NITROGEN TRANSLOCATION IN WHEAT INOCULATED WITH *AZOSPIRILLUM* AND FERTILIZED WITH NITROGEN[1]

OSMAR RODRIGUES[2], AGOSTINHO DIRCEU DIDONET[3], JORGE A. GOUVEIA[4] and RITA DE CÁSSIA SOARES[4]

ABSTRACT - The productivity and the translocation of assimilates and nitrogen (N) were compared after inoculation of wheat (*Triticum aestivum* L., cv. BR-23) seeds with two strains of *Azospirillum brasilense* (strains 245 and JA 04) under field conditions. The inoculation of wheat seeds was done with a peat inoculant at sowing time. Plant material for evaluations were collected at anthesis and maturity. No differences in grain yield and in the translocation of assimilates resulting from inoculation were detected. Differences were observed in relation to N rates (0, 15, and 60 kg ha^{-1}). N content in the grain increased significantly in the bacteria-inoculated treatments in which N was not added. This increase in N content in the grain with inoculation was probably due to higher N uptake after anthesis without any significant contribution on the grain yield. Such increment was of 8.4 kg ha^{-1} of N representing 66% more N than in no inoculated treatment. Regardless of the inoculation and the rate of N applied, it was observed that about 70% of the N accumulated at anthesis was translocated from vegetative parts to the grain.

Index terms: *Triticum aestivum*, nitrogen fertilization, nitrogen metabolism, assimilation, nitrogen fixing bacteria.

TRANSLOCAÇÃO DE NITROGÊNIO EM TRIGO INFECTADO POR *AZOSPIRILLUM* E ADUBADO COM NITROGÊNIO

RESUMO - Com o objetivo de verificar o efeito da inoculação na produtividade, na translocação de assimilados e de N, durante o desenvolvimento da planta de trigo (*Triticum aestivum* L. cv. BR-23), foi realizado este estudo em condições de campo, com duas estirpes de *Azospirillum brasilense* (245 e JA 04). A inoculação foi efetuada no momento da semeadura, com inoculante turfoso. As coletas dos materiais para as avaliações foram efetuadas na antese e na maturação. Não foi detectado efeito da inoculação na produção de grãos e na translocação de assimilados. Diferenças foram observadas em relação às doses de N (0, 15 e 60 kg ha^{-1}). O teor de N no grão aumentou significativamente nos tratamentos com bactérias, sem adição de N. Esse aumento foi provavelmente decorrente da maior absorção de N após a antese, sem reflexo significativo no rendimento de grãos. Tal aumento no N absorvido após a antese foi superior em 66% ao tratamento sem inoculação, representando um incremento de cerca de 8,4 kg ha^{-1}. Independentemente da inoculação e da dose de N estudadas, observou-se que aproximadamente 70% do N acumulado na antese foi translocado das partes vegetativas para o grão.

Termos para indexação: *Triticum aestivum*, adubação nitrogenada, metabolismo do nitrogênio, assimilação, bactéria fixadora de nitrogênio.

INTRODUCTION

The increase of root growth in wheat, as well as in other species, promoted by *Azospirillum* sp., seems to be related to hormonal effects caused by the bacterium associated with root cells (Zimmer et al., 1988; Zaady et al., 1993; Dubrovsky et al., 1994). The presence of the bacterium in wheat roots causes an increase in proton efflux in such a way that the bacterium is not directly in contact with the cell membrane,

---

[1] Accepted for publication on December 7, 1999.

[2] Agronomist, M.Sc., Embrapa-Centro Nacional de Pesquisa de Trigo (CNPT), Caixa Postal 569, CEP 99001-970 Passo Fundo, RS. E-mail: osmar@cnpt.embrapa.br

[3] Agronomist, Dr., Embrapa-Centro Nacional de Pesquisa de Arroz e Feijão (CNPAF), Caixa Postal 179, CEP 75375-000 Santo Antônio de Goiás, GO. E-mail: didonet@cnpaf.embrapa.br

[4] Agronomist, Instituto de Ciências Biológicas, Universidade de Passo Fundo, CEP 99001-970 Passo Fundo, RS. E-mail: cominfor@ upf.tche.br

thus requiring a stimulus to break out such action (Bashan et al., 1989; Bashan, 1990; Didonet, 1993). This phenomenon may be associated with the acidification of the cell wall, loosening the structure of the wall and, consequently, causing cell elongation (Cleland, 1983; Masuda, 1990). The increase in root volume favors the absorption of nutrients, mainly N (Kapulnik et al., 1985, 1987) resulting in greater plant growth. On the other hand, Ferreira et al. (1987), reported that wheat plants inoculated with *Azospirillum* showed greater activity of the nitrate reductase enzyme in the leaf compared to non inoculated plants. Corn trials also showed an increase in both dry matter and grain production by inoculation of seeds with *Azospirillum* (Fulchieri & Frioni, 1994).

Many studies have shown that the inoculation of wheat, and other cereals seeds, with bacteria of the genus *Azospirillum* resulted in an increase in both volume and number of roots (Bashan & Levanony, 1990; Didonet, 1993; Didonet & Magalhães, 1993). That improved the uptake of nutrients and water (Lin et al., 1983; Kapulnik et al., 1987) and, in certain instances, increased grain production (Okon, 1985; Okon & Labandera-Gonzalez, 1994). However, it has been difficult to obtain statistical differences in grain production because the deviations are in the 5% to 10% range (Zimmer et al., 1987; Okon & Labandera-Gonzalez, 1994). It should also be considered that the predominance of *Azospirillum* in the roots takes place at anthesis (Magalhães et al., 1979), when a considerable portion of reproductive sinks have already been defined. Thus, since wheat production seems to be more limited by sinks (Fischer, 1985; MacManey et al., 1986) such as the number of kernels m^{-2}, than by the source (cropping capacity to supply those sinks), it has been reported that the bacteria effect on grain yield is small. Although the increase in N content by bacterial activity has been shown significant (Baldani et al., 1987); no impact on grain yield occurred because it was absorbed after anthesis.

The objective of this study was to evaluate under field conditions, the effects of inoculating wheat seeds with bacteria of the genus *Azospirillum,* on the productivity and on the translocation of assimilates and N during plant development.

MATERIAL AND METHODS

The experiment was carried out in an area of native pasture in the region of Passo Fundo, State of Rio Grande do Sul, Southern Brazil. The soil had the following characteristics: pH (water 1:1) = 5.7; 0.5 $cmol_c$ dm^{-3} of Al^{+3}; 1.58 $cmol_c$ dm^{-3} of Mg^{2+}; 4.68 $cmol_c$ dm^{-3} of Ca^{2+}; 3.9 mg L^{-1} of P; 128 mg L^{-1} of K and 3.2% of organic matter. Sowing was done mechanically on June 16, and emergence occurred 12 days later. Three hundred and thirty viable seeds by square meter were sowed in 10 m^2 (2 m x 5 m) plots. The experiment was treated with fungicides for disease control and weeds were pulled out manually when present. Fertilizer was applied according to the recommendations of the Southern-Brazilian Wheat Research Commission. Two strains of *Azospirillum brasilense*, 245 (resistant to 20 g L^{-1} of spectinomycin) and JA 04 (isolated from sterilized wheat roots from the region of Passo Fundo, RS), were used for inoculating wheat seeds, cultivar BR 23. The seeds were inoculated at sowing time using a peat inoculant in the proportion of 1.8 g of inoculant per 150 g of seeds. The inoculant was prepared with autoclaved peat (pH=6.5), and had a population of 1,0 x 10^9 cells g^{-1} (MPN) of the two strain. The experiment was carried out in a factorial 3 x 3 in a completely randomized block design, with six replicates per treatment as follow: 1) control without inoculation and without N; 2) inoculation with *A. brasilense*, strain 245; 3) inoculation with *A. brasilense*, strain JA 04; 4) 15 kg ha^{-1} of N (as urea) at sowing; 5) inoculation with *A. brasilense*, strain 245, plus 15 kg ha^{-1} of N at sowing; 6) inoculation with *A. brasilense*, strain JA 04, plus 15 kg ha^{-1} of N at sowing; 7) 15 kg ha^{-1} of N at sowing plus 45 kg ha^{-1} of N as topdressed; 8) inoculation with *A. brasilense*, strain 245, plus 15 kg ha^{-1} of N at sowing and 45 kg ha^{-1} of N as topdressed; 9) inoculation with *A. brasilense*, strain JA 04, plus 15 kg ha^{-1} of N at sowing and 45 kg ha^{-1} of N as topdressed. Analysis of variance and comparison of means for rates of N (0, 15 and 60 kg ha^{-1}) and *A. brasilense* inoculation (none, strain 245 and strain JA 04) were performed using the F-test at the 0.05 level of probability.

The source of N was urea. The topdressed N was applied at the beginning of tillering. At both anthesis and maturity the dry matter weights of stems, leaves and spikes, with and without grains, were evaluated separately in one linear meter of plants/plot, after oven drying at 70°C for four days. Plant parts were then ground and total N content was determined by semi micro-Kjeldahl method (Tedesco et al., 1995). At harvest, in addition to the grain yield, 1,000 grain weight, test weight, number of spikes m^{-2} and total N in the straw and grain were also determined.

The N absorbed between anthesis and maturity was calculated by the difference of total N in the biomass between these periods.

The contribution of preanthesis assimilates to grain yield was determined, by calculating the difference between grain yield and total shoot dry matter accumulation from anthesis to maturity (Gallagner et al., 1975). The contribution of N produced in preanthesis to grain yield was calculated using the same methodology.

RESULTS AND DISCUSSION

Grain yield was directly associated with the amount of N applied and there were no differences between inoculated and non inoculated treatments (Fig. 1), regardless of the rate of N used. Increases in cereal production by inoculation with *Azospirillum* have been observed in many field experiments (Sarig et al., 1984; Baldani et al., 1986, 1987; Boddey et al., 1986; Kapulnik et al., 1987). However, it has been difficult to detect statistical differences in the increase of grain production since the deviations are around 5-10% (Zimmer et al., 1987). On the other hand, in Israel wheat cultivars responded to the inoculation with *Azospirillum* under field conditions. The success of such behavior has been attributed to the absence of competition of *Azospirillum* with other native bacteria and interaction between wheat cultivars and *Azospirillum* (Mertens & Hess, 1984).

No effects of seed inoculation with *Azospirillum* were observed on the 1,000 grain weight and number of spikes m^{-2} (Fig. 1). When the rate of applied N was 60 kg ha^{-1}, 1,000 grain weight was reduced, char-

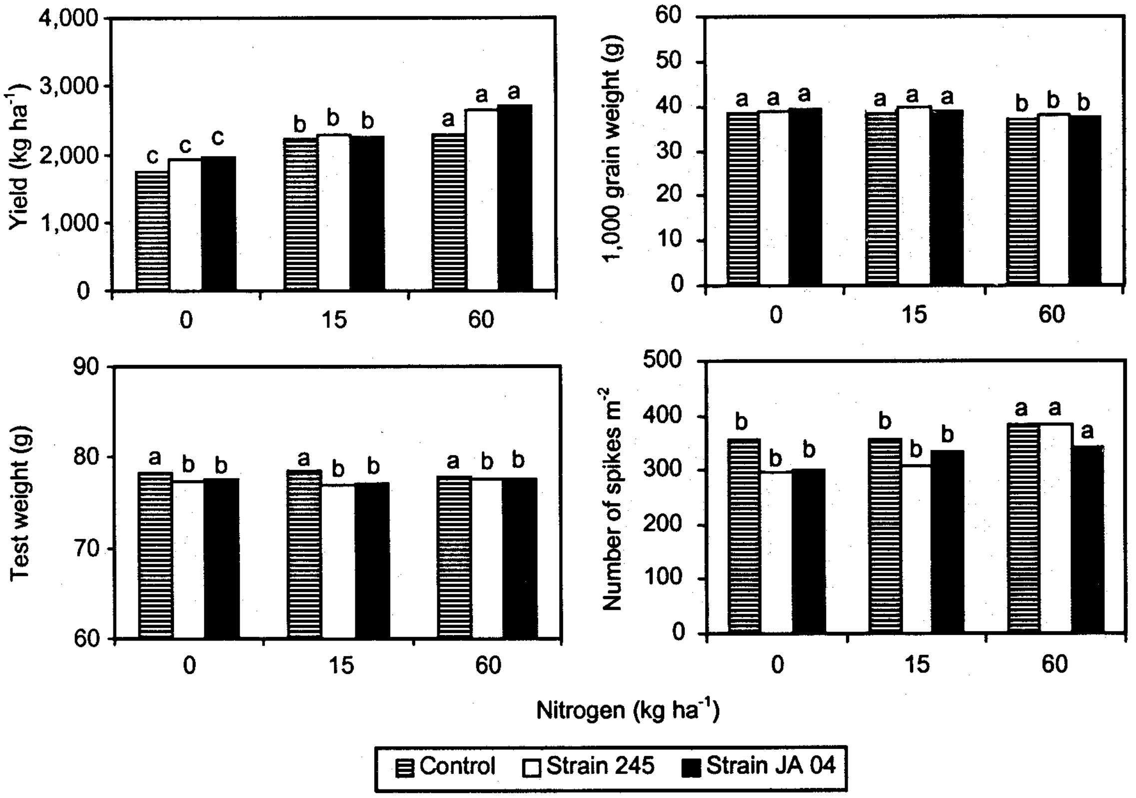

FIG. 1. Effect of wheat (*T. aestivum* L., cv. BR 23) inoculation with *Azospirillum brasilense*, strains 245 and JA 04, and doses of nitrogen on grain yield (kg ha^{-1}), 1,000 grain weight (g), test weight (g) and number of spikes m^{-2}. Letters refer to comparison among N rates. Bars followed by the same letters did not differ statistically at $P<0.05$.

acterizing the compensatory effect played by the higher number of spikes per square meter observed in these treatments. This was not the case for the test weight, which was reduced by inoculation (Fig. 1).

No significant effect of inoculation was observed on the production of dry matter from straw (biomass-grains) and from spikes at anthesis and at maturity, in the respective N fertilizer rates used (Fig. 2). Nitrogen increased dry matter (straw and spike) production at both periods. The inoculation with *Azospirillum* has often caused an increase only on dry matter production and this response has been attributed to the effect of growth substances produced by the bacteria (Tien et al., 1979; Lin et al., 1983; Zimmer et al., 1988). Nevertheless, those effects were not observed in this work.

The difference in straw dry weight between anthesis and maturity indicates that the organic material produced at preanthesis was translocated to the grain (Table 1). However, the difference in dry matter accumulation during this period does not include the dry matter losses that occurred either by senescence of tissues nor by respiration (Austin et al., 1980; Hall et al., 1989). Austin et al. (1977) found that 73% of the loss of vegetative dry matter was allotted to the grain. In this work it was observed that as the rate of applied N increased, the participation of the material produced before anthesis in final grain weight (translocation post-anthesis) also increased, contributing with as much as 52% (in the 60 kg ha^{-1} of N treatment) for the final grain weight, without adjusting dry matter losses proposed earlier (Table 1). Moreover, if the adjustments developed by Austin

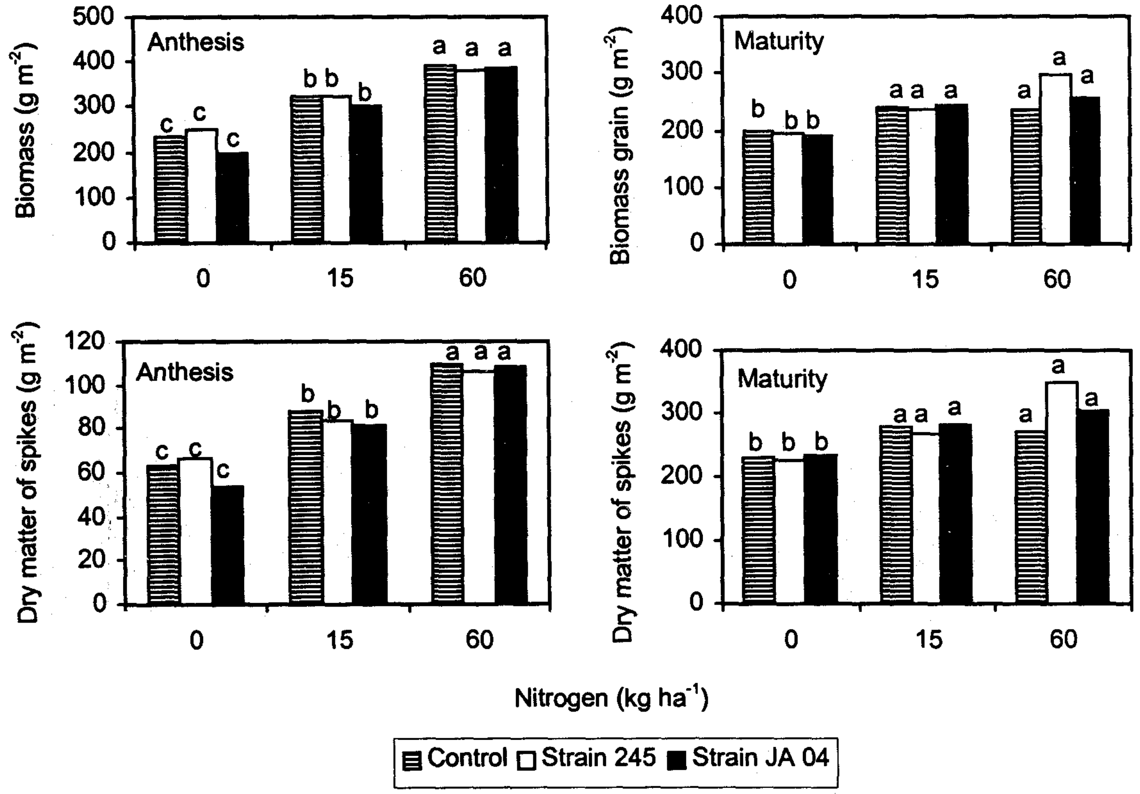

FIG. 2. Effect of wheat (*T. aestivum* L., cv. BR 23) inoculation with *Azospirillum brasilense* strains 245 and JA 04, and doses of nitrogen on the production of dry matter (g m^{-2}), at anthesis and maturity. Letters refer to comparisons among N rates. Bars followed by the same letters did not differ statistically at P<0.05.

et al. (1977) were applied, a contribution of up to 38% of the material produced in preanthesis to the final grain weight would be obtained. Bidinger et al. (1977) reported that the assimilates produced in preanthesis may contribute 13% to 43% of the final grain weight, under stress-free conditions over the grain filling period. On the other hand, no significant effect of increasing the rate of applied N on the production of assimilates at post-anthesis (current photosynthesis) was observed, indicating the importance of both capacity and quantity of the reproductive sinks defined at preanthesis for grain yield.

Total N content, quantified at anthesis, both in straw (stems + leaves) and in spikes (Table 2) followed the same correspondence, in relation to N rates, observed in grain yield, indicating that the higher the amount of applied N, the higher the absorption and, consequently, greater grain production. At maturity no effect of inoculation was found on N content in straw (Table 3). The increase in straw N content observed in this stage resulted from the effect of N rates (Table 3). On the other hand, N percent in the grain increased significantly in the treatments inoculated with bacteria, without adding N fertilizer (Table 3). This increase in N content in the grain with inoculation without N was markedly higher than in the treatments that received additional N, probably due to higher N uptake after anthesis (Table 4). Such increment was above 66%, representing about 8.4 kg ha^{-1} of N. This effect was not observed in the treatments receiving N fertilizer, which could be attributed to the inhibitory effect of N compounds on the nitrogenase activity of the bacterium (Turpin et al., 1984). In this regard, Zimmer et al. (1988) commented that the growth of the bacterium with nitrite or nitrate suppresses the biosynthesis of nitrogenase, since the cells synthetize nitrite reductase and nitrate reductase instead of using their limited energy in the nitrogenase reaction. Although, the occurrence of N fixation process by *Azospirillum* in wheat is not clear, apparently the dismissal of N fixation as a mechanism has been pointed out as premature (Bashan & Holguin, 1997). As a result of this uncertainty, it can not be concluded that the increase in N incorporation (Table 4) is due to biological fixation, since root growth could have been stimulated by bacterial activity (Tien et al., 1979) and, as a result, increased the influx of nitrate and phosphorus (Hallmark & Barber, 1984). Therefore, regardless of how the process originated (absorption or fixation), N incorporation took place after anthesis (Table 4) and behaved like late cycle absorbed N, and could have influenced the dry matter and probably the duration of leaf area without changing grain production (Thomas et al., 1978; Gifford et al., 1984). Probably both capacity and quantity of reproductive sinks (grain) became a limiting factor, since they are defined at a stage much earlier than the extra availability of N (Moll et al., 1982; Harper et al., 1987; Mac Kown & Sanford, 1988).

A positive effect of inoculating wheat seeds with *Azospirillum* on total N accumulation in aboveground plant parts and in the grain was also observed by Boddey et al. (1986). In this work, the N was incorporated in the late cycle and had no impact on grain yield since more than 70% of the N found in the grain was originated from the remobilization of vegetative parts (Table 5). This is in agreement with

TABLE 1. Effect of rates of nitrogen applied on wheat (*T. aestivum*, cv. BR 23) on the contribution of assimilates produced at preanthesis to grain production[1].

Treatments (kg ha^{-1} of N)	Grain weight (g m^{-2})	Difference in straw dry matter weight (biomass-grains) between anthesis and maturation (g m^{-2})	Assimilates produced in post-anthesis (actual photosynthesis) (g m^{-2})	Translocation of assimilates to grain %)
0	162.8c	33.0	129.9	20.3
15	197.2b	76.0	121.2	38.5
60	232.3a	122.4	109.9	52.7

[1] Treatment means with and without inoculation; means followed by different letters are statistically different at $P<0.05$.

TABLE 2. Effect of wheat (*T. aestivum* L. cv. BR 23) inoculation with *Azospirillum brasilense*, strains 245 and JA 04, and application of N (0, 15, and 60 kg ha^{-1} of N) on the percentage of nitrogen in the straw (stems + leaves) and spikes at anthesis[1].

Strains	Nitrogen rates (kg ha^{-1} of N)			Mean
	0	15	60[2]	
		Straw (CV = 11.1 %)		
Control	1.10	1.14	1.29	1.18
Strain 245	1.12	1.15	1.35	1.21
Strain JA 04	1.21	1.14	1.37	1.24
Mean	1.15B	1.14B	1.34A	
		Spikes (CV = 7.5 %)		
Control	1.82	1.79	1.92	1.84
Strain 245	1.76	1.74	1.94	1.81
Strain JA 04	1.71	1.90	1.95	1.86
Mean	1.76B	1.81B	1.94A	

[1] Means followed by the same letters did not differ statistically at $P<0.05$.
[2] 15 kg ha^{-1} of N were applied at sowing and 45 kg ha^{-1} of N were applied at tillering, as topdressing.

TABLE 3. Effect wheat (*T. aestivum* L. cv. BR 23) inoculation with *Azospirillum brasilense*, strains 245 and JA 04, and application of N (0, 15, and 60 kg ha^{-1} of N) on the percentage of nitrogen in the straw (stem + leaves + straw of spikes) and grains at maturity[1].

Strains	Nitrogen rates (kg ha^{-1} of N)			Mean
	0	15	60[2]	
		Straw (CV = 15.2%)		
Control	0.45	0.48	0.57	0.50
Strain 245	0.48	0.47	0.51	0.49
Strain JA 04	0.46	0.47	0.56	0.50
Mean	0.47B	0.48B	0.55A	
		Grains (CV = 5.5%)		
Control	2.13bB	2.27aB	2.44aA	2.28
Strain 245	2.38aA	2.26aA	2.36aA	2.38
Strain JA 04	2.35aAB	2.30aB	2.47aA	2.37
Mean	2.29B	2.28B	2.42aA	

[1] Means followed by the same capital and lowercase letters, within lines and columns, respectively, did not differ statistically at $P<0.05$.
[2] 15 kg ha^{-1} of N were applied at sowing and 45 kg ha^{-1} of N were applied at tillering, as topdressing.

TABLE 4. Effect of wheat (*T. aestivum* L., cv. BR 23) inoculation with *Azospirillum brasilense*, strains 245 and JA 04, on the N uptake (kg ha^{-1}) between anthesis and maturity[1].

Strains	Nitrogen rates (kg ha^{-1} of N)			Mean
	0	15	60	
Control	12.6 (0)	14.3 (0)	13.2 (0)	13.4
Strain 245	20.9 (66)	15.9 (11)	11.2 (15)	16.0
Strain JA 04	21.0 (67)	18.5 (29)	13.6 (3)	17.7
Mean	18.2	16.2	12.7	

[1] Values between parantheses indicate the percentage of variation in relation to the control in each rate of applied nitrogen.

TABLE 5. Effect of wheat (*T. aestivum* L., cv. BR 23) inoculation with *Azospirillum brasilense*, strains 245 and JA 04, on the N accumulated (g m^{-2}) between anthesis and maturity[1].

Strains	Nitrogen rates (kg ha^{-1} of N)			Mean
	0	15	60	
Control	2.57 (69)	3.64 (70)	5.18 (72)	3.80
Strain 245	2.70 (68)	3.63 (71)	5.12 (71)	3.82
Strain JA 04	2.17 (66)	3.38 (68)	5.33 (72)	3.63
Mean	2.50	3.50	5.20	

[1] Values obtained by evaluating total N accumulated in the plant at anthesis less total N accumulated in the straw (stem + leaf + straw from spike) at maturity; values between parantheses indicate the percentage of translocation to the grains of nitrogen accumulated at anthesis.

the results obtained by Moll et al. (1982) and Spiertz & Vos (1983). In general, there is a consensus that, in wheat, the amount of N remobilized from vegetative parts after anthesis is considerably higher than the amount of N assimilated after anthesis (Cox et al., 1985).

Usually, the higher availability of late cycle absorbed N is initially associated with a greater root activity (absorption) and, secondly, with an increase in protein content of the grain (Eilrich & Hageman, 1973; Blacklow, 1982; Spiertz & Vos, 1983; Ugalde & Jenner, 1990). Such ability to absorb N after anthesis depends on N availability and soil moisture and may represent 20-44% of total N in the plant; both the translocation and deposition of this N in the grain is limited by the grain itself (Perez et al., 1983). Therefore, when N availability in the soil as well as environmental conditions are favorable, the increase in both volume and number of roots, due to the presence of *Azospirillum*, is probably a decisive factor for a greater absorption of N after anthesis (Bashan et al., 1989; Bashan & Levanony, 1990). Since the capacity of translocation and accumulation of N in the grain is limited, and is defined before anthesis, most of this late cycle assimilated N has no influence on the increase of yield.

CONCLUSIONS

1. The inoculation of wheat seeds with *Azospirillum brasilense* has no effect on yield nor on translocation of assimilates.
2. Both yield and translocation of assimilates are affected by N rates.
3. The inoculation of wheat seeds with *Azospirillum brasilense* increases N content in the grain in those treatments in which N is not added.

ACKNOWLEDGEMENTS

To Rio Grande do Sul Research Support Foundation (FAPERGS), for the partial financial assistance; to Mr. C. Campos and Drs. R. A. Kochhann, J. C. Haas, E. S. Roman, for extensive support and help; to Ms. T.T. Sava (Nitral Co.) for her assistance with the preparation of the culture of *A. brasilense* strain 245 and JA 04.

REFERENCES

AUSTIN, R.B.; EDRICH, J.A.; FROD, M.A.; BLACKWELL, R.D. The fate of the dry matter, carbohydrates and ^{14}C lost from leaves and stems of wheat during grain filling. **Annals of Botany**, London, v.41, p.1309-1321, 1977.

AUSTIN, R.B.; MORGAN, C.L.; FORD, M.A.; BLACKWELL, R.D. Contributions to grain yield from preanthesis assimilation in tall and dwarf barley phenotypes in two contrasting seasons. **Annals of Botany**, London, v.45, p.309-319, 1980.

BALDANI, V.L.D.; ALVAREZ, M.A. de B.; BALDANI, J.I.; DÖBEREINER, J. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. **Plant and Soil**, Dordrecht, v.90, p.35-46, 1986.

BALDANI, V.L.D.; BALDANI, J.I.; DÖBEREINER, J. Inoculation of field grown wheat with *Azospirillum* spp. in Brazil. **Biology and Fertility of Soils**, Berlin, v.4, p.37-40, 1987.

BASHAN, Y. Short exposure to *Azospirillum brasilense* Cd inoculation enhanced proton efflux of intact wheat roots. **Canadian Journal of Microbiology**, Ottawa, v.36, p.419-425, 1990.

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

BASHAN, Y.; LEVANONY, H. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. **Canadian Journal of Microbiology**, Ottawa, v.36, p.591-608, 1990.

BASHAN, Y.; LEVANONY, H.; MITIKU, G. Changes in proton efflux of intact wheat roots induced by *Azospirillum brasilense* Cd. **Canadian Journal of Microbiology**, Ottawa, v.35, p.691-697, 1989.

BIDINGER, F.; MUSGRAVE, R.B.; FISCHER R.A. Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. **Nature**, London, v.270, p.431-433, 1977.

BLACKLOW, W.M. ^{15}N moved to the grain of winter wheat when applied as nitrate to senescing flag leaves. **Australian Journal of Plant Physiology**, Collingwood, v.9, p.641-646, 1982.

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**, Dordrecht, v.95, p.109-121, 1986.

CLELAND, R.E. The capacity for acid-induced wall loosening as a factor in the control of *Avena* coleoptile cell elongation. **Journal of Experimental Botany**, Oxford, v.34, p.676-680, 1983.

COX, M.C.; QUALSET, C.O.; RAINS D.W. Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein. **Crop Science**, Madison, v.25, p.435-440, 1985.

DIDONET, A.D. **Aspectos do mecanismo de ação fisiológica associados à promoção do crescimento radicular de trigo (*Triticum aestivum* L.) por bactérias do gênero *Azospirillum*.** Campinas : Unicamp, 1993. 68p. Tese de Doutorado.

DIDONET, A.D.; MAGALHÃES A.C. The role of auxin-like compounds in plant growth promoting rhyzobacteria: the wheat-*Azospirillum* association. **Revista Brasileira de Fisiologia Vegetal**, Londrina, v.5, p.179-183, 1993.

DUBROVSKY, J.G.; PUENTE, M.E.; BASHAN, Y. *Arabidopsis thaliana* as a model system for the study of the effect of inoculation by *Azospirillum brasilense* Sp-245 on root hair growth. **Soil Biology & Biochemistry**, Oxford, v.26, p.1657-1664, 1994.

EILRICH, G.L.; HAGEMAN, R.H. Nitrate reductase activity and its relationship to accumulation of vegetative and grain nitrogen in wheat (*Triticum aestivum* L.). **Crop Science**, Madison, v.13, p.59-66, 1973.

FERREIRA, M.C.B.; FERNANDES, M.S.; DÖBEREINER, J. Role of *Azospirillum brasilense* nitrate reductase in nitrate assimilation by wheat plants. **Biology and Fertility of Soils**, Berlin, v.4, p.47-53, 1987.

FISCHER, R.A. Number of kernels in wheat crops and influence of solar radiation and temperature. **Journal of Agricultural Science**, Cambridge, Great-Britain, v.105, p.447-461, 1985.

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

GALLAGNER, J.N.; BISCOE, P.V.; SCOTT, R.K. Barley and its environment stability of grain weight. **Journal of Applied Ecology**, Oxford, v.12, p.319-336, 1975.

GIFFORD, R.M.; THORNE, J.H.; HITZ, W.D.; GIAQUINTA, R.T. Crop productivity and photoassimilate partitioning. **Science**, Washington, v.225, p.801-808, 1984.

HALL, A.J.; CONNOR, D.J.; WHITFIELD, D.M. Contribution of preanthesis assimilates to grain filling in irrigated and water stressed sunflower crops. I. Estimates using labelled carbon. **Field Crops Research**, Amsterdam, v.20, p.95-112, 1989.

HALLMARK, W.B.; BARBER, S.A. Root growth and morphology, nutrient uptake, and nutrient status of early growth of soybeans as affected by soil P and K. **Agronomy Journal**, Madison, v.76, p.209-212, 1984.

HARPER, L.A.; SHARPE, R.R.; LANGDALE, G.W.; GIDDENS, J.E. Nitrogen cycling in a wheat crop: soil, plant, and aerial nitrogen transport. **Agronomy Journal**, Madison, v.79, p.965-973, 1987.

KAPULNIK, Y.; GAFNY, R.; OKON, Y. Effect of *Azospirillum* spp. inoculation on root development and NO_3 uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponic systems. **Canadian Journal of Botany**, Ottawa, v.63, p.627-631, 1985.

KAPULNIK, Y.; OKON, Y.; HENIS, Y. Yield response of spring wheat (*Triticum aestivum*) to inoculation with *Azospirillum brasilense* under field conditions. **Biology and Fertility of Soils**, Berlin, v.4, p.27-35, 1987.

LIN, W.; OKON, Y.; HARDY, R.W.F. Enhanced mineral uptake by *Zea mays* and *Sorghum bicolor* roots inoculated with *Azospirillum brasilense*. **Applied and Environmental Microbiology**, Washington, v.45, p.1775-1779, 1983.

Mac KOWN, C.T.; SANFORD, D.A. van. Nitrogen allocation with altered sink demand in wheat. **Crop Science**, Madison, v.28, p.133-136, 1988.

MacMANEY, M.; DIAZ, M.; SIMON, C.; GIOIA, A.; SLAFER, G.A.; ANDRADE, F.H. Respuesta a la reducción de la capacidad fotosintética durante el llenado de granos en trigo. In: CONGRESO NACIONAL DEL TRIGO, 1., 1986, Pergamino. **[Anales]**. Pergamino : AINBA, 1986. p.178-190.

MAGALHÃES, F.M.M.; PATRIQUIN, D.; DÖBEREINER, J. Infection of field grown maize with *Azospirillum* spp. **Revista Brasileira de Biologia**, Rio de Janeiro, v.39, p.587-596, 1979.

MASUDA, Y. Auxin-induced cell elongation and cell wall changes. **Botanical Magazine**, Tokyo, v.103, p.345-370, 1990.

MERTENS, T.; HESS, D. Yield increases in spring wheat (*Triticum aestivum* L.) inoculated with *Azospirillum lipoferum* under greenhouse and field conditions of temperate region. **Plant and Soil**, Dordrecht, v.82, p.87-99, 1984.

MOLL, R.H.; KAMPRATH, E.J.; JACKSON, W.A. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. **Agronomy Journal**, Madison, v.74, p.562-564, 1982.

OKON, Y. *Azospirillum* as a potential inoculant for agriculture. **Trends in Biotechnology**, Oxford, v.3, p.223-228, 1985.

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

PEREZ, P.; MARTINEZ-CARRASCO, R.; PUENTE, L.S. de la. Uptake and distribution of nitrogen in wheat plants supplied with different amounts of nitrogen after stem elongation. **Annals of Applied Biology**, Wellesbourne, v.102, p.399-406, 1983.

SARIG, S.; KAPULNIK, Y.; NUR, I.; OKON, Y. Response of non-irrigated *Sorghum bicolor* to *Azospirillum* inoculation. **Experimental Agriculture**, Cambridge, Great Britain, v.20, p.59-66, 1984.

SPIERTZ, J.H.J.; VOS, N.M. de. Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. **Plant and Soil**, Dordrecht, v.75, p.379-391, 1983.

TEDESCO, M.J.; GIANELLO, C.; BISSANI, C.A.; BOHNEN, H.; VOLKWEISS, S.J. **Análises de solo, plantas e de outros materiais**. Porto Alegre : UFRGS, 1995. 174p. (UFRGS. Boletim Técnico, 5).

THOMAS, S.M.; THORNE, G.N.; PEARMAN, I. Effect of nitrogen on growth, yield and photorespiratory activity in spring wheat. **Annals of Botany**, London, v.42, p.827-837, 1978.

TIEN, T.M.; GASKINS, M.H.; HUBBELL, D.H. Plant growth substances produced by *Azospirillum brasiliense* and their effect on growth of pearl millet (*Pennisetum americanum* L.). **Applied and Environmental Microbiology**, Washington, v.37, p.1016-1024, 1979.

TURPIN, D.H.; EDIE, S.A.; CANVIN, S.T. In vivo nitrogenase regulation by ammonium and methylamine and effect of MSX on ammonium transport in *Anabaena flosaguae*. **Plant Physiology**, Rockville, v.74, p.701-704, 1984.

UGALDE, T.D.; JENNER, C.F. Substrate gradients and regional patterns of dry matter deposition within developing wheat endosperm. II. Amino acids and protein. **Australian Journal of Plant Physiology**, Collingwood, v.17, p.395-406, 1990.

ZAADY, E.; PEREVOLOTSKY, A.; OKON, Y. Promotion of plant growth by inoculation with aggregated and single cell suspension of *Azospirillum brasilense* Cd. **Soil Biology & Biochemistry**, Oxford, v.25, p.819-823, 1993.

ZIMMER, W.; ROEBEN, K.; BOTHE, H. An alternative explanation for plant growth promotion by bacteria of the genus *Azospirillum*. **Planta**, Berlin, v.176, p.333-342, 1988.

ZIMMER, W.; ROEBEN, G.; DANNERBERG, G.; BOTHE, H. The bacterial genus *Azospirillum* and its potential application. In: WRICH, W.R.; APARICIO, P.J.; SYPETT, P.J.; CASTILLO, F. (Ed.). **Inorganic nitrogen metabolism**. Berlin : Springer, 1987. p.177-182.