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PHYSIOLOGICAL QUALITY OF CORN SEEDS REINOCULATED WITH DIAZOTROPHIC BACTERIA

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ABSTRACT - This work aimed to evaluate physiological characteristics of corn seeds provenient from cultivation associated with *Azospirillum brasilense* and *Herbaspirillum seropedicae* and reinoculated with *Azospirillum brasilense*. The seeds were coming from cultivation in field conditions, and the treatments were: T1 - control; T2 - 60 kg hectare^{-1} N, T3 - inoculation (*Azospirillum brasilense* - AbV5); T4 - inoculation (*Azospirillum brasilense*) + 60 kg hectare^{-1} N; T5 - inoculation (*Herbaspirillum seropedicae* - SMR1) and T6 - inoculation (*Herbaspirillum seropedicae*) + 60 kg hectare^{-1} N. The treatments were divided in two separate blocks, being one reinoculated with *A. brasilense* (AbV5). The experimental design was completely randomized, in factorial scheme (6x2), being six treatments inoculated and reinoculated. The seeds reinoculated showed greater length, volume and dry matter of root and aerial part, concluding that the seeds reinoculation promotes an increase in initial seedling growth.

Key words: *Azospirillum brasilense*, *Herbaspirillum seropedicae*, seeds physiology, reinoculation, *Zea mays*.

Qualidade fisiológica de sementes de milho reinoculadas com bactérias diazotróficas

RESUMO - O objetivo deste trabalho foi avaliar a qualidade fisiológica de sementes de milho provenientes do cultivo associado com *Azospirillum brasilense* e *Herbaspirillum seropedicae* e reinoculadas com *A. brasilense*. As sementes utilizadas foram provenientes de cultivo em condições de campo, sendo os tratamentos: T1 - testemunha; T2 - 60 kg hectare^{-1} de N; T3 – inoculação (*Azospirillum brasilense* - AbV5); T4 - inoculação (*Azospirillum brasilense*) + 60 kg hectare^{-1} de N; T5 - inoculação (*Herbaspirillum seropedicae* - SmR1) e T6 - inoculação (*Herbaspirillum seropedicae*) + 60 kg hectare^{-1} de N. Foram separados dois lotes dos tratamentos, sendo um reinoculado com *A. brasilense* (AbV5). O delineamento foi inteiramente ao acaso com esquema fatorial (6x2), sendo seis tratamentos; com inoculação e reinoculadas. As sementes reinoculadas apresentaram maiores comprimentos, volumes e matéria seca de raízes e parte aérea, concluindo que a reinoculação com sementes promove um incremento no desenvolvimento inicial das plântulas.

Palavras-chave: *Azospirillum brasilense*, *Herbaspirillum seropedicae*, fisiologia de sementes, reinoculação, *Zea mays.*

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INTRODUCTION

The economic and environmental costs related to nitrogen fertilization have stimulated the search for alternatives that can reduce the use of fertilizer without any decrease in production (ROESCH et al., 2005). Thus, due to the growing demand for sustainability in agricultural production systems, some authors have presented as an alternative to the economy of nitrogen fertilizer, the biological nitrogen fixation (BNF), which can supplement or even substitutes the use of those fertilizers (BERGAMASCHI, 2007; REIS JÚNIOR et al., 2008).

One possibility to make possible the production at lower costs without damaging the environment would be the use of the genetic potential of plants, combined with the biological resources of the soil, like the diazotrophic bacteria, which are seen as promoting plant growth by having the ability to fix nitrogen (biological nitrogen fixation) for the plant, and produce growth hormones like auxins and gibberellins, which stimulate plant growth mainly roots, working in greater absorption of nutrients and water (DOBBELAERE et al., 2002).

Various diazotrophic bacteria were isolated from the corn crop, especially the species *Azospirillum lipoferum*, *A. brasilense* and *Herbaspirillum seropedicae*, being the most studied species of the genus *Azospirillum* (REIS et al., 2000).

Reis Júnior et al. (2008) observed an increase in the dry mass of corn plants inoculated with *Azospirillum* spp., and considered that the highest yield of dry matter and nutrient accumulation in inoculated plants are owed to the production of growth-promoting substances by bacteria. Bashan and Holguin (2004) reported that phytohormones, mainly indolacetic acid (IAA), excreted by *Azospirillum* perform an essential role in promoting the growth of plants in general.

Studies in Argentina and Brazil in recent decades have been compiled and the most of them indicate benefits of inoculation with *Azospirillum* on plant growth and/or increased productivity (CASSAN; GARCIA DE SALAMONE, 2008). However, the results of inoculation with diazotrophic bacteria in non-leguminous plants are not very consistent, although significant effects on grain yield and total N of the plant have been reported (BALDANI, 2005, GUIMARÃES et al., 2007).

Significant increases in grain yield were observed in bean crops in soils of the cerrado, in the second year of cultivation, using reinoculated seeds (HUNGRIA et al., 2000). Roesch et al. (2005) observed that bacteria reinoculated in host plant wheat, promoted root elongation of plants grown in vitro, and these bacteria were able to fix N2 and transfer it to the plant, supplying their needs until 21 days after germination.

However, Campos and Gnatta (2006), working with the reinoculation of different formulations of inoculants on soybean cultivated by tillage system 8-12 years did not observe any answers to reinoculation in three consecutive harvest seasons in the studied parameters.

Information from reinoculation diazotrophic bacteria in non-leguminous plants are restricted, particularly in relation to seed physiological quality. Therefore, it is important to evaluate the effect of reinoculation diazotrophic bacteria that was efficient in promoting the BNF in crops such as corn, in view of the advantages attributable to these bacteria.

In this context, the objective of this study was to evaluate the physiological quality of corn seed from cultivation associated by *Azospirillum brasilense* and *Herbaspirillum seropedicae* and reinoculated with *A. brasilense*.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Biological Control, equipped with a controlled environment, belonging to the Center for Experimental Stations of the State University of West Paraná, *Campus* Marechal Cândido Rondon, Paraná State.

The seeds that were used were from the cultivar DKB 390®, previously grown in field conditions, in the 2010/2011 season. The following treatments were performed: T1 - without nitrogen and without inoculation; T2 - fertilization with 60 kg N ha^{-1} and without inoculation; T3 - without nitrogen fertilization and inoculation with *Azospirillum brasilense* (strain AbV5), T4 - fertilization with 60 kg ha^{-1} of N and inoculation with *A. brasilense*, T5 - without nitrogen fertilization and inoculation with *Herbaspirillum seropedicae* (strain SMR1) T6 - fertilization with 60 kg ha^{-1} of N and inoculation with *H. seropedicae*.

Two lots were separated from these seeds from each treatment, and one of the lots were reinoculated to *A. brasilense* (strain AbV5) at a dosage of 150 mL per 50 kg of seed. The inoculant was provided by the Federal University of Paraná (UFPR), being prepared from a solution of bacteria in pure concentration of 1×10^8 CFU (Colony Forming Unit) mL^{-1}.

The addition of the inoculant occurred with the use of automatic pipette at the recommended dosage, with the seed and bacteria completely homogenized after the addition, ensuring a homogeneous distribution of the inoculant, moments before sowing, in an environment with controlled temperature and lighting (HUNGRIA, 2011).

For performing the tests, was used a completely randomized design factorial (6x2) and six treatments from the previous management of the seeds; inoculated and reinoculated with four replications of 25 seeds. Using as substrate was sand of particle size less than 2 mm sterilized by autoclaving at 120 °C for 15 min, humidified with water in the amount of 60% of sand retention capacity (BRASIL, 2009).

For sowing, we used plastic boxes (42 x 28 x 6 cm), which were seeded four replicates of each treatment. The seeds were placed over an uniform layer of moist sand and covered with loose sand so as to obtain a layer of approximately one centimeter on seeds and then subjected to the constant temperature of 25 °C with 12 h photoperiod.

In the test of viability of the seeds, germination percentage corresponded to the proportion of the number of seeds germinated and was considered done from the moment in which we observed a stabilization of seedling germination (BRASIL, 2009).

Conducted with the germination test was realized the vigor test (first count) counting the percentage of seedlings considered normal (normal seedlings are those that show potential to continue their development and give rise to normal plants when grown under conditions favorable) for four days after sowing (DAS) (BRASIL, 2009).

The seedlings were removed from the substrate after germination cessing. These were washed and then performed to measurement of the number and length of roots and plant height for this using a ruler. After that, the roots were cut and washed again to remove excess substrate and subsequently were immersed in water in a graduated cylinder for estimating the root volume (ROSSIELLO et al., 1995). Put up samples of shoots and roots in drying oven with forced air circulation at 65° C, until constant weight. Subsequently it was determined the dry matter thereof.

Was verified normality of the data and these were submitted to analysis of variance (ANOVA) and means were compared by Tukey test at 5% probability, with statistical program support SISVAR 5.0 (Ferreira, 2003).

RESULTS AND DISCUSSION

The results presented in Table 1 show that there were no significant differences in the interaction between treatment and inoculation of bacteria, indicating that there was no influence of the tested treatments on seed germination of corn. It is observed that the difference was significant only in the mean of the inoculated, and the seeds reinoculated with *A. brasilense* showed germination at 11.9%, compared to seeds from inoculation.

TABLE 1. Final germination analyzed 10 DAS and first count of seed corn (4 DAS) (DKB 390®), from inoculation with diazotrophs and nitrogen fertilization, and reinoculated with *Azospirillum brasilense*.

Treatments*	Final germination (%)			First count of the germination (%)		
	Inoculated	Reinoculated	Mean	Inoculated	Reinoculated	Mean
0 N	72.00	91.00	81.50	51.00	72.00	61.50 a
60 N	84.00	87.00	85.50	45.00	63.00	54.00 ab
AbV5	81.00	87.00	84.00	48.00	63.00	55.50 ab
AbV5 + 60 N	80.00	86.00	83.00	30.00	47.00	38.50 c
SmR1	84.00	98.00	91.00	49.00	44.00	46.50 bc
SmR1 + 60 N	86.00	96.00	91.00	52.00	67.00	59.50 ab
Mean	81.17 B	90.83 A		45.83 B	59.33 A	
CV (%)	9.90			17.27		

Means followed by the same letter, lowercase in the column and uppercase on the line do not differ significantly by Tukey test (P ≤ 0.05). *0 N - without nitrogen and without inoculation; 60 N - fertilization with 60 kg N ha^{-1} and without inoculation; AbV5 - without nitrogen fertilization and inoculation with *Azospirillum brasilense* (strain AbV5), AbV5 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *A. brasilense*, SmR1 - without nitrogen fertilization and inoculation with *Herbaspirillum seropedicae* (strain SMR1) SmR1 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *H. seropedicae*.

TABLE 2. Length and volume of root of corn seedlings (DKB 390®) analyzed 10 DAS, from inoculation with diazotrophs and nitrogen fertilization, and reinoculated with *Azospirillum brasilense*.

Treatments*	Length of root (cm)			Volume of root (mL)		
	Inoculated	Reinoculated	Mean	Inoculated	Reinoculated	Mean
0 N	16.58 abA	18.21 aA	17.39	1.11 aB	1.31 aA	1.21
60 N	14.57 abcA	15.82 aA	15.19	1.08 abB	1.29 aA	1.18
AbV5	12.02 cB	16.37 aA	14.19	0.94 cB	1.23 abA	1.08
AbV5 + 60 N	17.78 abA	15.03 aA	16.40	1.11 aA	1.18 bA	1.14
SmR1	13.34 bcB	16.62 aA	14.98	1.00 bcB	1.18 bA	1.09
SmR1 + 60 N	17.90 aA	14.79 aB	16.34	1.00 bcB	1.30 aA	1.15
Mean	15.36	16.14		1.04	1.24	
CV (%)	13.28			4.14		

Means followed by the same letter, lowercase in the column and uppercase on the line do not differ significantly by Tukey test (P ≤ 0.05). *0 N - without nitrogen and without inoculation; 60 N - fertilization with 60 kg N ha^{-1} and without inoculation; AbV5 - without nitrogen fertilization and inoculation with *Azospirillum brasilense* (strain AbV5), AbV5 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *A. brasilense*, SmR1 - without nitrogen fertilization and inoculation with *Herbaspirillum seropedicae* (strain SMR1) SmR1 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *H. seropedicae*.

With respect to test first germination counting of seeds, there is no statistical difference in the interaction between treatments and forms of inoculation, however it is observed in the average that the seeds reinoculated also were higher than those inoculated at 29.46%. For treatments, the control 0 N was in the highest value of the first count germination. Treatment with *A. brasilense* associated with 60 kg ha^{-1} N, was what gave the worst performance in seed vigor.

Conceição et al. (2008) also observed that inoculation with diazotrophic bacteria did not affect the germination of corn. Ramamoorthy et al. (2000) found that inoculation with *Azospirillum* increased the germination of rice seeds from two loads with different vigor levels at 14 days of installation.

In seeds of *Catharanthus roseus* (L.), inoculation with diazotrophic bacteria (*Azotobacter* and *Azospirillum*), isolated from the rhizosphere and root of this plant, favored germination and vigor in gnotobiotics conditions (KARTHIKEYAN et al., 2007).

It has been found in root length (Table 2) of the seedlings from inoculated seeds, the highest yield was provided when the inoculation of *H. seropedicae* plus 60 kg ha^{-1} of nitrogen already when the bacteria *A. brasilense* inoculated without nitrogen addition, this treatment is that provided the worst result. The root length was not affected when there was a reinoculation of the seeds with *A. brasilense*. For interaction between the inoculated seeds and reinoculated the reinoculation provided increments in root length for treatments with bacteria without the addition of nitrogen, as for treatment with *H. seropedicae* associated with 60 kg N ha^{-1}, the reinoculation provided opposite effect, reducing the root length.

Roesch et al. (2005), studying the reinoculation diazotrophic bacteria in wheat plants, found that the bacteria reinoculated in the host plant promoted root elongation in plants grown *in vitro*.

These results can be explained if we consider that the reinoculation of seed with a strain AbV5 of *A. brasilense* might have optimized the synthesis of hormones, which stimulated the development of the root system of seedlings. Dobbelaere (2001), working with bacteria inoculation concludes that the use of these bacteria promote gains in both metabolism and vigor of these plants.

For the root volume of corn seedlings, it is observed that the treatments control 0 kg ha^{-1} and *A. brasilense* associated with 60 kg N ha^{-1}, provided higher values in seedlings originating from seed inoculation. In the reinoculated seeds, the treatments involving the controls (0 and 60 kg ha N^{-1}) as well as the seeds inoculated with strain SMR1 (*Herbaspirillum*) provide higher volumes of the seedlings root. Already in the interaction, the corn seedlings reinoculated with *A. brasilense* had their root volume significantly affected by up to 30.9% higher compared to inoculated (Table 2), except treatment with AbV5 inoculation + 60 kg ha^{-1} of N, even showing similar behavior, showed an increase of 6.3% in the root volum when reinoculated with *A. brasilense*.

Various authors have observed that plants infected with bacteria of the genus *Azospirillum* promote a significant increase of biomass and root volume mainly in the early stages of plant development (OKON and LABANDERA-GONZALEZ, 1994).

This fact can be explained by the synthesis of hormones, mainly auxin which enables greater development of the root system, resulting in greater absorption of water and nutrients to the physiological needs of the plant. Another factor driven by reinoculation and consequently higher rate of colonization of these bacteria, a factor that combined with higher nitrogen fixation provide a better root development, roots thickest, consequently more efficient and a larger volume of the same (BERTICELLI and NUNES, 2008).

For the number of roots (Table 3), it is verified that no significant interaction between seed inoculation and from reinoculated. As for the treatment averages, it is observed that the without N and without inoculation, the seedlings showed the highest number of roots. The accumulation of dry matter in roots, coming from the inoculation showed similarity, since when were reinoculated, treatment with 60 kg N ha^{-1} resulted in the highest amount of dry matter. The interaction between the inoculated seeds and reinoculated when seeds were inoculated with diazotrophic bacteria, these showed no significant differences with the seeds reinoculated only in treatments witness, 0 and 60 kg ha^{-1} provided significant effect for the reinoculation increasing the value of dry matter seedling root.

It is noteworthy that treatment with diazotrophic bacteria without added nitrogen, showed the lowest values of root dry matter when reinoculated. Reis Júnior et al. (2008) showed that insufficiency of N in the soil, resulting in deficiency in tissues of shoots, inhibiting the growth of roots, resulting in lower dry weight of roots. The results may be explained by Majerowicz (2002), where the reinoculation of seeds from treatments with an absence of N stimulates greater efficiency in N uptake per unit of root mass, where the those cases, the bacteria act as a compensatory mechanism between N in the soil and root development.

Based on the values of seedling height (Table 4), we find that among the inoculated seed treatment with strain AbV5 (*Azospirillum*) and 60 kg ha^{-1} showed the best average being higher than 51% on average the witness 0 kg ha^{-1} N. As the seeds reinoculated, the witness and treatment with *H. seropedicae* (SMR1), presented the highest height, taking into account treatments with both bacteria associated with 60 kg N ha^{-1} had the lowest heights.

For the interaction between seed inoculated with reinoculated, it was proved behavior except where the treatment involving strains AbV5 and SMR1 with 60 kg ha^{-1} of nitrogen, all other means reinoculated were higher.

Observe that for matter of the aerial part of corn seedlings that treatments witness 0 kg ha^{-1} and *H. seropedicae* (SMR1), presented the highest values in seedlings from inoculated seeds, when the seeds have been reinoculated with *A. brasilense*, treatment with *H. seropedicae* associated with 60 kg N ha^{-1}, provided the highest dry matter accumulation of shoots. The interaction between treatment and inoculation, showed that the reinoculation of the seeds provided increments of up to 70.4% in the amounts of dry matter in the aerial part except for treatments with *A. brasilense* associated with 60 kg ha^{-1}, and *H. seropedicae*, that showed similar behavior compared seedlings grown from seeds inoculated.

These informations are consistent with Berticelli and Nunes (2008), in which working with roots reinforcers in corn, claimed that reinoculation with bacteria dormant in the seed provides a greater synthesis of hormones as growth promoters, which causes an acceleration in the metabolism of seedlings and rate of cell division. Already Ferreira (2008), working with two varieties of rice and reinoculation in the seed with *H. seropedicae*, did not find statistical difference as the dry matter of the aerial part among inoculated and reinoculated.

TABLE 3. Number and root dry matter of corn seedlings (DKB 390®) analyzed 10 DAS, from inoculation with diazotrophs and nitrogen fertilization, and reinoculated with *Azospirillum brasilense*.

Treatments*	Number of root			Root dry matter (g)		
	Inoculated	Reinoculated	Mean	Inoculated	Reinoculated	Mean
0 N	4.98	4.73	4.85 a	0.31 aB	0.35 abA	0.33
60 N	4.75	4.55	4.65 ab	0.33 aB	0.39 aA	0.36
AbV5	4.40	4.20	4.30 b	0.34 aA	0.33 bA	0.33
AbV5 + 60 N	4.83	4.63	4.73 ab	0.32 aA	0.34 abA	0.33
SmR1	4.23	4.23	4.23 b	0.33 aA	0.31 bA	0.32
SmR1 + 60 N	4.10	4.38	4.24 b	0.32 aA	0.35 abA	0.33
Mean	4.55	4.45		0.33	0.34	
CV (%)	7.59			6.99		

Means followed by the same letter, lowercase in the column and uppercase on the line do not differ significantly by Tukey test ($P \leq 0.05$). *0 N - without nitrogen and without inoculation; 60 N - fertilization with 60 kg N ha^{-1} and without inoculation; AbV5 - without nitrogen fertilization and inoculation with *Azospirillum brasilense* (strain AbV5), AbV5 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *A. brasilense*, SmR1 - without nitrogen fertilization and inoculation with *Herbaspirillum seropedicae* (strain SMR1) SmR1 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *H. seropedicae*.

TABLE 4. Height and aerial part dry matter of corn seedlings (DKB 390®) analyzed 10 DAS, from inoculation with diazotrophs and nitrogen fertilization, and reinoculated with *Azospirillum brasilense*.

Treatments*	Height of seedling (cm)			Aerial part dry matter (g)		
	Inoculated	Reinoculated	Mean	Inoculated	Reinoculated	Mean
0 N	10.66 bB	13.07 aA	11.86	0.29 aB	0.36 bA	0.33
60 N	7.85 bcB	10.19 abA	9.02	0.24 bB	0.32 bcA	0.28
AbV5	6.89 cB	10.66 abA	8.78	0.18 cB	0.31 bcA	0.24
AbV5 + 60 N	16.16 aA	8.12 bB	12.14	0.28 abA	0.30 cA	0.30
SmR1	9.29 bcB	11.98 aA	10.64	0.29 aA	0.28 cA	0.29
SmR1 + 60 N	8.47 bcA	8.14 bA	8.31	0.27 abB	0.46 aA	0.37
Mean	9.89	10.36		0.26	0.34	
CV (%)	15.25			8.79		

Means followed by the same letter, lowercase in the column and uppercase on the line do not differ significantly by Tukey test ($P \leq 0.05$). *0 N - without nitrogen and without inoculation; 60 N - fertilization with 60 kg N ha^{-1} and without inoculation; AbV5 - without nitrogen fertilization and inoculation with *Azospirillum brasilense* (strain AbV5), AbV5 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *A. brasilense*, SmR1 - without nitrogen fertilization and inoculation with *Herbaspirillum seropedicae* (strain SMR1) SmR1 + 60 N - fertilization with 60 kg ha^{-1} of N and inoculation with *H. seropedicae*.

Cereglioli (2005) verifying the efficiency of diazotrophic bacteria says that auxin and indole acetic acids are primarily responsible for the increase in dry weight of seedlings from seeds reinoculated with bacteria, promoting more vigorous root systems, providing support for plants from greater heights (RAMOS, 2010).

According Reis Jr. (2008) in a general aspect, the reinoculation with *A. brasilense* promotes increments in both characteristics due primarily to morphological and physiological changes in seedlings of these seeds, leading to an increase in the uptake of water and nutrients. Actually, the highest values of dry matter occurs due to bacteria production of growth promoting substances, mainly indole acetic acid (IAA) (HOLGUIN, 2004).

This relationship between of water content and plant growth is altered in the presence of bacteria, because they provide greater water absorption (KAPPES et al., 2013), generating greater juiciness tissues, as reflected in the relationship between height and dry mass.

CONCLUSIONS

The reinoculation of corn seeds with *Azospirillum brasilense* promoted increase in length, volume and root dry matter and height and dry weight of shoots of maize seedlings.

Reinoculated seeds showed better early seedling development.

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REFERÊNCES

BALDANI, V.L.D.; BALDANI, J.I.; DOBEREINER, J. Inoculation of rice plants with endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. **Biology and Fertility of Soils**, v.30, p.485-491, 2000.

BASHAN, Y.; HOLGUIN, G; DE-BASHAN, L.E. Azospirillum-plant relations physiological, molecular, agricultural, and environmental advances (1997-2003). **Canadian Journal of Microbiology**, v.50, p.521-577, 2004.

BERGAMASCHI, C.; ROESCH, L.F.W.; QUADROS, P.D.; CAMARGO, F.A.O. Ocorrência de bactérias diazotróficas associadas a cultivares de sorgo forrageiro. **Ciência Rural**, v.37, p.727-733, 2007.

BERTICELLI, E.; NUNES, J. Avaliação da eficiência do uso de enraizador na cultura do milho. **Cultivando o Saber**, v.1, p.34-42, 2008.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento**. Regras para análise de sementes.** Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. – Brasília: Mapa/ACS, 2009. 399 p.

CAMPOS, B.C.; GNATTA, V. Inoculantes e fertilizantes foliares na soja em área de populações estabelecidas de *Bradyrhizobium* sob sistema de plantio direto. **Revista Brasileira de Ciência do Solo,** v.30, p.69-76, 2006.

CASSÁN, F.D.; SALAMONE, G. ***Azospirillum* spp.: cell physiology, plant interactions and agronomic research in Argentina.** I. (Ed.) Argentina: Asociación Argentina de Microbiologia, 2008. 268 p.

CERIOGLI, M.M. **Diversidade de bactérias endofíticas de raízes de milho (*Zea mays* L.) e potencial para promoção de crescimento** – Tese Doutorado São Carlos: UFSCar, 2005. 132 p.

CONCEIÇÃO, P.M.; VIEIRA, H.D.; CANELLAS, L.P.; MARQUES JUNIOR, R.B.; OLIVARES, F.L. Recobrimento de sementes de milho com ácidos húmicos e bactérias diazotróficas endofíticas. **Pesquisa Agropecuária Brasileira**, v.43, p.545-548, 2008.

DOBBELAERE, S. CROONENBORGHS, A.; THYS, A.; PTACEK, D.; OKON, Y.; VANDERLEYDEN, J. Effect of inoculation with wild type *Azospirillum brasilense* and *A. irakense* strains on development and nitrogen uptake of spring wheat and grain corn. **Biology and Fertility of Soils**, v.36, p.284-297, 2002.

DOBBELAERE, S.; CROONENBORGHS, A.; THYS, A., PTACEK, D., VANDERLEYDEN, J., DUTTO, P. Responses of Agronomically important crops to inoculation with *Azospirillum*. **Australian Journal of Plant Physiology,** v.28, p.871-879, 2001.

FERREIRA, J.S. **Inoculação de *Herbaspirillum seropedicae* em duas variedades de arroz irrigado: qualidade do inoculante e necessidade da reinoculação.** UFRRJ, Tese Doutorado. 2008. 85 p.

FERREIRA. D.F. **SISVAR – Sistemas de análises estatísticas**. Lavras, UFLA. 2003.

GUIMARÃES, S.L.; BALDANI, J.I.; BALDANI, V.L.D.; JACOB-NETO, J. Adição de molibdênio ao inoculante turfoso com bactérias diazotróficas usado em duas cultivares de arroz irrigado. **Pesquisa Agropecuária Brasileira**, v.42, p.393-398, 2007.

HUNGRIA, M.; CAMPO, R.J.; MENDES, I.E. **Fixação biológica do nitrogênio com a cultura de soja**. Workshop Nitrogênio na sustentabilidade de sistemas intensivos de produção agropecuária, Dourados-MS, p.51-75. 2000.

HUNGRIA, M. **Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo.** Documentos Embrapa Soja N.325. 36 p.

KAPPES, C.; ARF, O.; ARF, M.V.; FERREIRA, J.P.; DAL BEM, E.A.; PORTUGAL, J.R.; VILELA, R.G. Inoculação de sementes com bactéria diazotrófica e aplicação de nitrogênio em cobertura e foliar em milho. **Semina: Ciências Agrárias,** v.34, n.2, p.527-538, 2013.

KARTHIKEYAN, B.; JALEEL, C.A.; GOPI, R.; DEIVEEKASUNDARAM, M. Alterations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seed priming with native diazotrophs. **Journal of Zhejiang University**, v.8, p.453-457, 2007.

MAJEROWICZ, N.; PEREIRA, J.M.S.; MEDICI, L.O.; BISON, O.; PEREIRA, M.B.; SANTOS JÚNIOR, U.M. Estudo da eficiência de uso do nitrogênio em variadedes locais e melhoradas de milho. **Revista Brasileira de Botânica**, v.25, p.129-136, 2002.

OKON, Y.; LABANDERA-GONZALEZ, C.A. Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. **Soil Biology & Biochemistry**, v.26, p.1591-1601, 1994.

RAMAMOORTHY, K.; NATARAJAN, N.; KILLIKULAM, T.N.A.U.; VALLANAD, P.O. Seed biofortification with *Azospirillum* spp. for improvement of seedling vigour and productivity in rice (*Oryza sativa* L.). **Seed Science and Technology**, v.28, p.809-815, 2000.

RAMOS, A.S; SANTOS, dos.; T.M.C; SANTANA, T.M.; GUEDES, E.L.F.; MONTALDO, Y.C. Ação do *Azzospirillum lispoferum* no desenvolvimento de plantas de milho. **Revista verde**, v.5, n.4, p.113-117, 2010.

REIS, V.M.; BALDANI, V.L.D.; BALDANI, J.I.; DÖBEREINER, J. Biological nitrogen fixation in gramineae and palm trees. **Critical Review in Plant Sciences**, v.19, p.227-247, 2000.

REIS JÚNIOR, F.B.; MACHADO, C.T.T.; MACHADO, A.T.; MENDES, I.C.; MEHTA, A. **Isolamento, caracterização e seleção de estirpes de *Azospirillum amazonense* e Herbaspirillum seropedicae associadas a diferentes variedades de milho cultivadas no Cerrado.** Planaltina: EMBRAPA-CERRADOS, 2008. 36 p.

REIS JÚNIOR, F.B.; MACHADO, C.T.T.; MACHADO, A.T.; SODEK, L. Inoculação de *Azospirillum amazonense* em dois genótipos de milho sob diferentes regimes de nitrogênio. **Revista Brasileira de Ciência do Solo**, v.32, n.3, p.1139-1146, 2008.

ROESCH, L.F.; CAMARGO, F.O.; SELBACH, P.A.; SÁ, E.S. Reinoculação de bactérias diazotróficas aumentando o crescimento de plantas de trigo. **Ciência Rural**, v.35, p.1201-1204, 2005.

ROSSIELLO, R.O.P.; ARAÚJO, A.P.; MANZATTO. C.V. Comparação dos métodos fotoelétricos e da interseção na determinação da área, comprimento e raio médio radicular. **Pesquisa Agropecuária Brasileira**, v.30, p.633-638, 1995.