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Effects of *Azospirillum brasilense* on growth and yield components of maize grown at nitrogen limiting conditions

Efeitos de *Azospirillum brasilense* sobre o desenvolvimento e produtividade do milho cultivado sob redução da adubação nitrogenada

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ABSTRACT

This study aimed to evaluate the agronomic performance of different doses of *Azospirillum brasilense* in peat formulation on maize crop grown under limited N fertilization, using a completely randomized design with four replicates. The treatments tested consisted of absence of N fertilizer, the full N recommendation and a combination of the half of recommended N associated with the inoculation of three doses of *A. brasilense* in peat formulation (100, 150 or 200 g per 25 kg of seeds). The following variables were evaluated: insertion height of the first ear height, number of kernel per row, number of row per ear, shoot dry biomass, root dry biomass, chlorophyll content, thousand seed weight, grain N content, shoot N content and grain yield. Half the dose of N fertilizer combined with 150 g per 25 kg of seeds of *A. brasilense* in peat formulation provided significantly superior results in agronomic performance of maize, particularly regarding grain yield, thousand seed weight and dry biomass of both shoot and root.

Keywords: *Zea mays*, inoculation, *Azospirillum brasilense*, nitrogen.

RESUMO

O objetivo do presente trabalho foi avaliar a eficiência agronômica do inoculante turfoso a base de *Azospirillum brasilense* nos componentes de produtividade da cultura do milho safrinha sob redução da adubação nitrogenada. Para tanto, foi conduzido o ensaio no delineamento em blocos casualisados com quatro repetições. Os tratamentos consistiram em uma testemunha absoluta; dose total de N recomendada para a cultura e 1/2 dose de N + inoculação com *A. brasilense* na formulação líquida; 1/2 dose de N + inoculante turfoso em três proporções por 25 kg de sementes (100, 150 and 200 g). Foram avaliadas as seguintes características agronômicas: altura de inserção da primeira espiga, número de grãos por fileira, número de fileiras por espiga, biomassa seca da parte aérea, biomassa seca das raízes, teor de clorofila, peso de mil sementes, teores de nitrogênio no grão e na parte aérea e rendimento de grãos. A aplicação de metade da dose de nitrogênio associada a 150 g do inoculante turfoso por 25 kg de sementes propiciou resultados significativamente superiores no desempenho agronômico do milho, principalmente no que diz respeito ao rendimento de grãos, massa de mil grãos, massa seca da parte aérea e das raízes.

Palavras-chave: *Zea mays*, inoculação, *Azospirillum brasilense*, nitrogênio.

INTRODUCTION

Brazil currently ranks as world's third-largest maize producer and second-largest exporter. The national projection by the year 2024 is of about 103.1 million tons of grains, reaching a total area of around 22.1 million hectares (Conab, 2014).

Since nitrogen (N) is the first major nutrient responsible for growth of plants, this means that high amounts of this nutrient will be required to meet the needs of maize crop. Further, N supply is known as one of the largest variable cost for maize production (Embrapa, 2015).

In this context, *Azospirillum* spp., nitrogen-fixing microorganisms found in the rhizosphere of various grass species, have been reported as a way of providing grass-crops with the part of the N needed, through the bacterium biological N fixation process (Döbereiner and Day, 1976; Pedraza *et al*., 2009; Hungria *et al*., 2010; Piccinin *et al*., 2012; Piccinin *et al*., 2015).

Brazil has tradition in research with *Azospirillum*. Numerous studies have been carried out in the country since the discovery by the Brazilian researcher Johanna Döbereiner (1924-2000) of the biological N fixation capacity of these bacteria when in association with grasses. However, the conflicting responses to *Azospirillum* spp. inoculation in maize that have been reported in field conditions raised demands about the effective dose of this bacterium able to induce a statistically significant increase in crop's growth and yield.

An effective inoculant must be prepared with a strain of rhizobium selected for high N fixation efficiency and competitive ability for nodulation. The strain must survive in the inoculant formulation, maintain its properties during storage and be tolerant to stress factors such as acidity, desiccation, high temperature and chemical pesticides (Ben Rebah *et al*., 2007).

In this way, the quality of an inoculant can be associated to the guarantee of recommended strains' minimum concentration combined with the easiness provided by its physical state.

Peat has been commonly used as support and, at the same time, as a substrate for most of the inoculants produced, due to bacteria's protection against drying, both in the storage process and in seed coating after inoculation (Stephens and Rask, 2000). On this point, this study aimed to evaluate the agronomic performance of different doses of *A. brasilense* in peat inoculant on the maize grown under limited N fertilization.

MATERIAL AND METHODS

The experiment was carried out during the crop year of 2015 at Iguatemi Research Station (FEI) of the State University of Maringá (UEM), in Maringá in northwestern Paraná State, Brazil, located at latitude 23°25′ south and longitude 51°57′ west of Greenwich and with an average altitude of 540 m.

The region's climate and soil are classified, respectively, as Cfa (humid mesothermal, with abundant rains in warm summer and dry winter), according to Köppen classification (Caviglione *et al*., 2000) and Typical Red Dystrophic Argisol according to the Brazilian Classification System (Embrapa, 2013).

With the exception of N fertilizer, after physical and chemical analyses (Embrapa, 2011), soil correction and fertilization were adopted in order to achieve an average production of around 6000 kg ha^{-1} as stated in Embrapa (2015).

An amount of 250 kg ha^{-1} of fertilizer 0-20-20 formulation (N-P-K rating system) was applied at sowing. The N supply took place at three levels (0, 90 and 180 kg ha^{-1}) using ammonium sulphate (21% of N) in furrow. Based on Fageria and Baligar (2005), the N recommendation was applied in two split applications with one-third at sowing and the remaining two-third at the tillering, stage in which urea (46% of N) was used as N source.

The tested treatments consisted of a combination of three levels of nitrogen fertilization (0, 90 and 180 kg ha^{-1}) associated with the inoculation of three doses of *A. brasilense* in peat formulation (100, 150 and 200 g per 25 kg of seeds). Treatment 4 (commercial product – Total Biotecnologia®) was inserted to compare the doses of peat inoculant (T5, T6 and T7 – Grap Agrocete®) with a product whose efficiency has already been proven and has registration in the Ministry of Agriculture, Livestock and Food Supply. A detailed scheme of the treatments is shown in Table 1.

Table 1 - Detailed scheme of the treatments describing the N fertilizer levels and *A. brasilense* doses adopted

Treatment labels	Doses of N fertilizer * (kg ha^{-1})	*A. brasilense* doses in peat formulation (mL or g per 25 kg of seeds)
T1	0	0
T2	180	0
T3	90	0
T4	90	100 **
T5	90	100
T6	90	150
T7	90	200

* 1/3 applied at sowing and the remaining 2/3 at the crop tillering stage.
** Commercial dose (ml) in liquid formulation as registered in the Ministry of Agriculture, Livestock and Food Supply.

Inoculation with the liquid inoculant was performed by mixing and homogenizing seeds to the inoculant. The inoculant was at a concentration of 2×10^8 CFU (colony-forming units) per milliliter of the AbV5 and AbV6 strains, as it is approved and accredited by the Brazilian agricultural authorities. The dose used was of 100 ml per 25 kg of maize seed, according to the manufacturer's technical recommendation.

For better adhesion of the peat inoculant, a 10% sugar solution with the tested inoculant doses was employed as a way of obtaining a uniform coating. The inoculation process consisted of the application of the sugar solution on the seeds, followed by the peat inoculant. After homogenization, the seeds were dried in the shade for a period of 15 minutes (Brandão Junior and Hungria, 2000a).

Using the tillage conservation system, sowing took place on March 4th, 2015 using the completely randomized design with four replicates. Each experimental unit was composed of six rows of 5 m, spaced 0.90 m apart, with a population of about 60,000 plants ha^1 of the early maturity hybrid 30F53 YH. However, since the lateral rows and the end boundaries of the central portion were not considered, the harvesting area consisted of only 9 m^2.

As overviewed in Embrapa (2015), manual or chemical methods of control were carried out to deal with weeds, as well to keep disease and pest infestations below their injury levels.

Agronomic characteristics and yield evaluation

Harvest took place when the maturity of the hybrid (point from which kernel dry weight no longer increase) was visually evaluated by using the formation of an abscission layer or 'black layer' at the base of the kernel proposed by Daynard and Duncan (1969).

The following variables were evaluated: insertion height of the first ear height, number of kernel per row, number of row per ear, shoot dry biomass, root dry biomass, chlorophyll content, thousand seed weight, grain N content, shoot N content and grain yield. Detailed information on each variable are described below:

Insertion height of the first ear (IHFE): it was estimated based on ten randomly chosen plants from the usable area of each plot, at the crop physiological maturity. Results are the average measurement of the first ear insertion height from ground level (in cm).

Number of kernels per row (NKR) and number of row per ear (NRE): within the useful area, ten *ears at* physiological maturity *were randomly* selected and then the NKR and the NGS were manually counted.

Root dry biomass (RDB): At the R2 growth stage, ten plants were randomly collected from the harvesting area of each plot (Veloso *et al*., 2006). The roots were collected in each plot using a shovel to excavate a block of soil of 0.40×0.40 m by 0.20 m deep and then identified and taken to the laboratory, where they were washed and then taken to a forced-air oven at 65ºC until constant weight was achieved. Average data were expressed in g plant^1.

Shoot dry biomass (SDB) and Shoot N content (SNC): SDB and SNC were determined by collecting ten plants per plot at the pre-flowering stage. The plants were placed in paper bags, identified and dried in a forced-air oven and then weighted. The average SDB data were expressed in g plant^{-1}. Dried shoots were then ground (18 mesh) and subjected to sulfuric digestion to determine total SNC just as described in AOAC (2000).

Chlorophyll content (CC): Five plants from the central rows of the plot were selected randomly as samples. For each leave, CC was measured twice using the portable reader ClorofiLOG 1030®. Readings were performed directly on the leaves of the middle third portion of the plant as described in Piekielek *et al*. (1995). The mean chlorophyll levels were shown in Clorofilog Reading.

Thousand seed weight (TSW): After the moisture adjustment as previously mentioned, it was determined by weighing eight subsamples of 100 seeds for each field plot, with an analytical scale accurate to 1 mg. For all plots the coefficient of variation was less than four, and the results were multiplied by 10 (Brasil, 2009).

Grain N content (GNC): From the cleaned seeds portion, it was determined through the method of sulfuric acid digestion using a micro Kjeldahl distillation apparatus, as described in (AOAC, 2000).

Grain yield (GY): On 22 July, 2015, at crop physiological maturity, harvest was manually performed collecting the ears from the evaluation area of each plot. Ears were mechanically husked and then the impurities were manually removed in order to obtain a very clean lot. After proper moisture content adjustment of 13% (Brasil, 2009), the cleaned grains were weighed and the average data were converted into kg ha^{-1}.

Statistical analysis: All analyses were performed using the statistical software *Sisvar (*Ferreira, 2011). The data were submitted to the Shapiro-Wilk test ($p<0.01$) and Levene test ($p<0.01$) to verify the normal distribution and homoscedasticity, respectively. Each variable was subjected to analysis of variance at 1% probability and when it was significant, the means were compared by Fishers' protected t-test LSD (Least Significant Difference) ($p\leq0.01$) according to Banzatto and Kronka (2006).

RESULTS AND DISCUSSION

Table 2 shows the results of agronomic characteristics and yield evaluation of the hybrid 30F53 YH grown in Maringá, Paraná State (Brazil) during the second-crop maize of 2015.

Insertion height of the first ear (IHFE)

Overall, at half the dose of N associated to *A. brasilense* inoculation provided an increase in IHFE, regardless of the inoculant doses. Further, Table 2 indicates that higher values of the evaluated variable were found in T5 and T6.

Zsubori *et al*. (2002) summarized that the height of the main ear can be correlated to plant height, feature which has showed a significant positive correlation to plant yield. Further, the author pointed out that the ear height depends on the genetic background of the varieties, but is also influenced by the environment. On this point, while Lana *et al*. (2012) and Araújo *et al*. (2015) found that in maize the IHFE was not modified by N fertilization and inoculation rates, on the other hand *A. brasilense* provided superior IHFE in Portugal *et al*. (2016).

It is believed that one of the motives for the contrasting results could be attributed to the difference in the crop stage in which IHFE was assessed. In the present trial, such as in Portugal *et al*. (2016), the evaluations were performed at the stage of complete physiological maturity, point in which limited environmental resources rather than the genetic compound are more likely to affect the plant's architecture (Viégas and Peeten, 1987; Gratani, 2014).

Number of kernels per row (NKR) and number of rows per ear (NRE)

Such as for IHFE, at half the dose of N associated to *A. brasilense* inoculation provided an increase in NKR and NRE, regardless of inoculant dose. However, for both variables the highest results were found in the T5 and T6 (Table 2), which statically did not differ from each other.

Table 2 - Average results of the insertion height of the first ear (IHFE), number of kernel per row (NKR), number of row per ear (NRE), shoot dry biomass (SDB), root dry biomass (RDB), chlorophyll content (CC), thousand seed weight (TSW), grain N content (GNC), shoot N content (SNC) and grain yield (GY) of hybrid 30F53YH (Maringá – PR, Brazil, 2015)

Treat-ments	IHFE (cm)		NKR (unit row-1)		NRE (unit ear^{-1})		SDB (g)		RDB (g)	
T1	52.7	A	26.05	AB	14.75	A	152.63	A	110.4	A
T2	66.55	BCD	32.08	CD	16.25	BC	256.22	BC	224.8	DE
T3	59.9	B	28.43	AB	15.5	AB	200.56	AB	123.35	AB
T4	64.45	BC	30.35	C	15.75	ABC	229.98	BC	166.45	BC
T5	72.55	E	33.8	E	16	C	381	D	251.12	E
T6	77.55	E	35.63	E	16.75	C	502.93	E	309.07	F
T7	69	CD	32.05	D	15.5	AB	280.91	C	191.1	CD

Treat-ments	CC (g dm^{-2})		TSW (g)		GNC (%)		SNC (%)		GY (kg ha^{-1})	
T1	43.07	A	305.93	A	1.2	A	2.243	A	2245.13	A
T2	51.77	CD	345.02	CD	1.61	A	2.713	CD	4020.56	D
T3	47	B	326	B	1.38	A	2.465	B	2618.33	B
T4	49.17	BC	337.32	BC	1.51	A	2.618	C	3286.73	C
T5	53.08	D	359.8	CD	1.92	AB	2.828	D	5202.54	F
T6	56.54	E	375.62	E	2.91	B	3.035	E	6224.03	G
T7	51.3	CD	343.73	C	1.74	A	2.768	D	4547.23	E

Within columns, means followed by same letter do not significantly differ from each other ($p < 0.01$, LSD).

Row number ear and kernel number per row are two of the several components in maize yield. For Milander (2015) the NRE is determined strongly by plant genetics and less by the environment. However, as mentioned in Fancelli and Dourado Neto (2004), these features are consolidated at the early stage of the plant development, i.e. between the emission of 7th and 9th leaf. Therefore, the N supply in this stage is decisive to ensure ears with high number of grains.

Similar to the findings of the present work, Novakowiski *et al.* (2011) found increase in the number of rows, kernels per row and grains per ear under N fertilization associated to inoculation with *A. brasilense*.

Shoot dry biomass (SDB) and Root dry biomass (RDB)

Regarding the SDB and the RDB, increases of around 2.3 and 1.8 were respectively observed in the dry matter values of T6, when compared to the control (Table 2). Further, T5 and T6, in which the half of N dose was associated with higher doses of *A. brasilense*, presented superior values for both variables, when they are compared to T2, in which the full recommendation of N was adopted. Nevertheless, the growth conditions of T7 showed lower RDB than T2.

Interestingly, differently from the other treatments in which *A brasilense* was associated with the half of the N dose, T3 showed lower SDB and RDB values than those found in T2, in which the full N dose was supplied, due to nutritional deficiency.

Comparable results were presented by Domingues Neto *et al*. (2013), whom studying maize, found an increase in the dry biomass of both the shoots and roots as a result of foliar application of *Azospirilum* spp., in the absence of nitrogen fertilizer. On this point, according to Ramos *et al*. (2010) when inoculation of *Azospirilum* spp. is associated with adequate N fertilization, SDB and RDB can increase by 80%, compared to maize plants treated only with mineral N.

Bashan and Dubrovsky (1996) suggested that by affecting root roles, *Azospirillum* spp. participates in the partitioning of the carbon and minerals at the entire plant level. Later, Bashan *et al*. (2004) found a positive response of dry matter accumulation to *A. brasilense* inoculation as a result of the phytohormones synthesized by the bacteria, substances which modify the metabolism and morphology of the root system, improving thus the plant absorption rates of minerals and water.

Chlorophyll content (CC)

Leaf CC is used to predict the nutritional level of N in plants, since this nutrient participates directly in the synthesis of pigments (Piekielek *et al*., 1995 and Kappes *et al*., 2013). Further, Argenta *et al*. (2001) affirmed that CC is an adequate indicator for N fertilization recommendation in cereals, especially when associated with other soil indicators.

In the present trial, the highest CC was reported in T6, in which the half of the N dose was combined with the inoculation of *A. brasilense* at 150 g 25 kg^{-1} of seeds. Except when using the commercial dose of the inoculant (T4), once more all the treatments in which the inoculant was combined with N fertilizer had higher or equal CC values than the treatment in which the full recommendation of N fertilizer was employed (T2). Such as in the present work, Quadros *et al*. (2014), Costa *et al*. (2015) and Araújo *et al*. (2015) also reported increasing in CC after seed or foliar inoculation in maize.

Thousand seed weight (TSW)

The TSW is a key yield parameter that has direct impact on the crop's final yield and seed quality. By observing the average values in Table 2, it can be concluded that there were significant differences between the tested treatments, in which the highest result was obtained with the combination of the half of N dose with inoculation of *A. brasilense* at 150 g per 25 kg of seeds (T6).

Contrasting results have been recorded about the effects of the inoculation on TSW. In this regard, while in Biari *et al*. (2008), Braccini *et al*. (2012), Costa *et al*. (2015) and Morais *et al*. (2016) found an increase in seed weigh of maize after plant inoculation combined to N fertilizer rates, on the other hand, in Novakowiski *et al*. (2011) there was no significant interaction between N levels and inoculation for TSW. However, while the later authors used the bacteria strain Sp245, Hungria *et al*. (2010) pointed out that only the strains AbV4, AbV5, AbV6 and AbV7 are the most effective for maize.

Grain N content (GNC)

Only the GNC of treatment T6 was influenced by the combination of the inoculant doses with the half of the N dose, confirming the results described in Araújo *et al*. (2015). However, the inoculant doses used in T4 and T7 promoted comparable GNC values to those treatments conducted in the absence of *A. brasilense*, regardless of the N rate. Authors such as Dobbelaere *et al*. (1999) reported that inoculation with *A. brasilense* did not impact GNC. However, those authors used a wild bacteria strain, whereas only the strains AbV4, AbV5, AbV6 and AbV7 are the most effective in maize as reported in Hungria *et al*. (2010).

Shoot N content (SNC)

The full N recommendation (T2) as well the half of the N dose combined with inoculant application at 100 g per 25 kg of seeds or higher (T5, T6 and T7) showed SNC values ranked within the range considered as suitable for maize just as described in Martinez *et al*. (1999). But, visual deficiency symptoms were only observed in plots conducted in the absence of N (T1).

The findings pointed out for SNC in this trial corroborate those of the Araújo *et al*. (2015). Just as stated in Kappes *et al*. (2013), overall SNC revealed the same trend above observed in CC variable, i.e. the bigger the CC average the bigger the SNC.

Grain yield (GY)

The dose of *A. brasilense* combined with the half of N recommendation employed in T6 provided significant yield increase compared to the other treatments, mainly to those non-inoculated. Interestingly, overall GY revealed the same trend observed above in SDW and RDM, in which the highest values were observed in T6, followed in a decreasing order by treatments 5, 7, 2, 4, 3 and 1, with significant differences among them. Such as observed here, in Pedraza *et al*. (2009), Hungria *et al*. (2010), Piccinin *et al*. (2012) and Piccinin *et al*. (2015), *A. brasilense* combined N fertilizer in non-leguminous species promoted increasing in the plant growth and yield compounds.

However, from the results shown in present work, the dose of inoculant used played a crucial role on the crop response since (excluding GNC) in all the analyzed variables T4 presented lower values than that observed in T2 (full recommendation of N). For the variable NRE, T7 performed the same.

Brandão Júnior and Hungria (2000b) further affirmed that for *Rhizobium* spp. establishment in substrate, the minimum concentration of viable bacteria in the inoculant have to be hundred times higher than that naturally found in the soil. Based on these findings, it is plausible to suggest that the inoculant doses used in T4 and T5 could not have provided the minimum bacteria concentration *to* form a robust soil colony. It is important to point out, moreover, that in T5 there was an increase *in* GY when compared to the full N recommendation (T2).

Treatment T7, however, had the lowest GY and SDB among the tested non-commercial dose of the inoculant (T5, T6 and T7). In this case, based on the understanding of Hallmann *et al*. (1997) and Saubidet *et al*. (2002) it is guessed that under the employed N fertilizer level, plants were not able to provide *A. brasilense* with an adequate carbon compounds supply to sustain maximal cultivar's growth and yield. As discussed in Araújo *et al*. (2015), under limited N availability plants cannot produce sufficient root exudates, which act as *signal* to influence the ability of strains to colonize soil by horizontal movement or to survive in the rhizosphere (Mark *et al*., 2005; Bashan and Levanony, 1987).

The increase in growth and yield in inoculated plants with *A. brasilense* might not only be attributed to the biological N fixation. Under lower level of oxygen, the bacterium realized high N fixation rate, however it could also improve productivity through the excretion of hormones such as auxin, which induces the propagation of roots and thus, intensifying plant nutrient absorption (Döbereiner and Day, 1976; Tien *et al*., 1979; Dobbelaere *et al*., 1999).

CONCLUSION

At half the dose of N fertilizer combined with 150 g per 25 kg of seed of *A. brasilense* in peat formulation provided significantly superior results in agronomic performance of maize, particularly regarding grain yield, thousand seed weight and dry biomass of both shoot and root.

REFERENCES

AOAC (2000) – *Official methods of the Association of Official Analytical Chemist* 15.ª ed. Washington, D.C., Association of Official Analytical Chemists, 230 p.

Argenta, G.; Silva, P. R. F.; Bortolini, C. G.; Forsthofer, E. L. & Strieder, M. L. (2001) – Relação da leitura do clorofilômetro com os teores de clorofila extraível e de nitrogênio na folha de milho. *Revista Brasileira de Fisiologia Vegetal*, vol. 13, n. 2, p. 158-167. https://dx.doi.org/10.1590/S0103-31312001000200005

Araújo, E.O.; Mercante, F.M. & Vitorino, A.C.T. (2015) – Effect of nitrogen fertilization associated with inoculation of *Azospirillum brasilense* and *Herbaspirillum seropedicae* on corn. *African Journal of Agricultural Research*, vol. 10, n. 3, p. 137-145. http://dx.doi.org/10.5897/AJAR2014.8866

Banzatto, D.A.; Kronka, S.N. (2006) – *Experimentação Agrícola,* 4.ª ed. FUNEP, Jaboticabal. Brazil, 249 p.

Bashan, Y. & Dubrovsky, J.G. (1996) – *Azospirillum* spp. participation in dry matter partitioning in grasses at the whole plant level. *Biology and Fertility of Soils*, vol. 23, n. 4, p. 435-440. http://dx.doi.org/10.1007/BF00335919

Bashan, Y.; Holguin, G. & De-Bashan, L.E. (2004) – *Azospirillum*-plant relations physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of Microbiology*, vol. 50, n. 8, p. 521-577. http://dx.doi.org/10.1139/w04-035

Bashan, Y. & Levanony, H. (1987) – Horizontal and vertical movement of *Azospirillum brasilense* Cd in the soil and along the rhizosphere of wheat and weeds in controlled and field environments. *Journal of General Microbiology*, vol. 133, p. 3473-3480. http://dx.doi.org/10.1099/00221287-133-12-3473

Ben Rebah, F.; Prévost, D.; Yezza, A. & Tyagi, R.D. (2007) – Agro-industrial waste materials and wastewater sludge for rhizobial inoculant production: A review. *Bioresource Technology*, vol. 98, n. 18, p. 3535-3546. http://dx.doi.org/10.1016/j.biortech.2006.11.066

Biari A.; Gholami, A. & Rahmani H.A. (2008) – Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. *Journal of Biological Science*, vol. 8, n. 6, p. 1015-1020. http://dx.doi.org/10.3923/jbs.2008.1015.1020

Braccini, A.L.; Dan, L.G.M.; Piccinin, G.G.; Albrecht, L.P.; Barbosa, M.C. & Ortiz, A.H.T. (2012) – Seed inoculation with the use of bioregulators in maize. *Revista Caatinga*, vol. 25, n. 2, p. 58-64.

Brandão Junior, O. & Hungria, M. (2000a) – Efeito de concentrações de solução açucarada na aderência do inoculante às sementes de soja. *Revista Brasileira de Ciência do Solo*, vol. 24, n. 3, p. 515-526. http://dx.doi.org/10.1590/S0100-06832000000300005

Brandão Junior, O. & Hungria, M. (2000b) – Efeito de doses de inoculante turfoso na fixação biológica do nitrogênio pela cultura da soja. *Revista Brasileira de Ciência do Solo*, vol. 24, n. 3, p. 527-535. http://dx.doi.org/10.1590/S0100-06832000000300006

Brasil (2009) – *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento, Mapa/ACS – Secretaria de Defesa Agropecuária. Brasília, Brazil, 395 p.

Caviglione, J.H.; Kiihl, L.R.B.; Caramori, P.H. & Oliveira, D. (2000) – Cartas climáticas do Paraná. Londrina. *Instituto Agronômico do Paraná*. Compact Disc (CD).

Conab (2014). *Projections of agribusiness: Brazil 2013/14 to 2019/20 Long-term Projections,* Brazil Ministry of Agriculture – Livestock and Food Supply. Brazil, 98 p.

Costa, R.R.G.F.; Quirino, G.S.F.; Naves, D.C.F.; Santos, C.B. & Rocha, A.F.S. (2015) – Efficiency of inoculant with *Azospirillum brasilense* on the growth and yield of second-harvest maize. *Pesquisa Agropecuária Tropical*, vol. 45, n. 3, p. 304-311. https://dx.doi.org/10.1590/1983-40632015v4534593

Daynard, T.B. & Duncan, W.G. (1969) – The black layer and grain maturity in com. *Crop Science*, vol. 9, n. 4, p. 473-476. http://dx.doi.org/10.2135/cropsci1969.0011183X000900040026x

Döbereiner, J. & Day, J.M. (1976) – Associative symbiosis in tropical grasses: characterization of microorganisms and dinitrogen-fixing sites. In: Newton W.E. & Nyman, C.T. (Ed.) *Proceedings* 1^{st} *international symposium on nitrogen fixation*, USA, Washington State University Press, vol. 2, p. 518-538.

Dobbelaere, S.; Croonenborghs, A.; Trys, A.; Vande Broek, A. & Vanderleyden, J. (1999) – Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. *Plant and Soil*, vol. 212, n. 2, p. 155-164. http://dx.doi.org/10.1023/A:1004658000815

Domingues Neto, F.J.; Yoshimi, F.K.; Garcia, R.D.G.; Miyamoto,Y. R. & Domingues, M.C.S. (2013) – Desenvolvimento e produtividade do milho verde safrinha em resposta à aplicação foliar com *Azospirillum brasilense. Enciclopédia Biosfera*, vol. 9, n. 17, p. 1030-1040.

Embrapa (2011) – *Manual de métodos de análises de solos*. 2.ª ed. Rio de Janeiro, Empresa Solos. 230 p.

Embrapa (2013) – *Sistema brasileiro de classificação de solos*. 3.ª ed. Brasília, Empresa Brasileira de Pesquisa Agropecuária, 353 p.

Embrapa (2015) – *Cultivo do Milho.* Sistema de Produção 1, 9.ª ed. Embrapa Milho e Sorgo. [cit. 2016-07-24]. https://www.spo.cnptia.embrapa.br/conteudo?p_p_id=conteudoportlet_WAR_sistemasdeproducaolf6_1ga1ceportlet&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_p_col_id=column-2&p_p_col_count=1&p_r_p_-76293187_sistemaProducaoId=7905&p_r_p_-996514994_topicoId=8658

Fageria, N.K. & Baligar, V.C. (2005) – Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy*, vol. 88, n. 1, p. 97-185. http://dx.doi.org/10.1016/S0065-2113(05)88004-6

Fancelli A.L. & Dourado Neto, D. (2004) – *Produção de milho.* Guaíra, Ed. Agropecuária Ltda, 360 p.

Ferreira, D.F. (2011) – Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia,* vol. 35, n. 6, p. 1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001

Gratani, L. (2014) – Plant phenotypic plasticity in response to environmental factors. *Advances in Botany,* vol. 2014, art. 208747. http://dx.doi.org/10.1155/2014/208747

Hallmann, J.; Quadt–Hallmann, A.; Mahaffee, W.F. & Kloepper, J.W. (1997) – Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, vol. 43, n. 10, p. 895-914. http://dx.doi.org/10.1139/m97-131

Hungria, M.; Campo. R.J.; Souza, E.M. & 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*, vol. 331, n. 1, p. 413-425. http://dx.doi.org/10.1007/s11104-009-0262-0

Kappes, C.; Arf, O.; Arf, M.V.; Ferreira, J.P.; Dal Bem, J.A.; Portugal, J.R. & Vilela, R.G. (2013) – Inoculação de sementes com bactéria diazotrófica e aplicação de nitrogênio em cobertura e foliar em milho. *Semina: Ciências Agrárias*, vol. 34, n. 2, p. 527-538. http://dx.doi.org/10.5433/1679-0359.2013v34n2p527

Lana, M. do C.; Dartora, J.; Marini, D. & Hann, J.E.H. (2012) – Inoculation with *Azospirillum,* associated with nitrogen fertilization in maize. *Revista Ceres*, vol. 59, n. 3, p. 399-405. http://dx.doi.org/10.1590/S0034-737X2012000300016

Mark, G.L.; Dow, J.M.; Kiely, P.D.; Higgins, H.; Haynes, J.; Baysse, C. & O'Gara, F. (2005) – Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, n. 48, p. 17454-17459. http://doi.org/10.1073/pnas.0506407102

Martinez, H.E.P.; Carvalho, J.G. de & Souza, R.B. de (1999) – Diagnose foliar. In: Ribeiro, A.C.; Guimarães, P.T.G. & Alvarez, V.V.H. (Eds.) *Recomendação para uso de corretivos e fertilizantes em Minas Gerais: 5.ª Aproximação*. Viçosa, Comissão de Fertilidade do Solo do Estado de Minas Gerais, p.143-168.

Milander, J.J. (2015) – *Maize Yield and Components as Influenced by Environment and Agronomic Management.* Master thesis. Lincoln, University of Nebraska, 118 p.

Morais T.P.; Brito C.H.; Brandão A.M. & Rezende W.S. (2016) – Inoculation of maize with *Azospirillum brasilense* in the seed furrow. *Revista Ciência Agronômica*, vol. 47, n. 2, p. 290-298. http://dx.doi.org/10.5935/1806-6690.20160034

Novakowiski, J.H.; Sandini, I.E.; Falbo, M.K.; de Moraes, A., Novakowiski, J.H. & Cheng, N.C. (2011) – Efeito residual da adubação nitrogenada e inoculação de *Azospirillum brasilense* na cultura do milho. *Semina: Ciências Agrárias*, vol. 32, n. 4, p. 1687-1698. http://doi.org/10.5433/1679-0359.2011v32Suplp1687

Pedraza, R.O.; Bellone, C.H.; de Bellone, S.; Sorte, P.M.B. & Teixeira, K.R.D. (2009) – *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. *European Journal of Soil Biology,* vol. 45, n. 1, p. 36-43. http://dx.doi.org/10.1016/j.ejsobi.2008.09.007

Piccinin, G.G.; Braccini, A.L.; Dan, L.G.M.; Scapim, C.A.; Ricci, T.T. & Bazo, G.L. (2012) – Efficiency of seed inoculation with *Azospirillum brasilense* on agronomic characteristics and yield of wheat. *Industrial Crops and Products*, vol. 43, p. 393-397. http://dx.doi.org/10.1016/j.indcrop.2012.07.052

Piccinin, G.G.; Braccini, A.L.; Scapim, C.A.; Suzukawa, A.K.; Dan, L.G.M. & Godinho, F.B. (2015) – Agronomic performance of maize in response to seed inoculation with *Azospirillum brasilense* associated with nitrogen doses and bioregulator. *International Journal of Food, Agriculture and Environment*, vol. 13, n. 3-4, p. 67-73.

Piekielek W.P.; Fox R.H.; Toth, J.D. & Macneal, K.E. (1995) – Use of a chlorophyll meter at the early dent stage of corn to evaluate nitrogen sufficiency. *Agronomy Journal,* vol. 87, n. 3, p. 403-408. http://dx.doi.org/10.2134/agronj1995.00021962008700030003x

Portugal, J.R.; Arf, O.; Peres, A.R.; Gitti, D.C.; Rodrigues, R.A.F.; Garcia, N.F.S. & Garé, L.M. (2016) – *Azospirillum brasilense* promotes increment in corn production. *African Journal of Agricultural Research*, vol. 11, n. 19, p. 1688-1698. http://dx.doi.org/10.5897/AJAR2015.10723

Quadros, P.D.; Roesch; L.F.W.; Silva, P.R.F.; Vieira, V.M.; Roehrs, D.D. & Camargo, F.A. de O. (2014) – Desempenho agronômico a campo de híbridos de milho inoculados com *Azospirillum*. *Revista Ceres*, vol. 61, n. 2, p. 209-218. https://dx.doi.org/10.1590/S0034-737X2014000200008

Ramos, A.S.; Santos, T.M.C.; Santana, T.M.; Guedes, E.L.F. & Montaldo, Y.C. (2010) – Ação do *Azospirillum lipoferum* no desenvolvimento de plantas de milho. *Revista Verde,* vol. 5, p. 113-117.

Saubidet, M.I.; Fatta, N. & Barneix, A.J. (2002) – The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant and Soil*, vol. 245, n. 2, p. 215-222. http://dx.doi.org/10.1023/A:1020469603941

Stephens, J.H.G. & Rask, H.M. (2000) – Inoculant production and formulation. *Field Crops Research*, vol. 65, n. 2-3, p. 249-258. http://dx.doi.org/10.1016/S0378-4290(99)00090-8

Tien, T.; Gaskin, M. & Hubbel, D. (1979) – Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology*, vol. 37, n. 5, p. 1016-1024.

Veloso, C.A.C.; Viégas, I. de J.M.; Oliveira, R.F. de & Botelho, S.M. (2006) – *Amostragem de solo e planta para análise química*. Documentos 266, 1.ª ed. Embrapa Amazônia Oriental, 39 p. [cit. 2016.07.24]. http://ainfo.cnptia.embrapa.br/digital/bitstream/item/69534/1/Doc266.pdf

Viégas, G.P. & Peeten, H. (1987) – Sistemas de produção. In: Paterniani, E. & Viégas, G.P. (Eds.). *Melhoramento e produção do milho.* Campinas, Fundação Cargill, p. 453-538.

Zsubori Z.; Gyenes-Hegyi Z.; Illés O.; Pók I.; Rácz F. & Szıke C. (2002) – Inheritance of plant and ear height in maize (*Zea mays* L.). *Acta Agraria Debreceniensis*, vol. 8, p. 34-38.