

Sodium and chloride accumulation and repartition differed between the cultivated tomato (*Solanum lycopersicum*) and its wild halophyte relative *Solanum chilense* under salt stress

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ABSTRACT

Salinity is of growing global concern and affects the yield of several crop species, including tomato (*Solanum lycopersicum*). Halophyte wild relatives could be used to improve salt tolerance of crop species. Among wild tomato relatives, *Solanum chilense* was shown to be NaCl-tolerant but its strategy for Na⁺ accumulation and repartition remained poorly understood, and its Cl⁻ accumulation was never investigated. In this study, both species were cultivated under salinity (0, 60 and 120 mM NaCl) during vegetative and reproductive phases. We investigated the ion (Na⁺, K⁺, Cl⁻) accumulation and repartition in the different organs (leaves, stems, roots, inflorescences), the expression of Na⁺ and Cl⁻ transporters in vegetative organs and the role of these ions in osmotic management. *Solanum chilense* accumulated mainly Na⁺ in the shoots while *S. lycopersicum* accumulated it mainly in the roots so that the Na⁺ shoot/root ratio was 10 times higher in *S. chilense* than in *S. lycopersicum*. This suggests that *S. chilense* had an includer strategy while *S. lycopersicum* had an excluder strategy towards Na⁺. The excluder behavior of *S. lycopersicum* was linked to the high expression of *HKT1;2* and *SOS* pathway genes in roots. In contrast to Na⁺, both species accumulated Cl⁻ in a similar way and Cl⁻ content was higher in the shoots than in the roots in both species. In addition, both species limited the entry of Na⁺ and Cl⁻ in the inflorescences. The Na⁺ and Cl⁻ concentrations were respectively about 3 and 2 times lower in the inflorescences than in the leaves. Ions also contributed to osmotic adjustment, mainly Cl⁻ in *S. lycopersicum* and Na⁺ and K⁺ in *S. chilense*. Overall, our results highlight the salt-tolerance characteristics of *S. chilense* compared to the cultivated tomato.

1. Introduction

Salinity affects the yield of several plant species and is a growing global concern in the world (Costan et al., 2020; Yadav et al., 2020). According to FAO, 2.1% of the dryland agriculture and 19.5% of the irrigated lands are affected by salinity (FAO 2021). Soil salinity is described as an increase in soluble salts (mainly NaCl) concentrations in the soil caused by excessive evaporation compared to precipitations (Yadav et al., 2020). Soils are classified as saline when their electrical conductivity is equal to or higher than 4 dS.m⁻¹, which is equivalent to 40 mM NaCl (Munns and Tester 2008). Most crop species are glycophytes and their growth and development are strongly hampered by salinity. Salt stress induces both osmotic and ionic constraints on plants. A decrease in osmotic potential of the soil solution compromises plant

water uptake while accumulation of non-essential ions leads to toxicity by affecting cellular structures and plant metabolism (Munns and Tester 2008; Nieves-Cordones et al., 2016). Improving the tolerance of glycophyte crops to salinity is a complex task. Salinity tolerance is a polygenic trait which is influenced by genetic and environmental factors (Assaha et al., 2017; Zsögön et al., 2017). Conventional breeding by introgression of traits of interest is thus difficult because of the risk of loss of polygenic traits during backcrossing (Frank 2003). Moreover, domesticated plant species have been selected mainly for their high yields, resulting in low variation in salinity tolerance within species. Another strategy to develop tolerant crops is *de novo* selection from wild species (Zsögön et al., 2017; Li et al., 2018). Crop wild relatives have in general a higher level of tolerance to abiotic and biotic stress than cultivated plants.

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The cultivated tomato (*Solanum lycopersicum*) is a glycophyte species, but the tomato clade also includes 12 related wild species growing in the Andean region of South America. These wild relatives constitute a potential high source of genetic diversity, especially for stress resistance (Chetelat et al., 2009; Rothan et al., 2019). Among them, *Solanum chilense* is a halophyte species originating from Atacama and Peruvian deserts and different populations have been identified in a variety of environments (Peralta et al., 2008; Chetelat et al., 2009; Bøndel et al., 2015). Although some studies investigated the response of *S. chilense* to salinity (Zhou et al., 2011; Martínez et al., 2014, 2020; Gharbi et al., 2016, 2017a, 2017b, 2018; Martín-Davison et al., 2017; Kashyap et al., 2020), its salt tolerance mechanisms remain largely unknown.

Halophytes adopt a wide range of strategies to cope with salt. As far as Na^+ accumulation is concerned, some halophyte species (especially plants from the Poaceae or Amaranthaceae families), are able to accumulate Na^+ in specialized papillae or trichomes (Smaoui et al., 2011; Yuan et al., 2016). Many halophyte species thus behave as « includer » species, i.e. they accumulate high amount of Na^+ in the shoots. Among wild tomato species *S. cheesmaniae*, *S. peruvianum* and *S. pennellii* are capable of such a mechanism (Tal et al., 1979; Tal and Shannon 1983; Almeida et al., 2014). Gharbi et al. (2017a) showed that *S. chilense* behaved similarly when subjected to short-term salt stress during vegetative growth, unlike *S. lycopersicum*. Sodium is translocated to the aerial parts of the plant via the transpiration flow in the xylem, but some transporters are known to mitigate Na^+ accumulation in the shoot. Genes from the SOS (Salt Overly Sensitive) pathway, *SOS1*, *SOS2* and *SOS3*, are involved in Na^+ exclusion from the cell in several glycophyte crop species, including tomato (Asins et al., 2013; Assaha et al., 2017; Basu et al., 2020). The tomato *HKT1;2*, which belongs to class I-*HKT* transporters, is known to play a role in Na^+ transport from root to shoot since it removes Na^+ from xylem sap (Almeida et al., 2014; Romero-Aranda et al., 2020).

Most studies investigating the impact of salinity on plants focused on the accumulation and the toxicity of Na^+ and neglected the impact of Cl^- . Nevertheless, a few studies investigated the impact of Na^+ and Cl^- separately in Poaceae by adding a mixture of Na^+ or Cl^- salts in culture media (Tavakkoli et al., 2011; Khare et al., 2020; Proddjino et al., 2021). Those studies showed that barley and rice reacted differently to the two types of ion, particularly in terms of oxidative stress management and photosynthesis-related parameters. However, their results suggested that the toxic effect of these ions was additive. Although a few studies investigated the response of *S. lycopersicum* to Cl^- , nothing is known about Cl^- regulation in *S. chilense*. Kafkafi et al. (1982) suggested that Cl^- toxicity could be related to nitrate deficiency in *S. lycopersicum* and Estañ et al. (2005) observed that NaCl induced an increase in leaf Na^+ and Cl^- contents in this species. Rush and Epstein (1981) observed that the salt-tolerant *S. cheesmaniae* was able to accumulate more Cl^- in shoots than *S. lycopersicum*. Chloride is a nutrient required for many functions, such as regulation of leaf size or involvement in the oxygen-evolving complex in photosystem II (Kawakami et al., 2009; Franco-Navarro et al., 2016). Chloride can even be necessary for osmotic adjustment (Chen et al., 2016; Cui et al., 2020). However, the toxicity of high Cl^- concentration is less well understood than that of high Na^+ concentration. Chloride tolerant species seem to accumulate less Cl^- in leaves than sensitive species, although the opposite is sometimes observed (Teakle and Tyerman 2010; Li et al., 2017). As for Na^+ , few species are able to excrete Cl^- in salt glands or in trichomes (Litalien et al., 2020). Chloride entry into the cell is active (Teakle and Tyerman 2010); some transporters are involved in Cl^- transport, such as *NPF2.4* (NRT1/PTR family transporter). Indeed, a loss of function of *NPF2.4* induced a decrease of Cl^- in *Arabidopsis thaliana* shoots (Li et al., 2016).

In this study, we compared the response of *S. chilense* and *S. lycopersicum* to salinity (0, 60, 120 mM NaCl) during both vegetative and reproductive stages with a special focus on osmotic and ionic (Na^+ , K^+ , Cl^-) management. As previously indicated, the physiological response of *S. chilense* to NaCl and its Na^+ management has mainly been

studied in response to short-term salt stress during vegetative growth (Gharbi et al., 2016, 2017a, 2017b, 2018). Furthermore, Cl^- accumulation and distribution has never been investigated in this species. This study will show whether the mechanisms put in place in the short term remain valid when the plant is subjected to salinity during both vegetative and reproductive stages. It aims to answer the following questions: 1) How Na^+ , K^+ and Cl^- are distributed in the different plant organs of both species and especially what is the management of these ions in the inflorescences? Do these species behave as “includer” or “excluder” towards these elements and especially towards Cl^- ? 2) Is the expression of Na^+ and Cl^- transporters genes linked to Na^+ and Cl^- distribution in the plant? 3) Do Na^+ , K^+ and Cl^- contribute to osmotic adjustment?

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *S. lycopersicum* L. cv Ailsa Craig (accession LA2838A) and *S. chilense* Dunal (accession LA4107) were obtained from the Tomato Genetics Resource Center (TGRRC, University of California, Davis, CA, USA) and INIA-La Cruz (La Cruz, Chile), respectively. After 6 days pre-germination in Petri dishes on moistened Whatman filter paper (25 °C, 12 h photoperiod), seeds of *S. chilense* were sown in peat compost (DCM, Amsterdam, The Netherlands) in a temperate greenhouse. Seeds of *Solanum lycopersicum* were sown in the same peat compost and in the same greenhouse 13 days after the sowing of *S. chilense*, so that the same developmental stage was reached at the start of stress application for the two species. Environmental conditions in the greenhouse were as following: 24 °C $\pm$ 1.5 °C / 21 °C $\pm$ 0.8 °C day/night temperature, 63% $\pm$ 8% / 67% $\pm$ 5% day/night relative humidity, 16h-photoperiod provided by LED LumiGrow lights (650 W, red-blue) additional lighting (minimum mean light in the middle of the day 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$). At the two-leaf stage, plants were individually transplanted in pots (2.5 L) in perlite/vermiculite (50% v/v) and watered three times a week with a half strength Hoagland's nutrient solution according to Bigot et al. (2022). After four days of acclimation, plants were randomly divided into three groups receiving 0, 60 or 120 mM NaCl (respectively, 0.96, 7.33 and 9.38 mS cm^{-1}). In total, there were 25 plants per treatment and per species. The plants were arranged in a split-plot design with 5 plants per tray (each plant in a tray receiving the same treatment) but the treatments varied between neighboring trays. The NaCl was added in the nutrient solution and the plants were watered with the solutions three times a week according to Bigot et al. (2022). The NaCl concentrations were selected based on previous experiments to simulate a moderate and a strong salt stress.

2.2. Growth measurements

The number of leaves and inflorescences on the main stem was counted once a week on 6 plants per treatment and per species throughout the experiment. Three plants per treatment and per species were harvested at two developmental stages: i) 35 days after stress imposition (DAST) at early flowering stage, and ii) 85 DAST, at full bloom. These plants were used to determine the total number of leaves per plant (excluding falling leaves), the total leaf area per plant, and the plant fresh (FW) and dry (DW) weights. Each leaf was photographed (Lumix, Panasonic DMC SZ-10) and the pictures were analysed with ImageJ (v1.53a) to determine the leaf area of each leaf. The total leaf area per plant was calculated by adding up the individual area of each leaf. For each plant, the different organs (leaves, stems and roots) were separated and the FW of each organ was measured separately. The DW of each organ was measured after drying 72 h in an oven at 70 °C.

2.3. Plant water status and photosynthesis-related parameters

Based on the FW and DW measurements, the water content (WC) of the different organs (leaves, stems, roots) was calculated for plants harvested at 35 and 85 DAST, using the formula $WC = (FW - DW) / FW \times 100$. Leaf osmotic potential (Ψ_s) was quantified on the extracted tissular sap from the first fully expanded leaf (counted from the apex) of three plants per treatment and per species (plants harvested at 85 DAST), using a vapor pressure osmometer (Wescor 5500, Oceanside, CA, USA), as previously described (Prodjinoto et al., 2021).

Physiological parameters were measured on the fourth fully expanded leaf (counted from the apex) of six plants per treatment and per species at early flowering stage and during full bloom. The instantaneous CO_2 assimilation under ambient conditions (400 ppm CO_2) (A), the instantaneous transpiration (E) and intercellular CO_2 concentration (Ci) were quantified using an infrared gas analyzer (ADC BioScientific LCI-SD System Serial 33,413, Hoddesdon, UK). Gas exchanges were 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} . Chlorophyll fluorescence parameters (maximum quantum yield of photosystem II, F_v/F_m , non-photochemical quenching, NPQ, photochemical quenching, qP, efficiency of photosystem II, Φ_{PSII}) were measured using a fluorimeter (FMS II, Hansatech Instruments, Norfolk, UK) according to Prodjino et al. (2021). Chlorophyll content index (CCI) was measured using a chlorophyllometer (Opti-Sciences, CCM-200, Hudson, USA) on the same leaves (fourth fully expanded leaf of six plants per treatment and per species). Three measurements were taken per leaf and averaged to avoid heterogeneity due to the leaf portion.

2.4. Leaf anatomy

Transversal sections of leaflets were performed on the first expanded leaf (from the top) of three plants per treatment and per species harvested at 85 DAST. Samples were fixed in FAA (70% ethanol:acetic acid:35% formaldehyde 18:1:1 vol.), dehydrated in a graded ethanol series, embedded in paraffin and sectioned at 7 μm according to Quinet et al. (2006). Serial transversal sections were stained with safranin/fast green and observed with a light microscope (Polyvar Reichert-Jung) equipped with camera (SCMEX-6, Euromex). The thickness of the leaf blade, palisade parenchyma and spongy parenchyma, as well as the diameter of the xylem vessel elements were measured using Image-FocusAlpha software. The ratio between the thickness of the spongy parenchyma and the mesophyll (palisade + spongy parenchyma) was calculated.

2.5. Soluble sugars and proline quantification

Leaves of three plants per treatment and per species were harvested at 85 DAST and grounded separately in liquid nitrogen. Total soluble sugars were spectrophotometrically quantified from 100 mg fresh material using anthrone method (Yemm and Willis 1954) after extraction with ethanol 70% (v/v). The absorbance was measured at 625 nm using a UV-1800 spectrophotometer (Shimadzu, 's-Hertogenbosch, the Netherlands) and soluble sugars quantification was determined using a standard curve of glucose.

Proline was quantified according to Bates et al. (1973) from 200 mg of frozen fresh grounded material. Briefly, proline was extracted in 3% (w/v) sulfosalicylic acid with incubation 30 min at 70 °C. After filtration (Whatman, 11 μm), 2 mL of ninhydrin solution (1.25 g of ninhydrin in 30 mL glacial acetic acid and 20 mL of H_3PO_4 6 M) were added to the same volume of supernatant and kept at 100 °C for 1 hour. Reaction was stopped on ice then 2 mL toluene were added. Absorbance was read at 520 nm after strong vortex of the samples.

2.6. Mineral quantification

Na^+ , K^+ and Cl^- were quantified on stems, leaves and roots of three plants per treatment and per species (harvested at 35 and 85 DAST) and on inflorescences harvested throughout the experiment. Mineralization and quantification were performed according to Prodjino et al. (2021). Material was oven-dried at 70 °C for 72 h. For Na^+ and K^+ determination, 50 to 100 mg DW were weighted and digested in 4 mL of warm 68% (v/v) HNO_3 . After complete dissolution, minerals were dissolved in aqua regia (HCl 37%: HNO_3 68% 3:1), filtered (Whatman, 11 μm) and quantified by flame atomic absorption spectrophotometry (ICE 3300, Thermo Scientific, Waltham, MA) using standards (Spectracer-CPACHEM; accredited through ISO/IEC17025). For Cl^- determination, 20 mg DW were stirred during 48 h in 40 mL HNO_3 0.05% (v/v) according to Hamrouni et al. (2011). After filtration (Whatman, 11 μm), samples were quantified by liquid chromatography (HPLC-Dionex ICS2000, Dionex Corporation, Sunnyvale, Calif., USA) according to Irakoze et al. (2019). The ion concentrations were expressed on DW basis. To calculate the ratio of the ion quantities between organs, the ion quantity for a specific organ was calculated as followed: ion concentration of the organ x DW of the same organ.

2.7. SEM-EDX

The first fully expanded leaf of 3 plants grown at 0 and 120 mM NaCl of both species was harvested at 85 DAST. The elemental composition (Na^+ , Cl^- , K^+) of the epidermis surface (0.5 cm^2 leaf squares) was analyzed by scanning electron microscopy and energy dispersive X-Ray spectroscopy (SEM-EDX, Jeol 7500F Field Emission SEM) (Luyckx, 2021). Samples were freeze-dried, mounted on slides with double-sided carbon tape before coating with gold. Back-scattered electron SEM images were acquired at an accelerating voltage of 5 - 15 kV and EDX spectra acquisition time was 983 s. General views of 600×800 μm were taken, as well as spot analyses on the most basal cell of a trichome and on an epidermal cell. ZAF correction was applied to convert ray peak intensity into semi-quantitative concentrations. Semi-quantitative results were given by the ratio between the mass percentage of each element and the sum of the C and O percentages (% mass element / (% mass C + % mass O)).

2.8. Expression of genes coding for Na^+ and Cl^- transporters

The expression of six genes coding for Na^+ and Cl^- transporters was analyzed by qRT-PCR (quantitative real-time polymerase chain reaction). Genes were selected according to literature: *SOS1* (Solyc01g005020), *SOS2* (Solyc012g009570) and *SOS3* (Solyc06g051970) (Olías et al., 2009; Belver et al., 2012; Ji et al., 2013), *HKT1;2* (Solyc07g014680) (Almeida et al., 2014), and *NPF2.4* (Solyc11g072580) (Li et al., 2016). In order to verify that the primers allowed amplification in both tomato species, the sequences of the different tomato species were identified by nucleotide BLAST in the databases of the national center for biotechnology information (NCBI) and the solanaceae genomics network (SGN) and the identified sequences were aligned using BioEdit (Hall 1999). Primers were selected from the literature (Asins et al., 2013; Romero-Aranda et al., 2020) or designed using Primer3Plus (Untergasser et al., 2007). The analyzed genes, reference IDs in *S. lycopersicum* and *S. chilense* and primer sequences are available in Table S1.

The sixth youngest leaf, the part of stem in front of this leaf and the secondary root from the first node of 3 plants per treatment and per species were collected in liquid nitrogen at 85 DAST for RNA extraction (RNA extractions were performed separately for each plant and organ). RNA extraction was performed using TRI Reagent Solution (Ambion, Austin, TX, USA) with DNase treatment (RQ1 DNase 1 U/ μg Promega, Leiden, The Netherlands) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 μg RNA using the Revertaid H

Minus First Strand cDNA Synthesis Kit (ThermoFischer). The concentration and purity of the RNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Villebon-sur-Yvette, France). The quality of cDNA was checked by semi quantitative PCR using the tomato housekeeping genes *LeEF1-α* (Soly06g005060) and checked on gel prior qPCR analysis. Transcript levels were quantified in two independent qPCR (in triplicates for each of the three biological replicates) using the GoTaq qPCR Master Mix (Promega) in a StepOne-Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Cycling conditions were initial denaturation 10 min at 95 °C, then 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Results were expressed according to calibration curve method using the tomato housekeeping genes *LeEF1-α* (Elongation factor 1-α, Soly06g005060) and *TIP41* (TIP41-like protein, Soly10g049850) as reference genes (Leelanawit et al., 2017) and the leaves of *S. lycopersicum* under control conditions as control. A melt-curve analysis was performed to check the specific amplification of each primer.

2.9. Statistical treatments

All statistical treatments were analyzed using RStudio (R Core Team, 2017). Normality distribution and homoscedasticity were verified using respectively Shapiro-Wilk and Levene's test and data were transformed when required. When possible, two-ways analysis of variance (ANOVA II) was used to compare species, NaCl treatments and their interactions. Comparisons between the two species were analyzed using Student's test, permutation Student's *t*-test (if normality was not met) or Wilcoxon test (if homoscedasticity was not met). For a same species, comparisons between NaCl treatments were analyzed using one-way analysis of variance (ANOVA I), ANOVA I using permutation test (if normality was not met) or Kruskal-Wallis test (if homoscedasticity was not met) followed by appropriate post-hoc tests. To compare the evolution of parameters between the two dates of measurements, a Student's test was applied. If normality or homoscedasticity were not met, a permutation Student's test or a Wilcoxon test were respectively applied. Data were represented by the means ± standard deviation. Statistical results are presented in Table S2.

3. Results

3.1. Impact of salinity on plant growth

Plant DW was recorded higher in *S. lycopersicum* than in *S. chilense*, even under salt conditions (Fig. 1A, Table S2). However, the distribution of DW between organs differed little between species with the exception of roots (Fig. 1B, Table S2). The total leaf area per plant was also higher in *S. lycopersicum* compared to *S. chilense*, despite a smaller number of leaves per plant at the end of the experiment (Fig. 1C, D, Table S2). *Solanum lycopersicum* produced also more inflorescences but less ramifications on the main stem than *S. chilense* (Fig. 1E, F, Table S2).

The negative impact of salt on plant development significantly increased with time in both species (Fig. 1). At 35 DAST, salt had only a slight effect on plant DW at 120 mM NaCl in *S. lycopersicum*, whereas at 85 DAST, plant DW significantly decreased with NaCl concentrations in both species although more drastically in *S. lycopersicum* than in *S. chilense* (Fig. 1A, Table S2). Moreover, salt affected the DW distribution among organ only in *S. lycopersicum* at 85 DAST (Fig. 1B, Table S3). On the same way, salt reduced the total leaf area per plant in *S. lycopersicum* at 35 DAST and in both species at 85 DAST (Fig. 1C). However, the total number of leaves per plant was only reduced by salt at 85 DAST in *S. chilense* (Fig. 1D, Table S2). The number of inflorescences on the main stem slightly increased with salt in *S. chilense* at 35 DAST but decreased with NaCl concentrations in both species at 85 DAST (Fig. 1E, Table S2). In contrast to the other parameters, the number of ramifications on the main stem decreased with NaCl in both species as early as 35 DAST (Fig. 1F, Table S2).

3.2. Impact of salinity on mineral concentrations

3.2.1. Sodium

Both species differed by their accumulation and repartition of Na⁺. NaCl increased Na⁺ concentrations in both species, irrespective of date and organ (Fig. 2A - D, Table S2). *Solanum chilense* accumulated more Na⁺ than *S. lycopersicum* at the plant level, with greater Na⁺ accumulation at root level in *S. lycopersicum* and at shoot level in *S. chilense*. Consequently, the highest Na⁺ concentration in *S. lycopersicum* was

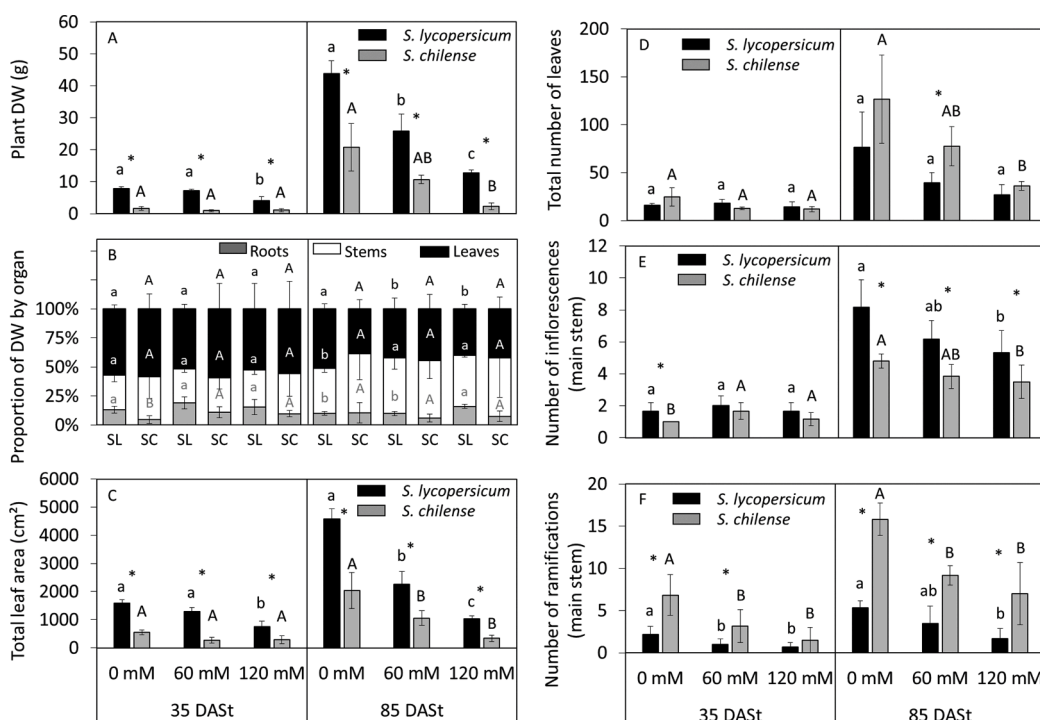


Fig. 1. Effect of salt stress (0, 60 and 120 mM NaCl) on growth parameters of *Solanum lycopersicum* and *Solanum chilense* at 35 and 85 days after stress imposition (DAST). (A) Plant dry weight, (B) proportion of dry weight according to the organs, (C) total foliar area (of all leaves) per plant, (D) total number of leaves per plant, (E) number of inflorescences on the main stem per plant, (F) number of ramifications on the main stem per plant. Data are means ± SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment on the same date. DAST, days after stress imposition, DW, dry weight, SC, *S. chilense*, SL, *S. lycopersicum*.

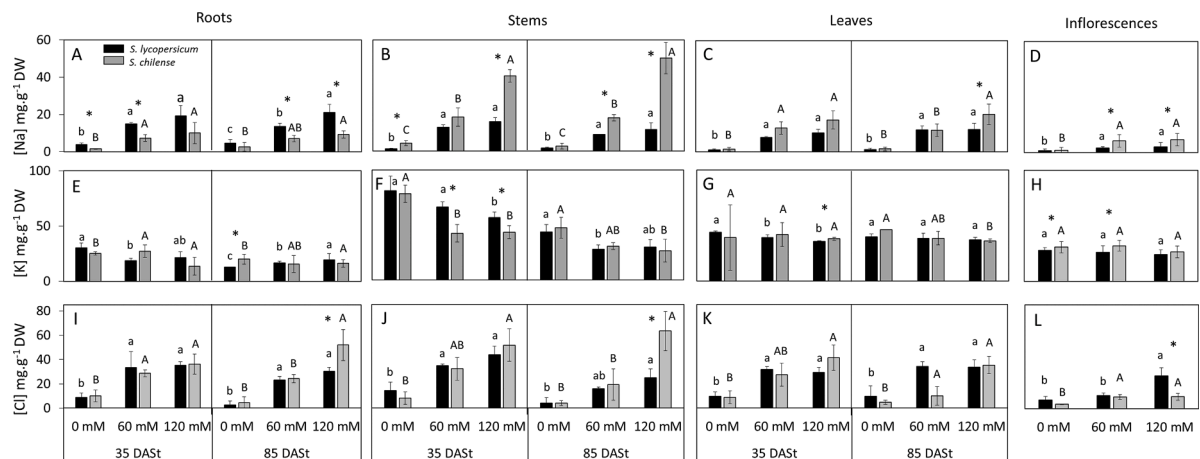


Fig. 2. Effect of salt stress (0, 60 and 120 mM NaCl) on mineral (Na^+ , K^+ , Cl^-) concentrations in *Solanum lycopersicum* and *Solanum chilense* at 35 and 85 days after stress imposition (DAST). (A-D) Na^+ concentrations, (E-H) K^+ concentrations, (I-L) Cl^- concentrations in the roots (A, E, I), stems (B, F, J), leaves (C, G, K) and inflorescences (D, H, L). Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment on the same date.

observed in roots and the highest Na^+ concentration in *S. chilense* was observed in stems (Fig. 2A, B). The ratio of Na^+ quantities between shoots and roots was 10 times higher in *S. chilense* than in *S. lycopersicum* at any date (Fig. 3A, Table S2). However, the ratio of Na^+ quantities between leaves and stems was 2 times higher in *S. lycopersicum* than in *S. chilense* at 85 DAST (Fig. 3D, Table S2). The Na^+ concentration in the inflorescences was lower than in the other organs in both species but it was higher in *S. chilense* than in *S. lycopersicum* under salinity (Fig. 2D, Table S2). The ratio of Na^+ quantities between leaves and inflorescences increased significantly with salt in *S. lycopersicum* but not in *S. chilense* (Tables 1, S2).

3.2.2. Potassium

Regardless the date, and considering all NaCl conditions, the K^+ concentration was higher in the shoots than in the roots in both species (*S. lycopersicum*, $F = 27.653$, $p < 0.001$, *S. chilense*, $F = 18.82$, $p < 0.001$, Fig. 2E, F, G). Differences between species for K^+ concentration was only observed in stems at 35 DAST (Fig. 2F, Table S2). The K^+ concentration in the inflorescences was lower than in the other aerial organs

(*S. lycopersicum*, $F = 2.855$, $p < 0.05$, *S. chilense*, $F = 12.895$, $p < 0.001$), and higher in *S. chilense* than in *S. lycopersicum* (Fig. 2H, Table S2). NaCl affected the K^+ concentration in the different organs, following a different pattern depending on species, date and organ (Fig. 2E, F, G, Table S2). It decreased K^+ concentration in stems of both species at all dates, in roots of *S. lycopersicum* at 35 DAST, in leaves of *S. lycopersicum* at 35 DAST, and in leaves of *S. chilense* at 85 DAST. However, the K^+ concentration in the inflorescences was only slightly affected by salinity (Fig. 2H, Table S2). Regarding organ ratios, salinity only decreased the ratio of K^+ quantities between shoots and roots in *S. lycopersicum* at 85 DAST (Fig. 3B, Table S2).

Regarding the Na^+/K^+ ratio, it was higher in *S. chilense* than in *S. lycopersicum* for leaves and stems at 35 DAST while it was higher in *S. lycopersicum* than in *S. chilense* for the roots at both dates (Tables 1, S2). In inflorescences, Na^+/K^+ ratio was similar between species (Tables 1, S2). NaCl increased the Na^+/K^+ ratio in all organs of both species, except in inflorescences of *S. lycopersicum* and in roots of *S. chilense* (Tables 1, S2).

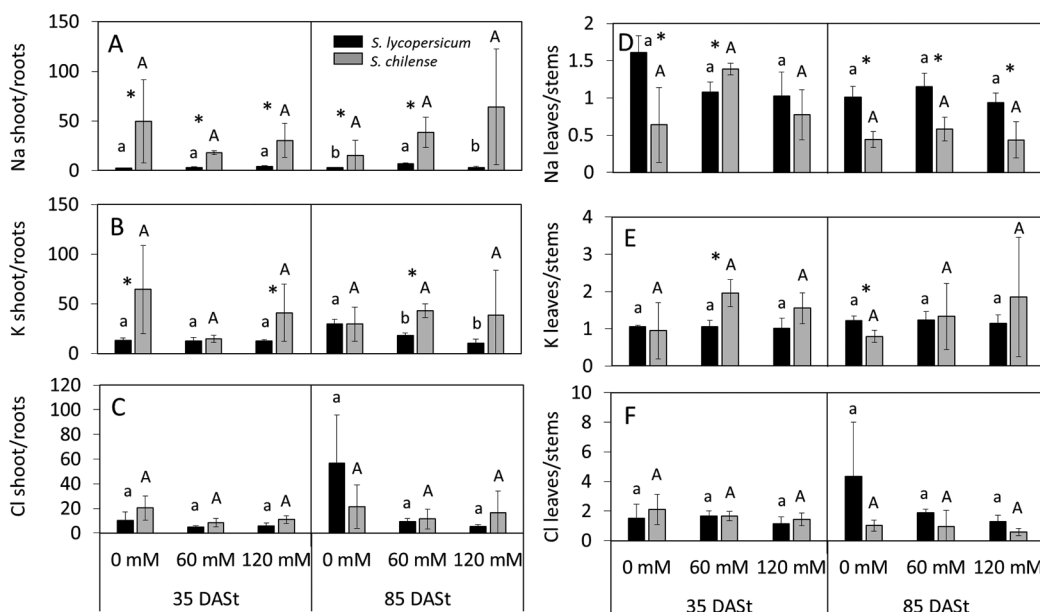


Fig. 3. Effect of salt stress (0, 60 and 120 mM NaCl) on the mineral distribution between organs in *Solanum lycopersicum* and *Solanum chilense* at 35 and 85 days after stress imposition (DAST). (A-C) Ratio of the element quantities between shoots and roots per plant and (D-F) ratio of the element quantities between leaves and stems per plant for (A, D) Na^+ , (B, E) K^+ and (C, F) Cl^- . Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment on the same date.

3.2.3. Chloride

Regardless of the date, and considering all the conditions, the Cl^- concentration was similar among vegetative organs ($F = 0.398$, $p = 0.672$, Fig. 2I, J, K). However, the Cl^- concentration was lower in the inflorescences than in the other organs (Fig. 2L). NaCl increased Cl^- concentration in all organs of both species regardless of the date (Fig. 2I–L, Table S2). At 120 mM NaCl, the Cl^- concentration was higher in the roots and the stems of *S. chilense* 85 DAST while it was lower in their inflorescences compared to *S. lycopersicum*. Salinity did not affect Cl^- ratios between shoots and roots, leaves and stems, or leaves and inflorescences in any species (Fig. 3C, F, Table 1, Table S2).

The Na^+/Cl^- ratio was higher in *S. chilense* stems at 35 DAST and in leaves at 85 DAST compared to *S. lycopersicum* while it was higher in the roots of *S. lycopersicum* than in those of *S. chilense* (Tables 1, S2). NaCl increased the Na^+/Cl^- ratio in leaves and stems of *S. lycopersicum* at 35 DAST and in leaves of *S. chilense* at 85 DAST (Tables 1, S2). This ratio also increased with NaCl in *S. chilense* inflorescences but decreased in *S. chilense* roots at 85 DAST (Tables 1, S2).

3.3. Impact of salinity on elemental composition of epidermal cells and trichomes

SEM-EDX was used to quantify elements (Na^+ , K^+ , Cl^-) on the leaf surface (Figure S1, Table 2). The ratio of Cl^- content between epidermal cells and trichomes was higher in *S. chilense* than in *S. lycopersicum* (Tables 2, S2). This ratio was only increased by salt in *S. chilense*. In this species, Cl^- content was more than 3 times higher in epidermal cells than in trichomes (Tables 2, S2). However, salinity did not affect the ratio between epidermal and trichome cells for any other element (Tables 2, S2).

3.4. Impact of salinity on the expression of Na^+ and Cl^- transporters

The expression of four genes coding for Na^+ transporters (*SOS1*, *SOS2*, *SOS3*, *HKT1;2*) and a gene coding for Cl^- transporter (*NPF2.4*) was compared in the different organs (roots, stems, leaves) of *S. lycopersicum* and *S. chilense* (Fig. 4).

Regarding the SOS pathway, SOS genes were expressed in all organs in both species although some differences were observed among organs and species (Fig. 4A–C, Table S2). Their expression was affected by salinity, particularly in *S. lycopersicum* (Fig. 4A–C). Indeed, NaCl increased the expression of *SOS1* in all organs of *S. lycopersicum* but not in *S. chilense* (Fig. 4A, Table S2). NaCl increased the expression of *SOS2* in stems of both species and in leaves of *S. lycopersicum* while it decreased it in leaves of *S. chilense* (Fig. 4B, Table S2). NaCl also

Table 2

Effect of salt stress (0 and 120 mM NaCl) on the ratio of element content between trichome basal cells and epidermal cells measured by scanning electron microscopy and energy dispersive X-Ray spectroscopy.

ratio	ion	<i>S. lycopersicum</i>		<i>S. chilense</i>	
		0 mM	120 mM	0 mM	120 mM
epidermal cell/ trichome	Na	1.20 $\pm 1.42^a$	1.17 $\pm 0.17^a$	0.72 $\pm 0.90^A$	0.71 $\pm 0.67^A$
epidermal cell/ trichome	K	1.26 $\pm 0.27^a$	1.08 $\pm 0.18^a$	1.07 $\pm 0.05^A$	1.90 $\pm 1.10^A$
epidermal cell/ trichome	Cl	1.27 $\pm 0.28^a$	1.53 $\pm 0.36^a$	1.51 $\pm 0.39^B$	3.12 $\pm 0.57^A$

Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species.

increased *SOS3* expression in roots and leaves of *S. lycopersicum* and decreased the expression of *SOS3* in the leaves of *S. chilense* (Fig. 4C, Table S2).

In contrast to the SOS pathway, the expression of *HKT1;2* was almost not affected by salinity but its expression strongly depended on the organs (Fig. 4D). *HKT1;2* was mainly expressed in the roots regardless of the species.

Regarding Cl^- transport, the expression of *NPF2.4* was higher in roots than in the other organs in *S. chilense*, ($F = 8.679$, $p < 0.001$) but was similar in all organs in *S. lycopersicum* ($F = 0.626$, $p = 0.538$, Fig. 4E). Its expression was not affected by salinity except in the leaves of *S. chilense* (Fig. 4E, Table S2).

3.5. Impact of salinity on physiological parameters

3.5.1. Photosynthesis-related parameters

The chlorophyll content (CCI) was higher in *S. lycopersicum* than in *S. chilense* at all dates (Tables 3, S2). It was not affected by salinity except for *S. lycopersicum* where it increased with salt at 70 DAST (Tables 3, S2).

Some chlorophyll fluorescence parameters differed between species (Table 3, S2): F_v/F_m was lower in *S. lycopersicum* than in *S. chilense* at all dates, NPQ was lower in *S. lycopersicum* than in *S. chilense* at 27 DAST, and qP was higher in *S. lycopersicum* than in *S. chilense* at 27 DAST. Salinity did not affect chlorophyll fluorescence parameters except F_v/F_m that decreased under salt stress in *S. lycopersicum* at 70 DAST (Table 3, S2).

Regarding gas exchanges, A was higher in *S. chilense* than in *S. lycopersicum* at 27 DAST but this difference was not observed at 70 DAST (Fig. 5A, Table S2). However, Ci was similar in both species at all dates (Fig. 5B, Table S2). Salinity decreased Ci in *S. lycopersicum* 27 DAST

Table 1

Effect of salt stress (0, 60 and 120 mM NaCl) on ratios of ion concentrations between organs at 35 and 85 days after stress imposition (DAST).

organs	ion	DAST	<i>S. lycopersicum</i>			<i>S. chilense</i>		
			0 mM	60 mM	120 mM	0 mM	60 mM	120 mM
leaves/inflorescences	Na	85	2.04 $\pm$ 1.26 ^b	4.49 $\pm$ 1.41 ^a	2.37 $\pm$ 2.40 ^{ab}	1.14 $\pm$ 0.46 ^A	2.13 $\pm$ 2.00 ^A	6.03 $\pm$ 3.17 ^A
	Cl	85	1.70 $\pm$ 1.97 ^a	3.23 $\pm$ 0.49 ^a	1.28 $\pm$ 0.17 ^a	1.34 $\pm$ 0.53 ^A	1.15 $\pm$ 0.92 ^A	3.87 $\pm$ 1.77 ^A
	K	85	1.52 $\pm$ 0.10 ^a	1.51 $\pm$ 0.47 ^a	1.18 $\pm$ 0.17 ^a	1.61 $\pm$ 0.09 ^A	1.21 $\pm$ 0.18 ^A	1.34 $\pm$ 0.33 ^A
leaves	Na/K	35	0.02 $\pm$ 0.01 ^b	0.20 $\pm$ 0.02 ^a	0.29 $\pm$ 0.05 ^a	0.05 $\pm$ 0.02 ^C	0.31 $\pm$ 0.03 ^B	0.45 $\pm$ 0.12 ^A
		85	0.03 $\pm$ 0.02 ^b	0.31 $\pm$ 0.04 ^a	0.32 $\pm$ 0.08 ^a	0.03 $\pm$ 0.02 ^B	0.31 $\pm$ 0.12 ^A	0.55 $\pm$ 0.13 ^A
stems	Na/K	35	0.02 $\pm$ 0.00 ^c	0.19 $\pm$ 0.01 ^b	0.28 $\pm$ 0.06 ^a	0.06 $\pm$ 0.02 ^C	0.43 $\pm$ 0.04 ^B	0.94 $\pm$ 0.07 ^A
		85	0.04 $\pm$ 0.02 ^b	0.33 $\pm$ 0.05 ^a	0.39 $\pm$ 0.09 ^a	0.06 $\pm$ 0.05 ^B	0.59 $\pm$ 0.02 ^{AB}	2.20 $\pm$ 1.26 ^A
roots	Na/K	35	0.12 $\pm$ 0.03 ^b	0.81 $\pm$ 0.12 ^a	0.89 $\pm$ 0.04 ^a	0.06 $\pm$ 0.01 ^C	0.27 $\pm$ 0.08 ^B	0.78 $\pm$ 0.12 ^A
		85	0.37 $\pm$ 0.16 ^b	0.81 $\pm$ 0.13 ^a	1.10 $\pm$ 0.08 ^a	0.15 $\pm$ 0.14 ^A	0.51 $\pm$ 0.20 ^A	0.60 $\pm$ 0.20 ^A
inflorescences	Na/K	85	0.03 $\pm$ 0.02 ^a	0.10 $\pm$ 0.02 ^a	0.27 $\pm$ 0.18 ^a	0.01 $\pm$ 0.01 ^B	0.27 $\pm$ 0.18 ^A	0.13 $\pm$ 0.02 ^{AB}
leaves	Na/Cl	35	0.12 $\pm$ 0.04 ^c	0.25 $\pm$ 0.01 ^b	0.35 $\pm$ 0.02 ^a	0.24 $\pm$ 0.25 ^A	0.56 $\pm$ 0.39 ^A	0.42 $\pm$ 0.06 ^A
		85	0.17 $\pm$ 0.08 ^a	0.35 $\pm$ 0.03 ^a	0.38 $\pm$ 0.17 ^a	0.29 $\pm$ 0.11 ^B	0.73 $\pm$ 0.08 ^A	0.57 $\pm$ 0.06 ^A
stems	Na/Cl	35	0.09 $\pm$ 0.02 ^b	0.37 $\pm$ 0.04 ^a	0.37 $\pm$ 0.04 ^a	0.72 $\pm$ 0.47 ^A	0.64 $\pm$ 0.37 ^A	0.81 $\pm$ 0.13 ^A
		85	0.75 $\pm$ 0.69 ^a	0.57 $\pm$ 0.05 ^a	0.48 $\pm$ 0.09 ^a	0.66 $\pm$ 0.17 ^A	0.64 $\pm$ 0.06 ^A	0.81 $\pm$ 0.09 ^A
roots	Na/Cl	35	0.49 $\pm$ 0.32 ^a	0.50 $\pm$ 0.22 ^a	0.54 $\pm$ 0.14 ^a	0.16 $\pm$ 0.06 ^A	0.26 $\pm$ 0.07 ^A	0.30 $\pm$ 0.18 ^A
		85	3.37 $\pm$ 2.71 ^a	0.58 $\pm$ 0.06 ^a	0.69 $\pm$ 0.08 ^a	0.65 $\pm$ 0.23 ^A	0.29 $\pm$ 0.08 ^{AB}	0.19 $\pm$ 0.08 ^B
inflorescences	Na/Cl	85	0.13 $\pm$ 0.14 ^a	0.27 $\pm$ 0.09 ^a	0.28 $\pm$ 0.12 ^a	0.09 $\pm$ 0.06 ^B	0.91 $\pm$ 0.61 ^A	0.41 $\pm$ 0.25 ^{AB}

Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date.

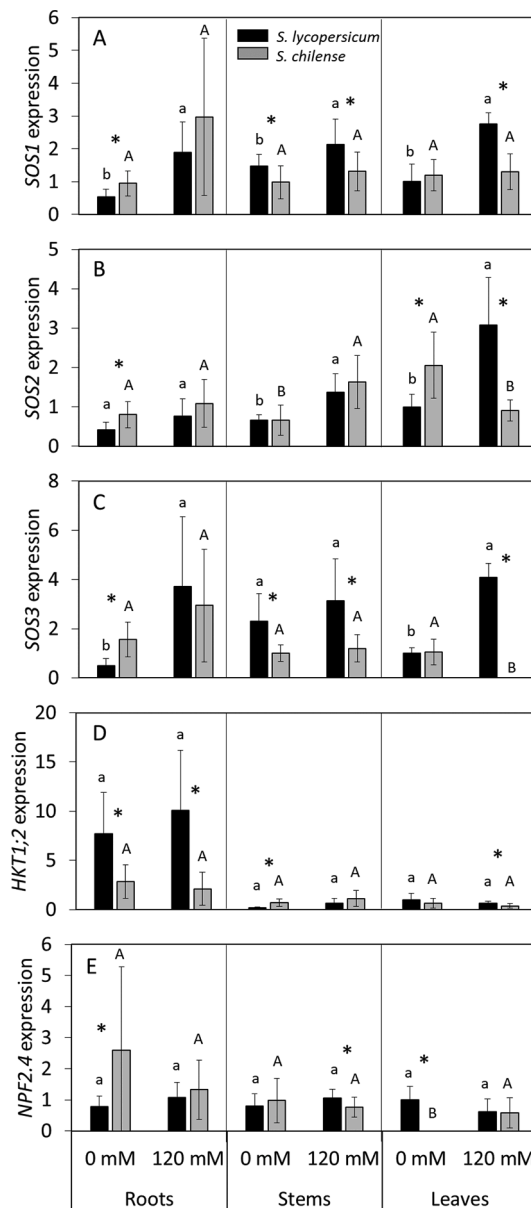


Fig. 4. Effect of salt stress (0 and 120 mM NaCl) on the relative expression of genes coding for Na^+ or Cl^- transporters in roots, stems and leaves of *Solanum lycopersicum* and *Solanum chilense*. (A) SOS1 (Salt Overly Sensitive 1, Solyc01g005020), (B) SOS2 (Salt Overly Sensitive 2, Solyc12g009570), (C) SOS3 (Salt Overly Sensitive 3, Solyc06g051970), (D) HKT1;2 (class I - High affinity K^+ transporter 2, Solyc07g014680), (E) NPF2.4 (NRT1/PTR 2.4, Solyc11g072580). The tomato elongation factor gene (*LeEF-1 α* , Solyc06g005060) and TIP41-like protein (*TIP41*, Solyc10g04985) were used as reference genes. Gene expression was expressed relative to expression in the leaves of *S. lycopersicum* grown at 0 mM NaCl, to which a value of 1 was assigned. Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species and organ. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment and organ.

and A in both species at 120 mM NaCl 70 DAST (Fig. 5A, B, Table S2).

3.5.2. Plant water status

Instantaneous transpiration (E) and stomatal conductance (g_s) were about 2 times higher in *S. chilense* than in *S. lycopersicum* whatever the date (Fig. 5C, D, Table S2). Both parameters decreased with salinity in the two species whatever the date (Fig. 5C, D, Table S2).

The WC varied between species depending on the organs (Fig. 6A-C,

Table S2) and it decreased with time in both species whatever the organ (Fig. 6, Table S4). The effect of salt on WC depended on the organ and the species (Fig. 6A-C, Table S2). Salinity decreased root WC 35 DAST in *S. chilense* and 85 DAST in *S. lycopersicum* (Fig. 6A), stem WC 35 DAST in *S. lycopersicum* (Fig. 6B), but did not affect leaf WC whatever the species (Fig. 6C).

Osmotic potential was lower in *S. lycopersicum* than in *S. chilense* and decreased progressively with salinity in both species (Fig. 6D, Table S2). In response to osmotic stress, proline concentration increased with salt in both species (Fig. 6E, Table S2). This increase was more gradual in *S. chilense* than in *S. lycopersicum*. In contrast, the concentration of soluble sugars was not affected by salinity (Fig. 6F, Table S2).

3.6. Impact of salinity on mesophyll structure

Histological modifications were observed in response to salinity in the leaves, but these differed between species (Fig. 7). The leaf blade was thicker in *S. lycopersicum* than in *S. chilense* due to a greater thickness of the palisade and spongy parenchyma (Fig. 7A-C, Table S2). The xylem vessels were also wider in *S. lycopersicum* than in *S. chilense* (Fig. 7E, Table S2). Salt increased leaf blade thickness in *S. chilense* but not in *S. lycopersicum* (Fig. 7A, Table S2). While the thickness of spongy parenchyma was not affected, salinity decreased the thickness of palisade parenchyma in *S. lycopersicum* and increased it in *S. chilense* (Fig. 7B-C, Table S2). The relative importance of the spongy parenchyma thus increased only in *S. lycopersicum* (Fig. 7D, Table S2). The diameter of xylem vessels decreased with salt in both species, although more markedly in *S. lycopersicum* (Fig. 7E, Table S2).

4. Discussion

A schematic representation of the response of both species to salinity regarding ionic and osmotic components is available in Fig. 8.

4.1. Growth is affected differently by NaCl in both species

Our results showed that salinity reduced plant growth in both species, although the response differed between species depending on the stress duration. In accordance with previous results (Gharbi et al., 2016, 2017b; Martínez et al., 2014), no decrease in *S. chilense* growth was observed at vegetative stage. However, at reproductive stage, DW was reduced in both species. This shows that the response of *S. chilense* to salinity differed between short- and long-term stress. Even halophyte plants need to produce osmotic protectants and antioxidants: a reallocation of resources to metabolism at the expense of growth is therefore inevitable (Flowers et al., 2015; Arbelet-Bonnin et al., 2020).

The present work demonstrates that the reduction of plant growth induced by salt was related to a reduction in leaf production, but above all to a reduction in branching and leaf area. Salt decreased plant branching and leaf area in other plants such as *Limonium linifolium* or *Lippia gracilis* (Tabot and Adams 2014; Oliveira et al., 2019). We could hypothesize that the reduction of branching could enable to decrease the strength of Na sinks in order to limit the entry of toxic ions. Indeed, the entry of Na^+ ions via the xylem is linked to the transpiration stream (Munns and Tester 2008). Thus, decreasing the number of Na sinks, or transpiring leaf area, would limit the entry of Na^+ in the aerial parts. The decrease of growth in *S. lycopersicum* could also be explained by the strong decrease in the diameter of the xylem vessels which can affect the transport of water and nutrients and consequently growth (Naz et al., 2022).

Salt affected reproductive growth in both species, as illustrated by the decrease in the number of inflorescences on the main stem. The reproductive phase is affected in numerous plants subjected to salinity, including true halophytes such as *Crithmum maritimum* (Ventura et al., 2014). This could be explained by the competition for assimilates between reproductive organ development and plant defense mechanisms

Table 3

Effects of salt stress (0, 60 and 120 mM NaCl) on physiological parameters of *Solanum lycopersicum* and *Solanum chilense* at 27 and 70 days after stress imposition (DAST).

parameter	DAST	<i>S. lycopersicum</i> 0 mM	60 mM	120 mM	<i>S. chilense</i> 0 mM	60 mM	120 mM
CCI	27	9.69±9.57 ^a	5.05±6.74 ^a	6.58±10.68 ^a	4.31±2.12 ^A	4.66±1.05 ^A	2.05±0.17 ^A
CCI	70	20.79±3.06 ^b	37.69±12.3 ^a	29.63±12.34 ^a	7.96±4.54 ^A	5.59±3.44 ^A	8.47±5.73 ^A
Fv/Fm	27	0.881±0.004 ^a	0.883±0.004 ^a	0.878±0.007 ^a	0.908±0.012 ^A	0.893±0.004 ^A	0.894±0.011 ^A
Fv/Fm	70	0.883±0.010 ^{ab}	0.888±0.004 ^a	0.870±0.009 ^b	0.897±0.006 ^A	0.888±0.013 ^A	0.884±0.010 ^A
φPSII	27	0.818±0.015 ^a	0.827±0.02 ^a	0.81±0.015 ^a	0.822±0.054 ^A	0.754±0.108 ^A	0.751±0.104 ^A
φPSII	70	0.830±0.011 ^a	0.788±0.066 ^a	0.744±0.092 ^a	0.808±0.048 ^A	0.797±0.047 ^A	0.786±0.094 ^A
NPQ	27	0.21±0.06 ^a	0.18±0.03 ^a	0.24±0.06 ^a	0.39±0.16 ^A	0.35±0.14 ^A	0.28±0.05 ^A
NPQ	70	0.19±0.03 ^a	0.23±0.13 ^a	0.27±0.22 ^a	0.17±0.04 ^A	0.3±0.14 ^A	0.33±0.08 ^A
qP	27	0.96±0.01 ^a	0.96±0.02 ^a	0.96±0.01 ^a	0.91±0.06 ^A	0.88±0.12 ^A	0.87±0.12 ^A
qP	70	0.97±0.01 ^a	0.91±0.07 ^a	0.86±0.08 ^a	0.92±0.06 ^A	0.93±0.03 ^A	0.93±0.09 ^A

Data are means ±SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date.

CCI, chlorophyll content index; DAST, days after stress imposition; Fv/Fm, maximum quantum efficiency of PSII photochemistry; NPQ, non-photochemical quenching; φPSII, quantum efficiency of PSII; qP, photochemical quenching.

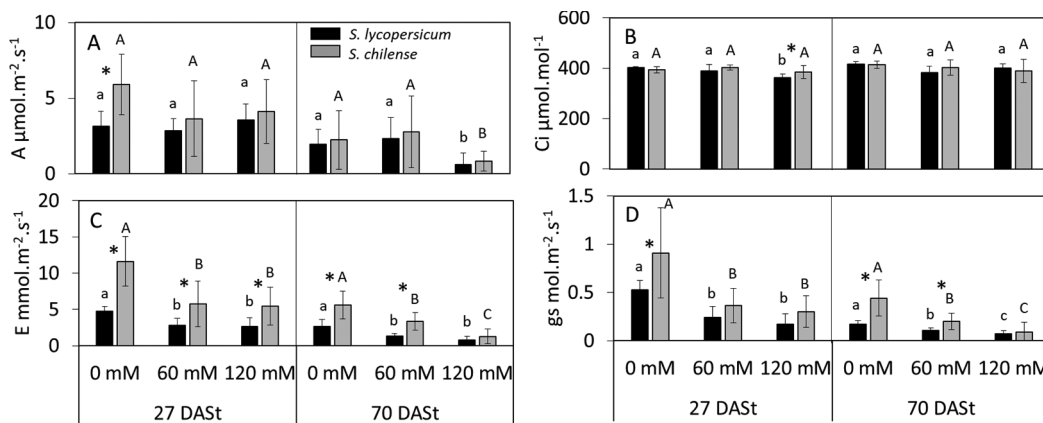


Fig. 5. Effect of salt stress (0, 60 and 120 mM NaCl) on photosynthesis related parameters in *Solanum lycopersicum* and *Solanum chilense* at 27 and 70 days after stress imposition (DAST). (A) Net photosynthesis (A), (B) inter-cellular CO₂ concentration (Ci), (C) net transpiration (E), and (D) stomatal conductance (gs). Data are means ± SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment on the same date.

(Ventura et al., 2014; Liu et al., 2019). Another explanation can be found in water management: the high water cost during anthesis could not be achieved due to decreased transpiration (Roddy et al., 2016), especially in *S. lycopersicum*.

4.2. Ion distribution is differently managed in both species

To limit the negative impact of toxic elements, some species limit their transfer to the shoot (“excluder” strategy) while other species cope with the accumulation and sequestration of the toxic elements within the shoot (“includer” strategy). Here, we observed that *S. lycopersicum* and *S. chilense* differed regarding their accumulation of Na⁺ and Cl⁻ and the expression of genes coding for transporters. No salt gland or bladder were found on the adaxial leaf surface in either species.

4.2.1. *S. lycopersicum* behaved as an excluder of Na⁺ and *S. chilense* as an includer

Our results showed that Na⁺ was mainly accumulated in the roots in *S. lycopersicum* and in the shoots in *S. chilense* throughout the experiment. Gharbi et al. (2017b) previously observed a similar behavior under short-term stress (7 days at 125 mM NaCl). This led us to conclude that *S. lycopersicum* and *S. chilense* were “excluder” and “includer” species respectively. The two types of strategy require energy and thus have a metabolic cost. It occurs that a given plant species may shift from one strategy to the other considering the duration of stress exposure and the cost of regulation of ion transport and sequestration, especially in relation to the capacity of Na⁺ storage in aerial parts (Munns et al., 2020). Nevertheless, according to our results, excluder and includer strategies of the two considered species seem to be maintained in long-term stress.

The includer strategy was observed in other tomato wild species in

response to salinity (Tal et al., 1979; Tal and Shannon 1983; Almeida et al., 2014). For example, *Solanum pennellii* was shown to accumulate more Na than *S. lycopersicum* under salt stress, and it was linked to a different affinity of *HKT1;2* in both species toward Na⁺ (Almeida et al., 2014). In the same way, in *S. cheesmaniae*, silencing of *HKT1;2* led to an enhanced Na accumulation in shoots (Romero-Aranda et al., 2021). In our study, *HKT1;2* expression was very high in roots of *S. lycopersicum* compared to other organs and was also higher than in *S. chilense*. *HKT1;2* is involved in retrieval of Na⁺ from the xylem sap (Almeida et al., 2014). Thus, *S. lycopersicum* would be able to limit Na⁺ accumulation in shoots, notably thanks to the high root expression of *HKT1;2*. Moreover, salt increased the expression of genes from the SOS pathway in the roots of *S. lycopersicum* but not in the roots of *S. chilense*. The SOS pathway is involved in Na⁺ extrusion (Ji et al., 2013). The increase of *SOS1* and *SOS3* expression in roots of *S. lycopersicum* suggests that Na⁺ was excluded from the roots by the SOS pathway, in addition to an exclusion out of aerial parts. However, despite the increase of expression of SOS pathway genes in roots of *S. lycopersicum*, Na⁺ concentration increased in this organ, thus suggesting that the exclusion process ensured by *SOS1* might be saturated in the glycophyte species for the considered NaCl doses (Nieves-Cordones et al., 2016).

As an includer species, *S. chilense* accumulated more Na⁺ in the shoot than *S. lycopersicum*, particularly in stems. This suggests that *S. chilense* protected the photosynthetically active tissues by sequestering Na⁺ in the stems, a strategy that was not observed in *S. lycopersicum* in our study. Olías et al. (2009) however reported that the Na⁺ sensitivity of *SOS1* silenced plants correlated with higher accumulation of Na⁺ in leaves and roots, but lower contents in stems. However, despite its role in the partitioning of the toxic ion between plant organs and the exclusion of Na⁺ out of the leaves (Ji et al., 2013), the expression of SOS

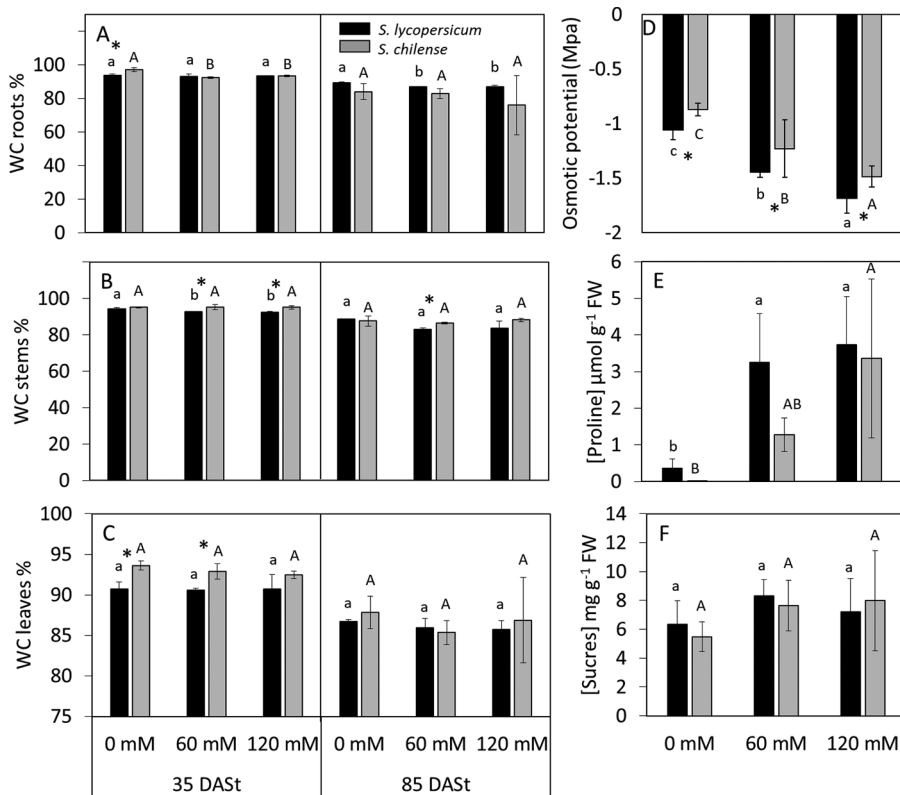


Fig. 6. Effect of salt stress (0, 60 and 120 mM NaCl) on water status-related parameters in *Solanum lycopersicum* and *Solanum chilense* at 35 and 85 days after stress imposition (DAST). (A-C) Water content in (A) roots, (B) stems, and (C) leaves. (D) Osmotic potential, (E) concentration of proline and (F) concentration of soluble sugars. Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species on the same date. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment on the same date.

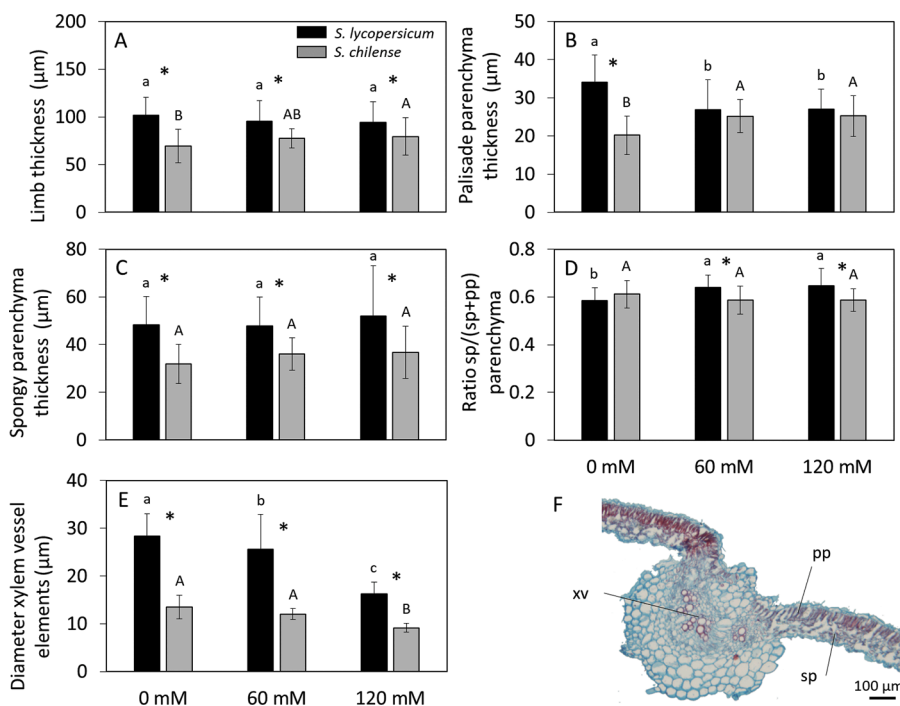


Fig. 7. Effect of salt stress (0, 60 and 120 mM NaCl) on mesophyll characteristics of *Solanum lycopersicum* and *Solanum chilense* (A) Thickness of the limb, (B) thickness of the palisade parenchyma, (C) thickness of the spongy parenchyma, (D) ratio between the thickness of the spongy parenchyma (sp) and the sum of the spongy and the palisade (pp) parenchyma thickness, (E) diameter of the xylem vessel elements, (F) transversal section of a leaflet of *S. lycopersicum* grown at 0 mM NaCl. pp, palisade parenchyma, sp, spongy parenchyma, xv, xylem vessel elements. Data are means $\pm$ SD. Different letters indicate significant differences ($p < 0.05$) between treatments for a same species. Asterisks indicate significant differences ($p < 0.05$) between species for a same treatment.

genes was not significantly increased by salt in *S. chilense*, except for *SOS2* in the stems. We surprisingly observed that salinity decreased the expression of *SOS2* and *SOS3* in the leaves of *S. chilense*. Sodium uptake by plant cells is a passive process and its exclusion out of mesophyll cells occurs through active transporters and require energy (Shabala and Shabala 2011). Other processes may be involved in the tolerance of *S. chilense* leaf tissue: vacuolar sequestration is especially important

considering that it will allow the use of Na^+ as a cheap osmoticum to regulate cell turgor. Transporters involved in Na^+ transfer to the vacuole (mainly those of the NHX family) still need to be studied in *S. chilense*. In *S. lycopersicum*, the high salt activation of SOS pathway in leaves seem not to be sufficient to afford tissue tolerance to Na^+ . The quantification of total Na^+ content at the whole leaf level did not provide us information regarding distribution of this element between apoplastic and

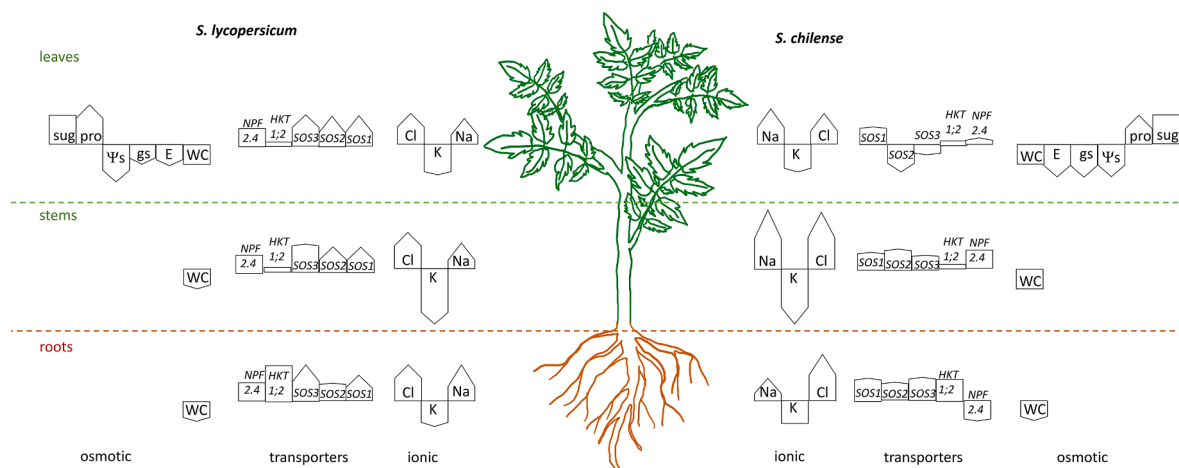


Fig. 8. Schematic representation of the response of *Solanum lycopersicum* and *Solanum chilense* to salinity regarding ionic and osmotic components and gene expression. Plants were grown in a mixture of perlite:vermiculite supplied with 0, 60 and 120 mM NaCl. The relative size of the boxes shows the difference among organs and species. The size of the arrow shows the size of the increase or decrease due to NaCl. sug: sugar, pro: proline, Ψ_s : osmotic potential, gs: stomatal conductance, E: transpiration, WC: water content, Cl: chloride, K: potassium, Na: sodium, SOS1: Salt Overly Sensitive 1 (Solyc01g005020), SOS2: Salt Overly Sensitive 2 (Solyc12g009570), SOS3: Salt Overly Sensitive 3 (Solyc06g051970), HKT1;2: class 1 - High affinity K^+ transporter 2 (Solyc07g014680), NPF2.4: NRT1/PTR 2.4 (Solyc11g072580).

symplastic compartments. It could not be excluded that a re-excretion of high amounts of Na^+ to the apoplast at the leaf level is not necessarily useful if it induces a significant osmotic constraint at the whole cell level (including vacuoles). The SOS pathway interact with other transporters to regulate Na^+ homeostasis (Xie et al., 2022) and we may not exclude that some systems are predominant over others depending on the species. Moreover, regulatory proteins (SOS2, SOS3,...) may have additional functions that interactions with SOS1 and SOS2. For example, they were shown to be involved in ROS signaling and H_2O_2 metabolism (Kumar et al., 2022).

4.2.2. Salinity decreased the K^+/Na^+ ratio in both species

Under salt stress, avoidance of K^+ deficiency is of paramount importance. Potassium concentration decreased with salinity mainly in the stems and the K^+/Na^+ ratio decreased in both species. In contrast to Na^+ , K^+ is an essential element used in different functions, such as in various steps of cell metabolism, photosynthesis and turgor pressure maintenance (Nieves-Cordones et al., 2016). Maintaining a high K^+/Na^+ ratio in shoots is thus necessary. Total exclusion of both Na^+ and K^+ from the shoots would be deleterious to K^+ nutrition, but managing sufficient K^+ transfer to the shoots may also contribute to increase Na^+ since both ions could be transported by the same ion channels and transporters with low ionic selectivity. For example, class 1 HKT transporters are usually Na^+ selective, even if some of them have been shown to transport also K^+ (Nieves-Cordones et al., 2016). Another example is the CNGC (cyclic nucleotide-gated channels) which contribute to cations transport across the plasma membrane (Ghorbani et al., 2019). Non-selective cation channels, and consequently CNGC genes are up-regulated under salinity treatments, which can lead to an increase of K^+ uptake but also of Na^+ uptake, because of their poor selectivity towards K^+ (Jin et al., 2015; Nieves-Cordones et al., 2016; Ghorbani et al., 2019). Under salinity, competition between Na^+ and K^+ for transporters could be in favor of Na^+ and thus explain the decrease in K^+ concentration we observed. It has however to be mentioned that in both species, the decrease of K^+ concentration due to salinity was mainly observed in the stems and remained limited in the leaves. It can therefore be hypothesized that selective K^+ transporters favor K^+ accumulation in the leaves rather than in the stems in response to salinity in our experiment.

4.2.3. Chloride accumulated more in shoots than in roots in both species under salinity

In the context of salt stress, the impact of excess Cl^- is less studied than the impact of Na^+ excess or K^+ deficiency (Tavakkoli et al., 2011; Franco-Navarro et al., 2016; Li et al., 2017). We observed that salinity increased Cl^- concentrations in all organs of both species and that Cl^- accumulated more in shoots than in roots. This contrasts with the results of Rush and Epstein (1981) who observed that *S. lycopersicum* accumulated Cl^- mainly in roots in response to a short-term stress. We nevertheless observed that, under salinity, the Cl^- shoot/root ratio was higher in *S. chilense* than in *S. lycopersicum*, meaning that *S. chilense* transferred more Cl^- in the shoot than *S. lycopersicum*. This could be explained by the higher expression of NPF2.4 in the roots of *S. chilense* than in those of *S. lycopersicum*. It was indeed reported that the NPF2.4 transporter is involved in Cl^- loading in root xylem sap and is specific to this anion in *Arabidopsis* (Li et al., 2016). However, the expression of NPF2.4 was not affected by salinity in our study, in contrast to what was observed in roots of *Arabidopsis thaliana* grown under salt stress (down-regulation by salt in roots).

As observed for Na^+ , Cl^- was present at a higher concentration in the stems than in the leaves of *S. chilense* and the Cl^- leaves/stems ratio decreased with time. Chloride avoidance in the photosynthesis active organs could be a strategy of the wild species to limit Cl^- toxicity. Indeed, despite the need for Cl^- in several metabolic processes, such as in the oxygen transformation complex in PSII, it has been shown in barley that excess Cl^- has a deleterious effect, especially on photosynthetic capacity (Kawakami et al., 2009; Tavakkoli et al., 2011). However, *S. lycopersicum* does not seem to be capable of such an exclusion mechanism, as Cl^- concentration was higher or similar in leaves than in stems. Although NPF2.4 could be involved in the Cl^- transfer from root to shoot in *S. chilense*, it seems not involved in the exclusion of Cl^- out of the leaves in *S. lycopersicum* since its expression was increased by salinity only in *S. chilense*. Other transporters are involved in Cl^- homeostasis in plants (Li et al., 2017) and their involvement in salt tolerance of cultivated and wild tomato species require further investigation.

4.2.4. Both species limited the accumulation of Na^+ and Cl^- in the inflorescences

Regardless of the 'includer' or 'excluder' strategy of the studied species, both limited Na^+ and Cl^- accumulation in the inflorescences compared to the other organs. To the best of our knowledge, it is the first

time that the concentration of Cl^- was investigated in the reproductive organs of tomato. Regarding Na^+ , our results contrast with the results of Ghanem et al. (2009) who showed a high Na^+ concentration in tomato inflorescences in response to salinity compared to the other organs in *S. lycopersicum*. However, they confirm our previous results showing that *S. lycopersicum* accumulated less Na^+ in the flowers than *S. chilense* (Bigot et al., 2022). Such discrepancy could be explained by the timing of stress application. Indeed, in the experiment of Ghanem et al. (2009), salt was applied specifically at the flowering stage for 10 days, when the inflorescence developed while in our experiment salt was applied since seedling stage and maintained for 85 days. The response of the plant may therefore differ depending on the stage of development at which the stress is applied.

4.3. Osmotic balance is differently adjusted in both species

We observed that *S. lycopersicum* and *S. chilense* differed in their physiological responses, especially concerning gas exchanges and mesophyll structure. *Solanum chilense* is reported to be a drought tolerant species and has developed several adaptations to arid habitat (Tapia et al., 2016; Beddows et al., 2017; Blanchard-Gros et al., 2021). We observed that *S. chilense* had a smaller leaf area than *S. lycopersicum* but a higher transpiration rate, photosynthesis rate and stomatal conductance even under control conditions. Leaf anatomy also differed between both species as *S. chilense* had thinner leaves with smaller xylem vessels than *S. lycopersicum*. Small xylem vessels were reported to be an adaptation to saline sites in halophytes species such as *Suaeda vera* (Naz et al., 2022). Salinity decreased diameter of xylem vessels in both tomato species but more drastically in *S. lycopersicum*. Change in xylem anatomy affect xylem hydraulic conductance and determine its relevant ability to regulate water loss (Jerszurki et al., 2020). A decrease in xylem vessel diameter is a long-term plant response to environmental stress such as salinity that will decrease transpiration rate, as observed in our experiment. Water losses are also regulated at the stomata level and we observed that stomatal conductance decreased with salinity in both species. The decrease in stomatal conductance via stomatal closure is one of the earliest and most important plant responses to stress conditions, but it also affect photosynthesis by limiting CO_2 uptake (Jerszurki et al., 2020), which was observed in both species at 70 DAS at 120 mM NaCl. It was suggested that the combination of a decrease in stomatal conductance with a same parenchyma thickness could lead the plant to use less efficiently the CO_2 under salt stress (Delfine et al., 1998). Intercellular CO_2 (Ci) decreased with salt and time in *S. lycopersicum* although the thickness of the spongy parenchyma was not affected, and therefore probably the volume of intercellular spaces as well. Delfine et al. (1998) observed that both mesophyll and stomatal conductance decreased in salt-stressed spinach leaves, which contrasts with our results for *S. lycopersicum* but agrees with our data in *S. chilense*. However, we observed a difference in the proportion of spongy and palisade mesophyll in *S. lycopersicum* which could also affect mesophyll conductance. Measuring the mesophyll conductance in both species would improve our understanding of CO_2 diffusion in the leaves under salinity.

Despite a higher transpiration rate, the WC was higher in the aerial organs of *S. chilense* than in those of *S. lycopersicum*. Leaves of *S. chilense* have been described as succulent by Martínez et al. (2014). Succulence enables to protect plants against NaCl stress, especially by diluting toxic ions (Martínez et al., 2014). Moreover, an increase in the leaf thickness under salinity was reported to contribute to the succulence of leaves in saline sites (Naz et al., 2022). We observed that salinity increased leaf and palisade parenchyma thickness in *S. chilense*, confirming this hypothesis.

Despite anatomical differences between *S. lycopersicum* and *S. chilense*, both species maintain their leaf WC under salt stress conditions. Another strategy to maintain water content under salinity is osmotic adjustment (Munns and Tester 2008; Munns et al., 2020). In

tomato, osmotic adjustment is usually achieved by synthesis of proline and soluble sugars (Balibrea et al., 1997; Kashyap et al., 2021). We observed that salinity decreased osmotic potential in both species, as previously observed (Gharbi et al., 2017c). Surprisingly, in our experiment, soluble sugars concentration did not increase with NaCl in either species, whereas Gharbi et al. (2017c) observed an increase in soluble sugars in *S. chilense* submitted to a short-term salt stress. It is not excluded that in our long-term stress, sugars contributed mainly to plant growth and development and not to osmotic adjustment. However, proline concentration increased in leaves of both species, and especially in *S. lycopersicum*, underlying the importance of this amino acid in osmotic response to salt stress (Gharsallah et al., 2016). Accumulation of inorganic ions are energetically cheaper than the synthesis of organic osmolytes and may also contribute to osmotic adjustment (Flowers et al., 2015). Gharbi et al. (2017c) observed that inorganic ions (Na^+ and K^+) were the main contributors to osmotic adjustment in *S. chilense* exposed for a short-term salt stress. The 3 major inorganic ions contributing to osmotic adjustment are K^+ , Na^+ and Cl^- (Shabala and Shabala 2011). We observed that Cl^- contributed to 45% and 32% of the osmotic adjustment while Na^+ contributed to 30% and 41% of the osmotic adjustment in *S. lycopersicum* and *S. chilense*, respectively. However, because of its high toxicity to cytosolic enzymes, Na^+ must be compartmented inside vacuoles. Active transport of Na^+ to this compartment has a metabolic cost since it requires the contribution of V-ATPase (EC 3.6.1.3) or V-PPase (EC 3.6.1.1) (Mohammed et al., 2012). Potassium is the most cytosolically abundant ion and is thus suitable for osmotic adjustment although its concentration often decreases under salinity (Shabala and Shabala 2011). In our experiment, K^+ contributed to 15.1% of the osmotic adjustment in *S. chilense* and only to 2.1% in *S. lycopersicum*. It should be noted that the calculations of the contribution of the different compounds to the osmotic adjustment should be interpreted with caution, since they assume a vacuolar volume corresponding to 90% of the cell and that the toxic elements are sequestered in the vacuole. In barley, Na^+ and Cl^- contribution to osmotic adjustment were similar between salt tolerant and sensitive genotypes while K^+ and organic compounds mainly contributed to osmotic adjustment in tolerant and sensitive genotypes, respectively (Chen et al., 2007). We observed a similar situation regarding the contribution of K^+ and proline in our tomato species.

Author contribution statement

SB, SL, JPM and MQ conceived and designed research. SB and MF conducted experiments. SB, SL and MQ analyzed data. SB and MQ wrote the manuscript. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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