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Electrostatic changes in root plasma membrane of glycophytic and halophytic species of tomato☆

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Abstract

We investigated the effect of salt stress on the electrostatic properties of plasma membrane vesicles obtained from both the glycophytic, cultivated tomato, *Lycopersicon esculentum* (Mill, cvs. Heinz-1350 and VF 36) and the halophytic, wild species, *L. cheesmanii* (Hook, C.H. Mull, ecotype 1401) grown under control and saline conditions to determine if there exists a difference in the modulation of the membrane surface charge between the glycophytic and halophytic tomato. In agreement with earlier findings, fluorescence titration of vesicles indicated that salinity stress resulted in the modulation of the plasma membrane surface potential to more positive values in the glycophytic tomato species. However, a much smaller shift was measured in the halophytic tomato. Membrane surface potentials (Ψ_0) of −20.0 and −7.6 mv were calculated for control and salt-stressed vesicles from the glycophytic Heinz-1350 cultivar, respectively, and −22.0 and −3.3 mv, respectively, from the glycophytic VF 36 cultivar. For the halophytic species, Lc-1401, we measured membrane surface potentials (Ψ_0) of −24.8 and −19.7 mv for control and salt-stressed vesicles, respectively. BTP-Cl^- stimulation of ΔpH was greater in vesicles isolated from control roots obtained from the wild Lc-1401 as compared with the cultivated Heinz-1350. However, the reverse was true in vesicles isolated from salt-stressed roots. Cl^- stimulated proton pumping to a greater degree in vesicles from Heinz-1350 than Lc-1401. Both K^+ or Na^+ (added as K^+- or Na^+-Mes along with 100 mM BTP-Cl^-) stimulated H^+-pumping activity in plasma membrane vesicles from both Heinz-1350 and Lc-1401. In most cases, K^+ stimulation of H^+-pumping activity was higher in vesicles isolated from salt-stressed than from non-stressed plants.

Keywords: *Lycopersicon esculentum*; *L. cheesmanii*; Tomato; Plasma membrane; Surface charge; Salinity

1. Introduction

In an earlier study on salt-induced changes in plasma membrane function, we measured salinity-induced changes in plasma membrane electrostatic properties. Fluorescence titration of vesicles with 8-anilino-1-napthalenesulphonic acid (ANS) indicated that membrane surface potentials (Ψ_0) shifted from −26.0 mv in controls to −13.7 mv in salt-stressed membranes of the glyophytic, cultivated tomato, Heinz-1350 [1]. These findings suggested that changes in membrane surface potential might be an adaptive mechanism of glycophtic species and that halophytic species might respond differently.

It is generally accepted that one important aspect of salt tolerance involves the processes limiting net ion uptake across the plasma membrane to a rate compatible with the capacity to compartmentalize Na^+ and Cl^- in the vacuole [2,3]. Current models suggest that the removal of Na^+ from the cytoplasm may occur in some plants via an energy-dependent Na^+/H^+ antiporter [4–6]. Cl^- would then efflux electrophoretically through an outward rectifying channel [7]. A working model of salt tolerance must integrate not only the transport of ions across the plasma membrane and tonoplast of different cell types, but also the biophysical and biochemical processes involved in regulating these fluxes.

At the molecular level, the regulatory mechanisms involved in salt adaptation in plants are presently under

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intensive investigation ([8] and the references cited therein). Recent findings indicate that responses to salt stress in a normally sensitive plant may be mediated by the expression of specific gene(s) or enzymes [9–14]. In experiments using transgenic plants [15], it has been shown that tolerance to salinity stress can be imparted to tobacco plants by expression of a gene involved in the synthesis of the polyol, mannitol. Other recent studies support the view that changes in metabolism may confer salt tolerance to plants [16–20].

Niu et al. [3] suggested earlier that regulating ion fluxes across the plasma membrane to a rate that allows for adequate vacuolar compartmentation is an important aspect of salinity adaptation. Support for this idea comes from observations using salt-adapted tobacco plants, in which significant tolerance to saline stress was correlated to the sequestration of Na^+ and Cl^- in the vacuole and low concentrations of the same ions in the cytosol [2]. Therefore, any mechanism regulating net ion influx at either the plasma membrane and the tonoplast would, presumably, be important in salinity tolerance.

With this in mind, we investigated the effect of salt stress on the electrostatic properties of the plasma membrane [1,21–23] vesicles obtained from both the glycophytic, cultivated tomato, *Lycopersicon esculentum* (Mill, cv. Heinz-1350), and the halophytic, wild species, *L. cheesmanii* (Hook, C.H. Mull, ecotype 1401), grown under control and saline conditions in order to determine if there exists a difference in the modulation of the membrane surface charge between the glycophytic and halophytic tomato which may affect the regulation of net ion influxes. Additionally, to ascertain if the isolation method employed would alter the results, we used a different membrane isolation procedure, the aqueous polymer two-phase technique rather than the two-step sucrose gradient method used by Suhayda et al. [1]. Finally, we included another cultivated variety, VF-36, in our study to determine if the observed shift in membrane surface potential to more positive values was consistent among glycophytic cultivars.

Our measurements of membrane surface potential from the glycophytic tomatoes are in agreement with our earlier findings and indicate that salt stress induces changes in the electrostatic properties of the plasma membrane, rendering them more positive. However, our results with the halophytic, wild species *L. cheesmanii*, indicate that the more tolerant plant has little change in electrostatic properties. We suggest that the capacity to minimize changes in membrane surface charge in response to saline conditions may play a role in salt tolerance of the halophyte.

2. Materials and methods

2.1. Plant material

Two glycophytic, cultivated tomato varieties, *L. esculentum* Mill., cv. Heinz-1350 and VF-36, and the halophytic, wild tomato, *L. cheesmanii*, ecotype 1401, were germinated in vermiculite for 1 week and then placed into aerated solution culture (0.5× Hoagland solution, pH 6.0). All plants were maintained in a controlled-environment chamber under a 30 °C day/25 °C night temperature cycle and a 16 h photoperiod at a light intensity of 250 $\mu E\ m^{-2}\ s^{-1}$. Salt stress was applied 10 days after transplanting at a rate of 0.1 MPa day^{-1} (15 mM NaCl, 3 mM $CaCl_2$) for four consecutive days resulting in a 72 mM applied salt stress at a Na:Ca ratio of 5:1 as described by Suhayda et al. [1]. Experimental plants were 35 days old.

2.2. Membrane isolation

Plasma membrane-enriched vesicles were isolated using the aqueous two-phase technique described by Larsson [24]. Briefly, tomato roots were harvested and immediately washed (two times) in ice-cold grinding buffer. The buffer-washed roots (1 g roots:1.5 ml buffer) were homogenized in an Omnimixer[1] at maximum speed for 3 × 20 s. The grinding buffer consisted of 40 mM Hepes-KOH, pH 7.8, 250 mM sucrose, 3 mM EDTA, 0.65% (w/v) polyvinylpyrrolidone, 5 mM ascorbic acid, 3.6 mM cysteine, 1 mM PMSF (dissolved in 95% ethanol), and 2.5 mM DTT, and 0.5% BSA. The homogenate was filtered through eight layers of cheesecloth and then centrifuged at 10,000 × *g* for 10 min. The supernatant was centrifuged at 85,000 × *g* for 40 min. The resulting microsomal pellet was gently resuspended in buffer containing 5 mM K_2HPO_4, 5 mM KH_2PO_4, 3 mM KCl, 330 mM sucrose, 1 mM PMSF, and 2.5 mM DTT, pH 7.8. Plasma membrane vesicles were prepared by partitioning of the microsomal fraction in 7.8% dextran–polyethylene glycol two-phase system, using a three-step batch procedure. The microsomal suspension (2 × 1.0 ml) was added to two 10 g phase mixtures to give an 11 g phase system and partitioned as described by Widell et al. [25] to the U_3 fraction. The phase mixture consisted of 7.8% Dextran T-500 and 7.8% PEG-3350 in 50 mM potassium phosphate buffer, 3 mM KCl, pH 7.8. Plasma membrane vesicles obtained from the U_3 fraction were washed twice by dilution and resuspended in buffer containing 1 mM BTP-Mes, 250 mM sucrose and 10% glycerol, pH 7.0, and isolated by centrifugation at 100,000 × *g* for 2 h. All solutions were kept at either 4 °C or on ice. After washing, no proteolytic enzyme activity was detected using the diffusion plate method of Gallagher et al. [26] (data not shown). Protein concentrations were determined using the colorimetric assay of Peterson [27]. Membrane vesicles were either used immediately or stored in liquid N_2 for up to 1 week for the following assays.

2.3. Measurement of surface potential

The measurements of membrane surface potential were performed using the method of Gibrat et al. [23] as modified by Suhayda et al. [1]. Plasma membrane vesicles were

titrated with the anionic, fluorescent probe 8-anilino-1-naphthalene-sulphonate (ANS) from Kodak. Excitation and emission wavelengths for the probe were 360 and 480 nm, respectively. Cutoff filters were used to minimize scattered light. The titrations were made in a solution containing 75 μg/3 ml in a buffered system consisting of 25 mM BTP-Mes (pH 7.0), 250 mM sorbitol by successive additions of ANS (5–5000 μM) to the stirred vesicle suspension. Data were recorded by signal averaging over 2 min after allowing 1 min for dye equilibration. The optical density of the dye plus sample was kept at less than 0.05 to minimize inner filter effects [28].

2.4. Measurement of ΔpH

The pH gradient formation experiments were performed using the methods of Giannini and Briskin [29] and Suhayda et al. [1] with some modifications. Proton transport activity in plasma membrane vesicles was measured in the presence of different salts by the quenching of quinacrine fluorescence using a SLM 8000C spectrofluorometer at 25 °C with excitation and emission wavelengths set at 430 and 500 nm, respectively. The assay buffer contained 25 mM BTP-Mes (pH 7.0), 250 mM sorbitol and the following: 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), and 50 mM monovalent ions, when present. Quinacrine was present for all assays at a final concentration of 2.5 μM. Fluorescence quenching assays were quantitated by determining the initial rate of quench (%Q min^{-1} mg $protein^{-1}$), or by ionophore reversible quench at the steady state (%Q mg $protein^{-1}$) determined after the addition of 5 μM gramicidin [30].

3. Results

3.1. Effect of salt stress on membrane surface potential

Suhayda et al. [1] demonstrated that root cells from glycophytic tomatoes modulate the electrostatic properties of the plasma membrane in response to high salt. The present experiments were designed to investigate the effect of salt stress on membrane surface charge of tomato root cells from a halophytic species. We used a different membrane isolation procedure, the aqueous polymer two-phase technique rather than the two-step sucrose gradient method used by Suhayda et al. [1], to determine if the isolation method employed would alter the results.

In agreement with the response obtained from 'Heinz-1350' using the two-step sucrose gradient method, salt stress shifted the membrane surface potential in plasma membrane vesicle derived from salt-stressed roots to more electro-positive values compared with control roots (Table 1). We measured surface potentials (Ψ_0) of −7.0 and −20.0 mv for membranes isolated from salt-stressed and control roots, respectively. Using the same cultivar, Suhayda et al. [1] reported values of −13.7 and −26.0 mv for membranes isolated from salt-stressed and control, respectively. In our case, salt stress reduced Ψ_0 by 62%. Both isolation methods yielded similar results.

We included another cultivated variety, VF-36, in our study to determine if the observed shift in membrane surface potential to more positive values was consistent among glycophytic cultivars. As was the case in Heinz-1350, plasma membrane vesicles from control VF-36 displayed similar values (Ψ_0 = −20.0 versus −22.0 mv, respectively) and responded similarly to salt-stress by shifting the membrane surface potential to more electro-positive value. However, the VF-36 membrane vesicles derived from salt-stressed roots reduced the membrane surface potential by 85%, consistently displaying slightly more electro-positive values compared with Heinz-1350.

Since it appears that salt stress affects root membranes from glycophtytic tomatoes by shifting the membrane surface potential to more electro-positive values, we next investigated the effect of salt stress on the halophytic, wild tomato *L. cheesmanii*. The measured membrane surface potential from control roots of *L. cheesmanii* was measured as −24.8 mv. This was in the same range as the two *L. esculentum* cultivars. In sharp contrast to the glycophytic tomatoes, we were able to measure only a small shift in

Table 1
Surface potential-dependent bulk ion concentrations ($C_{surface}$) at the surface of control and salt-stressed plasma membranes

	Surface potential	$[C^+]$ (mol m^{-3})[a]	$[C^{++}]$ (mol m^{-3})[b]	$[A^-]$ (mol m^{-3})[a]
L. esculentum (Heinz-1350)				
Control	−20.0 ± 5.7	108.8	23.7	23.0
Salt-stressed	−7.6 ± 3.8	67.2	9.0	37.2
L. esculentum (VF-36)				
Control	−22.0 ± 4.8	117.6	27.7	21.3
Salt-stressed	−3.3 ± 2.5	56.8	6.5	44.0
L. cheesmanii (Lc-1401)				
Control	−24.8 ± 2.2	131.1	34.4	19.1
Salt-stressed	−19.7 ± 2.8	107.5	23.2	23.3

Surface potential values equals mean ± S.E.M.; $N = 5$.
[a] Calculated for bulk ion concentration of 50 mol m^{-3} using the Boltzman equation.
[b] Calculated for bulk ion concentration of 5 mol m^{-3} using the Boltzman equation.

Table 2
Effect of Cl^- on ΔH in tomato root plasma membrane vesicles

	Control %Q mg protein^{-1}	Salt-stressed %Q mg protein^{-1}
L. esculentum (Heinz-1350)		
Mg ATP[a]	75	74
25 mol m^{-3} Cl^-	85	143
50 mol m^{-3} Cl^-	106	187
100 mol m^{-3} Cl^-	116	224
L. cheesmanii (Lc-1401)		
Mg ATPa	58	49
25 mol m^{-3} Cl^-	86	79
50 mol m^{-3} Cl^-	101	99
100 mol m^{-3} Cl^-	118	151

[a] Mg-ATP assays were carried out in the presence of 250 mol m^{-3} sorbitol, 3.75 mol m^{-3} ATP (BTP salt, pH 6.5), 3.75 mol m^{-3} $MgSO_4$, 25 mol m^{-3} BTP-Mes (pH 6.5), and 100 μg of membrane protein. Fluorescence quenching assays were quantitated by determining the ionophore reversible quench at steady state (%Q mg protein^{-1}) determined after the addition of 5 mmol m^{-3} gramicidin.

Table 3
Effect of K^+ or Na^+ on ΔH in tomato root plasma membrane vesicles

	Control %Q mg protein^{-1}	Salt-stressed %Q mg protein^{-1}
L. esculentum (Heinz-1350)		
Mg-ATP[a]	75	83
25 mol m^{-3} K^+	99	145
50 mol m^{-3} K^+	105	157
100 mol m^{-3} K^+	119	224
25 mol m^{-3} Na^+	68	102
50 mol m^{-3} Na^+	91	106
100 mol m^{-3} Na^+	101	112
L. cheesmanii (Lc-1401)		
Mg ATP[a]	58	49
25 mol m^{-3} K^+	101	112
50 mol m^{-3} K^+	111	123
100 mol m^{-3} K^+	146	175
25 mol m^{-3} Na^+	111	118
50 mol m^{-3} Na^+	121	124
100 mol m^{-3} Na^+	151	163

[a] Mg-ATP assays were carried out in the presence of 250 mol m^{-3} sorbitol, 3.75 mol m^{-3} ATP (BTP salt, pH 6.5), 3.75 mol m^{-3} $MgSO_4$, 25 mol m^{-3} BTP-Mes (pH 6.5), and 100 μg of membrane protein.

membrane surface potential in vesicles obtained from the salt-stressed roots ($\Psi_0 = -19.7$ mv).

If Ψ_0 values from control and salt-stressed plasma membranes are known, one can estimate the ion concentration at the membrane surface using the Boltzman equation [31]:

$$C_{\text{surface}} = C_{\text{bulk}} \exp\left(-\frac{ZF\Psi_0}{RT} \right)$$

where Z is the valency of the ion, F the Faraday constant, C_{bulk} the bulk ion concentration, and R and T stand for the universal gas constant and temperature, respectively. Calculations for a bulk concentration of 50 mM monovalent cations and anions, and 5 mM divalent cations are shown in Table 1. In the case of the cultivated varieties, salinity was calculated to have reduced monovalent and divalent cations at the surface of Heinz-1350 by 38 and 62%, respectively, and 52 and 77%, respectively, for VF-36. In contrast, monovalent anion concentrations would increase by 61 and 207%, for Heinz-1350 and VF-36, respectively.

However, the results for *L. cheesmanii* were much less dramatic. For the halophytic tomato, the concentration of monovalent and divalent cations at the surface of the salt-stressed membranes would be reduced by only 18 and 33%, respectively, while the monovalent anion concentrations would increase by only 22%.

3.2. Effect of salt stress on ΔpH

Since the Boltzman equation predicts an increase in the surface concentration of monovalent anions, we decided to further our investigations by examining the effect of Cl^- on proton pumping. Table 2 illustrates the effect of increasing concentration of BTP-Cl^- on ΔH^+ in tomato-root plasma membrane vesicles. In both the cultivated and wild species of tomato, Cl^- stimulation above the level of Mg-ATP increased with increasing concentrations of BTP-Cl^-. In *L. esculentum* the percent stimulation of ΔpH by Cl^- addition was 13% for 25 mM, 41% greater for 50 mM, and 55% greater for 100 mM Cl^- in vesicles from control plants. With vesicles from salt-stressed plants, the percent stimulation of ΔpH by Cl^- addition was 93% for 25 mM, 253% greater for 50 mM, and 303% greater for 100 mM Cl^-. In *L. cheesmanii*, the percent stimulation of ΔpH by Cl^- addition was 48% for 25 mM, 74% greater for 50 mM, and 203% greater for 100 mM Cl^- in vesicles from control plants. With vesicles from salt-stressed plants, the percent stimulation of ΔpH by Cl^- addition was 61% for 25 mM, 202% greater for mM, and 308% greater for 100 mM Cl^- (Table 2).

In contrast to anions, the Boltzman equation predicts a decrease in monovalent and divalent cations. Therefore, we examined cation stimulation of the Mg-ATPase. In these experiments, we held Cl^- concentrations constant at 100 mM using BTP-Cl^- and added various concentrations of K^+- or Na^+-Mes. In order to determine if BTP or Mes had any effect on proton transport, we compared the effect of 100 mM KCl and 100 mM K^+-Mes + 100 mM BTP-Cl^-. In agreement with Giannini and Briskin [29], our transport assay showed no difference between the two additions indicating that these two buffers did not differentially affect proton transport in our vesicles (data not shown). Based on percent quenching, both K^+ or Na^+ stimulated H^+-pumping activity in plasma membrane vesicles from both tomato species (Table 3). In most cases, K^+ stimulation of H^+-pumping activity was higher in root membrane vesicles isolated from salt-stressed than from non-stressed plants. For example, stimulation of quenching due to 100 mM K^+ was

170% higher in salt-stressed Heinz-1350 vesicles, but only 60% higher in vesicles isolated from non-stressed plants.

Na^+-Mes added with 100 mM BTP-Cl^- was also found to stimulate proton pumping above the levels measured with 100 mM BTP-Cl^- alone in vesicles from salt-stressed tissue. However, any comparison with K^+-Mes is complicated by the possible presence of a Na^+/H^+ antiport mechanism operating in the plasma membrane [6].

4. Discussion

In an earlier investigation [1], we reported changes in the electrostatic properties of the plasma membrane of a glycophytic tomato during salt adaptation. In the present study, we investigated the changes in membrane surface charge of plasma membrane vesicles isolated from both the cultivated glycophytic tomato (*L. esculentum*, cv. Heinz-1350 and VF-36) and its wild halophytic relative (*L. cheesmanii*). In agreement with earlier findings, salinity stress caused a dramatic shift in membrane surface potential to a more electro-positive value. We measured this salinity-induced shift of the electrostatic plasma membrane potential in both glycophytic cultivated varieties examined. Examination of the wild species, *L. cheesmanii*, indicated that the membrane surface potential of this halophytic plant changed only slightly. Based on measurement of surface charge alone, it appears that the membranes of the halophyte are more resistant to alterations brought about by changes in salinity.

Consistent with our observations on membrane surface charge were our findings that in salt-stressed vesicles, BTP-Cl^- stimulation of proton pumping was greater in the Heinz-1350 plasma membrane vesicles as compared with Lc-1401. These findings are in agreement with the prediction by the Boltzman equation that a larger positive shift in membrane surface potential would attract more monovalent anions to the membrane surface and possibly increase their likelihood of transport.

In the case of K^+ or Na^+ stimulation of proton pumping, the situation is less clear. We found that K^+ stimulation of H^+-pumping activity was generally higher in root membrane vesicles isolated from salt-stressed than from non-stressed plants. This should not be the case given the prediction by the Boltzman equation. One possibility is that salt stress may be affecting the amount of ATPase present in the plasma membrane or the amount of membrane proteins other than ATPase. Either case would result in a change in the ATPase-specific activity. Other investigators reported have found that NaCl treatment induced plasma membrane H^+-ATPase mRNA accumulation [3,32,33]. Alternatively, the membrane surface potential may only be a part of a complex set of regulatory mechanism affecting the plasma membrane H^+-ATPase. In addition, Na^+-Mes added with 100 mM BTP-Cl^- also stimulated proton pumping in vesicles from salt-stressed tissue above the levels measured with 100 mM BTP-Cl^- alone. However, any comparison with K^+-Mes is complicated by the possible presence of a Na^+/H^+ antiport mechanism operating in the plasma membrane [6]. If such an antiporter were operating, the observed quenching rate in the presence of Na^+ would be an underestimate of the true value due to the simultaneous efflux of H^+ from the vesicles via the antiporter.

It is useful to point out that with respect to *L. esculentum*, our findings obtained by measuring proton pumping are consistent with those reported earlier by this laboratory using ATP hydrolysis rates (6). This earlier work reported an increase in both ATP hydrolysis in the plasma membranes of both cultivated and wild species of tomato grown under saline conditions. Further, this previous work indicated that both K^+ and Na^+ stimulated Mg-ATP hydrolysis. As to *L. cheesmaii*, salinity treatment also increased proton gradient formation. However, K^+ and Na^+ stimulated Mg-ATP hydrolysis (6) was much more dramatic than indicated by the proton gradient formation data presented here (Table 3).

An interesting outcome of this study was the reasonably close agreement of the present data obtained from vesicles using the aqueous polymer two-phase isolation technique with those gathered earlier by the sucrose-density gradient method [1]. This agreement was noted for vesicles obtained from roots grown under both control and salt-stressed conditions. The aqueous polymer two-phase technique has been reported to produce plasma membrane preparations of very high purity [34]. Our data indicate that either method should produce similar results with respect to the electrostatic properties of the plasma membrane. In addition, we observed that membrane vesicles isolated from roots of salinized tomato plants had a slightly higher degree of latency compared with non-salinized controls. We found this difference in both glycophytic varieties as well as *L. cheesmanii*, although the difference in the wild species was smaller.

It is unclear from our work exactly what the consequence of a shift in the membrane surface charge to a more positive value would be with respect to the regulation of ion homeostasis. One possibility of a large shift in plasma membrane surface potential would be a decrease in the surface concentration of monovalent and divalent cations and an increase in divalent anions [1]. Thus, a more positive membrane surface would, presumably attract more anions thereby increasing their likelihood of transport. Conversely, the attraction of monovalent cations would diminish as would their transport. Equally important, the monovalent cation stimulation of the ATPase would decrease also. However, this interpretation of the data is far from certain. An alternative explanation might be that the ions attracted by these surface charges might remain in the neighborhood of these fixed charges and thus, be prevented from entering some channel and subsequent transport. However, data presented by Shannon et al. [35] on Cl^- content of roots and leaves in various tomato species grown under saline conditions do not support this view.

It might also be possible that the observed changes in surface potential is a sign of other important changes in

membrane function. For example, the idea that membrane surface charge is involved in the operation of ion channels was first investigated by Frankenhauser and Hodgkin [36] who concluded that Ca^{2+} binds to the external membrane surface and the removal of Ca^{2+} from the external solution shifts the squid axon Na^+ channel's activation curve towards a more negative transmembrane voltage. Later, Bell and Miller [37] demonstrated by reconstituting sacroplasmic reticulum K^+ channels into artificial membranes of differing surface charge densities that K^+ conduction through this channel does respond to the lipid surface charge. Additionally, Moczydlow et al. [35] concluded that both K^+-conduction and Ca^{2+} activation sense the lipid surface charge. Their observed effects were interpreted by an increase in the local K^+ and Ca^{2+} concentration in the vicinity of the conduction and activation sites as predicted by the Gouy-Chapman double-layer theory.

In the case of Na^+ uptake into plant cells, we now know that Na^+ can act as a competitor of K^+ influx [38,39]. Additionally, Schachtman et al. [40] indicated that Na^+ uptake can occur via outward-rectifying channels. Thus, the shifting of the membrane surface potential to more electropositive values may serve an adaptive mechanism in the cultivated tomato. It is possible that the decrease in surface concentrations of Na^+ could decrease the open probability of these outward-rectifying channels and, thereby, decrease Na^+ influx into the cell down its electrochemical potential. Such would not be the case with *L. cheesmanii*. However, the presence of an active Na^+/H^+ antiporter would, presumably, limit net intracellular uptake of Na^+ [6].

In conclusion, our present results agree with our earlier findings that the membrane surface potential of glycophytic tomato roots is modulated toward a more positive value as a result of salinity stress. However, our data with the halophytic species indicate that the magnitude of this shift is much greater for the glycophytic cultivated varieties than for the wild species. The capacity of *L. cheesmanii* to maintain the membrane surface potential to a more negative value may be a salt tolerance determinant of the halophytic tomato, although the reason for this is unclear based on the presented data. Whatever the case, it is possible that our observed changes in surface potential might be an important hint to still unknown mechanisms of adaptation.

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References

[1] C.G. Suhayda, J.L. Giannini, D.P. Briskin, M.C. Shannon, Electrostatic changes in *Lycopersicon esculentum* root plasma membrane resulting from salt stress, Plant Physiol. 93 (1990) 471–478.

[2] M.L. Binzel, F.D. Hess, R.A. Bressan, P.M. Hasegawa, Intracellular compartmentation of ions in salt adapted tobacco cells, Plant Physiol. 86 (1988) 607–614.

[3] X. Niu, R.A. Bressan, P.M. Hasegawa, J.M. Pardo, Ion homeostasis in NaCl environments, Plant Physiol. 109 (1995) 735–742.

[4] J. Garbarino, F.M. DuPont, NaCl induces a Na^+H^+ antiport in tonoplast vesicles from barely roots, Plant Physiol. 86 (1988) 231–236.

[5] L. Reinhold, Y. Braun, M. Hassidim, H.R. Lerner, The possible role of various membrane transport mechanisms in adaptation to salinity, in: J. Cherry (Ed.), Biochemical and Physiological Mechanisms Associated with Environmental Stress Tolerance, Springer-Verlag, Berlin, 1989, pp. 121–130.

[6] C. Wilson, M.C. Shannon, Salt-induced Na^+/H^+ antiport in root plasma membrane of a glycophytic and halophytic species of tomato, Plant Sci. 107 (1995) 147–157.

[7] M. Tester, Plant ion channels, whole-cell and single-cell channel studies, New Phytologist 114 (1990) 305–340.

[8] J.-K. Zhu, P.M. Hasegawa, R.A. Bressan, Molecular aspects of osmotic stress in plants, Crit. Rev. Plant Sci. 16 (1997) 253–277.

[9] E.A. Bray, Molecular response to water deficit, Plant Physiol. 103 (1993) 1035–1040.

[10] E.J. DeRocher, H.J. Bohnert, Development and environmental stress employ different mechanism in the expression of a plant gene family, Plant Cell 5 (1993) 1611–1625.

[11] H. Hayashi, H. Alia, L. Mustardy, P. Deshnium, M. Ida, N. Murata, Transformation of *Arabidopsis thaliana* with the codA gene for choline oxidase; accumulation of glycinebetaine an enhanced tolerance to salt and cold stress, Plant J. 2 (1997) 133–142.

[12] R.D. Locy, C.-C. Chang, B.L. Nielsen, N.K. Singh, Photosynthesis in salt-adapted heterotrophic tobacco cells and regenerated plants, Plant Physiol. 110 (1996) 321–328.

[13] P.S. Summers, E.A. Weretilnyk, Choline synthesis in spinach in relation to salt stress, Plant Physiol. 103 (1993) 1269–1276.

[14] J.C. Thomas, H.J. Bohnert, Salt stress perception and plant growth regulators in the halophyte *Mesembryanthemum crystallinum*, Plant Physiol. 103 (1993) 1299–1304.

[15] M.C. Tarczynski, R.G. Jensen, H.J. Bohnert, Stress protection of transgenic tobacco by production of the osmolyte mannitol, Science 259 (1993) 508–510.

[16] G.A. Gilbert, C. Wilson, M.A. Madore, Root-zone salinity alters raffinose oligosaccharide metabolism and transport in *Coleus*, Plant Physiol. 115 (1997) 1267–1276.

[17] G.A. Gilbert, M.V. Gadush, C. Wilson, M.A. Madore, Amino acid accumulation in sink and source tissue of *Coleus blumei* Benth. during salinity stress, J. Exp. Bot. 49 (1998) 107–114.

[18] P.B. Kavi Kishor, Z. Hong, G.H. Miao, C.-A.A. Hu, D.P. Verma, Overexpression of Δ-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants, Plant Physiol. 108 (1995) 1387–1394.

[19] G. Lilius, N. Holmberg, L. Bülow, Enhanced NaCl stress in transgenic tobacco expressing bacterial choline dehygrogenase, Biotechnology 14 (1996) 177–180.

[20] E.A.H. Pilon-Smits, M.J.M. Ebskamp, M.J. Paul, M.J.W. Jeuken, P.J. Weisbeek, S.C.M. Smeekens, Improved performance of transgenic fructan-accumulating tobacco under drought stress, Plant Physiol. 107 (1995) 125–130.

[21] V.C.K. Chiu, D. Mouring, B.D. Watson, D.H. Haynes, Measurement of surface potential and surface charge densities of sarcoplasmic reticulum membranes, J. Membr. Biol. 56 (1980) 121–132.

[22] R. Gibrat, J.P. Grouzis, J. Rigaud, C. Grigon, Electrostatic characteristics of corn root plasmalemma, effect of Mg^{2+}-ATPase activity, Biochim. Biophys. Acta 816 (1985) 349–357.

[23] R. Gibrat, C. Romieu, C. Grigon, A procedure for estimating the surface potential of charged or neutral membranes with 8-anilino-1-naphthalene-sulphonate probe, Biochim. Biophys. Acta 736 (1983) 196–202.

[24] C. Larsson, Plasma membranes, in: H.F.D Linskens (Ed.), Modern Methods of Plant Analysis (New Seriers), Cell Components, vol. 1, Springer-Verlag, Berlin, 1985, pp. 85–104.
[25] S. Widell, T. Lundborg, C. Larsson, Plasma membranes from oats prepared by partition in an aqueous polymer two-phase system, Plant Physiol. 70 (1982) 1429–1435.
[26] S.R. Gallagher, E.J. Carroll Jr., R.T. Leonard, A sensitive diffusion plate assay for screening inhibitors of protease activity in plant cell fractions, Plant Physiol. 81 (1985) 869–874.
[27] G.L. Peterson, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem. 83 (1977) 346–356.
[28] J. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, 1983.
[29] J.L. Giannini, D.P. Briskin, Proton transport in plasma membrane and tonoplast vesicles from red beet (*Beta vulgaris* L.) storage tissue, Plant Physiol. 84 (1987) 613–618.
[30] D.P. Briskin, W.R. Thornley, R.E. Wyse, Membrane transport in isolated vesicles from sugarbeet taproot. I. Isolation and characterization of energy-dependent, H^+-transporting vesicles, Plant Physiol. 78 (1985) 865–870.
[31] B.H. Honig, W.L. Hubbell, R.F. Flewelling, Electrostatic interactions in membranes and proteins, Ann. Rev. Biophys. Biophys. Chem. 15 (1986) 163–193.
[32] X. Niu, M.L. Narasimhan, R.A. Salzman, R.A. Bressan, P.M. Hasegawa, NaCl regulation of plasma membrane H^+-ATPase gene expression in a glycophyte and a halophyte, Plant Physiol. 103 (1993) 713–718.
[33] X. Niu, J.-K. Zhu, M.L. Narasimhan, R.A. Bressan, P.M. Hasegawa, Plasma-membrane H^+-ATPase gene expression is regulated by NaCl in cells of the halophyte *Atriplex nummularia* L., Planta 190 (1993) 433–438.
[34] C. Larsson, S. Widell, P. Kjekkbom, Preparation of high-purity plasma membranes, in: L. Packer, R. Douce (Eds.), Methods in Enzymology, Plant Cell Membranes, vol. 148, Academic Press, New York, 1987, pp. 558–568.
[35] M.C. Shannon, J.W. Gronwald, M. Tal, Effects of salinity on growth and accumulation of organic and inorganic ions in cultivated and wild tomato species, J. Am. Soc. Hortic. Sci. 112 (1987) 416–423.
[36] B. Frankenhauser, A.L. Hodgkin, The action of calcium on the electrical properties of squid axons: determination of surface charge, J. Physiol. 137 (1957) 218–244.
[37] J.E. Bell, C. Miller, Effects of phospholipid surface charge on ion conduction in the K^+ channel of sarcoplasmic reticulum, Biophys. J. 45 (1984) 279–287.
[38] J.I. Schroeder, J.M. Ward, W. Gassmann, Perspectives on the physiology and structure of inward-rectifying K^+ channels in higher plants: biophysical implications for K^+ uptake, Ann. Rev. Biophys. Biomol. Struct. 23 (1994) 441–471.
[39] A.A. Watad, M. Reuveni, R.A. Bressan, P.M. Hasegawa, Enhanced net K^+ uptake capacity of NaCl-adapted cells, Plant Physiol. 95 (1991) 1265–1269.
[40] D.P. Schachtman, S.D. Tyerman, B.R. Terry, The K^+/Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species, Plant Physiol. 97 (1991).