Water relations and yield in *Azospirillum-*inoculated wheat exposed to drought in the field

Cecilia M. Creus, Rolando J. Sueldo, and Carlos A. Barassi

Abstract: There are scarce data connecting water relations in *Azospirillum*-inoculated wheat suffering drought during anthesis with the yield and mineral content of grains. *Azospirillum brasilense* Sp245-inoculated seeds of *Triticum aestivum* 'Pro INTA Oasis' were sown in nonirrigated and control plots. Water potential, water content, and relative water content were determined on flag leaves. Plant water status was calculated from pressure–volume curves. At maturity, grain yield and its components were determined. P, Ca, Mg, K, Fe, Cu, and Zn were determined in dried grains. Even though the cultivar underwent osmotic adjustment, significantly higher water content, relative water content, water potential, apoplastic water fraction, and lower cell wall modulus of elasticity values were obtained in *Azospirillum*-inoculated plants suffering drought. Grain yield loss to drought was 26.5% and 14.1% in noninoculated and *Azospirillum*-inoculated plants, respectively. Grain Mg and K diminished in nonirrigated, noninoculated plots. However, grains harvested from *Azospirillum*-inoculated plants had significantly higher Mg, K, and Ca than noninoculated plants. Neither drought nor inoculation changed grain P, Cu, Fe, and Zn contents. A better water status and an additional "elastic adjustment" in *Azospirillum*-inoculated wheat plants could be crucial in promoting higher grain yield and mineral quality at harvest, particularly when drought strikes during anthesis.

Key words: Azospirillum, wheat, drought, pressure–volume curves, yield, mineral content.

Résumé : Chez le blé inoculé avec l'*Azospirillum*, il y a peu de donnés concernant les relations hydriques, le rendement et la teneur en minéraux des grains, lorsqu'une sécheresse survient au cours de l'anthèse. Les auteurs ont ensemencé des graines de *Triticum aestivum* 'Pro INTA Oasis', inoculées avec l'*A. brasilense* Sp245, dans des parcelles non irriguées et des parcelles témoins. Ils ont déterminé le potentiel hydrique, la teneur en eau et les teneurs relatives en eau des feuilles terminales. Ils ont de plus calculé le statut hydrique de la plante à partir des courbes pression–volume. Ils ont également déterminé le rendement en grains et ses composantes, à maturité. Ils ont finalement mesuré les P, Ca, Mg, K, Fe, Cu et Zn, dans les grains secs. Bien que le cultivar ait connu des ajustements osmotiques, les plantes inoculées avec l'*Azospirillum* en état de sécheresse montrent une teneur en eau, une teneur relative en eau, un potentiel hydrique et une fraction hydrique apoplastique significativement plus élevés, alors que les valeurs des modules d'élasticité pariétales sont significativement moindres. La perte de rendement en grain par la sécheresse est de 26,5 % et 14,1 %, chez les plantes non-inoculées et inoculées avec l'*Azospirillum*, respectivement. La teneur en Mg et K des grains diminue dans les parcelles non irriguées et non inoculées. Cependant, les grains récoltés sur les plants inoculés avec l'*Azospirillum* ont des teneurs en Mg, K et Ca significativement plus élevées que ceux provenant des plants non inoculés. Ni la sécheresse, ni l'inoculation n'ont modifié les teneurs des grains en P, Cu, Fe et Zn. Un statut hydrique amélioré et un ajustement élastique, chez les plants de blé inoculés avec l'*Azospirillum,* pourraient être déterminants pour assurer un rendement accru en grain et en qualité minérale, surtout lorsque la sécheresse survient au moment de l'anthèse.

Mots clés : Azospirillum, blé, sécheresse, courbes pression–volume, rendement, teneur en minéraux.

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Introduction

Attention has been drawn to effective management of the rhizosphere because of increased interest in using microorganisms to achieve sustainable agriculture and to avoid expensive and environmentally hazardous agrochemicals (Bowen and Rovira 1999). *Azospirillum* is a well-known plant growth promoting bacteria that can be found in a wide range of habitats associated with various types of plants (Bashan and Holguin 1998). Under certain environmental and soil conditions, *Azospirillum* was shown to exert beneficial effects on plant growth, nitrogen-content, and crop yield (Okon and Labandera González 1994; Steenhoudt and Vanderleyden 2000). Apart from fixing atmospheric N_2 (Boddey and Döbereiner 1988), the bacteria can produce hormone-like substances, including auxins, gibberellins, and cytokinins (Horemans et al. 1986; Bottini et al. 1989; Vande Broek et al. 1999), and stimulate both rates of root elongation and appearance of lateral and adventitious roots (Fallik et al. 1994).

Although there is a growing number of basic and techno-

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logical reports on the subject, the main features of plant–*Azospirillum* interactions leading to increased plant growth are not completely understood. Besides, poorly controlled experiments performed in the field apparently accounted for the inconsistency of results regarding grain yield enhancement exerted by *Azospirillum* inoculation (Okon and Labandera González 1994).

An early review on the benefits a plant could obtain following *Azospirillum* inoculation stressed the importance of improving plant–water relationships in increasing grain yield (Okon 1985). Indeed, field experiments performed with *Azospirilla*-inoculated crops in different countries have shown significantly increased yields accompanied by better water and mineral uptake and a improvement in root morphology and growth (Sarig et al. 1988; Dobbelaere et al. 2001). Wheat and maize seedlings inoculated with live *Azospirillum* and later exposed to water stress had better water status than controls inoculated with autoclaved cells (Creus et al. 1998; Casanovas et al. 2002). In this regard, other plant growth promoting bacteria microorganisms have been useful in ameliorating water stress effects in plants. While Razi and Sen (1996) used *Klebsiella* spp. to study these effects in rice, Timmusk and Wagner (1999) found that *Paenibacillus polymyxa* could increase the expression of *Arabidopsis thaliana* genes associated with plant defenses to both biotic and abiotic stress. Detailed tissue water relations in plants can be determined by pressure–volume (P–V) analysis (Turner 1981; Parker and Colombo 1995). These data can be useful to determine plant adaptation to water stress, such as osmotic or elastic adjustment (Saliendra and Meinzer 1991). Both effects have been associated in plants with the ability to maintain cell turgor under water stress (Tyree and Jarvis 1982). In fact, an increase in both apoplastic water and cell wall elasticity in coleoptiles of *Azospirillum*-inoculated wheat seedlings growing under osmotic stress was interpreted as part of the physiological mechanisms stimulated in the plant by bacterial action (Creus et al. 1998).

On the other hand, the flag leaf is the main source of photosynthates to filling grains in wheat ear during and after anthesis (Thome 1982; Patrick and Wardlaw 1984). While drought during such a phenological period would affect both flag leaf physiology and grain production, a better water status in *Azospirillum*-inoculated plants could imply lower grain mass loss at harvest. Moreover, an increased root surface in *Azospirillum*-inoculated plants could improve both water and mineral absorption, which, in turn, could benefit crops growing in water-deficient soils (Okon 1985). Regarding all these factors, there is still a lack of information connecting water relations in *Azospirillum*-inoculated wheat plants exposed to water stress during anthesis, yield at harvest, and mineral content in grains. Even though the results presented here correspond with 1-year experimentation in the field, they extend previous studies performed in a growth chamber (Creus et al. 1998).

The objective of this work was to observe whether *Azospirillum*-inoculated wheat plants grown in the field and exposed to controlled drought during anthesis would improve (*i*) flag leaf water relations during the stress period and (*ii*) grain yield and mineral content at harvest.

Materials and methods

Bacterial inoculum and seed inoculation

Azospirillum brasilense Sp245 was grown on agar Congo red medium (Rodríguez Cáceres 1982) during 4 d, transferred to NFb liquid medium containing 0.1% NH_4Cl (Döbereiner and Day 1976), and incubated for 48 h at 30 °C with orbital agitation (100 r/min). Cells were harvested by centrifugation (10 min at 8142g) in a SS34 Sorvall rotor and resuspended in 66 mmol·L^{-1} phosphate buffer (pH 7), to 10^8 cells·mL^{-1}. This bacterial concentration was determined by measuring absorbance at 600 nm.

Seeds of *Triticum aestivum* 'Pro INTA Oasis' were surface sterilized in 3% NaOCl for 2 min and washed twice with sterile distilled water. Seeds were inoculated as previously described (Creus et al. 1996), air dried in a laminar flow cabinet to 14% humidity, and stored for 2 d at 15 °C in the dark until sown. Control seeds were inoculated with phosphate buffer. Reinoculation of plants was performed 30 d after sowing by injecting 10 mL of 10^7 *Azospirillum* cells·mL^{-1} phosphate buffer in the soil surrounding each plant. Control plots received the same quantity of phosphate buffer.

Experimental conditions in the field

The experiment was conducted at the Estación Experimental Agropecuaria Instituto Nacional de Tecnología Agropecuaria Balcarce (37°45′S; 58°18′W) during the 1994–1995 winter season. The soil was an Argiudol typic (5.4% organic content; 11.5 ppm P; 8.85 ppm NO_3-N; pH 5.7). Plots were 1.4 m × 3 m containing seven rows sowed at a density of 380 plants·m^{-2}. Control and drought-exposed plots were fertilized with $(NH_4)_2HPO_4$ (100 kg·ha^{-1}) at sowing and covered with parabolic transparent plastic tents to avoid rain during the stress period. Drought was imposed by water withholding, while control plots were watered weekly with 500 L of water each. Stress was initiated when 20% of the crop was at anthesis (84 d after sowing) and maintained for 25 d. Thereafter and until harvest, plots were watered weekly up to field capacity. Soil matric potential (Ψ_m) at each sampling time was estimated from gravimetric data of percent humidity (%H) obtained at 20 cm depth and the characteristic water retention curve of the soil:

$$[1] \qquad \log(\%H) = -0.16 \log(-\psi_m) + 1.85, \qquad r^2 = 0.96$$

Colonization assessment

Following surface sterilization, inner bacterial colonization of roots was determined at 15, 45, 84, 96, and 109 d after sowing, as follows. A 0.5-g portion of root tissue was immersed in 1% chloramine-T for 2 min and washed twice for 10 min with sterile distilled water. After homogenization in a mortar with 4.5 mL of 66 mmol·L^{-1} phosphate buffer (pH 7.0), serial dilutions up to 10^{-9} were obtained. Three 0.1-mL replicates from each dilution were cultured in semisolid NFb medium (Döbereiner and Day 1976), and most probable numbers of bacteria per gram of fresh mass (MPN·g FM^{-1}) were estimated according to Postgate (1969). Bacterial growth was then transferred to agar Congo red medium to detect stained colonies similar to those obtained with pure *A. brasilense* cells (Rodríguez Cáceres 1982). Col-

onies developed in this medium during 5 d at 30 °C were subcultured in Okon, Albrecht, and Burris (OAB) medium (Bashan et al. 1993) for 16 h at 30 °C, with orbital stirring (100 r/min). Harvesting was performed by centrifugation at 8142*g* (Sorvall SS34) for 10 min, and the pellet was washed thrice with sterile distilled water. Approximately 100 mg of this material was used to obtain whole-cell fatty acid methyl ester derivatives.

Fatty acid extraction, methylation, and analysis

Bacterial pellets (100 mg) were saponified by mixing the cells with 1 mL of 1.2 mol·L^{-1} NaOH in a 50% aqueous methanol solution and heating for 30 min in a boiling water bath. Samples were neutralized by adding 0.5 mL of 6 mol·L^{-1} HCl and esterified by adding 1 mL 12% boron trichloride – methanol and heating for 5 min at 85 °C. Saponification and methylation were performed into sealed Teflon-capped tubes, under N$_2$ atmosphere. The fatty acid methyl esters were then extracted with 1 mL hexane–diethylether (1:1) and cleaned up by washing the extract with 3 mL of 0.3 mol·L^{-1} NaOH. The extracts were transferred to 2-mL vials and stored under N$_2$ at –20 °C until GLC analyses. Then the extract was dried under N$_2$ and immediately resuspended in 100 μL chlorophorm. Fatty acid methyl esters were analyzed by a Shimadzu GC-14B gas chromatograph equipped with flame ionization detectors and a Chromatopac C-R6A integrator. One-microlitre samples were injected both into polar SP-2330 and nonpolar SPB-5 (Supelco Inc., State College, Penna.) 30-m columns. Solvent blanks were checked periodically for impurities. Operating conditions were as follows: nitrogen carrier gas flow, 30 mL·min^{-1}; injector temperature, 250 °C; initial column temperature, 130 °C; final temperature, 230 °C; temperature program rate, 4 °C·min^{-1}; detector, 280 °C. Fatty acids between 8 and 20 carbons long were identified by co-chromatography with reference standards. Three replicate analyses were made for each field isolate and pure *A. brasilense* Sp245 cells.

Water status determinations

Flag leaves of mother shoots were excised on days 0, 5, 12, 15, and 25 after initiation of stress and used for fresh (FM), turgid (TM), and dry mass (DM) determinations. To determine turgid mass, the 1-cm ends of leaves were removed under distilled water. Immediately after, leaves were left to rehydrate in darkness for 12 h in a humidity chamber at room temperature, by keeping the freshly cut ends immersed in water. Dry mass was obtained after drying leaves in an oven at 60 °C until constant mass. These data were used to calculate water content (WC) and relative water content (RWC), as follows:

[2] $WC = (FM - DM) \times 100 \times DM^{-1}$

[3] $RWC = (FM - DM) \times 100 \times (TM - DM)^{-1}$

Water potential of flag leaf (Ψ_w) was measured before dawn with a pressure chamber (PMS Instruments Co., Corvallis, Oreg.) (Scholander et al. 1964).

Pressure–volume curves and water relations

Eight to 10 flag leaves from each treatment were excised at random 25 d after initiation of stress, and transported to the lab in sealed plastic bags. Turgid mass was determined as above. Immediately after, leaves were allowed to dehydrate by free transpiration in a humidity chamber for about 10 h (Talbot et al. 1975; Richter 1978). During this period, leaves were alternatively inserted in a pressure chamber to determine Ψ_w and, immediately after, FM. This process was repeated at least 10 times. Finally, leaves were dried in an oven at 60 °C, until they achieved constant mass. Leaf RWCs were calculated as indicated above. After plotting Ψ_w^{-1} versus RWC data pairs (Tyree and Richter 1982), all the points were fitted by least squares, using the statistics table curve package (Jandel Scientific 1992). The curve with the highest r^2 was selected in each case. The data pairs that were obtained after the point of turgor loss were used to determine the linear regression portion of the curves. The equation representing this regression was used to estimate osmotic potential at full turgor (π_{100}) and symplastic water fraction (SWF). Apoplastic water fraction (AWF) was calculated from

[4] $AWF = 100 - SWF$

Relative water content at zero turgor (RWC$_0$) and osmotic potential at zero turgor (π_0) were determined by extrapolating the intercepting point between the nonlinear and linear portions of the curves to the abscissa and ordinate, respectively.

Quantitative analyses of cell wall elasticity were also made from P–V curves, and volumetric cell wall modulus of elasticity values (ε_v) were obtained according to Stadelmann (1984).

Yield and yield components

At maturity, grain yield (kg·ha^{-1}) and yield components, spikes·m^{-2}, grains·m^{-2}, and 1000-grain mass, were determined on a 1-m length of the five central rows in each plot.

Macro- and micro-nutrient grain content

Grains were dried and ground in a mortar. For Ca, Mg, K, and P determinations, samples were digested with nitric and perchloric acids, 3:2 (*v/v*). For Fe, Cu, and Zn determinations, digestions were carried out with nitric – sulphuric – perchloric acids, 2:3:2 (by volume). Ca, Mg, K, Fe, Cu, and Zn content were determined by atomic absorption spectroscopy (Perkin-Elmer 5100PC). P content was determined by measuring absorbance at 820 nm (Chen et al. 1956).

Experimental design and statistical analysis of data

The experiment was a two-factor factorial in a completely randomized blocks design, with four replications. Two bacterial isolates obtained from three plants taken at random from each plot were used to determine MPN·g FM^{-1}. Five leaves per plot were used as replicates for WC, RWC, and Ψ_w determinations. Eight to 10 leaves per treatment were used as replicates for P–V curve analyses. Variance two-factor analyses were performed on the raw data for each variable tested through PROC GLM procedure, using the SAS statistical package (SAS Institute Inc. 1988). When in-

Table 1. Most probable number (MPN·g FM^{-1}) of microaerophilic bacteria in roots of *Triticum aestivum* 'Pro INTA Oasis' plants before and during a drought period.

Azospirillum brasilense inoculated	Drought exposed	MPN·g FM^{-1} for the following periods after sowing:				
		15 d[a]	45 d[a]	84 d[b]	96 d[b]	109 d[b]
No	No	$2.5 \times 10^3 \pm 10^2$	$3.5 \times 10^3 \pm 10^2$	$2.5 \times 10^3 \pm 10^2$	$1.5 \times 10^3 \pm 10^2$	$3.5 \times 10^3 \pm 10^2$
No	Yes	—	—	$3.5 \times 10^2 \pm 10$	$2.5 \times 10^3 \pm 10^2$	$1.0 \times 10^2 \pm 10$
Yes	No	$3.5 \times 10^4 \pm 10^3$	$4.5 \times 10^6 \pm 10^5$	$4.5 \times 10^6 \pm 10^5$	$1.1 \times 10^6 \pm 10^5$	$9.5 \times 10^5 \pm 10^4$
Yes	Yes	—	—	$4.5 \times 10^6 \pm 10^5$	$1.5 \times 10^6 \pm 10^5$	$7.5 \times 10^5 \pm 10^4$

Note: Results are means ± SD from three replicates.
[a]Before drought.
[b]During drought.

teractions were significant, Duncan's test ($P < 0.05$) was used to compare means (SAS Institute Inc. 1988). The model was as follows:

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the observed value, μ is the general media, τ_i is the effect of the *i*th inoculum, β_j is the effect of the *j*th drought, $(\tau\beta)_{ij}$ is the effect of the interaction between the *i*th inoculum and the *j*th drought, and ε_{ijk} is the aleatory error term.

Results

Bacterial samples isolated from surface-sterilized roots in NFb media and transferred to agar Congo red medium developed only one type of colonies, stained similarly to the ones obtained with pure *A. brasilense* cells (Rodríguez Cáceres 1982). The whole-cell fatty acid methyl ester profiles obtained from these colonies did not differ from those obtained with the initial *A. brasilense* Sp245 inoculum grown in the same media (data not shown).

Table 1 shows the most probable number of endophytic, N_2-fixing microorganisms present in roots of wheat plants inoculated with *A. brasilense* Sp245. Forty-five days after sowing, MPN was $4.5 \times 10^6 \pm 10^5$. Even though water deficit may reduce the bacterial population in roots (Lal and Rao 1990), in the present work its number was maintained nearly constant throughout the entire drought period (Table 1). In addition, 45 d after sowing noninoculated plants contained at least 10^2 fewer bacteria than inoculated ones. Furthermore, when transferred to agar Congo red medium, these bacteria did not develop stained colonies as those obtained from pure *Azospirillum* spp. cultures (Rodríguez Cáceres 1982) (data not shown).

The drought period lasted 25 d, during which, Ψ_m decayed from –0.04 to –0.56 MPa, indicating a moderate water stress development (Hsiao 1973). Figure 1 shows flag leaf WC and RWC versus Ψ_m during the whole drought period. After 12 d of withholding water, when soil Ψ_m was –0.22 MPa, a significantly higher WC and RWC revealed a better water status in inoculated plants than in noninoculated controls (Fig. 1). In addition, Fig. 2 shows the effect of *A. brasilense* inoculation on flag leaf Ψ_w as soil was drying. During the entire stress period, the Ψ_w of inoculated plants was significantly higher than that of noninoculated controls. On the other hand, Ψ_w of plants remained within a –0.5 to –0.2 MPa range in wa-

Fig. 1. Flag leaf water content (WC) (A) and relative water content (RWC) (B) in *Triticum aestivum* 'Pro INTA Oasis' plants, in a drying soil. Data are means ± SD of five samples in each of four blocks. Ψ_m, soil matric potential.
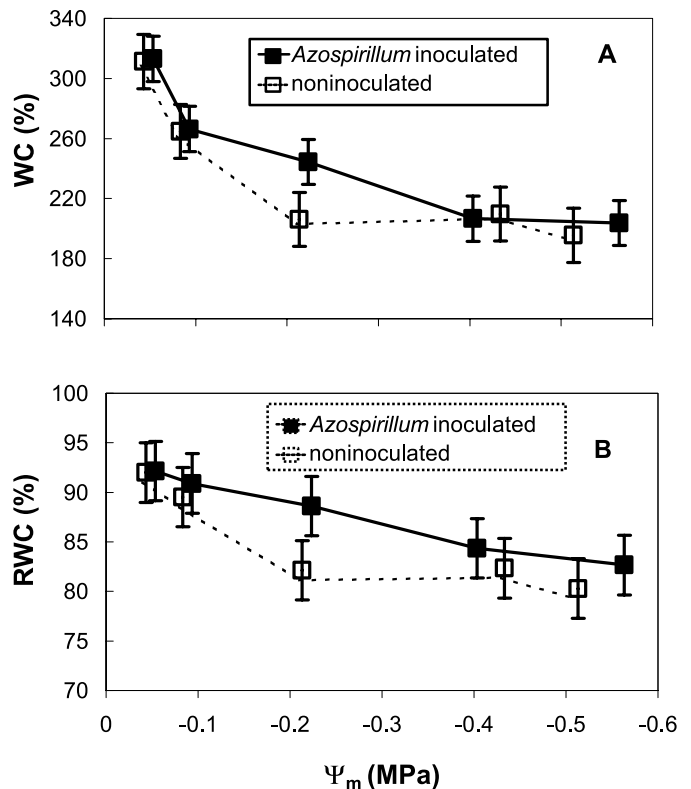
tered plots, irrespective of whether they were inoculated or not (data not shown).

A more detailed view of plant water status at the end of the water stress period was provided by the construction of P–V curves (Fig. 3) and its subsequent variance analysis. Table 2 shows π_{100}, π_0, RWC_0, ε_v, and AWF data obtained from these analyses. The π_{100} and RWC_0 parameters were not affected by inoculation treatment, but were significantly reduced by drought. The significant drop in π_{100} indicates the osmotic adjustment capability of the cultivar after the reduction in soil water content. The π_0 was not affected either
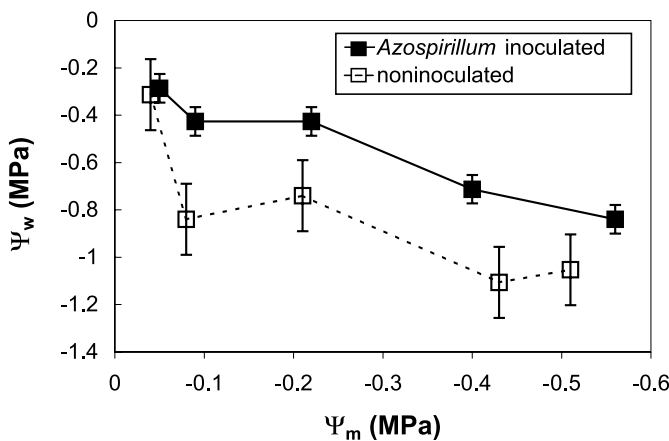
Table 2. Water status parameters in flag leaves of *Azospirillum brasilense* Sp245 inoculated and noninoculated *Triticum aestivum* 'Pro INTA Oasis' plants, obtained from variance analyses of pressure–volume curves.

A. brasilense inoculated	Drought exposed	π_{100} (MPa)[a]	π_0 (MPa)[b]	RWC_0 (%)	ε_v (MPa)	AWF (%)
No	No	−0.82±0.09	−2.72±0.94	71.7±4.4	23.3±1.9	34.4±6.5
No	Yes	−1.17±0.02	−2.56±0.61	62.6±7.0	22.9±1.1	32.8±4.0
Yes	No	−0.83±0.05	−2.14±0.61	74.2±4.4	23.0±1.1	41.7±5.5
Yes	Yes	−1.14±0.09	−2.74±0.26	68.0±3.0	13.9±1.1	42.48±7.6
F-test						
A. brasilense		ns	ns	ns	$p < 0.05$	$p < 0.05$
Drought		$p < 0.05$	ns	$p < 0.05$	$p < 0.05$	ns
A. brasilense × drought		ns	ns	ns	$p < 0.05$	ns

Note: Results are means ± SD from 8 to 10 curves. RWC_0, relative water content at zero turgor; ε_v volumetric cell wall modulus of elasticity; AWF, apoplastic water fraction; ns, not significant.

[a]Osmotic potential at full turgor.

[b]Osmotic potential at zero turgor.

Fig. 2. Flag leaf water potential (Ψ_w) in *Triticum aestivum* 'Pro INTA Oasis' plants, in a drying soil. Data are means ± SD of five samples in each of four blocks. Ψ_m, soil matric potential.
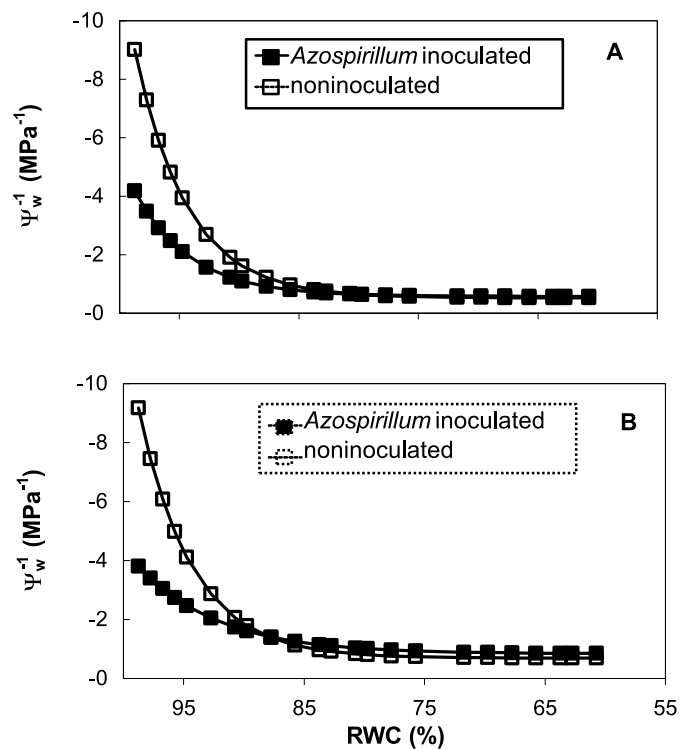

Fig. 3. Representative pressure–volume curves obtained from flag leaves of *Triticum aestivum* 'Pro INTA Oasis' plants grown under full irrigation (A) or drought conditions (B). Each curve was generated from 8 to 10 leaves as replicates. Ψ_w, flag leaf water potential; RWC, relative water content.

by inoculation or drought. Since statistical analysis of ε_v showed a significant interaction ($P < 0.05$) among factors, ε_v means were compared on the basis of inoculum factor only (Table 2). Significantly lower ε_v was observed in inoculated plants after drought, indicating a high cell wall elasticity in leaves of *Azospirillum*-inoculated plants. On the contrary, when plants were well watered, no significant effect of *Azospirillum* inoculation was seen (Table 2). *Azospirillum* inoculation caused a significant increase in AWF, irrespective of water availability (Table 2).

Table 3 shows the effects of *Azospirillum* inoculation and drought on yield, and yield components. Total yield was significantly reduced by drought impairing grains per square metre and 1000-grain mass. *Azospirillum* inoculation significantly increased total yield, both at high soil water content and at drought. The component of grain yield most affected by inoculation was grains per square metre, which increased by 0.9% and 7.5% in well-watered and drought conditions, respectively (Table 3).

Mg and K content significantly diminished in grains harvested from noninoculated wheat plants exposed to water

stress during anthesis and early grain filling (Fig. 4). However, grains harvested from *Azospirillum*-inoculated wheat plants had significantly higher content of Mg, K, and Ca than those harvested from noninoculated plants (Fig. 4). Drought during grain filling could affect nutrient uptake or translocation in wheat (Dubey and Pessarakli 1995). However, this drought effect could be ameliorated by *Azospirillum* inoculation (Fig. 4). P, Cu, Fe, and Zn grain

Table 3. Grain yield and yield components of *Azospirillum brasilense* Sp245 inoculated and noninoculated *Triticum aestivum* 'Pro INTA Oasis' plants exposed to drought during flowering.

A. brasilense	Drought	Yield (kg·ha^{-1})	Spikes·m^{-2}	Grains·m^{-2}	1000-grain mass (g)
No	No	3700.6	270.3	10 069.6	41.1
No	Yes	2717.7	273.3	8 800.3	37.6
Yes	No	3891.3	277.4	10 159.5	39.1
Yes	Yes	3176.5	308.8	9 460.5	36.6
***F*-test**					
A. brasilense	$p < 0.01$	ns	$p < 0.01$	$p < 0.05$	
Drought	$p < 0.01$	ns	$p < 0.05$	$p < 0.01$	
A. brasilense × drought	ns	ns	ns	ns	

Note: ns, not significant.

Fig. 4. Ca (A), Mg (B), and K (C) content in grains of *Triticum aestivum* 'Pro INTA Oasis' plants. Data are means ± SD of three samples in each of four blocks. DM, dry mass.
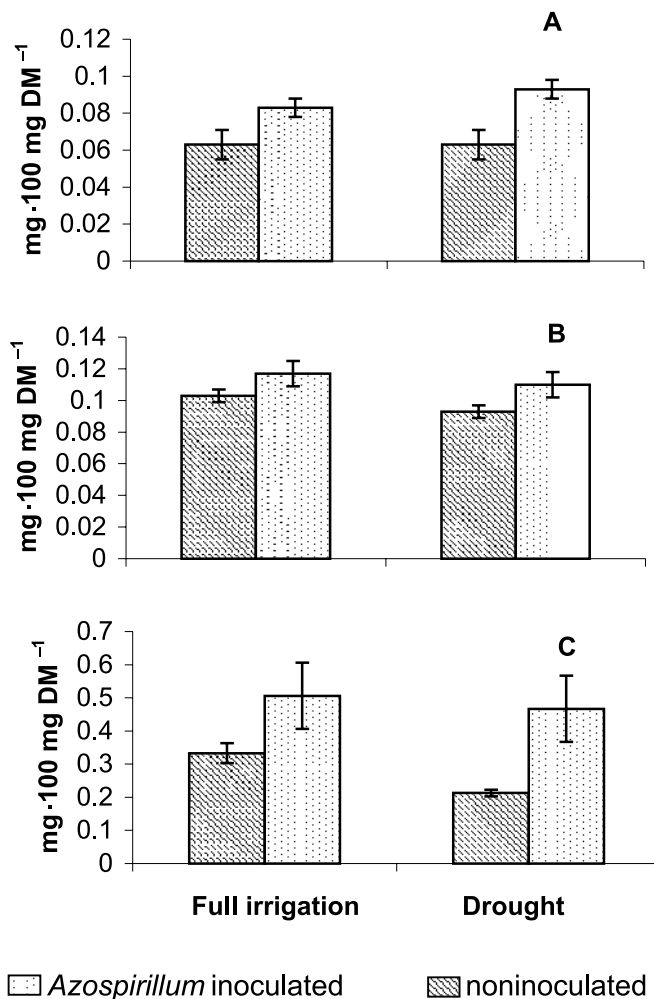
content was not affected by either drought or *Azospirillum* inoculation (data not shown).

Discussion

Plant colonization by *Azospirillum* spp. could be better assessed if strains capable of infecting the interior of roots are used as inocula. Root samples could be surface sterilized, and homogenates could be cultured in different media to determine both MPN and bacterial identity. Our inoculum choice, *A. brasilense* Sp245, was formerly isolated from wheat roots and has a proven ability to infect the interior of roots (Baldani et al. 1986; Schloter et al. 1994; Pinheiro et al. 2002). According to the criteria proposed by Hallmann et al. (1997), we could consider that MPN of bacteria isolated from surface-sterilized roots of 'Pro INTA Oasis' plants corresponded mainly to endophytic cells. Within these restrictions and 45 d after sowing, MPN inside the roots (Table 1) was within the optimum suggested by Okon (1985) for *Azospirillum* to exert its growth-promoting effect in wheat.

The maintenance of tissue turgor pressure is essential to several crucial processes upon which plant life depends. Higher plants have developed adaptive mechanisms to cope with water deficit. One of the most recognized mechanisms is osmotic adjustment, which involves active solute accumulation in plant tissues thus enabling plants to extract water at low soil water potential and maintaining cell turgor (Morgan 1984). A π_{100} reduction after drought indicates a net increase of solute contents at full turgor, thus providing a clear evidence of osmotic adjustment (Girma and Krieg 1992). High cell tissue elasticity is another mechanism that allows turgor maintenance even in a decreasing RWC situation. While in this case solutes are being slightly concentrated, turgor is maintained by elastic changes in cell volume (Al-Dakheel 1991). Indeed, the relationship among osmotic potential, turgor, and cell volume depends on the volumetric cell wall modulus of elasticity (ε_v) (Stadelman 1984). This mechanism of "elastic adjustment" is associated with the relative partitioning of water into apoplastic or symplastic fractions (Girma and Krieg 1992). In this regard, Lawlor and Leach (1985) stated that AWF could serve as a water reservoir to avoid significant variations in symplast volume during dehydration.

If water stress develops rather slowly, osmotic adjustment could compensate the reduction in plant growth resulting from a lower water potential (Kramer 1983). In the present work, osmotic adjustment was evident in *T. aestivum* 'Pro INTA Oasis' after drought, as revealed by a significant reduction in π_{100} (Table 2).

Further evidence of the cultivar's ability to withstand drought was provided by its RWC_0 decrease after drought (Table 2). In fact, dehydration postponement, that is, the

ability to maintain tissue hydration, could be a useful mechanism to drought tolerance in nonirrigated crops. This delay extends the lethal RWC_0 occurrence in a tissue (Flower and Ludlow 1986), thus lengthening the period of maximum physiological activity of the plant. This effect could reduce the stress impact on the duration of grain-filling period, and (or) contribute to root extension (Morgan and Condon 1986).

On the other hand, *Azospirillum* inoculation produced no effect either in π_{100} or in RWC_0 but significant increases in ε_v in water-stressed plants (Table 2). These data may indicate that somehow physiological and (or) biochemical changes elicited in the plant in response to water stress go along with an "elastic adjustment" triggered by the presence of *Azospirillum*. This plant–bacteria association could thus represent an additional advantage to the stressed crop.

Independent of water stress, *Azospirillum* inoculation stimulated a significant AWF increase in the plant (Table 2). While this latter response could be inherent to the colonization process, ε_v increase could represent a more specific and convenient requirement of the plant when exposed to drought. More details related to the value of water status parameters, obtained by P–V curves in the study of *Azospirillum*-inoculated wheat exposed to water stress, have been published before (Creus et al. 1998).

The role of reserves of preanthesis assimilates has long been recognized in maintaining grain filling when water deficits develop. Turner and Begg (1981) concluded from a survey of the literature that preanthesis assimilates contributed little to grain yield in cereals when the water supply was abundant, but could contribute up to 30% of the final grain yield when water deficit develops during grain filling. In the present work, grain yield loss to drought was 26.5% and 14.1% in noninoculated and *Azospirillum*-inoculated plants, respectively (Table 3). On this basis, *Azospirillum* inoculation mitigated 16.9% of the negative effects drought provoked on grain yield. This value is similar to the one obtained by Sarig et al. (1988) in experiments performed in the field with *Azospirillum*-inoculated sorghum. The relative increase we observed was mainly due to the bacterial effect on the number of grains per square metre (Table 3). For the same reason, *Azospirillum* inoculation also increased the absolute grain yield in nonstressed plants (Table 3). On the other hand, it has been shown that water stress during anthesis induces floral abortion (Murphy et al. 1992). In the present work, noninoculated plants experienced a 13% decrease in grains per square metre but only a 7% decrease in *Azospirillum*-inoculated plants (Table 3). Therefore, the possibility that *Azospirillum* could mitigate yield loss to drought by means of a lower floral abortion could not be discarded.

Low water availability has been recognized as a direct or indirect factor that affects mineral nutrition of plants. In particular, drought during grain filling could affect nutrient uptake or translocation in wheat (Dubey and Pessarakli 1995). In this regard, Mg and K content, but not Ca, were significantly diminished in water-stressed plants (Fig. 4). However, inoculation treatment increased the content of the three macronutrients in both control and stressed plants (Fig. 4). Grains with a higher Mg content could become seedlings with better photosynthetic capability during the first growth stages. One crucial step in seedlings establishment is that

which follows coleoptile full growth and the protrusion of the first leaf above the soil. In this moment, the reserves are almost depleted, and the seedlings need the photosynthetic booster to continue their growth without interruptions. If the Mg grain reserves needed to form chlorophyll molecules are low, then seedling establishment could be in danger. Moreover, Ca is extremely important in regulating cellular responses to environmental stresses (Palta 1996). A higher than normal concentration of Ca in seeds, as that promoted by *Azospirillum* inoculation in water-stressed plants, could mean future seedlings with better performance under drought. In this regard, the *Azospirillum* effect could be very important in promoting not only a higher grain yield, but also a higher seed quality.

Even though the wheat cultivar used here displayed an inherent osmotolerance to drought, the extra elasticity associated with the presence of *Azospirillum* could be accounting for the observed higher grain yield and mineral content.

Acknowledgements

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