Influência da inoculação de *Azospirillum sp*. em trigo, cevada e aveia

Influence of *Azospirillum sp*. inoculation in wheat, barley and oats

Osmar Roberto Dalla Santa[1]
Herta Stutz Dalla Santa[2]
Ramona Fernández[3]
Georgina Michelena[4]
Pedro Ronzelli Júnior[5]
Carlos Ricardo Soccol[6]

Abstract

The aim of this study has been to evaluate the influence of inoculation with *Azospirillum sp*. RAM-7 in wheat, barley and oats on yields, density and N total of grains. The experiments were conducted under field conditions. The treatments involved bacterial inoculation with or without different levels of nitrogen fertilization and absolute control. The inoculation was carried out by the seed coating method with bacterial suspension containing 1010 CFU.mL-1. Statistically significant effects on grain yield in wheat were obtained at the inoculated treatment without any nitrogen fertilizer, increasing the yield by 23.9% over de control ($p<0.05$). In the inoculated treatment associated with 48 kg of N.ha-1, the yield was similar to that obtained at 60 kg of N.ha-1 with or without inoculation. In barley, the grain yields of the inoculated treatment associated with 32 kg of N.ha-1 was statistically equivalent to that obtained with 40 kg de N.ha-1 with or without inoculation ($p<0,05$). In oats, there was no

1 Dr.; Professor do Departamento de Engenharia de Alimentos da Universidade Estadual do Centro-Oeste-UNICENTRO; E-mail: ordallasanta@yahoo.com.br

2 Drª.; Bióloga; Professora do Departamento de Engenharia de Alimentos da Universidade Estadual do Centro-Oeste-UNICENTRO; E-mail: hdalsanta@yahoo.com

3 Drª.; Bióloga; Instituto Cubano de Investigação de Derivados de Cana-de-açúcar-ICIDCA.

4 Drª.; Engenheira Química; Instituto Cubano de Investigação de Derivados de Cana-de-açúcar-ICIDCA.

5 Dr.; Engenheiro Agrônomo; Universidade Presbiteriana Mackenzie.

6 Dr.; Engenheiro Químico; Professor do Departamento de Engenharia Química da Universidade Federal do Paraná-UFPR; E-mail: soccol@ufpr.br

Recebido para publicação em 28/11/2006 e aceito em 19/12/2007

significant difference between treatments and their respective controls for yields, density and N total of grains. The results of the present investigations showed the possibility of the partial substitution of the inorganic nitrogen fertilizer with the *Azospirillum sp*. RAM-7 inoculation in wheat, barley and oats.

Key words: wheat; barley; oats; nitrogen fixation; *Azospirillum*.

Resumo

O objetivo deste estudo foi avaliar a influência da inoculação com *Azospirillum sp*. RAM-7 em trigo, cevada e aveia no rendimento, densidade e nitrogênio total nos grãos. Os experimentos foram conduzidos em condições de campo. Os tratamentos envolveram a inoculação de bactérias, com a presença ou não de diferentes doses de fertilização nitrogenada e o controle absoluto. A inoculação foi feita pelo método de contaminação das sementes com uma suspensão de bactérias contendo 1010 UFC.mL-1. Efeitos estatisticamente significativos sobre a produtividade de grãos de trigo foram obtidos no tratamento inoculado e sem a adição de fertilização nitrogenada, aumentando o rendimento em 23,9% em relação ao controle ($p<0,05$). No tratamento inoculado associado a 48 kg de N.ha-1, a produtividade foi similar à obtida no tratamento com 60 kg de N.ha-1 com ou sem inoculação. Na cevada, o rendimento de grãos do tratamento inoculado associado a 32 kg de N.ha-1 foi semelhante ao que recebeu 40 kg de N.ha-1 com ou sem inoculação ($p<0,05$). Na aveia, os tratamentos não diferiram de seus controles em relação ao rendimento, densidade e nitrogênio total dos grãos. Os resultados obtidos nesta pesquisa indicam a possibilidade da substituição parcial da fertilização nitrogenada inorgânica pela inoculação de *Azospirillum sp*. RAM-7 nas culturas de trigo, cevada e aveia.

Palavras-chave: trigo; cevada; aveia; fixação de nitrogênio; *Azospirillum*.

Introduction

Many diazotrophic bacteria were isolated from rhizosphere soil or the rhizoplane of a big variety of non-leguminous plants. The first species of *Azospirillum* was isolated by Beijerinck in 1925 from N-poor sandy soil and was originally named *Spirillum lipoferum*. This bacterium was later isolated from soil and from rhizosphere of roots of a variety of grasses (TARRAND et al., 1978; SUNDARAM et al., 1987; KIRCHHOF et al., 1997; PANDEY et al., 1998). Nitrogen-fixing bacteria of the genus *Azospirillum* colonize the rhizosphere of economically important crops exerting beneficial effects on

plant growth, although the mechanism of colonization by *Azospirillum* species is not clear (STEENHOUDT and VANDERLEYDEN, 2000; RAMOS et al., 2002). At present, five species have been described: *Azospirillum lipoferum, A. brasilense, A. amazonense, A. halopraeferens* and *A. irakense* (DÖBEREINER and PEDROSA, 1987; FANI et al., 1995; BASHAN and HOLGUIN, 1997; ALEXANDRE et al., 1999; HAUWAERTS et al., 2002).

Inoculation of plants with *Azospirillum* can result in significant change in various plant growth parameters, which may or may not affect yield. Increases in the amount of N total, grains weight, germination rates, plant dry weight, increase in the density and length of root hairs, number of spikes and grains per spike were reported (KAPULNIK et al., 1981; BASHAN and LEVANONY, 1990; OKON and VANDERLEYDEN, 1998; JOFRÉ et al., 1998; DALLA SANTA et al., 2004a).

Plant growth promotion by *Azospirillum* has been demonstrated in field and greenhouse experiments (SUMNER, 1990). Success in crop yield enhancement by microorganisms, depends the ability of *Azospirillum* to survive and to colonize germinated seeds successfully in the presence of a large number of other indigenous rhizosphere microorganisms. The abundance and the activity of theses microorganisms depend yet on the soil properties (JACOUD et al., 1998; PEREG-GERK et al., 1998; ALEXANDRE et al., 1999; JAMES, 2000; CHOTTE et al., 2002). The successful colonization of the plant root surface by the bacterium is thought to be dependent on active motility and chemotaxis toward root exudates (BASHAN and LEVANONY, 1990; CREUS et al., 1996; BASHAN and HOLGUIN, 1997; HAUWAERTS et al., 2002).

Yield increases have been attributed to mechanisms such as nitrogen fixation, nitrate reduction, protection from pathogenic plant microorganisms and production of plant-growth promoting substances (BARBIERI et al., 1986; FULLCHIERI and FRIONI, 1994; REIS et al., 2000; FISCHER et al., 2003). In several field experiments, with different cultures, found increases of the grains yield after inoculation with different bacteria strain of *Azospirillum*. (RAO, 1981; SUMNER, 1990; DIDONET et al., 1996).The effect on the total yield increase of field-grow plants generally ranged from 5 to 30%. However, the reported effects on yield, due to bacterial inoculations, have not always been positive, many negative or no-effect results of inoculation were rarely reported (BASHAN and LEVANONY, 1990; SUMNER, 1990; BASHAN and HOLGUIN, 1997).

In this work, three experiments were carried out in field conditions, with the aim to evaluate the contribution of inoculation with *Azospirillum sp.* RAM-7 in wheat, barley and oats on grain yield and grain quality.

Material and methods

Bacterial strain, media and growth conditions: *Azospirillum sp.* RAM-7 isolated from roots of sugar cane, was obtained from Instituto Cubaño de Investigaciones de los Derivados de la

Canã de Azúcar (ICIDCA). *Azospirillum* were grown in medium containing ($g.L^{-1}$) fructose: 12; yeast extract: 1.0; Na_2PO_4: 4.2; $(NH_4)2HPO_4$: 3.5; $MgSO_4.7H_2O$: 0.2; KCl: 0.02; $CaCl_2$: 0.01; $NaMoO_4.2H_2O$: 0.01; $FeCl_3$: 0.015. The initial pH was adjusted to 7.3. After autoclaving the medium at 121 °C for 20 min the culture was added in the medium and incubated for 18 h at 37 °C, 250 rpm at 0.9 vvm (volume of air/volume of media by minute) of aeration in a fermenter MDL Marubishi (FERNÁNDEZ et al., 1999). Final cells concentration in the fermenter was the 10^{10} $CFU.mL^{-1}$. The bacterial suspension was stored at 4 °C and used as seeds inoculation.

Treatments: For the experiments of wheat the treatments were: (1) control (uninoculated and no N), (2) *Azospirillum* sp. RAM-7 inoculation (IN) and no N, (3) IN plus 12 kg of $N.ha^{-1}$, (4) IN plus 24 kg of $N.ha^{-1}$, (5) IN plus 36 kg of $N.ha^{-1}$, (6) IN plus 48 kg of $N.ha^{-1}$, (7) IN plus 60 kg of $N.ha^{-1}$, and (8) 60 kg of $N.ha^{-1}$ and uninoculated. For the experiments of barley and oats the treatments were: (1) control (uninoculated and no N), (2) *Azospirillum sp*. RAM-7 inoculation (IN) and no N, (3) IN plus 8 kg of $N.ha^{-1}$, (4) IN plus 16 kg of $N.ha^{-1}$, (5) IN plus 24 kg of $N.ha^{-1}$, (6) IN plus 32 kg of $N.ha^{-1}$, (7) IN plus 40 kg of $N.ha^{-1}$, and (8) 40 kg of $N.ha^{-1}$ and uninoculated.

Inorganic nitrogen was applied during sowing and as cover fertilizer; phosphorous (70 $Kg.ha^{-1}$) and potassium (40 $Kg\ ha^{-1}$) were similar for all treatments. The inoculation was carried out by the seed coating method with bacterial suspension (10^{10} $CFU.\ mL^{-1}$). The final bacterial count in seeds was 10^6 $CFU.g^{-1}$.

Experimental designs and analysis: The treatments were distributed in complete randomized blocks with four replicate blocks per treatment. The individual replicated plot size was 1.0 per 5.0 m with six plant rows. For the evaluation, two external rows and 0.5 m in each extremity were considered borders. The effect of the *Azospirillum sp.* RAM-7 inoculation in wheat, barley and oats was analyzed on grain yield, grain density and total nitrogen grain by Kjeldahl methods (AOAC, 1990). The yield plot measurements for each treatment were transformed to $kg.ha^{-1}$. The data were statistically analyzed using Tukey multiple test at significance P = 0.05.

Results and discussion

Effect of *Azospirillum sp.* RAM-7 inoculation in wheat OR-1: The response of *Azospirillum sp*. RAM-7 inoculation in wheat OR-1 at different levels of nitrogen is presented in table 1. In this trial, significant differences on grain yield were obtained by the inoculation or by the nitrogen level. On the other hand, the influence of the treatment on grain density and the total nitrogen in grains were not statistically significant.

The simple seeds inoculation without any nitrogen additions increased in 23.9% wheat grain yield, over the corresponding control. Increase on crops yield derived from *Azospirillum* inoculation have been previously also obtained in field conditions at low levels of nitrogen fertilization or without any nitrogen additions (MILLET et al., 1984; BODDEY and DÖBEREINER, 1988; DIDONET et al., 1996). In this experiment, significant positive response

Table 1. Effects of *Azospirillum sp.* RAM-7 inoculation at different nitrogen level in wheat OR-1 on grain yield, grain density and grain nitrogen total

Treatments	Grain yield ($kg.ha^{-1}$)	Grain density ($kg.hl^{-1}$)	Grain nitrogen total (%)
Control	1.058 d	69.1 a	1.77 a
Inoculated (IN)	1.311 c	70.6 a	1.76 a
IN plus 12 kg $N.ha^{-1}$	1.472 c	70.4 a	nd
IN plus 24 kg $N.ha^{-1}$	1.793 b	70.1 a	nd
IN plus 36 kg $N.ha^{-1}$	1.955 b	70.3 a	nd
IN plus 48 kg $N.ha^{-1}$	2.262 a	71.0 a	nd
IN plus 60 kg $N.ha^{-1}$	2.420 a	71.1 a	1.71 a
60 kg $N.ha^{-1}$	2.245 a	69.6 a	1.70 a

[1]Means followed by the different letters, in de same column are statistically different (P<0,05) according Tukey's multiple range test.
IN - inoculated
nd – not determined.

due to inoculation on grain yield, were observed even at the higher level of nitrogen. The inoculated treatment associated with 60 kg of $N.ha^{-1}$ increased the grain yield by 7.8% over the control with 60 kg of $N.ha^{-1}$ without inoculation, but no statistical difference was observed. The yield in the inoculated treatment associated with 48 kg of $N.ha^{-1}$ was statistically equivalent to that obtained with 60 kg of $N.ha^{-1}$ with or without inoculation. In this case, the inoculation with *Azospirillum sp.* RAM-7 replaced about 20% of the inorganic nitrogen recommended for this experiment.

The present results, together with previous reports (RAO, 1981; BODDEY et al., 1986; RAI and GUAR, 1988; SUMNER, 1990) clearly indicated that wheat inoculation with *Azospirillum sp.* increased the grain yield. That increase on grain yield has been explained through *Azospirillum sp*. enhancing root branching and root hair formation and, therefore, increased root uptake capacity. This effect on the root systems was probably due to growth hormones secreted by bacteria (KAPULNIK et al., 1981, KUCEY, 1988; KENNEDY et al., 1997; HECHT-BUCHHOLZ, 1998).

Effect of *Azospirillum sp.* RAM-7 inoculation in barley BR-2: The results on grain yield, grain density and grain nitrogen total of barley in the experiment with *Azospirillum sp*. RAM-7 were shown in table 2. In this experiment, the seed inoculation increased the grain yield. The yield obtained in the treatment inoculated by *Azospirillum sp.* without any nitrogen level was 19% higher as compared to the control uninoculated and without nitrogen. The beneficial effect of inoculation with *Azospirillum sp.* on grain yield was yet observed when combined with inorganic nitrogen level. The inoculated treatment associated with 40 kg of $N.ha^{-1}$ increased the grain yield in 8% above that with 40 kg of N. ha^{-1}, without inoculation, but the yield differences were not statistically significant. However, the effects of inoculation, in general were significantly

Table 2. Effects of *Azospirillum sp.* RAM-7 inoculation at different nitrogen level in barley BR-2 on grain yield, grain density and grain nitrogen total

Treatments	Grain yield ($kg.ha^{-1}$)	Grain density ($kg.hl^{-1}$)	Grain nitrogen total (%)
Control	1.178 e	58.9 b	1.31 b
Inoculated (IN)	1.401 de	60.7 a	1.32 b
IN plus 8 kg $N.ha^{-1}$	1.372 e	60.4 ab	nd
IN plus 16 kg $N.ha^{-1}$	1.713 cd	60.2 ab	nd
IN plus 24 kg $N.ha^{-1}$	1.839 bc	59.8 ab	nd
IN plus 32 kg $N.ha^{-1}$	2.158 ab	59.9 ab	nd
IN plus 40 kg $N.ha^{-1}$	2.355 a	60.1 ab	1.39 a
40 kg $N.ha^{-1}$	2.184 a	60.1 ab	1.36 ab

[1]Means followed by the different letters in de same column are statistically different (P<0,05) according Tukey's multiple range test.
IN - inoculated
nd – not determined.

higher to that of control. Similar beneficial responses of yield were observed by other authors (RAI and GUAR, 1988; BASHAN and LEVANONY, 1990; FALLIK and OKON, 1996; REIS et al., 2000; DALLA SANTA, et al., 2004b).

The increased amount of nitrogen application resulted in significantly higher yield than the preceding amount. In this experiment, the yield of the inoculated treatment associated with 32 kg of $N.ha^{-1}$ and those with 40 kg of $N.ha^{-1}$, were statistically equivalent, this result show that the inoculation replace 20% of inorganic nitrogen fertilization recommended for this experiment, without any negative effects on yield.

The beneficial effects of inoculation with *Azospirillum sp.* were also manifested in the grain density. The grain density in the inoculated treatment without any nitrogen was higher than compared to the control ($P<0.05$). *Azospirillum sp.* inoculation can increase nitrogen contents in grain. These responses have been attributed to the capacity of *Azospirillum* in nitrogen fixation, nitrate reduction and production of plant growth substances (BODDEY et al., 1986; BODDEY and DÖBEREINER, 1988; SUMNER, 1990; BASHAN, 1998). In this experiment, no difference was observed in the concentration of nitrogen when compared the treatments inoculated and the respective control. However, the concentration of nitrogen in grain was higher in the inoculated treatment associated with 40 kg $N.ha^{-1}$ above the treatments without nitrogen.

Effect of *Azospirillum sp.* RAM-7 inoculation in oats FAPA-1: The response of *Azospirillum sp.* RAM-7 inoculation in oats FAPA-1 at different levels of inorganic nitrogen is presented in table 3. In general, simple seed inoculation with *Azospirillum* increased yields of crops, although such responses varied depending on the chemical and physical factors of the soil, the bacterial strain, the environmental conditions,

Table 3. Effects of *Azospirillum sp.* RAM-7 inoculation with different nitrogen level in oats FAPA-1 on grain yield, grain density and gain nitrogen total

Treatments	Grain yield ($kg.ha^{-1}$)	Grain density ($kg.hl^{-1}$)	Grain nitrogen total (%)
Control	1.711 d	46.3 a	1.27 a
Inoculated (IN)	1.688 d	45.9 a	1.28 a
IN plus 8 kg $N.ha^{-1}$	1.762 d	46.3 a	nd
IN plus 16 kg $N.ha^{-1}$	2.039 cd	46.2 a	nd
IN plus 24 kg $N.ha^{-1}$	2.189 bc	45.7 a	nd
IN plus 32 kg $N.ha^{-1}$	2.443 ab	45.7 a	nd
IN plus 40 kg $N.ha^{-1}$	2.796 a	46.7 a	1.27 a
40 kg $N.ha^{-1}$	2.668 a	46.9 a	1.24 a

[1]Means followed by the different letters in de same column are statistically different (P<0,05) according Tukey's multiple range test.
IN - inoculated
nd – not determined

the variety planted and the initial inorganic N status of the soil (RAO, 1981; PANDEY et al., 1998; RAMOS et al., 2002). In the experiment with oats, increases in grain yield were obtained by inoculation associated with 40 kg of $N.ha^{-1}$ over the corresponding control, but these were not statistically significant. For the simple seed inoculation, no effects on grain yield were detected. The grain yield in the treatment inoculated associated with 32 kg of $N.ha^{-1}$ was approximately equivalent to those obtained by 40 kg of $N.ha^{-1}$, in this case the inoculation replaced 20% of the inorganic nitrogen recommended for this experiment.

The inoculation of *Azospirillum sp.* RAM-7 in wheat, barley and oats increased grain yield, but did not replace the total inorganic nitrogen fertilizer recommended for each culture. Different responses were observed among the three cultures by the inoculation with *Azospirillum sp.* RAM-7, indicating some specificity between the bacteria and the culture genotype. This specificity has been previously demonstrated (MILLET et al., 1984; FALLIK and OKON, 1996; PANDEY et al., 1998).

Many reports have shown positive interaction between nitrogen-fixing rhizosphere bacteria with grasses and grain crops roots. Yield improvement by inoculation with associative bacteria requires on efficient screening system aiming at selecting constantly the most successful combinations between the plant genotype and a particular bacterial strain (MILLET et al., 1984; FAGES, 1994; SUMNER, 1990; OKON and VANDERLEYDEN, 1998; PANDEY et al.,1998). The results of the present investigations showed the possibility of the partial substitution of the inorganic nitrogen fertilizer with the *Azospirillum sp.* RAM-7 inoculation in wheat, barley and oats.

References

ALEXANDRE G.; RENÉ R.; RENÉ B. A phase variant of *Azospirillum lipoferum* lackes a polar flagellum and constitutively expresses mechanosensing lateral flagela. *Apllied and Environmental Microbiology*, v. 65, p. 4701-4704, 1999.

AOAC. Association of Official Analytical Chemists. *Official Methods of Analysis*. 15th ed. Washington DC. 1298p, 1990.

BARBIERI P.; ZANELLI T.; GALLI E.; ZANETTI G. Wheat inoculation with *Azospirillum brasilense* Sp6 and some mutans altered in nitrogen fixation and indole-3-aceticacid prodution. *Microbiology Letters*, v. 36**,** p. 87-90, 1986.

BASHAN, Y. *Azospirillum* plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton, and wheat. *Canadian Journal of Microbiology*, v. 44, p.168-174, 1998.

BASHAN Y.; LEVANONY H. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. *Canadian Journal of Microbiology*, v. 36, p. 591-603 1990.

BASHAN, Y.; HOLGUIN G. *Azospirillum*-plant relationships: environmental and physiological advances (1990 – 1996). *Canadian Journal of Microbiology*, v. 43, p. 103-121, 1997.

BODDEY R.M.; BALDANI V.L.D.; BALDANI J.I.; DÖBEREINER J. Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field-grown wheat. *Plant and Soil*, v. 95, p. 109-121 1986.

BODDEY R.M.; DÖBEREINER J. Nitrogen fixation associeted with grasses and cereals: Recent results and perspectives for future research. *Plant and Soil*, v. 108, p. 53-65, 1988.

CHOTTE J.; SCHWARTZMANN A.; BALLY R.; MONROZIER L.J. Changes in bacterial communities and *Azospirillum* diversity in soil fractions of a tropical soil under 3 or 10 years of natural fallow. *Soil Biology and Biochemistry*, v. 34, p. 1083-1092, 2002.

CREUS C.M.; SUELDO R.J.; BARASSI C.A. *Azospirillum* inoculation in pregerminating wheat seeds. *Canadian Journal of Microbiology*, v. 42, p. 83-86, 1996.

DALLA SANTA, O.R.; FERNÁNDEZ, R.; MICHELENA, G.; RONZELLI Jr., P.; SOCCOL, C.R. *Azospirillum sp.* Inoculation in wheat, barley and oats seeds in greenhouse experiments. *Brazilian Archives of Biology and Technology*, v. 47, p. 843-849, 2004a.

DALLA SANTA, O.R.; SOCCOL, C.R.; RONZELLI Jr.,P.; FERNÁNDEZ, R.; MICHELENA, G.; DALLA SANTA, H.S.; PANDEY, A. Effects of inoculation of *Azospirillum* sp. in maize seeds under field conditions. *Journal of Food, Agriculture & Environment*, v. 2, p. 242-238, 2004b.

DIDONET A.D.; RODRIGUES O.; KENNER M.H. Acúmulo de nitrogênio e de massa seca em plantas de trigo inoculadas com *Azospirillum brasilense*. *Pesquisa Agropecuária Brasileira*, v. 31, p. 645–651, 1996.

DÖBEREINER J.; PEDROSA F.O. *Nitrogen–fixing bacteria in nonleguminous croup plants*. Springer–Verlag, EUA, 120p, 1987.

FAGES J.: *Azospirillum* inoculants and field experiments. In Y. OKON (Ed): *Azospirillum Plant Associations*. CRC Press, USA, p. 88-105, 1994.

FALLIK E.; OKON Y. The response of maize (*Zea mays*) to *Azospirillum* inoculation in various types of soils in the field. *World Journal of Microbiology & Biotecnology*, v. 12, p. 511–515, 1996.

FANI R.; BANDI C.; BAZZICALUPO M.; DAMIANI G.; DI CELLO F.; FANCELLI S.; GALLORI E.; GERACE L.; GRIFONI A.; LIÒ P.; MORI E. Phylogenetic studies of the genus *Azospirillum*. In I. FENDRIK et al (Eds*):* Azospirillum *and related microorganisms*. Springer Verlag, Germany, pp. 59-75, 1995.

FERNÁNDEZ R.; DALLA SANTA O.R.; SOCCOL C.R.; MICHELENA G. Obtención de un inoculante comercial a partir de *Azospirillum sp*. en condiciones brasileñas. *Revista Sobre los Derivados de la Caña de Azucar – ICIDCA*, v. 23, p. 30-36, 1999.

FISCHER S.E.; MIGUEL M.J.; MORI G.B. Effect of root exudates on the exopolysaccharide composition and the lipopolysaccharide profile of *Azospirillum brasilense* Cd under saline stress. *FEMS Microbiology Letters*, v. 219**,** p. 53-62, 2003.

FULCHIERI M.; FRIONI L. *Azospirillum* inoculation on maize (*Zea mays*): Effect on yield in a field experiment in central Argentina. *Soil Biology and Biochemistry*, v. 26, p. 921-923, 1994.

HAUWAERTS D.; ALEXANDRE G.; DAS S.K.; VANDERLEYDEN J.; ZHULIN I.B. A major chemotaxis gene cluster in *Azospirillum brasilense* and relationships between chemotaxis operons in alfa-proteobacteria. *FEMS Microbiology Letters*, v. 208**,** p. 61-67, 2002.

HECHT-BUCHHOLZ C. The apoplast-habitat of endophytic dinitrogen-fixation bacteria and their significance for te nitrogen nutrition of nonleguminous plants. *Z. Pflanzenernähr. Bodenk*, v. 161, p. 509-520, 1998.

JACOUD C.; FAURE D.; WADOUX P.; BALLY R. Development of a strain-specific probe to follow inoculated *Azospirillum lipoferum* CRT1 under field conditions and enhancement of maize root development by inoculation. *FEMS Microbiology Ecology*, v. 27, p. 43-51, 1998.

JAMES E.K. Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Research*, v. 65, p. 197-209, 2000.

JOFRÉ E.; FISCHER S.; RIVAROLA V.; BELEGNO H.; MORI G. Saline stress affects the attachment of *Azospirillum brasilense* Cd to maize and wheat roots. *Canadian Journal of Microbiology***,** v. 44, p. 416-422, 1998.

KAPULNIK J.; KIGEL J.; OKON Y.; NUR I.; HENIS Y. Effect of *Azospirillum* inoculation on some growth parameters and N-content of wheat, sorghum and panicum. *Plant and Soil*, v. 61, p. 65-70, 1981.

KENNEDY I. R.; LILY L.; PEREG-GERK L.; WOOD C.; DEAKER R.; GILCHRIST K.; KATUPITIYA S. Biological nitrogen fixation in non-leguminous field crops: Facilitating the evolotion of na effective association between *Azospirillum* and wheat. *Plant and Soil*, v. 194, p. 65–79, 1997.

KIRCHHOF G.; REIS V.M.; BALDANI J.I.; ECKERT B.; DÖBEREINER J.; HARTMANN A. Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. *Plant and Soil*, v. 194, p. 45-55, 1997.

KUCEY R.M.N. Alteration of size of wheat root systems and nitrogen fixation by associative nitrogen-fixing bacteria measured under field conditions. *Canadian Journal of Microbiology*, v. 34, p. 735-739, 1988.

MILLET E.; AVIVI Y.; FELDMAN M. Yield response of various wheat genotypes to inoculation with *Azospirillum brasilense*. *Plant and Soil*, v. 80, p. 261-266, 1984.

OKON Y.; VANDERLEYDEN J. Root-associated *Azospirillum* species can stimulate plants. *Features*, v. 63, p. 366–370, 1998.

PANDEY A.; SHARMA E.; PALNI L.M.S. Influence of bacterial inoculation on maize in upland farming systems of the sikkim himalaya. *Soil Biology and Biochemistry*, v. 30, p. 379–384, 1998.

PEREG-GERK L.; PAQUELIN A.; GOUNON P.; KENNEDY I.; ELMERICH C. A transcriptional regulator of the luxR-UhpA family, FIcA, controls flocculation and wheat root surface colonization by *Azospirillum brasilense* Sp7**.** *Molecular Plant-Microbe Interactions*, v. 11, p. 177-187, 1998.

RAI S.N.; GUAR A.C. Characterization of *Azobacter spp.* and effect of *Azobacter* and *Azospirillum* as inoculant on the yield and N-uptake of wheat crop. *Plant and Soil*, v. 109**,** p. 131-134, 1988.

RAMOS H.J.O.; RONCATO-MACCARI L.D.B.; SOUZA E.M.; SOARES-RAMOS J. R.L.; HUNGRIA M.; PEDROSA F.O. Monitoring *Azospirillum*-wheat interactions using the gfp and gusA genes constitutively expressed from a new broad-host rang vector. *Journal of Biotechnology*, v. 97**,** p. 243-252, 2002.

RAO N.S.S. Response of crops to *Azospirillum* inoculation in India. In VOSE, P.B.; RUSCHEL, A.P. (Eds): *Associative N_2-Fixation*. CRC Press, Florida, pp. 137–144, 1981.

REIS V.M.; BALDANI J.I.; BALDANI V.L.D.; DOBEREINER J. Biological dinitrogen fixation in gramineae and palm trees. *Plant Science*, v. 19, p. 227-274, 2000.

STEENHOUDT O.; VANDERLEYDEN J. *Azospirillum*, a free living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews*, v. 24**,** p. 487-506, 2000.

SUMNER M.E. Crop responses to *Azospirillum inoculation*. In STEWAT, B.A (Ed): *Advances in Soil Science*. Springer-Verlag, New York, pp. 52-123, 1990.

SUNDARAM S.; ARUNAKUMARI A.; KLUCAS R. Characterization of *Azospirilla* isolated from seeds and roots of turf grass. *Canadian Journal of Microbiology*, v. 34, p. 212-217, 1987.

TARRAND J.J.; KRIEG N.R.; DÖBEREINER J.A. Taxonomic study of the *Spirillum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. *Canadian Journal of Microbiology*, v. 24, p. 967–980, 1978.