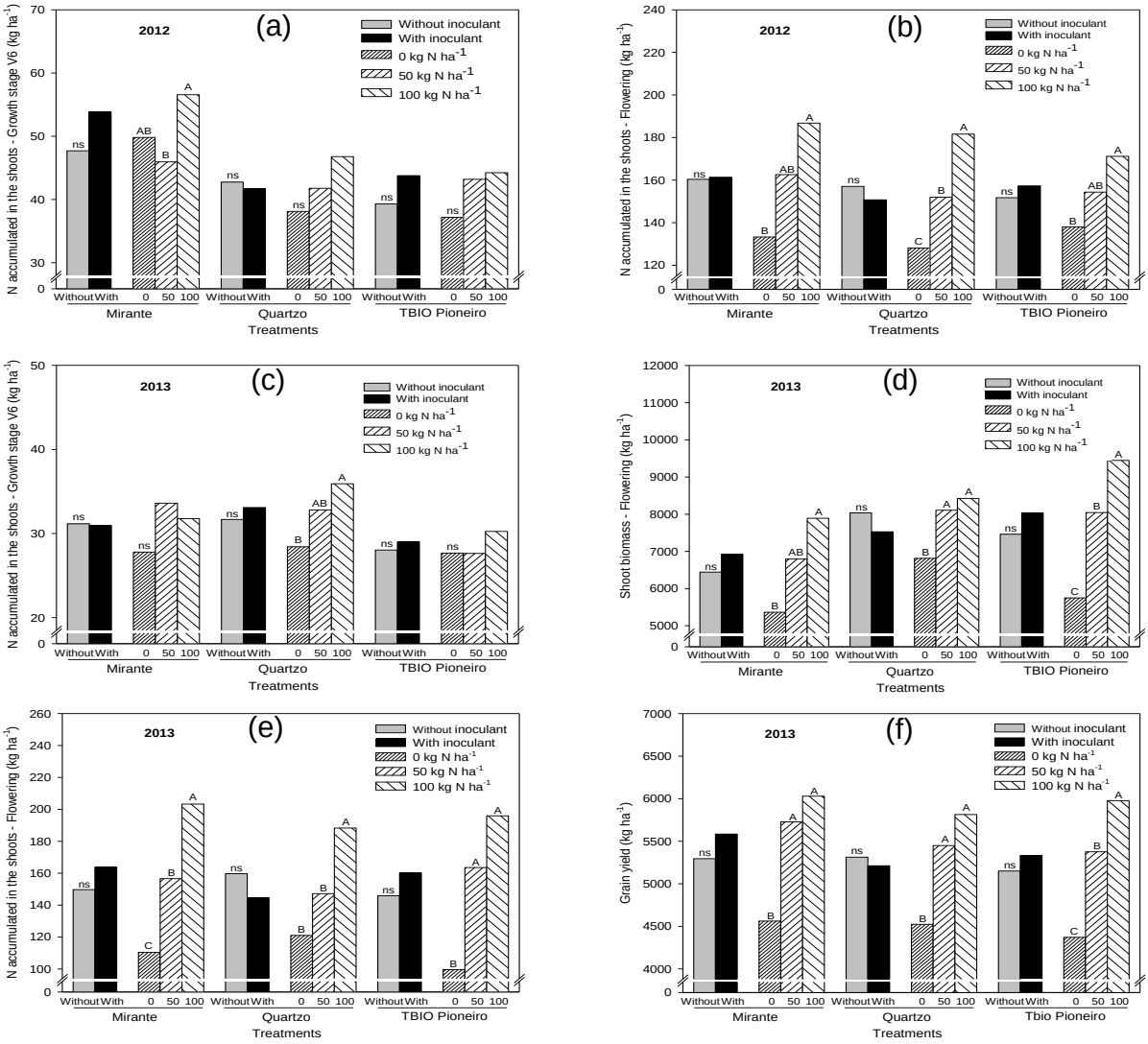

Figure 1 - N accumulated in the shoots in growth stage V_6 (1a and 1c) and flowering (1b and 1e), shoot biomass at flowering (1d and 1e) and grain yield (1f) of wheat cultivars, as affected by N application and seed inoculation with *Azospirillum brasilense*, in Itapiranga (SC) in 2012 and 2013. Lowercase letters compare inoculant levels and uppercase letters compare N rates in each cultivar (Tukey´s test, $p \leq 0.05$). ns - not significant.

The variability of soil fertility characteristics, especially the higher organic matter content of the experimental site in Itapiranga (SC) in relation to Eldorado do Sul (RS), may explain the different responses to N application in both environments. In Itapiranga (SC), the organic matter contents were 78.9 and 125% higher than those of Eldorado do Sul (RS) in 2012 and 2013, respectively. Notwithstanding, even in this condition, in 2013, the application of 100 kg N ha^{-1} provided increases in grain yield of approximately 1467, 1290 and 1607 kg ha^{-1}, respectively, for cv. Mirante, Quartzo and TBIO Pioneiro, in relation to the control without nitrogen fertilization (Figure 2c).

The response of the three wheat cultivars to seed inoculation with two strains of *A. brasilense* (Ab-V5 and Ab-V6) varied depending on year and growing environment. In 2012, in Itapiranga (SC), with the use of inoculant, cv. Mirante and cv. TBIO Pioneiro showed an increase in shoot dry biomass production at the V6 growth stage of 6 and 11%, respectively, compared to the treatment without seed inoculation (Figure 2a). Cv. TBIO Pioneiro also showed, as a function of seed inoculation, an increase of 8% in shoot dry biomass at flowering in this same year and site (Figure 2b). However, the higher shoot biomass production was not reflected in an increase in grain yield in Itapiranga (SC). This was probably due to adverse climatic conditions at this site, such as irregular rainfall distribution, and low precipitation and high air temperatures from the period before flowering until the end of the grain filling stage. Dobbelaere et al. (2001) also found that positive effects, such as increased shoot biomass production and N accumulated in biomass in the early stages of wheat growth promoted by the inoculation of diazotrophic bacteria, were not necessarily reflected in increased grain yield.

A significant increase in grain yield by seed inoculation, in relation to the treatment without inoculation, occurred only in Eldorado do Sul (RS) with cv. Mirante, in the 2012 growing season (Figure 2c). Inoculation increased grain yield by approximately 200 kg ha^{-1}, representing about 7.0% increase. This increase may be associated to the higher amount of N accumulated in the shoots at flowering with inoculation and application of 100 kg N ha^{-1} (Figure 2d) (increase of 37.3%) and, among the grain yield components, to the highest number of ears m^{-2} (Figure 2e) (7.2% increase, averaging the three N rates). In 2013, only the amount of N accumulated in the shoots at flowering for cv. Mirante with the dose of 100 kg N ha^{-1} showed significant effect of seed inoculation (Figure 2f), representing an increase of 31.2% in relation to the treatment without inoculation. Sala et al. (2007) also reported higher shoot dry matter production and higher amount of N accumulated with the inoculation of *Azospirillum* in wheat, attributing this effect to the production of phytohormones, such as auxins, and to the biological nitrogen fixation.

Considering that only the cultivar Mirante, in 2012 and in Eldorado do Sul (RS), showed a significant increase in grain yield by seed inoculation with *A. brasilense* (Figure 2c), obtaining this single result of the effect of seed inoculation in relation to the increase in grain yield is in contrast to the results reported by Hungria et al. (2010). These authors observed an increase in wheat grain yield of up to 31%, using the same strains of *A. brasilense* used in this experiment (Ab-V5 and Ab-V6). However, the wheat cultivars used in that study (BR-18 and EMBRAPA-16) were different from those used in the present one, which may explain the difference in the results. Dias-Zorita & Canigia (2008) observed a mean increase in wheat grain yield of 8% (260 kg ha^{-1}) in 70% of the 297 experiments conducted in the Argentine Pampa region. On the other hand, in regional and recent trials carried out in southern Brazil, many results without statistical contrast in the wheat growth promotion by inoculation of the two strains of *A. brasilense* were also obtained for different wheat cultivars and growing environments (Mello, 2012; Pires et al., 2014).

The variability obtained in the present study regarding the wheat responses to the inoculation of bacteria may be due to the fact that the two strains of *A. brasiliense* were isolated from corn plants (Hungria et al., 2010). It has been demonstrated that, in wheat plants, isolates of *Azospirilum* obtained from this same plant species were more efficient than those isolated from plants of different species (Baldani et al., 1986). In other poaceae, such as corn (Salamone et al., 1996) and rice (Hahn et al., 2016), the effect of genotype on the interaction with bacteria has also been demonstrated.

The results of the present study indicate that the technique of inoculation of wheat seeds with *A. brasilense* can be improved considering genotypes and the different environments. It is necessary to study new strains and inoculant formulations to increase inoculation efficiency so that this practice may eventually result in a reduction in the rate of nitrogen fertilizers applied and in an increase of wheat grain yield.

Conclusions

Nitrogen application up to the rate of 100 kg ha^{-1} increases growth and grain yield of wheat, even in soils with high fertility levels.

The response to seed inoculation with *Azospirillum brasilense* is associated with wheat genotype and cultivation on low organic matter soil (Eldorado do Sul-RS).

The wheat cultivar Mirante presented more consistent positive results regarding the inoculant use.

In a few situations, there was statistical significance for the interaction between seed inoculation and applied nitrogen rates, showing that the inoculation effect is independent of the amount of N provided by mineral fertilization.

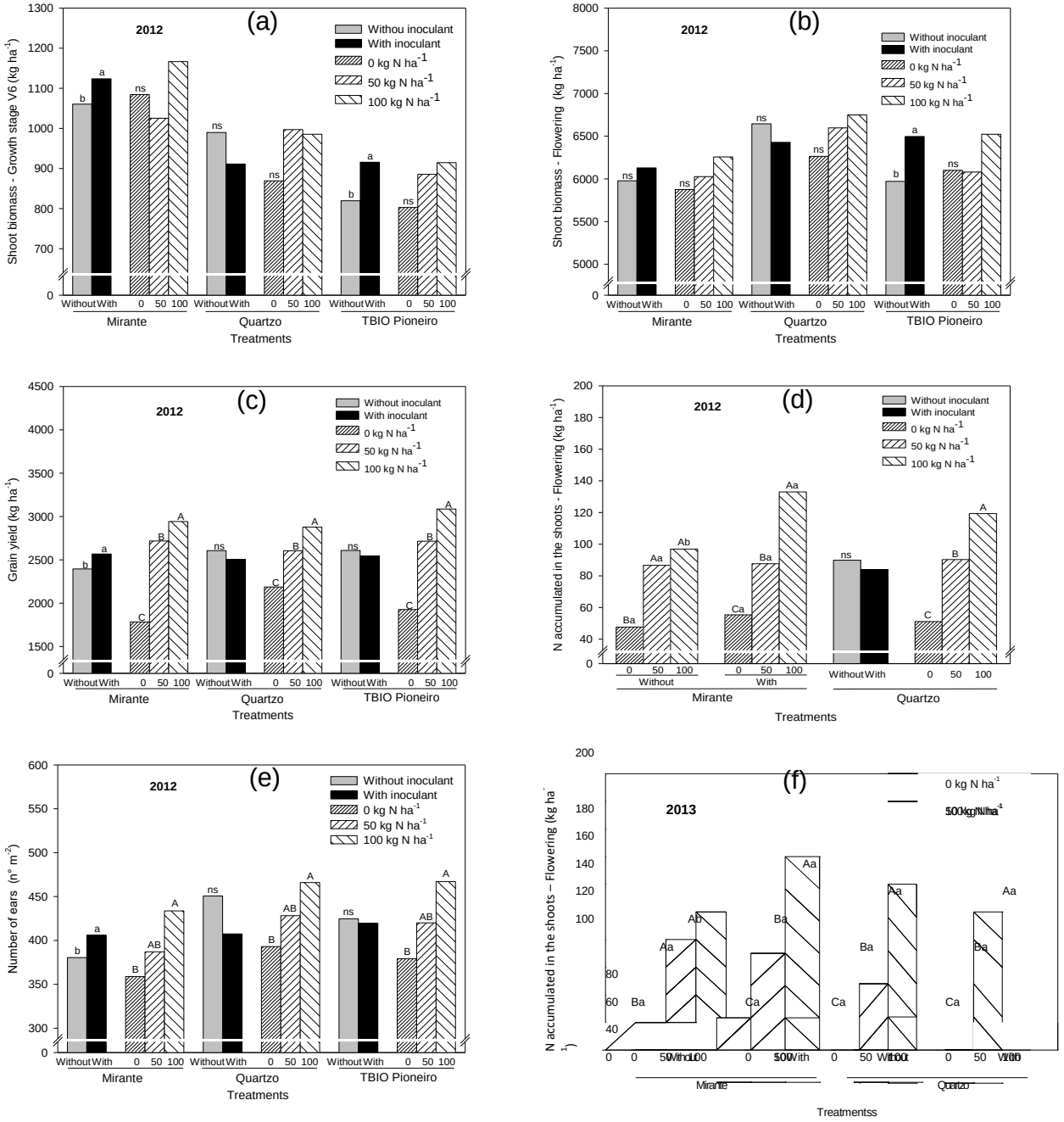

Figure 2 - Shoot biomass in growth stage V_6 (2a) and flowering (2b) in Itapiranga (SC) in 2012 and grain yield (2c), N accumulated in the shoots at flowering (2d and 2f) and number of ears m^{-2} (2e) in Eldorado do Sul (RS) in 2012 and 2013, as affected by N application and seed inoculation with *Azospirillum brasilense*. Lowercase letters compare inoculant levels and uppercase letters compare N rates in each cultivar (Tukey´s test, p≤0.05). ns - not significant.

Acknowledgements

To the Coordination for the Improvement of Higher Education Personnel (CAPES), for the scholarship granted to the first author.

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