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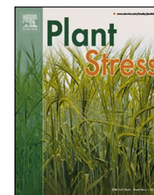

SlNRT1.5 transporter and the SlSKOR K^+ channel jointly contribute to K^+ translocation in tomato plants

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A B S T R A C T

Accumulation of K^+ in shoots is largely dependent on K^+ transport via the xylem and has important implications not only for K^+ nutrition but also for stress tolerance. In tomato plants, the K^+ channel SlSKOR contributed to K^+ translocation but the decrease in the shoot K^+ content in *slskor* mutants was only ~15 %, indicating that additional K^+ transport systems operated in the tomato stele. Here, we studied the physiological roles of the transporter SlNRT1.5 in tomato plants, whose homolog in Arabidopsis, AtNRT1.5, contributed to xylem K^+ load. By using heterologous expression of SlNRT1.5 in Xenopus oocytes and a *slnrt1.5* knock-out mutant, we have gained insights into its role in shoot K^+ nutrition. Expression of SlNRT1.5 in Xenopus oocytes resulted in K^+ efflux, similar to that mediated by AtNRT1.5, which could indicate that SlNRT1.5 operates as a K^+ transport system. Plants lacking *slnrt1.5* accumulated less K^+ in shoots than WT plants under low external pH (4.5), and low supply of K^+ (0.05 mM) and N (0.5 mM). Interestingly, *slnrt1.5* plants accumulated less Na^+ and Cl^- in shoots than WT plants. Further analyses on *slskor slnrt1.5* double mutant plants revealed an overlapping role of SlSKOR and SlNRT1.5 in shoot K^+ accumulation. Double mutants showed a 40 % decrease in shoot K^+ content in comparison with *slskor* and *slnrt1.5* single mutants. Altogether, this study showed that SlNRT1.5 and SlSKOR are major players in shoot K^+ accumulation in tomato plants.

Introduction

Delivery of mineral nutrients to the shoot from the root is a crucial process for plant growth and tolerance to abiotic stresses (Lambers, 2010). In vascular plants, load of mineral nutrients into the xylem vessels is a tightly controlled process in which nutrient transport systems contribute to the xylem sap composition (White, 2012). Plants are equipped with a vast number of nutrient transport systems with different transport properties in terms of substrate selectivity and sensitivity to cellular factors. Among plant mineral nutrients, K^+ is the most abundant cation in plant cells and its transport to shoots is not only important for K^+ nutrition but for acclimation to different abiotic stresses such as salinity (Wu et al., 2018). K^+ accumulation in the xylem prevents large accumulation of Na^+ in shoots and is positive for plant salt tolerance (Albacete et al., 2009; Alejandro et al., 2007; Wu et al., 2018).

K^+ translocation to aerial parts has been deeply studied in Arabidopsis plants (Raddatz et al., 2020). The K^+ channel SKOR and the NRT/NPF transporter NRT1.5 have been demonstrated to contribute to K^+ transport to shoots. While SKOR operates as an outward-rectifying K^+ channel (Gaymard et al., 1998), NRT1.5 works as a K^+/H^+ antiporter (Li et al., 2017). NRT1.5 was originally proposed to mediate NO_3^- translocation to shoots as a NO_3^- transporter (Lin et al., 2008), and a coordinated transport of K^+ and NO_3^- by NRT1.5 remains unclear. The aforementioned transport properties allowed NRT1.5 and SKOR to contribute to K^+ translocation under distinct physiological conditions. SKOR is relevant under sufficient K^+ supply or high N supply whereas NRT1.5 is important under low K^+ supply (Drechsler et al., 2015; Li et al., 2017). As other NRT/NPF transporters, NRT1.5 mediated

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hormone transport, in particular it transported IBA (Watanabe et al., 2020). The activity of NRT1.5 is not only important for K^+ transport but also for salt and heavy metal stress tolerance (Chen et al., 2012). Moreover, NRT1.5 interacted with the H^+ ATPase AHA2, so NRT1.5 activity seems to be coordinated with H^+ pumping in root cells (Sena and Kunze, 2023). It is worth noting that the large number of studies conducted in Arabidopsis contrasts with the scarcity of reports regarding the function of NRT1.5 homologs in other plant species.

Research on crops as tomato is necessary as the models obtained in Arabidopsis for K^+ transport are not always conserved in tomato plants. For example, a contribution of the CIPK23 kinase to Na^+ uptake was observed in tomato plants but not in Arabidopsis (Amo et al., 2021). In addition, the SKOR K^+ channel from different plant species including tomato, lettuce, medicago and grapevine were inhibited by CIPK23 whereas the same was not true for the Arabidopsis SKOR channel (Nieves-Cordones et al., 2023).

The study of the tomato *skor* mutant plants revealed a mild phenotype for K^+ translocation (Nieves-Cordones et al., 2023). Therefore, functional redundancy with other K^+ transport systems seemed plausible. Thus, taking into account the information available in Arabidopsis, we pursued the identification and characterization of the NRT1.5 homolog in tomato, SlNRT1.5. Functional assays and phenotyping of *slnrt1.5* mutant plants showed that SlNRT1.5 contributed to K^+ translocation under low K^+, nitrogen (N) and pH conditions. A remarkable degree of functional redundancy was observed for SlNRT1.5 and SlSKOR. Thus, shoot K^+ accumulation in tomato plants relies largely on the activity of these two transport systems.

Results

SlNRT1.5 is highly expressed in the tomato root stele

In tomato, there are 90 genes encoding for NRT/NPF transporters (Léran et al., 2014). To identify the closest tomato homolog of AtNRT1.5, we carried out a phylogenetic analysis of the NRT1-like transporters from Arabidopsis and tomato (Fig. S1). Two homologous proteins to AtNRT1.5 were identified in tomato (SlNPF7.7 and SlNPF7.6) (Fig. S1a). Expression analysis added relevant information regarding the putative orthologue of AtNRT1.5 in tomato. While expression of SlNPF7.6 was low in all plant tissues, SlNPF7.7 was highly expressed in tomato roots, where the function of the AtNRT1.5 orthologue was expected (Fig. S1b). Thus, we focused on the study of SlNPF7.7 as the orthologue of AtNRT1.5 and we denominated it SlNRT1.5.

The expression of the gene encoding the SlNRT1.5 transporter was studied by real-time quantitative PCR (qRT-PCR). Plants of the WT line were grown in 1.4 mM K^+ and root samples were separated into cortex and stele tissues, their RNA isolated and reverse transcribed (Fig. 1a). The results showed that *SlNRT1.5* exhibited high expression in cortex and stele, being the expression of *SlNRT1.5* higher in the stele than in the cortex (Fig. 1a). *SlNRT1.5* transcript levels were also checked in other plant tissues, and found to be highly expressed in tomato roots, floral stems, sepals and stamens, with intermediate levels of expression in stems, leaves, petals and carpels (Fig. 1b).

SlNRT1.5 mediated K^+ efflux in Xenopus oocytes

A previous report showed that AtNRT1.5 mediated K^+ efflux in Xenopus oocytes (Li et al., 2017). To check whether SlNRT1.5 also shared this transport capacity, we expressed SlNRT1.5 and AtNRT1.5 in Xenopus oocytes. Oocytes injected with water or with cRNAs encoding for SlNRT1.5 or AtNRT1.5 were incubated in a K^+-free modified ND96 solution at pH 5.5 for 3 h and then, the K^+ concentration in the external solution was measured. As shown in Fig. 2, oocytes injected with SlNRT1.5 exhibited higher K^+ efflux rates than oocytes injected with water. It is also worth noting that oocytes injected with AtNRT1.5

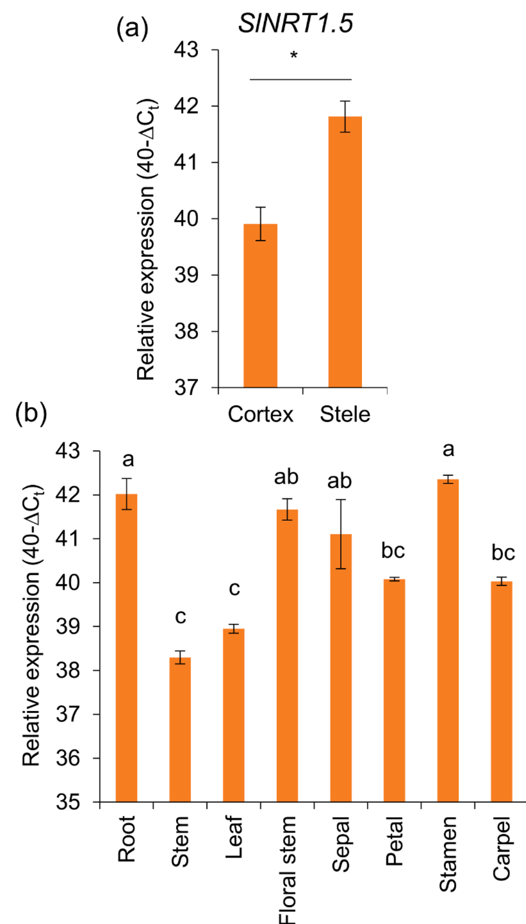

Fig. 1. The *SlNRT1.5* gene is expressed in the stele of tomato roots. (a) Relative expression of *SlNRT1.5* in isolated cortex and stele tissues was measured using qRT-PCR. (b) Expression levels of the *SlNRT1.5* gene were studied in different organs of WT plants. WT plants were grown in a medium containing 1.4 mM K^+, with their specified organs separated, RNA extracted, and cDNA synthesized. qPCR was then carried out on the cDNA using specific primers targeting *SlNRT1.5*. Gene expression was quantified using the $40-\Delta C_t$ method. Data are presented as mean values ± SE ($n = 3$). * denotes $p < 0.05$ based on Student's *t*-test. Letters represent homogeneous groups of data at $p < 0.05$, according to LSD post-hoc test following ANOVA.

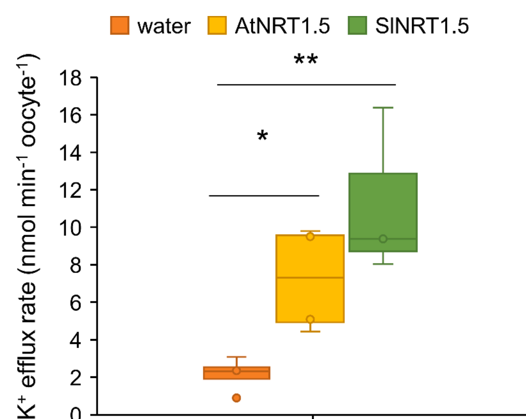

Fig. 2. SlNRT1.5 mediates K^+ efflux in Xenopus oocytes. K^+ efflux rates of Xenopus oocytes injected with AtNRT1.5, SlNRT1.5 or water as negative control and incubated in K^+- free medium. Data are presented as mean values ± SE ($n = 3$), each sample (n) contained seven oocytes. * indicates $p < 0.05$ and ** $p < 0.01$ in Student's t-test.

exhibited comparable K^+ efflux to oocytes injected with the tomato transporter. Therefore, both SlNRT1.5 and AtNRT1.5 share the capacity to mediate K^+ efflux when expressed in Xenopus oocytes.

SlNRT1.5 contributes to ion homeostasis under K^+-limiting conditions

The following step was to elucidate the physiological role of SlNRT1.5 in tomato plants. For this purpose, *slnrt1.5* knock-out mutants were produced with CRISPR-Cas using two sgRNAs that targeted the *SlNRT1.5* locus (Fig. S2a). After tomato plant transformation, two independent *slnrt1.5* knock-out alleles, *slnrt1.5–1* and *slnrt1.5–2*, were obtained. These mutants harbored frameshift mutations in exon 4 (Fig. S2b). T2 plants were cultivated hydroponically to evaluate the impact of the *slnrt1.5* mutation. Plants were grown for 21 days in a 1/5 Hoagland solution with a sufficient K^+ concentration (1.4 mM K^+, +K solution). In this experiment, no differences were observed regarding plant dry weight, K^+ and NO_3^- content between wild-type (WT) and *slnrt1.5* plants (Fig. S3).

The experimental design was modified in the next experiment: a first harvest of plants growing in the +K solution at 14 d (+K treatment) and a second harvest of plants growing in a –K solution (no K^+ added) for 7 d (21 d of growth in total) in the presence or absence of 50 mM NaCl (–K and –K +Na treatments, respectively) were conducted. No significant differences with respect dry weight were observed among the mutants *slnrt1.5* and the WT when grown in the +K solution (Fig. 3a). However, under K^+ starvation, both with and without Na^+, a significant decrease in the dry weight of roots and leaves of the *slnrt1.5* mutants was observed when compared to the WT (Fig. 3a). It is worth noting that the relative growth rate also decreased in the mutants compared to the wild type (Fig. 3b). Regarding ion content, root and shoot K^+ contents were similar in WT and *slnrt1.5* plants in all treatments except for roots in the –K treatment where *slnrt1.5* roots had higher K^+ content than WT plants (Fig. 3c). Interestingly, *slnrt1.5* plants exhibited lower Na^+ and Cl^- shoot contents than WT plants (Fig. 3d and Fig. 3e), which indicated that SlNRT1.5 prevented massive NaCl accumulation in shoots. Although this experiment revealed a function for SlNRT1.5 in tomato ion homeostasis, the involvement of this transporter in K^+ translocation was not evident.

AtNRT1.5 was originally described as a NO_3^- transporter involved in NO_3^- translocation (Lin et al., 2008). However, under standard NO_3^- concentration (2.8 mM), *slnrt1.5* plants did not show any significant difference with WT plants regarding NO_3^- content (Fig. S3). Therefore, we tested the effect of variable NO_3^- supply (0.5 mM or 10 mM NO_3^-) on the accumulation of NO_3^- and K^+ in WT and *slnrt1.5* plants (Fig. 4). Analyses of plants grown at 0.5 or 10 mM NO_3^- revealed no significant differences in dry weight, K^+ and NO_3^- between WT and *slnrt1.5* plants except that mutant plants had lower shoot K^+ content than WT plants at 0.5 mM NO_3^- (Fig. 4). It is worth noting that shoot K^+ content was lower in 0.5 N plants than in the 10 N ones (Fig. 4b). Therefore, SlNRT1.5 contributed to K^+, but not to NO_3^- translocation under low N supply.

K^+ translocation was reduced in slnrt1.5 plants grown at pH 4.5

Since it was proposed that AtNRT1.5 operates as a H^+-dependent transport system (Li et al., 2017), we explored the effect of external pH on SlNRT1.5 function in tomato plants. Thus, a set of experiments was carried out to test the effect of growing WT and *slnrt1.5* plants in the +K solution at different pH values: 4.5, 5.5 and 7.5. Dry organ weight did not reveal any impact of pH on plant growth (Fig. 5a). By contrast, K^+ content was higher in roots and lower in shoots in *slnrt1.5* plants than in the WT ones at pH 4.5 (Fig. 5b). However, no significant differences in K^+ content were observed at pH 5.5 or 7.5 (Fig. 5b). Therefore, the low K^+ content in *slnrt1.5* shoots and high in roots indicated that SlNRT1.5 contributed to K^+ translocation in tomato plants at pH 4.5 but not at higher pH values.

SlNRT1.5 and SlSKOR cooperate in K^+ translocation in tomato plants

The mild effect of *SlNRT1.5* (Fig. 3, 4 and 5) and *SlSKOR* mutations (Nieves-Cordones et al., 2023) on K^+ translocation could be indicative of function redundancy between these two transport systems. So, we obtained *slnrt1.5 slskor* double mutants: *slnrt1.5 slskor #1 and slnrt1.5 slskor #2* by crossing *slnrt1.5–1* plants with *slskor-1* plants. The *slnrt1.5 slskor #1* line was obtained using *slnrt1.5–1* pollen on *slskor-1* emasculated flowers and the *slnrt1.5 slskor #2* line was obtained using *slskor-1* pollen on *slnrt1.5–1* emasculated flowers. The double mutants, along with the single mutants *slnrt1.5–1* and *slskor-1*, were grown in *a* +K solution at pH 4.5 or 5.5 during 21 d (Fig. 6). Then, dry weight and K^+ content were measured. With respect to dry weight, no significant differences were observed for shoot biomass among the plant genotypes (Fig. 6a). In contrast, roots from *slnrt1.5 slskor* double mutants showed lower dry weight than WT roots at pH 4.5 but not at pH 5.5. Regarding K^+ content, as in the previous experiment, the single mutant *slnrt1.5* exhibited lower K^+ content in shoots and higher in roots than WT plants only at pH 4.5 (Fig. 6b). The double *slnrt1.5 slskor* mutant lines showed a further decrease in shoot K^+ content, that accounted for a ∼40 % decrease in shoot K^+ content when compared to the WT, and this occurred at both pH values (Fig. 6b). Thus, the effect of the double mutation was greater than the single ones, which indicated a high degree of functional redundancy between SlSKOR and SlNRT1.5 in K^+ translocation.

SlNRT1.5 supports plant growth at a low external K^+ concentration

As impaired K^+ translocation rendered Arabidopsis plants sensitive to low K^+ supply (Alejandro et al., 2007; Li et al., 2017), we tested the effect of such conditions on *slnrt1.5* and *slskor* single and double mutants. Thus, the aforementioned single and double mutants were grown under low K^+ conditions (0.05 mM K^+) during 21 d Regarding plant dry weight, *slnrt1.5* single and *slnrt1.5 slskor* double mutants showed lower root and shoot biomass than WT and *slskor* mutants (Fig. 7a and 7b). With respect to K^+ content, *slnrt1.5* single and double mutants exhibited lower K^+ content in shoots than WT and *slskor* plants (Fig. 7c). In the case of roots, only double mutants showed higher K^+ content in this organ in comparison with WT and single mutants (Fig. 7c). Therefore, the contribution of SlNRT1.5 to K^+ translocation under low K^+ was greater than that of SlSKOR as the phenotypes of the double mutants regarding dry weight and K^+ content resembled more to those of *slnrt1.5* excepting K^+ accumulation in roots.

Discussion

Our results showed that SlNRT1.5 is an important component of K^+ translocation systems in tomato plants with a high degree of functional redundancy with SlSKOR. Among the 90 *NRT/NPF* genes in the tomato genome, SlNRT1.5 is the orthologue of AtNRT1.5 since: (i) it has the highest protein homology among tomato NRTs when compared to AtNRT1.5 (Fig. S1), (ii) it is preferentially expressed in the root stele (Fig. 1a), (iii) it mediates K^+ efflux in Xenopus oocytes (Fig. 2), (iv) it takes part in K^+ translocation with no evident contribution to NO_3^- accumulation (Fig. 4) and (v) it prevents massive Na^+ accumulation in shoots (Figs. 4-7). These five results are in agreement with the information available for AtNRT1.5 (Chen et al., 2012; Drechsler et al., 2015; Li et al., 2017; Lin et al., 2008).

In tomato plants, SlNRT1.5 contribution to K^+ translocation is not evident under control conditions (Fig. S3), but it is under low pH and low K and N supply (Figs. 4-7). In contrast, SlSKOR contribution to K^+ translocation is observed under control conditions and it was independent of pH and N supply (Fig. 6 and 8) (Nieves-Cordones et al., 2023). These results indicated that the relevance of SlNRT1.1 and SlKOR to K^+ translocation depended on the growth conditions. However, the fact that a dramatic reduction of K^+ content in shoots was only observed when both transport systems were mutated (Fig. 6) supports that under those

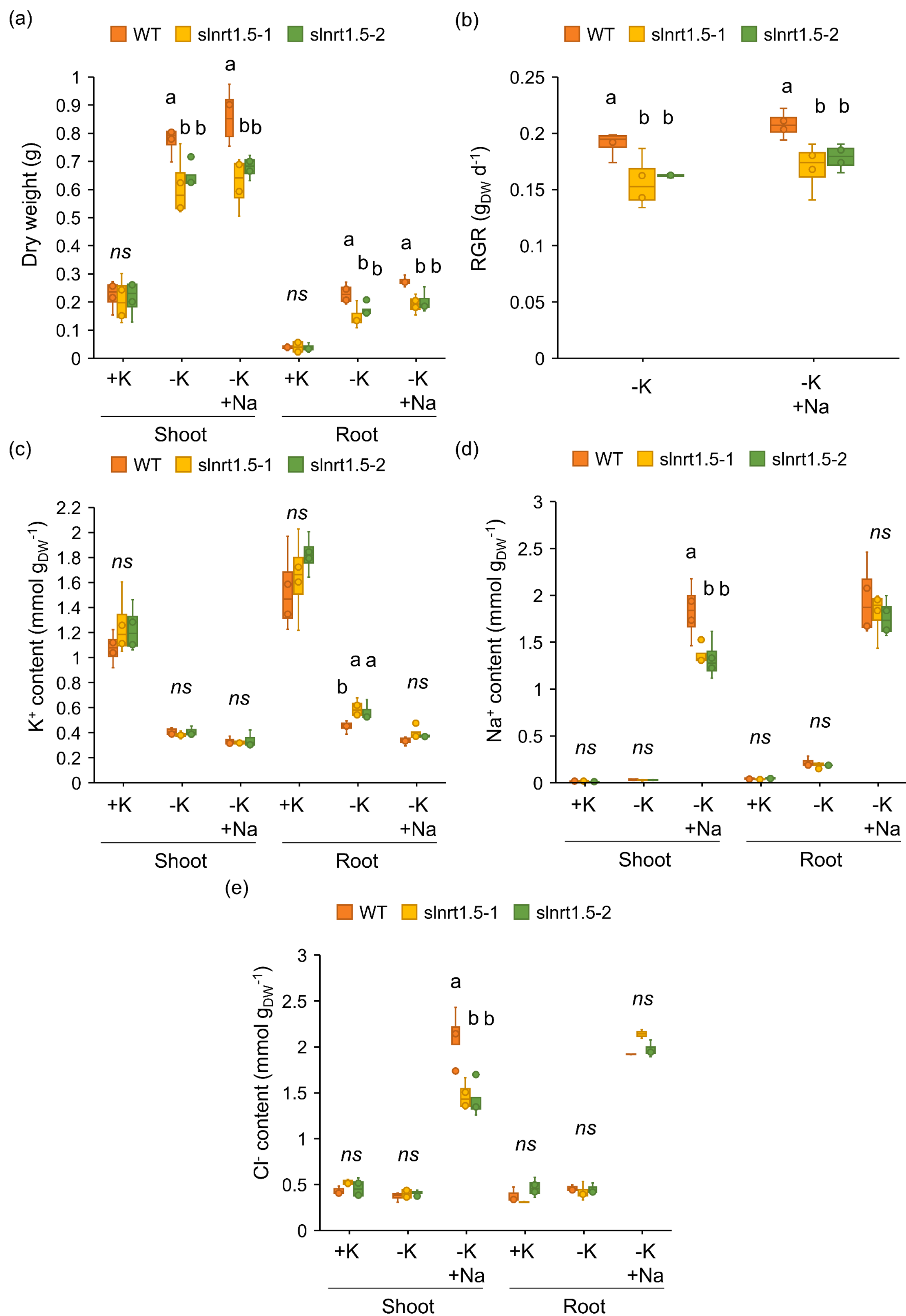

Fig. 3. The function of SlNRT1.5 is relevant under K^+ starving conditions with/without high Na^+. (a) Dry weight of WT, *slnrt1.5–1* and *slnrt1.5–2.* (b) Relative growth rate of WT, *slnrt1.5–1* and *slnrt1.5–2.* (c) K^+, (d) $Na^+$and (e) Cl^- content in shoots and roots of WT, *slnrt1.5–1* and *slnrt1.5–2* plants. Plants were grown at 1.4 mM for 14 d in hydroponic culture and then transferred to 0 mM K^+or 0 mM K^+ 50 mM Na^+ for 7 d Data are presented in boxplots ($n = 4$). Letters represent different groups of data at $p < 0.05$ based on LSD's post hoc test following ANOVA and ns denotes not significant.

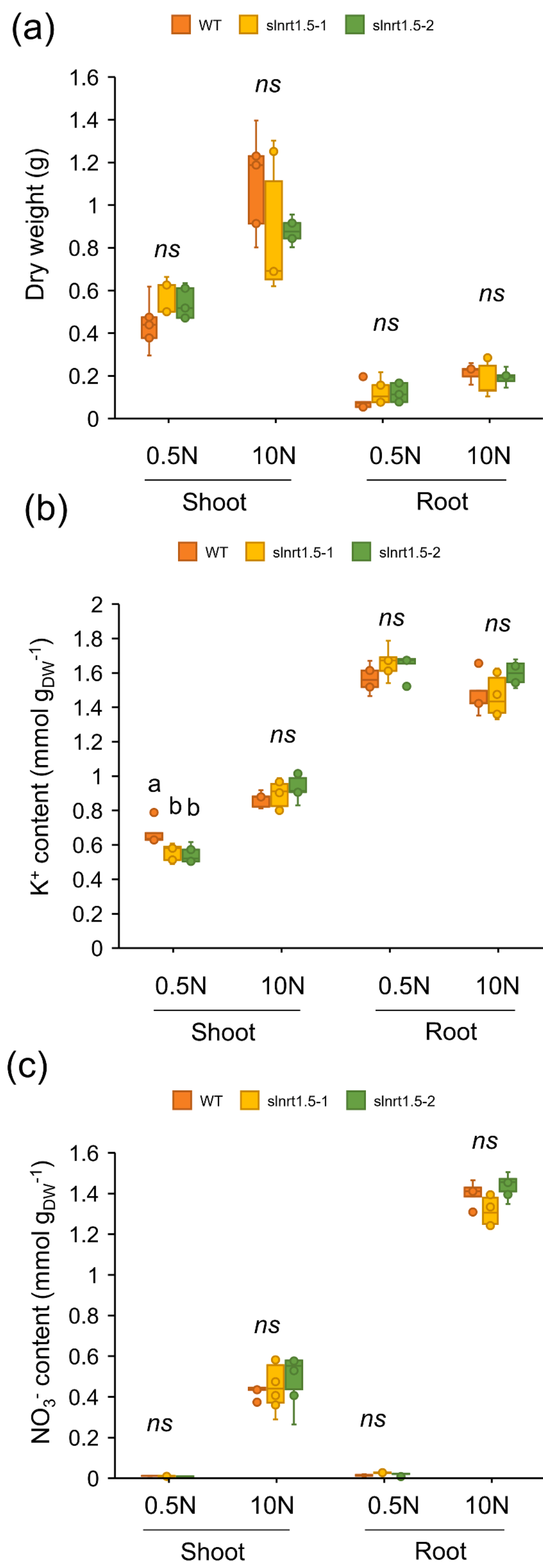

Fig. 4. SlNRT1.5 is involved in K^+ translocation in presence of low NO_3^-. (a) Dry weight of WT, *slnrt1.5–1* and *slnrt1.5–2* roots and shoots. (b) K^+ (c) NO_3^- content in roots and shoots of WT, *slnrt1.5–1* and *slnrt1.5–2* plants. Plants were grown in K^+ sufficient conditions (1.4 mM K^+) with 0.5 mM NO_3^- or 10 mM NO_3^- in hydroponic culture for 21 d Data are presented in boxplots ($n = 5–6$). Letters represent different groups of data at $p < 0.05$ based on LSD's post hoc test following ANOVA and ns denotes not significant.

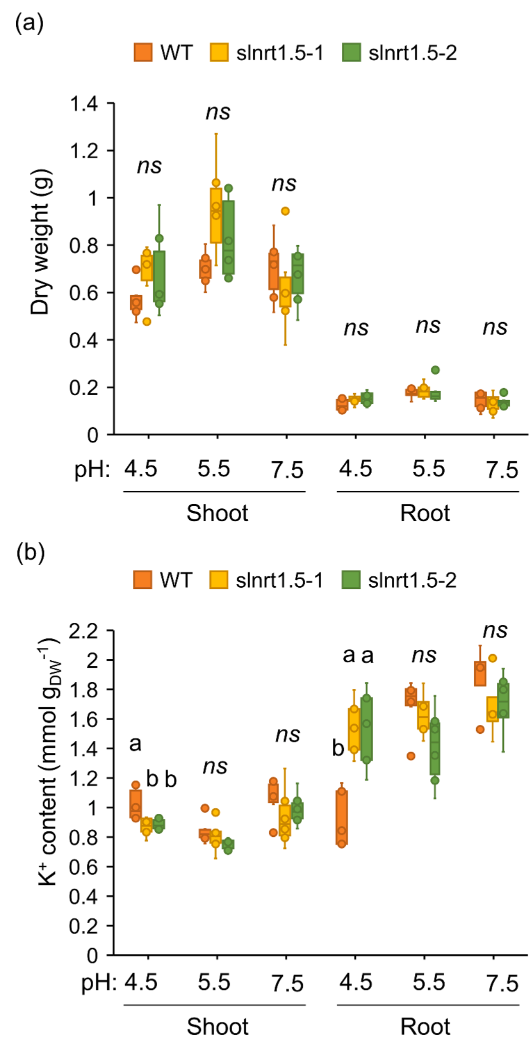

Fig. 5. K^+translocation is affected in *slnrt1.5* plants at pH 4.5. (a) Dry weight of WT, *slnrt1.5–1* and *slnrt1.5–2* roots and shoots. (b) K^+ content in roots and shoots of WT, *slnrt1.5–1* and *slnrt1.5–2* plants. Plants were grown at 1.4 mM at pH 4.5, 5.5 or 7.5 in hydroponic culture for 21 d Data are presented in boxplots ($n = 6$). Letters represent different groups of data at $p < 0.05$ based on LSD's post hoc test following ANOVA and ns denotes not significant.

conditions there is a significant overlap in their function (Fig. 8). The Arabidopsis counterpart of SlNRT1.5, AtNRT1.5, was involved in NO_3^- transport to shoots (Lin et al., 2008), however we did not observe any effect of the *slnrt1.5* mutation on NO_3^- accumulation in shoots (Fig. 4 and S3). Thus, the contribution of SlNRT1.5 to NO_3^- transport deserves further investigation. The K^+ translocation model described here for the Micro-tom variety will be validated in the future on commercial tomato varieties to assess the effect of different genetic backgrounds on the contribution of SlSKOR and SlNRT1.5.

It is important to highlight that SKOR K^+ channels and NRT1.5 transporters mediate K^+ efflux by different transport mechanisms. This is a key aspect from thermodynamic and physiological point of views. SKOR channels are opened by membrane depolarization and inhibited by acidic extracellular pH and high external K^+ concentrations (Drain et al., 2020; Gaymard et al., 1998; Lacombe et al., 2000; Nieves-Cordones et al., 2023; Villette et al., 2019). By contrast, AtNRT1.5 has been proposed to operate as a K^+/H^+ antiporter (Li et al., 2017). As a K^+/H^+ antiporter, AtNRT1.5 function is dependent on the pH and K^+ gradients. It has been reported that low K^+ supply gave rise to hyperpolarized membrane potentials in root cells (De Boer, 1999; Nieves-Cordones et al., 2008; Rubio et al., 2014). Therefore, under low K^+

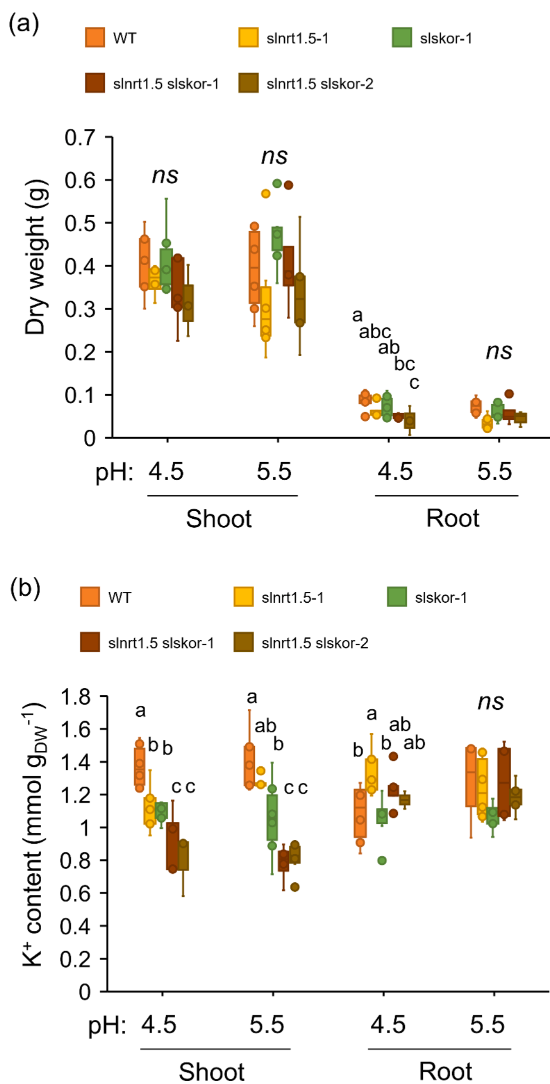

Fig. 6. SlNRT1.5 and SlSKOR cooperate in K^+ translocation in tomato plants. (a) Dry weight of WT, *slnrt1.5–1, slskor-1, slnrt1.5 slskor #1 and slnrt1.5 slskor #2* roots and shoots. (b) K^+ content in roots and shoots. Plants were grown at 1.4 mM K^+ at pH 4.5 or 5.5 in hydroponic culture for 21 d Data are presented in boxplots ($n = 4$–6). Letters represent different groups of data at $p < 0.05$ based on LSD's post hoc test following ANOVA and ns denotes not significant.

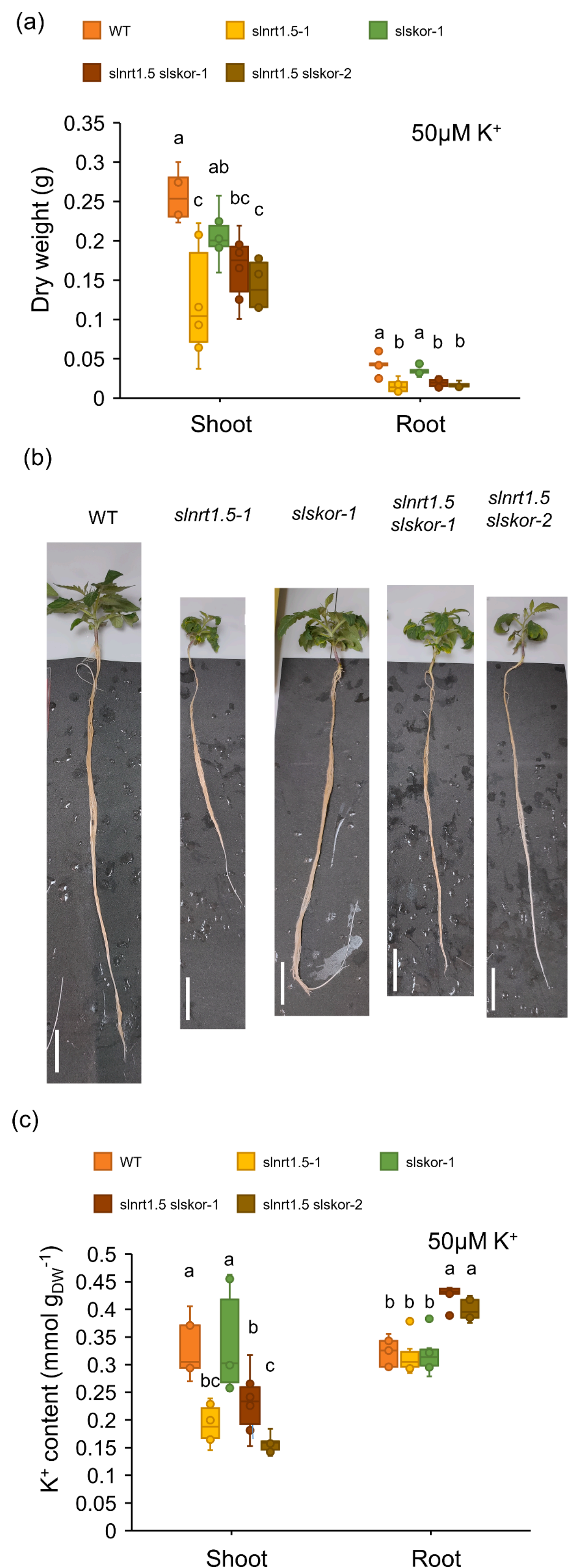

Fig. 7. SlNRT1.5 supports plant growth at a low external K^+ concentration. (a) Dry weight of WT, *slnrt1.5–1* and *slskor-1* single mutants and *slnrt1.5 slskor #1 and slnrt1.5 slskor #2* double mutants in roots and shoots. (b) Representative picture of the plants. Scale bar = 4 cm. (c) K^+ content in roots and shoots. These plants were grown in hydroponic at 0.05 mM K^+ for 21 d Data are presented in boxplots ($n = 5$–6). Letters represent different groups of data at $p < 0.05$ based on LSD's post hoc test following ANOVA and ns denotes not significant.

supply, K^+ efflux into xylem vessels by SKOR channels is not possible as the channel closes at hyperpolarized membrane potentials. However, NRT1.5 transporters could still mediate K^+ efflux at hyperpolarized membrane potentials which is in agreement with its role in K^+ translocation under low K^+ supply in Arabidopsis and tomato plants (Li et al., 2017) (Fig. 7). A working model for K^+ translocation in tomato plants would consist of the SlSKOR channel and the SlNRT1.5 transporter operation to release K^+ to xylem vessels under K^+ sufficient conditions (Fig. 6 and 8), whereas, under low K^+ supply, SlNRT1.5 would be a major component of xylem K^+ load (Fig. 7 and 8). A significant amount of K^+ was translocated to shoots in *slnrt1.5 slskor* double mutants which indicated that additional transport systems contribute to K^+ translocation in tomato plants (Fig. 8).

Besides K^+ translocation, the different transport mechanism of SlNRT1.5 transporter (K^+/H^+ antiport) and SlSKOR channel (outward-rectifying K^+ channel) may underlie the opposed effects of their mutation on Na^+ and Cl^- accumulation in shoots (Nieves-Cordones et al., 2023) (Fig 5). While *slskor* mutant accumulated more Na^+ and Cl^- in shoots than WT plants (Nieves-Cordones et al., 2023), *slnrt1.5* mutant accumulated less Na^+ and Cl^- in shoots than WT plants (Fig. 5). Although

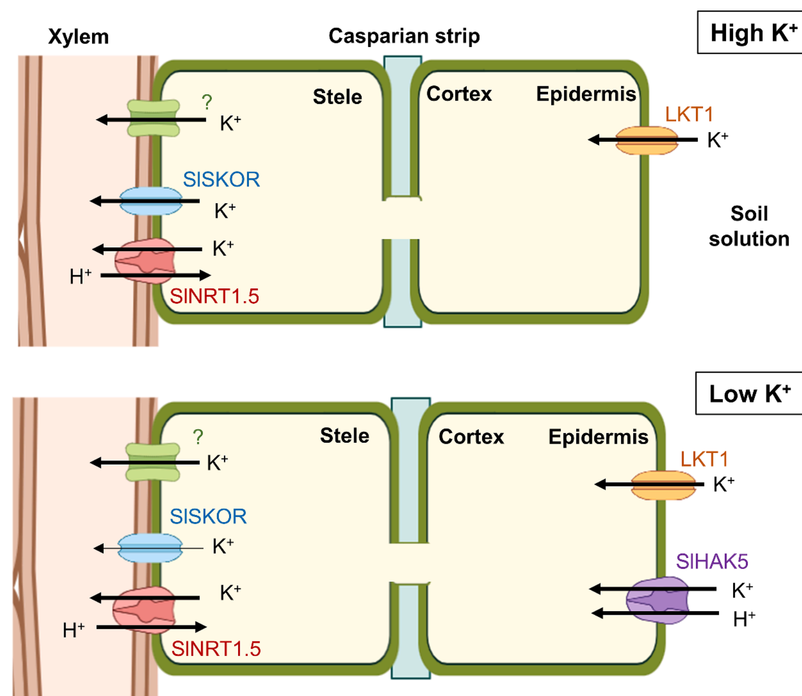

Fig. 8. Model for K^+uptake and translocation in tomato plants. Under K^+-sufficient conditions (1.4 mM K^+) the LKT1 channel mediates K^+ uptake in the epidermis and cortex. The translocation of K^+ from stele to the xylem is mediated by SlSKOR, together with SlNRT1.5 through different transport mechanisms (SlSKOR mediates K^+ efflux and SlNRT1.5 is expected to mediate K^+/H^+ antiport). Under K^+-deficient conditions (<0.1 mM K^+), the transporter SlHAK5 contributes, along with the LKT1 channel, to K^+ uptake in the epidermis and cortex. In the stele, under low K^+ conditions, SlNRT1.5 plays a fundamental role in K^+ translocation to the xylem, while SlSKOR does not seem be crucial to this process (Fig. 7). It is worth noting that a yet unidentified third system may be involved in K^+ translocation under both K^+-sufficient and K^+-limiting conditions. Icons were made with Biorender (https://www.biorender.com/).

the main cause for the different effect on Na^+ and Cl^- translocation of *SlSKOR* and *SlNRT1.5* mutations remains unclear at this moment, it can be hypothesized that changes in membrane potential or pH gradient due to SKOR or NRT1.5 activity are likely to be involved. In agreement with this idea, it has been reported that Na^+ and K^+ transport into xylem vessels are tightly linked and have a strong pH dependence in soybean (Lacan and Durand, 1996). In fact, in this plant species, it was proposed that K^+ release into xylem vessels was mediated by a K^+/H^+ antiport (Lacan and Durand, 1996), which could be reminiscent of NRT1.5-like transporter activity.

SlNRT1.5 and SlSKOR are major components involved in K^+ translocation in tomato plants and they should be regarded as relevant biotechnological targets to improve nutrient use efficiency and salt tolerance in tomato plants. Further research in this topic in crops, as the case of this study in tomato Micro-tom, will definitively provide a solid ground to design better strategies for crop breeding in the current context of climate change.

Material and methods

Plant material and growth treatments

In this study, tomato plants (*Solanum lycopersicum* L. var. Micro-Tom) were employed. Germination of seeds was performed in 0.8 % agar plates with 1/5 Hoagland solution. After six days, the seedlings were transferred to plastic containers containing a modified 1/5 Hoagland solution (+K solution), which included the following macronutrients (mM): 1.4 KCl,1.4 $Ca(NO_3)_2$, 0.35 $MgSO_4$ and 0.1 $Ca(H_2PO_4)_2$. The micronutrients (μM) were as follows: 50 $CaCl_2$,12.5 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.5 $CuSO_4$, 0.1 H_2MoO_4, 0.1 $NiSO_4$ and 10 Fe-EDDHA. The –K solution was obtained by avoiding the addition of KCl. The 0.5 N solution contained 0.25 mM $Ca(NO_3)_2$ and 1.15 $CaCl_2$. The 10 N solution was obtained by adding 8.6 mM $Ca(NO_3)_2$ to the +K solution. pH of the nutrient solution was adjusted with H_2SO_4 or $Ca(OH)_2$ as indicated in each experiment in a daily basis. The plants were cultivated in a growth chamber under a 16/8 h light/dark photoperiod, with temperatures set at 25 °C during the day and 20 °C at night, 65 % relative humidity, and a light intensity of 360 μmol m^{-2} s^{-1}.

Ion content determination in plant tissues

To determine the ion content, plant organs were harvested at the end of experiments and their fresh weight was measured. The dried plant material was digested in a HNO3 solution (5:3 v:v) using a microwave digestion system (CERM MarsXpress, North Carolina), and the cation content was analyzed through inductively coupled plasma (ICP) mass spectrometry with an Iris Intrepid II ICP spectrometer (Thermo Electron Corp., Franklin, MA, USA). NO_3^- and Cl^- contents were measured using a Dionex Aquion ion chromatographer (Thermo Fisher Scientific, Waltham, MA, USA) after incubating dried tissues in MilliQ water.

Real time qPCR

The Macherey-Nagel NucleoSpin RNA Plant Kit (Neumann-Neander, Germany) was used to extract total RNA from tomato tissues. Then, in order to remove any contaminating DNA, the RNA samples were treated with the DNA-free™ kit (Thermo Fisher Scientific, Waltham, MA, USA). qPCR was carried out on a 7500 Real-Time PCR System (Thermo Fisher Scientific). Expression levels of target genes relative to the expression level of the endogenous gene encoding the elongation factor SlEF1α are given as 40 - ΔC_t ($\Delta C_t = C_t^{target} - C_t^{SlEF1\alpha}$) (Nieves-Cordones et al., 2020). The primers used were designed with Primer3 Plus online server. Primers are listed in Table S1.

Production of slnrt1.5 plants with CRISPR-Cas and slnrt1.5 slskor double mutants

Knock-out (KO) mutants of *slnrt1.5* were produced by targeting two sgRNAs at exon 4 of the *SlNRT1.5* locus (Solyc08g007060) (Fig. S2). Highly-specific sgRNA sequences were designed with Breaking Cas tool (Oliveros et al., 2016). Target sequences were selected based on their elevated specificity (cut probability in off-target sites < 0.7 %). *SlNRT1.5* sgRNAs were cloned in A59 and A60 plasmids (cut probability in off-target sites < 0.7 %). *SlNRT1.5* sgRNAs were cloned in A59 and A60 plasmids (property of Abiopep Plant Health S.A.) as described previously (Amo et al., 2021; Nieves-Cordones et al., 2023, 2020) (Table S1). *Agrobacterium tumefaciens* GV3101 transformed with the plasmid A60 harboring *SlNRT1.5* sgRNAs, and Cas9 was used to infect WT Micro-Tom cotyledons (Van Eck et al., 2019). In vitro culture was subsequently used to achieve the regeneration of whole plants (T0 generation). The resulting plants were genotyped by PCR and sequencing. Allele sequences were inferred with the DECODR tool (Bloh et al., 2021) and verified by sequencing cloned sequences in an entry plasmid. Plant lines exhibiting frameshift mutations were chosen for additional analysis. Further analyses did not show edits in off-target sites (Table S2). T1 and T2 plants were used in the experiments after verifying their genotypes. Double mutants for *SlSKOR* and *SlNRT1.5* genes were obtained by crossing. Emasculated *slskor* flowers received pollen from *slnrt1.5* plants and vice versa. Genotyping was conducted as indicated previously. Two double mutant lines were propagated, one with *slnrt1.5–1* as pollen donor (*slnrt1.5 slskor #1*) and the other with *slskor-1* as pollen donor (*slnrt1.5 slskor #2*).

K^+ efflux assays in Xenopus oocytes

SlNRT1.5 cDNA was amplified from Micro-Tom roots total RNA by reverse transcription and PCR (RT-PCR) (Table S1). subsequently, this cDNA was cloned into the PCR2.1 TOPO vector (Invitrogen) and confirmed by Sanger sequencing. cRNA were transcribed in vitro using mMACHINE T7 Ultra kit (Ambion; http://www.ambion.com) as described previously (Amo et al., 2021; Nieves-Cordones et al., 2023, 2014). *Xenopus laevis* oocytes in stage V-VI were injected with water (negative control) or 25 ng of cRNA of *SlNRT1.5* or *AtNRT1.5* (positive control) using a Nanoliter 2010 microinjector (World Precision Instruments). Oocytes were stored at 18 °C for 72 h in ND96 solution. Then, oocytes were washed three times with a K^+-free modified ND96 solution (in mM: 98 NaCl, 1.8 CaCl2, 1 MgSO4, 5 HEPES, 0.05 bis-tris-propane, pH 5.5) and incubated in this solution for 3 h Seven oocytes incubated in each Petri dish represented one sample. After that, external K^+ concentration was measured using an AAnalyst 400 Perkin-Elmer spectrometer and the rate of K^+ efflux calculated as the total K^+ nmol in the external solution per minute per oocyte.

CRediT authorship contribution statement

Almudena Martínez-Martínez: Writing – original draft, Methodology, Investigation. **Maria Ángeles Botella:** Writing – review & editing, Investigation. **Manuel Francisco García-Legaz:** Investigation. **Elvira López-Gómez:** Investigation. **Jesus Amo:** Methodology, Investigation. **Lourdes Rubio:** Writing – review & editing, Methodology. **Jose Antonio Fernández:** Methodology. **Vicente Martínez:** Resources, Methodology. **Francisco Rubio:** Writing – review & editing, Supervision, Funding acquisition. **Manuel Nieves-Cordones:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100689.

Data availability

Data will be made available on request.

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