

Water relations in *Azospirillum*-inoculated wheat seedlings under osmotic stress

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Abstract: *Azospirillum* has been shown to improve coleoptile growth in seedlings growing in darkness under osmotic stress. However, the changes in water relations that may occur in this experimental system have not yet been studied. Two-centimetre long *Triticum aestivum* cv. Buck Pucará and *Triticum durum* cv. Balcarceño-INTA seedlings were inoculated with viable or autoclaved (control) *Azospirillum brasilense* Sp. 245 bacteria, at approximately 10^8 cells per seedling. Three days after inoculation, seedlings were exposed to osmotic stress by immersing their roots in 20% polyethylene glycol 6000 for up to 72 h. Germination and seedling growth were at 20°C in darkness. Shoots were excised after 72 h of stress, and water-status parameters were determined through pressure–volume analyses. While osmotic potential at full turgor remained constant, *Azospirillum*-stimulated growth in Buck Pucará seedlings was accompanied by significant decreases in osmotic potential and relative water content at zero turgor, in volumetric cell wall modulus of elasticity, and in absolute symplastic water volume and by a significant rise in apoplastic water fraction parameters. Except for a constant volumetric cell wall modulus of elasticity, similar results were obtained with Balcarceño-INTA seedlings. However, bacterial growth promotion was evident only in the less tolerant cv. Buck Pucará. Turgor at low water potential was higher in inoculated seedlings in both wheat cultivars under osmotic stress. These results are consistent with a better water status in *Azospirillum*-inoculated wheat seedlings under water stress, where both effects on cell wall elasticity and (or) apoplastic water are evident.

Key words: *Azospirillum*, drought, seedlings, water status, wheat.

Résumé : Il a été démontré que l'*Azospirillum* améliore la croissance du coléoptile chez des plantules se développant à l'obscurité sous un stress osmotique. Toutefois, les modifications des relations hydriques qui peuvent survenir dans ce système expérimental n'ont jamais été étudiées. Des plantules du *Triticum aestivum* cv. Buck Pucará et du *Triticum durum* cv. Balcarceño-INTA mesurant 2 cm de long ont été inoculées avec la bactérie, viable ou autoclavée (témoin), *Azospirillum brasilense* Sp. 245, à raison d'environ 10^8 cellules par plantule. Trois jours après l'inoculation les plantules ont été exposées à un stress osmotique en immergeant leurs racines dans du polyéthylène glycol 6000 à 20% pendant 72 h. La température de germination et de croissance était de 20°C, à l'obscurité. Les tiges ont été excisées après 72 h d'exposition au stress, et les paramètres de l'état hydrique ont été mesurés par analyses de pression–volume. Alors que le potentiel osmotique en pleine turgescence demeure constant, la croissance stimulée par l'*Azospirillum*, chez le cv. Buck Pucará, est accompagnée de pertes significatives du potentiel osmotique et de la teneur relative en eau à turgescence zéro, en termes de module volumétrique d'élasticité de la paroi cellulaire et de volume hydrique symplastique absolu, ainsi que par une élévation significative des paramètres de la fraction hydrique apoplastique. Sauf pour un module d'élasticité de la paroi cellulaire constant, on obtient des résultats comparables avec les plantules du cv. Balcarceño-INTA. Cependant, la stimulation de la croissance d'origine bactérienne est évidente uniquement chez le cv. Buck Pucará, moins tolérant. À un faible potentiel hydrique, la turgescence est plus élevée chez les plantules inoculées des deux cultivars soumis au stress osmotique. Ces résultats sont congruents avec un meilleur statut hydrique chez les plantules de blé inoculées avec l'*Azospirillum* soumis au stress hydrique, où les deux effets sur l'élasticité de la paroi cellulaire et (ou) l'eau apoplastique sont évidents.

Mots clés : *Azospirillum*, sécheresse, plantules, statut hydrique, blé.

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Abbreviations:

AWF, apoplastic water fraction; cv., cultivar; DW, dry weight; FW, fresh weight; MPN, most probable number; NFb, nitrogen-free basal; π_{100} , osmotic potential at full turgor; π_0 , osmotic potential at zero turgor; ψ_p , pressure potential; $P-V$, pressure–volume; RWC_0 , relative water content at zero turgor; SDW, sterile distilled water; SV, absolute symplastic water volume; SWF, symplastic water fraction; TW, turgid weight; ϵ_v , volumetric cell wall modulus of elasticity; ψ_w , water potential.

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Introduction

Seedling establishment is a phenological stage at which drought could be particularly harmful to annual plants. Even though wheat is generally grown in water stress prone parts of the world (Hanson et al. 1982), soil water potential strongly affects seedling emergence (Lafond and Fowler 1989). In wheat, successful seedling establishment is highly dependent on proper coleoptile development, which in turn could be affected by osmotic stress. This specialized tissue could thus have an important role not only in seedling survival when drought strikes shortly after seeding but also in much later stages such as grain yield at harvest (Gan et al. 1992).

Grain yield can be improved by *Azospirillum* inoculation (Okon and Labandera 1994). Root association with *Azospirillum* spp. has been shown to promote vegetative growth in several plant species (Gaskins et al. 1984; Baldani et al. 1987). Apart from fixing atmospheric nitrogen under limited conditions (Boddey and Döbereiner 1988), *Azospirillum*, in general, stimulated both rates of root elongation and appearance of lateral and adventitious roots (Okon et al. 1988). As a consequence of deeper plant rooting, inoculated plants showed enhanced mineral and water uptake, which in turn could benefit crops growing in water-deficient soils (Okon 1985). However, other studies have shown that changes in the balance and (or) content of minerals are not the sole mechanism by which *Azospirillum*-inoculated plants could grow better than non-inoculated ones (Bashan et al. 1990). Moreover, in a hydroponic system where no nutrients were present, *A. brasilense* Sp. 245 inoculation could partially reverse the negative effects that water stress had on wheat seedlings, as it was observed in the growth rate of coleoptiles (Alvarez et al. 1996). Furthermore, a correlation between coleoptile length and osmoregulation among genotypes of wheat has been reported (Morgan 1988). However, little or no work has been undertaken to determine if *Azospirillum* has a significant impact on plant water relations.

An overall view of these properties can be obtained in the same tissue by pressure–volume (P – V) curves (Hellkvist et al. 1974). In this sense, the ability of a given plant cell to tolerate a restricted water supply depends on three known physiological mechanisms of adaptation: (i) active or passive solute accumulation in vacuoles, (ii) changes in cell wall elasticity, and (iii) changes in the relative partitioning of water into apoplastic and symplastic fractions (Girma and Krieg 1992).

The objective of this work was to study the effects of *A. brasilense* Sp. 245 inoculation on water relations in two wheat cultivars, *T. durum* cv. Balcarceño-INTA and in *T. aestivum* cv. Buck Pucará, seedlings growing in darkness under osmotic stress.

Materials and methods

Bacterial inoculum

Azospirillum brasilense Sp. 245 cells were grown on agar – Congo red medium (Rodríguez Cáceres 1982) during 4 days, transferred to NFB liquid medium containing 0.1% NH_4Cl (Döbereiner and Day 1976), and incubated for 48 h at 30°C with agitation (100 rpm). Cells were harvested by centrifugation (10 min at $8142 \times g$) in a SS34 Sorvall rotor and resuspended in 66 mM phosphate buffer (pH 7), to 10^8 cells·mL⁻¹. The bacterial concentration was determined by spectrophotometric measurements of the absorbance of samples at

600 nm. This was previously compared with the cell MPN method, determined by bacterial growth in semisolid cultures of serial dilutions according to Postgate (1969).

Seed germination, bacterial inoculation of seedlings, and stress treatments

Seeds of *T. aestivum* cv. Buck Pucará and *T. durum* cv. Balcarceño-INTA were selected for uniformity in weight and size, surface sterilized in 3% NaOCl for 5 min, and washed thrice with SDW. Seeds were then soaked for 3 h in SDW and germinated in a moist chamber at 20°C for 4 days in the dark. Subsequently, enough seedlings with 20 ± 1.2 mm coleoptile growth were selected under green safety light and grouped into experimental and control lots. Seedlings were transferred to racks made with bottomless ELISA plates, where roots could freely protrude downwards (Blum et al. 1980). Inoculation was performed by immersing roots for 3 h in the inoculum. After that, the inoculum was replaced by SDW and the seedlings allowed to grow for up to 3 days. Sterile distilled water was replaced by 20% polyethylene glycol (PEG) 6000 (Sigma, St. Louis, Mo.) after 72 h. The osmotic potential of the PEG 6000 solution (–0.54 MPa) was calculated from Michel and Kaufmann (1973) data. All the operations were performed under green safety light at 20°C. The same procedure was followed for the controls, except that the inoculum was autoclaved prior to application. After 72 h of treatment, roots and shoots were separated and used for colonization assessment and water status determination, respectively. Shoot length was measured with a ruler.

Colonization assessment

Internal bacterial colonization of roots was determined 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 SDW. After homogenization in a mortar with 4.5 mL 66 mM phosphate buffer (pH 7), nine 1:10 serial dilutions up to 10^{-9} were obtained. Three 0.1-mL replicates from each dilution were cultured in semisolid NFB medium containing 0.1% NH_4Cl (Döbereiner and Day 1976), and bacterial most probable numbers (MPN) per gram of fresh weight (FW) were estimated according to Postgate (1969). Bacterial colonies were then transferred to ACR medium to detect typical *A. brasilense* growth (Rodríguez-Cáceres 1982). Excepting chloramine-T treatment, the same procedure was followed to determine total colonization of roots.

Growth and water status determinations in coleoptile

Shoot samples (coleoptile and primary leaves) were measured for length and then excised under distilled water 72 h after the initiation of treatments. Shoots were rehydrated in darkness for 12 h in a humid chamber, with the cut end immersed in distilled water. After determining TWs, shoots were allowed to dehydrate by free transpiration in a humid chamber for about 10 h (Talbot et al. 1975; Richter 1978). During this period, shoots were alternatively inserted in a pressure chamber (PMS Instruments Co., Corvallis, Oreg.) for determining ψ_w and, then immediately afterwards, FW. This process was repeated at least 10 times. The same person performed all the measurements in the same chamber. Finally, the shoots were dried in an oven at 60°C until they achieved a constant weight (DW). Data for TW, intermediate fresh weights corresponding to values for ψ_w , and DW were used to calculate RWCs. Data pairs of RWCs and the corresponding ψ_w were plotted as $\psi_w - 1$ versus RWC transformations (Tyree and Richter 1982). All the points were fitted by least squares, using the statistics table curve package (Jandal Scientific 1992). The curve with the highest r^2 was selected in each case. The data of paired readings determined after the point of turgor loss during shoot dehydration was the set used to generate the linear regression. The equation representing this regression was used to estimate π_{100} and symplastic water fraction (SWF). Apoplastic water fraction (AWF) was calculated as

$$[1] \text{AWF} = 100 - \text{SWF}$$

Relative water content at zero turgor and π_0 were determined by ex-

Table 1. *Azospirillum* colonization (MPN·g⁻¹ fresh weight) of roots of wheat seedlings grown for 72 h in the dark, in 20% PEG 6000.

Species	Inoculum	Internal	Total
<i>T. aestivum</i> ^a	Live	2.5 × 10 ⁶ ± 0.5 × 10 ⁶	5.0 × 10 ⁷ ± 4.5 × 10 ⁷
	Autoclaved	<10 ²	<10 ²
<i>T. durum</i> ^b	Live	5.0 × 10 ⁶ ± 2.5 × 10 ⁶	5.1 × 10 ⁷ ± 4.5 × 10 ⁷
	Autoclaved	<10 ²	<10 ²

^aCultivar Buck Pucar.^bCultivar Balcarceo-INTA.

trapolating the intercept of the nonlinear and linear portions of the curves with the abscissa and ordinate, respectively. Pressure potential was calculated as the difference between the predetermined levels of ψ_w and π_0 . Absolute symplastic water volume was calculated according to Saliendra and Meinzer (1991). The formula used was

$$[2] \quad SV = \frac{SWF (FW - DW)}{DW}$$

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

Statistical analysis of data

The experiment was a two-factor factorial with a completely randomized design. All treatments were replicated four times, with not less than 10 plants for each combination of factors. Five to 10 coleoptiles and primary leaves were used as replicates for $P-V$ curve analyses. Two-factor analyses of variance were performed on the raw data for each variable tested. When interactions were significant (only for ϵ_v), Duncan's test ($p < 0.05$) was used to compare means (SAS Institute Inc. 1988). The model was as follows:

$$[3] \quad y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk}$$

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

Results

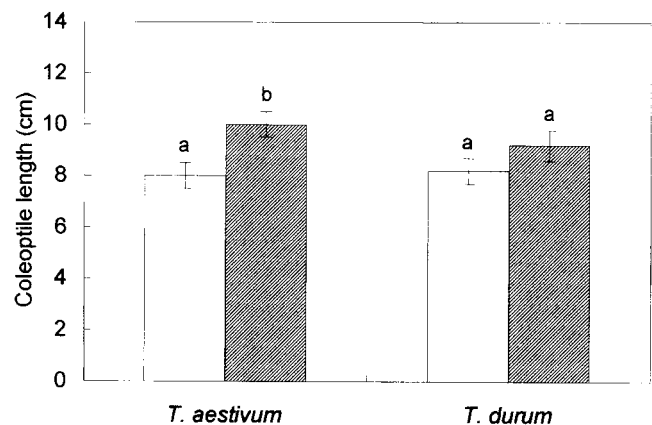
Total and internal *Azospirillum* colonization of roots of both *T. aestivum* cv. Buck Pucar and *T. durum* cv. Balcarceo-INTA seedlings grown in darkness under osmotic stress were ca. 10⁷ and 10⁶ cells·g⁻¹ fresh weight, respectively (Table 1). Less than 10² bacterial cells were detected in root samples from seedlings previously inoculated with autoclaved *Azospirillum* cells (controls).

Figure 1 shows the effects of *A. brasilense* Sp. 245 inoculation on the length of seedlings growth in darkness, in 20% PEG 6000. After 72 h, coleoptile size was increased 22% compared with controls in Buck Pucar seedlings, but no significant changes were observed in Balcarceo-INTA seedlings (Fig. 1).

Figure 2 shows representative $P-V$ curves obtained for 5–10 coleoptiles of *T. aestivum* cv. Buck Pucar and *T. durum* cv. Balcarceo-INTA seedlings previously inoculated with live or autoclaved *A. brasilense* Sp. 245, after 72 h growth in darkness, in 20% PEG 6000. Even though the curves from both cultivars cannot be compared directly, slopes are more pronounced in Buck Pucar than in Balcarceo-INTA, indicating a different cell wall elasticity for each one.

Table 2 shows water status parameters obtained from analysis of variance of $P-V$ curves presented in Fig. 2. The π_{100} remained unmodified by *Azospirillum* inoculation in seed-

Fig. 1. Shoot height of *Azospirillum*-inoculated *T. aestivum* cv. Buck Pucar and *T. durum* cv. Balcarceo-INTA seedlings grown for 72 h in the dark, in 20% polyethylene glycol (PEG) 6000. □, seedlings inoculated with autoclaved *A. brasilense* Sp. 245 cells; ■, seedlings inoculated with live *A. brasilense* Sp. 245 cells. Results are shown as mean ± SD, obtained from quadruplicates of at least 10 samples each. Different letters at the top of the bars indicate significant differences ($p < 0.05$).



lings; however, osmotic potential at zero cell turgor and RWC_0 were significantly lower than controls from both cultivars exposed to osmotic stress. 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 shoots from *Azospirillum*-inoculated Buck Pucar seedlings exposed to osmotic stress than in controls (Table 2). No significant changes in this parameter in relation to *Azospirillum* inoculation were observed in Balcarceo-INTA (Table 2). *Azospirillum* inoculation caused a significant rise in AWF in both wheat cultivars (Table 2).

Pressure potential and SV changes as a function of ψ_w loss in coleoptiles from *T. aestivum* cv. Buck Pucar and *T. durum* cv. Balcarceo-INTA seedlings inoculated with *A. brasilense* Sp. 245 growing in 20% PEG 6000 are shown in Fig. 3. A decline in the ψ_p concomitant with the fall in ψ_w was observed in *T. aestivum* cv. Buck Pucar both in control and inoculated seedlings (Fig. 3A). On the other hand, SV fell in inoculated seedlings but remained constant in controls, concomitant with the loss in ψ_w . In spite of ψ_w loss due to osmotic stress in *T. durum* cv. Balcarceo-INTA, ψ_p remained constant in coleoptiles from *Azospirillum*-inoculated seedlings (Fig. 3B), while ψ_p fell to zero in seedlings inoculated with autoclaved

Table 2. Water status parameters obtained from analyses of variance of P - V curves of shoots sampled from *Azospirillum*-inoculated wheat seedlings grown for 72 h in the dark in 20% PEG 6000.

Species	Inoculum	π_{100} (MPa)	π_0 (MPa)	RWC ₀ (%)	ϵ_v (MPa)	AWF (%)
<i>T. aestivum</i> ^a	Live	-0.43±0.09	-0.56±0.09	91.9±1.0	7.6±1.5	35.7±4.0
	Autoclaved	-0.41±0.05	-0.47±0.06	96.0±1.1	18.1±2.4	27.8±3.0
<i>T. durum</i> ^b	Live	-0.25±0.07	-0.36±0.10	89.6±0.8	1.1±0.3	34.0±3.8
	Autoclaved	-0.17±0.03	-0.24±0.04	92.0±2.9	1.5±0.3	24.5±6.4
<i>F</i> test						
Cultivar (C)		*	*	*	*	ns
Inoculum (I)		ns	*	*	*	*
C × I		ns	ns	ns	*	ns

Note: Results are mean ± SD of 5–10 determinations obtained from the analyses of 5–10 P - V curves. AWF, apoplastic water fraction; π_{100} , osmotic potential at full turgor; π_0 , osmotic potential at zero turgor; RWC₀, relative water content at zero turgor; ϵ_v , volumetric cell wall modulus of elasticity.

*, $p < 0.05$; ns, not significant.

^aCultivar Buck Pucara.

^bCultivar Balcarceno-INTA.

Fig. 2. Representative P - V curves of shoots sampled from *Azospirillum*-inoculated (A) *T. aestivum*, cv. Buck Pucara; (B) *T. durum*, cv. Balcarceno-INTA wheat seedlings grown for 72 h in the dark, in 20% polyethylene glycol (PEG) 6000. \circ , seedlings inoculated with autoclaved *A. brasilense* Sp. 245 cells; \bullet , seedlings inoculated with live *A. brasilense* Sp. 245 cells. Each curve was generated from 5–10 coleoptiles and primary leaves as replicates.

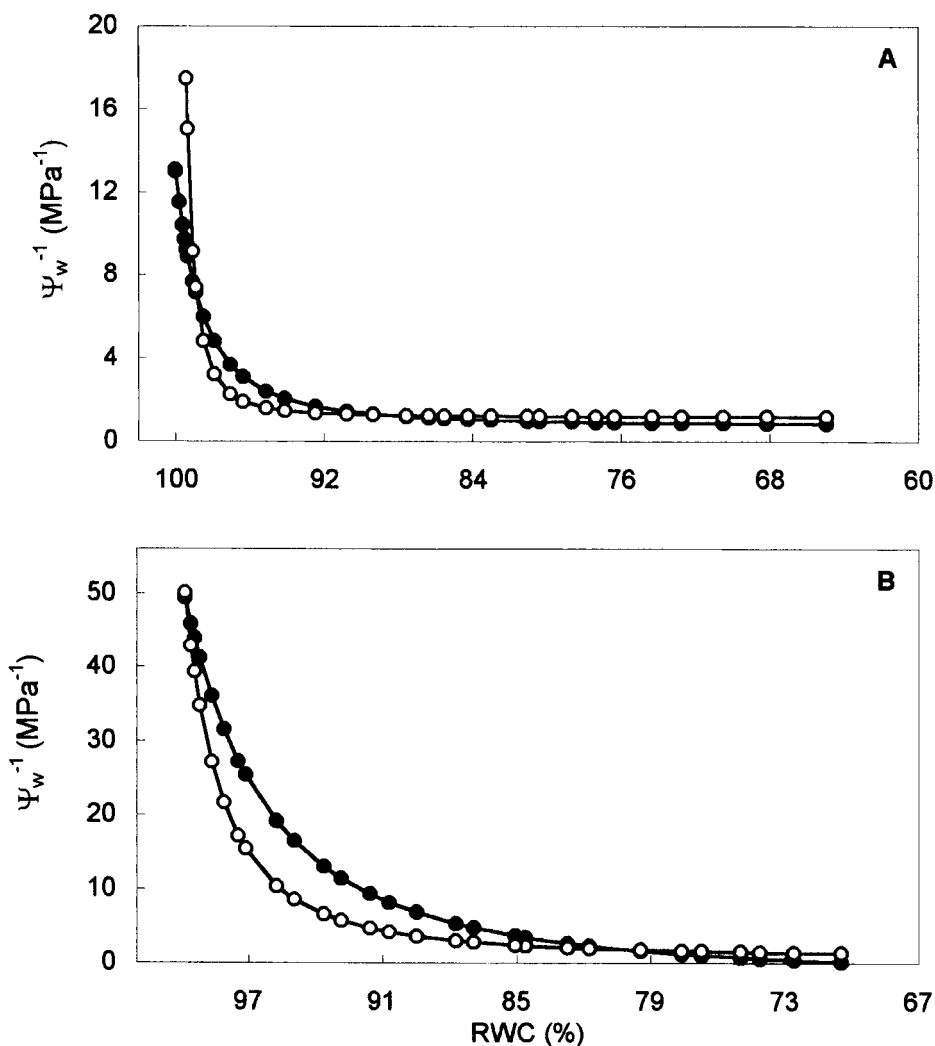
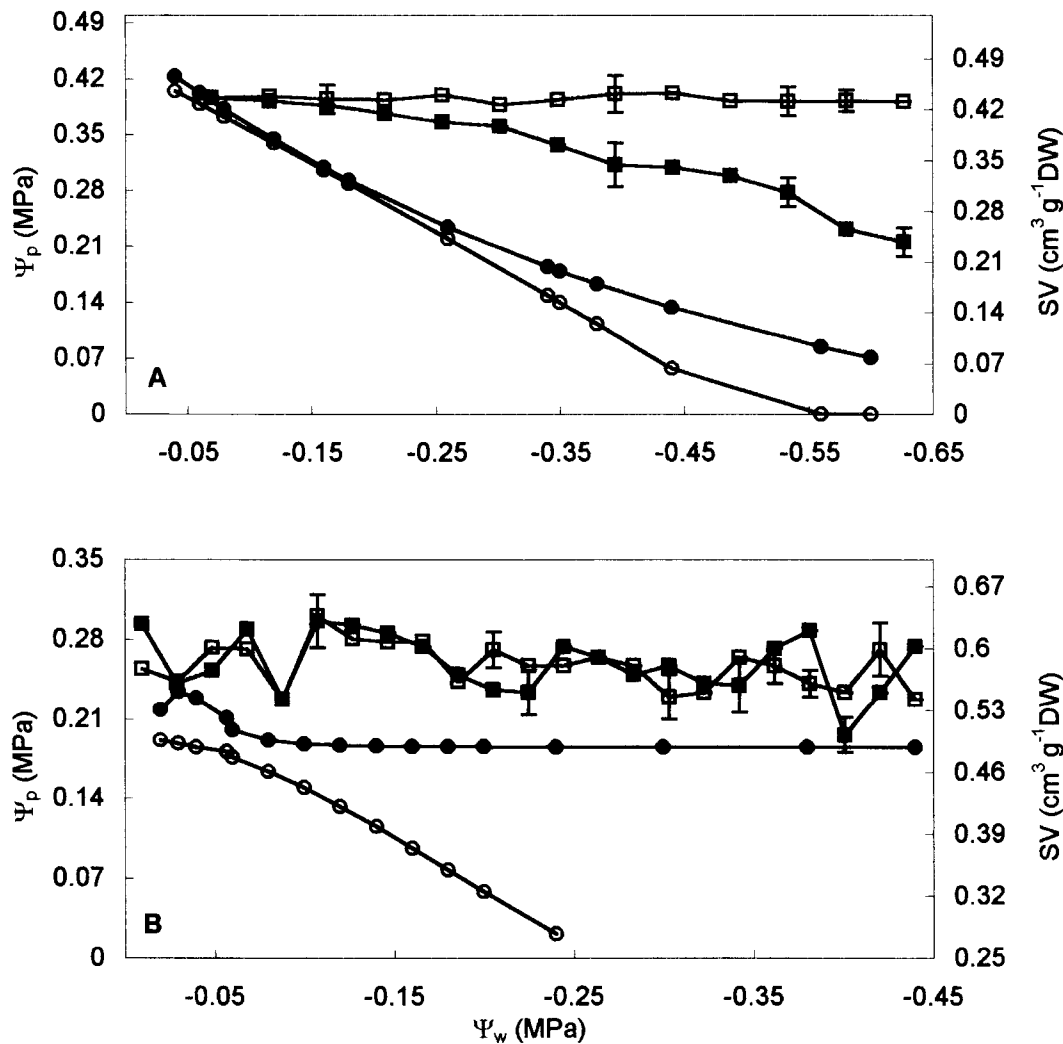


Fig. 3. Pressure potential (Ψ_p ; circles) and absolute symplastic water volume (SV; squares) in relation to water potential of shoots sampled from *Azospirillum*-inoculated (A) *T. aestivum*, cv. Buck Pucara; (B) *T. durum*, cv. Balcarceno-INTA wheat seedlings grown for 72 h in the dark, in 20% polyethylene glycol (PEG) 6000. \circ and \square , seedlings inoculated with autoclaved *A. brasilense* Sp. 245 cells; \bullet and \blacksquare , seedlings inoculated with live *A. brasilense* Sp. 245 cells. Error bars are 1 SD.



bacteria, at -0.25 MPa of Ψ_w (Fig. 3B). The SV remained relatively constant at 0.50 – 0.64 $\text{mL}\cdot\text{g}^{-1}$ DW in coleoptiles of inoculated and control seedlings of *T. durum* cv. Balcarceno-INTA and showed no significant differences (Fig. 3B).

Discussion

Since the seedlings were grown in darkness, the growth promotion of *T. aestivum* cv. Buck Pucara seedlings under osmotic stress induced by *Azospirillum* (Fig. 1) was independent of photosynthesis. Furthermore, no nutrients were present in the hydroponic media, suggesting that the effect of inoculation on coleoptile size could be related to either one or all of higher water absorption by roots, better nutrient translocation from endosperm reserves to aerial parts, or effects on cellular mechanisms of adaptation to water stress.

The analyses of P – V curves provide data on water status parameters, including π_{100} , π_0 , RWC_0 , ϵ_v , AWF, and SV, that can be useful to determine plant adaptation to water stress,

such as osmotic adjustment, elastic adjustment, and selective partitioning of water into apoplastic and symplastic fractions (Saliendra and Meinzer 1991).

A simultaneous decrease in π_{100} and π_0 , and to a lesser extent, in π_{100} alone, has been considered as indicative of osmotic adjustment in plants suffering water deficits (Eamus and Narayan 1990; Saliendra and Meinzer 1991). Since π_{100} is determined at full cell turgor, a decrease in this parameter is directly related to a higher solute concentration in the symplast (Meier et al. 1992). In our experimental system, π_{100} remained constant while π_0 fell in coleoptiles from *Azospirillum*-inoculated wheat seedlings from both cultivars (Table 2). On the other hand, π_0 is influenced by the cell wall elasticity (Cheung et al. 1975), which could be the situation reported here. Moreover, a decrease in RWC_0 has been associated more with elastic adjustment than to osmoregulation (Cutler et al. 1977). This kind of effect on RWC_0 was observed in coleoptiles from both wheat cultivars, when seedlings inoculated with *A. brasilense* Sp. 245 were exposed to osmotic stress (Table 2). However, only *Azospirillum*-inoculated Buck Pucara coleoptiles

exposed to osmotic stress had lower ϵ_v than control coleoptiles (Table 2), indicating higher cell wall elasticity. This has been associated in plants with the ability to maintain cell turgor under water stress (Tyree and Jarvis 1982). Both osmotic and elastic adjustment have been considered to be components of the more general turgor adjustment response and as such, while triggered by the same stress situation, could be quite unrelated processes (Kikuta and Richter 1986). An increase in tissue elasticity accompanied by an increase in solute accumulation could have resulted in a slight or nonexistent decrease in π_{100} (Saliendra and Meinzer 1991). In this regard, the results presented here are insufficient to exclude a possible effect of *Azospirillum* inoculation on osmotic adjustment under water stress in Buck Pucará. Thus, both osmotic and elastic adjustments could account for the effect that *Azospirillum* exerts on coleoptile growth in seedlings under water stress (Fig. 1).

In *T. durum* cv. Balcarceño-INTA seedlings exposed to osmotic stress, the effect of *Azospirillum* inoculation on π_0 (Table 2) could be the result of passive solute accumulation. In *Solanum melongena*, an increased bound water fraction has been linked to a decreased water potential associated with zero turgor (Eamus and Narayan 1990). In fact, passive solute concentration is faster in plant tissues with higher AWFs (Girma and Krieg 1992), which means low π_0 values under drying conditions. Similarly, the π_0 fall in Balcarceño-INTA, which was not accompanied by a ϵ_v fall (Table 2), could imply an effect on AWF. In general, data on changes in AWF accompanied by changes in the ψ_p/ψ_w ratio in plants acclimating to water stress are scarce (Gunasekera and Berkowitz 1992). To our knowledge, this kind of study in *Azospirillum*-inoculated wheat seedlings under osmotic stress has not been done so far. *Azospirillum* inoculation caused a significant rise in AWF in both wheat cultivars (Table 2). This could delay cellular water loss during plant dehydration (Tyree and Jarvis 1982).

A fall in the ψ_p was observed concomitant with the fall in ψ_w in *T. aestivum* cv. Buck Pucará both in control and inoculated seedlings (Fig. 3A). However, coleoptiles from inoculated seedlings maintained ψ_p better than controls at the lowest ψ_w reached during the experiment (Fig. 3A). On the other hand, SV fell in inoculated seedlings but remained constant in controls according to the loss in ψ_w . A change in cell wall elasticity would alter the ratios at which ψ_p and SV decrease as a function of ψ_w fall. *Azospirillum* inoculation provoked this kind of response in Buck Pucará seedlings grown in 20% PEG 6000 (Fig. 3A). That, plus a positive correlation among higher RWC_0 and AWF and lower ϵ_v (Table 2), supports the idea that the bacterial inoculation enhanced cell wall elasticity under water deficit conditions. Both higher cell wall elasticity and AWF (Table 2) could help cells to keep SV constant (Fig. 3A) and, thus, to provide a possible explanation as to why *Azospirillum* improves coleoptile growth in Buck Pucará seedlings under osmotic stress (Fig. 1).

The results presented here show differential effects of *Azospirillum* on the wheat seedlings' capability to withstand water stress between the genotypes used. Even though the effect of *Azospirillum* inoculation on coleoptile turgor was more evident in Balcarceño-INTA than in Buck Pucará (Fig. 3), this was not reflected on a larger coleoptile (Fig. 1). In this regard, it has been reported that growth can be impaired even when turgor remains high (Schultz and Matthews 1993). In any case, the effect of *Azospirillum* inoculation on ψ_p in Balcarceño-

INTA (Fig. 3B) could be related mainly to the increase in AWF (Table 2). A high AWF is usually found in plants adapted to drought (Cutler et al. 1977). In this regard, Balcarceño-INTA is a *T. durum* cultivar and, as such, is better adapted to arid regions than *T. aestivum* (Hanson et al. 1982). In fact, greenhouse experiments have shown that *T. durum* cv. Balcarceño-INTA could tolerate drought better than *T. aestivum* cv. Buck Pucará (Borgo 1990). The results obtained with *T. durum* cv. Balcarceño-INTA inoculated with autoclaved bacteria show considerably lower π_{100} , π_0 , and ϵ_v than their counterparts in *T. aestivum* cv. Buck Pucará (Table 2) but about the same coleoptile length as these latter seedlings (Fig. 1). These results could indicate a better capacity to tolerate osmotic stress in Balcarceño-INTA than in Buck Pucará. Whether or not *Azospirillum* inoculation could improve water status and growth in less drought tolerant wheat genotypes better than in more tolerant ones remains to be investigated.

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