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ORIGINAL ARTICLE

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Cellular and whole-plant chloride dynamics in barley: insights into chloride–nitrogen interactions and salinity responses

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Abstract The first analysis of chloride fluxes and compartmentation in a non-excised plant system is presented, examining ten ecologically pertinent conditions. The short-lived radiotracer couple $^{38}Cl/^{39}Cl$ was used as a Cl^- tracer in intact barley (*Hordeum vulgare* L. cv. Klondike) seedlings, which were cultured and investigated under four external [Cl^-], from abundant (0.1 mM) to potentially toxic (100 mM). Chloride–nitrogen interactions were investigated by varying N source (NO_3^- or NH_4^+) and strength (0.1 or 10 mM), in order to examine, at the subcellular compartmentation level, the antagonism, previously documented at the influx level, between Cl^- and NO_3^-, and the potential role of Cl^- as a counterion for NH_4^+ under conditions in which cytosolic [NH_4^+] is excessive. Cytosolic [Cl^-] increased with external [Cl^-] from 6 mM to 360 mM. Cl^- influx, fluxes to vacuole and shoot, and, in particular, efflux to the external medium, also increased along this gradient. Efflux reached 90% of influx at the highest external [Cl^-]. Half-times of cytosolic Cl^- exchange decreased between high-affinity and low-affinity influx conditions. The relationship between cytosolic [Cl^-] and shoot flux indicated the presence of a saturable low-affinity transport system ('SLATS') responsible for xylem loading of Cl^-. N source strongly influenced Cl^- flux to the vacuole, and moderately influenced Cl^- influx and shoot flux, whereas efflux and half-time were insensitive to N source. Cytosolic pool sizes were not strongly or consistently influenced by N source, indicating the low potential for Cl^- to act as a counterion to hyperaccumulating NH_4^+. We discuss our results in relation to salinity responses in cereals.

Keywords Compartmental analysis · Efflux · *Hordeum* · Ion transport · Salinity · Translocation

Abbreviations $[Cl^-]_{cyt}$ cytosolic chloride concentration · $[Cl^-]_o$ external chloride concentration

Introduction

Chlorine is an essential micronutrient for plants, although it often accumulates in soils to levels much higher than required for optimal plant growth. In field soil water, it is typically found at concentrations of 1–5 mM (Eaton 1966), and in some cases [Cl^-] can reach phytotoxic levels, particularly in coastal environments, such as salt marshes, where it can exceed 800 mM (Jefferies et al. 1983). Given the increasing occurrence of saline conditions in soils of agricultural and natural ecosystems, it is not surprising that salt tolerance in plants has become a major research concern (Zhu 2001; Apse and Blumwald 2002). However, much of the recent focus in this regard has been on the transport and accumulation characteristics of sodium, while the role of chloride, its frequently occurring counterion, has been relatively ignored. Little is known concerning the primary acquisition mechanisms of Cl^- by plants, and knowledge about its subcellular distribution and flux dynamics is scarce and afflicted by controversy. For example, Cram (1983) proposed that accumulation and influx of Cl^- are homeostatically maintained by an "error-actuated feedback system", a mechanism later questioned by Glass and Siddiqi (1985) and Deane-Drummond (1986). Another proposition invoked the flux to the shoot as a major regulator of influx across the plasma membrane of roots (Drew and Saker 1984), a

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relationship that is important given that Cl^- accumulation in the shoot appears to be a major determinant of Cl^- sensitivity in plants (Greenway 1965; Downton and Millhouse 1983). The elucidation of mechanisms of Cl^- toxicity also requires knowledge of the subcellular localization and quantification of Cl^- pools; for instance, one may ask whether the toxic effect is due primarily to the osmotic effect of high $[Cl^-]$ in the cell walls (Oertli 1968), or to Cl^- accumulation in the cytosol, where it can affect protein synthesis (Gibson et al. 1984) and enzyme activity (Flowers et al. 1977; Gimmler et al. 1984). In general, there appears to be no consensus regarding either the regulation of Cl^- uptake (Flowers 1988; Xu et al. 2000), or the mechanisms of Cl^- toxicity. This is partly due to much previous work on this subject having been performed using excised roots (Elzam et al. 1964; Macklon 1975; Jacoby and Rudich 1980; Cram 1983; Felle 1994), which is problematic in that that there are pronounced differences in Cl^- fluxes between these systems and roots of intact plants (Collins and Abbas 1985; also see White and Broadley 2001). Also problematic is a scarcity of studies that have used intact plants growing with complete nutrient solutions, or varied steady-state chloride regimes. The present work seeks to redress this lack, by using compartmental analysis with the short-lived radiotracer couple $^{38}Cl/^{39}Cl$ in intact barley plants, to study whole-plant Cl^- fluxes and compartmentation under a wide range of ecologically realistic conditions.

In particular, we were interested in the influences that external $[Cl^-]$ ($[Cl^-]_o$), and the source and external concentration of inorganic nitrogen, exert on chloride fluxes and compartmentation. It is well established that the presence of nitrate (NO_3^-) in soils can ameliorate the toxic effect of excess Cl^- (Bar et al. 1997), ostensibly due to the suppressive effect NO_3^- has on Cl^- fluxes into the plant (Glass and Siddiqi 1985), and on Cl^- accumulation in plant tissues (Kafkafi et al. 1982; Adler and Wilcox 1995). Similarly, increased NaCl sensitivity and tissue accumulation under NH_4^+ nutrition (Speer et al. 1994) may be due to the increased Cl^- flux observed with this N form (Sanders 1984; Peuke et al. 1998). We had previously demonstrated that barley plants, when challenged with toxic external concentrations of NH_4^+, displayed very high fluxes, and accumulated very high concentrations of this ion within the cytosolic compartment of root cells (Britto et al. 2001); however, the chemical nature of the counterion(s) required to bring about electroneutrality in that compartment were not investigated. The present study was designed in part to test the hypothesis that Cl^- could accumulate cytosolically to provide sufficient negative charge under high-NH_4^+ regimes. Finally, we wanted to investigate Cl^- fluxes and compartmentation under high (100 mM) $[Cl^-]_o$, at which growth reduction in glycophytes, including barley, is known to become pronounced (White and Broadley 2001). In particular, we hypothesized that, under this condition, barley roots might be unable to prevent this ion from entering the plant in excessive quantities, and could thus display the type of very high, toxicity-related, fluxes, which are seen with NH_4^+ in barley (Britto et al. 2001).

Materials and methods

Plant culture

Seeds of barley (*Hordeum vulgare* L. cv. Klondike) were surface-sterilized for 15 min in 1% sodium hypochlorite and germinated under acid-washed sand for 3 days. Seedlings were then transferred to 8-l hydroponic tanks for a growth period of 4 days prior to experimentation, in controlled-environment chambers set at a 16 h/8 h light/dark cycle, 20 ± 2°C temperature, 70% relative humidity, and approximately 250 μmol m^{-2} s^{-1} photon flux at plant level provided by fluorescent tubes with a spectral composition similar to sunlight (Vita-Lite Duro-Test, Fairfield NJ, USA). Solutions in hydroponic tanks contained aerated 0.25-strength modified Johnson's solution, with NH_4^+ provided as $(NH_4)_2SO_4$ or NO_3^- as $Ca(NO_3)_2$, at pH 6. NH_4^+ or NO_3^- concentrations in solution were 0.1 mM or 10 mM, while Cl^- was varied from 0.1 to 100 mM (see Fig. 2 for the ten nutritional permutations used) by variable addition of $CaCl_2$.

Isotope preparation

The chlorine isotopes ^{38}Cl ($t_{1/2} = 37.2$ min) and ^{39}Cl ($t_{1/2} = 55.6$ min) were produced by irradiation of natural argon gas with 41 MeV protons accelerated by the CP42 cyclotron located at the Tri-University Meson Facility (TRIUMF) in Vancouver, Canada. A water-cooled aluminum target body of 75 cm^3 volume was filled with approximately 250 psi (1.7 MPa) of Ar gas and irradiated with protons at currents between 5 and 7 μA. A typical run at a current of 5 mA for 30 min yielded approximately 555 MBq of Cl radioactivity, as measured 15 min following irradiation. The Cl isotopes adhered to the walls of the target and were rinsed off with a slightly alkaline aqueous solution after the target gas was released, then caught on a Sepak strong anion-exchange column. The only other radioisotope produced in appreciable quantities was ^{38}K ($t_{1/2} = 7.6$ min), which was removed by passage through the exchange column. The Cl isotopes were eluted from the Sepak with 20 ml of 10 mM $CaSO_4$, and then used immediately for labelling experiments.

Flux experiments

Seedlings were transferred from growth solutions to $^{38}Cl/^{39}Cl$ loading solutions for 60 min, and then attached to efflux funnels, as described elsewhere (Wang et al. 1993). This time period was chosen so that the plants' influx mechanisms could allow extensive radiolabelling of cytosolic compartments of root cells, but was short enough that the labelling of, and potentially sizable effluxes from, the vacuole (Cram 1968; Cram and Laties 1971) and the phloem (Peuke et al. 1998; Zhong et al. 1998; White and Broadley 2001) were minimal. Efflux was measured by desorbing radioactivity from the roots into a timed series of 20-ml aliquots of non-labelled solution. These eluates were collected following the time course 10 s (3 times), 15 s (6 times), 30 s (4 times), 1 min (4 times), 2 min (13 times), yielding a total desorption time of 34 min. Loading and desorption solutions were chemically identical to the growth solutions described above to ensure that the plants were at a nutritional steady state throughout the experimental period. Roots and shoots were separated after desorption was completed, surface water was removed from roots by low-speed centrifugation for 45 s, and the plant organs were then weighed. Radioactivity in roots, shoots, and eluates was counted in a γ-counter (Minaxi δ Auto-γ 5000 series; Canberra–Packard, Mississauga, Ontario, Canada). The combined signal from the two Cl isotopes was

corrected for differential radioactive decay using two simultaneous equations based on the unique decay constants for each isotope, and multiple recounting of high-activity $^{38}Cl/^{39}Cl$ samples. The change in tracer efflux was plotted logarithmically over time (see Fig. 1), and linear regression was used to resolve the slow phase of efflux corresponding to the cytosolic compartment (as distinct from either extracellular or vacuolar phases; see Results and discussion). The exponential rate constant k for this phase (related to the phase half-time by $k = 0.693/t_{1/2}$) was used to determine the extent of cytosolic labelling, and for flux calculations (for calculation details, see Siddiqi et al. 1991, and Kronzucker et al. 2003). In brief, fluxes are denoted by the symbol φ and are in units of μmol Cl^- (g FW root)$^{-1}$ h^{-1}; these fluxes consist of influx from the external solution to the plant (φ_{oc}), efflux from the cytosol to the external solution (φ_{co}), and net flux from the external solution to the plant (φ_{net}). The latter flux consists of a flux from cytosol to vacuole (φ_{vac}), and a flux from root (cytosol) to xylem (φ_{xylem}).

Cytosolic concentrations of Cl^- were derived from unidirectional influxes and cytosolic kinetic exchange constants, according to the flux-turnover equation $[Cl^-]_{cyt} = \Omega\ \varphi_{oc/k}$, where Ω is a proportionality constant accounting for the cytosolic compartment comprising 5% of tissue volume (see Britto and Kronzucker 2003). Experiments were repeated three to eight times.

Statistical treatment

Compoundly exponential efflux plots were parsed into separate phases by a linear regression in which points were added to a line from left to right on the graph, and a new regression was generated with each addition. When the coefficient of determination (r^2) of the line dropped three times with each successive addition, a new phase was considered to have begun.

One-way ANOVA was performed on all data sets, signifying differences among groups. Student's t-test was used to compare means of paired samples of treatments that differed only in nitrogen source, with and without assumptions of heteroscedasticity. When comparing all treatments, the ANOVA was followed by a test of least significant difference (LSD). In all cases, the alpha level was set at 0.05.

The transformations used to derive kinetic parameters in Table 2 were the Lineweaver–Burk ($1/V = 1/V_{max} \cdot 1/[S] + 1/V_{max}$) and Eadie–Hofstee ($V = K_M/V_{max} \cdot 1/[S] + 1/V_{max}$). Regression analysis was performed on the transformed lines to extract the parameters, and coefficients of determination (r^2) are given in the table.

Results and discussion

$^{38}Cl/^{39}Cl$ efflux traces: half-times and compartment identification

Figure 1 shows two representative $^{38}Cl/^{39}Cl$ efflux traces from radiolabelled barley roots, which had been grown under low (0.1 mM) or high (100 mM) $[Cl^-]_o$. The multiphasic nature of these traces was consistently observed in all treatments, and was similar, both in the number of phases, and in their slopes, to efflux traces documented with other ions such as NO_3^-, NH_4^+, and K^+ (see, e.g., Siddiqi et al. 1991; Kronzucker et al. 1995; and Memon et al. 1985, respectively). The mean half-times for the slowest phase (Fig. 1, Table 1) fell into two distinct categories, depending on $[Cl^-]_o$ supply: one of 18–20 min at low $[Cl^-]_o$ (0.1 mM), and the other of 11–14 min at high $[Cl^-]_o$ (≥1 mM). This trend was independent of external nitrogen source or concentration. This phase was attributed to tracer eluted from the collective cytosolic phase of the labelled roots, for several reasons. Most importantly, the half-times for Cl^- exchange for this phase (Table 1) were very similar to those assigned as cytosolic in previous Cl^- efflux analyses, in a wide variety of plant systems (Cram 1968; Hill 1970; Pierce and Higinbotham 1970; Pitman 1971; Binzel et al. 1988; Hajibagheri et al. 1988). Secondly, the range of values found in the present study for cytosolic concentrations of Cl^- ($[Cl^-]_{cyt}$) fell well within the majority of literature values found by efflux analysis (Sanders 1984, and references therein; Binzel et al. 1988), and, more importantly, by other methods such as X-ray microanalysis (Harvey 1985; Seemann and Critchley 1985; Hajibagheri et al. 1988, 1989; Huang and van Steveninck 1989; Flowers and Hajibagheri 2001) and Cl^--selective microelectrodes (Gerson and Poole 1972).

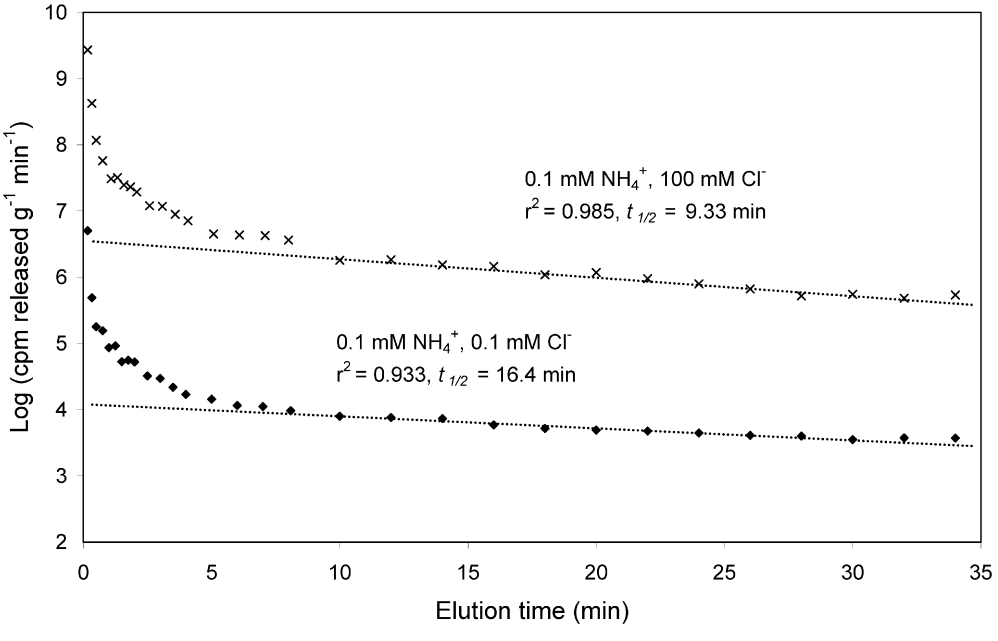

Fig. 1 Representative semi-logarithmic plots of $^{38}Cl^-/^{39}Cl^-$ efflux from radiolabelled roots of intact barley (*Hordeum vulgare*) seedlings, grown and monitored for γ release under two steady-state nutrient regimes. *Dashed lines* represent tracer released from the cytosolic phase of the root cells. Plots have been normalized for specific activity of Cl^- isotopes. Note differences in half-times (derived from slopes of dashed lines) between the two conditions

Table 1 Cytosolic chloride concentrations and half-times of Cl^- exchange in barley (*Hordeum vulgare*) roots. Values in*bold* indicate the shorter half-times associated with low-affinity chloride fluxes.*Superscript letters* indicate significantly differing treatment classes (LSD,$P < 0.05$).*Asterisks* (*) indicate significant differences between treatments with equal N and Cl supply, but differing in N source (Student's *t*-test, $P < 0.05$)

Condition (mM)	$[Cl^-]_{cyt}$ (mM)	Half-time (min)
0.1 NH_4^+/0.1 Cl^-	9.48 ± 0.64^a	18.72 ± 1.81^a
0.1 NO_3^-/0.1 Cl^-	7.99 ± 1.17^a	18.92 ± 0.63^a
10 NH_4^+/0.1 Cl^- *	12.05 ± 1.22^a *	19.22 ± 2.07^a
10 NO_3^-/0.1 Cl^- *	5.67 ± 0.58^a *	19.02 ± 2.28^a
10 NH_4^+/1 Cl^-	21.37 ± 3.84^b	$\mathbf{13.80 \pm 0.49}^b$
10 NO_3^-/1 Cl^-	17.09 ± 1.8^b	$\mathbf{14.07 \pm 1.69}^b$
10 NH_4^+/10 Cl^- *	70.50 ± 11.1^b *	$\mathbf{12.31 \pm 0.76}^b$
10 NO_3^-/10 Cl^- *	36.53 ± 4.3^b *	$\mathbf{11.92 \pm 0.79}^b$
0.1 NH_4^+/100 Cl^-	357.15 ± 31.4^c	$\mathbf{11.14 \pm 1.03}^b$
0.1 NO_3^-/100 Cl^-	333.29 ± 96.9^c	$\mathbf{13.38 \pm 1.46}^b$

The variability of this putative cytosolic phase in response to physiological stimuli (see below) adds additional certitude as to the subcellular nature of the tracer-releasing compartment. Several studies have estimated the half-time for vacuolar Cl^- exchange to be 1–2 orders of magnitude longer than the labelling time used in the present experiments (Hill 1970; Pierce and Higinbotham 1970; Pitman 1971; Macklon 1975, 1976; Cram 1983; Binzel et al. 1988), which indicates that the efflux of tracer from the vacuole will have little, if any, influence on the efflux data presented here. This explains why no deviations from first-order kinetics were observed in the slow phase of our efflux traces.

Fluxes and energetics of Cl^-

Efflux, half-time, and tissue tracer-retention data allowed us to determine the unidirectional components of total Cl^- influx into the root tissue. Figure 2 shows the breakdown of these fluxes under the ten nutritional conditions tested. In general, Cl^- influx into the cytosol, flux from cytosol to vacuole, flux from the root to the shoot, and efflux from the cytosol to the external medium, all increased with increasing $[Cl^-]_o$. Of these, efflux increased to the greatest extent, such that the ratio of efflux to influx strongly increased with $[Cl^-]_o$, approaching unity at the highest $[Cl^-]_o$ (Fig. 2). By contrast, the shoot and, especially, the vacuolar fluxes did not change greatly between 10 and 100 mM $[Cl^-]_o$. This finding agrees with previous studies that indicate that above 10 mM $[Cl^-]_o$, vacuolar fluxes are saturated, while the linear, low-affinity, component of Cl^- influx continues to rise (Cram and Laties 1971; Cram 1973). The increase in efflux in this range of $[Cl^-]_o$, both in absolute terms, and relative to influx, thus reflects a phenomenon in which the influx capacity of the root system is greatly in excess of the capacity of the plant to accumulate chloride. Cl^- flux dynamics thus showed strong similarities to what we have shown previously with NH_4^+ in barley roots, where downregulation of NH_4^+ entry into the plant system was impaired, a situation linked to toxicity of NH_4^+ (Britto et al. 2001). In the latter case, thermodynamic calculations and oxygen-exchange experiments strongly suggested that the toxic flush of NH_4^+ ions through the root system was an energy-demanding process that could contribute to the toxicity of ammonium (Kronzucker et al. 2001). In the

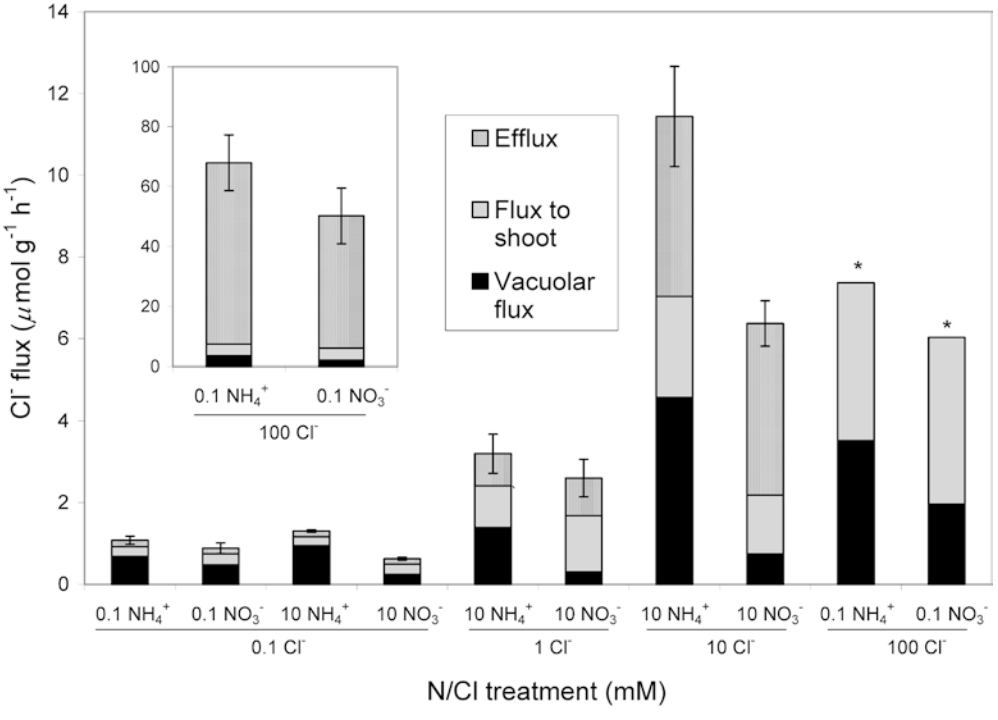

Fig. 2 Breakdown into component fluxes (flux to vacuole, flux to shoot, efflux) of Cl^- influx in roots of intact barley plants, under 10 steady-state nutrient regimes, as determined by $^{38}Cl/^{39}Cl$ tracer analysis. *Asterisks* (*) indicate that the truncated bars in the main figure are shown in full in the inset. Units in inset are as in larger graph. Error bars indicate ± SE of influx means

case of Cl^-, however, influx across the plasma membrane of roots is most likely active even at high external concentrations (given the still higher cytosolic concentrations observed—see below), so the high fluxes seen at these concentrations may be expected to deplete significant amounts of metabolic energy from the plant. However, preliminary investigations have shown that plants under these conditions show no higher respiratory rates than plants grown at a lower $[Cl^-]_o$ (not shown). A possible explanation for this is that the influx process for Cl^- is much more efficient, energetically speaking, than the efflux process for NH_4^+ (Kronzucker et al. 2001); alternatively, it may be that some of the energy liberated in the Cl^- efflux process is conserved, and coupled to Cl^- influx. The mechanisms underlying the energetics of this condition invite further investigation.

In all cases, Cl^- influx at equimolar external N was greater for NH_4^+-grown plants, as compared to NO_3^--grown plants (Fig. 2), although this difference was of high significance ($P < 0.05$) only at 10 mM external [N] and when external $[Cl^-]$ was 0.1 or 10 mM. The effect of N source on Cl^- efflux and flux to the shoot was not generally significant, but N source had a pronounced effect on the Cl^- flux across the tonoplast into the vacuole; this parameter in NH_4^+-grown plants was significantly higher ($P < 0.05$) in all cases except at the highest $[Cl^-]_o$ (Fig. 2). Moreover, this effect was particularly strong when the N supply was high, and it accounted for most of the difference between total plasma-membrane influx values for NH_4^+- vs. NO_3^--grown plants. These large differences in vacuolar fluxes strongly suggest that vacuolar accumulation of Cl^- is subject to substantial variation. Thus, our findings support the proposal by Glass and Siddiqi (1985) that the plant, rather than having a rigidly defined set-point or homeostat for Cl^- accumulation (Cram 1983), may instead exert control over total anionic contents in the vacuole, chiefly the sum of Cl^- and NO_3^- in this instance.

Cytosolic Cl^- pool sizes

Cytosolic chloride pool sizes ($[Cl^-]_{cyt}$) were determined using the well-established method detailed in Lee and Clarkson (1986) and Siddiqi et al. (1991), to provide tissue-averaged values for the entire root system (see Kronzucker et al. 2003 for a discussion on the utility and special merits of such averaging techniques). Like influx, $[Cl^-]_{cyt}$ increased pronouncedly with $[Cl^-]_o$ (Table 1). At both low and high $[Cl^-]_o$, our $[Cl^-]_{cyt}$ estimates are in excellent agreement with results obtained by Cl^--selective microelectrodes (Gerson and Poole 1972) and X-ray microanalysis (Huang and van Steveninck 1989; Flowers and Hajibagheri 2001). NH_4^+-grown plants showed consistently higher $[Cl^-]_{cyt}$, although this difference was of high significance ($P < 0.05$) in only two cases. Moreover, because $[Cl^-]_{cyt}$ was moderate when the external $[NH_4^+]$ was high (10 mM), with values falling far below the very high $[NH_4^+]_{cyt}$ values observed for barley at this external $[NH_4^+]$ (Britto et al. 2001), the hypothesis that Cl^- could act as a cytosolic counterion with NH_4^+, and possibly contribute to ammonium toxicity as a result, was not supported. In general, however, the $[Cl^-]_{cyt}$ values determined by compartmental analysis in the present work agree well with a large number of studies using widely divergent methodologies (see above). Although we report some apparently very high values for $[Cl^-]_{cyt}$ under the threshold-salinity, 100-mM $[Cl^-]_o$, condition, these are in fact lower than some values reported in studies using X-ray microanalysis (e.g. Harvey and Thorpe 1986, who report $[Cl^-]_{cyt}$ to be in excess of 1 M Cl^-). Interestingly, Hajibagheri et al. (1988) found that compartmental analysis gave much higher values for $[Cl^-]_{cyt}$ in maize roots as compared to X-ray microanalysis of the same plant system. These authors attributed this difference to the absence, in their excised root system, of a flux from the cytosol to the shoot; this indicates the importance of using intact systems, as in the present study.

As shown in Fig. 3, the increase in cytosolic $[Cl^-]_{cyt}$ along the external $[Cl^-]$ gradient, when recalculated in terms of the Nernst potential for Cl^- across the plasmalemma, indicates that the energetics for Cl^- accumulation become more favorable (on a Cl^--equivalency basis) with increasing $[Cl^-]_o$. An analysis of a compilation by Sanders (1984) of previously published work on Cl^- fluxes shows that this condition appears to be generally true for transmembrane Cl^- ratios, in a wide variety of higher plant and other systems (Fig. 3, inset). It is important to note, however, that, at the toxicity threshold of 100 mM $[Cl^-]_o$, extraordinarily high bidirectional plasma-membrane Cl^- fluxes are associated with this cytosolic Cl^- accumulation. Based on the present study, and on previous work we have undertaken studying NH_4^+ fluxes and compartmentation, we propose that large bidirectional fluxes and cytosolic hyperaccumulation may be a universal feature of incipient, or ongoing, ion toxicity in plant cells.

Half-time variability of the cytosolic Cl^- pool

The half-times for cytosolic Cl^- exchange, in contrast to $[Cl^-]_{cyt}$, decreased over the Cl^- gradient, falling into two populations of values (Table 1); this indicates that cellular Cl^- dynamics differ in a fundamental way from inorganic N dynamics, which are characterized by the maintenance of constant half-times over a wide range of external N provision (Britto and Kronzucker 2001). The longer and shorter half-times appear to be associated with high- and low-affinity Cl^- fluxes, respectively (Cram and Laties 1971; Cram 1973), and might be shortened, in response to the larger Cl^- fluxes in the high-capacity, low-affinity range, reflecting an attempt by the plant to lower cytosolic Cl^- accumulation (see Britto and Kronzucker 2003 for a discussion of the relationships between influx, half-times, and pool sizes).

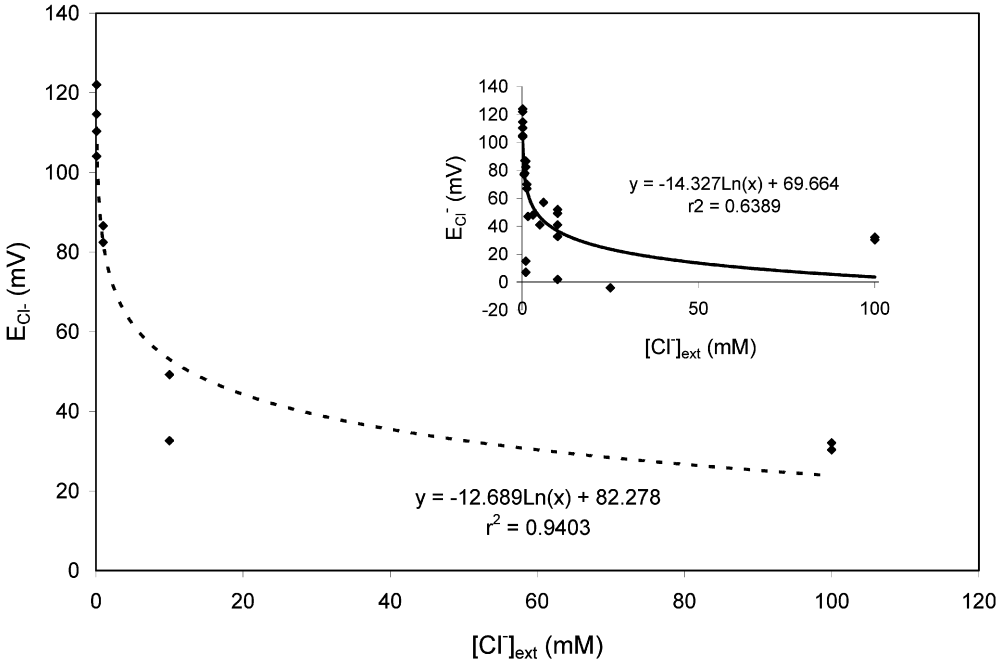

Fig. 3 Nernst potential (E) for passive equilibration of Cl^- across the plasma membrane of barley root cells, as a function of external [Cl^-], and based on cytosolic [Cl^-] as determined using compartmental analysis by $^{38}Cl/^{39}Cl$ efflux. *Inset* Nernst potentials for transmembrane Cl^- distribution plotted from compiled data in Sanders (1984), Fig. 3.1. Logarithmic regression equations, with coefficients of determination (r^2), are given

Mechanism and significance of Cl^- translocation from root to shoot

The cytosolic exchange half-times were used to calculate fluxes to the shoot (see Materials and methods), which can be a significant component of total efflux from the cytosol of root cells (Hajibagheri et al. 1988; Jeschke et al. 1995). The underlying assumption that the cytosolic compartment of xylem-loading cells obeys similar exponential exchange kinetics as other root cells is supported by a study that showed that the half-time of $^{36}Cl^-$ labelling of the glandular exudate of *Limonium vulgare* leaf tissue was identical to the half-time of "cytoplasmic" $^{36}Cl^-$ efflux (Hill 1970; see also Cram 1968). An analysis of cytosolic pool sizes and associated shoot fluxes under the two N regimes revealed that Michaelis–Menten kinetics appear to govern the xylem-loading step of long-distance transport (Table 2). This transport system appears to be unlike most saturable systems, in that the K_M is very high while the V_{max} is quite modest. This novel finding can be understood in the context of cytosolic accumulation of Cl^- generally being in the mid- to high-millimolar range, and hence this mechanism might be termed a saturable, low-affinity transport system ('SLATS'). That the K_M and V_{max} values for this system are considerably higher under NH_4^+ nutrition relative to NO_3^- nutrition is a further indication of the suppressive effect of NO_3^- on both $[Cl^-]_{cyt}$ and Cl^- fluxes in general (Glass and Siddiqi 1985). It also reflects one aspect of the protective role of NO_3^- in Cl^- toxicity, given that potential osmotic problems associated with Cl^- accumulation are less easily counteracted in the leaves than in the roots (Flowers 1988).

Table 2 Results of linear transformations of concentration-dependence curves for shoot translocation of Cl^-. A Hanes–Wolf transformation was also generated, but because a clustering of points led to a misleadingly high r^2, this transformation was omitted from the table

	NH_4^+			NO_3^-		
	K_M	V_{max}	r^2	K_M	V_{max}	r^2
Lineweaver–Burk	84	5.1	0.99	27	3.2	0.74
Eadie–Hofstee	73	4.7	0.97	36	3.9	0.66

Conclusions

As $[Cl^-]_o$ increased from moderate levels to the threshold of salinity stress, the following key effects were observed:

1. $[Cl^-]_{cyt}$ was not homeostatically maintained, but increased by nearly two orders of magnitude.
2. Cl^- influx, and efflux as a percentage of total influx, rose continuously, such that futile cycling of chloride across the plasma membrane was observed at threshold salinity.
3. The flux to the shoot, as a function of $[Cl^-]_{cyt}$, followed Michaelis–Menten-type kinetics, indicating the operation of a saturable, low-affinity transport system ('SLATS') for xylem loading.
4. The half-time parameter for cytosolic Cl^- exchange showed two set-points, reflective of two distinct modes of cellular Cl^- transport, coincident with high- and low-affinity influx systems for Cl^-.

Our study also examined the effects of N source and strength upon Cl^- exchange, showing that:

1. NO_3^- generally suppressed Cl^- fluxes and accumulation, in particular the flux to the vacuole.
2. V_{max} and K_M for φ_{xylem} were higher for plants grown on NH_4^+ as compared to NO_3^-.
3. The half-time of exchange remained unaffected by N source.
4. Futile Cl^- cycling, at the threshold of salinity stress, was unaffected by N source.
5. Cytosolic Cl^- accumulation was insufficient to account for charge balancing of the excessive cytosolic NH_4^+ concentrations seen under NH_4^+ toxicity conditions.

Our observation of futile cycling of Cl^- across the plasma membrane, at the threshold of salinity stress, in conjunction with our previous demonstration of futile cycling under NH_4^+ toxicity conditions, suggests that a loss of efficiency in primary ion acquisition, coupled to the inability to exclude these ions from concentrated external pools, may be a general feature of ion toxicity in plants. This idea, along with the first evidence for a saturable transport system responsible for translocation of Cl^- to the shoot, requires further investigation, particularly in the context of breeding salt-tolerant plants (Noble and Rogers 1992).

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