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Inoculation of Maize Seeds With *Azospirillum* and Magnesium Through Foliar Application to Enhance Productive Performance

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Abstract

The present study aimed to evaluate the productive performance of maize crop when seed inoculated with *A. brasilense*, associated with different foliar doses of magnesium in the crop vegetative stages. For this, two essays were conducted in field conditions, one located in Laranjeiras do Sul-PR and the other in Entre Rios do Oeste-PR. A randomized blocks scheme was used, with a 3 × 2 factorial, being the treatments with magnesium (Mg): magnesium sulphate; magnesium oxide and without magnesium, and the presence or absence of seed inoculation with *A. brasilense*. The magnesium sources were supplied via foliar at the V_4 stage of the crop, using doses of 6 kg ha^{-1}. Evaluations were carried at R_1 determining the SPAD index and stem diameter and, at the end of the productive cycle, were evaluated production components and yield. In both sites no significant effects of foliar application with Mg were observed over the evaluated parameters. The *A. brasilense* inoculation provided an increase of 9.66% and 6.32% in stem diameter and of 6.8% and 6.24% in the SPAD index in Laranjeiras do Sul and Entre Rios do Oeste respectively, however, they did not increase production components and yield. It is concluded that the inoculation with *A. brasilense* increases of stem diameter values and SPAD index, in turn the foliar fertilization with different sources of magnesium do not interfere in the development and productivity of corn crop.

Keywords: *Zea mays* L., plant growth promoting bacteria, productivity

1. Introduction

Maize is the main cereal cultivated worldwide with a total production in 1.07 billion tons (USDA, 2018), with Brazil having a representability of about 7.62% of its global production with 82 million tons (CONAB, 2018), filling the third place in the global scenario of maize production.

Generally, maize yield is limited by series of factors among them are the environmental factors, pests, diseases, weeds, the management used and the plants mineral nutrition.

Thus, seeking an increase in the crop yield it is considered the seed inoculation with growth promoting bacteria (GPB), among them stands out the *Azospirillum brasilense* (Novakowiski et al., 2011; Dartora et al., 2013; Costa et al., 2015) which have many beneficial effects, for example, stimulus to plants development from the provision of vegetal hormones such as auxins, gibberellins and cytokinin (Kuss et al., 2007), providing in many cases more vigorous and productive plants (Cassán & Diaz-zorita, 2016), promoting higher nutrients absorption such as nitrogen, potassium and phosphorus (Hungria et al., 2010), besides reducing adverse effects caused by environmental conditions such as hydric deficit, by stimulus in the antioxidative vegetal system (Bulegon et al., 2016) and the crops gas exchanges (Bulegon et al., 2017).

Considering mineral fertilization for maize, the plants show better response to nitrogen, followed by potassium, phosphorus, calcium, magnesium and sulfur, when considering the essential nutrients (Cruz et al., 2008). The fertilization management with magnesium under commercial cultivation conditions is generally associated with liming by using dolomitic lime which have magnesium in its composition (Andreotti et al., 2001).

In the physiological and biochemical system, magnesium has an important rule for the photosynthesis, constituting the chlorophyll molecule and activating enzymes, being the ribulose 1,5-biphosphate (RuBP) (Vitti et al., 2006) the most relevant. The deficit of magnesium cases degradation of chloroplasts, thus, compromises photosynthesis, directly affecting the CO_2 diffusion.

More recently, some researchers started to consider the possibility of providing Mg by the foliar application, with results still in the beginning. In study with the foliar supply of Mg doses in soybean and maize was evidenced the potential of its use (Altarugio et al., 2017), these authors found for the maize crop that the supply in the reproductive stages increases crop yield, even though its application in vegetative stages did not show the same responses. In the soybean crop, the use of foliar magnesium increased the SPAD index and the crop photosynthesis, resulting in increases of yield (Teklic et al., 2009).

Therefore, the *A. brasilense* inoculation associated to magnesium fertilization may assist the plants development with lower chlorophyll relative content and higher development, providing higher yield, given that both of them have an influence in important biochemical and physiological reactions on plants.

Thus, the present study aimed to evaluate the productive performance of maize crop when seed inoculated with *A. brasilense*, associated with different foliar doses of magnesium in the crop vegetative stages.

2. Material and Methods

This study was conducted in two environments, the first corresponded to the municipality of Laranjeiras do Sul, located in the Paraná state, Brazil, in a private farm with main focus on grain production, located at 25°23′ S, 52°28′ W, with altitude of 719 m. The second environment corresponded to the municipality of Entre Rios do Oeste, located in the Paraná state, Brazil, at the experimental station Professor Alcibiades Luiz Orlando belonging to Universidade Estadual do Oeste do Paraná, located at 24°40′ S and 54°17′ W, with altitude of 242 m, both places are straight distance of 201 km. The meteorological data for both environments are shown at (Figure 1).

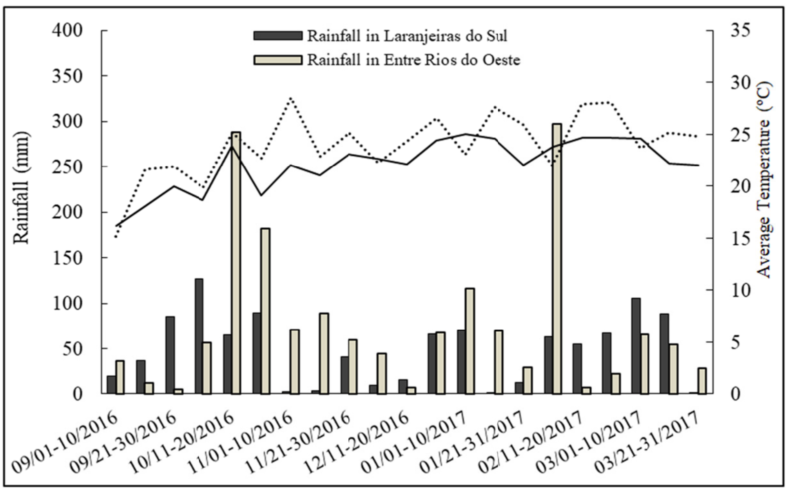

Figure 1. Rainfall conditions (vertical bars) and average temperature (lines) every ten days during the experiments conduction in Laranjeiras do Sul-PR and Entre Rios do Oeste-PR

The soil of both places is characterized as an Oxisol with clayey texture (Santos et al., 2013). The physical and chemical characteristics determined by previous sampling and implementation of the 0-20 cm layer are presented at Table 1. In both environments, the predecessor crops were soybean in the summer, and oats in the winter for production of straw. The previous count of diazotrophic bacteria prior to sowing resulted in 7.5×10^4 colony forming units (CFU) g^{-1} of soil.

Table 1. Chemical and physical characteristics on the 0-20 cm layer of an Oxisol in Laranjeiras do Sul (LDS) and Entre Rios do Oeste (ERO), 201/2017

Site	P	OM	pH	H+Al	Al^{3+}	K^+	Ca^{2+}	Mg^{2+}	BS	CEC	V
	mg dm^{-3}	g dm^{-3}	$CaCl_2$	-----------------------			$cmol_c$ dm^{-3}			------------------------	%
LDS	12.20	67.01	6.20	2.96	0.00	0.70	8.14	3.45	12.29	15.25	80.60
ERO	8.99	25.97	5.72	4.53	0.00	1.96	5.95	1.19	9.10	13.63	66.76

Note. P and K ($Mehlich^{-1}$); Ca, Mg and Al^{3+} (KCl 1 mol); Al+H (calcium acetate 0.5 mol L^{-1}).

In both experiments was used a blocking randomized design, with factorial scheme 3 × 2, with four replicates. The first factor consisted in the magnesium (Mg) fertilizer sources: magnesium sulphate ($MgSO_4$); magnesium oxide (MgO) and control (without Mg) and the second factor was represented by the presence or absence of seed inoculation with *A. brasilense.*

The maize was sowed in 09/10/2016 at Laranjeiras do Sul, in direct sowing system, using a row spacing of 0.45 m with a density of three plants per meter, totalizing 66.6 thousand plants per hectare, the base fertilization was made with 400 kg ha^{-1} of the commercial formula 12-32-18 NPK. The experimental plots were of 2.25 m wide and 7.0 m long, totalizing an area of 15.75 m².

For the environment at Entre Rios do Oeste, the sowing was made in 10/06/2016, using a row spacing of 0.70 m, with density of 4.2 plants per meter, totalizing 60 thousand plants per hectare, the base fertilization was made with 320 kg ha^{-1} from the commercial formula 10-15-15 NPK. The experimental plots were of 4.2 m wide and 5.0 m long, totalizing an area of 21.00 m².

In both areas was used the commercial simple hybrid 30F53VYHR® from the seed company Piooner, which has the interaction of two transgenic characteristics, the Leptra® technology with protection against insects and the Roundup Ready® gene, with high productive potential and highly responsive to the applied management. The seeds were treated with the combination of Fipronil (65.2 g a.i. for 100 kg of seeds), Piraclostrobina (6.25 g a.i. for 100 kg of seeds) and Tiofananto-metilico (52.25 g a.i. for 100 kg of seeds).

Prior sowing, seed were inoculated with *Azospirillum brasilense* strains AbV5 + AbV6, from commercial product which had a concentration of 2.00×10^8 CFU mL^{-1}, about 30 min before sowing, using a dose of 100 mL of inoculant to 60 thousand seeds.

For the supply of foliar Mg in both places was performed the application at the phenological stage V_4, using a dose of 6 kg ha^{-1} of magnesium (Jezek et al., 2015), for this process the applications were made at the end of the day with a backpack sprayer equipped with a 3 meters spray lance with capacity for 6 jet stream nozzles 110-02, calibrated for a flow rate of 300 L ha^{-1}.

For cover nitrogen fertilization it was made at the phenological stage V_6 with a urea source (45% of N) seeking the supply of 185 kg ha^{-1} of N in both places. During the crop development was necessary to control weeds using the weedcide Atrazine with dose of 2.5 kg a.i. ha^{-1}, to control stink bug during the initial crop development was used Tiametoxam (35.25 g a.i. ha^{-1}) + Lambda-Cialotrina (26.5 g a.i. ha^{-1}), at the V_{10} a fungicide was sprayed preventively with Picoxistrobin (90 g a.i. ha^{-1}) + Ciproconazole (36 g a.i. ha^{-1}).

The non-destructive evaluations were carried at R_1 when was determined the SPAD index, with aid of apparel SPAD-502 Minolta, by the measuring of 10 opposite leaves to the ear, that is, the diagnose leave for foliar sampling, being performed three samplings per leaf, one in the lower third, one in the medium third and one in the superior third from the plants of the central rows. Still in that moment were measured the stem diameter of the same 10 plants, being this measurement made at 0.15 m above ground, with a digital caliper.

Plants were harvested when they reached maturation, being considered a useful area of 1.35 × 5 m in Larangeiras, totalizing 6.75 m² and at Entre Rios do Oeste 2.8 × 3 m, totalizing 8.4 m². Plants were harvested manually, removing the ears. Then, 10 random ears were chosen which had the straw removed and manually counted of lines of grains in the center of the ear, measured the ear diameter with a digital caliper and the ear length was measured with a meter graduated ruler.

The ears were then placed back on their respective parcels, being submitted to mechanical threshing for posterior determination of 1000 grains mass and yield. To determine the 1000 grains mass was used the methodology from the Brazilian Rules for Seed Analysis (MAPA, 2009). The yield was determined by the total grain mass within the parcel useful area, correcting it to 13% humidity content and the expressed in kg per hectare.

The data from both conditions were submitted to variance analysis at 5% of probability and, when pertinent, the means were compared by the Tukey test at 5% of error probability, with aid of statistical software Sisvar (Ferreira, 2011).

3. Results and Discussion

In field conditions it is observed among the parameters evaluated that at the reproductive stage (R_1) no significant interaction between Mg sources and seed inoculation occurred ($p > 0.05$) in both sites studied. The parameter magnesium fertilization did not influence any of the tested parameters ($p > 0.05$) in both sites with experiment. For the parameter inoculation with *A. brasilense,* there was a significant effect on stem diameter and SPAD index for plants cultivated in both locations (Table 2).

The absence of results obtained for magnesium foliar fertilization may be attributed to the soil initial condition of Mg, since the soil contents for this nutrient were between 0,8 and 1 $cmol_c$ dm^{-3} which are considered elevated (SBCS, 2004). However, in research of Altarugio et al. (2017), it is reported that even in soil with adequate levels of Mg, its foliar application increases the SPAD index and the maize production components when applied at V_4 and R_2. Thus, as Mg is a component of the chlorophyll molecule, it was expected an increase in the SPAD index of fertilized plants, in the same way as it was evidenced in Teklic et al. (2009) studies, where the foliar fertilization with Mg in soybean increased the SPAD index and, consequently, the chlorophyll values and the crop photosynthetic rates.

The absence of Mg responses in this study may be attributed to two different conditions, the time of foliar application and the doses used. In Altarugio et al. (2017) studies, the positive results from the foliar fertilization with Mg were obtained at R_2, and in turn at V_4 the responses were inconstant, in a similar way to the present study. When considering the dose used Altarugio et al., (2017) obtained as the highest dose used 1.5 kg ha^{-1}, being the productivity peaks at R_2 obtained with 0.0883 kg ha^{-1}, well below the 6 kg ha^{-1} (Jezek et al., 2015) used in this study.

Table 2. Variance analysis summary, square means values, for the chlorophyll relative content (SPAD), stem diameter (SD), at reproductive stage (R_1) and number of row of grains per ear (NGRE), number of grains per row (NGR), ear diameter (ED), ear length (EL), thousand seeds mass (TSM), grains productivity (GP) of maize, hybrid 30F53VYHR®, in function of *A. brasilense* inoculation, associated to foliar fertilization with magnesium in Laranjeiras do Sul-PR and Entre Rios do Oeste-PR, 2016/2017

VF	SPAD	DC	NGRE	NGR	ED	EL	TSM	GP
Laranjeiras do Sul								
Block	1.45 ^{ns}	0.53 ^{ns}	0.38	0.83 ^{ns}	1.69 ^{ns}	0.36 ^{ns}	12.50 ^{ns}	256.21 ^{ns}
Fertilization (F)	1.84 ^{ns}	0.01 ^{ns}	0.85 ^{ns}	0.38 ^{ns}	0.81 ^{ns}	0.01 ^{ns}	0.97 ^{ns}	13.48 ^{ns}
Inoculation (I)	98.17**	47.82**	0.01 ^{ns}	1.08 ^{ns}	0.27 ^{ns}	0.35 ^{ns}	2.82 ^{ns}	27.32 ^{ns}
F×I	0.11 ^{ns}	0.14 ^{ns}	0.62 ^{ns}	2.16 ^{ns}	1.90 ^{ns}	0.54 ^{ns}	7.04 ^{ns}	166.80 ^{ns}
Residual	0.87	0.18	0.38	3.43	2.20	0.83	13.05	285.30
VC (%)	1.52	1.42	4.00	4.30	2.92	4.52	1.11	7.96
Entre Rios do Oeste								
Block	0.89 ^{ns}	0.30 ^{ns}	2.15**	29.23**	1.20 ^{ns}	8.24**	23.65 ^{ns}	404.39 ^{ns}
Fertilization (F)	0.18 ^{ns}	0.12 ^{ns}	0.03 ^{ns}	15.16 ^{ns}	0.79 ^{ns}	2.48 ^{ns}	9.57 ^{ns}	8.75 ^{ns}
Inoculation (I)	99.43**	20.16**	0.06 ^{ns}	9.12 ^{ns}	0.11 ^{ns}	0.91 ^{ns}	31.05 ^{ns}	138.59 ^{ns}
F×I	3.45 ^{ns}	0.40 ^{ns}	0.06 ^{ns}	1.06 ^{ns}	0.01 ^{ns}	0.001 ^{ns}	21.66 ^{ns}	483.09 ^{ns}
Residual	3.67	0.52	0.59	8.21	1.23	1.03	23.01	444.31
VC (%)	3.11	2.39	4.77	7.92	2.09	5.44	1.43	11.52

Note. ** Significant at 1% of probability by the F test. ^{ns} non-significant by the F test with limit of 5% of probability.

When considered the isolate effects of the inoculation over the stem diameter it was evidenced increases in inoculated plants of 9.6% and 6.24% in relation to non-inoculated in Laranjeiras do Sul-PR and Entre Rios do Oeste-PR, respectively (Figure 2).

The present study corroborates with the ones shown by Inagaki et al. (2015), which mention a 7% increase in stem diameter of plants inoculated with *A. brasilense* + *H. seropedicae* using the hybrid 30F53VYHR.

Thus, the increases found in stem diameter are attributed to inoculation with PGPB, because they provide vegetal hormones to plants (Bashan et al., 2004), resulting in greater capacity of nutrients absorption (Cassán & Diaz-Zorita, 2016) and accumulation of reserves, which increases stem diameter (Dartora et al., 2013), contributing to greater development of plants.

The biggest stem diameter of maize plants is important because it contributes with lowers susceptibility to lodging and, also, collaborate with higher accumulation of organic solutes on plants (Fancelli and Dourado Neto, 2000). Thus, allows the increase in yield, once these photoassimilates can contribute to grain-filling (Kappes et al., 2011).

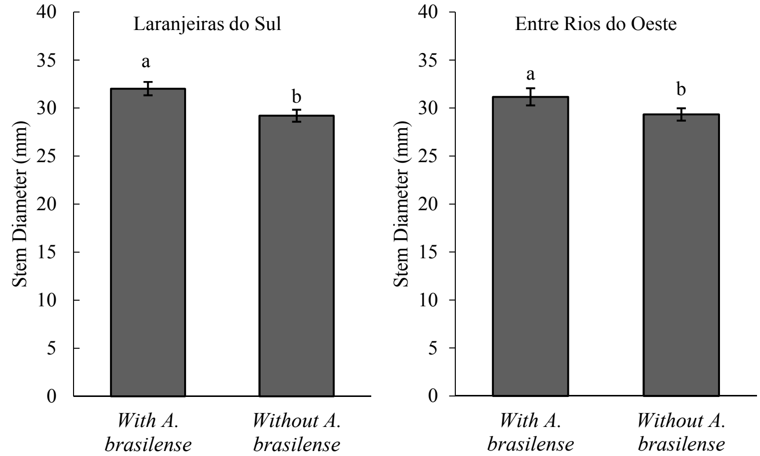

Figure 2. Stem diameter averages of maize plants with and without *A. brasilense* inoculation at the reproductive stage (R_1), hybrid 30F53VYHR®, in field conditions in Laranjeiras do Sul-PR and Entre Rios do Oeste-PR, 2016/2017

Note. Averages followed by upper case letters in the line do not differ from each other by the Tukey test at 5% probability.

The inoculation over the SPAD index highlight increases in plants inoculated of 6.80% and 6.32% in relation to non-inoculated, in the experimental areas of Laranjeiras do Sul-PR and Entre Rios do Oeste-PR, respectively (Figure3).

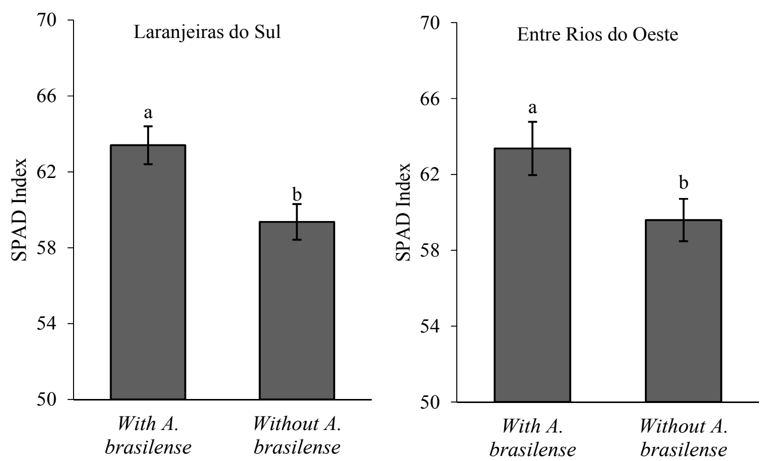

Figure 3. SPAD index averages of maize plants with and without *A. brasilense* inoculation, at reproductive stage (R_1), hybrid 30F53VYHR®, over field conditions in Laranjeiras do Sul-PR and Entre Rios do Oeste-PR, 2016/2017

Note. Averages followed by upper case letters in the line do not differ from each other by the Tukey test at 5% probability.

The data from the SPAD index provide a value which is correlated with the chlorophyll content present in plat leaves because in the center of the chlorophyll molecule are four atoms of nitrogen (Taiz & Zeiger, 2013). Thus, this evaluation allows an estimative of the foliar nitrogen content, once there is a strong correlation of its content with the foliar chlorophyll content (Zuffo et al., 2012). Similarly, Argenta et al. (2001) and Zotarelli et al. (2003), in studies with SPAD-502 Minolta, the same used in this experiment, concluded that raises in SPAD index is correlated to increases in foliar nitrogen of maize plants. So, as in the present study were used the diagnose leaves to evaluate the SPAD index, it is possible to conclude that plants inoculated with *A. brasilense* showed higher accumulation of foliar N to non-inoculated, however, this higher contribution of nitrogen did not correspond with increases in productivity for the crop (Table 3).

The higher SPAD index and consequently higher accumulation of foliar N may be a result of greater root development (Reis Jr et al., 2008), caused by the supply of hormones which stimulate vegetal growth (Kuss et al., 2007), providing higher absorption area of soil nutrients (Hungria et al., 2010) and higher membrane permeability which facilitates the entry of water and nutrients (Vurukonda et al., 2016).

Evaluating the maize production components, it was not verified isolate significant effect of inoculation to number of grain rows per ear, number of grains per row, ear diameter, ear length, thousand grains mass and grains yield in both places of evaluation, Laranjeiras do Sul-PR and Entre Rios do Oeste (Table 3). Similar result was found by Mello (2012), evaluating the contribution of *Azospirillum* sp. inoculation in yield and grain yield components of maize in high fertility soils, associated to nitrogen doses in cover, no influence of inoculation was verified for any of the production components, as like as in this study. However, the absence of results cannot be attributed exclusively to soil fertility. In this sense, the studies carried out on corn cultivation inoculated with *A. brasilense* seed in soil with fertility were classified on average, with 17% increments in crop yield, compared to uninoculated plants (Cavallet et al., 200).

Table 3. Productivity averages of maize with fertilization of magnesium oxide, magnesium sulphate and without fertilization associated with inoculation to treatments with and without inoculation with *A. brasilense*, of the hybrid 30F53VYHR®, in Laranjeiras do Sul – PR and Entre Rios do Oeste-PR, 2016/2017

Treatment	Laranjeiras do Sul			Entre Rios do Oeste		
	With *A. brasilense*	Without *A. brasilense*	Average	With *A. brasilense*	Without *A. brasilense*	Average
	Productivity (kg ha^{-1})					
Control	12554.4	13053.0	12803.7	10395.0	11656.2	11025.6
Mg oxide	12907.8	12388.2	12648.0	10809.6	11011.2	10910.4
Mg sulphate	12913.8	12550.8	12732.3	11310.0	10712.4	11011.2
Average	12792.0	12664.0		10838.2	11126.6	

It is valid to stand out that researches related to *Azospirillum* inoculation show in many cases increases in maize yield in different crop conditions (Díaz-Zorita et al., 2015). As an example, Lana et al. (2012), found 7 and 15% yield increase of maize plants inoculated, evaluating the crop responses to inoculation with *Azospirillum* associated to nitrogen fertilization. Thereby, it is observed that normally the inoculation of bacteria from the genus *Azospirillum* contributes to the obtainment of plants with higher development (Cassán & Diaz-Zorita, 2016), providing plants with higher photosynthetic capacity and, consequently, higher grain productivity (Bashan et al., 2004).

Another aspect to be approached is that the grain production, for the maize crop, is related to a series of characters (Lopez et al., 2007), Thus, the production components are characteristics that together with the plants genotype determine the productivity (Ohland et al., 2005). However, the availability of nutrients and climate conditions are important in the yield determination (Pereira et al., 2009). Being the character of grain production very influenced by environmental factors, the use of inoculation has its importance enlarged. While collaborating for mitigation of hydric stress (Bulegon et al., 2016), also contributes to avoid stress by salty soils (Nia et al., 2012) and higher nutrients absorption area (Morais et al., 2016).

However, prolonged adverse conditions were not observed in the present experiments in both locations (Figure 1). So, with favorable environmental conditions to development and grain productivity of maize, elevated yields were observed in both sites studied (Table 3).

The absences of responses in relation to crops productivity, besides environmental conditions, may be associated to interactions between maize genotypes and diazotrophic bacteria (Salomone & Dobereiner, 1996). In the same way, for rice was also demonstrated that there is a differential response between cultivars and *Azospirillum* strains (Okumura et al., 2013).

In spite of the inoculation of *A. brasilense* in maize seeds, it was possible to obtain a higher SPAD index and stem diameter, in field conditions, it was not observed yield increases or even increases in the production components. However, the inoculation must be used in the maize productive system, because the studies have been showing better development over stress conditions (Cassán & Diaz-zorita, 2016).

Therefore, studies with plant growth promoting bacteria are important for the improvement of inoculation techniques, to provide its best efficiency and, consequently, better development and yield of inoculated plants.

4. Conclusion

Maize seed inoculation with *A. brasilense* increases the values of stem diameter and SPAD index in both sites studied at R_1, however, they did not increase production components and maize yield.

The foliar fertilization with different magnesium sources at V_4 do not interfere in the development and yield of maize, in none of the studied sites.

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