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Response to saline stress and aquaporin expression in *Azospirillum*-inoculated barley seedlings

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Abstract The ability of two strains of *Azospirillum brasilense* to mitigate NaCl stress in barley plants was evaluated. Barley seedlings were inoculated and subjected to 200 mM NaCl for 18 days. Several days after NaCl treatment, a significant decline in biomass as well as in height was observed in uninoculated plants. However, smaller reductions in biomass and height were detected in plants inoculated with strain Az39. All the stressed plants showed significantly higher Na^+ but lower K^+ contents in their shoots. The growth rate of uninoculated plants was adversely affected by saline treatment, which was associated with higher putrescine content and lower levels of *HvPIP2;1* transcripts in the roots. *Azospirillum* inoculation triggered the transcription of this gene. Our results suggest that barley plants inoculated with *A. brasilense* may be better prepared to thrive under saline conditions. To our knowledge, this is the first report showing an effect of *Azospirillum* inoculation on the expression of *PIP2;1*, a gene involved in the synthesis of root water channels.

Keywords *Azospirillum* · Barley · Saline stress · Aquaporins · Polyamines

Introduction

Soil salinization is one of the major threats to global agriculture. Apart from the osmotic component that may inhibit plant growth, salinity entails ion toxicity effects mainly through perturbations in protein and membrane structure (Cushman 2001).

Attempts to improve salt tolerance of cultivated plants through conventional selection-based breeding strategies have met very limited success, due to the complex nature of this trait (Flowers 2004). Under this scenario, the possibility of alleviating the deleterious effects of salinity on plant growth through the action of root-associated microorganisms has emerged as a new field of interest. Inoculating plants with non-pathogenic bacteria can provide "bioprotection" against biotic stresses, and some root-colonizing bacteria increase tolerance against abiotic stresses such as drought, salinity, and metal toxicity (Dimkpa et al. 2009).

The associative rhizobacterium *Azospirillum* has extensively been investigated in the last decades (Bashan et al. 2004). Besides its well-known abilities of promoting root growth mainly by AIA production and fixing atmospheric nitrogen, *Azospirillum* also mitigates salt, water, and osmotic stresses in different plant species (Hamaoui et al. 2001; Casanovas et al. 2002; Creus et al. 2004; Barassi et al. 2006; Cassan et al. 2009). Some mechanisms proposed to explain these effects include changes in ion selectivity at the root level resulting in higher K^+/Na^+ ratios (Hamdia et al. 2004; Mayak et al. 2004; Nadeem et al. 2007), changes in the saturation patterns of membrane phospholipids resulting in lower membrane potential and bacteria-mediated changes in the elasticity of root cell membranes (Creus et al. 1998, 2004; Pereyra et al. 2006), and also a better osmotic adjustment involving higher levels of compatible solutes such as proline, glycine betaine, and trehalose (Tripathi et al. 1998; Yuwono et al. 2005; Rodríguez-Salazar et al. 2009).

Aquaporins are channel proteins that mediate the transport of water and small neutral molecules across

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cellular membranes. In plants, they can modulate transmembrane water transport in situations where adjustment of water flow is physiologically critical (Martinez-Ballesta et al. 2006). In fact, it has been argued that gating of aquaporins could represent a rapid pathway of response to environmental constraints such as anoxia, salt, and water stress (Chaumont et al. 2005). In barley roots, three plasma membrane type aquaporin genes (*PIP*) have been established (Katsuhara et al. 2002). *HvPIP1* genes (*HvPIP1;3*, *HvPIP1;5*) were less abundantly expressed than *HvPIP2;1* (Katsuhara and Shibasaka 2007). Chaumont et al. (2000) reported that aquaporins in the PIP1 subgroup have no or very low water transport activity.

Although some researchers have focused on plant aquaporin gene expression under symbiotic interactions (Marjanovic et al. 2005; Uehlein et al. 2007; Aroca et al. 2007), information concerning the effects of associative rhizobacteria on root aquaporin gene expression is lacking.

The purpose of this work was to compare the ability of two *Azospirillum* strains currently used in Argentinian biofertilizer formulations to mitigate the adverse effects of salt stress in barley seedlings growing under controlled conditions. Additionally, we tried to link the observed results to possible changes in root polyamine patterns and expression of aquaporin genes.

Materials and methods

Plant material, inoculation, and treatments

Barley (*Hordeum vulgare* L.) seeds were surface-disinfected with 30% sodium hypochlorite for 15 min, thoroughly rinsed in sterile distilled water, and germinated at 20°C. Germinated seeds were transferred to plastic pots filled with a mixture of sterile vermiculite and perlite (1:1) previously saturated with half strength Hoagland solution (Hoagland and Arnon 1950) and inoculated either with *Azospirillum brasilense* strain Az39 (provided by the Strains Collection Laboratory of IMYZA-INTA) or with an *A. brasilense* strain isolated from the *Azospirillum*-based biological fertilizer "Graminante™ trigo", manufactured by Laboratorio Alquimia S.A. (Venado Tuerto, Santa Fe, Argentina). According to label information, this product is formulated in a carrier of natural components, calcium and magnesium carbonates. To isolate this strain from the commercial product, published routine procedures were followed (Döbereiner et al. 1995). The identification of this isolate as *A. brasilenese* was based on Gram staining, motility, cell shape, colony morphology, and biochemical tests (Tarrand et al. 1978). Additionally, the assignment of the partial 16S ribosomal RNA (rRNA) gene sequence to the new phylogenetically consistent higher-order bacterial taxonomy using the naive Bayesian rRNA classifier utility from the Ribosomal Database Project Release 10 (Wang et al. 2007) showed that the isolate belongs to the genus *Azospirillum* (100% confidence). The comparison of the 16S rDNA partial sequence of the strain isolated from the commercial product against sequences deposited in public databases using the BLAST (MegaBlast) utility from the NCBI website showed that the two closest relative sequences (100% identity) correspond to the type strain of *A. brasilense* [*Azospirillum brasilense* strain DSM 1690 16S ribosomal RNA gene, partial sequence (GU256438.1) and *Azospirillum brasilense* (T) ATCC 29145 NCIMB 11860 (AY324110)].

Inoculation was accomplished by adding 0.1 ml of an exponential phase culture obtained in NFb medium (Döbereiner 1980) at bacterial titers ranging between 1.6 and 1.8×10^{8} cfu ml^{-1} to each germinating seed. A third group of pots that remained uninoculated served as controls.

Five days later, pots were thinned to six seedlings each and half of the pots of each group were watered from this day onwards with half strength Hoagland solution containing 200 mM NaCl. Plants were grown in a growth chamber at 22–24°C and 70% relative humidity under a 16-h light/8-h dark photoperiod. Before starting NaCl treatment (day 0) and at days 4, 8, 12, and 18 of salt treatment, two pots representing each treatment were kept aside, and their plants were used to measure plant height, shoot, and root fresh biomass and to determine chlorophyll content by standard spectrophotometric procedure. Fresh root samples were processed to evaluate the contents of polyamines (PAS) and to extract RNA in order to perform reverse transcriptase polymerase chain reaction (RT-PCR) to amplify *HvPIP2;1* gene, as described below. Shoots were dried at 80°C to calculate water content and to further analyze Na^{+} and K^{+} concentrations. At days 8 and 18, fresh root samples (100 mg) were processed to estimate *Azospirillum* numbers by the most probable number MPN technique, as previously described (Döbereiner et al. 1995).

Analysis of polyamines in inoculated barley plants

Roots (300 mg FW) were homogenized with 5% (*v*/*v*) perchloric acid, kept 30 min on ice, and centrifuged at 5,000 rpm for 10 min. The supernatants were derivatized using the dansylation method described by Smith and Meeuse (1966) and 1,6 hexanediamine was used as an internal standard. Standards of polyamines putrescine (Put), spermidine (Spd), spermine (Spm), and cadaverine (Cad) were dansylated simultaneously. The dansylated derivatives were extracted with 1 ml ethyl acetate. Polyamines were separated and identified by TLC, performed on high resolution silica gel plates (JT Baker, silica gel plates IB 2-F) using *n*-hexane/ethyl acetate (1:1) as first solvent. Dansylated polyamines were identified by comparing the

Rf values of dansylated standards. Silica plates were observed under UV light and bands corresponding to the polyamines in the samples and standards were scraped off the plates and eluted with 1 ml ethyl acetate. Their fluorescence was measured at 365 nm excitation and 510 nm emission in a spectrofluorometer (Aminco Bowman, USA).

Analysis of polyamines in *Azospirillum* liquid cultures

Polyamine production by *Azospirillum* growing in the presence or absence of 200 mM NaCl was investigated by processing 1 ml aliquots of stationary-phase cultures grown in NFb medium containing 0.3 g l^{-1} of NH_4Cl in a rotary shaker at 28°C and 100 rpm (72 h). Each aliquot was centrifuged at 12,000 rpm for 20 min at 4°C, and the resulting supernatant acidified with perchloric acid to obtain a 5% *v/v* final solution. These fractions were further processed as described above.

RNA isolation and RT-PCR procedure

Total RNA was extracted from root samples (100 mg) using a modified TRIzol (Invitrogen; Carlsbad, CA, USA) procedure and the extract was then treated with DNase I (Sambrook et al. 1989). It was converted to complementary DNA (cDNA) with random hexamers using the RevertAid™ MMuLV Reverse Transcriptase (Fermentas, Argentina).

For *HvPIP2;1* amplification, primers described by Katsuhara and Shibasaka (2007) were used. PCR reactions were performed using a programmable Minicycler (Ivema T-18, Argentina). Cycling conditions were 94°C for 2 min, then 35 cycles at 94°C denaturing for 30 s, 59°C annealing for 1 min, and 72°C extension for 1 min, and then a final step of 72°C for 10 min. Amplification of 18S ribosomal cDNA (forward primer: 5′-GGCTACCACATCCAAGGAA-3′; reverse primer: 5′-CTATTGGAGCTGGAATTACCG-3′) was used as an internal control for the amount of RNA and RT efficiency. The PCR products were electrophoresed through 2% agarose gel and visualized with ethidium bromide. Gels were photographed with a gel documentation system (Fotodyne, USA) and analyzed with GelPro software, and data were expressed as arbitrary units (assuming control value equal to 1), based on absolute integrated optical density of each band.

Na^+ and K^+ determination

Shoots were rinsed with deionized water and dried at 80°C to constant weight. Dry powder samples of 0.1 g were properly digested and diluted to further determine Na^+ and K^+ contents in an atomic absorption flame photometer Shimadzu AA-6800.

Statistics

Data shown in tables and figures are mean values of two independent experiments. Standard errors of the means (SEM) are presented. Differences among treatments were analyzed by unpaired *t* test or one-way ANOVA followed by Tukey's multiple range test using InStat™ software (Graph Pad Software, San Diego, CA, USA), taking $P < 0.05$ as significant.

Results

Plant growth and ionic balance

In order to assess if *Azospirillum* inoculation could mitigate the effects of moderate but sustained salt stress to barley seedlings, several growth parameters were measured. The differences in the fresh weight of the shoots in unstressed and salt-stressed plants were not visible until the 12th day of NaCl treatment. By this time, shoot fresh biomass of all salt-stressed plants was significantly reduced as compared to their non-stressed counterparts, with a reduction of about 62% for uninoculated plants (UI-s) and plants inoculated with the strain isolated from the commercial biofertilizer (CS-s) and of 27% for plants inoculated with strain Az39 (Az39-s) (Fig. 1a).

The roots of plants that had not been inoculated showed a significant weight reduction as a consequence of NaCl treatment from day 8 onwards (Fig. 1b). In line with that observed for shoots, the growth rate of roots under salt stress was also less affected in plants inoculated with strain Az39. In fact, the roots of these plants continued to grow at least until day 12 of NaCl treatment (last measurement for roots), while the roots of uninoculated stressed plants stopped growing after day 4 and the roots of plants inoculated with the commercial strain did so after day 8. In this way, Az39-s plants showed a 25% decrease in fresh weight in comparison with their non-stressed controls by day 12 of salt treatment, while UI-s and CS-s plants showed reductions of 50% and 36% compared to their untreated controls, respectively. By day 18, inoculated barley seedlings had significantly more fresh and dry shoot biomass than uninoculated plants (Fig. 1a, c). Under saline stress, however, this advantage disappeared; nevertheless, Az39-s treatment was the least affected by this abiotic stress.

Plant height was also affected by NaCl addition to the nutrient solution from day 12 onwards (Table 1). On comparing uninoculated and inoculated treatments, the same trend was observed: plants inoculated with Az39 exposed to 200 mM NaCl showed less reduction than UI-s

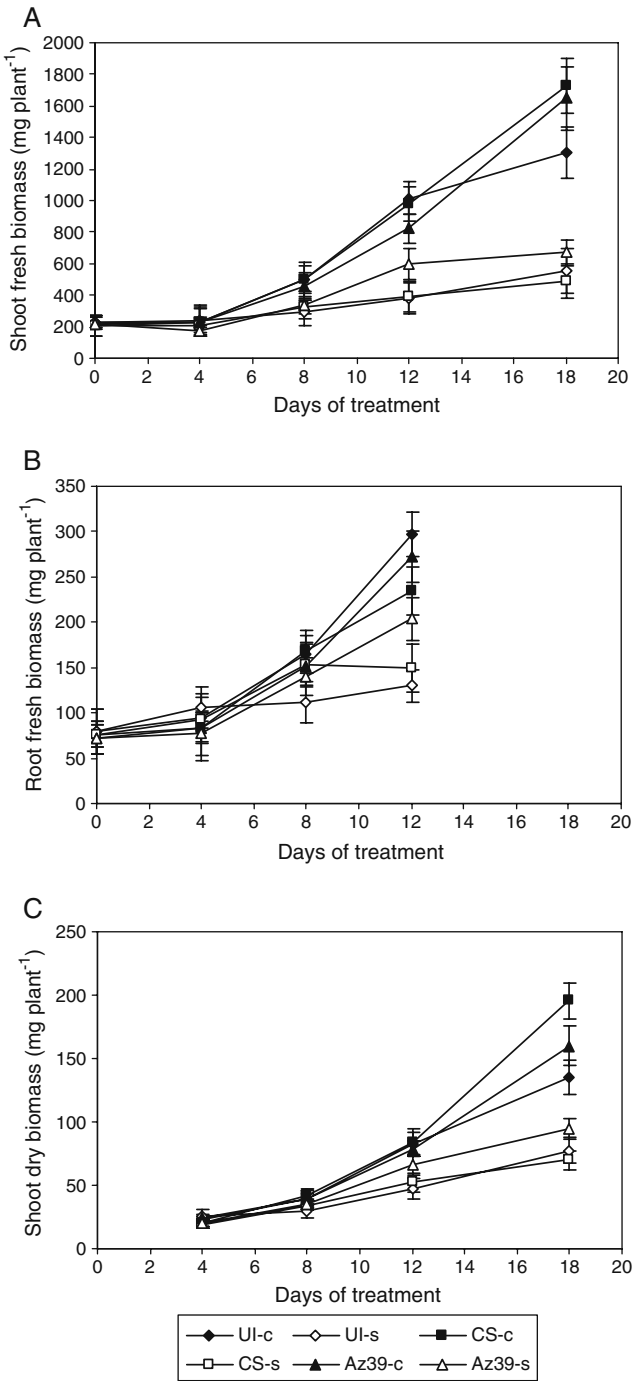

Fig. 1 Biomass of control (*filled symbols*) and saline-stressed (*open symbols*) barley plants uninoculated or inoculated with *A. brasilense*. Plants were grown as described in the "Materials and methods" section. **a** Shoot fresh weight, **b** root fresh weight, **c** shoot dry weight. *UI* uninoculated control, *CS* plants inoculated with a commercial strain of *A. brasilense*, *Az39* plants inoculated with Az39 strain, *c* control plants, *s* salt-treated plants. Data are means ± SEM

and CS-s plants. By day 12 and 18 of NaCl treatment, significant decreases with respect to their corresponding unstressed controls were detected for UI-s and CS-s plants, but not for Az39-s.

Na^+ and K^+ contents of barley shoots were also investigated (Table 1). Na^+ significantly increased while K^+ significantly decreased in salt-stressed plants with respect to untreated ones, irrespective of the inoculation treatments.

Shoot water contents showed no significant differences between inoculated or non-inoculated treatments. All unstressed plants had a mean of 90% humidity at any sampling time. Salt-stressed plants began to show a slight and non-significant water content reduction by day 8 of salt treatment, with a mean water content of 89% at day 8, of 87.5–88% at day 12 (insignificant), and of 85.5–86.5% at day 18 (significant), irrespective of their inoculation status. Chlorophyll contents did not show any significant difference all along the experiment (data not shown). *Azospirillum* counts in inoculated plants ranged between 8.0×10^3 and 2.5×10^4 cfu g^{-1} without any significant difference on comparing strains, treatments, or sampling times (data not shown).

Polyamines in barley roots and in *Azospirillum* liquid cultures

Figure 2 shows polyamine patterns along the time of the experiment. Although the differences in Spm and Spd levels among treatments were not significant, root Put levels diminished and reached their lowest levels by day 4 in salt-treated plants and by day 8 in control plants. From this time onwards, root Put levels showed different patterns among treatments. By days 12 and 18, Put contents in the roots of uninoculated salt-treated plants were significantly greater than those observed for Az39-s inoculated plants. No cadaverine was found in barley roots at any sampling time.

Polyamine release by *Azospirillum* strains in NFb liquid medium containing 0.3 g l^{-1} of NH_4Cl was investigated in the presence or absence of 200 mM NaCl. Putrescine was the most abundant polyamine synthesized by strain Az39, irrespective of the presence of 200 mM NaCl in the culture medium; this production was approximately tenfold greater than that observed in the strain isolated from the commercial biofertilizer (Fig. 3). Spermidine and spermine were far less abundant in both strain cultures. Salt addition slightly reduced all PAS synthesis in strain Az39, with a significant drop only for Spd. For the other strain, no significant changes in PAS patterns were detected due to salt addition to the culture media; however, Spm was detectable only in salt-amended flasks. Cadaverine was not found under these experimental conditions.

Transcripts of the root aquaporin gene *HvPIP2;1*

We detected that, before salt treatment, uninoculated plants did not express *HvPIP2;1*, a barley gene involved in the

Table 1 Plant height and ionic balance for uninoculated and *Azospirillum*-inoculated barley seedlings after different times of salt treatment

	UI-c	UI-s	CS-c	CS-s	Az39-c	Az39-s
Height (cm)						
Day 4	14.9±2.6	12.8±1.0	12.4±1.7	15.3±3.6	14.0±1.4	12.3±1.9
Day 8	24.6±1.4	19.1±2.3	25.2±2.0	20.6±4.0	23.6±3.0	20.6±3.8
Day 12	34.9±3.2	22.5±1.9*	33.6±3.7	22.2±1.9*	32.6±4.5	28.4±2.8
Day 18	36.8±2.8	27.1±2.0*	37.5±2.4	26.0±2.4*	34.4±3.1	31.5±2.9
Na^+ (mg g^{-1} dw)						
Day 4+8	0.55±0.06	8.40±0.75*	0.48±0.03	9.73±0.45*	0.77±0.02	9.82±0.65*
Day 12	0.86±0.09	9.80±0.64*	0.16±0.02	9.47±0.89*	0.35±0.05	10.11±0.86*
Day 18	0.26±0.04	10.44±0.76*	0.16±0.03	10.45±0.73*	0.32±0.04	12.41±0.92*
K^+ (mg g^{-1} dw)						
Day 4+8[a]	23.31±1.22	14.32±0.84*	26.04±2.45	17.07±2.03*	24.57±1.55	17.07±1.76*
Day 12	18.52±1.03	12.20±0.98*	24.70±1.99	10.81±1.33*	21.48±1.20	13.58±1.34*
Day 18	18.47±2.01	10.80±1.15*	24.29±2.19	12.97±1.56*	22.45±1.81	11.43±0.84*

Asterisks indicate significant differences with respect to the corresponding unstressed control

UI uninoculated control, *CS* plants inoculated with a commercial strain of *A. brasilense*, *Az39* plants inoculated with Az39 strain, *c* control plants, *s* salt-treated plants, *dw* dry weight

[a] Plants harvested at day 4 were gathered with plants harvested at day 8 and processed together for K^+ and Na^+ determinations

synthesis of the most abundant root aquaporin. Plants inoculated with the strain isolated from the commercial biofertilizer had moderate expression and those inoculated with strain Az39 showed the maximum expression (Fig. 4a). Salt treatment triggered the transcription of this gene in uninoculated plants, which sustained a moderate level of expression until the end of the experiment (Fig. 4c). Inoculated plants exposed to 200 mM NaCl kept their initial levels of *HvPIP2;1* transcripts practically unchanged until day 8, with a significant increase thereafter (Fig. 4c). After 12 days of salt treatment, expression of *HvPIP2;1* in the roots of Az39-s barley plants doubled that of UI-s plants and was approximately 33% greater than that of CS-s. In the roots of inoculated plants not exposed to salt, this gene was significantly less expressed; however, transcript abundance of *HvPIP2;1* in plants inoculated with strain Az39 was always above that observed in plants inoculated with the commercial strain (Fig. 4b, c).

Discussion

During the last decade, several authors have reported mitigating effects of *Azospirillum* on different plant species growing under salt, drought, and osmotic stresses (Dimkpa et al. 2009 and references therein). The underlying mechanisms to account for these effects, however, are just beginning to be unraveled.

It is well known that reduction in growth rates is one of the earliest responses of plants to salt stress (Parida and Das 2005; Munns and Tester 2008). Although barley is considered a salt-tolerant species (Munns et al. 1988), we found a significant growth reduction after 18 days of 200 mM NaCl treatment. However, plants previously inoculated with the strain Az39 of *A. brasilense* were less affected than uninoculated plants. The mitigating effect displayed by this strain was demonstrated by greater shoot and root biomasses at final sampling times as compared to uninoculated plants subjected to the same stress. The greater root development of those plants, involving better water and mineral uptake, may be, at least in part, a consequence of phytohormone production by *Azospirillum*. AIA and other phytohormones were detected in liquid cultures of *A. brasilense* strain Az39 (Perrig et al. 2007).

Populations of *Azospirillum* in inoculated barley roots estimated by the MPN method were slightly below those reported by some authors in inoculation experiments involving other plant species (Ribaudo et al. 2006; Cassan et al. 2009). To our knowledge, there are no reports in which survival of *Azospirillum* cells in barley roots had been evaluated; in fact, few data on *Azospirillum* inoculation in barley plants are available (Dalla Santa et al. 2004).

Hamdia et al. (2004) observed that *Azospirillum* inoculation markedly altered the selectivity for ion uptake in maize plants exposed to NaCl, restricting Na^+ uptake and enhancing K^+ uptake and translocation, especially in the most sensitive cultivar assayed. In barley, we did not observe any significant difference between uninoculated and inoculated salt-stressed plants regarding Na^+ and K^+ shoot contents along the experiment. It may be noticed that

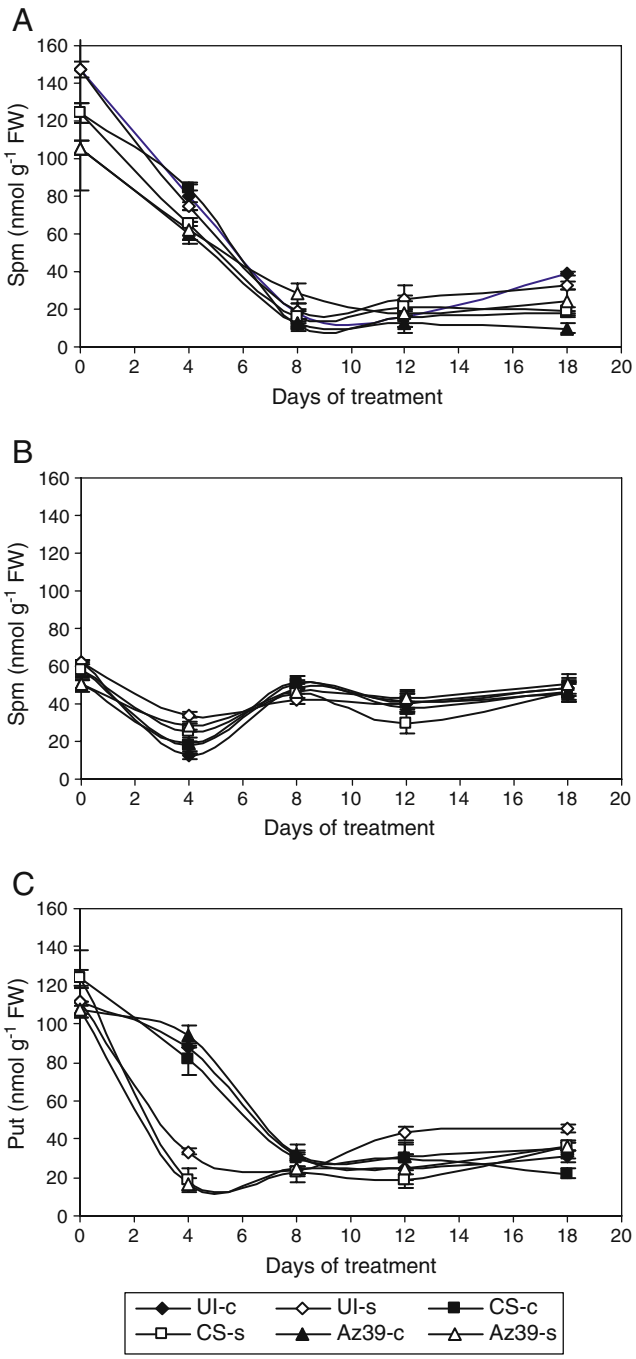

Fig. 2 Root polyamine content of control (*filled symbols*) and saline-stressed (*open symbols*) barley plants uninoculated or inoculated with *A. brasilense*. Plants were grown as described in the "Materials and methods" section. **a** Spermine, **b** spermidine, **c** putrescine. *UI* uninoculated control, *CS* plants inoculated with a commercial strain of *A. brasilense*, *Az39* plants inoculated with Az39 strain, *c* control plants, *s* salt-treated plants. Data are means ± SEM

salt addition led to an enhancement of Na^+ and to a reduction of K^+ in aerial tissues of all plants. This response to NaCl stress is widely documented in the literature (Hasegawa et al. 2000; Viégas et al. 2001; Taleisnik and Grunberg 2006). Based on these results, we can say that the smaller decline in growth rate observed in barley plants inoculated with strain Az39 exposed to 200 mM NaCl was not the consequence of an active Na^+ exclusion mechanism or a lesser rate of Na^+ translocation to shoots. It is noticeable that unstressed plants inoculated with the commercial strain had higher translocation rates of K^+ to aerial parts and displayed higher accumulation of dry matter compared to plants inoculated with strain Az39 and to uninoculated plants.

Polyamine concentrations in barley roots seem to have been affected by developmental stage rather than by the presence of *Azospirillum* or the addition of NaCl to the nutrient solution, and no relationship between PAS levels in *Azospirillum* liquid cultures and PAS levels in the roots of inoculated plants was found.

It is known that PAS are involved in plant responses against several environmental stress conditions. Under salt stress, PAS levels increased in mung bean, rice, maize, and sorghum (Friedman et al. 1989; Krishnamurthy and Bhagwat 1989; Rodriguez-Kessler et al. 2006). It was recently reported that after 3 weeks of salt treatment only the most sensitive of two barley cultivars showed elevated putrescine levels, consistent with the suggestions that Put accumulation correlated with slower growth and/or necrosis rather than being an adaptive response to salinity (Widodo et al. 2009). In line with this report, we observed that putrescine was the sole polyamine which had significantly increased in uninoculated plants—the most affected by NaCl treatment under our experimental conditions—and in plants inoculated with the commercial strain, but not in those inoculated with Az39. This result is in agreement with the observations that elevated contents of Put are related with plant toxicity and not with plant tolerance

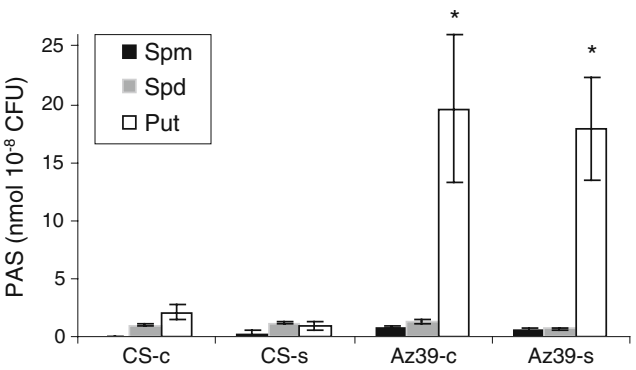

Fig. 3 Polyamine content in *Azospirillum* liquid cultures grown in NFb medium containing 0.3 gl^{-1} of NH_4Cl with or without the addition of 200 mM NaCl, as described in the "Materials and methods" section. *CS* commercial strain of *A. brasilense*, *Az39* Az39 strain, *c* control culture, *s* salt-amended culture. Data are means ± SEM. *Asterisks* indicate significant differences according to post hoc Tukey's multiple range test ($P<0.05$)

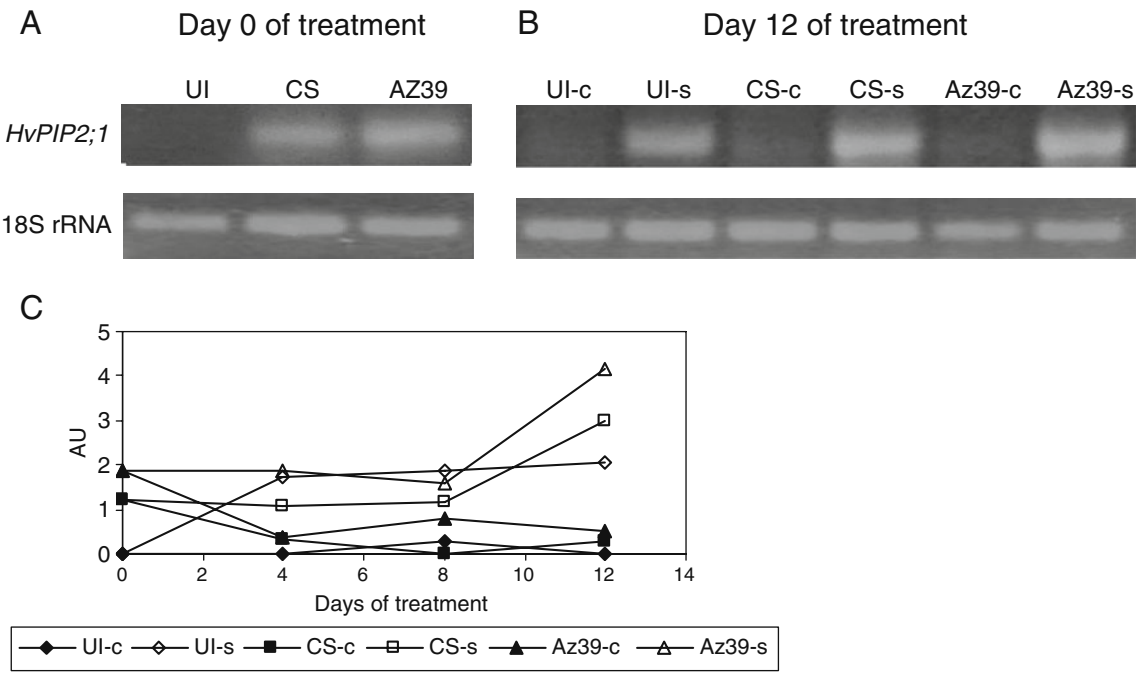

Fig. 4 Expression of *HvPIP2;1* gene. Transcript levels of *HvPIP2;1* in the roots of uninoculated or *A. brasilense*-inoculated barley plants: **a** before salt treatment, **b** at day 12 of salt treatment (gels shown are representative of three replicates). **c** Changes in *HvPIP2;1* transcript amounts along time. RT-PCR was performed as described in the "Materials and methods" section. Data are expressed as arbitrary units (AU) assuming control value equal to 1 based on absolute integrated optical densities. *UI* uninoculated control, *CS* plants inoculated with a commercial strain of *A. brasilense*, *Az39* plants inoculated with Az39 strain, *c* control plants, *s* salt-treated plants

against different abiotic stresses (Alcázar et al. 2006; Groppa and Benavides 2008). In our experiment, the strain Az39 of *Azospirillum* would be mitigating the effect of salt stress on barley plants by preventing putrescine rise.

In recent years, there has been growing evidence that plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) may be important for plants to cope with abiotic stresses. However, little is known about the possible influence of these microorganisms on key components of plant salt tolerance, such as root hydraulic conductance (*L*) and aquaporin regulation. Regulation of root aquaporin genes of the PIP family in the symbiosis of *Phaseolus vulgaris* (Aroca et al. 2007) and *Lactuca sativa* (Alguacil et al. 2009) with the AMF *Glomus intraradices* has been already reported. In this last report, the effect of the PGPR *Pseudomonas mendocina* was also assessed, and it was found that this rhizobacterium improved relative water content and helped lettuce plants to alleviate drought stress. This was not related to an increased *PIP2* gene expression, as it was observed when plants were inoculated with *G. intraradices*. Nevertheless, *PIP2* expression was significantly higher in *P. mendocina*-inoculated plants under non-stressed conditions than in uninoculated controls. Likewise, maize plants inoculated with a *Bacillus megaterium* strain, previously characterized as a PGPR, were found to exhibit higher *L* values under both unstressed and salt-stressed conditions. These higher *L* values in inoculated plants correlated with higher plasma membrane type two (PIP2) aquaporin amount in their roots under salt-stressed conditions (Marulanda et al. 2010). In line with these findings, we found that inoculation of barley with both strains of *A. brasilense* triggered the transcription of the barley root aquaporin *HvPIP2;1*. In unstressed plants, this gene induction was only observed at the first sampling time; therefore, a transient effect resulting from *Azospirillum* establishment in barley roots is suspected to be involved. The effect of salt treatment on aquaporin expression is a controversial issue. In several plant species, down-regulation of aquaporins expression is the principal cause of salt-induced reduction in hydraulic conductivity (Martinez-Ballesta et al. 2003). On the other hand, Katsuhara et al. (2002) observed that 200 mM NaCl added to hydroponic grown barley plants produced down-regulation of *HvPIP2;1*, and this was associated to a complete inhibition of root growth. However, with 100 mM NaCl, no reduction in root elongation and a slight increase in *HvPIP2;1* gene expression was observed (Katsuhara and Shibasaka 2007). In agreement with this last result, we observed upregulation of *HvPIP2;1* in barley roots under chronic salt stress. This response was more pronounced in inoculated plants and may be partly responsible for the mitigating effect of *Azospirillum*.

To sum up, our results suggest that barley plants inoculated with *A. brasilense* may be better shaped to thrive under saline conditions. The ability of a given *Azospirillum* strain to mitigate NaCl stress seems to partly rely on their particular metabolic properties. As far as we know, this is the first report to inform an impact of *Azospirillum* inoculation on the expression of *PIP2;1*, a gene involved in the synthesis of root water channels.

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