Regulation of K^+ Transport in Tomato Roots by the *TSS1* Locus. Implications in Salt Tolerance[1]

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The *tss1* tomato (*Lycopersicon esculentum*) mutant exhibited reduced growth in low K^+ and hypersensitivity to Na^+ and Li^+. Increased Ca^{2+} in the culture medium suppressed the Na^+ hypersensitivity and the growth defect on low K^+ medium of *tss1* seedlings. Interestingly, removing NH_4^+ from the growth medium suppressed all growth defects of *tss1*, suggesting a defective NH_4^+-insensitive component of K^+ transport. We performed electrophysiological studies to understand the contribution of the NH_4^+-sensitive and -insensitive components of K^+ transport in wild-type and *tss1* roots. Although at 1 mM Ca^{2+} we found no differences in affinity for K^+ uptake between wild type and *tss1* in the absence of NH_4^+, the maximum depolarization value was about one-half in *tss1*, suggesting that a set of K^+ transporters is inactive in the mutant. However, these transporters became active by raising the external Ca^{2+} concentration. In the presence of NH_4^+, a reduced affinity for K^+ was observed in both types of seedlings, but *tss1* at 1 mM Ca^{2+} exhibited a 2-fold higher K_m than wild type did. This defect was again corrected by raising the external concentration of Ca^{2+}. Therefore, membrane potential measurements in root cells indicated that *tss1* is affected in both NH_4^+-sensitive and -insensitive components of K^+ transport at low Ca^{2+} concentrations and that this defective transport is rescued by increasing the concentration of Ca^{2+}. Our results suggest that the *TSS1* gene product is part of a crucial pathway mediating the beneficial effects of Ca^{2+} involved in K^+ nutrition and salt tolerance.

Most plant cells accumulate K^+ and exclude Na^+. The resulting high K^+ to Na^+ ratios in the cells enable K^+ to perform essential functions that Na^+ cannot fulfill (Hasegawa et al., 2000; Rodriguez-Navarro, 2000). This selectivity in favor of K^+ is especially important in the arid and semiarid regions of the world, where the high sodium salts concentrations in the soil cause severe problems in crop production (Epstein, 1998). However, K^+ nutrition, particularly at low K^+, is not only impaired by an excess of Na^+ but also by the presence of NH_4^+ in the growth media (Santa-María et al., 2000).

K^+ uptake occurs through different transport mechanisms depending on the external K^+ concentration. Carriers that exhibit a high affinity for K^+ and some K^+ inward-rectifying (KIR) channels work at low K^+ (<1 mM K^+), whereas different types of channels, such as KIR channels or nonselective cation channels (NSCCs), are involved when external K^+ concentration is in the millimolar range (Rodriguez-Navarro, 2000; Schachtman, 2000; Demidchik et al., 2002; Véry and Sentenac, 2003). Although some KIR channels (AKT type) may function at low K^+ (Hirsch et al., 1998), carriers are thought to be responsible for the majority of K^+ uptake in the micromolar range (Maathuis and Sanders, 1997; Rubio et al., 2000). As previously reported in Arabidopsis (Spalding et al., 1999), in tomato (*Lycopersicon esculentum*) there appear to be two distinct mechanisms involved in K^+ uptake (Borsani et al., 2001). The first mechanism is insensitive to inhibition by NH_4^+ and, by analogy to Arabidopsis, may correspond to inward-rectifying K^+ channels such as the AKT1 channel (Spalding et al., 1999). The second mechanism is inhibited by NH_4^+ and may correspond to an active K^+ transport coupled to the H^+ electrochemical potential gradient (Santa-María et al., 2000). In addition to its effect on K^+ uptake, NH_4^+ can result toxic for most plant species, including tomato. Different explanations for this toxicity besides K^+ transport inhibition have been suggested. These include intracellular pH disturbance, carbon deprivation, and ionic imbalance associated with a decrease in the internal concentration of K^+, Mg^{2+}, and Ca^{2+} (Britto et al., 2001; Kronzucker et al., 2001).

Ca^{2+} plays multiple roles in root hair development, ionic homeostasis, and stress-induced responses (Sanders et al., 1999; Reddy, 2001). A rise in the external Ca^{2+} concentration can improve the K^+/

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Na^+ selectivity (Hasegawa et al., 2000), increasing the K^+ uptake under salt stress. An inhibitory effect on the activity of KIR channels by Ca^{2+} has been reported (White, 1997). In addition, different responses of NSCCs to Ca^{2+} such as activation (Ca^{2+}-activated NSCCs), partial inhibition (voltage-insensitive NSCCs), and increased permeability (hyperpolarization-activated NSCCs) have been shown (Demidchik et al., 2002). Ca^{2+}-mediated responses involve temporal and spatial oscillations in cytosolic free Ca^{2+} activity (Evans et al., 2001). For this reason, the free Ca^{2+} concentration in the cytosol, usually in the nanomolar range, is strongly regulated. Interaction between Ca^{2+} concentrations, K^+ transport, and salinity has been reported in Arabidopsis (Liu and Zhu, 1998; Hasegawa et al., 2000; Zhu, 2002). A myristoylated Ca^{2+}-binding protein encoded by SOS3 presumably senses the salt-elicited calcium signal and translates it to downstream responses (Liu and Zhu, 1998; Ishitani et al., 2000). SOS3 interacts with and activates SOS2, a Ser/Thr protein kinase (Halfter et al., 2000; Liu et al., 2000). SOS2 and SOS3 regulate the expression level of SOS1, a salt tolerance effector gene encoding a plasma membrane Na^+/H^+ antiporter (Shi et al., 2000). More importantly, SOS2 and SOS3 are required for the activation of SOS1 transport activity (Qiu et al., 2002; Quintero et al., 2002).

We have isolated tomato mutants in the search for salt and osmotic hypersensitive mutants using a molecular genetic approach (Borsani et al., 2001, 2002). The *tss1* mutant behaves as a phenocopy of the Arabidopsis *sos3* mutant because it is hypersensitive to Na^+ and exhibits a growth defect on low K^+ (Borsani et al., 2001). K^+-dependent membrane potential depolarizations indicated impaired K^+ uptake in *tss1*. As occurs is *sos3*, increased Ca^{2+} concentration in the culture medium can partially suppress the Na^+ hypersensitivity of *tss1* seedlings and completely suppress the growth defect on low K^+ medium (Borsani et al., 2001). These phenotypes suggest that, as SOS3, TSS1 is required to amplify a Ca^{2+} signal during Na^+ stress and K^+ starvation.

In this work, we show that the hypersensitivity to Na^+ and Li^+ and the reduced growth in low K^+ of *tss1* is suppressed when the growth medium does not contain NH_4^+. Interestingly, this NH_4^+-dependent phenotype was not mimicked by the Arabidopsis *sos3* mutant. The aim of this work is to study the effect of Ca^{2+} and the influence of NH_4^+ on both root growth and K^+ transport in wild-type and *tss1* root seedlings, with special emphasis on K^+ transport kinetics.

RESULTS

Isolation of New Mutants at the TSS1 Locus

A previous screening of 600 M_2 seed families resulted in the isolation of three tomato mutants (*tss1-1*, *tss1-2*, and *tss2*) defining two genetic loci required for NaCl tolerance (Borsani et al., 2001). A further 2,000 ethylmethane sulfonate M_2 seed families were screened looking for hypersensitivity to NaCl. The screening resulted in the isolation of three additional mutants hypersensitive to NaCl. As shown in Table I, analysis of the mutant segregation within each M_2 family showed an approximately 3:1 segregation ratio of wild type:mutant (Table I). Thus, all new *tss* mutants were caused by single recessive nuclear mutations. Complementation analyses indicated that the three mutants were allelic to *tss1-1* (data not shown). We determined the degree of NaCl hypersensitivity from the five *tss1* alleles. Table I shows the concentrations of NaCl at which the root elongation rate decreased by 50% relative to medium without salt (I_{50}). *tss1-1*, *tss1-2*, and *tss1-3* showed similar I_{50} values (68, 70, and 67 mM NaCl, respectively). *tss1-4* and *tss1-5* were weaker alleles with I_{50} values of 125 and 115 mM NaCl, respectively. All subsequent physiological studies were performed using *tss1-3* (hereafter referred to as *tss1*).

Hypersensitivity of tss1 Seedlings to Na^+, Li^+, and the Growth Defect on Low K^+ Is Abolished in the Absence of NH_4^+

tss1 mutants were isolated based on their hypersensitivity to grow on Murashige and Skoog medium containing 125 mM NaCl (Borsani et al., 2001, this work). *tss1* was hypersensitive to all NaCl concentrations analyzed (Borsani et al., 2001). We also deter-

Table I. *Frequency of the mutation in each M_2 family and values of their I_{50} to NaCl*

Mutants	I_{50} to NaCl[a]	Frequency of Mutant[b]	χ^{2c}	Reference
	mM			
tss1-1	68	36/7	1.75	Borsani et al. (2001)
tss1-2	70	32/10	0.03	Borsani et al. (2001)
tss1-3	67	34/10	0.4	This study
tss1-4	125	22/6	0.12	This study
tss1-5	115	31/6	0.12	This study

[a] I_{50} to NaCl was determined as previously described (Borsani et al., 2001). [b] Frequency of segregation in M_2 families; numerator, total no. screened in a family or a cross; denominator, no. of segregating mutants. [c] The values represent the fit of the data to an expected 3:1 (wild type:mutant) phenotypic segregation ($P < 0.001$).

mined that *tss1* was affected in K^+ uptake, which in turn would render the plant hypersensitive to NaCl. Surprisingly, we found that *tss1* was not hypersensitive to NaCl when a new growth medium was employed (see "Materials and Methods"). Several differences in salt concentrations are found between these two media. However, the main qualitative difference found is the absence of NH_4^+ in the new medium, whereas the concentration of NH_4^+ in the Murashige and Skoog medium is 20 mM. The inhibitory effects of NH_4^+ on K^+ uptake have been reported previously (Scherer et al., 1984; Vale et al., 1987; Spalding et al., 1999; Santa-María et al., 2000). Supplementing the medium with 5 mM NH_4^+ restored the *tss1* hypersensitivity to NaCl (Fig. 1), proving that the abolishment of NaCl hypersensitivity in the new medium was due solely to the lack of NH_4^+.

Next, we investigated whether *tss1* hypersensitivity was abolished in media lacking NH_4^+ at different concentrations of NaCl. As shown in Figure 2A, no differences between wild-type and *tss1* seedlings were observed when the media did not contain NH_4^+, whereas *tss1* seedlings remained hypersensitive in NH_4^+-containing media. We had shown previously that *tss1* was hypersensitive to Li^+ (Borsani et al., 2001). Li^+ is considered a more toxic analog of Na^+ and has been used to mimic Na^+ toxicity without the osmotic stress component associated with NaCl. We also determined that Li^+ hypersensitivity of *tss1* was dependent on the presence of NH_4^+ in the growth medium (Fig. 2B). *tss1* growth was impaired at low K^+ concentrations (<1 mM K^+), suggesting that *tss1* is most likely affected in an NH_4^+-insensitive K^+ transport mechanism (Borsani et al., 2001). When we analyzed the growth defect of *tss1* on low K^+ in the presence or absence of NH_4^+, reduced growth was observed only in media with NH_4^+. Removal of NH_4^+ also stimulated root growth of wild-type plants at increasing K^+ concentrations (Fig. 2C).

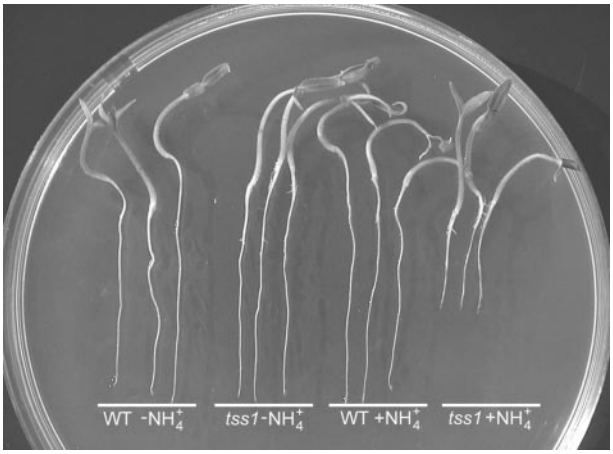

Figure 1. *tss1* is only hypersensitive to Na^+ only when the medium contains NH_4^+. Wild type (WT) and *tss1* 3-d-old seedlings with 1.5-cm-long roots grown on vertical agar plates on basal medium were transferred to new medium supplemented with 125 mM NaCl in the absence ($-NH_4^+$) or presence of 5 mM NH_4^+ ($+NH_4^+$) and allowed to grow for another 2 d.

sos3 Hypersensitivity to Na^+ and Li^+ Is Independent of NH_4^+

tss1 mutants resemble the *sos3* mutant isolated previously in Arabidopsis (Liu and Zhu, 1997, 1998). Both *sos3* and *tss1* plants exhibited normal growth except when challenged with low K^+ or salt stress. As in *sos3*, increasing external Ca^{2+} suppressed the growth defect on low K^+ medium and partially suppressed the salt hypersensitivity phenotype of *tss1*. Therefore, we analyzed whether *sos3* hypersensitivity to NaCl and LiCl was abolished in the absence of NH_4^+ as occurred with *tss1*. As shown in Figure 3A, the *sos3* mutant remained hypersensitive to NaCl whether or not the media contained NH_4^+. Moreover, the degree of NaCl hypersensitivity of *sos3* was similar in both media (Fig. 3A). It is interesting to note that Arabidopsis wild-type seedlings markedly improved their NaCl tolerance when the NH_4^+ was removed from the growth media (Fig. 3A). The improved NaCl tolerance of wild-type Arabidopsis seedlings without NH_4^+ was not observed in wild-type tomato (Fig. 2A). As for NaCl, *sos3* remained hypersensitive to LiCl in NH_4^+-free media. However, in contrast to Na^+, the addition of NH_4^+ to the growth media had a beneficial effect (Fig. 3B).

Effect of Ca^{2+} and NH_4^+ on K^+ Uptake

Our previous results suggest that the reduced growth on low K^+ medium and the hypersensitivity to both Na^+ and Li^+ could be due to a defective K^+ uptake of *tss1* (Borsani et al., 2001). Because these defects are abolished when the media lacks NH_4^+, we speculate that NH_4^+ is inhibiting a K^+ uptake system that become functional after NH_4^+ is removed from the media. Previous studies using the Arabidopsis *akt1* mutant indicated that at low K^+ concentrations (10–100 μM K^+), K^+ uptake is dependent on two classes of transport mechanisms operating in parallel (Hirsch et al., 1998; Spalding et al., 1999). The first mechanism is uninhibited by NH_4^+ and corresponds to the inward-rectifying K^+ channel AKT1 (Sentenac et al., 1992; Spalding et al., 1999). The second mechanism is NH_4^+ sensitive and corresponds to non-AKT1 transporters (Spalding et al., 1999). There is evidence indicating that HAK transporters may be involved in this NH_4^+-sensitive pathway (Santa-María et al., 2000). The reduced growth of *tss1* is only observed at concentrations of Ca^{2+} ranging from 0.15 to 1 mM. Increasing the external Ca^{2+} over this value completely suppressed the growth defect of *tss1* at low K^+ (Borsani et al., 2001). Therefore, we have studied the effect of two different external Ca^{2+} concentrations, 1 and 5 mM, in the

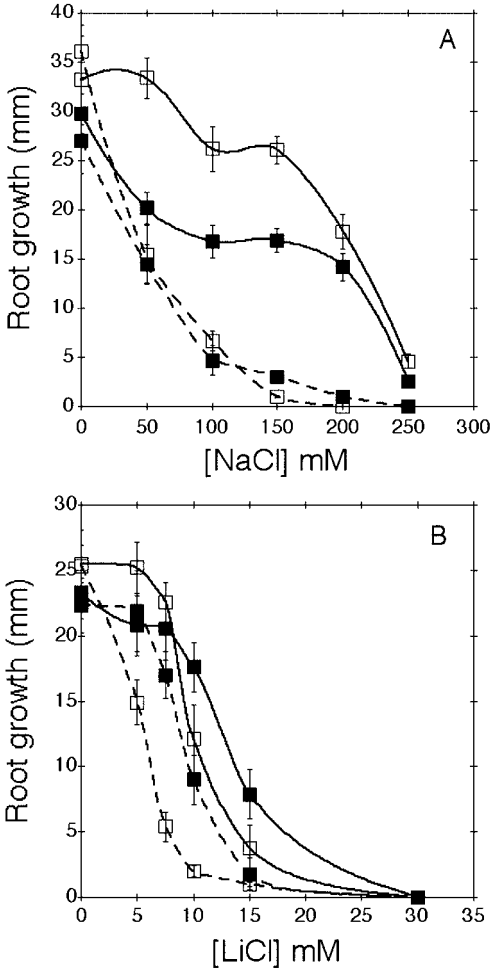

Figure 2. Removing NH_4^+ from the growth medium suppresses the hypersensitivity of *tss1* to Na^+ and Li^+ and the growth defect of *tss1* at low K^+. Wild-type (solid line) and *tss1* (dashed line) seedlings were grown on basal medium for 2 d. The seedlings were then transferred for 3 d to new medium supplemented with different external concentrations of NaCl (A) and LiCl (B), in the absence (white circles) or presence (solid circles) of 5 mM NH_4. C, Wild-type (solid line) and *tss1* (dashed line) seedlings were germinated and grown for 3 d on K^+- and Ca^{2+}-free medium. Then, seedlings were transferred to new K^+- and Ca^{2+}-free medium supplemented with the indicated concentrations of KCl. Root growth in the first 2 d was not measured so that the effect of residual K^+ carried over from the germination medium was minimized. Root elongation was measured 3 d later. Error bars = SD (n = 10).

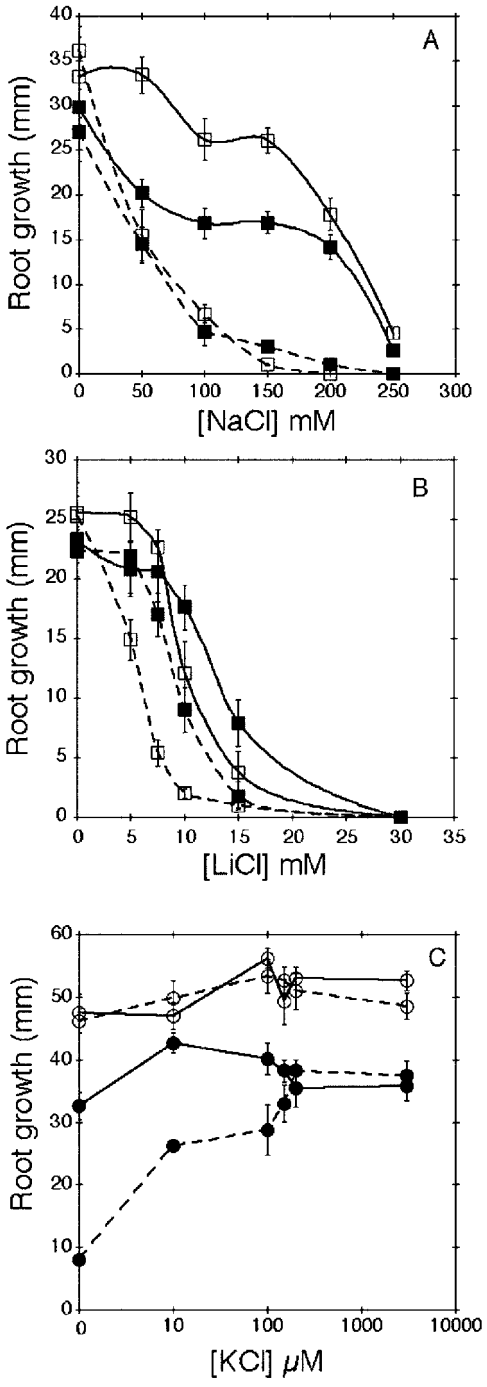

Figure 3. Hypersensitivity to Na^+ and Li^+ of *sos3* is independent of the presence of NH_4^+ in the growth medium. Arabidopsis seedlings were grown on basal medium for 4 d and then transferred for 7 additional d to new medium supplemented with different external concentrations of NaCl (A) and LiCl (B), in the absence (white squares) or presence (solid squares) of 5 mM NH_4^+. Solid line, Wild type; dashed line, *sos3*. Error bars = SD (n = 10).

transport kinetics of K^+ in wild-type and *tss1* root seedlings in the absence or presence of NH_4^+.

Electrophysiological experiments revealed similar resting membrane potentials (E_m) at 1 and 5 mM external Ca^{2+} concentrations both in wild-type and *tss1* root cells either in the absence (−160 ± 12 mV) or presence (−153 ± 12 mV) of 1 mM NH_4^+ (ANOVA, α = 0.01). In medium without NH_4^+, increasing K^+ concentrations (from 0.1–1000 μM KCl) elicited rapid membrane depolarizations in both wild-type and *tss1* seedlings grown at 1 or 5 mM external Ca^{2+} concentrations (Fig. 4, A and B). The membrane depolarization values showed a saturation kinetics model and were fitted to the Michaelis-Menten equation (Table II). Wild-type seedlings exhibited similar K_m values whether the media contained 1 or 5 mM external

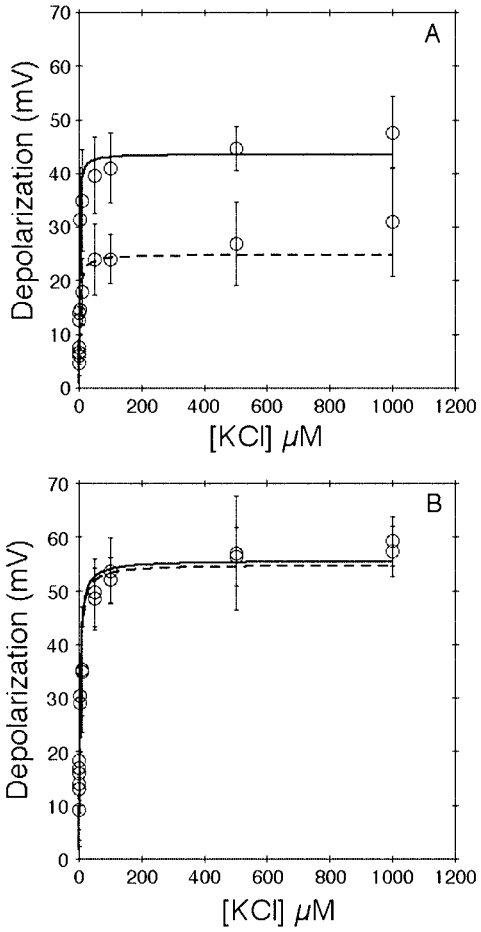

Figure 4. Membrane potential depolarizations induced by increasing K^+ concentrations in wild-type (solid line) and *tss1* (dashed line) root cells. Seeds were germinated in distilled water and grown for 6 d in the absence of NH_4^+ at 1 mM NaCl and at 1 (A) or 5 (B) mM $CaCl_2$. Values showed a saturation kinetics model and were fitted to the Michaelis-Menten equation. The calculated kinetics parameters are shown in Table II. Data are means ± SD ($n = 5$).

Ca^{2+}, although a slight increase in D_{max} value was reported in seedlings adapted to 5 mM external Ca^{2+} (Table II). Root cells of *tss1* seedlings grown at 1 mM external Ca^{2+}, showed a similar K_m value but approximately 50% of the D_{max} value obtained for the wild type in similar conditions (Table II). However, when wild-type and *tss1* seedlings were grown at 5 mM external Ca^{2+}, no differences were observed in kinetics parameters, indicating that K^+ transport was re-established (two way ANOVA, $\alpha = 0.01$).

Evidence for an inhibitory effect of NH_4^+ on K^+ transport has been reported in other plant species on the basis of short-term radiometric studies (Scherer et al., 1984; Vale et al., 1987, 1988; Wang et al., 1996; Spalding et al., 1999; Santa-María et al., 2000). In medium containing NH_4^+, increasing K^+ concentrations also evoked rapid membrane depolarizations in both wild-type and *tss1* seedlings (Fig. 5, A and B). As before, the membrane depolarization values showed a saturation kinetics model that could be fitted to the Michaelis-Menten equation. However, the K^+ concentration needed to reach saturation was around 10 mM. When kinetics parameters for K^+ uptake in the presence of 1 mM NH_4^+ were calculated, clear differences between wild type and *tss1* were observed (Table II). In wild-type and *tss1* seedlings, the K_m value for K^+ increased at least 100-fold, regardless of the external Ca^{2+} concentration, indicating that the affinity for K^+ uptake is greatly dependent on the presence of NH_4^+ (Table II). At 1 mM Ca^{2+}, *tss1* root cells exhibited a higher K_m and a lower D_{max} value than wild type (Fig. 5A; Table II). However, the addition of 5 mM Ca^{2+} to the medium suppressed the kinetics differences between wild-type and *tss1* seedlings (Fig. 5B; two-way ANOVA, $\alpha = 0.01$).

DISCUSSION

Identification of New tss1 Alleles

In plants, K^+ plays an essential role as an osmoticum and charge carrier (Rodriguez-Navarro, 2000). The capacity of plants to maintain a high cytosolic K^+ to Na^+ ratio is likely to be one of the key determinants of plant salt tolerance (Maathuis and Amtmann, 1999). The *tss1* mutant is specifically hypersensitive to growth inhibition by Na^+ or Li^+ and has impaired growth at low K^+ (<1 mM K^+) when external Ca^{2+} concentration is low. We have shown that all phenotypes cosegregated, indicating that mutations in *TSS1* were responsible for all three phenotypes (Borsani et al., 2001). Five mutants have been identified in the *TSS1* locus and three of them, *tss1-1*, *tss1-2*, and *tss1-3*, appeared very similar in their sensitivity to NaCl (Table I), which suggests that they are null mutants.

Root Growth of tss1 in Low K^+ or High Na^+ Is Dependent on Ca^{2+} and NH_4^+

Based on previous data (Borsani et al., 2001), tomato seems to have two distinct mechanisms for K^+ uptake similar to those reported previously in Arabidopsis and barley (*Hordeum vulgare*; Spalding et al., 1999; Santa-María et al., 2000). The first one is a mechanism insensitive to inhibition by NH_4^+ that, by analogy to Arabidopsis, may correspond to inward-rectifying K^+ channels such as the AKT1 channel. The second is a mechanism inhibited by NH_4^+, which may correspond to an active K^+ transport coupled to the H^+ electrochemical gradient. Based on previous data (Borsani et al., 2001), we can speculate that the NH_4^+-sensitive component of *tss1* is functional (at least partially) because this reduced growth of *tss1* in low K^+ and low Ca^{2+} is reverted in media lacking NH_4^+. However, removing NH_4^+ from the growth media not only suppressed the growth defect at low K^+ but also the *tss1* hypersensitivity to Na^+

Table II. *Kinetics parameters of K^+ transport in wild-type and tss1 root cells*

K^+-induced membrane depolarizations were fitted to a Michaelis-Menten equation, and maximum depolarization (D_{max}, expressed in millivolts) and K_m (micromolar) were calculated. The coefficient of determination (r^2) is also indicated.

	No NH_4^+		1 mM NH_4^+	
	Ca^{2+} 1 mM	Ca^{2+} 5 mM	Ca^{2+} 1 mM	Ca^{2+} 5 mM
WT				
K_m (μM KCl)	1.3a[a] ± 0.4	2.9a ± 1.2	335.8b ± 68.9	253.4b ± 159.2
D_{max} (mV)	43.6d ± 2.7	55.6e ± 2.9	39.2d ± 2.9	21.7f ± 3.4
r^2	0.98	0.95	0.98	0.94
tss1				
K_m (μM KCl)	2.5a ± 0.8	2.9a ± 1.2	617.0c ± 158	247.0b ± 69
D_{max} (mV)	24.9f ± 3.2	54.8e ± 2.5	29.6g ± 2.3	18.2f ± 0.83
r^2	0.95	0.94	0.86	0.96

[a] a to g, Values in which significant differences were not found (ANOVA $\alpha = 0.01$).

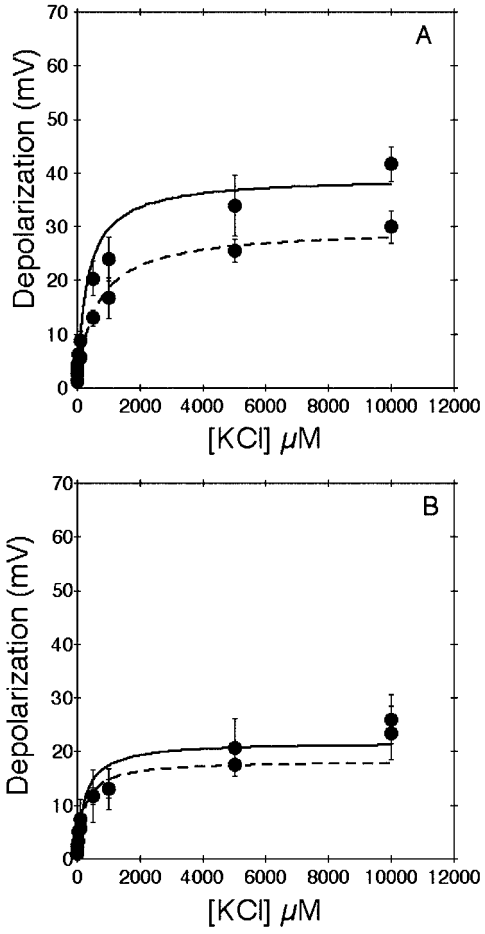

Figure 5. Membrane potential depolarizations induced by increasing K^+ concentrations in wild-type (solid line) and *tss1* (dashed line) root cells. Seeds were germinated in distilled water and grown for 6 d in the presence of 1 mM NH_4Cl at 1 mM NaCl and at 1 (A) or 5 (B) mM $CaCl_2$. Values showed a saturation kinetics model and were fitted to the Michaelis-Menten equation. The calculated kinetics parameters are shown in Table II. Data are means ± SD ($n = 5$).

and Li^+ (Fig. 2, A–C). These results suggest that the hypersensitivity to Na^+ and Li^+ of *tss1* can be due to a defective in K^+ uptake in the presence of these ions that is rectified when the NH_4^+-sensitive component becomes active.

tss1 tomato mutants resemble the Arabidopsis *sos3* mutant. This prompted us to speculate that *TSS1* could be a *SOS3* ortholog in tomato, therefore encoding some type of Ca^{2+} sensor (Liu and Zhu, 1997, 1998). However, the hypersensitivity to Na^+ and Li^+ of *sos3* was not overcome by removing NH_4^+ from the growth media (Fig. 3). It is possible that *TSS1* and *SOS3* are not ortholog genes and, despite similarities in phenotype, they regulate different targets. Alternatively, Arabidopsis may lack or not express the genes encoding the NH_4^+-sensitive K^+ transporters required for growth at low K^+ or high salt. It is known that SOS3, a Ca^{2+} sensor, together with SOS2, encoding a protein kinase, regulates SOS1, an Na^+/H^+ antiporter (Zhu, 2002). Therefore, the Na^+ hypersensitivity of *sos3* also could be explained by factors other than K^+ nutrition. Despite the advantages of Arabidopsis as a model plant, it is not clear if it can be used as a universal system to understand the physiology of K^+ uptake or Na^+ tolerance. As a consequence, additional studies in other plants might be necessary to understand the whole picture of K^+ transport and Na^+ tolerance.

K^+ Uptake Mechanisms Affected in tss1

Our results revealed that tomato takes up K^+ from low micromolar K^+ concentrations when the plants are grown in conditions of K^+ deficiency (Table II). Because relatively similar kinetics parameters for K^+ uptake were found in wild-type seedlings in media lacking NH_4^+ at 1 mM Ca^{2+} and 5 mM Ca^{2+}, we can speculate that the same type of K^+ transporters are active regardless of external Ca^{2+} concentration. However, at 1 mM Ca^{2+}, *tss1* exhibits a defective K^+ uptake (Fig. 4A). In these conditions, root cells of both *tss1* and wild type showed a similar K_m value, but *tss1* exhibited a lower D_{max}, an estimation of K^+ transport V_{max} (Fig. 4A; Table II). This may be interpreted as a decrease in the number of active K^+ transporters (Malhotra and Glass, 1995). A higher external Ca^{2+} concentration (5 mM Ca^{2+}) suppressed

the K^+ transport defect exhibited by *tss1* (Fig. 4B; Table II), most likely by increasing the number of active K^+ transporters.

The addition of NH_4^+ drastically reduced the affinity for K^+ in wild-type tomato (Table II), indicating the inhibition of NH_4^+-sensitive K^+ transport systems. A reduced affinity for K^+ induced by NH_4^+ has been reported in other plant species (Santa-María et al., 2000). However, NH_4^+ not only affected the K_m but also reduced the D_{max} of K^+ transport at 5 mM Ca^{2+}, suggesting that NH_4^+-sensitive components account for an important proportion of the K^+ taken by tomato seedlings in this condition.

Increasing the Ca^{2+} concentration in the presence of NH_4^+ has two effects. First, there is a reduction in D_{max} both in *tss1* and wild type. Because this reduction in D_{max} occurs only in the presence of NH_4^+ it is likely that the increase of Ca^{2+} specifically inhibits NH_4^+-insensitive K^+ transporters. Second, it recovers the K^+ affinity of *tss1* to a similar value than that observed in the wild type. Thus, *tss1* shows a Ca^{2+} requirement for a normal regulation of K^+ transport systems in K^+-starved conditions, irrespective of NH_4^+. The effect of this latter ion demonstrates that NH_4^+-sensitive and -insensitive transport mechanisms are involved in K^+ uptake in tomato root cells in conditions of K^+ starvation.

TSS1 would play an essential role in the regulation of K^+ transport at low Ca^{2+} concentration. Thus, it is proposed that this increase in the external Ca^{2+} concentration could lead to an increase in the internal Ca^{2+} sufficient to overcome the defective activity of *TSS1* and to allow a normal regulation of the activity K^+ transporters, both NH_4^+-sensitive and -insensitive mechanisms.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds were surface sterilized with 10% (v/v) commercial bleach for 30 min and washed several times with sterile water. The seeds were first germinated in sterile water until radicle emergence because we found that this method improved germination uniformity. The basal agar medium used for growth curves contained 0.5% (w/v) Suc and 0.7% (w/v) agar. The medium was based on the one described by Spalding et al. (1999) and consisted of the following: 2.5 mM $NaNO_3$, 2.5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 0.1 mM NaFeEDTA, 80 μM $Ca(H_2PO_4)_2$, 25 μM $CaCl_2$, 25 μM H_3BO_4, 2 μM $ZnSO_4$, 2 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM Na_2MoO_4, 0.01 μM $CoCl_2$, 2.5 mM KCl (unless otherwise indicated), and 2.5 mM MES. The pH of the mixture was adjusted to 5.7 with NaOH and autoclaved for 10 min. The K^+- and Ca^{2+}-free medium consisted of the same components with the exception of K^+ and Ca^{2+} salts. NH_4^+ was added as NH_4NO_3. The NO_3^- concentration was compensated by adding $NaNO_3$. The K^+ and Ca^{2+} were as KCl and $CaCl_2 \cdot 2H_2O$, respectively. Different salinity conditions used in this work were obtained by adding the appropriate amount of NaCl and LiCl to the molten basal medium. Seedlings were grown under a 16-h-light: 8-h-dark photoperiod at 50 μmol m^{-2} s^{-1} and 70% relative humidity.

Growth Measurements

Ten seeds were used per treatment, and three replicates were run for each treatment. Increases in root length were measured with a ruler after 2 d of treatment as described by Borsani et al. (2001). The only modification was in the experiments with low K^+, in which case the root tip was marked 2 d after transfer, and growth was measured after 3 d.

Electrophysiological Experiments

To investigate K^+ transport at low K^+, wild-type and *tss1* seeds were surface sterilized, germinated in distilled water, and grown for 6 d in K^+-deficient assay medium containing 10 mM MES-Bis Tris propane and 1 mM NaCl (pH 5.7). To study the effect of Ca^{2+} on K^+ uptake at low K^+, this medium was supplemented with 1 or 5 mM $CaCl_2$. To analyze the effect of NH_4^+ on K^+ uptake, 1 mM NH_4Cl was added to the assay media described above.

Membrane potentials (E_m) were measured using the standard glass microelectrode technique (Felle, 1981; Fernández et al., 1999). Excised roots were mounted in a Plexiglas chamber (1.1-mL volume). Continuous perfusion of the assay medium was maintained at a flux of approximately 10 mL min^{-1}. Epidermal root cells were impaled with single barrel microelectrodes inserted into root hairs approximately 5 to 10 mm from the apex, as described previously (Borsani et al., 2001). Increasing K^+ concentrations ranging from 0.1 μM to 10 mM were added sequentially to characterize the K^+ uptake in wild-type and *tss1* root cells.

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