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In vivo ^{23}Na and ^{31}P NMR measurement of a tonoplast Na^+/H^+ exchange process and its characteristics in two barley cultivars

(Na^+/H^+ antiport/intracellular pH/energy phosphates/salt tolerance/roots)

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ABSTRACT **A Na^+ uptake-associated vacuolar alkalinization was observed in roots of two barley cultivars (Arivat and the more salt-tolerant California Mariout) by using ^{23}Na and ^{31}P *in vivo* NMR spectroscopy. A NaCl uptake-associated broadening was also noted for both vacuolar P_i and intracellular Na NMR peaks, consistent with Na^+ uptake into the same compartment as the vacuolar P_i. A close coupling of Na^+ with H^+ transport (presumably the Na^+/H^+ antiport) *in vivo* was evidenced by qualitative and quantitative correlations between Na^+ accumulation and vacuolar alkalinization for both cultivars. Prolongation of the low NaCl pretreatment (30 mM) increased the activity of the putative antiport in Arivat but reduced it in California Mariout. This putative antiport also showed a dependence on NaCl concentration for California Mariout but not for Arivat. No cytoplasmic acidification accompanied the antiporter activity for either cultivar. The response of adenosine phosphates indicated that ATP utilization exceeded the capacity for ATP synthesis in Arivat, but the two processes seemed balanced in California Mariout. These comparisons provide clues to the role of the tonoplast Na^+/H^+ antiport and compensatory cytoplasmic adjustments including pH, osmolytes, and energy phosphates in governing the different salt tolerance of the two cultivars.**

Though not halophytes, some barley (*Hordeum vulgare* L.) cultivars are more salt tolerant than are many other crop plants (1–3). Vacuolar Na^+ accumulation, part of the response to salt stress (4, 5), is probably mediated through a Na^+/H^+ antiport that uses the pH gradient between the vacuole and cytoplasm established by means of a H^+-ATPase (6). Evidence consistent with the scheme, including the H^+-ATPase and Na^+/H^+ antiporter activities, has been obtained from isolated tonoplast vesicles of barley roots (7–10) and other plant tissues (11–13). In addition, the barley Na^+/H^+ antiport was shown in isolated tonoplast vesicles to be activated under short- and longer-term NaCl pretreatment (9, 14). The relevance of these *in vitro* findings for plant responses to salt stress needs to be clarified by observation of this process *in vivo*.

One inevitable compensatory consequence of the Na^+/H^+ exchange process at the tonoplast is at least a transient build-up of H^+ in the cytoplasm, which needs to be dissipated for normal functioning of the cytoplasm. The cytoplasm also requires separate osmotic adjustment, Na^+ being unsuitable for osmoregulation there (4). In both cases, the "energy" metabolism of the plant will likely be impacted and represent a limiting factor for salinity responses (15, 16). As with the Na^+/H^+ antiport itself, coordination among compartments is a key feature of the biochemistry accompanying the antiporter activity, hence the need for *in vivo* experimental approaches that maintain tissue integrity.

We have applied the *in vivo* NMR approach to the salt-sensitive barley cultivar Arivat and the more tolerant California Mariout (CM). The advantages of NMR as an noninvasive tool are well documented (17). For salinity studies of plants in particular, ^{23}Na and ^{31}P NMR is well suited for monitoring Na^+ transport and its impact on intracellular pH and energy metabolism. There already have been several such demonstrations (13, 18–20). The two barley cultivars differing in salt tolerance afforded the opportunity to examine whether the characteristics of their Na^+/H^+ antiport and cytoplasmic adjustments have any bearing on their difference in salt tolerance. Here, we present *in vivo* evidence for the operation of a tonoplast Na^+/H^+ exchange process in intact roots of these two barley cultivars, as monitored by ^{23}Na and ^{31}P NMR. We also provide preliminary evidence for differences in kinetic properties of this process. Finally, we describe effects of this process on cytoplasmic pH and phosphate metabolites.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Field-grown seeds of the two barley cultivars, Arivat and CM, were germinated in a growth chamber at 25°C and 60% relative humidity. The medium was an aerated 10% modified Hoagland solution (21) plus 0.1 mM NaCl. The solution was replenished after 4 days and changed the day before the experiment to a solution without the micronutrients, except for boron. The seedlings (6–7 days old) were kept in the dark until the roots were excised and placed in the NMR tubes.

NMR Experiments. The NMR instruments used were a Nicolet model NT-200 and NT-360 for the ^{23}Na and ^{31}P measurements, respectively.

A superfusion procedure was used for both *in vivo* NMR measurements. Mature root sections (≈4 cm) were excised from 6- to 7-day old etiolated seedlings and superfused in 12-mm NMR tubes (Wilmad) with a coaxial superfusion apparatus (22). The base medium (low NaCl treatment) contained 10 mM sodium acetate, 20 mM glucose in 10% modified Hoagland solution minus micronutrients except for boron (pH 6.0). For the high NaCl treatment, the NaCl concentration of the base medium was raised by 100 mM. The flow rate was adjusted to 15 ml/min, and the sample temperature was maintained at 25°C.

The high NaCl treatment protocol for the ^{23}Na NMR experiments involved three durations (96, 206, and 425 min) of low NaCl pretreatment in the base medium/2 mM $Na_{10}Dy(P_3O_{10})_2$, followed by the 100 mM NaCl increment; for the ^{31}P NMR experiments, pretreatment in the base medium was ≈200 min before high NaCl treatment was initiated. Extended low NaCl treatment for a duration comparable to

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Abbreviations: CM, California Mariout; Glc-6-*P*, glucose 6-phosphate; Na_i, intracellular Na.

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that for the high NaCl treatment was also done as a control for ^{31}P NMR measurements.

Each ^{23}Na NMR spectrum was averaged over 512 transients and acquired at 52.9 MHz using a 90° pulse (33.5 μs), 0.5-s interpulse delay, ±1000 Hz sweep width, and 2048 sampling points, resulting in 8.7 min of acquisition time per spectrum. The longitudinal relaxation time (T_1) for the intracellular ^{23}Na (Na_i) signal was determined to be 50.5 ms for Arivat and 52.1 ms for CM by using an inversion recovery pulse sequence. The 0.5-s interpulse delay was thus sufficient to fulfill a nonsaturating condition allowing fully relaxed ^{23}Na spectra to be obtained in all cases. An external standard of 0.5 M NaCl/50 mM $Na_{10}Dy(P_3O_{10})_2$ in a capillary was included as peak area reference in all ^{23}Na NMR experiments. Relative Na_i peak areas were obtained by normalizing the Na_i peak areas to that of the external standard and then correcting for run-to-run differences in tissue volumes. To accomplish this, superfusion was stopped, and two ^{23}Na NMR spectra (256 transients each) were acquired, one with and the other without root tissue. The extracellular Na peak areas from each spectrum were normalized against that of the external capillary standard and used to calculate the % vol occupied by tissue in the NMR probe sensing region (20, 23). All Na^+ accumulation rates were measured from a linear regression fit from plots of relative Na_i peak area vs. time.

The ^{31}P NMR spectra were recorded at 145.7 MHz with a 30–40° pulse (5–7 μs), a 231-ms recycle time, ±4000 Hz sweep width, and 2048 data points. Each spectrum was an average of 10,240 transients for 39.44 min. A polyethylene capillary tube containing 0.5 M methylene diphosphonate in Tris buffer (pH 8.9) was arranged coaxially with the root sample to serve as a reference at 0 ppm. The P_i and glucose 6-phosphate (Glc-6-*P*) standard titration curves were established by using a standard aqueous mixture containing 10 mM each of Glc-6-*P*, P_i, phosphocreatine, phosphoarginine, ATP, and $MgCl_2$ plus 100 mM KCl, titrated to various pH values between 4.5–9.0. Vacuolar and cytoplasmic pH were determined by comparing the chemical shifts of the vacuolar P_i and cytoplasmic P_i (or Glc-6-*P*) with those from the standard titration curves.

RESULTS

Na^+ Transport and Compartmentalized pH Shifts. Representative ^{23}Na NMR spectra of excised barley roots acquired before and at various times after the raise by 100 mM NaCl are shown in Fig. 1 for the Arivat cultivar. The spectral positions (chemical shifts) and separation between the two ^{23}Na signals were constant throughout all experiments, consistent with the exclusion of the shift reagent (Dy^{3+}) (see *Discussion*) from barley root cells. While the peak areas of extracellular Na remained constant throughout a given treatment, those of the Na_i in all treatments progressively increased, indicating transport of Na^+ ions into the root cells (compare Figs. 1 and 2). The CM cultivar exhibited qualitatively similar spectral characteristics and Na^+ accumulation (Fig. 2 and data not shown).

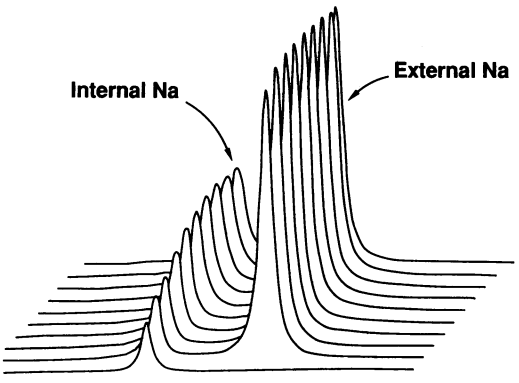

FIG. 1. *In vivo* ^{23}Na NMR spectra of barley Arivat roots. Each spectrum was processed with a 10 Hz line broadening and corresponded to 10.8 min of acquisition time. $Na_{10}Dy(P_3O_{10})_2$ (2 mM) was added 1.5 hr after superfusion in the base buffer; 0.5 hr thereafter the NaCl concentration was raised by 100 mM. The spectra from bottom to top correspond to 11 min before addition of the shift reagent and 16, 71, 163, 314, 465, 617, 768, 919, and 1070 min after the raise by 100 mM NaCl, respectively. The peaks at left represent the unshifted Na_i signals, whereas those at right represent the shifted external Na signals.

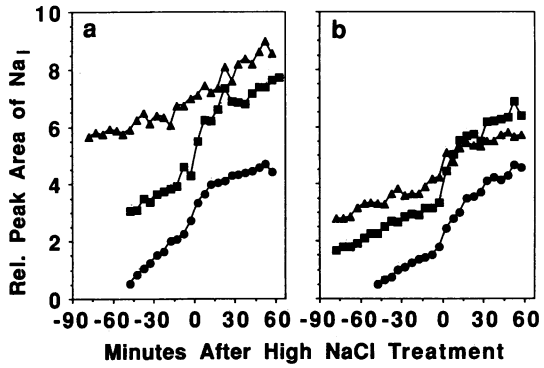

FIG. 2. Time courses of Na^+ accumulation into Arivat and CM roots. Relative (rel.) peak areas of Na_i were determined as described. Each time point corresponded to 8.7 min of acquisition time. Shift reagent was added at the start of superfusion, and high NaCl treatment began after three different periods of low NaCl pretreatment (●, 96 min; ■, 206 min; and ▲, 425 min). (*a*) Arivat roots; (*b*) CM roots.

Coincident with Na^+ accumulation under both low (30 mM) and high NaCl (130 mM) conditions, a progressive increase in the line width at half height of the Na_i peak was always observed for both cultivar roots (see Table 1). This broadening behavior was not observed in the extracellular Na peak, indicating that this phenomenon was specific to the Na_i peak and was not due to systematic instrument artifacts.

Peak area analyses of spectra such as those in Fig. 1 yielded time courses of Na^+ accumulation into Arivat and CM roots, as illustrated in Fig. 2. The three curves for each cultivar

Table 1. Time-dependent broadening of Na_i and vacuolar P_i line widths of two barley cultivars Arivat and CM

Time after 100 mM NaCl increment,* min	Line width, $\nu_{1/2}$, Hz†	
	Arivat	CM
Na_i		
−325		12.8
−110	16.6	17.6
−4	19.4	18.8
58	19.5	21.2
101	20.2	21.8
Vac P_i		
−161	68	
−138		82
459	108	
496		127

Vac P_i, vacuolar P_i peaks.

*For the ^{23}Na experiment, the preparation was superfused in the base buffer containing 20 mM glucose, 10 mM NaOAc, and 2 mM $Na_{10}Dy(P_3O_{10})_2$, followed by increase of 100 mM NaCl; for the ^{31}P experiment, the same protocol was used, except that the shift reagent was omitted.

†Line width at half height was determined according to the Lorentzian line-fit routine.

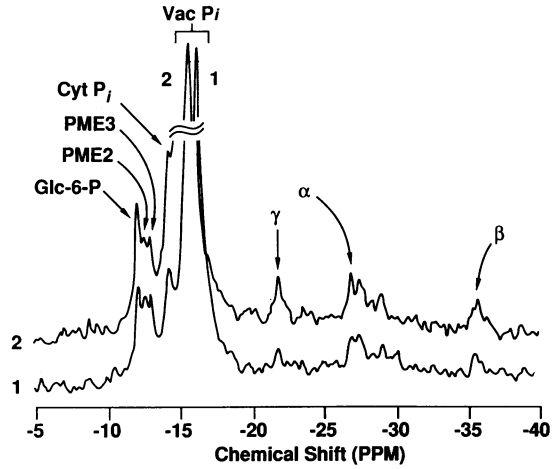

FIG. 3. *In vivo* ^{31}P NMR spectra of NaCl-treated Arivat roots. NaCl concentration was raised by 100 mM 165 min after start of superfusion. Each spectrum corresponded to 158 min of acquisition time, and chemical shifts were referenced to methylene diphosphonate (0 ppm). Spectrum 1 was taken under low NaCl conditions, whereas spectrum 2 represented an average of 5.5–8.0 hr after the raise by 100 mM NaCl. A line broadening of 30 Hz was included in processing both spectra. The vacuolar P_i (Vac P_i) peaks were plotted offscale for clear presentation of other metabolite peaks, with the top of the Vac P_i peaks included to illustrate their alkaline shift under high NaCl conditions. Cyt P_i, cytoplasmic P_i; PME, phosphomonoester; α, attributed to [αATP + αADP + αAMP]; γ, attributed to [γATP + βADP]; and β attributed to [βATP].

correspond to three different durations of low NaCl pretreatment, after which the NaCl concentration was raised by 100 mM at t_0. In all pretreatments, Na^+ accumulation rates were constant within the experimental error. After the NaCl concentration was raised, rates increased for ≈15 min and then continued at a different steady value.

The effects of NaCl on intracellular pH were also investigated *in vivo*, using ^{31}P NMR spectroscopy. Typical ^{31}P NMR spectra of Arivat roots taken at low NaCl concentration (spectrum 1) and after the raise by 100 mM NaCl (spectrum 2) are shown in Fig. 3. One notable spectral change consistently induced by the high NaCl treatment was a downfield shift of the vacuolar P_i peak (top of Fig. 3); a similar change also occurred under extended low NaCl treatment (Fig. 4*a*), indicating alkalinization of the vacuole. In contrast, the cytoplasmic pH probably did not change significantly with NaCl treatments as inferred from the position of the cytoplasmic P_i peak. The CM roots gave qualitatively similar spectra (data not shown) and vacuolar pH response to NaCl treatments (Fig. 4*a*).

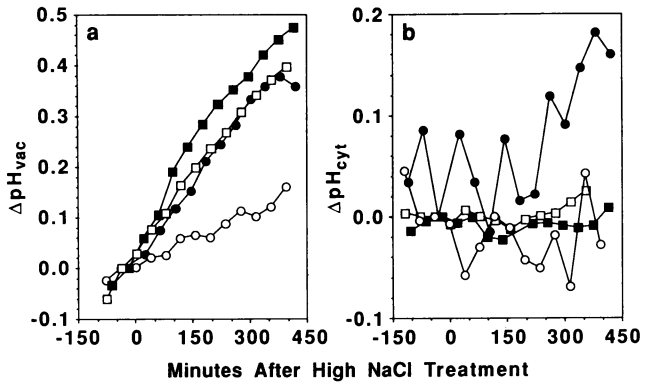

FIG. 4. Time course changes in intracellular pH of NaCl-treated barley roots. The vacuolar and cytoplasmic pH were determined as described. High NaCl treatment was the same as in Fig. 3, except for a 200-min low NaCl pretreatment period (■, Arivat; ●, CM; extended low NaCl treatment served as the control (□, Arivat; ○, CM). Each time point for the Arivat experiment was a sliding average of three files (119 min of acquisition time), whereas that for the CM experiment represented a single file (39.44 min of acquisition time). Vacuolar pH change (ΔpH_{vac}) (*a*) and cytoplasmic pH change (ΔpH_{cyt}) (*b*) for the high NaCl treatment were calculated by subtracting the pH value just before the 100 mM NaCl increment from each individual value along the time course; those changes for low-NaCl-only treatment were similarly calculated by subtracting the (t_0) pH value from each subsequent one over treatment time.

One other change in Fig. 3 was an increase in the line width at half height of the vacuolar P_i peak that accompanied the vacuolar alkalinization (see Table 1), which was akin to that seen for the Na_i peak under similar conditions. This finding is consistent with Na^+ accumulation primarily into the same compartment as vacuolar P_i. Such broadening phenomenon did not show up in the cytoplasmic P_i peak, whose line width was inherently smaller than that of the vacuolar P_i peak before the NaCl concentration was raised (data not shown).

Comparison of Salinity-Induced Responses Between Cultivars. Despite the qualitatively similar NaCl-elicited Na^+ accumulation and vacuolar alkalinization in both cultivars, several quantitative differences were seen. As Table 2 shows, the average initial Na^+ accumulation rate by Arivat roots during low NaCl pretreatment was nearly twice that by CM roots. The before NaCl line in Table 2 shows that in both cultivars Na^+ accumulation rates declined as pretreatment progressed. However, when switched to high NaCl treatment (Table 2, after NaCl), Na^+ accumulation rates for Arivat increased with longer pretreatment but decreased for CM. Thus, as the after NaCl/before NaCl ratio shows, Arivat responded to delayed high NaCl treatment with ever larger changes of Na^+ accumulation rate, while no such trend was evident with CM. This result occurred despite the higher Na_i level reached by Arivat during pretreatment (Table 2, relative Na_i level).

Differences between the cultivars in the intracellular pH response involved mainly the vacuolar compartment. The high

Table 2. Na^+ accumulation rates vs. relative Na_i levels of two barley cultivars under three different pretreatment durations

	Na^+ accumulation linear rate × 10^2, min^{-1}		
	96 min*	206 min*	425 min*
Arivat			
Initial† = 5.00 ± 0.50‡			
Before NaCl§	4.50	3.10	1.50‖
After NaCl¶	1.80	2.90	3.20‖
After NaCl/before NaCl	0.40	0.94	2.13
Relative Na_i level**	2.71	4.29	6.97
CM			
Initial† = 2.70 ± 0.01‡			
Before NaCl§	2.70	2.10	1.70‖
After NaCl¶	3.80	3.40	1.40‖
After NaCl/before NaCl	1.41	1.62	0.82
Relative Na_i level**	1.78	3.34	4.24

*Time in min of low NaCl pretreatment in base buffer/2 mM $Na_{10}Dy(P_3O_{10})_2$.

†Time when low NaCl pretreatment began.

‡Linear regression fit of the first 10 data points from Na^+ accumulation curves; each value is an average of two measurements.

§1.5–2 hr (10–14 data points) before the increase by 100 mM NaCl.

¶1.5–2 hr (10–14 data points) after the increase by 100 mM NaCl.

‖Linear regression fit of 10–14 data points in the linear regions of Fig. 2 (−80–0 and 15–60 min for before and after the increase by 100 mM NaCl, respectively); each value is a single measurement.

**Normalized and tissue volume-corrected (see *Materials and Methods*) area of the Na_i peak immediately before the increase by 100 mM NaCl.

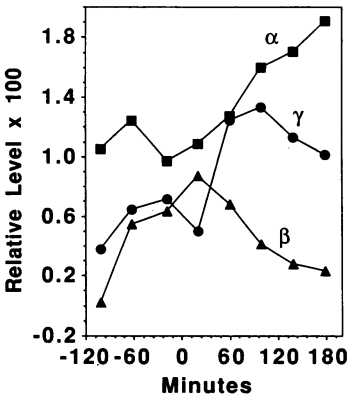

FIG. 5. Time courses of adenosine phosphates in high-NaCl-treated Arivat roots. High NaCl treatment constituted superfusion in the base buffer for 218 min, followed by the 100 mM NaCl increment (t_0). Relative levels were calculated by normalizing peak intensities to that of the external standard (methylene diphosphonate). Labels α, β, and γ correspond to those in Fig. 3.

NaCl treatment for both cultivars yielded similar rates of vacuolar alkalinization (Fig. 4*a*). Yet comparison of high and low NaCl treatments shows that the rate of vacuolar pH change (ΔpH_{vac}) differed by $\approx$ 2-fold in CM, but no difference was evident in Arivat roots. Lastly, the initial rate of ΔpH_{vac} in Arivat was nearly twice that in CM roots (Fig. 4*a*), consistent with the nearly 2-fold greater initial Na^+ accumulation rates for Arivat roots during the same interval. Thus, there was a positive correlation between the magnitude of alkaline ΔpH_{vac} and Na^+ accumulation rates. As for the cytoplasmic pH, no significant changes were seen except for a small alkaline shift for CM roots under high NaCl treatment (Fig. 4*b*).

In addition to the above, comparisons in the responses of phosphate metabolites between the cultivars were made. Because NMR spectroscopy reveals only the "free" metabolites, changes in the peak intensities of a given metabolite could represent, partly, changes in its mobilization. The spectral assignments yielding discrete chemical identities were made according to Jackson *et al.* (24). The adenosine phosphate peaks labeled α, γ, and β were attributed to [αATP + αADP + αAMP], [γATP + βADP], and [βATP], respectively. All three peaks from both cultivar roots increased in intensity after either high NaCl (e.g., for Arivat, as shown in Fig. 3) or extended low NaCl treatments (data not shown). Similar changes have been reported in mature corn roots (25) and in corn root tips treated with 160 mM NaCl (19).

Although the β peak intensity showed a net increase, a transient reduction was noted during the first 100 min of the high NaCl treatment for Arivat only (Fig. 5), but the γ and α peak intensities continued to rise, indicating that the levels of ADP, AMP, or both increased, whereas that of ATP declined in Arivat. Beyond 100 min, the α peak still rose, though the β and γ peaks both declined, suggesting that the AMP level increased, while the ATP level continued to decline and the ADP level either declined or stabilized. In all other experiments, the increase in intensities plus the constant ratios of adenosine phosphate peaks (data not shown) indicated that a rise in the ATP level alone was primarily responsible.

DISCUSSION

The permeability of the plant cell plasma membrane to the shift reagent dysprosium, and the contribution of the cell wall component to the Na_i peak were discussed in previous studies (18–20). In the plant systems investigated, dysprosium did not appear to traverse the plasma membrane, and the Na_i peak contained little contribution from the cell wall. In our experiments, a constant spectral separation was maintained between the dysprosium-shifted and -unshifted Na peaks, supporting the conclusion that the internal spaces of barley root cells were inaccessible to dysprosium. Thus, the unshifted and shifted peaks in Fig. 1 correspond to the Na_i and extracellular Na signals, respectively. The Na_i signal should comprise the sum of those from all dysprosium-inaccessible spaces, presumably dominated by the cytoplasm and vacuole.

Paramagnetic ions (e.g., Mn^{2+}) have been reported to be sequestered in root vacuoles and to result in selective broadening of the NMR signals of vacuolar components (26). Our finding of an inherently broader line width of the vacuolar P_i than of the cytoplasmic P_i peak is consistent with that report. The further broadening of both Na_i and vacuolar P_i peaks observed as Na^+ accumulation progressed is inexplicable at present. Two possibilities exist: (*i*) alterations in the exchange dynamics between the mobile Na^+ or P_i and the less mobile macromolecules, or (*ii*) mobilization of otherwise bound paramagnetic ions. In either case, the common broadening of the Na_i and vacuolar P_i peaks suggests that Na^+ is transported into the same compartment as the P_i—the vacuole.

For Na^+ transport into the vacuole, experiments with isolated tonoplast vesicles from barley roots suggest that a Na^+/H^+ antiport is involved (7, 9, 14). We presented two pieces of supporting *in vivo* evidence. (*i*) Na^+ influx into the root cells was consistently accompanied by vacuolar alkalinization (Figs. 3 and 4*a*). (*ii*) Changes in the Na^+ accumulation rates paralleled changes in the vacuolar alkalinization rates for both barley cultivars (Table 2 and Fig. 4*a*). Such coupled Na^+/H^+ exchange has also been reported in isolated tonoplast vesicles of several other plant species (6, 11) and in algae (27), but not in corn root tips under NaCl treatment (19).

Differences in the properties of the Na^+/H^+ antiport of the two cultivars may bear on their salt tolerance or sensitivity. One notable difference was the dependence of this antiporter activity during high NaCl treatment on starting Na_i level (Table 2). The significance of this difference for NaCl tolerance mechanisms of the cultivars is unclear. One scenario could be that roots of the more tolerant CM accumulate Na^+ as an osmoticum if challenged with NaCl stress at low Na_i level, whereas at higher Na_i level, they utilize other means of osmoregulation, such as synthesis of organic osmolytes. Conversely, the less tolerant Arivat roots rely more on Na^+ for osmotic adjustment, which leads to increasing activation of the antiport with higher starting Na_i level and, hence, increased sequestration of Na^+ in the vacuole.

If salinity tolerance of the cultivars depends, in part, on the activation control of their tonoplast antiporters, the compensatory processes in their cytoplasms must also be considered. One of the consequences of the large H^+ efflux from the vacuole should be, at least, a transient Brønsted–Lowry (protic) acid load in the cytoplasm. In this study, the apparent cytoplasmic pH was either stable (Arivat roots) or possibly became slightly *alkaline* (CM roots), so the cytoplasm has adequate means for regulating its pH. Adapting from a recent review of short-term regulation of pH in plants (28), four—rather than three listed by the review—cytoplasmic mechanisms seem to be applicable—namely, (*i*) immediate H^+ extrusion to the medium, (*ii*) an increase in the protic buffering capacity, (*iii*) "metabolic buffering" by H^+-consuming reactions, or (*iv*) some combination of the above. For the first, a Na^+/H^+ antiport at the plasma membrane (29) could be responsible for removing H^+ to the medium. Alternatively, the organism may regulate its cytoplasmic pH by synthesis of buffer species with neutral pK_a values or by activating H^+-consuming reactions. The latter, which falls under the Usanovich definition of acid–base equilibria (30), may also contribute to the former. Moreover, the products of these two processes could simultaneously serve as osmolytes

in the cytoplasm. Cytoplasmic pH regulation by means of ethanol and lactate production in hypoxic plant tissues is well documented (22, 25, 31, 32). Whether these fermentation processes also operate under salinity stress awaits further investigation.

Regardless of the response mechanisms discussed above, the energetics in the two cultivar roots were impacted by NaCl treatment. In Arivat, the high NaCl treatment elicited an energy demand exceeding the capacity of ATP production, as implied from the transient decline of ATP and increase in the ADP and AMP levels. As for CM, the lack of change in the ADP and AMP levels suggests that ATP production kept pace with its utilization. Probably in both cases a major portion of the ATP consumed is for cytoplasmic pH regulation and production of organic osmolytes. As for Na^+ uptake, the main driving force may come from existing Na^+ and H^+ gradients across the plasma and tonoplast membranes. A similar process has been shown in the activation of unfertilized sea urchin eggs (33, 34).

In conclusion, we have presented *in vivo* evidence for the operation of a tonoplast Na^+/H^+ antiport in mature roots of two barley cultivars, Arivat and the more salt-tolerant CM by using a combination of ^{23}Na and ^{31}P NMR monitoring. Comparison between the two cultivars revealed differences in the property of this antiport as well as in the associated cytoplasmic pH regulation and energy metabolism. These differences were detected under NaCl concentration regimes that may be expected in saline soil environments. Such information provides clues to the role of the tonoplast antiport and compensatory cytoplasmic adjustments in governing the different salt tolerance of the two cultivars. In a broader sense, the reported lack of vacuolar alkalinization in the even more sensitive corn root tips (19) may further test the relevance of these processes to *general* salt tolerance mechanisms. This line of inquiry should warrant further investigation by comparing salt-sensitive and tolerant plants, both of closely related genotypes and of widely disparate species.

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