



Inhibition of ethylene synthesis reduces salt-tolerance in tomato wild relative species *Solanum chilense*



Emna Gharbi^{a,c}, Juan-Pablo Martínez^b, Hela Benahmed^c, Gilles Lepoint^d,
Brigitte Vanpee^a, Muriel Quinet^a, Stanley Lutts^{a,*}

^a Groupe de Recherche en Physiologie végétale (GRPV), Earth and Life Institute – Agronomy (ELI-A), Université catholique de Louvain, Louvain-la-Neuve, Belgium

^b Instituto de Investigaciones Agropecuarias (INIA – La Cruz), La Cruz, Chile

^c Laboratoire d'Ecologie végétale, Faculté des Sciences, Université de Tunis El Manar, Tunisie

^d Laboratoire d'Océanologie, MARE Center, Université de Liège, Belgium

ARTICLE INFO

Article history:

Received 8 September 2016

Received in revised form

30 November 2016

Accepted 1 December 2016

Available online 7 December 2016

Keywords:

Ethylene

Halophyte

Salinity

Solanum chilense

Stomatal conductance

Tomato

ABSTRACT

Exposure to salinity induces a burst in ethylene synthesis in the wild tomato halophyte plant species *Solanum chilense*. In order to gain information on the role of ethylene in salt adaptation, plants of *Solanum chilense* (accession LA4107) and of cultivated glycophyte *Solanum lycopersicum* (cv. Ailsa Craig) were cultivated for 7 days in nutrient solution containing 0 or 125 mM NaCl in the presence or absence of the inhibitor of ethylene synthesis (aminovinylglycine (AVG) 2 μM). Salt-induced ethylene synthesis in *S. chilense* occurred concomitantly with an increase in stomatal conductance, an efficient osmotic adjustment and the maintenance of carbon isotope discrimination value ($\Delta^{13}\text{C}$). In contrast, in *S. lycopersicum*, salt stress decreased stomatal conductance and $\Delta^{13}\text{C}$ values while osmotic potential remained higher than in *S. chilense*. Inhibition of stress-induced ethylene synthesis by AVG decreased stomatal conductance and $\Delta^{13}\text{C}$ in *S. chilense* and compromised osmotic adjustment. *Solanum chilense* behaved as an includer and accumulated high amounts of Na in the shoot but remained able to maintain K nutrition in the presence of NaCl. This species however did not stimulate the expression of genes coding for high-affinity K transport but genes coding for ethylene responsive factor *ERF5* and *JREF1* were constitutively more expressed in *S. chilense* than in *S. lycopersicum*. It is concluded that ethylene plays a key role in salt tolerance of *S. chilense*.

© 2016 Published by Elsevier GmbH.

1. Introduction

Soil salinization is a major environmental stress affecting plant productivity. Salinity can cause nutritional imbalance, ion toxicity, water deficit and oxidative damage in plants (Munns 2002; James et al., 2011). Tomato (*Solanum lycopersicum*) is one of the most important crops in the world but is considered to be salt-sensitive and encounters drastic yield decrease in response to NaCl (Chen et al., 2015).

Some plants, however, are able to cope with high levels of NaCl in the surrounding media and for this purposes induce different mechanisms allowing adaptation against the stress (Ruan et al., 2010). Analysis of wild-related halophytes species can be consid-

ered as a useful tool to improve the salt tolerance of cultivated plant species (Munns, 2005). Several halophyte plant species are available within the genus *Solanum* and could be considered as a valuable source of salt tolerance genes for transfer to the cultivated tomato (Albacete et al., 2009; Fischer et al., 2013). In its natural environment (salt desert of Atacama, Chile), the wild tomato species *Solanum chilense* is frequently exposed to extreme temperatures, drought, and high salt concentrations in the soil (Chetelat et al., 2009; Nakazato et al., 2010). This species is considered as a good model to study adaptation to biotic stress and as a source of genes coding for resistance to several pathogens such as *Topovirus* species and *Pseudomonas syringae* (Dianese et al., 2011; Thapa et al., 2015). It also displays a promising behavior in relation to drought tolerance (Fischer et al., 2013; Tapia et al., 2016). Considering its halophytic nature, salinity resistance in *S. chilense* also recently started to be studied. Martínez et al. (2014) reported that salt-resistance in *S. chilense* can be related to the optimal management of the water and the oxidative status compared with *S. lycopersicum*.

* Corresponding author at: Groupe de Recherche en Physiologie végétale, Earth and Life Institute, Université catholique de Louvain, 5 (Bte 7.07.13) Place Croix du Sud, 1348, Louvain-la-Neuve, Belgium.

E-mail address: stanley.lutts@uclouvain.be (S. Lutts).

Our previous results confirmed that *S. lycopersicum* and *S. chilense* exhibited contrasting levels of salt-resistance. In *S. chilense*, salinity induces a marked burst in ethylene synthesis occurring concomitantly with ACCS2 gene induction (Gharbi et al., 2016). These data are contrasting with those obtained in the cultivated glycophyte *S. lycopersicum*. Indeed, Albacete et al. (2008) and Ghanem et al. (2008) demonstrated that the accumulation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) may explain the onset of salt-induced oxidative damage in the leaves of cultivated tomato preceding massive Na⁺ accumulation.

Ethylene involvement in salinity resistance is highly complex and still remains unclear. Although ethylene is commonly considered as a senescing stress hormone, some recent data also suggest that it may have a dual impact in salt resistance (Amjad et al., 2014; Kazan, 2015; Pan et al., 2016). Ethylene was reported to induce stomatal closure through NADPH oxidase activation leading to H₂O₂ in the guard cells (Desikan et al., 2006). According to Ge et al. (2015), ethylene-induced stomatal closure is mediated through the heterotrimeric G protein G α subunit GPA1. In contrast, Tanaka et al. (2005) reported that ethylene inhibits abscisic acid-induced stomatal closure in Arabidopsis. Benlloch-González et al. (2010) provided evidences that K⁺ starvation inhibits water-stress-induced stomatal closure via ethylene synthesis in sunflower plants.

Potassium deficiency is commonly observed as a consequence of salt stress in plants but Jiang et al. (2013) reported that in Arabidopsis, ethylene promotes soil-salinity tolerance via an improved Na/K homeostasis associated with elevated *HIGH-AFFINITY K⁺ transporter5* (*HAK5*) transcripts levels. Besides *HAK5*, the inward-rectifier Shaker K⁺ channel *AKT1* also mediates K⁺ uptake and the expression of the corresponding gene is mainly detected in the root epidermal cells (Alemán et al., 2011).

Gharbi et al. (2016) demonstrated that leaf sodium concentration of salt-treated plants was higher in *S. chilense* than in *S. lycopersicum*, while an opposite trend was recorded for the roots. According to this study, salicylic acid (SA) may positively influence K⁺ nutrition in salt-treated plants. Exogenous SA however decreased ethylene production in *S. chilense* but increased polyamine (PA) content. Polyamine and ethylene share a common precursor (*S*-adenosylmethionine) and the two corresponding biosynthetic pathways are therefore often considered to be competitive (Lutts et al., 2013). Polyamines are thought to assume key functions in mineral nutrition of salt-stressed plants and a specific interaction of PAs with several types of cation channels may contribute to salt stress resistance (Pandolfi et al., 2010). Both salicylic acid and PAs should thus be regarded as important determinants of salinity resistance in *S. chilense* but the influence of ethylene on their synthesis still requires further investigations.

Beside ethylene synthesis itself, transduction through ethylene responsive factors (ERFs) may also assume crucial functions in the tomato plant response to NaCl stress. Zhang et al. (2004) demonstrated that the ethylene- and NaCl responsive tomato transcription factor *JERF1* modulates expression of GCC box-containing genes and salt resistance in tobacco. Similarly, ectopic overexpression of tomato *JERF3* in tobacco activates downstream gene expression and enhances salt resistance (Wang et al., 2004) while *ERF5* is promoting adaptation to drought and salt tolerance in tomato (Pan et al., 2012). By contrast, mutants deficient for ethylene responses display salt hypersensitivity at different developmental stages (Achard et al., 2006; Lei et al., 2011).

The present work was undertaken in order to confirm the putative involvement of ethylene in salinity resistance exhibited by the halophyte wild tomato *Solanum chilense*. Since no mutants impaired in ethylene synthesis are available in this species, the potent inhibitor of ethylene production aminovinylglycine (AVG), which inhibits ACC synthase (Amrhein and Wenker, 1979) was applied on plants cultivated in the absence or presence of salt.

Stomatal conductance and water use efficiency estimated through carbon isotope discrimination were analyzed in relation to mineral nutrition, endogenous SA and PAs concentration and expression of genes coding for ethylene responsive factors.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of tomato *Solanum lycopersicum* L. cv. Ailsa Craig (TGRC accession number LA2838A) and of the wild species *Solanum chilense* (TGRC accession number LA4107) were obtained from the Tomato Genetics Resource Center (University of California, Davis, USA). Seeds were germinated in peat compost in a germination chamber at 25 °C. Sixteen-day-old seedlings of the two species were transferred to a hydroponic culture system into a growth chamber at 24 °C/22 °C under a 16 h day/8 h night period. Light intensity was 245 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by fluorescent lamps (Master TL-D reflex Super 80 58W/840 from Philips) and relative humidity was maintained at 70 \pm 5%. Seedlings were fixed on polystyrene plates floating on aerated half-strength Hoagland nutrient solution containing (in mM): 5 KNO₃, 5.5 Ca(NO₃)₂, 1 NH₄H₂PO₄, 0.5 MgSO₄, and (in μM) 25 KCl, 10H₃BO₄, 1 MnSO₄, 0.25 CuSO₄, 1 ZnSO₄, 10 (NH₄)₆Mo₇O and 1.87 g L⁻¹ Fe-EDTA. Solution was renewed every week and pH was adjusted daily to 5.5–6 using 5 M KOH. For each treatment, seedlings were distributed among four tanks (six seedlings per tank) containing 1.5 L of solution in a complete randomized block design. After 7 days of acclimatization in control conditions the seedlings were randomly divided into four groups: (1) control: plants grown in half-strength Hoagland solution, (2) NaCl: plants grown in half-strength Hoagland solution containing 125 mM NaCl, (3) AVG: plants grown in half-strength Hoagland solution containing 2 μM AVG, (4) NaCl + AVG: plants grown in half-strength Hoagland solution containing 125 mM NaCl + 2 μM AVG. Two actively growing leaves, present at the moment of treatment application (leaf number 3 and 4, numbering from the base of the plant) were tagged for subsequent physiological measurements and biochemical analysis. After 7 days of treatment, the plants (30 days old) were harvested and divided into roots and leaves for physiological and biochemical parameter determinations.

2.2. Plant growth, water content, and osmotic potential

Plant growth was determined on the basis of the shoot and root dry weight (DW) per plant (estimated on 6 individual plants per treatment). Roots of harvested plants were quickly rinsed in deionised water, blotted dry and weighed for fresh weight (FW) determination. For dry weight determination, roots and shoots were incubated in an oven at 70 °C for 72 h. Water content (WC) was calculated as WC = (FW – DW)/FW \times 100. To determine leaf osmotic potential (Ψ_s), leaves were cut into small pieces and immediately frozen into a perforated Eppendorf tube. After two thaw-freezing cycles, sap was collected after 15 min of centrifugation (15,000g) at 4 °C. Leaf and root osmotic potential was estimated on the extracted sap using a Wescor 5500 vapour pressure osmometer as previously detailed (Lutts et al., 1999).

2.3. Gas-exchange parameters, stomatal conductance and carbon isotope discrimination

The instantaneous CO₂ assimilation under ambient conditions (400 ppm CO₂) (*A*), the instantaneous transpiration (*E*) and inter-cellular CO₂ concentration (*C_i*) were taken from the fourth fully expanded leaf of six plants per treatment using an infrared gas analyser (LCA4 8.7 ADC, Bioscience, Hertfordshire, UK). The gas exchange was measured using a Parkinson leaf cuvette, on intact

leaves for 1 min (20 records min^{-1}) with an air flow of 300 mL min^{-1} . Leaf stomatal conductance (g_s) was measured on the fourth fully expanded leaf on 6 plants per treatment using an AP4 diffusion porometer (Delta-TDevices Ltd., Cambridge, UK). All measurements were performed on leaf 4 between 2 p.m. and 4 p.m.

For carbon isotope discrimination, approximately 5 mg of tissue for each sample were ground to a fine powder. Measurements were performed using an IsoPrime100 isotope ratio mass spectrometer (Isoprime, UK) coupled to a vario MICRO cube C-N-S elemental analyzer (Elementar Analysensysteme GMBH, Germany) for sample transformation and automated analysis. Isotopic ratios were expressed using the δ notation ($\delta^{13}\text{C}$) (‰). Certified Reference Materials (CRM) was IAEA-C6 (sucrose; $\delta^{13}\text{C} = -10.8 \pm 0.5\%$; mean \pm SD). CRM is calibrated against the international references Vienna Pee Dee Belemnite. Standard deviations on replicate measurements of a randomly selected sample were inferior to 0.2‰. Carbon isotope discrimination ($\Delta^{13}\text{C}$) was calculated according to the formula of Farquhar and Richards (1984): $\Delta^{13}\text{C} = [(\delta_a - \delta_p)/(1 + \delta_p)] \times 10^3$ where δ_p is the $\delta^{13}\text{C}$ of the leaf sample and δ_a is the $\delta^{13}\text{C}$ of the atmospheric CO_2 (-8%).

2.4. Chlorophyll and malondialdehyde quantification

Chlorophyll a, b and carotenoid concentrations were measured on the fourth leaf from 6 plants per treatment using 100 mg FW of leaf material placed in prechilled mortar in the presence of 8 mL of acetone 80% (v/v). The absorbance of the extract was read using a Shimadzu UV-1800 spectrophotometer (Kyoto-Japan), and pigment contents were calculated according to Lichtenthaler (1987).

Malondialdehyde (MDA) was quantified in the leaves and the roots of 6 plants per treatment using the method of Heath and Packer (1968). Frozen 250 mg were homogenized in prechilled mortar with a solution of 0.5% TBA in 20% trichloroacetic acid (TCA) and were heated to 95 °C for 30 min and then samples were cooled at room temperature. After centrifugation at 3000 rpm for 5 min the absorbance of supernatant was read at 532 nm, and the values of the non-specific absorbance were taken at 600 nm and subtracted from the original (532 nm).

2.5. Determination of Na^+ , K^+

For Na^+ and K^+ quantification, the third leaf and root tissues of three plants per treatment were oven-dried at 70 °C for 3 days and 50 mg of DW were incubated in 4 mL of 35% HNO_3 at 80 °C. The residue was dissolved with aqua regia (HCl 37%: HNO_3 65% 3:1) and filtered (Whatman, 11 mm). Elements were quantified by flame atomic absorption spectrophotometry (ICE 3300; Thermo Scientific; Waltham, MA).

2.6. Ethylene, salicylic acid and polyamine quantification

The ethylene production was measured by ethylene detector ETD-300 (Sensor Sense, Nijmegen, The Netherlands). Leaves harvested on treated plants were placed in glass bottle dishes on two layers of filter paper moistened with 5 mL of water. As a control from the obtained emission rates, the levels of ethylene were measured in a cuvette containing moistened filter papers with 5 mL of water without leaves. Samples were treated with the method detailed by Cristescu et al. (2002). The measurements were conducted as described in Gharbi et al. (2016). Measurements were performed in triplicates; each replicate consisted of a pool of 3 leaves.

Free PAs were extracted and dansylated according to Quinet et al. (2014) from approximately 500 mg FW of shoots and 250 mg FW of roots. Samples were re-suspended in methanol, filtered (Chromafil PES-45/15, 0.45 μm ; Macherey-Nagel, Duren, Germany)

and injected onto a Nucleodur C18 Pyramid column (125 \times 4.6 mm internal diameter, 5 μm particle size; Macherey-Nagel) maintained at 40 °C. Analyses were performed by a Shimadzu HPLC system coupled to a RF-20A fluorescence detector (Shimadzu, 's-Hertogenbosch, The Netherlands) with an excitation wavelength of 340 nm and an emission wavelength of 510 nm. The mobile phase consisted of a water/acetonitrile gradient from 40 to 100% acetonitrile and the flow was 1.0 mL min^{-1} . For a given sample, quantifications were performed in triplicates as described by Gharbi et al. (2016).

Endogenous SA was extracted according to Molinari and Loffredo (2006). The procedure was modified as described by Gharbi et al. (2016). Salicylic acid quantification was performed on 6 independent samples by high performance liquid chromatography (HPLC) (5 μL of sample was injected). The system consisted of an Agilent 1260 series equipped with an automatic injector and a column (Inertsil ODS-3; 250 \times 3.0 mm, 3 μm) oven both thermostated at 30 °C. Salicylic acid was detected by a fluorescence detector at 315 nm emission and 408 nm excitation wavelengths. The mobile phase was a water/ACN gradient from 10 to 100% ACN and the flow was 1.0 mL min^{-1} . Quantification of SA was performed by external calibration using SA standards with concentrations from 0.78 to 100 μM .

2.7. Reverse transcription-PCR (RT-PCR)

Total RNA was isolated from 150 mg tomato leaves or roots and cDNA was synthesized using 1 μg of total RNA as described in Quinet et al. (2014). Expression of *ERF5*, *ERF3*, *JERF1* and *ACCS2* was analyzed in the leaves and the expression of *HAK5* and *AKT1* was investigated in the roots. Amplifications (33 cycles) were conducted using GoTaq DNA polymerase (Promega Benelux b.v., Leiden, The Netherlands). Primers and annealing temperature conditions were listed in Table S1. The PCR products were resolved on agarose gels and expression differences were analyzed by gel densitometry using ImageJ software and expressed as relative values compared to *EF1* α expression (Table S1, Gharbi et al., 2016). For a given sample, gene expression analyses were repeated three times and gave similar results.

2.8. Statistical treatment

For each treatment, 6 individual plants were considered. Normality distributions and homoscedasticity were verified using Shapiro-Wilk and Levene's tests respectively and data were transformed when required. Data were analyzed using two-way analysis of variance (ANOVA). When the ANOVA was significant at $P \leq 0.05$, differences between means were scored for significance according to Student-Newman-Keuls test. Data were analyzed using SAS Enterprise Guide 6.1 (SAS 9.4 system for windows). Two independent experiments were performed and provided similar trends. Results hereafter are from one experiment and are presented as means \pm standard errors.

3. Results

3.1. Plant growth and water status

Salinity reduced the shoot DW of the salt-sensitive *S. lycopersicum* while it significantly increased the shoot DW of the halophyte wild tomato species *S. chilense*. Application of the ethylene biosynthesis inhibitor (AVG) in the absence of salt reduced the shoot DW in *S. lycopersicum* but not in *S. chilense* compared to control plants. In contrast, application of AVG in salt-treated plants suppressed the salt-induced growth stimulation in *S. chilense*. (Fig. 1A). All treatments induced a significant decrease in root DW in *S. lycopersicum*

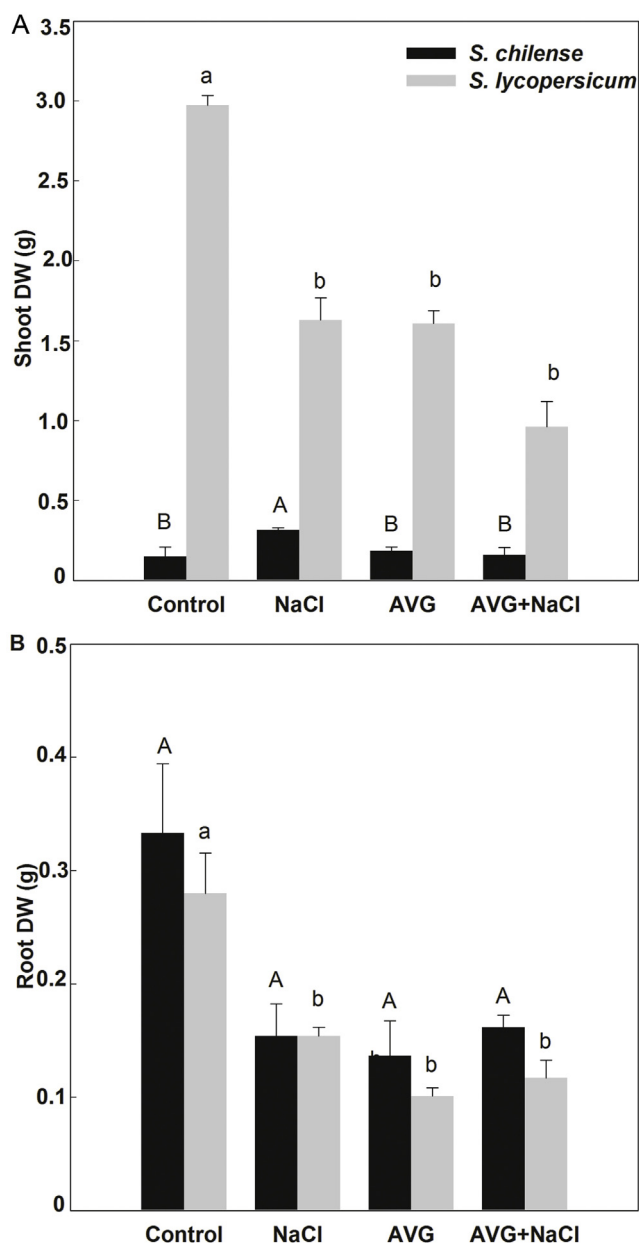


Fig. 1. Shoot (A) and root (B) dry weight of *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinyglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

(Fig. 1B) while the root DW remained statistically unaffected in *S. chilense* (Fig. 1B).

As shown in Table 1, leaf and root WC were not affected by salt stress in *S. chilense* and in *S. lycopersicum*. The AVG and AVG + NaCl treatment similarly had no impact on the leaf WC. Salinity reduced the leaf and root Ψ s to a higher extent in *S. chilense* than in *S. lycopersicum*. The addition of AVG to salt-containing solution had contrasting effect on Ψ s in the two species since it slightly increased it in *S. chilense* while it reduced it in *S. lycopersicum* for both shoots and roots.

3.2. Ethylene production, ACCS2 and ethylene responsive factor gene expression

Salinity had no impact on ethylene production in *S. lycopersicum* (Fig. 2A), while ethylene synthesis strongly increased in response

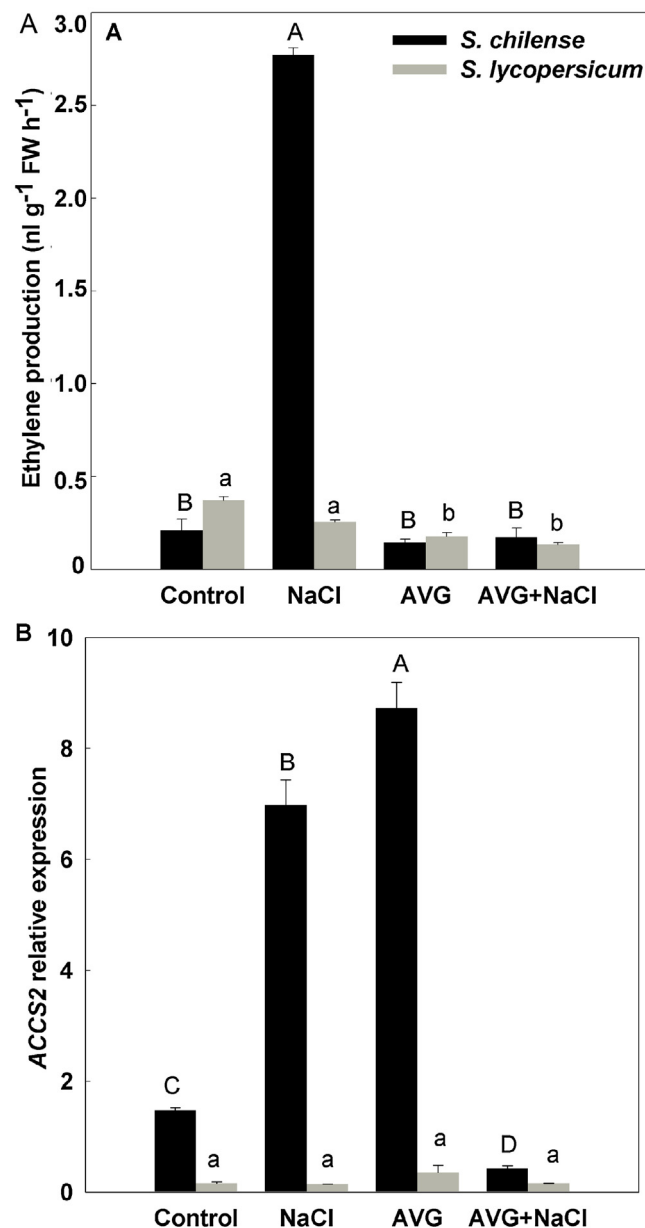


Fig. 2. Leaf ethylene production (A) and ACCS2 relative expression (B) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinyglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

to salt in *S. chilense*. In this species, salt-treated shoots produced up to 6 times more ethylene than controls. However, application of exogenous AVG alone or combined with NaCl strongly decreased the level of ethylene in *S. chilense* compared to salt stressed plants.

Relative expression of ACCS2, mainly involved in NaCl-induced synthesis in *S. chilense* (Gharbi et al., 2016) (Fig. 2B) remained always low in *S. lycopersicum*. In the absence of NaCl, this expression was higher in *S. chilense* than in *S. lycopersicum* and it was strongly increased in salt-treated plants of the halophyte plant species. Exogenous AVG slightly stimulated ACCS2 relative expression in *S. chilense* while the concomitant presence of AVG and NaCl completely abolished such ACCS2 expression in *S. chilense*.

As far as genes coding for Ethylene Responsive Factors are concerned (Fig. 3), *ERF5* was similarly expressed in the two species under control conditions but it then dropped to a low level in

Table 1
Water content (WC) and osmotic potential (Ψ_s) in the leaves and the roots of *Solanum chilense* and *Solanum lycopersicum* cv Ailsa Craig plants exposed during 7 in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinyglycine (AVG). Six different plants were considered for each treatment, and fourth leaves were pooled. Each value is thus the mean of 6 replicates \pm S.E. Values exhibiting different letters are significantly different at $P < 0.05$.

	Leaves		Roots	
	<i>S. chilense</i>	<i>S. lycopersicum</i>	<i>S. chilense</i>	<i>S. lycopersicum</i>
WC (%)				
Control	93.5 \pm 0.8 A	93.0 \pm 0.83 a	91.8 \pm 1.5 A	94.8 \pm 0.28 a
NaCl	94.8 \pm 2.4 A	92.9 \pm 1.2 a	94.7 \pm 1.7 A	94.7 \pm 0.33 a
AVG	91.2 \pm 0.64 A	93.1 \pm 1.2 a	94.4 \pm 0.16 A	96.9 \pm 0.42 a
AVG + NaCl	89.5 \pm 0.68 A	92.3 \pm 0.63 a	92.8 \pm 0.13 A	95.4 \pm 0.45 a
Ψ_s (MPa)				
Control	-0.90 \pm 0.02 D	-0.90 \pm 0.01 d	-0.73 \pm 0.004 D	-0.72 \pm 0.05 d
NaCl	-1.56 \pm 0.02 B	-1.32 \pm 0.002 b	-1.44 \pm 0.01 B	-1.25 \pm 0.009 b
AVG	-1.16 \pm 0.009 C	-1.13 \pm 0.01 c	-0.65 \pm 0.003 C	-0.84 \pm 0.004 c
AVG + NaCl	-1.46 \pm 0.003 A	-1.59 \pm 0.001 a	-1.39 \pm 0.01 A	-1.38 \pm 0.001 a

response to NaCl or to AVG in *S. lycopersicum* but not in *S. chilense*. In response to NaCl + AVG, however, *ERF5* expression was similar to control in *S. lycopersicum* and significantly decreased in *S. chilense*. A strong difference between the two considered species was recorded for *JERF1* whose expression was always higher in *S. chilense* than in *S. lycopersicum*. Salinity however decreased *JERF1* expression in *S. chilense* while AVG treatment increased it. No significant difference was recorded between the two considered species for *ERF3* expression which decreased in response to NaCl and AVG.

3.3. Sodium and potassium content

Sodium accumulated in response to salt stress in both species but to a significant higher extent in the shoot of *S. chilense* than in the shoot of *S. lycopersicum* (Fig. 4A). The application of AVG to salt-treated plants had no significant impact on the pattern of Na⁺ accumulation. In the presence of salt, the root Na⁺ concentration was higher in *S. lycopersicum* than in *S. chilense* but AVG abolished the difference between the two species (Fig. 4B).

The leaf potassium concentration (Fig. 4C) increased in response to NaCl in *S. chilense* while it decreased in *S. lycopersicum*. Exogenous application of AVG in the absence of salt increased the leaf K⁺ concentration in both species but the effect was more marked in *S. chilense* than in *S. lycopersicum*. In the presence of NaCl, however, AVG had the opposite effect and reduced the leaf K concentration in *S. chilense* but not in *S. lycopersicum* comparatively to salt-treated plants. The root K⁺ concentration (Fig. 4D) decreased in *S. lycopersicum* in response to NaCl and AVG had no impact on the root K⁺ concentration of salt treated plants. However, in *S. chilense* only AVG + NaCl treatment decreased root K⁺ compared to salt stressed plants.

3.4. Expression of genes coding for HAK5 and AKT1

Only a faint signal was detected for the high-affinity K⁺ transporter HAK5 transcripts in both species and its intensity was not modified by the various treatments (detailed data not shown). The expression of gene coding for the inward-rectifier K⁺ channel AKT1 was also investigated and its expression level depended on the species and the treatment. The AKT1 was expressed to a higher extent in *S. lycopersicum* than in *S. chilense* under control condition (Fig. 4E). Both NaCl and NaCl + AVG decreased *AKT1* transcript level in the root of *S. lycopersicum* compared to control plants. In the root of *S. chilense*, salt did not markedly affect *AKT1* expression compared to control plants (Fig. 4E), while exogenous AVG applied alone or in combination with NaCl increased the expression of *AKT1* compared with control and salt-stressed plants.

Table 2

Carbon isotope discrimination ($\Delta^{13}\text{C}$; in ‰) in the fourth leaf of *Solanum lycopersicum* and *S. chilense* exposed during 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinyglycine (AVG). Six different plants were considered for each treatment, and fourth leaves were pooled. Each value is the mean of 6 replicates \pm S.E. Values exhibiting different letters are significantly different at $P < 0.05$.

Treatment	<i>S. lycopersicum</i>	<i>S. chilense</i>
Control	25.78 \pm 0.05 a	25.62 \pm 0.04 a
NaCl	21.47 \pm 0.05 f	25.36 \pm 0.02 b
AVG	24.15 \pm 0.02 c	24.18 \pm 0.06 c
AVG + NaCl	23.18 \pm 0.04 d	22.64 \pm 0.03 e

3.5. Gas exchange parameters, stomatal conductance and carbon isotope discrimination

In *S. lycopersicum* salt stress decreased the net photosynthesis compared to control plants and the application of AVG under salt condition had a similar effect (Fig. 5A). In *S. chilense*, NaCl treatment had no significant impact on CO₂ assimilation compared to controls. The net CO₂ assimilation rate (A) remained unaffected by exposure to AVG + NaCl compared to salt stressed plants for both species. Salt stress also induced a decrease of instantaneous transpiration (E) comparatively to unstressed controls while AVG application under non saline condition increased E value in both species compared to salt stress condition (Fig. 5B). In contrast, AVG did not modify the transpiration rate of salt-treated plants. Instantaneous water use efficiency (WUE_i, defined as A/E) was higher in response to NaCl in both species compared to control (Fig. 5C) and application of AVG with NaCl decreased A/E in both species compared to salt stressed plants. Salt also decreased the intercellular CO₂ (Fig. 5D) in the two species compared to controls plant but the application of AVG reduced the impact of NaCl on this parameter.

In *S. lycopersicum* NaCl applied separately or with AVG decreased g_s by more than 93% compared to control plants (Fig. 5E). It is noteworthy that *S. chilense* displayed an opposite behavior and that salt stress strongly increased g_s values. Such an increase was completely abolished by the addition of AVG in the NaCl-containing solution.

Carbon isotope discrimination values ($\Delta^{13}\text{C}$; Table 2) were similar in the two species. However, $\Delta^{13}\text{C}$ dropped to low value in *S. lycopersicum* while only small differences were recorded between control and salt-treated plants in *S. chilense*. Exogenous application of AVG in the absence of salt also decreased $\Delta^{13}\text{C}$ which reached similar values in *S. lycopersicum* and *S. chilense*. It is noteworthy, however, that AVG application on salt-treated plants increased $\Delta^{13}\text{C}$ comparatively to plants exposed to NaCl in the absence of AVG in *S. lycopersicum* while it had an opposite effect in *S. chilense*. In this latter species, the lowest $\Delta^{13}\text{C}$ values were indeed recorded for NaCl + AVG-treated plants.

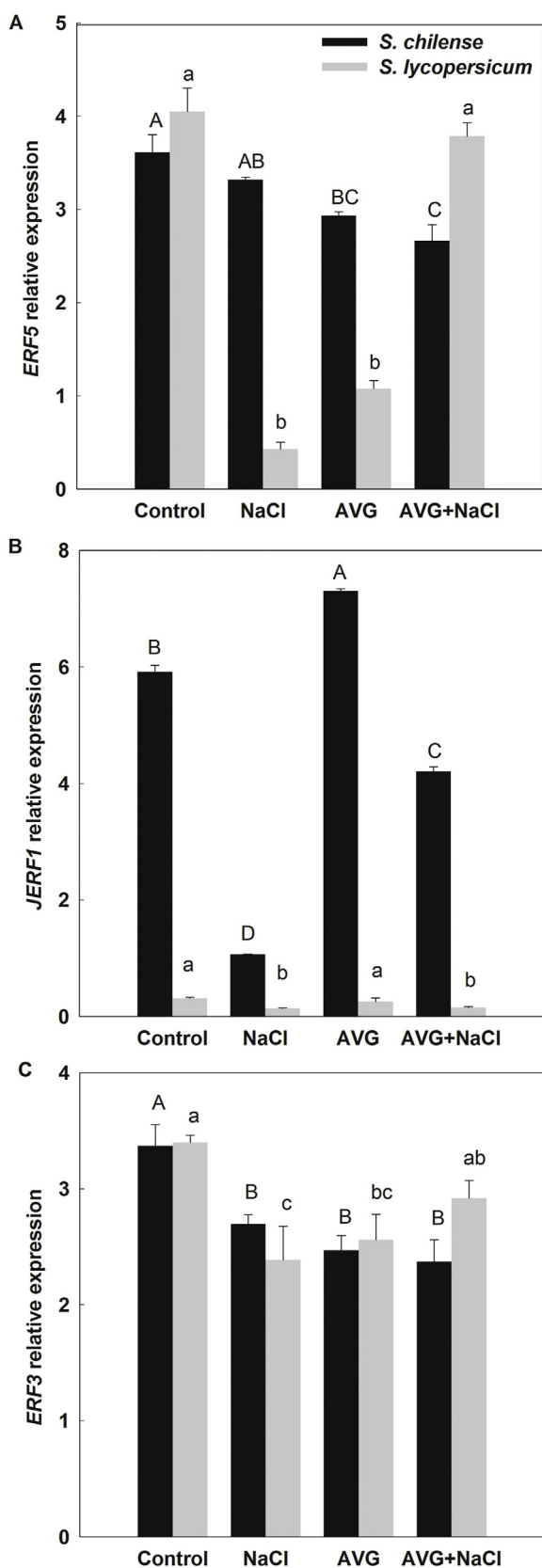


Fig. 3. Leaf relative expression of *ERF5* (A), *JRF1* (B) and *ERF3* (C) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinylglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

3.6. Chlorophyll and malondialdehyde content

Chlorophyll a (Chl a) increased in response to salt in *S. lycopersicum* and even more in response to AVG+NaCl application (Fig. 6A). In contrast, neither NaCl nor AVG had any impact on Chl a concentration in *S. chilense* (Fig. 6A). Chlorophyll b (Chl b) was not markedly affected by the treatments whatever the species (Fig. 6B).

The Chl a/Chl b ratio remained stable in response to NaCl and AVG+NaCl in both species (Fig. 6C). However in *S. lycopersicum* we noticed that application of AVG decreased Chl a/Chl b ratio compared with control plants. Carotenoids content decreased in response to NaCl compared to control in the salt sensitive species *S. lycopersicum* and AVG did not significantly abolished the negative impact of salt (Fig. 6D). In *S. chilense* salt slightly decreased the carotenoid content compared to control while AVG reduced this NaCl impact.

Salinity had no significant effect on leaf MDA content compared with control plant (Fig. 6E). Application of AVG in the absence of stress decreased MDA in *S. lycopersicum* but increased it in *S. chilense*. In the presence of NaCl, AVG increased leaf MDA content in both species. Salt stress decreased MDA in the root of *S. lycopersicum* (Fig. 6F) and application of AVG combined with NaCl increased MDA. In *S. chilense*, salt had no significant effect on root MDA content compared to control plant, while application of AVG and AVG+NaCl caused significant increase in MDA compared to salt stressed plants (Fig. 6F).

3.7. Polyamine and salicylic acid quantification

The leaf Put concentration was always higher in *S. chilense* than in *S. lycopersicum* (Fig. 7). It slightly decreased in response to salt stress in the former but not in the latter. Exogenous AVG application increased leaf and root Put concentration to a higher extent in the presence than in the absence of salt (Fig. 7A–B). The leaf Spd concentration was not affected by the treatments (Fig. 7C). The root Spd concentration was higher in *S. lycopersicum* than in *S. chilense* in control plants. Salt stress increased the root Spd (Fig. 7D) concentration in the halophyte *S. chilense* but decreased it in the glycophyte *S. lycopersicum*. Salt-induced increase in root Spd was completely abolished by AVG in *S. chilense*. Salt stress also clearly increased the leaf Spm in *S. chilense* but had no impact in *S. lycopersicum* (Fig. 7E). Exogenous application of AVG had no impact on this observed trend. The root Spm was in the same range for the two considered plant species and remained unaffected by the treatment (Fig. 7F).

The leaf SA concentration increased in response to NaCl in *S. chilense* but not in *S. lycopersicum* (Fig. 8A). Conversely, AVG increased leaf SA concentration in *S. lycopersicum* but not in *S. chilense*. Exposure to AVG abolished the NaCl-induced SA increase in *S. chilense*. Although NaCl did not increase root SA concentration in *S. chilense*, AVG significantly reduced SA concentration in the roots of this halophyte species exposed to the salt treatment (Fig. 8B).

4. Discussion

Improvement of salinity tolerance in tomato is a major goal for plant breeders. Interspecific crosses between cultivated *Solanum lycopersicum* and wild-relative halophytes may be considered as a promising option for this purpose. Fig. 9 is providing a comparative overview of the plant response exposed for 7 days to a moderate NaCl dose in *S. lycopersicum* and *S. chilense*. The present work confirms the halophytic nature of *Solanum chilense*. In this species, a moderate dose of salt indeed improved the shoot dry weight and had no deleterious impact on net photosynthesis in contrast

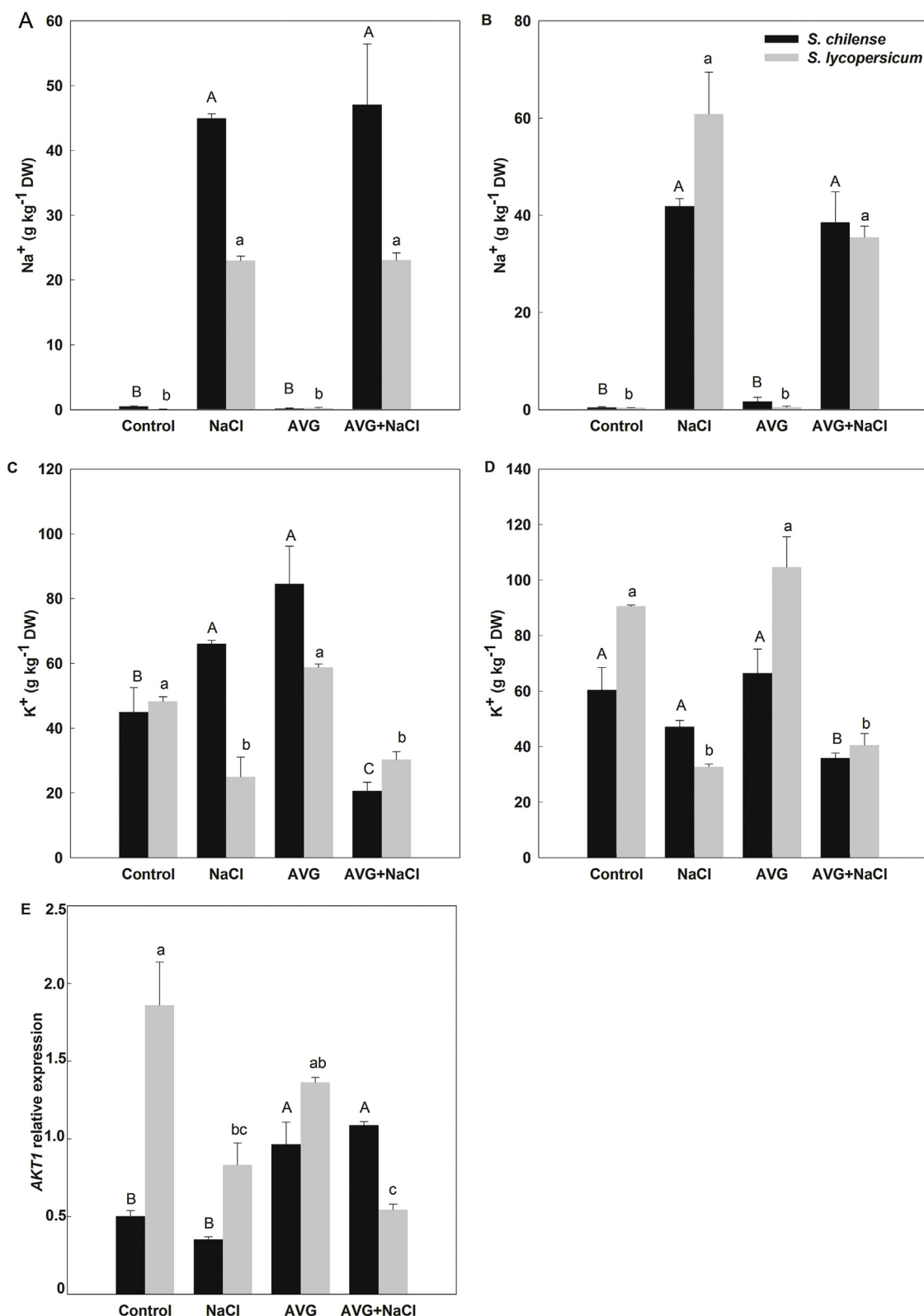


Fig. 4. Sodium concentration in shoots (A) and roots (B), potassium concentration in shoots (C) and roots (D) and AKT1 relative expression (E) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinyglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

to the domesticated *S. lycopersicum* (Fig. 9). Salt stress is considered to induce both an osmotic stress related to the decrease in water availability, and an ionic stress in relation to the accumulation of toxic Na⁺ and Cl⁻ ions (Munns, 2002). *Solanum chilense* was

able to efficiently perform osmotic adjustment when exposed to NaCl and the recorded Ψ_s values in salt-treated plants were lower in both roots and shoots of salt-treated plants of *S. chilense* than the values recorded for *S. lycopersicum*. *Solanum chilense* also typ-

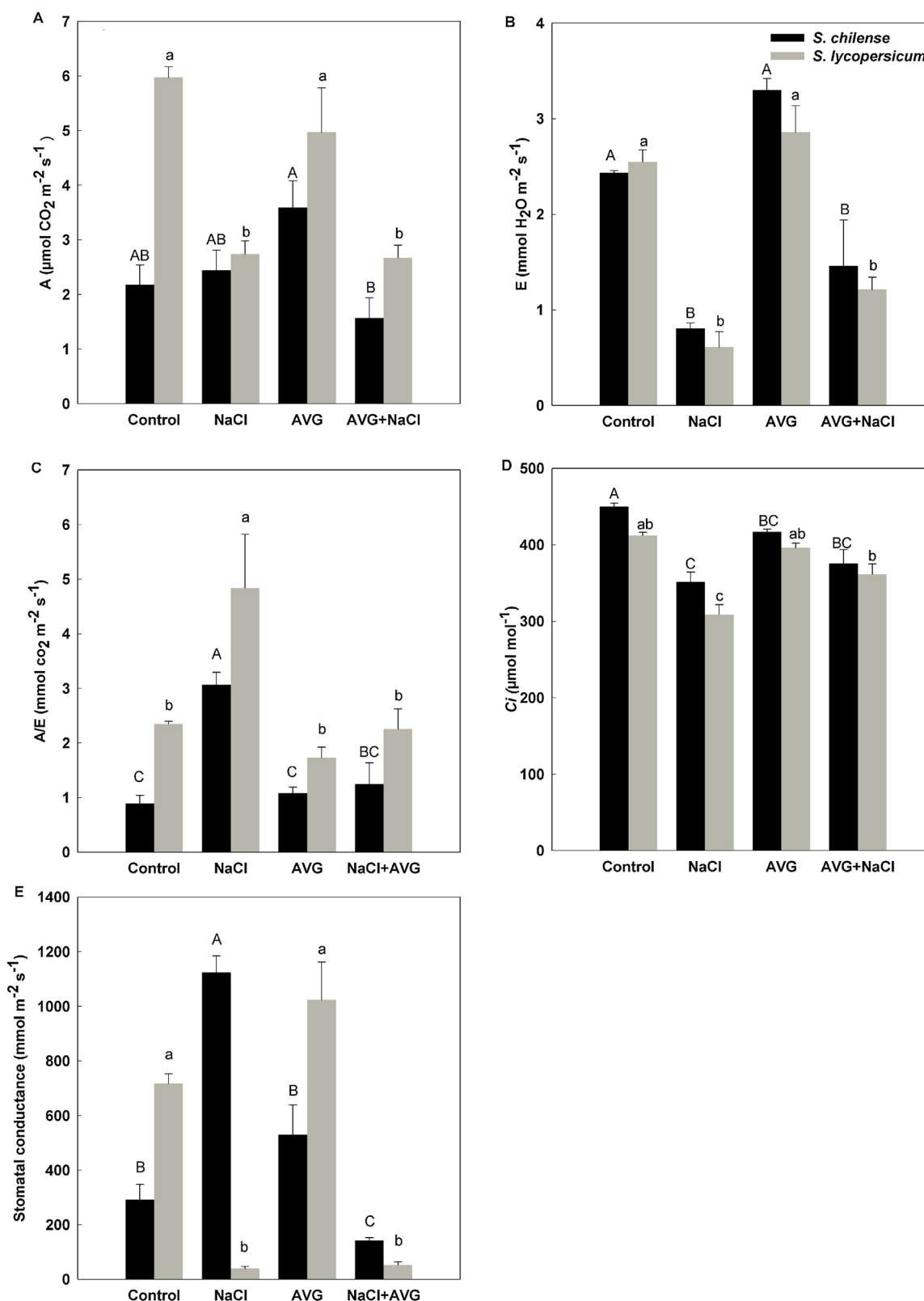


Fig. 5. Net photosynthesis (A; A), instantaneous transpiration (E; B), instantaneous water use efficiency (A/E; C), intercellular CO_2 concentration (C_i ; D) and stomatal conductance (g_s ; E) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μM aminovinylglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

ically behaves as an includer species and accumulates more Na^+ in the shoot parts than *S. lycopersicum*, suggesting that the halophyte species displayed interesting properties in terms of tolerance

to accumulated ions which could be, at least partly, related to its ability to also increase K absorption under NaCl conditions (Fig. 9). Although *Solanum chilense* produces small white fruits which are

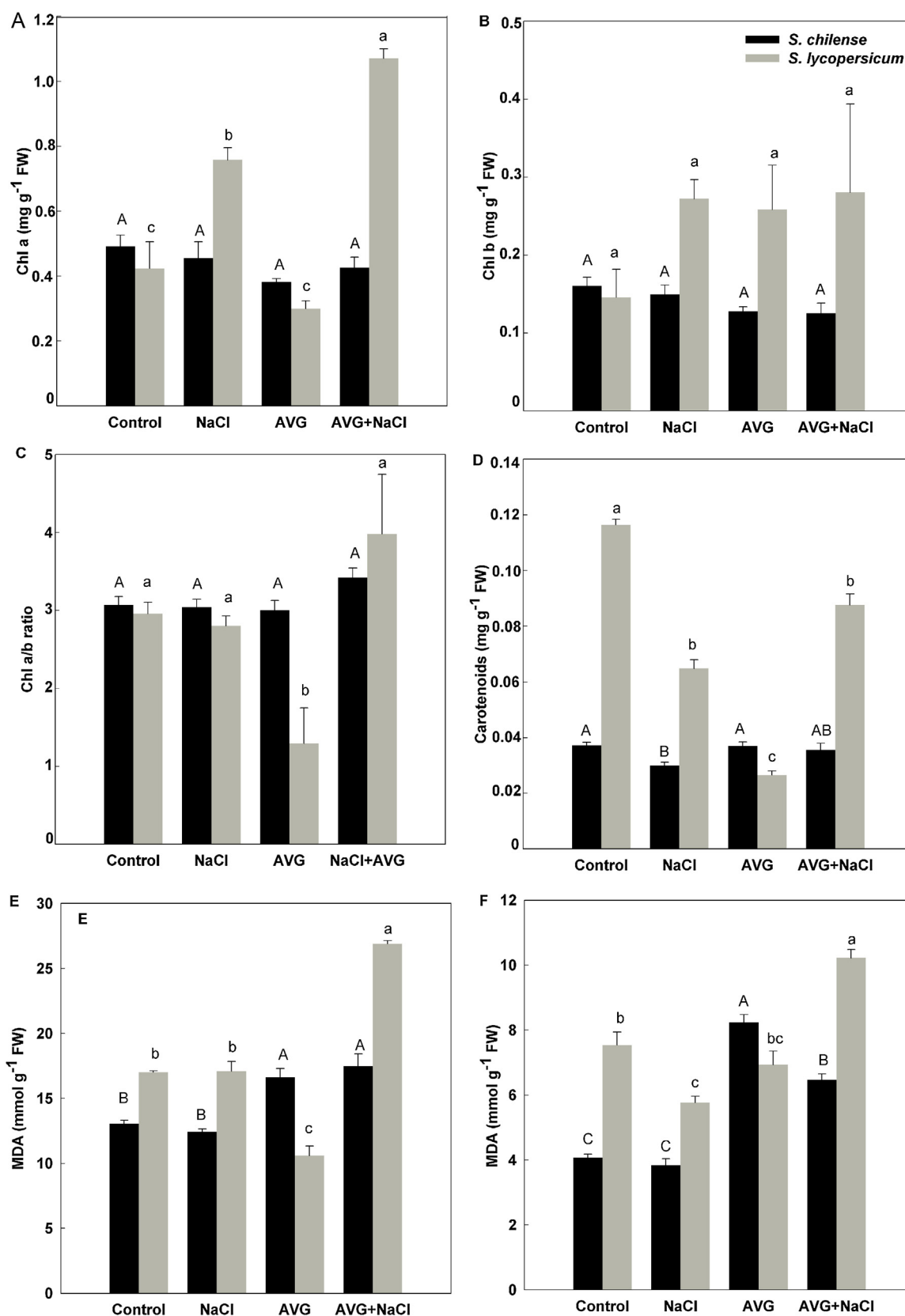


Fig. 6. Concentration in chlorophyll a (A), chlorophyll b (B), Chl a/Chl b ratio (C), carotenoid (D), leaf (E) and root malondialdehyde (F) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μ M aminovinylglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

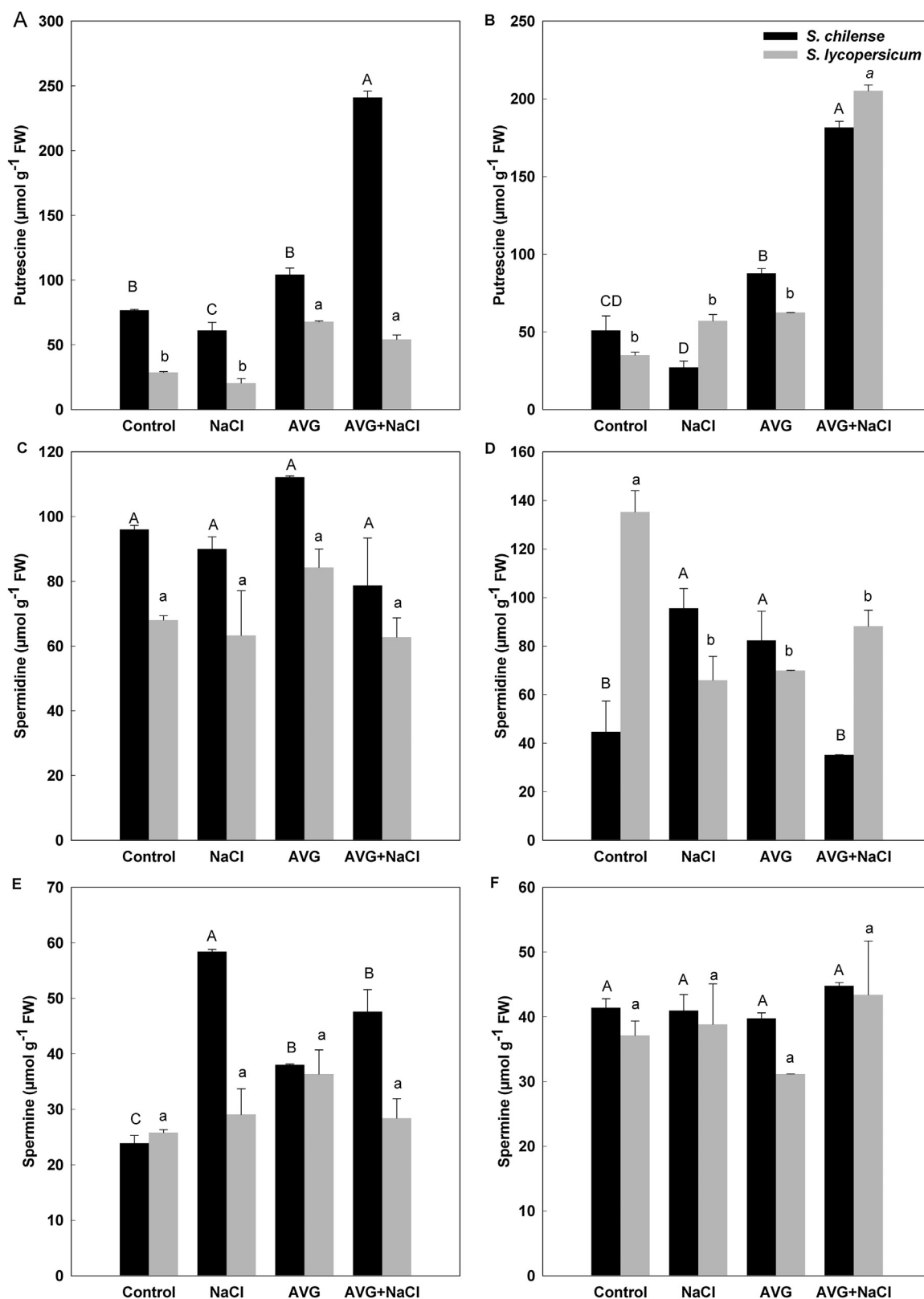


Fig. 7. Concentration of putrescine (leaf (A) and roots (B)), spermidine (leaf (C) and roots (D)) and spermine (leaf (E) and roots (F)) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μM aminovinylglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

not valuable from a commercial point of view, those fruits exhibit interesting qualitative properties in terms of antioxidant com-

pounds (Martínez et al., 2012) and this species thus appears as a promising candidate for improvement of cultivated *S. lycopersicum*.

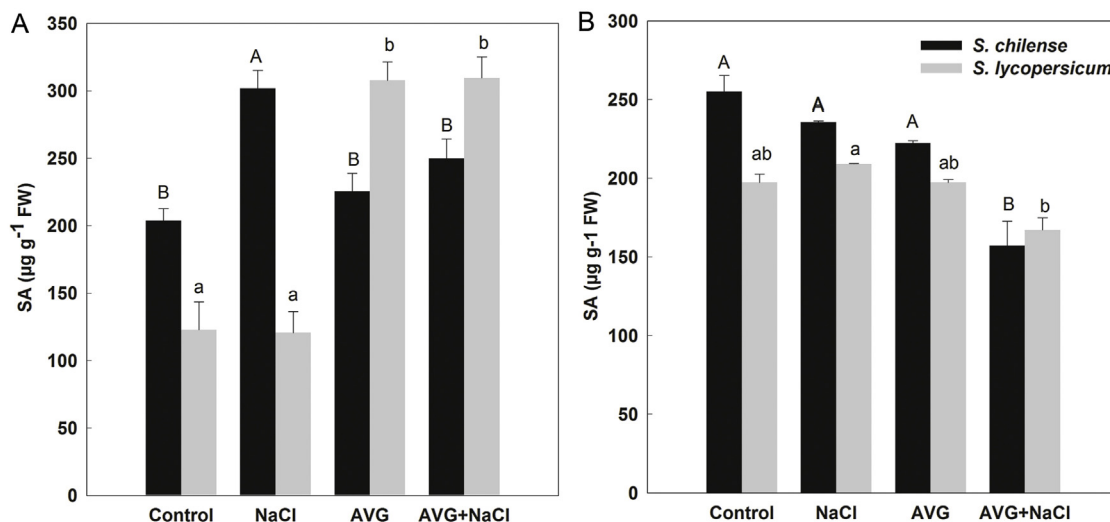


Fig. 8. Salicylic acid concentration in leaves (A) and roots (B) in *S. chilense* and *S. lycopersicum* cultivated for 7 days in control or salted nutrient solution (125 mM NaCl) in the absence or in the presence of 2 μM aminovinylglycine (AVG). Each value is the mean of 6 replicates and vertical bars are standard errors. For a given species, means exhibiting different letters are significantly different at $P < 0.05$.

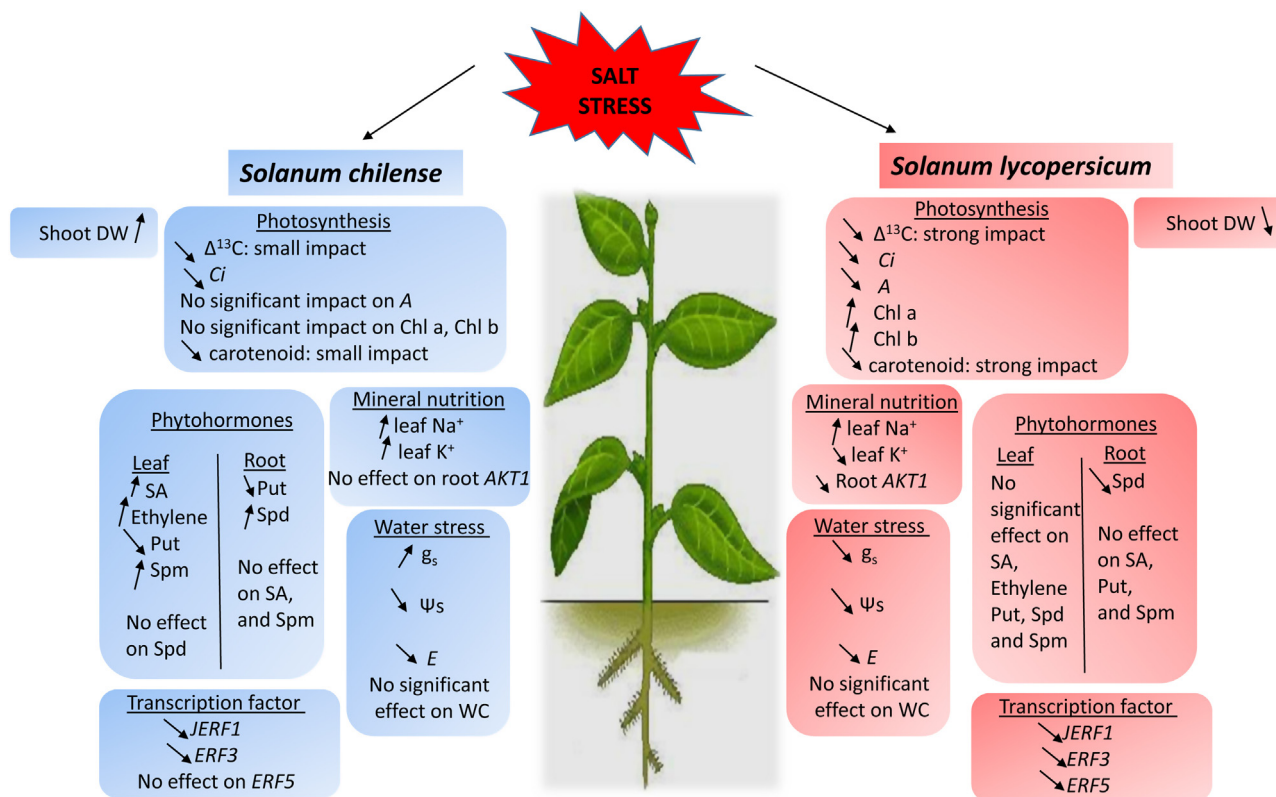


Fig. 9. Comparative overview of salinity response in the halophyte *Solanum chilense* (left) and glycophyte *S. lycopersicum* (right) exposed for 7 days to 125 mM NaCl in relation to photosynthesis-related parameters, mineral nutrition, regulation of the plant water status, phytohormones (salicylic acid (SA) and polyamines (Put, Spd and Spm) content and ethylene synthesis) and relative expression of genes coding for putative Ethylene Responsive Factor.

As previously noticed (Gharbi et al., 2016), the response of *S. chilense* to NaCl is directly associated with a salt-induced burst in ethylene synthesis in relation to ACC2 overexpression. The present work suggests that some of the properties sustaining plant resistance to NaCl in *S. chilense* are related to such ethylene over-synthesis since exogenous application of the ethylene synthesis inhibitor AVG mitigated the response. The present study considered a moderate NaCl dose directly relevant from salt concentration experienced by the plants in field conditions and it focused on a

one week exposure in order to identify the first events triggering subsequent responses in salt-treated plants. Under these circumstance, ethylene commonly known as senescing hormone was not yet overproduced in *S. lycopersicum*, which is in line with other data revealing the absence of other senescing symptoms such as chlorophyll breakdown or MDA accumulation. Moreover, the cultivated plant species did not experience any decrease in leaf water content but already closed its stomata and performed osmotic adjustment to maintain the plant water status (Fig. 9). Carbon isotope dis-

crimination ($\Delta^{13}\text{C}$) is providing a time-integrated estimation of water use efficiency in relation to coordination between stomatal conductance and photosynthesis (Cernusak et al., 2013). Carbon isotope discrimination drastically decreased in response to NaCl which could be related to a decrease in C_i/C_a resulting from partial stomatal closure as well as a decrease in carboxylation efficiency suggested by the NaCl-induced decrease in net photosynthesis (Sharkey, 2012; Cernusak et al., 2013). *Solanum chilense* displayed a different behavior: beside ethylene oversynthesis, salt-treated plants obviously increased their stomatal conductance but this had no deleterious impact on the leaf WC (Fig. 9). Similarly, $\Delta^{13}\text{C}$ was only slightly reduced by NaCl in *S. chilense* despite a decrease in C_i/C_a recorded in these plants after one week of treatment. Such a decrease could not be ascribed to stomatal closure but rather to a possible increase in carboxylation efficiency which is supported by the maintenance of net photosynthesis in salt-treated plants of *S. chilense* (Fig. 9).

The NaCl-induced increase in g_s recorded in *S. chilense* should be regarded as a transient response occurring on a short term basis. Tapia et al. (2016) reported that *S. chilense* efficiently closes its stomata when exposed to long term water stress. Although it does not modify stomatal density, the species is able to change stomatal distribution between adaxial and abaxial surfaces in new formed leaves experiencing water stress during their growing process (Tapia et al., 2016). On a short term basis, as it is the case in our work, the recorded increase in stomatal conductance correlated with an increase in ethylene synthesis, and the application of ethylene synthesis inhibitor AVG completely abolished the process suggesting that ethylene might be, to some extent, involved in stomatal regulation in the halophyte plant species. Chen et al. (2013) reported that ethylene may inhibit the ABA-stomatal closure in wheat. According to this study, stomata of older leaves are more sensitive to ethylene than those of younger leaves and the different behavior between leaves is due to altered stomatal sensitivity rather than to ethylene production. Tanaka et al. (2005) reported that in Arabidopsis, ethylene delays stomatal closure by inhibiting the ABA-signaling pathway. In the present study, only the fourth leaf was considered but the differences recorded between salt-treated species for g_s regulation were clearly related to differences in ethylene synthesis. In contrast, according to Desikan et al. (2006) and Ge et al. (2015), ethylene may induce stomatal closure via hydrogen peroxide synthesis generated by NADPH oxidase AtrbohK in Arabidopsis guard cells. Further works are required to assess if such a mechanism is absent or less efficient in *S. chilense* than in the model plant species. Whatever the underlying mechanisms involved, it could be hypothesized that maintenance of stomatal conductance during the first stages of stress exposure may allow a more efficient sugar synthesis for osmotic adjustment, as soluble sugars were recently reported to play a key role in osmotic adjustment of halophyte species *S. peruvianum* and *S. chilense* (Tapia et al., 2016).

Potassium starvation may also inhibit water stress-induced stomatal closure via ethylene synthesis (Benlloch-González et al., 2010). It is well established that salt stress compromises K nutrition (Munns, 2002, 2005). Salinity indeed reduced the leaf K concentration in *S. lycopersicum* but not in *S. chilense* (Fig. 9). Thus, K concentration was probably not involved in stomatal opening of salt-treated *S. chilense*. In fact, under our experimental conditions, even *S. lycopersicum* salt-treated plants did not suffer from K starvation. According to Britto and Kronzucker (2008), cytosolic concentration should be at least 100 mM to allow metabolic processes. Considering that cytosol occupies more or less 10% of the cell volume and taking into account the mean K concentration and WC of plants, the cytosolic K concentration always remained higher than 100 mM in our plants. The external K concentration in

the nutrient solution was 5 mM and these observation may explain the absence of clear influence of our treatments on gene expression coding for HAK5 and AKT1, which are the two major physiologically relevant molecular entities mediating high affinity K uptake into roots during young plant growth. Jiang et al. (2013) reported that ethylene promoted salinity tolerance via a decrease in Na accumulation and an improved K nutrition leading to an improved Na/K homeostasis. These authors demonstrated that gene coding for HAK5 was directly regulated by ethylene. This, obviously, should not have occurred in *S. chilense* as ethylene synthesis was associated with an increase and not with a decrease in Na content. Rubio et al. (2014) confirmed that a low K signal is required for transcriptional activation of HAK5 while both exogenous and endogenous K concentration were too high in the present study to directly impact this process. At an external K concentration higher than 500 μM , AKT1 is the major system mediating K uptake (Rubio et al., 2014). Even if K content increased in salt-treated *Solanum chilense*, AKT1 gene expression in salt-treated was not stimulated by salinity. Conversely, although AVG decreased K accumulation in salt-treated *S. chilense*, it paradoxically increased the AKT1 transcripts in this species (Fig. 4). It has to be mentioned, however, that AKT1 channels are mainly post-translationally regulated by a CBL interacting protein kinase and an impact of ethylene on this target cannot be ruled out (Jung et al., 2009). Beside ethylene, SA which is also produced by *S. chilense* in response to NaCl (Fig. 8) may also improve K nutrition in salt-treated plants (Jayakannan et al., 2013).

Inhibition of ethylene synthesis may have an impact on endogenous concentration of a plethora of hormonal compounds. Polyamine synthesis is especially related to ethylene synthesis as ethylene on the one hand, Spd and Spm on the other hand, share a common precursor S-adenosyl-methionine (SAM) (Lutts et al., 2013). Polyamines are known to assume numerous positive functions in stressed tissues contributing to redox homeostasis, protection of cellular structures, regulation of osmotic adjustment and mineral nutrition (Lutts et al., 2013). The present work shows that shoot Put and Spd were constitutively higher in *S. chilense* than in *S. lycopersicum*. The fact that Put accumulated in AVG-treated plants of *S. chilense* remains however puzzling as decarboxylated SAM (SAMdc) is expected to increase as a consequence of ACC synthase inhibition and allows subsequent conversion of Put to Spd under these circumstances. In salt-treated plants of *S. lycopersicum*, Spd was reported to assume photoprotective roles in relation to maintenance of the zeaxanthin content (Hu et al., 2014), regulation of chloroplast antioxidant system and chlorophyll metabolism (Li et al., 2015), and D1 protein stabilization (Hu et al., 2016). It is noteworthy, however, that in the halophyte *S. chilense* exposed to NaCl, the Spd content rose in the roots but not in photosynthetic organs (Fig. 7). Considering that polyamines, especially Spd and Spm, are regulating numerous aspect of ion transport (Pandolfi et al., 2010), and owing to the fact that Na^+ appeared more efficiently translocated to the shoots in *S. chilense* than in *S. lycopersicum*, the putative involvement of Spd in ion absorption by the roots of the halophyte plant species *S. chilense* requires further investigation. Spermine is also known to protect cellular structures against the stress, especially in the presence of high concentration of Na^+ toxic ions (Lutts et al., 2013). Spermine concentration clearly increased in NaCl treated shoots of the halophyte *S. chilense* comparatively to *S. lycopersicum*.

The Ethylene Responsive Factors (ERF) are important plant-specific transcription factors positioned downstream of the ethylene signaling pathway and modulating ethylene-responsive resistance gene. Pan et al. (2015) reported that ERF5 modulates high tolerance to drought and salt stress in tomato: we noticed that *ERF5* gene expression drastically decreased in *S. lycopersicum* exposed to NaCl (Fig. 3) but still remained highly expressed in *S. chilense*. Another transcription factor JERF1 is known to modulate

expression of GCC box-containing genes involved in salt tolerance of the glycophyte species *Nicotiana tabacum* (Zhang et al., 2004). In the absence of NaCl, this transcription factor was highly expressed in the halophyte *S. chilense* but NaCl drastically decreased *JERF1* expression in this species. Hence, it still remains possible that *JERF1* does not assume exactly similar function in glycophyte and halophyte species exposed to NaCl. Beside ethylene synthesis, the efficiency of ethylene perception and transduction of the signal also need to be analyzed in the future. In the specific case of *S. chilense*, Tapia et al. (2005) recently identified four members of *Ty1/copia*-like retrotransposon families (TLC1, TLC2, TLC3 and TLC4); these authors found that TLC1:1 is transcriptionally active and may be induced by high salt concentration. According to this study, ethylene is directly involved in salt induction through putative ethylene-responsive elements present in the U3 region.

5. Conclusion

Taken together, these data support the hypothesis that ethylene may assume positive function in salt-adaptation of *Solanum chilense* in relation to maintenance of stomatal conductance, water use efficiency and osmotic adjustment. However, one single accession of *S. chilense* was analyzed in the present work and further studies are thus required to precisely identify modalities of ethylene action in this species.

Acknowledgements

This work was supported by Wallonie Bruxelles International (WBI; 15/63179) and the “Fonds national de la Recherche Scientifique” (FNRS; Belgium). E.G. is grateful to CAI (Conseil de l'action internationale; Université catholique de Louvain) for the award of a research fellowship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jplph.2016.12.001>.

References

- Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J., Harberd, N.P., 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311, 91–94.
- Albacete, A., Ghanem, M.E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Lutts, S., Dodd, I.C., Pérez-Alfocea, F., 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *J. Exp. Bot.* 59, 4119–4131.
- Albacete, A., Martínez-Andújar, C., Ghanem, M.E., Acosta, M., Sánchez-Bravo, J., Asins, M.J., Cuartero, J., Lutts, S., Dodd, I.C., Pérez-Alfocea, F., 2009. Rootstock-mediated changes in xylem ionic and hormonal status are correlated with delayed senescence, and increased leaf area and crop productivity in salinized tomato. *Plant Cell Environ.* 32, 928–938.
- Alemán, F., Nieves-Cordones, M., Martínez, V., Rubio, F., 2011. Root K⁺ acquisition in plants: the *Arabidopsis thaliana* model. *Plant Cell Physiol.* 52, 1603–1612.
- Amjad, M., Akhtar, J., Anwar-ul-Haq, M., Yang, A., Akhtal, S.S., Jacobsen, S.E., 2014. Integrating role of ethylene and ABA in tomato plants adaptation to salt stress. *Sci. Hortic.* 172, 109–116.
- Amrhein, N., Wenker, D., 1979. Novel inhibitors of ethylene production in higher plants. *Plant Cell Physiol.* 20, 1635–1642.
- Benlloch-González, M., Romera, J., Critescu, S., Harren, F., Fournier, J.M., Benlloch, M., 2010. K⁺ starvation inhibits water-stress-induced stomatal closure via ethylene synthesis in sunflower plants. *J. Exp. Bot.* 61, 1139–1145.
- Britto, D.T., Kronzucker, H.J., 2008. Cellular mechanisms of potassium transport in plants. *Physiol. Plant.* 133, 637–650.
- Cernusak, L.A., Ubierna, N., Winter, K., Holtum, J.A.M., Marshall, J.D., Farquhar, G.D., 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytol.* 200, 950–965.
- Chen, L., Dodd, I.C., Davies, W.J., Wilkinson, S., 2013. Ethylene limits abscisic acid- or soil drying-induced stomatal closure in aged wheat leaves. *Plant Cell Environ.* 36, 1850–1859.
- Chen, T.W., Nguyen, T.M.N., Stützel, H., 2015. High temperature and vapor pressure deficit aggravate architectural effects but ameliorate non-architectural effects of salinity on dry mass production of tomato. *Front. Plant Sci.* 6, 887.
- Chetelat, R.T., Pertuzé, R.A., Faúndez, L., Graham, E.B., Jones, C.M., 2009. Distribution, ecology and reproductive biology of wild tomatoes and related nightshades from the Atacama Desert region of Northern Chile. *Euphytica* 167, 77–93.
- Cristescu, S.M., De Martinis, D., Te Lintel Hekkert, S., Parker, D.H., Harren, F.J.M., 2002. Ethylene production by *Botrytis cinerea* in vitro and in tomatoes. *Appl. Environ. Microbiol.* 68, 5342–5350.
- Desikan, R., Last, K., Harrett-Williams, R., Tagliavia, C., Harter, K., Hooley, R., Hancock, J.T., Neill, S.J., 2006. Ethylene-induced stomatal closure in *Arabidopsis* occurs via AtrbohF-mediated hydrogen peroxide synthesis. *Plant J.* 47, 907–916.
- Dianese, E.C., Fonseca, M.E.N., Inoue-Nagata, A.K., Resende, R.O., Boiteux, L.S., 2011. Search in *Solanum* (section *Lycopersicon*) germplasm for sources of broad-spectrum resistance to four Topovirus species. *Euphytica* 180, 307–319.
- Farquhar, G.D., Richards, R.A., 1984. Isotopic composition of plant carbon correlates with water-use efficiency in wheat genotypes. *Aust. J. Plant Physiol.* 11, 539–552.
- Fischer, I., Steige, K.A., Stephan, W., Mboup, M., 2013. Sequence evolution and expression regulation of stress-responsive genes in natural populations of wild tomato. *PLoS One* 8, e78182.
- Ge, X.M., Cai, H.L., Lei, X., Zhou, X., Yue, M., He, J.M., 2015. Heterotrimeric G protein mediates ethylene-induced stomatal closure via hydrogen peroxide synthesis in *Arabidopsis*. *Plant J.* 82, 138–150.
- Ghanem, M.E., Albacete, A., Martínez-Andújar, C., Acosta, M., Romero-Aranda, R., Dodd, I.C., Lutts, S., Pérez-Alfocea, F., 2008. Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). *J. Exp. Bot.* 59, 3039–3050.
- Gharbi, E., Martínez, J.P., Benahmed, H., Fauconnier, M.L., Lutts, S., Quinet, M., 2016. Salicylic acid differently impacts ethylene and polyamine synthesis in the glycophyte *Solanum lycopersicum* and the wild-related halophyte *Solanum chilense* exposed to mild salt stress. *Physiol. Plant.* 158, 152–167.
- Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 185–188.
- Hu, L., Xiang, L., Zhang, L., Zhou, X., Zou, Z., Hu, X., 2014. The photoprotective role of spermidine in tomato seedlings under salinity-alkalinity stress. *PLoS One* 10, e110855.
- Hu, L., Xiang, L., Li, S., Zou, Z., Hu, X.H., 2016. Beneficial role of spermidine in chlorophyll metabolism and D1 protein content in tomato seedlings under salinity-alkalinity stress. *Physiol. Plant.* 156, 468–477.
- James, R.A., Blake, C., Byrt, C.S., Munns, R., 2011. Major genes for Na⁺ exclusion, *Nax1* and *Nax2* (wheat *HKT1;4* and *HKT1;5*), decrease Na⁺ accumulation in bread wheat leaves under saline and waterlogged condition. *J. Exp. Bot.* 62, 2939–2947.
- Jayakannan, M., Bosse, J., Babourina, O., Rengel, Z., Shabala, S., 2013. Salicylic acid improves salinity tolerance in *Arabidopsis* by restoring membrane potential and preventing salt-induced K⁺ loss via a GORK channel. *J. Exp. Bot.* 64, 2255–2268.
- Jiang, C., Belfield, E.J., Cao, Y., Smith, A.C., Harberd, N.P., 2013. An *Arabidopsis* soil-salinity-tolerance mutation confers ethylene-mediated enhancement of sodium/potassium homeostasis. *Plant Cell* 25, 3535–3552.
- Jung, J.Y., Shin, R., Schachtman, D.P., 2009. Ethylene mediates response and tolerance to potassium deprivation in *Arabidopsis*. *Plant Cell* 21, 607–621.
- Kazan, K., 2015. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends Plant Sci.* 20, 219–229.
- Lei, G., Shen, M., Li, Z.G., Zhang, B., Duan, K.X., Wang, N., Cao, Y.R., Zhang, W.K., Ma, B., Ling, H.Q., Chen, S.Y., Zhang, J.S., 2011. EIN2 regulates salt stress response and interacts with a MA3 domain-containing protein ECIPI in *Arabidopsis*. *Plant Cell Environ.* 34, 1678–1692.
- Li, J., Hu, L., Zhang, L., Pan, X., Hu, X., 2015. Exogenous spermidine is enhancing tomato tolerance to salinity-alkalinity stress by regulating chloroplast antioxidant system and chlorophyll metabolism. *BMC Plant Biol.* 15, 303.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Meth. Enzymol.* 148, 350–382.
- Lutts, S., Bouharmont, J., Kinet, J.M., 1999. Physiological characterization of salt-resistant rice somaclones. *Aust. J. Bot.* 47, 835–849.
- Lutts, S., Hausman, J.F., Quinet, Lefèvre, I., 2013. Polyamines and their roles in the alleviation of ion toxicities in plants. In: Hakeem, K.R., Ahmad, P., Ozturk, M. (Eds.), *Crop Improvement: New Approaches and Modern Techniques*. Springer Science – Business Media LLC Publisher, New York, USA.
- Martínez, J.P., Antúnez, A., Petruzé, R., Acosta, M.D.P., Palma, X., Fuentes, L., Ayala, A., Araya, H., Lutts, S., 2012. Effects of saline water on water status: yield and fruit quality of wild (*Solanum chilense*) and domesticated (*Solanum lycopersicum* var. *cerasiforme*) tomatoes. *Exp. Agric.* 48, 573–586.
- Martínez, J.P., Antúnez, A., Araya, H., Petruzé, R., Acosta, M.D.P., Fuentes, L., Lizana, X.C., Lutts, S., 2014. Salt stress differently affects growth: water status and antioxidant enzyme activities in *Solanum lycopersicum* L. and its wild-relative *Solanum chilense* Dun. *Aust. J. Bot.* 62, 359–368.
- Molinari, S., Loffredo, E., 2006. The role of salicylic acid in defense response of tomato to root-knot nematodes. *Physiol. Mol. Plant Pathol.* 68, 69–78.
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* 25, 239–250.

- Munns, R., 2005. Genes and salt-tolerance: bringing them together. *New Phytol.* 3, 645–663.
- Nakazato, T., Warren, D.L., Moyle, L.C., 2010. Ecological and geographic modes of species divergence in wild tomatoes. *Am. J. Bot.* 97, 680–693.
- Pan, Y., Seymour, G.B., Lu, C., Hu, Z., Chen, X., Chen, G., 2012. An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. *Plant Cell Rep.* 31, 349–360.
- Pan, Y.J., Liu, L., Lin, Y.C., Zu, Y.G., Li, L.P., Tang, Z.H., 2016. Ethylene antagonizes salt-induced growth retardation and cell death process via transcriptional controlling of ethylene- BA- and senescence-associated genes in *Arabidopsis*. *Front. Plant Sci.* 7, 696.
- Pandolfi, C., Pottosin, I., Cuin, T., Mancuso, S., Shabala, S., 2010. Specificity of polyamine effects on NaCl-induced ion flux kinetics and salt stress amelioration in plants. *Plant Cell Physiol.* 51, 422–434.
- Quinet, M., Bataille, G., Dobrev, P.I., Capel, C., Gómez, P., Capezi, J., Lutts, S., Motyka, V., Angosto, T., Lozano, R., 2014. Transcriptional and hormonal regulation of petal and stamen development by STAMENLESS, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class APETALA3 gene. *J. Exp. Bot.* 65, 2243–2256.
- Ruan, C.J., Teixeira da Silva, J., Mopper, S., Qin, P., Lutts, S., 2010. Halophyte improvement for a salinized world. *Crit. Rev. Plant Sci.* 29, 329–359.
- Rubio, F., Fon, M., Ródenas, R., Nieves-Cordones, M., Alemán, J., Rivero, R.M., Martínez, V., 2014. A low K⁺ signal is required for functional high-affinity K⁺ uptake through HAK5 transporters. *Physiol. Plant.* 152, 558–570.
- Sharkey, T.D., 2012. Mesophyll conductance: constraint on carbon acquisition by C3 plants. *Plant Cell Environ.* 35, 1881–1883.
- Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N., Hasesawa, S., 2005. Ethylene inhibits abscisic acid-induced stomatal closure in *Arabidopsis*. *Plant Physiol.* 138, 2337–2343.
- Tapia, G., Verdugo, I., Yañez, M., Ahumada, I., Theoduloz, C., Cordero, C., Poblete, F., González, E., Ruiz-Lara, S., 2005. Involvement of ethylene in stress-induced expression of the TLC1: 1 retrotransposon from *Lycopersicon chilense* Dun. *Plant Physiol.* 138, 2075–2086.
- Tapia, G., Méndez, J., Inostroza, L., 2016. Different combinations of morpho-physiological traits are responsible for tolerance to drought in wild tomatoes *Solanum chilense* and *Solanum peruvianum*. *Plant Biol.* 18, 406–416.
- Thapa, S.P., Miyao, F.M., Davis, M.R., Coaker, G., 2015. Identification of QTLs controlling resistance to *Pseudomonas syringae* pv tomato race 1 strains from the wild tomato, *Solanum habrochaites* LA1777. *Theor. Appl. Genet.* 128, 681–692.
- Wang, H., Huang, Z., Chen, Q., Zhang, Z., Zhang, H., Wu, Y., Huang, D., Huang, R., 2004. Ectopic expression of *JERF3* in tobacco activates downstream gene expression and enhances salt tolerance. *Plant Mol. Biol.* 55, 183–192.
- Zhang, H., Huang, Z., Xie, B., Chen, Q., Tian, X., Zhang, X., Zhang, H., Lu, X., Huang, D., Huang, R., 2004. The ethylene, jasmonate-, abscisic acid- and NaCl-responsive tomato transcription factor *JERF1* modulates expression of GCC box-containing genes and salt-tolerance in tobacco. *Planta* 220, 262–270.