Comparison of the salt resistance of *Solanum lycopersicum* x *Solanum chilense* hybrids and their parents

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Introduction: Soil salinization is a major global problem. Tomato (*Solanum lycopersicum*) is one of the main crops produced in the world and is cultivated in areas affected by salinity. However, it is considered as a glycophyte species and is sensitive to salt stress. *Solanum chilense* is a wild tomato native to the Atacama Desert and is therefore adapted to harsh environmental conditions, including salinity. It is considered as a promising source of genes for tomato improvement. However, the physiology of abiotic stress resistance remains largely unknown in *S. chilense* and no studies have investigated the resistance of *S. lycopersicum x S. chilense* interspecific hybrids and the potential value of their use in harsh environments.

Methods: In this study, we compared the salt (0 vs. 100 mM NaCl) resistance of *S. lycopersicum, S. chilense* and their hybrids at vegetative and reproductive stages.

Results: Overall, hybrids showed an intermediate behavior between their parents and a higher salt resistance than *S. lycopersicum*. Their physiological behavior toward salt stress was sometimes closed to *S. lycopersicum* and sometimes closed to *S. chilense.* Their sodium accumulation was intermediate between parents, with a sodium concentration closer to *S. lycopersicum* than *S. chilense* in roots, but with an aerial concentration closer to *S. chilense* than to *S. lycopersicum*. In inflorescences and fruits, the sodium concentration of hybrids was closer to *S. lycopersicum* than to *S. chilense.* Despite a decrease in instantaneous transpiration, the photosynthetic nitrogen use efficiency of hybrids was not decreased by salt stress: our results suggest a greater tolerance to the osmotic phase of salt stress in hybrids compared to the parents. Regarding the reproductive stage, inflorescence production and fruit quality were not affected by salt in hybrids.

Discussion: This study highlights the potential use of hybrids in improving tomato for salt stress resistance.

KEYWORDS

salinity, tomato, interspecific crosses, reproduction, wild relative

1 Introduction

Soil salinization is a growing constraint in agriculture and tomato, as a glycophyte species, is not spared (Yadav et al., 2020). Global map of salt affected soils estimates that more than 833 Mha of soils are salt-affected (8.7% of terrestrial lands, representing 20 to 50% of irrigated soils) (FAO, 2021). Tomato is cultivated in arid and semi-arid areas where soil salinization may represent a tremendous constraint (FAOStats, 2022). Tomato is the main horticultural crop in the world (250 M tons produced in 2020) and its glycophyte status induces major yield losses in saline areas (decrease of tomato yield by in average 50% for an electrical conductivity of 5 dS.m^{-1}) (Campos et al., 2006; Assaha et al., 2017; FAOStats, 2022). In contrast to most of its wild relatives adapted to saline coastal regions, cultivated tomato probably lost its salt resistance properties during domestication (Guo et al., 2022). Indeed, as for several other crop species, improvement of quality traits induced a loss of abiotic stress resistance traits (Van Deynze et al., 2007; Rothan et al., 2019; Wang et al., 2020; Rawat et al., 2022). Despite its large distribution, cultivated tomato has a very low genetic diversity (Van Deynze et al., 2007; Tamburino et al., 2020). To struggle against abiotic stresses, increasing genetic diversity through the use of wild relative species constitutes a promising option (Rick and Chetelat, 1995; Zsögön et al., 2017; Rawat et al., 2022). Tomato could be improved with wild relatives *via* different strategies. The most common use of wild species is the selection of introgression lines (Chetelat et al., 2019). Wild species could also be domesticated *de novo* (Zsögön et al., 2017; Li et al., 2018). Moreover, a strategy closed to introgression is the "rewilding" of crops, by a more holistic action than introgression of single genes (Rawat et al., 2022). In addition, wild relatives or hybrids could also be used as rootstocks for grafting (Singh et al., 2020).

Solanum chilense is a wild halophyte species in the *Solanum* sect. *Lycopersicon* clade (Peralta et al., 2008). Its important genetic diversity makes *S. chilense* a promising source of genes for tomato improvement (Igic et al., 2007; Böndel et al., 2015; Tamburino et al., 2020). This species has indeed a wide distribution in a huge range of stressful environments, such as high altitude, salty and drought-affected soils, as well as areas prone to high or low temperatures (Moyle, 2008; Chetelat et al., 2009; Ramírez-Ojeda et al., 2021b). *Solanum chilense* is one of the most diverse species in terms of habitats (different soils and climates) among the *Solanum* sect. *Lycopersicon* clade (Ramírez-Ojeda et al., 2021a; Ramírez-Ojeda et al., 2021b). It has already been used to improve tomato resistance to biotic stresses such as Tomato Yellow Leaf Curl Virus (Ji et al., 2009) or Cucumber Mosaic Virus (Stamova and Chetelat, 2000) but also to improve fruit qualities, especially anthocyanin content (Schreiber et al., 2012). The drought, heat, cold and salinity resistance of this species has been investigated (Martínez et al., 2014; Nosenko et al., 2016; Tapia et al., 2016; Gharbi et al., 2018; Blanchard-Gros et al., 2021). However, breeding for abiotic stress resistance by crossing *S. lycopersicum* and *S. chilense* has never been attempted.

Crossing between species of the *Solanum* sect. *Lycopersicon* clade is possible despite several interspecific reproductive barriers.

In this clade, species are divided into "red-fruited" species (which comprises self-compatible species only) and "green-fruited" species (self-compatible and self-incompatible species) (Baek et al., 2015). Hybridization can be spontaneous between red-fruited species: it is the case of crossings in Galapagos islands between the invasive *S. pimpinellifolium* and the native *S. cheesmaniae* (Gibson et al., 2021). However, among green- and red-fruited species, interspecific reproductive barriers strongly hamper hybridization. Indeed, species in the tomato clade are dependent on the « SI x SC rule »: hybridization between self-incompatible and self-compatible species is only possible when the self-compatible species is used as female parent (Lewis and Crowe, 1958; Tovar-Méndez et al., 2014; Baek et al., 2015). Self-compatible pollen tubes are rejected in pistils of self-incompatible species (Tovar-Méndez et al., 2014; Baek et al., 2015). As *S. lycopersicum* is self-compatible and *S. chilense* is self-incompatible, hybridization can only be achieved in the direction *S. lycopersicum* x *S. chilense*. Nevertheless, the phenotype of F1 hybrids can be superior to that of the parents for one or more traits due to heterosis or hybrid vigor (Shivaprasad et al., 2012; Kumar et al., 2020).

To the best of our knowledge, the resistance to salinity of hybrids between *S. lycopersicum* and *S. chilense* was not described until now, although there is an obvious interest to assess the salinity resistance of such hybrids. In this study, *S. lycopersicum* (used as female parent) and *S. chilense* (used as male parent) were crossed and F1 interspecific hybrids were obtained. Salinity resistance of *S. lycopersicum*, *S. chilense* and their hybrids was quantified in plants exposed to100 mM NaCl for 150 days and growth, reproductive and physiological parameters were monitored.

2 Material and methods

2.1 Plant material and growth conditions

Seeds of *S. lycopersicum* L. cv Ailsa Craig (accession LA2838A) and of *S. chilense* Dunal (accession LA4107) were obtained from the Tomato Genetics Resource Center (TGRC, University of California, Davis, CA, USA) and INIA-La Cruz (La Cruz, Chile), respectively. Hybrids were obtained by crossing emasculated flowers of *S. lycopersicum* with pollen from one single plant of *S. chilense*. Flowers were emasculated in stage 15 of Brukhin et al. (2003) scale and pollinated at anthesis. After sowing of the obtained seeds, the hybrid status of the progeny was confirmed through the use of CAPS markers on leaf DNA according to Pérez de Castro et al. (2013) (Figure S1). Primers sequences, restriction enzymes and length of fragments are given in Table S1. For the sake of clarity, the term "plant groups" will be used to describe *S. lycopersicum*, *S. chilense* and the hybrids *S. lycopersicum* x *S. chilense*.

After 6 days pre-germination in Petri dishes (25°C, 12h photoperiod), seeds of *S. chilense* were sown in peat compost (DCM, Amsterdam, The Netherlands) in a temperate greenhouse (16h, 24 ± 1.5°C, 63 ± 8%/8h, 21 ± 0.8°C, 67 ± 5% day/night photoperiod, temperature and RH). LED LumiGrow lights (650 W, red-blue) to obtain a minimum mean light intensity in the middle of the day of 181.33 ± 63.42 μmol.m^{-2}.s^{-1} provided additional lighting.

Seeds of hybrids and of *S. lycopersicum* were sown in the same peat compost and in the same greenhouse respectively 8 and 12 days after the sowing of *S. chilense* to obtain plants at the same developmental stage. When the second-leaf stage was reached, plants were individually transplanted in 2.5 L pots filled with perlite/vermiculite (50% v/v) in the same greenhouse. Plants were watered three times a week with modified Hoagland solution (5 mM KNO_3, 5.5 mM $Ca(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 25 μM KCl, 10 μM H_3BO_4, 1 μM $MnSO_4$, 0.25 μM CuSO4, 1 μM $ZnSO_4$, 10 μM $(NH_4)_6Mo_7O$ and 1.87 g.L^{-1} Fe-EDTA, pH 5.5-6). After four days of acclimation, plants were randomly divided into 2 groups (10 to 15 plants per group) receiving 0 or 100 mM NaCl (respectively 0.86 and 10.82 mS cm^{-1}). NaCl was added to the Hoagland solution. Plants were watered three times a week with either Hoagland solution (control plants) or Hoagland solution containing NaCl (salt-treated plants), volume depending on the physiological stage of the plant.

2.2 Growth measurements

During 85 days of stress, numbers of leaves, inflorescences and ramifications were estimated weekly on the main stem of 10 plants per treatment. At 85 and 150 DASt (days after stress imposition), fresh (FW) and dry (DW) weights of the different organs (leaves, stems and roots) of respectively 3 and 5 plants was estimated before and after 72 hours at 70°C. The area of the sixth youngest leaf from the top of 3 plants per treatment was measured at 85 DASt using ImageJ (v1.53a) on the basis of pictures (Lumix, Panasonic DMC SZ-10). After measuring the DW of the same leaves, specific leaf area (SLA) was calculated as the ratio between leaf area and DW of the same leaf ($cm^2.g^{-1}$).

2.3 Flower development and fruit production

Flowering time of initial and sympodial segments was assessed by counting the number of leaves respectively below the first inflorescence and between the following inflorescences on 10 plants per treatment. The number of flower buds and flowers at anthesis per inflorescence was determined on the second inflorescence, enabling to determine the percentage of bud development in an inflorescence.

Ten developed flowers per treatment were harvested on the second inflorescence of the main stem and stored in FAA (5 mL formaldehyde 35%, 5 mL glacial acetic acid, 90 mL ethanol 70% v/v) to evaluate the number of floral organs and the length of sepals, petals, stamens and pistil. Pictures were taken under binocular magnifier (Leica,-MZ75, Moticam 10.0 MP) and organs were then measured using ImageJ (v1.53a). The style exertion was also monitored for *S. chilense* and the hybrids (subtraction of the length of the stamens to the length of ovary and style).

The number of pollen grains was estimated on two anthers of ten flowers per treatment according to Ayenan et al. (2020) using ImageJ (v1.53a) with a pollen size of 5-800 pixel² and a circularity of 0.3-1.0; pictures were taken under a microscope connected with a camera (Polyvar Reichert-Jung, sCMEX-6, Euromex). Pollen viability was assessed on two anthers of ten flowers at anthesis per treatment using Alexander's dye (Alexander, 1969). Pollen was considered viable when a red coloration appeared, while it was considered non-viable when its coloration was blue (a minimum of 100 pollen grains per anther was counted, in 2 anthers per flower). Stigma receptivity was assessed on 10 flowers per treatment by detecting peroxidase activity on the stigma surface, as described by Dafni and Maués (1998). Reddish-brown color developed on the surface was scored as 0% (no color = no receptive stigma) or 100% (reddish-brown color = receptive stigma).

Flowers of the self-compatible *S. lycopersicum* were self-pollinated and flowers of the self-incompatible *S. chilense* and hybrids were hand-pollinated with pollen of other plants from the same treatment. The fruit set per inflorescence was calculated as the ratio between the number of fruits and the number of pollinated flowers. Fruits were collected at maturity stage. The number of seeds per fruit, the fruit fresh weight (FWf) and the equatorial diameter of the fruit were measured on 10 fruits per treatment. Sugar concentration and pH of the juice of those fruits were analyzed respectively by refractometry in degrees Brix (Eclipse, Bellingham + Stanley, Tunbridge Wells, UK) and by pH paper indicator (Dosatest pH test strips pH 3.6 - 6.1, VWR). Fruit FW (FWfr) was measured on 5 fruits per treatment and the DW of the same fruits (DWfr) was measured after 3 days at 70°C: water content (WCfr, in %) was then calculated using the formula 100*(FWfr-DWfr)/FWfr.

2.4 Water status of vegetative parts and photosynthesis parameters

Water content (WC) of the vegetative organs (leaves, stems, roots) harvested at 85 DASt and 150 DASt on respectively 3 and 5 plants per treatment was calculated using the formula WC = ((FW-DW)/FW)*100. Leaf and root osmotic potentials (ψs) were quantified on the extracted sap from respectively the 5th youngest leaf and the first secondary root (counting from the root collar) of 3 plants per treatment harvested at 85 DASt, using a vapor pressure osmometer (Wescor 5500, Oceanside, CA, USA), as previously described (Lutts et al., 1999).

Photosynthetic and gas-exchange parameters were quantified at 28 and 70 DASt, on the 5th youngest leaf of 6 plants per treatment. The instantaneous CO_2 assimilation rate under ambient conditions (400 ppm CO_2) (*A*), the instantaneous transpiration rate (*E*), the intercellular CO_2 concentration (*Ci*) and the stomatal conductance (*g*s) were quantified using an infrared gas analyzer (ADC BioScientific LCI-SD System Serial 33413, 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}. Instantaneous water use efficiency (WUEi) was calculated as the *A/E* ratio. 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 analyzed using a fluorimeter (FMS II, Hansatech Instruments, Norfolk, UK) according to Mathieu et al. (2014). The

chlorophyll content index (CCI) was estimated using a chlorophyllometer (Opti-Sciences, CCM-200, Hudson, USA) and measurements were performed in triplicates on each leaf. Net photosynthesis was corrected by CCI to get photosynthetic nitrogen use efficiency (PNUE) according to Paponov and Engels (2003). At 28 and 70 DASt, the abaxial stomatal density (in number of stomata per mm^2) was measured on the same leaf than the one used for photosynthetic and gas-exchange parameters measurements for 3 plants per treatment by using polish nail method as described by Sun et al. (2014). The area of the same leaf was measured using ImageJ (v1.53a) from pictures (Lumix, Panasonic DMC SZ-10).

2.5 Biochemical parameters

At 85 DASt, the 6th youngest leaf and inflorescences of 3 plants per treatment were harvested and grounded separately in liquid nitrogen. Total soluble sugars were quantified in leaves and inflorescences from 100 mg of fresh material using anthrone method (Yemm and Willis, 1954) after extraction with ethanol 70% (v/v). The absorbance was read at 625 nm using a UV-1800 spectrophotometer (Shimadzu,'s-Herlogenbosch, the Netherlands) and soluble sugars quantification was determined using a standard curve of glucose.

Proline was extracted from 200 mg of leaves in 3% (w/v) sulfosalicylic acid with 30 min incubation at 70°C (Bates et al., 1973). Two mL of ninhydrin solution (1.25 g of ninhydrin in 30 mL glacial acetic acid and 20 mL of H_3PO_4 6M) were added to 2 mL of filtrate (Whatman, 11 μm) then water-bathed (1 h at 100°C). Reaction was stopped on ice then 2 mL toluene were added. Proline was quantified based to a standard curve. Absorbance was read at 520 nm after strong vortex of the samples.

Malondialdehyde (MDA) was quantified according to Heath and Packer (1968) method by thiobarbituric acid reaction from 250 mg leaves and inflorescences. Absorbance was read at 532 nm and 600 nm (non-specific values, subtracted to 532 nm values).

2.6 Mineral quantification

Sodium (Na^+) and potassium (K^+) were quantified on roots, stems and leaves at 85 DASt (3 plants per treatment) and 150 DASt (5 plants per treatment) and on inflorescences, pericarps and seeds harvested all along the experiment. Fifty to 100 mg DW were weighted and digested in 4 mL of hot 68% (v/v) HNO_3. After complete evaporation, 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).

2.7 Statistical treatments

The experiment was performed twice and gave similar results. We present the results of one repetition. All statistical treatments were analyzed using RStudio (R Core Team, 2018). Normality distribution and homoscedasticity were verified using respectively Shapiro-Wilk and Levene's test and data were transformed when required. Two-ways analysis of variance (ANOVA II) was used to compare species, NaCl treatment and their interactions. Data were represented by the means ± standard deviation. Statistical results are presented in Table S2. Principal component analysis (PCA) followed by vector projection was realized on the whole dataset using the package FactoMineR (Lê et al., 2008).

3 Results

To understand the impact of NaCl on plant growth and physiology of *S. lycopersicum*, *S. chilense* and their hybrids, differences among treatments were visualized using PCA (Figure 1). The PCA shows that 23.2% of the variance was explained by axis 1 and 21.0% by axis 2 while axis 3 only
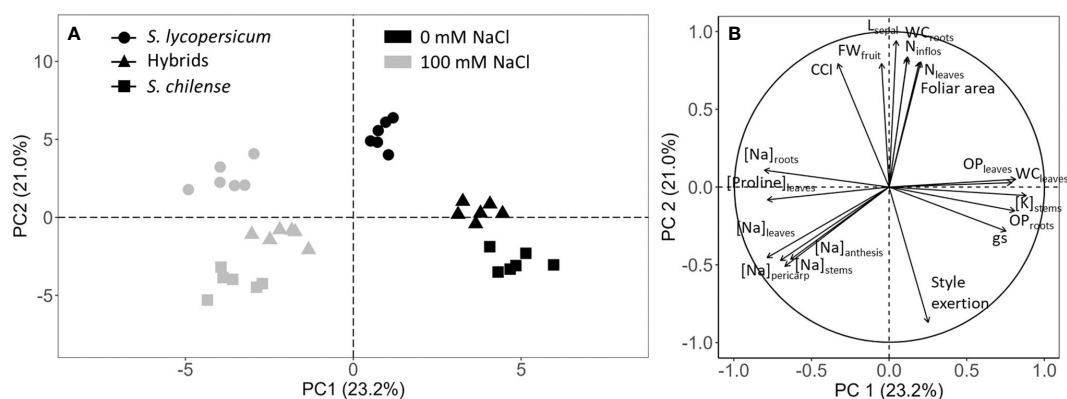
FIGURE 1
Principal component analysis (PCA) of vegetative and reproductive growth, physiological parameters and mineral concentrations in response to salt stress (0 mM and 100 mM NaCl) in *S. lycopersicum*, *S. chilense* and their hybrids. **(A)** Individual graph. **(B)** Variable graph of PCA (only variables with cos^2>0.6 are shown). PC1 and 2, Principal component 1 and 2; CCI, chlorophyll content index; FW$_{fruit}$, fruit fresh weight; Lsepal, sepal length; [Na] and [K], concentration of sodium and potassium in the different organs (roots, stems, leaves, flowers in anthesis, pericarp); Nleaves, number of leaves on the main stem; Ninflos, number of inflorescences on the main stem; OP, osmotic potential in leaves or roots; [Proline], proline concentration in the leaves; WC, water content of leaves and roots.

explained 9% of the variance. Plants firstly grouped according to the salt treatment and within each treatment according to the plant groups (Figure 1A). NaCl-stressed and control plants of the three plant groups were discriminated according to axis 1 which was mainly explained by stem K^+ concentrations, ψs of leaves and roots and root Na^+ concentration (Figure 1B). Axis 2 discriminated the plant groups mainly according to reproduction and flower morphology parameters (length of sepals, style exertion and number of inflorescences on the main stem) (Figure 1B). Hybrids exhibited an intermediate behaviour between the parents according to those parameters. However, according to the PCA, they were closer to *S. chilense* than to *S. lycopersicum*, at least under control conditions (Figure 1A).

3.1 Growth measurements

Solanum lycopersicum produced more biomass than the two other plant groups. At 85 DASt, the plant DW was higher for *S.*

lycopersicum than for the two other plant groups while at 150 DASt, hybrids had an intermediate DW between their two parents, especially at 100 mM NaCl (Figure 2A, Table S2). At 85 DASt, the stems represented the biggest part of the plant DW (44.5% ± 8.6%), followed by leaves (41.3% ± 7.3%) and roots (2.2% ± 4.6%) (Figures 2B–D). Although the proportion of root DW was similar between plant groups, stems were more developed in hybrids and *S. chilense* while leaves were more developed in *S. lycopersicum*, mainly at 150 DASt (Figures 2B–D; Table S2). *Solanum lycopersicum* produced more leaves than hybrids and *S. chilense* while *S. chilense* produced much more ramifications than the two other plant groups at 85 DASt (Figures 2E, F; Table S2).

Although NaCl decreased plant DW as early as 85 DASt only in *S. lycopersicum*, it decreased it in all three plant groups at 150 DASt (Figure 2A; Table S2). Nevertheless, NaCl had only a slight effect on the DW proportions of the different organs (Figures 2B–D; Table S2). The number of leaves on the main stem decreased with salinity only in *S. lycopersicum* at 85 DASt (Figure 2E; Table S2) and the
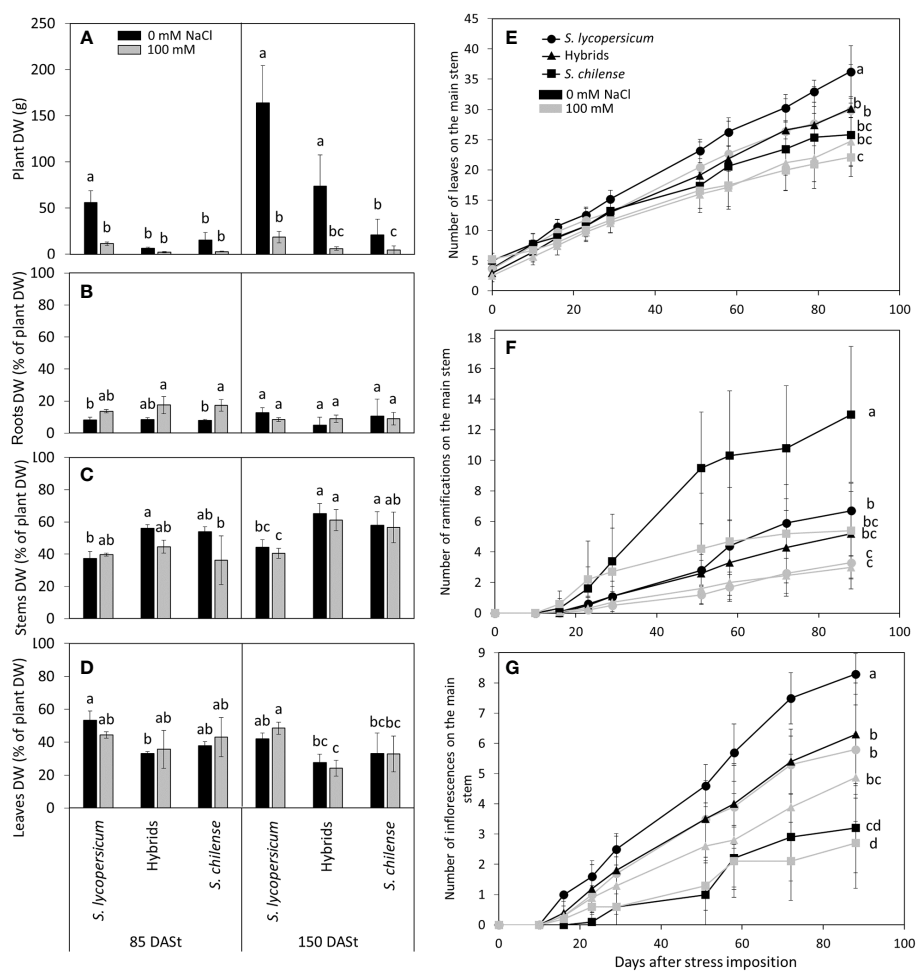
FIGURE 2
Effects of salt stress (0 mM and 100 mM NaCl) on *Solanum lycopersicum*, *Solanum chilense* and their hybrids. **(A)** Plant dry weight (DW) at 85 and 150 days after stress imposition (DASt) and **(B–D)** percentage of the DW represented by **(B)** the roots, **(C)** the stems and **(D)** the leaves. **(E–G)** Evolution of the number of **(E)** leaves, **(F)** ramifications and **(G)** inflorescences on the main stem up to 85 days after stress imposition. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05 for a same date.

number of ramifications decreased with salinity in both parents but not in the hybrids (Figure 2F, Table S2).

3.2 Flowers development and morphology

The flowering time of the initial segment was similar among plant groups while the flowering time of the sympodial segments was slightly higher in *S. chilense* than in the other plant groups (Table 1, Table S2). As a result, *S. lycopersicum* produced more inflorescences on the main stem than *S. chilense* while the hybrids had an intermediate behavior (Figure 2G, Table S2). Within an inflorescence, *S. chilense* and hybrids produced more floral buds and had a higher percentage of floral buds reaching anthesis than *S. lycopersicum* (Tables 1, S2). Salt did not affect flowering time nor anthesis percentage (Tables 1, S2) but decreased inflorescence production in *S. lycopersicum* only (Figure 2G, Table S2).

The flower morphology of the hybrids was intermediate between those of the parents (Figure S2C) Despite similar petal length between the three plant groups, sepal and stamen length of the hybrids were intermediate to those of *S. lycopersicum* and *S. chilense*. Hybrids presented a style exertion like *Solanum chilense* (Tables 1, S2). The only effect of NaCl on flower morphology was recorded on sepal length, which decreased in *S. lycopersicum* and hybrids but not in *S. chilense* (Tables 1, S2).

Regarding flower fertility parameters, stigma receptivity was affected neither by plant groups nor by salt stress (Tables 1, S2). The number of pollen grains per anther and pollen viability were affected by the plant group (Table S2) although no significant differences were observed under control conditions (Table 1). Salt did not alter pollen viability (Tables 1, S2) but tended to increase the number of pollen grains per anther (Table S2) although the difference was not significant inside a same plant group (Table 1).

3.3 Fruit production

The fruit set per inflorescence was slightly higher in the hybrids than in the parents while the seed set was 9 and 3 times lower in the hybrids than in *S. lycopersicum* and *S. chilense*, respectively (Tables 2, S2). The general appearance of hybrid fruits was closer to that of *S. chilense* than to *S. lycopersicum*, although this was not the case for all parameters (Figure S2A). Indeed, fruit FW, DW and diameter were intermediate in hybrids compared to parents but were closer to *S. chilense* than to *S. lycopersicum* (Tables 2, S2). Similarly, fruit sugar content was twice as high in hybrids than in *S. lycopersicum* but was slightly lower than in *S. chilense* (Tables 2, S2). However, hybrid and *S. lycopersicum* fruits had a higher WC and were more acidic than *S. chilense* fruits (Tables 2, S2).

Salt did not affect neither the fruit set per inflorescence nor the seed set in any of the 3 plant groups (Tables 2, S2). However, it affected fruit quality in parents but not in hybrids (Tables 2, S2). Indeed, salt decreased fruit FW in *S. lycopersicum*, fruit DW in *S. chilense* and fruit equatorial diameter in both species (Tables 2, S2). It also decreased fruit WC in *S. lycopersicum* but increased it in *S. chilense*. Salinity decreased fruit pH and sugar content in *S. chilense* and increased fruit sugar content in *S. lycopersicum* (Tables 2, S2).

TABLE 1 Effects of salt stress on flowering parameters and flower morphology and fertility of *Solanum lycopersicum*, *Solanum chilense* and their hybrids grown at 0 and 100 mM NaCl.

NaCl concentration	N	*Solanum lycopersicum*		Hybrids		*Solanum chilense*	
		0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Flowering time of initial segment[1]	10	10.0 ± 1.15[a]	10.90 ± 0.88[a]	10.10 ± 1.52[a]	9.75 ± 1.28[a]	12.0 ± 1.41[a]	10.89 ± 3.33[a]
Flowering time of sympodial segment[1]	10	2.90 ± 0.22[b]	3.17 ± 0.36[ab]	2.87 ± 0.45[b]	2.96 ± 0.12[ab]	3.28 ± 0.25[ab]	3.78 ± 1.44[a]
Floral buds per inflorescence	10	6.80 ± 1.62[c]	6.83 ± 1.27[c]	9.55 ± 3.91[bc]	9.07 ± 3.20[bc]	12.60 ± 2.22[ab]	15.50 ± 5.93[a]
Flower anthesis per inflorescence (%)[2]	10	70.32 ± 11.79[ab]	53.4 ± 19.3[b]	86.29 ± 9.42[a]	83.52 ± 17.81[a]	77.55 ± 13.64[a]	76.34 ± 18.05[a]
Length of petals (mm)	10	12.78 ± 1.88[ab]	14.76 ± 1.35[a]	14.93 ± 1.90[a]	13.61 ± 2.16[ab]	13.56 ± 0.97[ab]	12.05 ± 1.37[b]
Length of sepals (mm)	10	12.32 ± 1.43[a]	9.68 ± 1.64[b]	8.43 ± 0.96[b]	6.75 ± 0.81[c]	6.70 ± 0.52[c]	5.89 ± 0.66[c]
Length of stamens (mm)	10	9.18 ± 0.59[a]	8.91 ± 0.98[ab]	8.50 ± 0.92[ab]	8.04 ± 0.67[bc]	7.47 ± 0.34[c]	7.27 ± 0.5[c]
Style exertion (mm)	10	ND	ND	2.28 ± 0.35[ab]	1.95 ± 0.65[b]	2.28 ± 0.41[ab]	2.69 ± 0.51[a]
Stigma receptivity (%)	10	95 ± 16[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]
Pollen viability (%)	10	83.82 ± 3.67[a]	88.01 ± 7.83[a]	80.12 ± 8.78[ab]	70.85 ± 8.38[b]	86.63 ± 3.24[a]	89.49 ± 2.67[a]
Number of pollen grains per anther (x 1000)	10	55.17 ± 26.51[c]	91.88 ± 44.53[ac]	82.02 ± 46.41[bc]	117.92 ± 63.37[ab]	97.91 ± 38.68[ac]	145.63 ± 67.43[a]

[1] expressed in number of leaves below inflorescence.
[2] number of floral buds openned within an inflorescence.
Data are means ± standard deviation, different letters indicate significant difference at p < 0.05 within each row.

TABLE 2 Effects of salt stress on fructification parameters of *Solanum lycopersicum*, *Solanum chilense* and their hybrids grown at 0 and 100 mM NaCl.

NaCl concentration	n	*Solanum lycopersicum*		Hybrids		*Solanum chilense*	
		0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Fruit set per inflorescence (%)[1]	10	77 ± 26.40[ab]	25.79 ± 12.73[ab]	37.53 ± 42.76[b]	37.10 ± 43.61[b]	54.08 ± 43.64[ab]	76.81 ± 22.72[a]
Fruit FW (g)[2]	15	36.85 ± 15.09[a]	12.60 ± 6.48[b]	1.28 ± 0.48[c]	1.14 ± 0.33[cd]	0.66 ± 0.19[d]	0.89 ± 0.30[cd]
Fruit DW (g)[2]	5	1.40 ± 0.35[a]	1.46 ± 0.42[a]	0.21 ± 0.08[bc]	0.22 ± 0.07[b]	0.12 ± 0.03[c]	0.07 ± 0.01[d]
Fruit WC (%)[2]	5	93.81 ± 0.71[a]	80.91 ± 5.27[c]	88.20 ± 2.34[ab]	82.92 ± 2.44[bc]	81.14 ± 2.62[c]	88.20 ± 0.81[ab]
Number of seeds/fruit	10	63.20 ± 41.8[a]	49.80 ± 31.81[a]	7.63 ± 2.90[b]	10.20 ± 7.98[b]	21.22 ± 4.47[a]	25.90 ± 9.84[a]
Fruit diameter (mm)	10	43.95 ± 5.46[a]	28.69 ± 4.48[b]	12.45 ± 1.42[c]	11.41 ± 2.10[bc]	9.89 ± 0.76[d]	12.87 ± 1.03[c]
Fruit sugar content (°Brix)	10	6.45 ± 1.80[c]	10.39 ± 1.14[b]	13.70 ± 3.44[b]	10.70 ± 3.44[b]	18.40 ± 3.2[a]	10.50 ± 1.49[b]
Fruit pH	10	4.17 ± 0.25[b]	4.20 ± 0.26[b]	4.20 ± 0.24[b]	4.09 ± 0.29[b]	4.67 ± 0.26[a]	3.75 ± 0.24[c]

[1] percentage of pollinated flowers developing a fruit within an inflorescence.
[2] FW, fresh weight; DW, dry weight; WC, water content.
Data are means ± standard deviation, different letters indicate significant difference at p < 0.05 within each row.

3.4 Water status and photosynthesis parameters

Water content was similar among plant groups for a given organ and salt did not affect plant WC except for the stems of *S. lycopersicum* at 85 DASt (Tables 3, S2). Leaf ψs was intermediate in hybrids compared to parents and root ψs was lower in hybrids and *S. chilense* compared to *S. lycopersicum* under control conditions (Figures 3B, C, Table S2). NaCl decreased leaf ψs in *S. lycopersicum* and *S. chilense* but not in hybrids, while it decreased root ψs in all plant groups (Figures 3B, C, Table S2).

Under control conditions, the leaf area of the first mature leaf was the highest in *S. lycopersicum*, followed by the hybrids and the smallest in *S. chilense* (Figure 4A, Table S2). As a result, the specific leaf area (SLA) of hybrids was intermediate between their parents under control conditions (Figure 3A, Table S2). Salt decreased the leaf area of *S. lycopersicum* at 70 DASt (Figure 4B, Table S2) and the SLA of *S. chilense* at 85 DASt (Figure 3C, Table S2) without affecting these parameters in the hybrids. Stomatal density differed among plant groups but was not affected by salinity (Figure 4B, Table S2): it was intermediate in hybrids compared to parents at 28 DASt, but closer to that of *S. lycopersicum* at 70 DASt (Figure 4B, Table S2).

The photosynthetic nitrogen use efficiency (PNUE) was the highest in *S. chilense* under control condition (Figure 4C, Table S2) and salt increased PNUE of the hybrids at 28 DASt and decreased PNUE of *S. lycopersicum* at 70 DASt (Figure 4C, Table S2). Stomatal conductance (g_s), intercellular CO_2 concentration (Ci) and instantaneous transpiration (E) were only affected at 70 DASt (Table 3, Figure 4D, Table S2): g_s was the highest in *S. chilense* under control condition and decreased with salt in hybrids and *S. chilense*; Ci was the lowest in *S. lycopersicum* and decreased with salt in hybrids; E was the highest in *S. chilense* and decreased with salt in both *S. chilense* and hybrids. Instantaneous water use efficiency (WUEi) was the highest in *S. lycopersicum* under control condition at 70 DASt but decreased with salt in the latter while it increased with salt in hybrids (Tables 3, S2). All chlorophyll fluorescence-related parameters (F_v/F_m, NPQ, qP) were similar between plant groups and remained unaffected by NaCl, except ϕ_{PSII} at 28 DASt which was slightly lower in hybrids than in parents (Tables 3, S2).

3.5 Biochemical parameters

Soluble sugars were more than 2.5 times more concentrated in inflorescences than in leaves (Table 4, $t_{78.6}$ = 12.32, p < 0.001). Under control condition, the leaf soluble sugars concentration was similar between plant groups. However, as far as inflorescences were concerned, hybrids and *S. chilense* had higher soluble sugars concentrations than *S. lycopersicum* (Tables 4, S2). NaCl affected soluble sugar concentration only in leaves, where it increased mostly in *S. chilense* (Tables 4, S2). Proline concentration in leaves was low under control condition, especially in *S. chilense* and it increased with salt by 8.2, 3.3 and 161.5 times in *S. lycopersicum*, hybrids and *S. chilense*, respectively (Tables 4, S2). The MDA concentration was 1.5 times higher in inflorescences than in leaves (t_{70} = -5.071, p < 0.001). The MDA concentration in leaves was similar between plant groups while its concentration in inflorescences was slightly higher in *S. lycopersicum* and hybrids than in *S. chilense* (Tables 4, S2). Salt slightly increased MDA concentrations in the leaves (Tables 4, S2).

3.6 Mineral quantification

3.6.1 Sodium

The Na^+ concentration was similar among plant groups in control conditions. Considering the whole plant, under salinity condition at 85 DASt, the Na^+ concentration was the lowest in *S. lycopersicum* and the highest in hybrids (Figure 5A, Table S2). However, at 150 DASt, the difference between plant groups was not significant anymore (Figure 5A, Table S2). At 85 DASt, the increase of Na^+ concentration between control and salted conditions was the highest in hybrids (Figures 5B–D) while at 150 DASt, this relative

TABLE 3 Effects of salt stress on photosynthesis and water status parameters of *Solanum lycopersicum*, *Solanum chilense* and their hybrids grown at 0 and 100 mM NaCl.

NaCl concentration	n	*Solanum lycopersicum* 0 mM	100 mM	Hybrids 0 mM	100 mM	*Solanum chilense* 0 mM	100 mM
WC roots 85 DASt	3	89.61 ± 1.22^a	88.36 ± 0.33^{ab}	86.82 ± 1.09^{ab}	83.76 ± 1.55^b	84.51 ± 4.81^{ab}	83.02 ± 2.14^b
WC stems 85 DASt	3	91.81 ± 0.35^a	86.55 ± 1.36^b	92.64 ± 0.53^a	90.62 ± 1.79^a	90.25 ± 1.02^a	90.43 ± 2.03^a
WC stems 150 DASt	5	86.56 ± 1.92^a	86.8 ± 2.54^a	86.15 ± 2.78^a	88.86 ± 1.16^a	87.96 ± 1.92^a	88.07 ± 3.07^a
WC leaves 85 DASt	3	88.91 ± 0.82^a	86.73 ± 1.08^a	90.4 ± 1.37^a	86.23 ± 0.57^a	90.44 ± 2.36^a	87.22 ± 3.24^a
WC leaves 150 DASt	5	87.7 ± 0.83^a	88.51 ± 1.44^a	85.08 ± 1.70^a	83.89 ± 2.21^a	86.70 ± 5.14^a	89.52 ± 4.36^a
Ci 28 DASt	6	444.93 ± 19.14^a	437.81 ± 19.27^a	462.92 ± 2.42^a	408.33 ± 51.13^a	424.13 ± 90.7^a	378.14 ± 78.1^a
Ci 70 DASt	6	356.15 ± 27.03^{bc}	377.52 ± 28.35^{ac}	397.05 ± 14.49^a	351.2 ± 43.79^c	388.11 ± 17.38^{ab}	383.1 ± 57.3^{ac}
gs 28 DASt	6	0.55 ± 1.16^a	0.64 ± 0.97^a	0.77 ± 0.2^a	0.95 ± 1.15^a	1.06 ± 1.06^a	0.54 ± 0.56^a
gs 70 DASt	6	0.13 ± 0.06^{bc}	0.11 ± 0.06^c	0.20 ± 0.10^b	0.05 ± 0.02^c	0.37 ± 0.17^a	0.13 ± 0.10^{bc}
WUEi 28 DASt	6	0.90 ± 0.68^b	1.75 ± 1.99^{ab}	0.45 ± 0.15^b	2.80 ± 2.34^a	2.45 ± 5.09^{ab}	2.79 ± 3.15^a
WUEi 70 DASt	6	2.04 ± 0.59^a	1.05 ± 0.48^b	0.87 ± 0.33^b	2.05 ± 1.01^a	1.10 ± 0.69^b	1.15 ± 1.21^b
Fv/Fm 28 DASt	6	0.882 ± 0.016^a	0.893 ± 0.013^a	0.884 ± 0.019^a	0.888 ± 0.017^a	0.888 ± 0.041^a	0.897 ± 0.008^a
Fv/Fm 70 DASt	6	0.890 ± 0.008^a	0.874 ± 0.009^a	0.884 ± 0.004^a	0.883 ± 0.013^a	0.891 ± 0.014^a	0.875 ± 0.019^a
ϕPSII 28 DASt	6	0.812 ± 0.058^{ab}	0.841 ± 0.014^a	0.755 ± 0.076^b	0.791 ± 0.038^{ab}	0.828 ± 0.054^{ab}	0.833 ± 0.012^{ab}
ϕPSII 70 DASt	6	0.826 ± 0.015^a	0.817 ± 0.016^a	0.799 ± 0.024^a	0.839 ± 0.018^a	0.758 ± 0.14^a	0.804 ± 0.028^a
NPQ 28 DASt	6	0.19 ± 0.07^a	0.16 ± 0.04^a	0.28 ± 0.13^a	0.18 ± 0.08^a	0.19 ± 0.05^a	0.21 ± 0.14^a
NPQ 70 DASt	6	0.21 ± 0.17^a	0.16 ± 0.03^a	0.13 ± 0.04^a	0.16 ± 0.04^a	0.16 ± 0.06^a	0.14 ± 0.01^a
qP 28 DASt	6	0.94 ± 0.06^a	0.96 ± 0.01^a	0.89 ± 0.08^a	0.91 ± 0.03^a	0.94 ± 0.05^a	0.95 ± 0.02^a
qP 70 DASt	6	0.95 ± 0.03^a	0.96 ± 0.02^a	0.92 ± 0.03^a	0.96 ± 0.01^a	0.87 ± 0.16^a	0.94 ± 0.01^a
CCI 28 DASt	6	18.7 ± 11.09^{ab}	21.43 ± 5.22^a	10.16 ± 2.31^{cd}	14 ± 4.63^{bc}	7.57 ± 2.31^d	9.46 ± 2.77^d
CCI 70 DASt	6	26.9 ± 8.55^{ab}	37.68 ± 12.38^a	18.21 ± 7.04^c	18.86 ± 5.07^{bc}	9.27 ± 2.58^d	6.91 ± 2.93^d

CCI, chlorophyll content index; Ci, intercellular CO_2 concentration ($\mu mol.m^{-2}.s^{-1}$); DASt, days after stress imposition; Fv/Fm, maximum quantum yield of photosystem II, gs, stomatal conductance ($mol.m^{-2}.s^{-1}$); NPQ, non-photochemical quenching; ϕPSII, efficiency of photosystem II; qP, photochemical quenching; WC, water content (%); WUEi, instantaneous water use efficiency ($\mu mol.mmol^{-1}$). Data are means ± standard deviation, different letters indicate significant difference at p < 0.05.

increase was lower (Figures 5B–D). Such increase in Na^+ concentration was clearly lower in *S. lycopersicum* and *S. chilense*. As a result, at 85 DASt, the hybrids had a Na^+ concentration under salinity close to *S. lycopersicum* in roots and close to *S. chilense* in stems and leaves (Figures 5B–D, Table S2). In contrast, at 150 DASt, the Na^+ concentration was similar in stressed plants of all three plant groups in stems and leaves but lower in roots of *S. chilense* (Figures 5B–D, Table S2). The root/shoot ratio of Na^+ quantity differed among plant groups at both 85 and 150 DASt while salt affected it only at 150 DASt (Figure 5E, Table S2). Under control condition, the root/shoot ratio of Na^+ quantity was the highest in *S. lycopersicum* and the lowest in *S. chilense*. In hybrids, the ratio was intermediate between parents at 85 DASt and close to *S. chilense* at 150 DASt (Figure 5E). Comparing only stressed plants, the root/shoot ratio of Na^+ was similar in the three plant groups at 85 DASt (F = 1.685, p = 0.263) and at 150 DASt, it was intermediate in hybrids between *S. lycopersicum* (highest) and *S. chilense* (lowest)

(F = 6.914, p = 0.011), although such difference was not observed for *post-hoc* test comparing both salt conditions (Figure 5E).

As far as reproductive organs are concerned, Na^+ concentration in inflorescences of stressed plants was lower than in vegetative organs ($t_{27.255}$ = -4.509, p < 0.001). It was higher in *S. chilense* than in *S. lycopersicum* and hybrids (Figure 6A, Table S2). In the fruits, the Na^+ concentration was higher in pericarp than in the seeds (Figures 6B, C, F = 39.955, p < 0.001). In salt-treated plants, the Na^+ concentration in pericarp was higher in *S. chilense* than in the other plant groups (Figures 6A, B, Table S2) while seed Na^+ concentration was similar among the three plant groups (Figure 6C, Table S2).

3.6.2 Potassium

In control conditions, the K^+ concentration in the plant at 85 DASt was higher in hybrids than in *S. lycopersicum* while such a difference was not recorded at 150 DASt (Figure 7A, Table S2). Salinity decreased K^+ concentration in *S. chilense* at 85 and 150
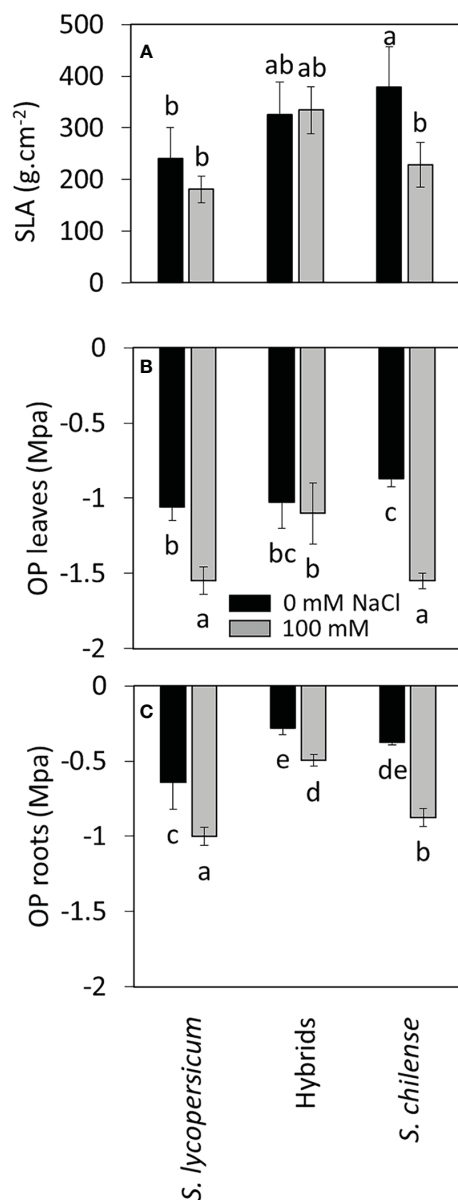
FIGURE 3
Effects of salt stress (0 mM and 100 mM NaCl) on specific leaf area and osmotic potential of *Solanum lycopersicum*, *Solanum chilense* and their hybrids 85 days after stress imposition. **(A)** Specific leaf area (SLA) of the 6th youngest leaf. **(B, C)** Osmotic potential (OP) of **(B)** the 5th youngest leaf and **(C)** the first secondary root. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05.

FIGURE 4
Effects of salt stress (0 mM and 100 mM NaCl) on leaf parameters of *Solanum lycopersicum*, *Solanum chilense* and their hybrids at 28 and 70 days after stress imposition (DASt). **(A)** Leaf area, **(B)** abaxial stomatal density (number/mm²), **(C)** photosynthetic nitrogen use efficiency (PNUE) and **(D)** instantaneous transpiration of the 5th youngest leaf. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05 for a same date.

DASt, in hybrids at 85 DASt but did not affect it in *S. lycopersicum* (Figure 7A, Table S2). The response of each plants group differed depending on the organ and the considered date (Figures 7B–D). In roots, K^+ concentration was higher in hybrids compared to the two other plant groups at 85 DASt under control condition and salt had only a limited impact (Figure 7B, Table S2). In stems, the highest K^+ concentration was observed in hybrids at 85 DASt and in *S. chilense* at 150 DASt and the lowest in *S. lycopersicum* under control condition while K^+ concentration was similar among plant groups under salinity (Figure 7C, Table S2). In leaves, K^+

concentration was neither affected by plant groups nor by NaCl (Figure 7D; Table S2).

The root/shoot K^+ ratio was similar whatever the plant groups and slightly increased with salt, mainly in hybrids and *S. chilense* at 85 DASt (Figure 7E; Table S2).

TABLE 4 Effects of salt stress on biochemical parameters of *Solanum lycopersicum*, *Solanum chilense* and their hybrids grown at 0 and 100 mM NaCl.

NaCl concentration	n	*Solanum lycopersicum*		Hybrids		*Solanum chilense*	
		0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Leaves							
[Soluble sugars] mg.g⁻¹ FW	3	6.34 ± 1.64^{bc}	6.75 ± 1.19^{b}	4.94 ± 1.62^{cd}	4.26 ± 0.21^{d}	5.48 ± 1.03^{bd}	11.49 ± 1.08^{a}
[MDA] nmol.g⁻¹ FW	3	8.72 ± 1.25^{ab}	12.86 ± 4.21^{a}	8.30 ± 2.37^{ab}	6.38 ± 1.47^{b}	5.95 ± 1.54^{b}	10.82 ± 6.88^{ab}
[Proline] nmol.g⁻¹ FW	3	0.36 ± 0.26^{bc}	2.94 ± 0.40^{a}	0.27 ± 0.39^{bc}	0.90 ± 0.15^{ac}	0.014 ± 0.005^{c}	2.26 ± 2.29^{ab}
Inflorescences							
[Soluble sugars] mg.g⁻¹ FW	3	11.80 ± 0.76^{c}	12.07 ± 5.42^{c}	17.49 ± 1.34^{ab}	15.08 ± 6.80^{bc}	19.97 ± 2.62^{ab}	21.77 ± 0.96^{a}
[MDA] nmol.g⁻¹ FW	3	13.41 ± 2.37^{ab}	13.88 ± 2.74^{ab}	13.19 ± 1.19^{ab}	16.95 ± 4.47^{a}	11.54 ± 1.37^{b}	10.43 ± 2.11^{b}

FW, fresh weight. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05.

In control conditions, K^+ concentration was slightly lower in reproductive than in vegetative organs ($t_{26.789}$ = -3.928, p < 0.001) and was not affected by plant groups and salt treatment (Figure 6D, Table S2). In fruits, K^+ concentration was higher in pericarp than in seeds (Figures 6E–F, F = 211.34, p < 0.001). In pericarp, salinity decreased K^+ concentration in the two parent species but not in hybrids (Figure 6E, Table S2). In seeds, *S. chilense* had a higher K^+ concentration than *S. lycopersicum* and hybrids and salt decreased K^+ concentration in *S. chilense* only (Figure 6F, Table S2).

4 Discussion

4.1 Hybrids exhibited an intermediate phenotype between parents

Figure 8 summarizes the response of the three plant groups to salinity. We observed that hybrids globally exhibited an intermediate phenotype between *S. lycopersicum* and *S. chil*ense. Similar results were previously reported for interspecific crosses within the tomato clade or between other species (Baek et al., 2016; Ghani et al., 2020). Ghani et al. (2020) crossed *S. lycopersicum* with three wild *Solanum* species (*S. pimpinellifolium*, *S. arcanum* and *S. pennellii*) and observed that most of the hybrids presented morphological parameters intermediate between parents, such as plant height, leaf length, number of fruits and fruit DW. However, this is not the case for all interspecific crosses: hybrids may show better or worse fitness compared to the parents (Hooftman et al., 2005; Rhode and Cruzan, 2005). In our study, even if an intermediate phenotype was globally observed, it was not the case for all recorded parameters. Morphologically, the general appearance of the hybrids was closer to *S. lycopersicum* than to the wild parent, especially regarding the vegetative parts. Indeed, the hybrids do not have the bushy appearance of *S. chilense* which has a high number of ramifications in the main stem and a decumbent profile (Peralta et al., 2008). Instead, the hybrids had a stem width and a leaf shape more similar to *S. lycopersicum* (Figure S2). Grafting is a technique more and more used in production and hybrids present thus a better morphological compatibility to *S. lycopersicum* than *S. chilense*. Thus, the use of hybrids, if they present a good resistance to salinity, could be an alternative to the grafting of thin wild tomato species (Singh et al., 2020). In terms of flower morphology, hybrids presented style exertion as observed in *S. chilense*. Style exertion is described as a marker of self-incompatibility (Rick et al., 1977; Chetelat et al., 2009) and *S. lycopersicum x S. chilense* hybrids are self-incompatible as their *S. chilense* parent (Martin, 1961). This is a feature already observed in others hybrids between self-compatible and self-incompatible species, as it has also been observed for hybrids between *S. lycopersicum* and *S. peruvianum* (McGuire and Rick, 1954) or *Petunia* hybrids (Ai et al., 1991). The fruits of the hybrids were also morphologically more similar to those of *S. chilense* than to *S. lycopersicum*: they remained green and smaller than the large red fruits of *S. lycopersicum* (Figure S2). In addition, the fruits of the hybrids contained fewer seeds than the parents despite the fact that flower fertility was similar in hybrids comparatively to parents, suggesting a reproductive barrier or inbreeding depression in the hybrids. The production of fruits with few seeds can be observed in self-compatible species x self-incompatible species crosses (Baek et al., 2016).

4.2 Tolerance to salt stress in hybrids

We observed that salinity decreased plant growth in the 3 plant groups although such decrease was only observed at 150 DASt in hybrids and *S. chilense* while plant growth decrease was already visible at 85 DASt in *S. lycopersicum*. This clearly suggests that hybrids and *S. chilense* were more salt resistant than *S. lycopersicum*. *Solanum lycopersicum* is described as a glycophyte species and *S. chilense*, as a halophyte species (Martínez et al., 2014; Gharbi et al., 2018). Since hybrids had an intermediate phenotype between parents and some characteristics were closer to *S. lycopersicum* and others to *S. chilense*, we could therefore suspect that the physiological behavior of hybrids could be sometimes closer to halophyte behavior and sometimes closer to glycophyte behavior (Cano et al., 1996; Uwimana et al., 2012; Aycan et al., 2021). In addition, interspecific hybrids may have better fitness for some traits than their parents (Rhode and Cruzan, 2005; Kumar et al., 2020).
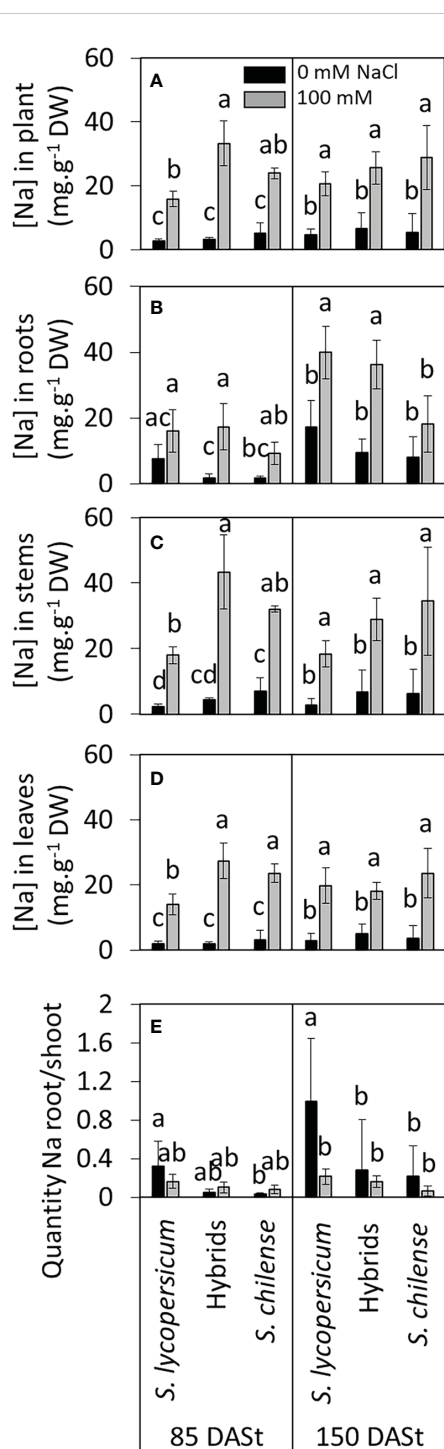
FIGURE 5
Effects of salt stress (0 mM and 100 mM NaCl) on Na+ accumulation in *Solanum lycopersicum*, *Solanum chilense* and their hybrids at 85 and 150 days after stress imposition (DASt). **(A–D)** Concentration of sodium in **(A)** the whole plant, **(B)** the roots, **(C)** the stems and **(D)** the leaves; **(E)** ratio of quantities of Na+ between roots and shoots. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05 for a same date.

As far as osmotic phase of salt stress is concerned, some accessions of *S. chilense* were reported to increase leaf succulence in response to salt stress, which is a characteristic enabling plant to cope with arid conditions (Martínez et al., 2014). In our study,

salinity did not increase the leaf WC of *S. chilense* but decreased the SLA without affecting leaf area, which could indicate succulence in this species. However, our results suggested that hybrids could have a higher constitutive leaf succulence than the parents, since salinity decreased neither SLA nor leaf osmotic potential of hybrids in contrast to their parents. Moreover, instantaneous water use efficiency (WUEi) increased with salinity in hybrids in contrast to their parents. Increase of WUEi is considered as a salt tolerance mechanism, improving the capacity of the plant to limit water loss despite salinity toxic effects (Martínez et al., 2003; Yan et al., 2015; Tarin et al., 2020). Together, these results suggested a greater tolerance to osmotic phase in hybrids compared to the parents.

Individual leaf area was not significantly decreased by salt in hybrids, in contrast to *S. lycopersicum*. A decrease in leaf area can be considered as an avoidance mechanism by reducing evapotranspiration and by extension the amount of Na+ in the shoots (Munns and Tester, 2008). It can also be considered a symptom of salt stress, which may reflect a decrease in cell expansion, common in osmotic stress, but a measurement of mesophyll cell diameter would be necessary to confirm this symptom (Tenhaken, 2015). However, instantaneous transpiration (*E*) was importantly decreased by salt in hybrids 70 DASt as observed for *S. chilense*, in contrast to *S. lycopersicum*. This decrease in *E* was not due to a regulation of the number of stomata but to a decrease in stomatal conductance (g_s). Plants close their stomata to avoid transpiration and water loss (Tapia et al., 2016; Hedrich and Shabala, 2018). In control conditions, *S. chilense* was the species with the lowest stomatal density but with the highest g_s. It could mean that this species would have stomata able to open efficiently, maybe because of bigger stomata than the other plant groups. Indeed, stomatal conductance is not always positively correlated with stomatal density, but may also be influenced by the mean stomatal size (Morales-Navarro et al., 2018). Stomatal conductance and density in hybrids were closer to *S. lycopersicum* than to *S. chilense* in control conditions although hybrids behave more like *S. chilense* under salinity regarding transpiration regulation.

Solanum chilense and hybrids were able to maintain their photosynthetic nitrogen use efficiency (PNUE) under salt stress, despite the decrease in transpiration. In contrast, the glycophyte *S. lycopersicum* was unable to maintain PNUE values in salt stress conditions. In terms of tolerance strategies, the stability of PNUE of hybrids under salt stress would enable them to synthesize proteins and osmoprotectants (Shabala, 2013; Slama et al., 2015). However, we observed no increase of soluble sugars nor proline concentrations in hybrids with salt stress. This could be related to the fact that salt did not decrease leaf osmotic potential in hybrids and that osmotic adjustment was not needed. Osmotic adjustment is performed with a high metabolic cost (Shabala, 2013). It is possible that hybrids directed their metabolism toward limiting salt stress-induced water loss rather than osmotic adjustment. In contrast, osmotic adjustment was observed in leaves of both parents; salt respectively increased sugar and proline concentrations in the leaves of *S. chilense* and *S. lycopersicum*. It was previously reported that proline contributed more to osmotic adjustment in *S. lycopersicum* than in *S. chilense* (Tapia et al., 2016;
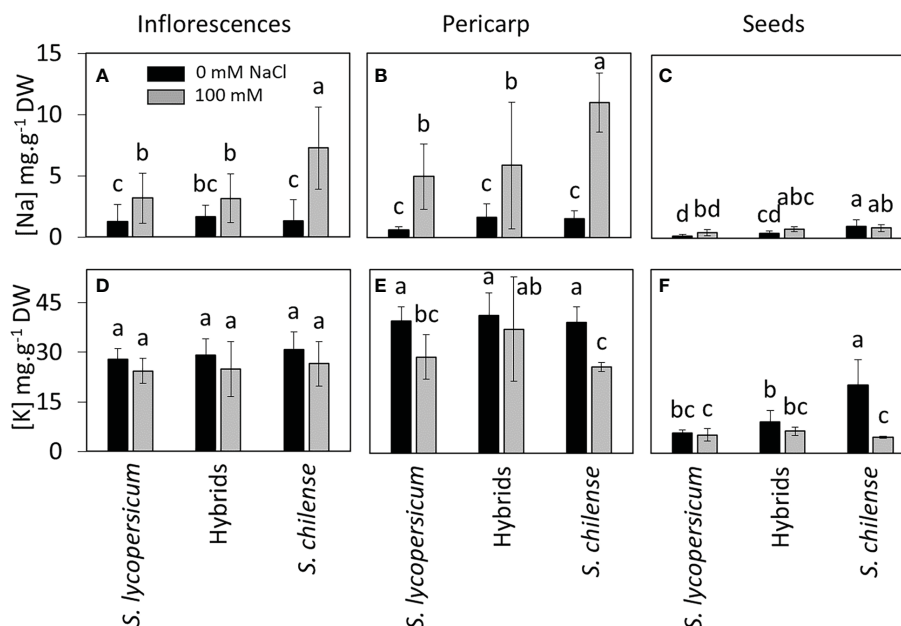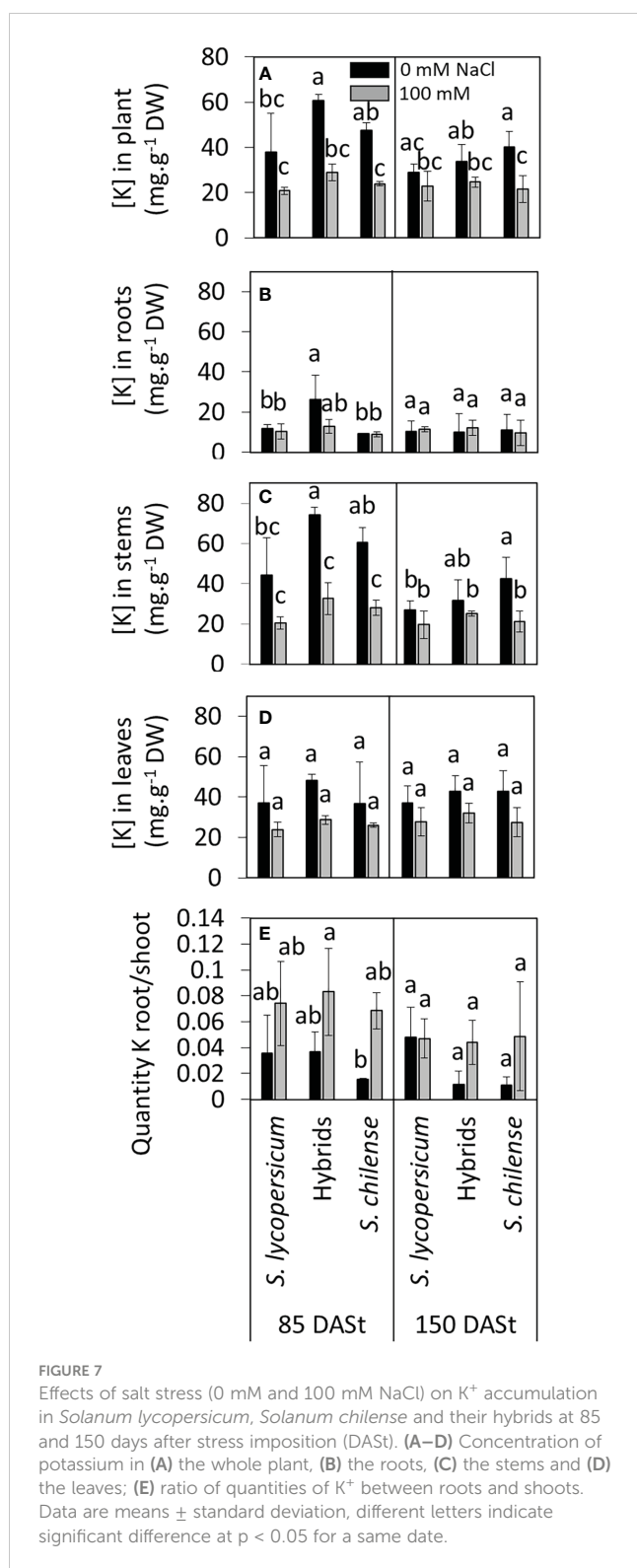
FIGURE 6
Effects of salt stress (0 mM and 100 mM NaCl) on Na^+ and K^+ concentrations in the reproductive organs of *Solanum lycopersicum*, *Solanum chilense* and their hybrids. Concentration of **(A–C)** sodium and **(D–F)** potassium in **(A, D)** inflorescences, **(B, E)** fruit pericarp and **(C, F)** seeds. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05.

Gharbi et al., 2017). Moreover, under salt stress, ions (K^+, Na^+) were shown to be the main contributors to osmotic adjustment in both species, mainly in *S. chilense* (Gharbi et al., 2017). In contrast to leaves, salt decreased root osmotic potential in the three plant groups, suggesting that osmotic adjustment was at least required in roots of the hybrids. It is likely that K^+ and Na^+ ions contribute to this osmotic adjustment as observed previously for *S. lycopersicum* and *S. chilense* (Gharbi et al., 2017). However, since Na^+ is highly toxic for cytosolic enzymes, including in halophytes, it has to be sequestered in vacuoles to display an efficient contribution to the whole cell osmotic adjustment (Flowers et al., 2015).

Despite an increase of Na^+ concentration in the three plant groups under salinity, its distribution and concentration in the plants were different between plant groups and between the two dates of measurements. Surprisingly, hybrids accumulated more Na^+ under salt stress than the parents at 85 DASt while the Na^+ concentration was similar between plant groups at 150 DASt. Na^+ concentration increased with time in *S. lycopersicum* under salinity but not in *S. chilense* and hybrids, suggesting that *S. lycopersicum* limited the Na^+ accumulation on a short-term basis but could not maintain this strategy on a long-term basis. In contrast the two other plant groups behave as typical includers and accumulated Na^+ since the beginning. It has been shown that at a very short exposure to NaCl (7 days of 125 mM NaCl), *S. lycopersicum* was able to retain a majority of Na^+ in its roots parts, avoiding exposure of aerial parts to Na^+ while *S. chilense* mainly accumulated Na^+ in the shoots (Gharbi et al., 2016). *Solanum lycopersicum* has thus been described as an excluder species and *S. chilense*, as an includer species (Gharbi et al., 2016). An excluder strategy enables the plant to protect its

photosynthetic apparatus from the Na^+ toxicity. However, to adjust osmotically, plants require the use of osmotic compounds, and notably osmoprotectants that could be energetically costly (Shabala, 2013; Flowers et al., 2015). An includer strategy enables the plant to use minerals instead of compatible osmolytes for osmotic adjustment, thereby reducing the cost in energy (Shabala, 2013; Almeida et al., 2014). Hybrids seemed to have an intermediate accumulator behavior compared to their parents as they accumulated more Na^+ in the roots than *S. chilense* (at 150 DASt) and in the shoots than *S. lycopersicum* (at 85 DASt) under salt stress, explaining the higher total Na^+ accumulation. Pérez-Alfocea et al. (1994) and Ali et al. (2021) showed that the salt-tolerant species *Solanum pennellii* also accumulated more Na^+ than *S. lycopersicum* in shoots and that their hybrids had intermediate Na^+ concentration in stems and leaves (Pérez-Alfocea et al., 1994; Ali et al., 2021). This suggests that halophyte wild species can maintain metabolism despite Na^+ accumulation and could be related to a more efficient Na^+ vacuolar compartmentation at the cell level.

A decrease of K^+ concentration is frequently observed in response to NaCl stress and often results from a lack of specificity of some transporters for Na^+ and K^+ (Ghanem et al., 2009; Gharbi et al., 2017). On the contrary, an increase in K^+ concentration has already been observed in *S. chilense* during a short-term exposure to salinity (Gharbi et al., 2017). However, in our experiment, salt decreased K^+ concentration with a stronger decrease in *S. chilense* than in *S. lycopersicum* and an intermediate decrease in the hybrids at the whole plant level; this decrease was mostly due to a decrease in K^+ concentrations in the stems. This suggests that the impact of salt on K^+ concentration in *S. chilense* may depend on the stress

Effects of salt stress (0 mM and 100 mM NaCl) on K$^+$ accumulation in *Solanum lycopersicum*, *Solanum chilense* and their hybrids at 85 and 150 days after stress imposition (DASt). **(A–D)** Concentration of potassium in **(A)** the whole plant, **(B)** the roots, **(C)** the stems and **(D)** the leaves; **(E)** ratio of quantities of K$^+$ between roots and shoots. Data are means ± standard deviation, different letters indicate significant difference at p < 0.05 for a same date.

lycopersicum. It has been discussed that halophytes can substitute K$^+$ for small amounts of Na$^+$ up to a certain concentration at which it becomes toxic for plants (Albaladejo et al., 2017; de Souza Mateus et al., 2019). Salinity also increased the K$^+$ leaves/stems ratio in *S. chilense* compared to control conditions by 3.3, suggesting that this species could use the mobile properties of K$^+$ to direct it in leaves, where K$^+$ is the most needed for photosynthesis and osmoregulation (Kanai et al., 2011). K$^+$ concentration in leaves was indeed not affected by salinity whatever the plant groups in our study.

At the reproductive level, salinity decreased inflorescence production in *S. lycopersicum* but not in *S. chilense* and had an intermediate impact in hybrids. Moreover, it affected neither flower anthesis nor flower fertility, and had only a slight effect on the number of pollen grains per anther in *S. chilense*. Such a high number of pollen grains could be a way to compensate a potential loss of pollen viability often induced by salinity (Ghanem et al., 2009; de Storme and Geelen, 2014). Moreover, Alexander staining could overestimate the rate of pollen viability because it only distinguish aborted from non-aborted pollen grains (Dafni and Firmage, 2000). Compensation between pollen grain production and pollen viability was previously reported in *S. chilense* under salt stress (Bigot et al., 2022). The decrease of flower production and flower fertility under stress is often explained by a decrease in sugar allocation to the reproductive structures (Ghanem et al., 2009). Here, we observed that the soluble sugar concentration was higher in the inflorescences of *S. chilense* and hybrids than in those of *S. lycopersicum* and that it was not affected by salt, maybe explaining the maintenance of flower fertility. The Na$^+$ accumulation in inflorescence was also lower than in the vegetative organs although it was higher in *S. chilense* than in *S. lycopersicum* and hybrids. Na$^+$ is a toxic ion for plants, which commonly exclude toxic ions from reproductive structures (Munns and Tester, 2008; Baby et al., 2016; Romero-Aranda et al., 2020). It has been shown that Na$^+$ repartition inside the flower differed between *S. lycopersicum* and *S. chilense*, (Bigot et al., 2022) but such analysis was not yet performed for hybrids. Salinity also decreased the length of sepals in *S. lycopersicum* and hybrids, as previously reported for *S. lycopersicum* (Bigot et al., 2022). Such decrease could limit the photosynthesis rate of flower and fruit (Herrera, 2005) as green reproductive organs play a role in fruit growth (Hetherington et al., 1998; Smillie et al., 1999). However, this decrease could not explain alone the decrease of fruit weight and size observed in *S. lycopersicum* since salt did not affect those parameters in hybrids. Hybrids had better yield-related parameters under salt stress than *S. lycopersicum*. Indeed, salt did not affect fruit parameters in hybrids while it decreased them in *S. lycopersicum* and mostly increased them in *S. chilense* as previously observed (Bigot et al., 2022, Martinez et al., 2012).

Salt also affected fruit quality. Water content and sugar content of hybrid fruits were not modified by salt. In contrast, salt decreased fruit WC and increased fruit sugar content in *S. lycopersicum*; maintaining a low ψs in fruits of *S. lycopersicum* by sugars is required for fruit growth (Bolarín et al., 2001; Balibrea
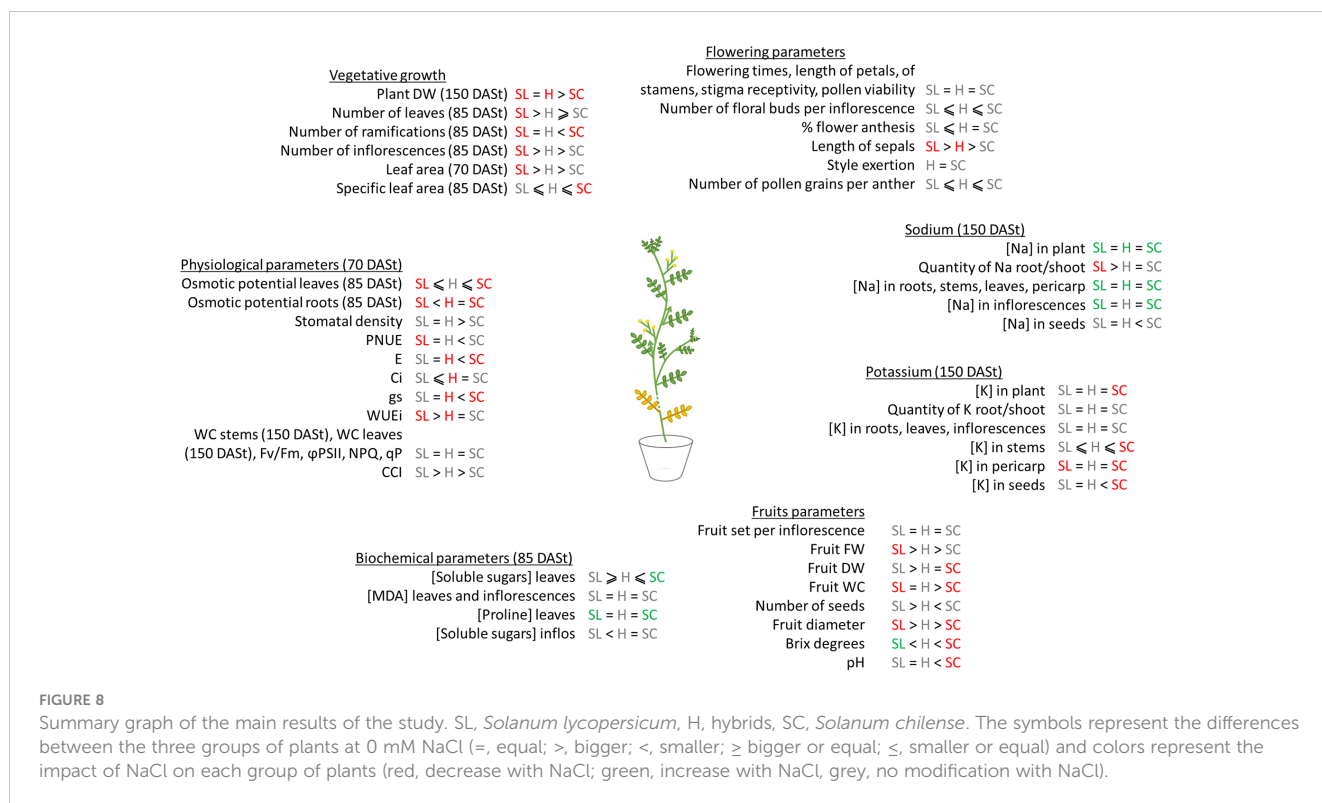
duration. Partly in accordance with our results, Pérez-Alfocea et al. (1994) did not observe a decrease in K$^+$ concentration in stems and leaves of *S. lycopersicum* but observed a decrease in those of *S. pennellii* and hybrids. Similarly, Albaladejo et al. (2017) observed a greater reduction in K$^+$ concentration in *S. pennellii* leaves than in *S.*

Vegetative growth
Plant DW (150 DASt) SL = H > SC
Number of leaves (85 DASt) SL > H > SC
Number of ramifications (85 DASt) SL = H < SC
Number of inflorescences (85 DASt) SL > H > SC
Leaf area (70 DASt) SL > H > SC
Specific leaf area (85 DASt) SL ⩽ H ⩽ SC

Flowering parameters
Flowering times, length of petals, of
stamens, stigma receptivity, pollen viability SL = H = SC
Number of floral buds per inflorescence SL ⩽ H ⩽ SC
% flower anthesis SL ⩽ H = SC
Length of sepals SL > H > SC
Style exertion H = SC
Number of pollen grains per anther SL ⩽ H ⩽ SC

Physiological parameters (70 DASt)
Osmotic potential leaves (85 DASt) SL ⩽ H ⩽ SC
Osmotic potential roots (85 DASt) SL < H = SC
Stomatal density SL = H > SC
PNUE SL = H < SC
E SL = H < SC
Ci SL ⩽ H = SC
gs SL = H < SC
WUEi SL > H = SC
WC stems (150 DASt), WC leaves
(150 DASt), Fv/Fm, φPSII, NPQ, qP SL = H = SC
CCI SL > H > SC

Sodium (150 DASt)
[Na] in plant SL = H = SC
Quantity of Na root/shoot SL > H = SC
[Na] in roots, stems, leaves, pericarp SL = H = SC
[Na] in inflorescences SL = H = SC
[Na] in seeds SL = H < SC

Potassium (150 DASt)
[K] in plant SL = H = SC
Quantity of K root/shoot SL = H = SC
[K] in roots, leaves, inflorescences SL = H = SC
[K] in stems SL ⩽ H ⩽ SC
[K] in pericarp SL = H = SC
[K] in seeds SL = H < SC

Fruits parameters
Fruit set per inflorescence SL = H = SC
Fruit FW SL > H > SC
Fruit DW SL > H = SC
Fruit WC SL = H > SC
Number of seeds SL > H < SC
Fruit diameter SL > H > SC
Brix degrees SL < H < SC
pH SL = H < SC

Biochemical parameters (85 DASt)
[Soluble sugars] leaves SL ⩾ H ⩽ SC
[MDA] leaves and inflorescences SL = H = SC
[Proline] leaves SL = H = SC
[Soluble sugars] inflos SL < H = SC

FIGURE 8

Summary graph of the main results of the study. SL, *Solanum lycopersicum*, H, hybrids, SC, *Solanum chilense*. The symbols represent the differences between the three groups of plants at 0 mM NaCl (=, equal; >, bigger; <, smaller; ≥ bigger or equal; ≤, smaller or equal) and colors represent the impact of NaCl on each group of plants (red, decrease with NaCl; green, increase with NaCl; grey, no modification with NaCl).

et al., 2006; Bonarota et al., 2022). In *S. chilense*, salt increased fruit WC but decreased sugar content, suggesting the other osmoprotectants than sugars may be involved in osmotic adjustment. In contrast to *S. chilense*, the wild tomato species *S. chmielewskii* and *S. cheesmaniae* accumulated more soluble sugars in ripe fruits than *S. lycopersicum* under salinity, as well as their hybrids (Balibrea et al., 2006). In our study, pericarp of *S. chilense* accumulated large amounts of Na$^+$, suggesting that *S. chilense* was able to use Na$^+$ for osmotic adjustment in fruits. However, hybrids did not accumulate as much Na$^+$ in pericarp than *S. chilense* but accumulated more K$^+$, suggesting that K$^+$ could assume a similar function. It was indeed shown that the salt-tolerant *S. pimpinellifollium* was able to use K$^+$ and Na$^+$ as osmotic compounds in fruits whereas *S. lycopersicum* was limited in its ability to use Na$^+$ as osmotic compound (Bolarín et al., 2001). Whatever the Na$^+$ accumulation in the fruits, the Na$^+$ content in the seeds was limited in the three plant groups, showing that the plants protected the next generation. This is a common feature in plants that exclude toxic ions from reproductive structures (Munns and Tester, 2008; Baby et al., 2016; Romero-Aranda et al., 2020).

In summary, the hybrids maintained their overall growth under salt stress, with an intermediate behavior between the parents. They accumulated intermediate concentrations of ions between the parents, but are more salt tolerant than their glycophyte parent both at the vegetative and reproductive stages. Their architecture is more similar to that of *S. lycopersicum*, making them suitable for grafting as salt-tolerant rootstocks. However, their self-incompatibility and post-zygotic barriers make them difficult to cross. Further studies on their compatibility and salinity tolerance are needed to see their potential for the improvement of cultivated tomato.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

SB, MQ, J-PM and SL contributed to conception and design of the study. SB, CL, CR performed the experiments and analyzed the data. SB performed the statistical analysis. SB and MQ wrote the first draft of the manuscript. CL, CR, J-PM, and SL wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fhort.2023.1130702/full#supplementary-material

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