Plant Physiology and Biochemistry 168 (2021) 282–293

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Role of Na^+ transporters HKT1;1 and HKT1;2 in tomato salt tolerance. I. Function loss of *cheesmaniae* alleles in roots and aerial parts

María Remedios Romero-Aranda [a,1], Jesús Espinosa [b,1], Paloma González-Fernández [b], Emilio Jaime-Fernández [a], José Ángel Traverso [c], María José Asins [d], Andrés Belver [b,*]

[a] Department of Plant Breeding and Biotechnology, La Mayora Institute for Mediterranean and Subtropical Horticulture, UMA/CSIC, Malaga, Spain
[b] Department of Biochemistry, Molecular and Cellular Biology of Plants,Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), C/ Prof. Albareda 1, 18008, Granada, Spain
[c] Department of Cellular Biology, Faculty of Sciences, University of Granada, 18071, Granada, Spain
[d] Plant Protection and Biotechnology Center, Valencian Institute of Agrarian Research (IVIA), 46113, Moncada, Valencia, Spain

ARTICLE INFO

Keywords index:
Fruit yield and quality
Grafting
K^+ and Na^+ homeostasis
Salinity
ScHKT1.1 and *ScHKT1;2* genes
Solanum cheesmaniae
Tomato

ABSTRACT

We analyzed the physiological impact of function loss on *cheesmaniae* alleles at the *HKT1;1* and *HKT1;2* loci in the roots and aerial parts of tomato plants in order to determine the relative contributions of each locus in the different tissues to plant Na^+/K^+ homeostasis and subsequently to tomato salt tolerance. We generated different reciprocal rootstock/scion combinations with non-silenced, single RNAi-silenced lines for *ScHKT1;1* and *ScHKT1;2*, as well as a silenced line at both loci from a near isogenic line (NIL14), homozygous for the *Solanum cheesmaniae* haplotype containing both *HKT1* loci and subjected to salinity under natural greenhouse conditions. Our results show that salt treatment reduced vegetative growth and altered the Na^+/K^+ ratio in leaves and flowers; negatively affecting fruit production, particularly in graft combinations containing single silenced *ScHKT1;2*- and double silenced *ScHKT1;1/ScHKT1;2* lines when used as scion. We concluded that the removal of Na^+ from the xylem by ScHKT1;2 in the aerial part of the plant can have an even greater impact than that on Na^+ homeostasis at the root level under saline conditions. Also, *ScHKT1;1* function loss in rootstock greatly reduced the Na^+/K^+ ratio in leaf and flower tissues, minimized yield loss under salinity. Our results suggest that, in addition to xylem Na^+ unloading, ScHKT1;2 could also be involved in Na^+ uploading into the phloem, thus promoting Na^+ recirculation from aerial parts to the roots. This recirculation of Na^+ to the roots through the phloem could be further favoured by *ScHKT1;1* silencing at these roots.

1. Introduction

Salinity in soils and in water available for irrigation is one of the main environmental factors limiting the growth and yields of crops, including tomato. Tomato, one of the world's most widely produced crops, is of great economic importance (FAOSTAT, 2017). Greenhouse tomato, which is mainly grown in arid and semi-arid Mediterranean regions, generates considerable revenue. However, this crop, which requires large amounts of water to grow, exerts intense pressure on water resources (Romero-Aranda et al., 2001). Given that the effects of climate change on the water cycle are expected to aggravate the problem of water scarcity in these areas, it will become increasingly necessary to use low quality irrigation water in terms of salt content. Hence, there is an urgent need to develop salt tolerant tomato crops that are capable of efficiently assimilating K^+ and other nutrients in the presence of excessive Na^+ and Cl^- (Cuartero et al., 2006).

After tomato domestication, improvements in new cultivar plants have greatly reduced the genetic pool used by breeders due to intensive selection, mostly under optimal production conditions, in the absence of soil, water or nutrient shortages among other factors (Mickelbart et al., 2015). Nevertheless, considerable genetic diversity in the tomato germplasm could be used to increase this crop's water and soil salinity resilience (Kashyap et al., 2021). The identification of quantitative trait loci (QTLs) that control salt tolerance is of paramount importance for breeding salt tolerant tomatoes (Cuartero et al., 2006). Exploiting the halotolerant genetic potential of related wild type species as donors of

* Corresponding author.
E-mail address: andres.belver@eez.csic.es (A. Belver).
[1] The authors contributed equally to this article.

https://doi.org/10.1016/j.plaphy.2021.10.018
Received 6 August 2021; Received in revised form 6 October 2021; Accepted 13 October 2021
Available online 14 October 2021

tolerance traits, identified by QTL analysis, has been postulated as an efficient strategy to reduce the impact of salinity on tomato (Cuartero et al., 2006; Asins et al., 2010). One important trait of salt tolerance in plants, including tomato, is the maintenance of cellular Na^+/K^+ homeostasis, essentially through Na^+ exclusion from the root, modulation of root-shoot transport and cellular compartmentalization of Na^+ (Van Zelm et al., 2020; Kashyap et al., 2021), even though increasing evidence suggests that K^+ channels and transporters are also crucial (Wang et al., 2020; Rubio et al., 2020). Tomato species display a broad genotypic diversity in regulating inter- and intra-cellular Na^+ transport under salt stress conditions. The most tolerant cultivars generally accumulate more salt in stems and leaves than in roots as compared to more sensitive cultivars (Estañ et al., 2008; Cuartero et al., 2006; Asins et al., 2010, 2015).

Previous genetic analyses of fruit yield and content of Na^+ and K^+ using two RIL populations derived from *Solanum lycopersicum* x *S. pimpinellifolium* and *S. lycopersicum* x *S. cheesmaniae* under salinity conditions indicate that a major QTL on chromosome 7 controlling leaf Na^+/K^+ homeostasis corresponds to two class I High-Affinity K^+ Transporters, HKT1;1 and HKT1;2, (Villalta et al., 2008; Asins et al. 2013, 2015), that have been shown to be Na^+ selective transporters (Asins et al., 2013; Almeida et al., 2014). Given the close linkage between *HKT1;1* and *HKT1;2* in tomato (Asins et al., 2013), a reverse genetic strategy based on loss of gene function was necessary to determine which HKT1 transporter, if any, plays the main role in regulating Na^+ and K^+ concentrations in shoots when cultivated under saline conditions. Thus, using two near-isogenic lines (NILs), which were homozygous for either the *S. lycopersicum* allele (NIL17) or the *S. cheesmaniae* allele (NIL14) at both *HKT1* loci, and transgenic lines derived from these NILs in which each *HKT1;1* and *HKT1;*2 had been silenced by stable transformation, showed that silencing either *S. lycopersicum* or *S. cheesmaniae* alleles at *HKT1;2* altered the leaf Na^+/K^+ ratio and caused hypersensitivity to salinity in plants cultivated under transpiration conditions, while silencing these alleles at *HKT1;*1 had a lesser effect (Jaime-Pérez et al., 2017). In these previous studies, we consistently found that *ScHKT1;2* expression levels were much lower in roots and higher in leaves, unlike *SlHKT1;2*, whose expression follows an opposite pattern (Asins et al., 2013(; Jaime-Pérez et al., 2017; Romero-Aranda et al., 2020). The differential *HKT1;2* expression levels in NIL17 and NIL14 roots and leaves were associated with differences in salt tolerance in terms of fruit yields under high salinity conditions (145.5 mM NaCl), which favoured NIL17 (Asins et al., 2013), while no such difference was found under moderate salinity conditions (70 mM NaCl) (Romero-Aranda et al., 2020). In addition, despite the negligible levels of *ScHKT1;1* and *SlHKT1;1* expression in all the tissues analyzed, as compared to those of *ScHKT1;2* and *SlHKT1;2* (Asins et al., 2013; Jaime-Pérez et al., 2017), under controlled growth chamber conditions, we observed a significant increase in the growth of the NIL14 line silenced for *ScHKT1, 1*, with respect to the non-silenced genotype, which achieved a growth rate similar to that for NIL17 in the absence of NaCl (Jaime-Pérez et al., 2017). Given that the *cheesmaniae* allele at *HKT1;1* is hyperactive in leaves and roots, unlike the *lycopersicum* allele, appears to indicate that the cultivated tomato species has diverged from these wild species by fixing a hyperactive allele in *HKT1;2*, and also by decreasing *HKT1;1* expression (Jaime-Pérez et al., 2017). Previous studies have shown that an efficient and profitable utilization of wild germplasm can be carried out through the improvement of rootstocks that confer salt tolerance in terms of fruit yield on the grafted variety which is always homozygous for the *S. lycopersicum* allele at both *HKT1;2* and *HKT1;1* (Estañ et al., 2008; Asins et al., 2015). In order to investigate the precise function of HKT1;1 and whether both *HKT1* loci play a similar role in the root and the aerial part of the tomato plant, we decided to determine the effects of silencing each *HKT1* locus only at the rootstocks or at the scion.

To clarify the role of tomato HKT1-like transporters in tomato salt tolerance, we analyzed the physiological impact of function loss on *cheesmaniae* alleles at the *HKT1;1* and *HKT1;2* loci in the roots and aerial parts of the tomato NIL14 line in order to determine the relative contribution of each isoform in the different tissues to tomato Na^+/K^+ homeostasis and subsequently to tomato salt tolerance. To do this, we carried out a salinity tolerance experiment using reciprocal grafting combinations of non-silenced NIL14, single RNAi-silenced lines for *ScHKT1;1* and *ScHKT1;2*, as well as a double silenced line at both loci.

2. Materials and methods

2.1. Plant material

The tomato near-isogenic line NIL14 used in this study was obtained as described elsewhere (Asins et al., 2013). Briefly, it was developed by selfing a segregating F6 line (RIL B157), which itself was obtained after 5 selfing generations of an F1 progeny from a cross between a salt sensitive genotype of *S. lycopersicum*, var. Cerasiform (E9) as the female parent, and the salt tolerant accession L2 (Asins et al., 1993) from *S. cheesmaniae* (L. Riley) Fosberg as a male parent (Monforte et al., 1997; Villalta et al., 2007, 2008). NIL14 and parents were genotyped for the 7720 SolCAP panel of SNP. Both parents (E9 and L2) share genotype at 4800 SNP loci. NIL14 presents E9 genotype at 1919 SNP loci, and *S. cheesmanieae* genotype at 981 SNP loci, including *HKT1;1* and *HKT1;2* loci. Regarding other genes involved in Na^+ homeostasis, this NIL is homozygous for the *S. cheesmaniae* allele at *SOS1*, and *NHX4*, and homozygous for the E9 allele at *SlHAK20* (Wang et al., 2020), as inferred from the genotypes at the flanking SNPs (solcap_snp_sl_64082 and solcap_snp_sl_21335) of Soly04g008450.2; therefore, differences found in this study cannot be attributed to line segregation at them. We used three homozygous transgenic lines derived from NIL14 in which the *cheesmaniae* alleles at *HKT1;1* (L1.2) and *HKT1;2* (L47.1) were silenced by stable transformation using RNAi, as described elsewhere (Jaime-Pérez et al., 2017). Initial transgenic lines (L1.2 and L47.1) were crossed, and three selected homozygous transgenic lines were derived from a single hybrid plant (plant 3.13) by successive selfings and marker-assisted selection at each selfing generation: line S8 (h1, silenced at *ScHKT1;1*), line S30 (h2, silenced at *ScHKT1;2)*, and line S110 (h1-h2, silenced at both loci, *ScHKT1;1* and *ScHKT1;2*), as shown in Fig. 1. As control for comparative purposes, the non-silenced NIL14 (Wt) was used. Reciprocal rootstock/scion graft combinations were performed with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1), *ScHKT1;2* (h2), as well as silenced lines at both loci (h1-h2). Non-silenced self-grafted Wt/Wt was used as control. Genotype codes of all tomato graft combination lines used in the present study are shown in Suppl. Table S1.

2.2. Grafting procedure, greenhouse growth conditions and salt treatments

Grafting was performed when seedlings had developed 3–4 true leaves (27 d after germination) in the Semilleros Saliplant SL facility (Carchuna, Granada, Spain). For reciprocal rootstock/scion combinations, seedlings of each line described above (section 2.1) were cut over the cotyledons, using the shoot as scion and the remaining plant part as rootstock. After grafting, plants were grown as described elsewhere (Romero-Aranda et al., 2020). Briefly, grafted plants with 6 leaves (45 d after germination, 18 d after grafting) were transferred to a 900 m^2 polyethylene greenhouse located at La Mayora Estación Experimental (IHSM-CSIC) in Malaga in Southern Spain (36°45′20.78″N 4°02′28.72″W) (Suppl. Fig. S1). At this developmental stage, 6-7 week-old plants were transplanted to 17 L plastic pots filled with vermiculite, with one single grafted plant per pot. Pots in the greenhouse were arranged in rows 1 m apart, corresponding to a density of two plants per square meter. The graft combinations were randomly arranged in the rows. Two extra plants at the beginning and end of each row and two rows of plants before and after the first and final experimental rows were positioned to prevent border effects. Plants were

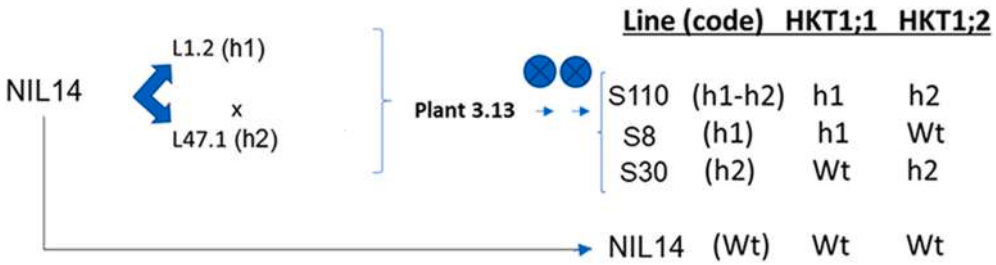

Fig. 1. Obtaining homozygous silenced lines S8, S30and S110 derived from NIL14, a homozygote for the *S. cheesmaniae* allele at both *HKT1* loci. Wt:, non-silenced wild type; h1: *ScHKT1;1*-silenced; h2: *ScHKT1;2*-silenced; h1-h2: *ScHKT1;1* and *ScHKT1,2*-double silenced lines.

grown under natural greenhouse light conditions without temperature control during the autumn-spring season (November 2018 to April 2019) (Suppl. Fig. S1). Pollination was performed manually from flowering start to fruit set. Pest and disease control was carried out according to standard commercial practices. All axillary vegetative buds were removed from the main stems which were trained vertically using plastic nets attached with string to the greenhouse ceiling. The plants were fertirrigated from the transplanting stage to the end of the experiment, with a standard Hoagland nutrient solution adapted to tomato (5, 9 mM N, 1 mM P, 6.6 mM K^+, 4.5 mM Ca^{2+}, 2 mM S, 2 mM Mg^{2+}, and microelements) (Hoagland and Arnon, 1950). Irrigation was computer-controlled to provide all plants with the same volume of nutrient solution, using an automatic drip irrigation system, with one dripper per plant discharging 2 L h^{-1} of the solution. Salt treatment was applied 8d after transplantation (53 d after germination, 26 d after grafting). Thirty plants per graft combination were irrigated with Hoagland nutrient solution supplemented with either 0 or 70 mM NaCl until completion of the experiment (166 d after starting salt treatment). The electrical conductivity (EC, dS m^{-1}) and pH of the irrigation solution provided by the drippers were measured weekly to confirm the stability of the irrigation conditions during the entire experimental period (CE = 2.9 dS m^{-1}, pH = 5.8–6.0).

2.3. Xylem sap collection, determination of plant dry biomass, and Na^+ and K^+ analysis

After 60 days of salt treatment, the aerial part of six plants per graft combination and saline treatment was severed at 1.5 cm above the base of the stems where a silicone tube was inserted to collect the xylem sap exudate. The volume of xylem sap collected during the first 10 min was discarded to rule out possible contamination from damaged cells and was then collected for 20–60 min and stored at 4 °C for further analysis (Romero-Aranda et al., 2020). After the xylem sap was collected, the aerial parts (stems, leaves and flowers) and washed roots were identified, placed in paper bags and dried at 65 °C for 72h in a forced-air oven, followed by measurement of dry mass. The dry biomass of the aerial part (APDW) was calculated as the sum of the dry biomasses of leaves + stems + flowers. The aerial part to root (AP/R) ratio was calculated by dividing aerial part dry weight (APDW) by root dry weight (RDW). Na^+ and K^+ content in the xylem sap; in the 3rd inflorescence (including peduncles, pedicels and flowers) and in the leaf directly below this inflorescence (9^{th} leaf), all sampled on d 60 of salt treatment, was analyzed for ion composition using a Varian 720-E inductively coupled plasma-optical emission spectrometer (ICP-OES; Scientific Instrumentation Service, EEZ, CSIC, Granada, Spain).

2.4. Determination of yield, fruit content of total soluble solids, fruit acidity, and fruit antioxidant content

Fruits from the first three trusses were harvested while maturing from the end of February 2019 up to the beginning of April 2019 (after between 105 and 145 d of salt treatment). Number of fruits and fruit weight were determined for six plants per graft combination and salt treatment. Total yield per plant was calculated by adding up the number and weight of fruits from all harvests to obtain the total fruit number (TFN) and total fruit weight (TFW). Fruits were washed and ground to a homogeneous liquid paste in a blender (Braun, Kronberg, Germany). Subsamples of the homogenates obtained were immediately frozen at −20 °C and stored for further analysis. Soluble sugar content (SSC-°Brix-), titrable acidity (A), total carotenoids (TCarot) and total phenolic compounds (phenolics) were determined as described elsewhere (Romero-Aranda et al., 2020).

2.5. Gene expression analysis by qRT-PCR

Total RNA was purified from roots, the 7^{th} leaf and the 1^{st} floral inflorescence, including peduncle, pedicel and floral receptacles, from all graft combinations. Three independent biological samples, with one plant per sample and treatment, were used for analysis in an initial purification step with CTAB extraction buffer, followed by separation in chloroform:isoamyl alcohol (24:1, v:v) and LiCl precipitation, according to the protocol described by Liao et al. (2004). Resuspended RNA was further purified using the Aurum™ total RNA mini kit (Bio-Rad Laboratories, S.A.), together with RNAse-free DNase in-column treatment (Promega Biotech Ibérica, SL), as well as RNAsecure™ resuspension solution (Ambion Europa Ltd, Austin, TX, USA) for elution and resuspension according to the manufacturer's instructions. First-strand cDNA synthesis from 1 μg of total RNA was performed with iScript™ Reverse T Supermix for RT-qPCR (Bio-Rad Laboratories, S.A.) using the oligo-dT and random hexamer primer blend provided according to the supplier's protocol. As described elsewhere (Asins et al., 2013; Jaime-Pérez et al., 2017), RT-qPCR was carried out using 1 μl of undiluted cDNA mixed with *iTaq™ universal SYBR green supermix* (BioRad) and 0.45 μM of forward and reverse primers (Suppl. Information Table S2) in a *QuantStudio™ 3 Real-Time PCR System* (Applied Biosystems, Thermo Fisher Scientific). Relative expression data (fold change) were calculated from the difference in the threshold cycle (ΔC_t) between the genes studied and DNA amplified by specific primers for the tomato elongation factor 1α (*LeEF1-α,* acc. AB061263) as housekeeping gene. In previous studies, *LeEF1-α* was shown to be highly stable regardless of genotype, tissue or treatment (Asins et al., 2013; Jaime-Pérez et al., 2017; Romero-Aranda et al., 2020). Relative expression levels were calculated with the aid of the $2^{-[\Delta\Delta Ct]}$ equation (Livak and Schmittgen, 2001) using the expression level of each gene in each tissue and the expression levels of the non-silenced, non-treated, self-graft combination Wt/Wt as the calibrating sample. Due to the heterogeneity of these data, ANOVA statistical analysis was carried out following transformation using the $\log_2$ Fold Change application (Rieu and Powers, 2009).

2.6. Statistical analysis

Data were analyzed by two-way ANOVA with the factors graft-combination (G), salt treatment (E) and the interactions of G × E (Statgraphics Centurion software, 2009). When one or both factors had a

significant impact but their interaction was insignificant, the means of the treatments of one or both factors were separated by Fisher's least significant difference (LSD) test ($P < 0.05$). The same test was used to separate the means of all treatments when the interactions were also significant.

3. Results

3.1. Graft combinations with ScHKT1;2 function loss showed a visible salt-hypersensitive phenotype in the vegetative stage associated with an altered Na^+/K^+ ratio in leaves and flowers as well as a sharp reduction in fruit yields

Transcript levels of *ScHKT1;2* were analyzed in root, leaf and flower tissues in selected reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double silenced lines at both loci (h1-h2), grown for 30 d under control and saline conditions, to confirm that these genes are actually silenced in the respective specific tissues (Fig. 2). *ScHKT1;2* gene expression in roots and aerial parts was strongly silenced where expected. In roots, these expression levels were significantly lower in rootstocks bearing *ScHKT1;2*-silenced lines, such as h2/Wt and h1-h2/Wt, including self-graft combinations h2/h2, h1-h2/h1-h2, than in the non-silenced self-graft Wt/Wt (Fig. 2A). In leaf and flower tissues, *ScHKT1;2* expression levels in scions bearing *ScHKT1;2*-silenced lines, such as the Wt/h2, Wt/h1-h2, and the respective self-grafts h2/h2, h1-h2/h1-h2, were also lower than in non-silenced self-graft Wt/Wt (Fig. 2B and C). However, in roots, *ScHKT1;2* gene expression was also low in non-silenced rootstocks when grafted under silenced aerial parts such as Wt/h2 and Wt/h1-h2 (Fig. 2A). Meanwhile, scarcely at all in

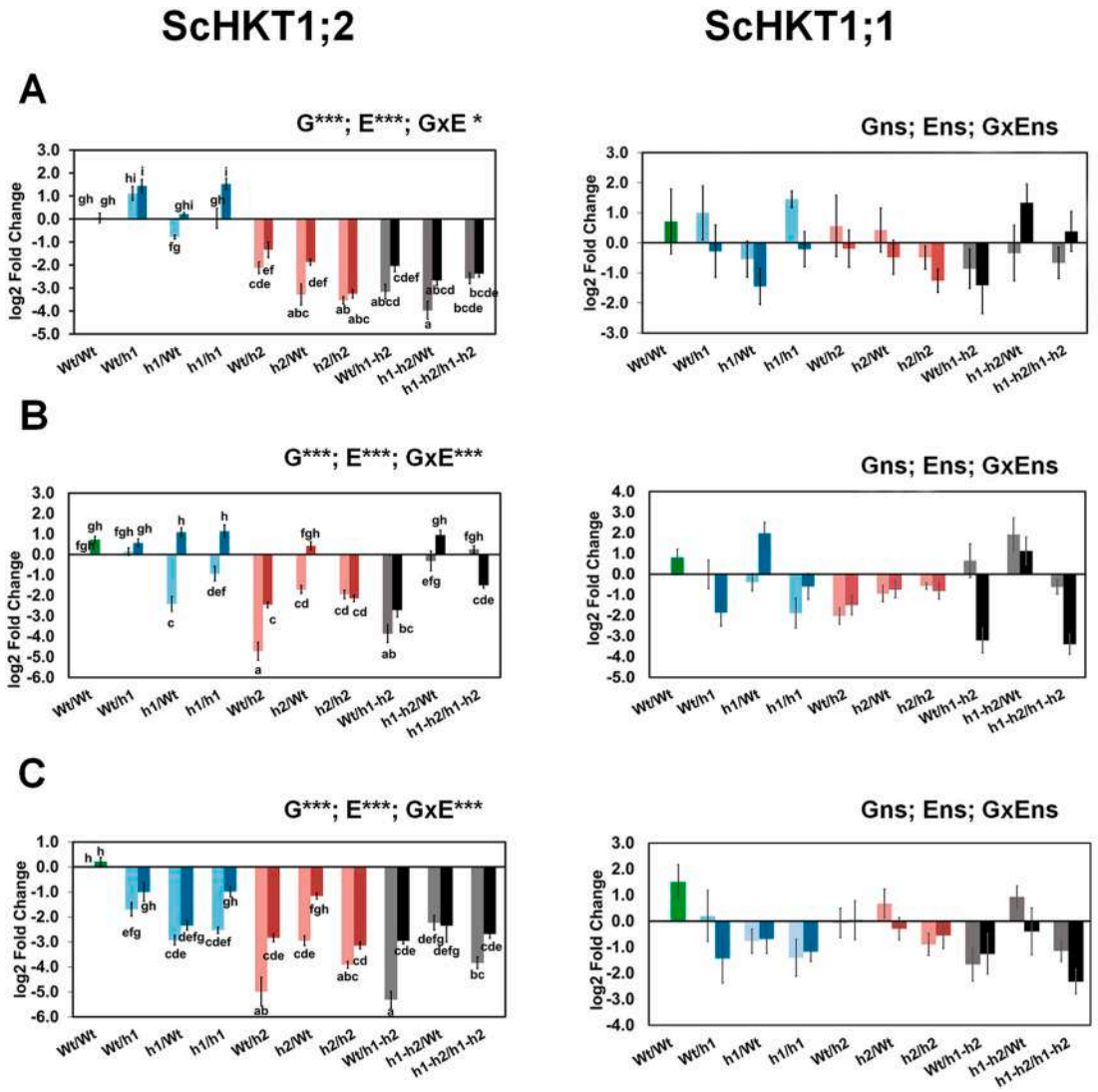

Fig. 2. Transcript levels of *ScHKT1;2 and* ScHKT1:1 in root (A), leaf (B) and flower (C) tissues in reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double-silenced lines at both loci (h1-h2) grown under control and saline conditions. Total RNA was purified from roots, the 7th leaf and 1st floral inflorescence, including the peduncle, pedicel and floral receptacle of grafted combinations cultivated in vermiculite in 17 L pots irrigated with 1x Hoagland solution and treated for 30 d with 0 mM NaCl (clear bars) and 70 mM NaCl (dark bars) under natural greenhouse conditions during the fall-spring season (November–April). The tomato elongation factor gene, LeEF-1α, was used as the reference gene. The results show an increase or decrease in the transcript levels of each *HKT1;2* gene in relation to the levels for roots, leaves and flowers of the Wt/Wt combination graft cultivated in the absence of stress, to which the value 0 was assigned. Each value is the mean ± the standard error of nine replicates for roots and flowers (three biological and three technical replicates). Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

leaves and particularly, in flowers, this unexpected silencing effect also occurred in non-silenced scions when grafted onto silenced rootstocks (h2/Wt and h1-h2/Wt), but to a lesser extent than in the case described above (Fig. 2B and C). This expression pattern could be due to small interference RNA (siRNA) fragments translocated from shoots to roots and vice versa via the phloem, which induces silencing machinery in these tissues (Brosnan and Voinnet, 2011). However, this subsequent silencing effect on *HKT1;2* gene expression after 30 d of salt treatment, when silenced lines are grafted under or onto non silenced organs, appears to be transient; the effect disappeared after 60 d of salt treatment when *HKT1;2* gene expression was measured in tissues, such as peduncles and flower receptacles of the third floral truss of selected graft combinations (Suppl. Fig. S2). This silencing effect may also have disappeared in roots, where gene expression was not, however, measured. As observed in previous studies of NIL14 Wt plants (Asins et al., 2013) and NIL 14 with *ScHKT1;2* silenced in the whole plant (Jaime-Pérez et al., 2017; Romero-Aranda et al., 2020), salt treatment with 70 mM NaCl for 30 d did not significantly affect *ScHKT1;2* expression in any tissue of self-grafted Wt/Wt, h2/h2 or h1-h2/h1-h2. Likewise, in most graft combinations with h2 lines, either as rootstock or scion, salt treatment increased *ScHKT1;2* expression levels to a certain extent in roots and aerial parts (leaves and flowers) as compared to the control, but never reached the transcript levels of non-silenced Wt/Wt. In graft combinations with *ScHKT1;1*-silenced lines, either as rootstock or scion, *ScHKT1;2* expression levels in roots and leaves remained unchanged with respect to non-silenced Wt/Wt under control and saline conditions, except for an unexpected decrease observed in the leaves of h1/Wt under control conditions and an increase in h1/h1 roots under saline conditions (Fig. 2A and B). In flowers, *ScHKT1;2* expression levels decreased in all graft combinations with *SHKT1;1*-silenced lines under control and saline conditions.

Growth in roots (RDW) and aerial parts (APDW), expressed as dry weight, and the visual appearance of reciprocal rootstock/scion graft combinations grown under control and saline conditions are shown in Fig. 3 and Suppl. Fig. S3, respectively. After 60 d of salt treatment, with 70 mM NaCl added to the irrigation solution, non-silenced self-grafted Wt/Wt plants showed a significant reduction in shoot growth (stems + leaves), with no significant changes in root biomass production observed. However, no remarkable visual symptoms were observed despite a significant increase in foliar Na^+ content and a decrease in K^+ content (Fig. 4). Salinity reduced APDW growth to different degrees in graft combinations, with either h2 or h1-h2 used as rootstock and scion (Fig. 3). Specific salt effects in graft combinations with h1 used as either rootstock or scion will be assessed in Section 3.2. As compared to the control, salt treatment for 60 d significantly reduced growth in the dry weight of both roots and aerial parts in graft combinations with h2 or h1-h2 used as scion, as well as self-grafted h2/h2 and h1-h2/h1-h2 (Fig. 3). All these graft combinations (Wt/h2, h2/Wt, h2/h2, Wt/h1-h2, h1-h2/Wt and h1-h2/h1-h2) showed a visible salt-hypersensitive phenotype in the vegetative stage (Suppl. Fig. S3). After 60 d of salt treatment, initial foliar necrosis at the edge of the leaves was observed; which then expanded to the whole leaf surface and from the leaves at the plant's base to those at the top. After 83 d of salt treatment, we observed total necrosis in basal leaves, marginal necrosis in young leaves and necrosis in inflorescences. Most flowers showed necrotic sepals and petals and even significant floral abscission; whose effects were exacerbated after 166 d by the end of the assay. Throughout the assay, non-silenced plants Wt/Wt showed green leaves with no necrotic symptoms.

The salt-hypersensitive phenotype observed in Wt/h2, h2/Wt, h2/h2, Wt/h1-h2, h1-h2/Wt and h1-h2/h1-h2 could be related to higher Na^+ accumulation and a higher Na^+/K^+ ratio recorded in leaves and flowers (Figs. 4 and 5). Xylem sap analysis showed an overall significant increase in Na^+ in all graft combinations, with h1, h2 or h1-h2 used as rootstock and scion grown with 70 mM NaCl; the highest values were recorded on the h1-h2/h1-h2, while, in this combination, xylem sap showed a slight reduction in K^+ content, and no significant change was

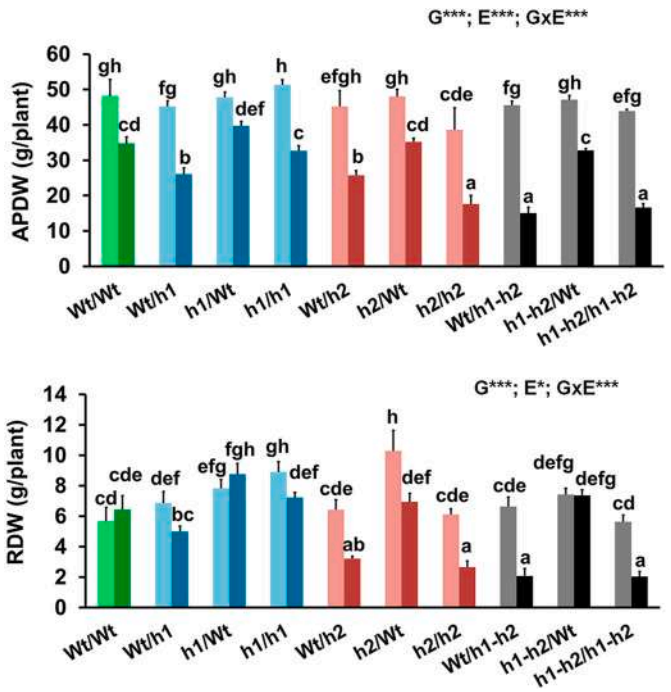

Fig. 3. Growth expressed as aerial part dry weight (APDW) and root dry weight (RDW) in reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double-silenced lines at both loci (h1-h2) grown under control and saline conditions. Grafted combinations were cultivated in vermiculite in 17L pots irrigated with 1x Hoagland solution and treated for 60 d with 0 mM NaCl (clear bars) and 70 mM NaCl (dark bars) under natural greenhouse conditions during the fall-spring season (November–April). Values represent mean ± standard error of six different samples. Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

detected in h2/h2, Wt/h1-h2, h1/Wt or h/h1 (Fig. 6). However, no relationship between Na^+ content in xylem sap and Na^+ content in leaves was observed with respect to the different graft combinations (Figs. 4–6).

Tomato yields measured as total fresh weight (TFW) and total fruit numbers (TFN) are shown in Fig. 7. Under non-saline conditions, yields were similar to Wt/Wt in most graft combinations except for h1/h1, h2/Wt and h1-h2/h1-h2, which showed a slight but significant increase, while a sharp reduction in h2/h2 self-grafts was observed. As expected, salinity reduced TFW in all graft combinations although this reduction was not significant in h1/Wt. This salt-induced reduction in TFW was even higher in graft combinations containing h2 and h1-h2 lines used as either rootstock or scion than in salt-treated Wt/Wt, except h2/Wt. Regarding the number of fruits per plant, there was a clear salt-induced reduction in TFN in Wt/h2, h2/Wt, and the three combinations containing h1-h2. Fruit quality parameters are shown in Fig. 8. Under non-saline growth conditions, the range of means for acidity (A, as % of citric acid), soluble sugar content (SSC) expressed as °Brix, and total phenolic compounds was quite narrow as compared to that under salinity conditions where an overall increment was observed in these quality traits. A notable exception was the self-grafted silenced line for *ScHKT1;2* (h2/h2), whose SSC and total phenolics did not increase. Total carotenoids remained unchanged (Fig. 8).

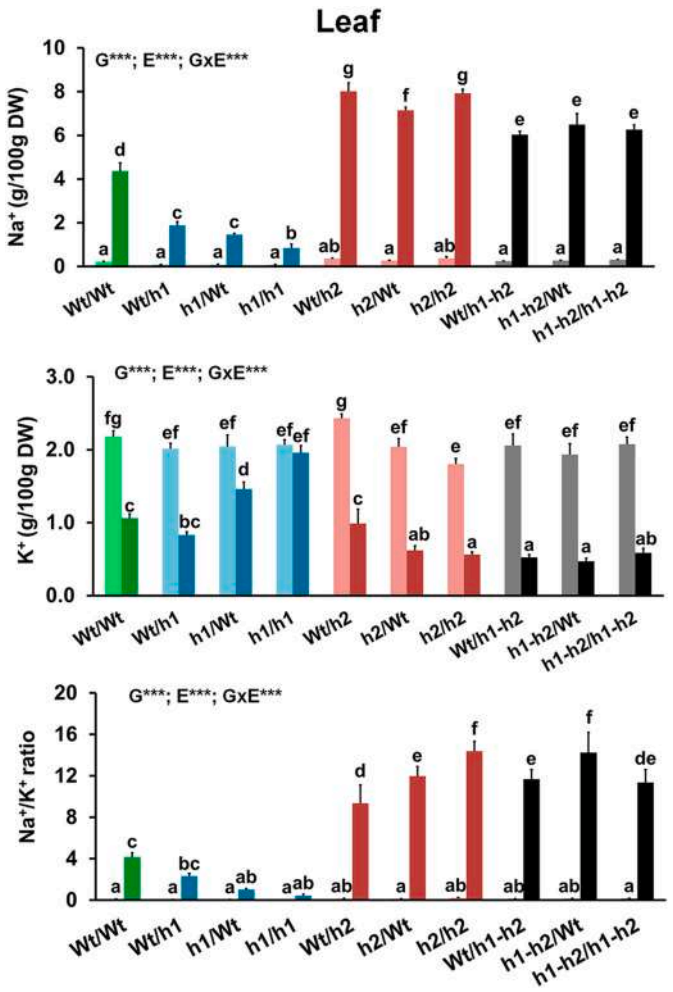

Fig. 4. Leaf Na^+ and K^+ content and the Na^+/K^+ ratio in the leaf immediately below the 3rd inflorescence (9th leaf) in reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double-silenced lines at both loci (h1-h2) grown under control and saline conditions. Na^+ and K^+ content in the 9th leaf of grafted combinations cultivated in vermiculite in 17 L pots irrigated with 1x Hoagland solution and treated for 60 d with 0 mM NaCl (clear bars) and 70 mM NaCl (dark bars) under natural greenhouse conditions during the fall-spring season (November–April). Values represent mean ± standard error of six different samples. Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

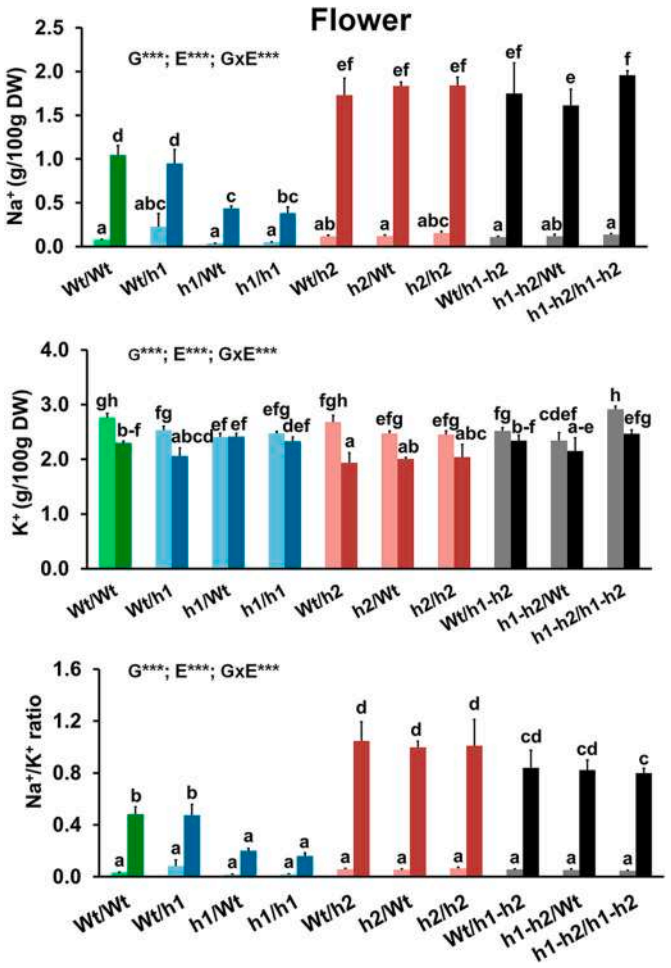

Fig. 5. Flower Na^+ and K^+ content and the Na^+/K^+ ratio in control and salt-treated reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double-silenced lines at both loci (h1-h2) grown under control and saline conditions. Na^+ and K^+ content in the 3rd floral inflorescence, including the peduncle, pedicel and floral receptacle, of grafted combinations cultivated in vermiculite in 17L pots irrigated with 1x Hoagland solution and treated for 60 d with 0 mM NaCl (clear bars) and 70 mM NaCl (dark bars) under natural greenhouse conditions during the fall-spring season (November–April). Values represent mean ± standard error of six different samples. Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

3.2. Na^+ accumulation with saline irrigation in leaves was lower in graft combinations with single ScHKT1;1-silenced lines, with no marked visual symptoms in the vegetative stage, and their fruit yields were similar to control plants but enhanced for SSC under salinity conditions

No significant differences in *ScHKT1;1* expression levels were observed among graft combinations with *ScHKT1;1*- or *ScHKT1;2*-silenced lines as compared to non-silenced lines in any tissue (root, leaf or flower), even after salt treatment with 0 and 70 mM NaCl for 30 days under commercial greenhouse conditions (natural light and no temperature control) (Fig. 2). This could be due to the highly variable data obtained, together with the low expression levels detected in all tissues from the silenced and non-silenced lines, as Ct levels exceeding 30 were systematically observed for *ScHKT1;1*, which is very close to the qPCR detection range for controls with no RNA (results not shown). Salt treatment over a period of 60 d slightly reduced shoot growth (APDW) in graft combinations bearing single *ScHKT1;1*-silenced lines as rootstock h1/Wt, and self-graft h1/h1 in a similar way to Wt/Wt (Fig. 3). In addition, growth of the root system (RDW) in h1/Wt was unaffected by salt treatment (such as Wt/Wt), and h1/h1 recorded growth comparable to that of self-grafted Wt/Wt. Moreover, the h1/Wt, and h1/h1 grafts showed lower Na^+ in the 3rd flower truss and in the leaf immediately below, resulting in a Na^+/K^+ ratio lower than that for Wt/Wt self-grafts under salinity (Figs. 4 and 5). They also showed no pronounced visual symptoms of deleterious salt effects (Suppl. Fig. S3). No significant salinity effect was detected for TFW in h1/Wt graft combination (Fig. 7), while the TFN of any combination bearing silenced *HKT1;1* either as scion or rootstock did not change significantly (such as Wt/Wt), and their means under salinity were similar to those of Wt/Wt. It is noteworthy that fruits from graft combinations Wt/h1, h1/Wt and h1/h1

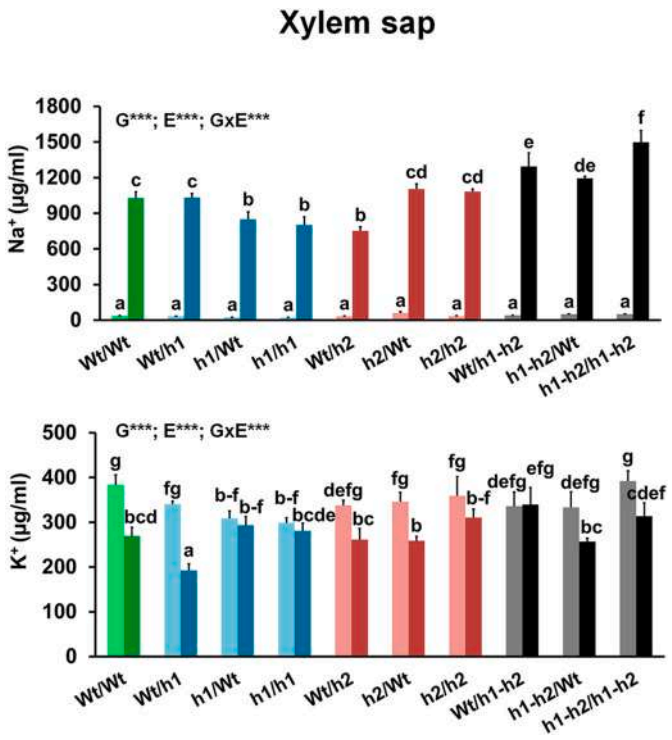

Fig. 6. Xylem sap Na^+ and K^+ content in reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double silenced lines at both loci (h1-h2) grown under control and saline conditions. Na^+ and K^+ content in xylem sap from grafted combinations cultivated in vermiculite in 17 L pots irrigated with 1x Hoagland solution and treated for 60 d with 0 mM NaCl (clear bars) and 70 mM NaCl (dark bars), under natural greenhouse conditions during the fall-spring season (November–April). Values represent mean ± standard error of six different samples. Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

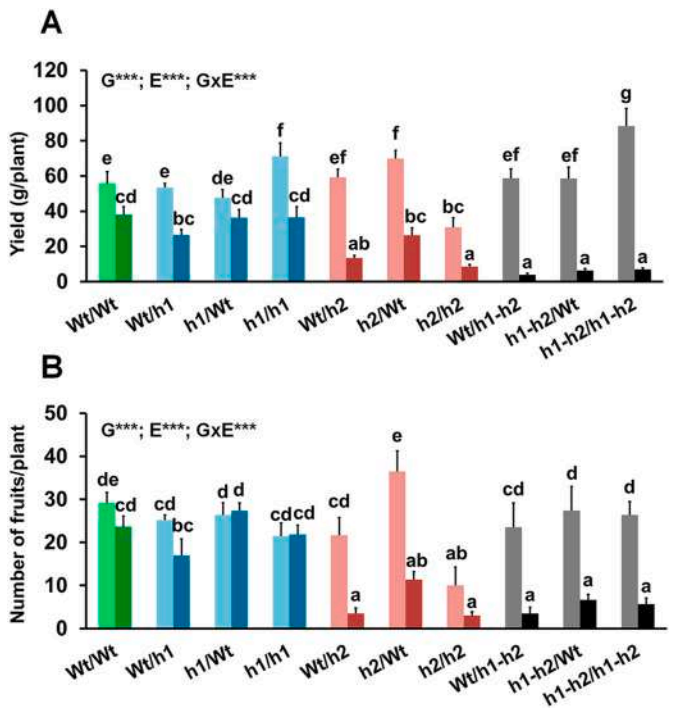

Fig. 7. Fruit yield measured as total weight per plant (A) and number of fruits per plant (B) of rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double silenced lines at both loci (h1-h2) grown under control and saline conditions. Grafted plants were cultivated in vermiculite pots and irrigated with 1x Hoagland solution in a commercial greenhouse and treated for 159 d with 0 mM (clear bars) and 70 mM NaCl (dark bars). Fruits were harvested from the three first trusses during a salt treatment period of 104 and 159 d. Values represent mean ± standard error of six different samples Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their statistical significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

contained higher SSC than those of Wt/Wt under salinity conditions (Fig. 8).

4. Discussion

As can be seen in Fig. 9, depicting a schematic representation of Na^+ homeostasis by different transport proteins in tomato, Na^+ concentrations in various tissues of tomato plants under salinity conditions, and consequently Na^+ (and K^+) homeostasis, are regulated by different Na^+ transporters including the plasma membrane Na^+/H^+ exchanger SOS1 and its regulatory proteins (Olías et al., 2009; Huertas et al., 2012; Fig. 9, points 2, 3), HKT1-like Na^+ transporters (Asins et al., 2013; Jaime-Pérez et al., 2017, Fig. 9, points 5, 6), as well as certain NHX cation/proton antiporter isoforms (Rodríguez-Rosales et al., 2009; Huertas et al., 2012, 2013, Fig. 9, points 3, 4). It is particularly important to investigate the role of genes encoding HKT1-like Na^+ transporters in Na^+/K^+ homeostasis in tomato under saline conditions to improve tomato salt tolerance. These transporters play an important role in Na^+ and K^+ homeostasis under saline conditions in many model and crop plants by removing Na^+ from the xylem and by controlling its accumulation in shoots, as well as through Na^+ recirculation via the phloem (Berthomieu et al., 2003; Ren et al., 2005; Sunarpi et al., 2005; Davenport et al., 2007; Hauser and Horie, 2010; Munns et al., 2012; Byrt et al., 2014; Kobayashi et al., 2017; Henderson et al., 2018). Tomato *HKT1;1* and *HKT1;2* genes encode class I HKT Na^+-selective transporters (Asins et al., 2013; Almeida et al., 2014), which are expressed in the vascular system, particularly in the xylem and possibly in the phloem of tomato root and shoot tissues (Jaime-Pérez et al., 2017). In previous studies, we demonstrated that the Na^+ transporter-encoding *HKT1;2* gene functionally underlies the major QTL controlling shoot Na^+/K^+ homeostasis and plays an important role in tomato salt tolerance in terms of vegetative growth (Jaime-Pérez et al., 2017; Romero-Aranda et al., 2020). However, the Na^+ transporter-encoding *HKT1;1* gene had little effect on tomato shoot Na^+/K^+ homeostasis under controlled growth chamber conditions (Asins et al., 2013; Jaime-Pérez et al., 2017). In this study, we carried out a reciprocal grafting experiment using the *cheesmaniae* allele-bearing NIL14 at the HKT1 locus and their transgenic silenced derivatives for both *ScHKT1;1* and *ScHKT1;2* in order to determine in which tissue loss of function of each isoform has the greatest impact on plant salt tolerance. It is also important to determine the relative contribution of each gene to the removal of Na^+ from the xylem at root level with respect to that of the aerial part of this NIL, its role in phloem loading and its impact on sink tissues.

4.1. The combined removal of Na^+ from the xylem and, possibly, Na^+ uploading into the phloem in the aerial part by ScHKT1;2 play an important role in preventing a negative saline impact on growth in the aerial part and on fruit yields

Function loss in *ScHKT1;2* throughout the plant in self-grafted h2/h2 under saline conditions resulted in a sharp increase in the Na^+/K^+ ratio

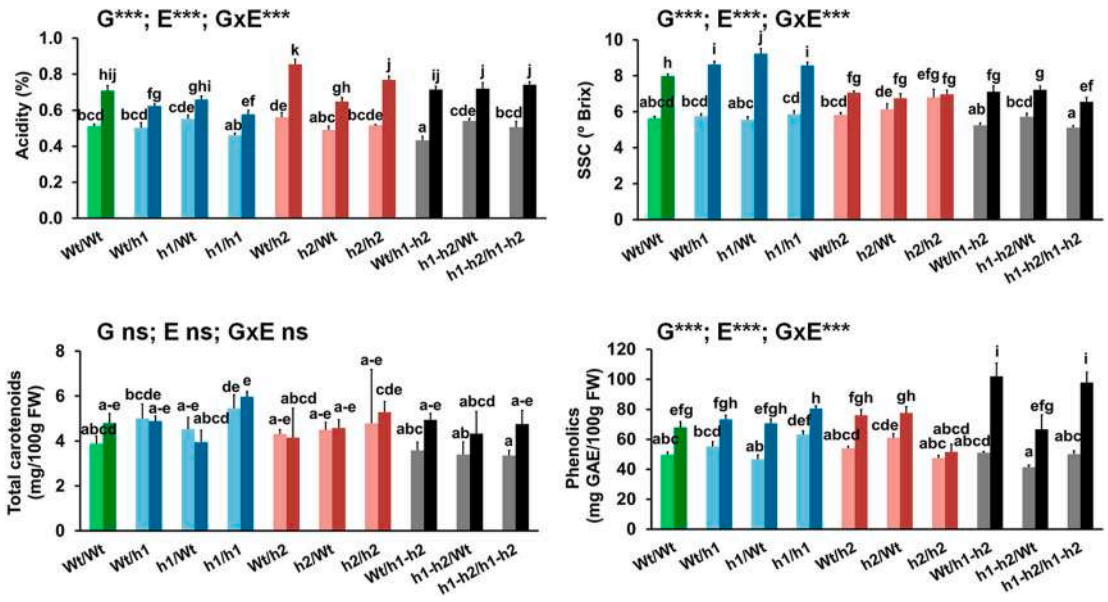

Fig. 8. Quality parameters, titrable acidity, sugars (°Brix), carotenoids and phenolics for fruits in reciprocal rootstock/scion combinations with non-silenced (Wt), single RNAi-silenced lines for *ScHKT1;1* (h1) and *ScHKT1;2* (h2), as well as double-silenced lines at both loci (h1-h2) grown under control and saline conditions. Grafted plants were cultivated in vermiculite pots and irrigated with 1x Hoagland solution and treated for 159 d with 0 mM (clear bars) and 70 mM NaCl (dark bars) under natural greenhouse conditions during the fall-spring season (November–April). Fruits were harvested from the three first trusses during a salt treatment period of 104 and 159 d. Values represent mean ± standard error of six different samples. Data were analyzed by two-way ANOVA using genotype (G), salt treatment (E) and their interaction (GxE) as sources of variation. Their significance was evaluated (P-value, not significant, ns, *P < 0.05, **P < 0.01, ***P < 0.001). Significant differences are indicated by different letters according to Fisher's LSD test (P < 0.05).

in tomato leaves as compared to the non-silenced self-grafted Wt/Wt (Figs. 4 and 5), being these effects associated with a sharper reduction in aerial part (APDW) and root (RDW) growth (Fig. 3), as well as a visible salt-hypersensitive phenotype in the vegetative stage (Suppl. Fig. S3). This salt-hypersensitive phenotype is similar to that of the Arabidopsis *hkt1;1* mutant, indicating that ScHKT1;2 and AtHKT1;1 are orthologues (Berthomieu et al., 2003; Sunarpi et al., 2005; Davenport et al., 2007). These data corroborate our previous results obtained with silenced lines throughout both plant grown under controlled conditions in a culture chamber (Jaime-Pérez et al., 2017), as well as under natural greenhouse conditions usual for commercial greenhouse tomato production (Romero-Aranda et al., 2020). We also found that salt treatment induces a Na^+ over-accumulation pattern in the 3rd floral truss similar to that in leaves after 60 d of salt treatment in self-grafted h2/h2. However, there was a slight but significant reduction in K^+ followed by a sharp increase in the Na^+/K^+ ratio, while salt-induced Na^+ accumulation in the flowers of non-silenced self-grafted Wt/Wt was much lower. This Na^+ over-accumulation in self-grafted h2/h2 flowers was associated with a sharper reduction in TFW than in the non-silenced self-grafted Wt/Wt (Fig. 7), which is in line with the almost totally silenced transcript levels of *ScHKT1;2* in flower tissues (Fig. 2). It has therefore confirmed that ScHKT1;2 plays an important role in limiting Na^+ flux to flowers in order to protect these organs against Na^+ toxicity and to mitigate the reduction in tomato fruit yields under salinity conditions as shown in a previous study (Romero-Aranda et al., 2020). Very similar results were obtained with the self-grafted h1-h2/h1-h2 combination (Figs. 3–5, Suppl. Fig. S3), which appear to indicate that function loss in *ScHKT1;1* did not alter the effects of the lack of function of *ScHKT1;2* under salinity conditions.

Results obtained from grafted combinations with function loss in *ScHKT1;2* in either rootstock or scion, single or double silenced with *ScHKT1;1* (Wt/h2, h2/Wt, Wt/h1-h2 and h1-h2/Wt) showed a visible salt-hypersensitive phenotype in the vegetative stage (Suppl. Fig. S3), associated with a high increase in Na^+ content and a sharp reduction in K^+ content in leaves and flowers, although the reduction in K^+ content was not significant in the flowers of h1-h2/Wt and Wt/h1-h2 (Figs. 4 and 5). However, Wt/h2 plants showed less growth and a sharper reduction in fruit yields caused by salinity than Wt/Wt plants, with the reductions in these parameters resembling those for Wt/Wt in h2/Wt plants (Figs. 3 and 7). Very similar results were obtained using the graft combinations h1-h2/Wt and Wt/h1-h2 with function loss in both *ScHKT1;2* and *ScHKT1;1* in either rootstock or scion (Figs. 3–5, Suppl. Fig. S3), except for the sharper reduction in fruit yields in the grafted combinations h1-h2/Wt and Wt/h1-h2 than in self-grafted Wt/Wt. Our previous studies have shown that *ScHKT1;2* expression levels are much lower in roots and considerably higher in leaves and flowers (Asins et al., 2013; Jaime-Pérez et al., 2017; Romero-Aranda et al., 2020). This gene expression pattern must determine the major QTL controlling Na^+/K^+ homeostasis in tomato shoots, rather than differences in functional proteins, as *cheesmaniae* and *lycopersicum HKT1;2* alleles share identical nucleotide-encoding sequences (Asins et al., 2013). The *cheesmaniae HKT1;2* allele is associated with larger amounts of Na^+ and smaller amounts of K^+ stored in the aerial part of NIL14 plants, while the *lycopersicum HKT1;2* allele has an opposite effect (Villalta et al., 2008; Asins et al., 2013). Thus, in roots, the low transcription levels of *ScHKT1;2* in NIL14 could mean that less Na^+ was transferred from the xylem to xylem parenchyma cells and that larger amounts of Na^+ were transported via the transpiration stream to the aerial part (Fig. 9, point 5). Meanwhile, the higher expression levels of *ScHKT1,2* in leaves from NIL14 may increase the withdrawal of Na^+ from the leaf xylem, thus promoting its intracellular accumulation in the mesophyll cells of expanding leaves (Asins et al., 2013; Jaime-Pérez et al., 2017; see Fig. 9, points 4 and 5). This physiological mechanism produced by Sc*HKT1;2* could result in improved and more inexpensive osmotic adjustments in leaves if safely stored in vacuoles; this also explains why salt-tolerant tomato varieties and species accumulate higher levels of Na^+ in aerial parts than in sensitive varieties without any major negative impact on growth and yields (Cuartero and Fernández-Muñoz, 1998; Cuartero et al., 2006). Given these differential gene expression levels, silencing *ScHKT1;2* in the scion could have a more negative effect on the removal of Na^+ from the xylem in the aerial part as compared to its impact in the rootstock on the removal of Na^+ from the xylem in the root in order to prevent any

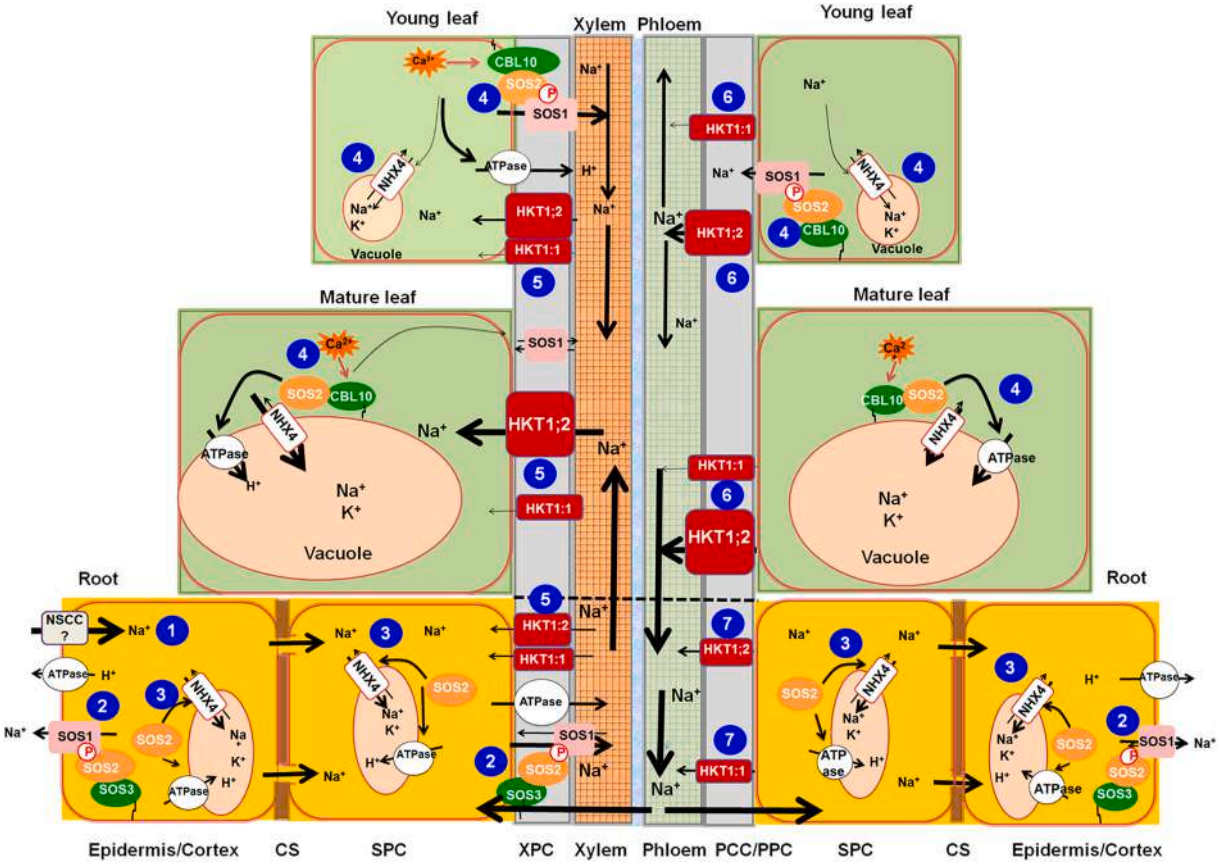

Fig. 9. A hypothetical model for the role of ScHKT1;1 and ScHKT1;2 Na^+ transporters in Na^+/K^+ homeostasis in tomato under salinity conditions. (1) Na^+ ions massively enter the cells via non selective cation channels (NSCCs) (Van Zelm et al., 2020). (2) The Na^+/H^+ antiporter SOS1, activated by the SOS2–SOS3 complex, promotes Na^+ extrusion out of the root in the epidermis and cortex cells, thus reducing the net influx of Na^+, Na^+ loading into the xylem, and Na^+ efflux from leaves at the XPC (Olías et al., 2009). (3) Activation of NHX4 and NHX2 by SOS2 causes Na^+ (and K^+) accumulation in the vacuolar and endosomal (not shown) compartments, respectively, in roots and leaves (Huertas et al., 2012, 2013). SOS2 also activates the plasma membrane and tonoplast H^+-ATPase responsible for energizing SOS1 antiporter activity, as well as NHX2 and NHX4 (Huertas et al., 2012; 2013). (4) In aerial parts, the CBL10/SlSOS2 kinase complex promotes cell Na^+ extrusion by SOS1 in young leaves and intracellular Na^+ compartmentalization by NHX transporters in mature leaf tissues (Egea et al., 2018). (5) At the root XPC, HKT1-like proteins retrieve Na^+ from the xylem sap, thereby restricting the amount of Na^+ reaching the photosynthetic tissues which is probably exchanged for K^+ through other K^+ channels (Rubio et al., 2020). In the case of NIL14, the hypoactive *cheesmaniae* allele of *HKT1;2* at the root level causes less withdrawal of Na^+ from the root xylem, again exchanged for K^+, which remains in the root (Asins et al., 2013; Jaime-Pérez et al., 2017). Thus, more Na^+ reaches the aerial part through the transpiration current, which, together with the presence of a ScHKT1;2 hyperactive allele in XPC from the aerial part, promotes the removal of Na^+ from xylem and subsequent vacuolar accumulation in the mesophyll cells through the NHX system (4) (Jaime-Pérez et al., 2017). This would functionally explain the QTL as defined by the *HKT1;2 cheesmaniae* allele which determines higher Na^+ and lower K^+ accumulation in the aerial part of tomato under saline conditions, while the ScHKT1;1 Na^+ transporter in XPC appears to play a minor role in Na^+/K^+ homeostasis in the aerial part of tomato (Jaime-Pérez et al., 2017). (6) To translocate Na^+ back to the root, ions unloaded from the xylem in the simplasm and apoplasm from leaf mesophyll cells may be transported into the phloem via HKT1;2 and HKT1;1 transporters and Na^+ is then transferred into roots via the phloem, preventing Na^+ over-accumulation in leaves and flowers. (7) This Na^+ recirculation from aerial parts to roots is further facilitated by *ScHKT1;1* silencing in root phloem cells, which promotes phloem Na^+ unloading and subsequent transport to other root compartments or extrusion back to the medium (results from this study). CS, casparian strip; SPC, stelar parenchyma cells; XPC, xylem parenchyma cells; PCC/PPC, phloem companion cells/phloem parenchyma cells. Arrow thickness indicates the hypothetical dominance of the ion flux. Gene box size indicates the gene expression levels reported in specific tissues (Asins et al., 2013; Jaime-Pérez et al., 2017).

negative impact on shoot growth under saline conditions. Tomato HKT1;2, whose role is analogous to that of other plant HKT1-like Na^+ transporters (Berthomieu et al., 2003; Sunarpi et al., 2005; Ren et al., 2005; Davenport et al., 2007; Byrt et al., 2014; Kobayashi et al., 2017), is localised in the vascular system, including the xylem and phloem (Jaime-Pérez et al., 2017). This means that the ScHKT1;2 transporter in leaf xylem cells could be more involved in promoting Na^+ uptake through the plasma membrane and its subsequent compartmentation through other secondary Na^+ transporters to large mesophyll cell vacuoles from growing/expanded leaves than in preventing Na^+ translocation from roots to aerial parts (see Fig. 9, points 4 and 5). Similarly, reciprocal grafting experiments have revealed that a hyperallelic *AtHKT1;1* in shoots determines the salt tolerance of salt-tolerant accession Tsu-1 by reducing floral Na^+ and by increasing fecundity under salt stress conditions; on the other hand, whereas hyperallelic *AtHKT1;1* in roots primarily regulates the salt tolerance of the moderately salt-tolerant wild-type accession Col-0 by reducing Na^+ translocation from roots to shoots (An et al., 2017). Although silencing *ScHKT1;2* at the rootstock or scion caused a similar visible salt-hypersensitive phenotype in leaves and flowers as a result of Na^+ over-accumulation in these tissues, silencing *ScHKT1;2* at the scion had a more negative effect on shoot growth under saline conditions than at the rootstock (Figs. 2, 4 and 5, Suppl. Fig. S3). This more extreme salt-hypersensitive phenotype due to *ScHKT1;2* function loss in the aerial parts could be due to the sharp increase in Na^+ accumulation in the apoplast, which hyperosmotically affects the mesophyll cells of expanding leaves and flowers, causing damage to these cells due to water leakage (physiological drought), as it was proposed for the salt hypersensitivity Arabidopsis mutant *sas1*

(Nublat et al., 2001). Hence, *ScHKT1;2* function loss can also contribute to Na^+ over-accumulation in the apoplast of expanded and young growing leaves as well as flowers (Figs. 4 and 5, see Fig. 9, points 4, 5), which showed symptoms of necrosis under long term salt treatment (Suppl. Fig. S3), leading to a reduction in fruit yields (Fig. 7). These results again confirm that Na^+ transporter HKT1;2 protects flowers from Na^+ toxicity and mitigates the typical reduction in tomato fruit yields under salinity conditions (Cuartero and Fernández-Muñoz, 1998), as demonstrated in a previous study (Romero-Aranda et al., 2020).

Additionally, several studies have shown that the rate of recirculation of Na^+ to roots via the phloem is an important factor in limiting Na^+ accumulation in leaves and plays a role in overall salt tolerance (Jacoby, 1979; Tester and Davenport, 2003; Tian et al., 2010, Wang et al., 2015). To prevent its over-accumulation in shoots, Na^+, which is sequestered in leaf vacuoles, can also be loaded into the phloem by HKT1-like transporters for transport back to the root (Van Zelm et al., 2020). Na^+ over-accumulation in the apoplast of leaves and flowers in graft combinations with *ScHKT1;2* silenced in the aerial part may also, to a great extent, be the result of its possible additional role in Na^+ loading into phloem sieves. The location of ScHKT1;2 in tomato phloem and xylem cells in the aerial parts of the plant (Jaime Perez et al., 2017) suggests that, together with xylem Na^+ unloading, HKT1;2 could be involved in Na^+ loading into the phloem sieves. Thus, silencing *ScHKT1;*2 might prevent the recirculation of Na^+ towards the root (see Fig. 9, point 6), which, according to previous findings for AtHKT1;1, means that ScHKT1;2 could be similarly involved in Na^+ redistribution towards sink organs and tissues (Mäser et al., 2002; Berthomieu et al., 2003; Sunarpi et al., 2005).

Moderate salinity levels improve the quality of tomato fruits, whose total soluble solids and antioxidant compounds typically increase under these conditions (Cuartero et al., 2006; Magán et al., 2008; Klee and Giovannoni, 2011). As expected, total soluble solids (°Brix) slightly increased in graft combinations bearing h2 or h1-h2 in rootstock and/or scion in fruits from plants grown under saline conditions, except h2/h2, although this increase was slightly lower than in self-grafted Wt/Wt (Fig. 8). This could be due to the smaller amounts of water in fruit, which induces an increase in sugar concentrations (Romero-Aranda et al., 2001). Similar results were obtained in a previous study using non-grafted NIL14 (similar to Wt/Wt) and *ScHKT1;2* silenced throughout the plant (similar to h2/h2) (Romero-Aranda et al., 2020). Antioxidant activity is associated with compounds such as phenolics, which act through several chemical mechanisms to prevent oxidative damage to plant tissues (Zhao et al., 2015). With regard to antioxidant activity, only total phenolics increased in all graft combinations under salinity conditions except for h2/h2. Although we did not measure ROS production or Na^+ content in fruits, the entry of Na^+ via the xylem due to *ScHKT1;2* silencing may increase ROS production in fruits and subsequently antioxidant synthesis.

4.2. *ScHKT1;1 function loss in rootstock sharply reduced the Na^+/K^+ ratio in leaves and flowers minimizing yield loss under salinity*

In previous studies, we found that *ScHKT1;1* silencing in a plant subjected to 0 and 100 mM NaCl for 15 days did not significantly inhibit growth or alter the Na^+/K^+ ratio of plants grown under controlled growth chamber conditions (Jaime-Pérez et al., 2017). We concluded that the *HKT1;1* gene, although expressed in the same type of vascular cells as *HKT1;2*, appears to play a minor role in Na^+ transport and Na^+/K^+ homeostasis in the aerial part of the plant (Jaime-Pérez et al., 2017; see Fig. 9, point 5). However, in light of the new data obtained in the present study, the role of *HKT1;1* needs to be reassessed. *HKT1;1* expression levels are always much lower than those for *HKT1;2*, or even negligible, irrespective of the allelic variant (*cheesmaniae* or *lycopersicum)* and tissue studied (Asins et al., 2013; Almeida et al., 2014; Jaime-Pérez et al., 2017; http://bar.utoronto.ca/eplant_tomato/), although *ScHKT1;1* gene expression levels have been reported to be significantly higher in NIL14 leaves and roots than in those of NIL17 (Jaime-Pérez et al., 2017). Moreover, data relating to *HKT1;1* transcription levels in NIL14 leaves suggest that, unlike *S. lycopersicum, the HKT1;1* gene expression of *S. cheesmaniae* in leaves is similar to that of *S. pimpinellifolium*in wild type species (http://bar.utoronto.ca/eplant_tomato/). It is therefore important to determine whether *HKT1;1* and *HKT1;2* loci play a similar role in all tissues, as well as the effects of silencing at the *HKT1;1* locus in the root alone or in the aerial part. Salt treatment significantly reduced the growth of the aerial part in self-grafted h1/h1, similar to that observed for non-silenced self-grafted Wt/Wt (Fig. 3). However, h1/h1 plants had lower Na^+ content and higher K^+ content in the aerial part and flowers than in the self-grafted Wt/Wt (Figs. 4 and 5) and no visible salt-sensitive symptoms (Suppl. Fig. S3). Very similar results were obtained in a previous study carried out under controlled growth chamber conditions using short-term salt treatments (Jaime-Pérez et al., 2017), although, in the present study, fruit yields were eventually reduced by salinity to levels similar to those of Wt/Wt (Fig. 7). Moreover, unlike graft combination wt/h1 and self-grafted h1/h1 and Wt/Wt, h1/Wt, showed no significant differences in fruit yield when comparing control and salinity (Fig. 7). However, these combinations with *ScHKT1;1*-silenced lines used as rootstock and scion showed a sharper increase in SSC and even more so in h1/Wt (Fig. 8). It is therefore important to determine the actual role played by ScHKT1;1 in Na^+/K^+ homeostasis in tomato, given that its function loss displays a similar plant performance under salinity conditions and improves fruit quality measured as total soluble solids. As indicated in the previous section, in order to reduce its accumulation in aerial parts, Na^+, which can be sequestered in leaf vacuoles, can also be loaded into the phloem by HKT1-like transporters for transport back to the root (Van Zelm et al., 2020). In a previous study, we obtained circumstantial evidence showing that tomato HKT1;1 and/or HKT1;2 could be involved in loading Na^+ into the phloem sap in leaves and in unloading the sap in sink organs such as fruits and roots (Asins et al., 2015). As indicated above, the possible localization of HKT1;1 in phloem cells also suggests that, in addition to unloading Na^+ in the xylem, however, *ScHKT1;1* expression in roots was undetectable using an *in situ* PCR protocol (Jaime-Pérez et al., 2017, see Fig. 9, points 6 and 7), probably due to its extremely low levels in roots (Asins et al., 2013; Almeida et al., 2014). Given the very low expression levels for *ScHKT1;1* in all the tissues analyzed as compared to those for *ScHKT1;2* (Asins et al., 2013; Jaime-Pérez et al., 2017), the role of ScHKT1;1 in uploading Na^+ to the phloem in the aerial parts could be less important than that of ScHKT1;2 in order to determine Na^+ recirculation from the aerial parts to the roots (see Fig. 9, point 6). Graft plant combinations bearing silenced *ScHKT1;1* (h1/Wt, h1/h1 and Wt/h1) display normal *ScHKT1;2* expression levels in both roots and aerial parts (Fig. 2). Surprisingly, *ScHKT1;1* silencing could therefore be involved in Na^+ exclusion in leaves (Fig. 4) through different mechanisms. These include unloading Na^+ in the xylem in roots and aerial parts via *ScHKT1;2* combined with uploading Na^+ to phloem cells in the aerial part through the normal transport function of ScHKT1;1 and particularly ScHKT1;2, as well as the uploading of Na^+ to the phloem in roots (see Fig. 9, points 5 and 6). Regardless of the mechanisms underlying phloem loading which connects phloem parenchyma to companion cells/sieve elements (Wei et al., 2021), we hypothesize that ScHKT1;1, and above all, ScHKT1;2 in the aerial parts are involved in the recirculation of Na^+ to roots, thus limiting its accumulation in leaves and flowers (see Fig. 9, point 6). Na^+ recirculation from aerial parts to roots could also be favoured by *ScHKT1;1* silencing in root phloem cells, which would facilitate phloem Na^+ unloading and subsequent transport to other root compartments or extrusion back to the medium (see Fig. 9, point 7). More precise subcellular and cellular localization assays would provide further insights into the role of HKT1;1 and HKT1;2 in Na^+ homeostasis in tomato under salinity conditions.

In conclusion, the results obtained in this study indicate that the removal of Na^+ from the xylem by ScHKT1;2 in the aerial part of the

plant could have an even greater impact than that on Na^+ homeostasis at the root level under saline conditions. Our results suggest that, in addition to xylem Na^+ unloading, ScHKT1;2 could also be involved in Na^+ uploading into the phloem, thus promoting Na^+ recirculation from aerial parts to roots. This recirculation of Na^+ to roots through the phloem could be additionally favoured by *ScHKT1;1* silencing at the roots. Appropriate rootstocks are well known to enhance the salt tolerance of commercial tomato cultivars by limiting the transport of Na^+ and Cl^- to shoots (Santa-Cruz et al., 2002; Cantero-Navarro et al., 2016). The use of tomato grafting is considered a suitable approach to improve salt tolerance, with grafted plants being widely used in greenhouse tomato production in the Mediterranean region (Singh et al., 2020). In light of our findings, the *ScHKT1;1* silenced line could be used as a rootstock prototype in order to maintain fruit yield levels and to enhance the soluble solid fruit content of commercial tomato varieties grafted under saline conditions, which has not yet been evaluated. If deemed useful, given current European constraints on GMO use in agriculture, following genetic editing to knock on/out the *ScHKT1;1* gene, and final approval as a non-GMO plant breeding technique (Gao, 2021), this rootstock could be used in a tomato salt tolerance strategy.

Contributions

MRRA: conceived and designed the research, conducted the experiments and analyzed the data, wrote the manuscript; supervised the design and interpretation of the experiments; **JE:** conducted the experiments and analyzed the data; **PGF:** conducted the experiments and analyzed the data; **EJF:** conducted the experiments and analyzed the data; **JAT:** conducted the experiments and analyzed the data; **MJA:** conceived and designed the research, wrote the manuscript and supervised the design and interpretation of the experiments; **AB:** conceived and designed the research, conducted the experiments and analyzed the data, wrote the manuscript; supervised the design and interpretation of the experiments.

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.

Acknowledgments

We wish to thank Elena Sánchez Romero (EEZ-CSIC) for her technical assistance, the Scientific Instrumentation Service at EEZ-CSIC, Granada, for their ICP-OES mineral analysis and Michael O'Shea for proofreading the manuscript. The study was funded by grant AGL2017-82452-C2-1R (A.B.) and grant AGL2017-82452-C2-2R (M.J.A.), both from the Spanish Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación, MCIN/AEI/10.13039/501100011033, and FEDER "*Una manera de hacer Europa*", as well as grant ACCESP2018 (J.A.T.) from the University of Granada. J.E. was supported by a JAE Intro-CSIC grant, JAEINT_19_00566.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2021.10.018.

References

Almeida, P.M.F., de Boer, G.J., de Boer, A.H., 2014. Assessment of natural variation in the first pore domain of the tomato HKT1;2 transporter and characterization of mutated versions of *SlHKT1;2* expressed in *Xenopus laevis* oocytes and via complementation of the salt sensitive *athkt1;1* mutant. Front. Plant Sci. 5, 600.

An, D., Chen, J.-G., Gao, Y.-Q., Li, X., Chao, Z.-F., Chen, Z.-R., et al., 2017. *AtHKT1* drives adaptation of *Arabidopsis thaliana* to salinity by reducing floral sodium content. PLoS Genet. 13, e1007085.

Asins, M.J., Bretó, M.P., Cambra, M., Carbonell, E.A., 1993. Salt tolerance in *Lycopersicon* species. I. Character definition and changes in gene expression. Theor. Appl. Genet. 86, 37–743.

Asins, M.J., Bernet, G.P., Villalta, I., Carbonell, E.A., 2010. QTL analysis in plant breeding. In: Jain, S., Brar, D. (Eds.), Molecular Techniques in Crop Improvement. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2967-6_1.

Asins, M.J., Villalta, I., Aly, M.M., Olías, R., Alvarez de Morales, P., Huertas, R., et al., 2013. Two closely linked tomato HKT coding genes are positional candidates for the major tomato QTL involved in Na^+/K^+ homeostasis. Plant Cell Environ. 36, 1171–1191.

Asins, M.J., Raga, V., Roca, D., Belver, A., Carbonell, E.A., 2015. Genetic dissection of tomato rootstock effects on scion traits under moderate salinity. Theor. Appl. Genet. 128, 667–679.

Berthomieu, P., Conéjéro, G., Nublat, A., Brackenbury, W.J., Lambert, C., Savio, C., et al., 2003. Functional analysis of AtHKT1 in Arabidopsis shows that Na^+ recirculation by the phloem is crucial for salt tolerance. EMBO J. 22, 2004–2014.

Brosnan, C.A., Voinnet, O., 2011. Cell-to-cell and long-distance siRNA movement in plants: mechanisms and biological implications. Curr. Opin. Plant Biol. 14, 580–587.

Byrt, C.S., Xu, B., Krishnan, M., Lightfoot, D.J., Athman, A., Jacobs, A.K., et al., 2014. The Na^+ transporter, TaHKT1;5-D, limits shoot Na^+ accumulation in bread wheat. Plant J. 80, 516–526.

Cantero-Navarro, E., Romero-Aranda, M.R., Fernández-Muñoz, R., Martínez-Andújar, C., Pérez-Alfocea, F., Albacete, A., 2016. Improving agronomic water use efficiency in tomato by rootstock-mediated hormonal regulation of leaf biomass. Plant Sci. 251, 90–100.

Cuartero, J., Bolarin, M.C., Asins, M.J., Moreno, V., 2006. Increasing salt tolerance in the tomato. J. Exp. Bot. 57, 1045–1058.

Cuartero, J., Fernández-Muñoz, R., 1998. Tomato and salinity. Sci. Hortic. 78, 83–125.

Davenport, R.J., Muñoz-Mayor, A., Jha, D., Essah, P.A., Rus, A., Tester, M., 2007. The Na^+ transporter AtHKT1 controls xylem retrieval of Na^+ in Arabidopsis. Plant Cell Environ. 30, 497–507.

Egea, I., Pineda, B., Ortíz-Atienza, A., Plasencia, F.A., Drevensek, S., García-Sogo, B., et al., 2018. The SlCBL10 calcineurin B-like protein ensures plant growth under salt stress by regulating Na+ and Ca^{2+} homeostasis. Plant Physiol. 176, 1676–1693.

Estañ, M.T., Villalta, I., Bolarín, M.C., Carbonell, E.A., Asins, M.J., 2008. Identification of fruit yield loci controlling the salt tolerance conferred by solanum rootstocks. Theor. Appl. Genet. 118, 305–312.

FAOSTAT, 2017. Food and agriculture organization of the united nations. http://faostat.fao.org/site/339/. (Accessed 28 November 2018).

Gao, C., 2021. Genome engineering for crop improvement and future agriculture. Cell 184, 1621–1635.

Hauser, F., Horie, T., 2010. A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. Plant Cell Environ. 33, 552–565.

Henderson, S.W., Dunlevy, J.D., Wu, Y., Blackmore, D.H., Walker, R.R., Edwards, E.J., et al., 2018. Functional differences in transport properties of natural HKT1;1 variants influence shoot Na^+ exclusion in grapevine rootstocks. New Phytol. 21, 1113–1127.

Hoagland, D.R., Arnon, D.I., 1950. The water culture method for growing plants without soil. Calif. Agric. Ext. Serv. Circ. 347, 1–39.

Huertas, R., Olías, R., Eljakaoui, Z., Gálvez, F.J., Li, J., Álvarez De Morales, P., Belver, A., Rodríguez-Rosales, M.P., 2012. Overexpression of SlSOS2 (SlCIPK24) confers salt tolerance to transgenic tomato. Plant Cell Environ. 35, 1467–1482.

Huertas, R., Rubio, L., Cagnac, O., García-Sánchez, M.J., Alché, J., Venema, K., Fernández, J.A., Rodríguez-Rosales, M.P., 2013. The K^+/H^+ antiporter LeNHX2 increases salt tolerance by improving K^+ homeostasis in transgenic tomato. Plant Cell Environ. 36, 2135–2149.

Jacoby, B., 1979. Sodium recirculation and loss from *Phaseolus vulgaris*. Ann. Bot. 43, 741–744.

Jaime-Pérez, N., Pineda, B., García-Sogo, B., Atares, A., Athman, A., Byrt, C.S., Belver, A., 2017. The Na^+ transporter encoded by the HKT1;2 gene modulates Na^+/K^+ homeostasis in tomato shoots under salinity. Plant Cell Environ. 40, 658–671.

Kashyap, S.P., Kumari, N., Mishra, P., Moharana, D.P., Aamir, M., 2021. Tapping the potential of *Solanum lycopersicum* L. pertaining to salinity tolerance: perspectives and challenges. Genet. Resour. Crop Evol. https://doi.org/10.1007/s10722-021-01174-9.

Klee, H.J., Giovannoni, J.J., 2011. Genetics and control of tomato fruit ripening and quality attributes. Annu. Rev. Genet. 45, 41–59.

Kobayashi, N.I., Yamaji, N., Yamamoto, H., Okubo, K., Ueno, H., Costa, A., Nayef, M.A., 2017. OsHKT1;5 mediates Na^+ exclusion in the vasculature to protect leaf blades and reproductive tissues from salt toxicity in rice. Plant J. 91, 657–670.

Liao, Z., Chen, M., Guo, L., Gong, Y., Tang, F., Sun, X., Tang, K., 2004. Rapid isolation of high-quality total RNA from taxus and ginkgo. Prep. Biochem. Biotechnol. 34, 209–214.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) method. Methods 25, 402–408.

Magán, J.J., Gallardo, M., Thompson, R.B., Lorenzo, P., 2008. Effects of salinity on fruit yield and quality of tomato grown in soil-less culture in greenhouses in Mediterranean climatic conditions. Agric. Water Manag. 95, 1041–1055.

Mäser, P., Eckelman, B., Vaidyanathan, R., Horie, T., Fairbairn, D.J., Kubo, M., et al., 2002. Altered shoot/root Na^+ distribution and bifurcating salt sensitivity in Arabidopsis by genetic disruption of the Na+ transporter AtHKT1. FEBS Lett. 531, 157–161.

Mickelbart, M.V., Hasegawa, P.M., Bailey-Serres, J., 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat. Rev. Genet. 16, 237–251.
Monforte, A.J., Asins, M.J., Carbonell, E.A., 1997. Salt tolerance in *Lycopersicon* species. 5. Does genetic variability at quantitative trait loci affect their analysis? Theor. Appl. Genet. 95, 284–293.
Munns, R., James, R.A., Xu, B., Athman, A., Conn, S.J., Jordans, C., et al., 2012. Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nat. Biotechnol. 30, 360–364.
Nublat, A., Desplans, J., Casse, F., Berthomieu, P., 2001. *sas1*, an Arabidopsis mutant over accumulating sodium in the shoot, shows deficiency in the control of the root radial transport of sodium. Plant Cell 13, 125–137.
Olías, R., Eljakaoui, Z., Li, J., De Morales, P.A., Marín-Manzano, M.C., Pardo, J.M., Belver, A., 2009. The plasma membrane Na^+/H^+ antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na^+ between plant organs. Plant Cell Environ. 32, 904–916.
Ren, Z.H., Gao, J.P., Li, L.G., Cai, X.L., Huang, W., Chao, D.Y., Zhu, M.Z., et al., 2005. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. 37, 1141–1146.
Rieu, I., Powers, S.J., 2009. Real-time quantitative RT-PCR: design, calculations, and statistics. Plant Cell 21, 1031–1033.
Rodríguez-Rosales, M.P., Gálvez, F.J., Huertas, R., Aranda, M.N., Baghour, M., Cagnac, O., Venema, K., 2009. Plant NHX cation/proton antiporters. Plant Signal. Behav. 4, 265–276.
Romero-Aranda, R., Soria, T., Cuartero, J., 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Sci. 160, 265–272.
Romero-Aranda, M.R., González Fernández, P., Pérez Tienda, J.R., López Díaz, M.R., Espinosa, J., Granum, E., Traverso, J.A., et al., 2020. Na^+ transporter HKT1;2 reduces flower Na^+ content and considerably mitigates the decline in tomato fruit yields under saline conditions. Plant Physiol. Biochem. 154, 341–352.
Rubio, F., Nieves-Cordones, M., Horie, T., Shabala, S., 2020. Doing 'business as usual' comes with a cost: evaluating energy cost of maintaining plant intracellular K^+ homeostasis under saline conditions. New Phytol. 225, 1097–1104.
Santa-Cruz, A., Martínez-Rodríguez, M.M., Pérez-Alfocea, F., Romero-Aranda, R., Bolarín, M.C., 2002. The rootstock effect on the tomato salinity response depends on the shoot genotype. Plant Sci. 162, 825–831.
Singh, H., Kumar, P., Kumar, A., Kyriacou, M.C., Colla, G., Rouphael, Y., 2020. Grafting tomato as a tool to improve salt tolerance. Agronomy 10, 263. https://doi.org/10.3390/agronomy10020263.
Sunarpi, Horie, T., Motoda, J., Kubo, M., Yang, H., Yoda, K., Horie, R., et al., 2005. Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na^+ unloading from xylem vessels to xylem parenchyma cells. Plant J. 44, 928–938.
Tester, M., Davenport, R.J., 2003. Na^+ tolerance and Na+ transport in higher plants. Ann. Bot. 91, 503–527.
Tian, H., Baxter, I.R., Lahner, B., Reinders, A., Salt, D.E., Ward, J.M., 2010. Arabidopsis NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance. Plant Cell 22, 3963–3979.
Van Zelm, E., Zhang, Y., Testerink, C., 2020. Salt tolerance mechanisms of plants. Annu. Rev. Plant Biol. 71, 403–433.
Villalta, I., Bernet, G.P., Carbonell, E.A., Asins, M.J., 2007. Comparative QTL analysis of salinity tolerance in terms of fruit yield using two Solanum populations of F7 lines. Theor. Appl. Genet. 114, 1001–1017.
Villalta, I., Reina-Sánchez, A., Bolarin, M.C., Cuartero, J., Belver, A., Venema, K., Carbonell, E.A., Asins, M.J., 2008. Genetic analysis of Na^+ and K^+ concentrations in leaf and stem as physiological components of salt tolerance in tomato. Theor. Appl. Genet. 116, 859–880.
Wang, R., Jing, W., Xiao, L., Jin, Y., Shen, L., Zhang, W., 2015. The rice high-affinity potassium transporter1;1 is involved in salt tolerance and regulated by an MYB-type transcription factor. Plant Physiol. 168, 1076–1090.
Wang, Z., Hong, Y., Zhu, G., Li, Y., Niu, Q., Yao, J., et al., 2020. Loss of salt tolerance during tomato domestication conferred by variation in a Na^+/K^+ transporter. EMBO J. 39, e103256.
Wei, X.-Y., Collings, D.A., McCurdy, D.W., 2021. More than sweet: new insights into the biology of phloem parenchyma transfer cells in Arabidopsis. Plant Sci. 310 https://doi.org/10.1016/j.plantsci.2021.110990.
Zhao, G.M., Han, Y., Sun, X., Li, S.H., Shi, Q.M., Wang, C.H., 2015. Salinity stress increases secondary metabolites and enzyme activity in safflower. Ind. Crop. Prod. 64, 175–181.