A web-accessible computer program for calculating electrical potentials and ion activities at cell-membrane surfaces

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Abstract

Aims. Increasing evidence indicates that plant responses to ions (uptake/transport, inhibition, and alleviation of inhibition) are dependent upon ion activities at the outer surface of root-cell plasma membranes (PMs) rather than activities in the bulk-phase rooting medium.

Methods. A web-accessible computer program was written to calculate the electrical potential (ψ) at the outer surface of root-cell PMs (ψ_{PM}). From these values of ψ_{PM}, activities of ion I with charge Z ($\{I^Z\}$) can be calculated for the outer surface of the PM ($\{I^Z\}_{PM}$). In addition, ψ and $\{I^Z\}$ in the Donnan phase of the cell walls (ψ_{CW} and $\{I^Z\}_{CW}$) can be calculated.

Results. By reanalysing published data, we illustrate how this computer program can assist in the investigation of plant-ion interactions. For example, we demonstrate that in saline solutions, both Ca deficiency and Na uptake are more closely related to $\{Ca^{2+}\}_{PM}$ and $\{Na^+\}_{PM}$ than to $\{Ca^{2+}\}_b$ and $\{Na^+\}_b$ (activities in the bulk-phase media). Additional examples are given for Zn and P nutrition, Ni toxicity, and arsenate uptake.

Conclusions. The computer program presented here should assist others to develop an electrostatic view of plant-ion interactions and to re-evaluate some commonly-held views regarding mechanisms of ion transport, toxicity, competition among ions, and other phenomena.

Keywords: electrical potential, mineral nutrition, plant growth, plant-ion interactions, plasma membrane, toxicity.

Introduction

When considering interactions between plants and ions, plant performance has been related predominantly to the concentration or activity of the ions in the bulk-phase rooting medium. However, these relationships are often weak or inconsistent. For example, cations in the rooting medium, especially Ca^{2+}, Mg^{2+}, and H^{+}, may promote or inhibit root elongation and may alleviate or enhance the phytotoxicities of ionic species of trace metals and metalloids such as Al (Brady et al. 1993; Kinraide 2003b), Cu (Luo et al. 2008), Zn (Pedler et al. 2004), Se (Kinraide 2003a), and As (Wang et al. 2008). In general, the interpretation of ion effects is clarified by a consideration of ion activities at root-cell plasma membrane (PM) surfaces in addition to ion activities in the rooting media.

The PM surface carries negative charges, thus creating a negative PM surface electrical potential (ψ_{PM}) (Kinraide and Wang 2010). This negative ψ_{PM} influences the distribution of ions at the PM surface by attracting cations and repelling anions. Hereafter, the activity of an ion *I* with charge Z in the bulk-phase rooting medium will be denoted $\{I^Z\}_b$ whilst its activity at the outer surface of the PM will be denoted $\{I^Z\}_{PM}$. $\{I^Z\}_{PM}$ is computed from ψ_{PM} and $\{I^Z\}_b$ as described later. The magnitude of ψ_{PM} is not static, rather, it is influenced by the composition of the rooting medium. Increases in the concentration of cations reduce the negativity of ψ_{PM} due to binding of the cations or by electrical screening (Wang et al. 2011); anions have relatively small effects upon ψ_{PM} because of their usually low concentration at negative PM surfaces and because of weak binding.

Whilst all cations reduce the negativity of ψ_{PM}, the magnitude of the reduction is a function of ion charge and the strength of binding to the PM surface. For ions commonly encountered in

biological systems, the ability to reduce the negativity of ψ_{PM} follows this order: $Al^{3+} > H^+ > Cu^{2+} > Ca^{2+} \approx Mg^{2+} > Na^+ \approx K^+$ (Kinraide 2006). ψ_{PM} is different from the transmembrane potential difference (E_m) from the bulk phase of the rooting medium to the bulk phase of the cell interior as commonly measured by microelectrodes (see Nobel (2009), see also Fig. 1 of Kinraide (2001) or Fig. 1 of Wang et al. (2011)).

Increasing evidence indicates that plant responses to ions (uptake/transport, inhibition, and alleviation of inhibition) are dependent upon $\{I^Z\}_{PM}$ rather than $\{I^Z\}_b$ (for examples, see Kinraide (2006), Kopittke et al. (2011a), Kopittke et al. (2011c), Wang et al. (2008), or Wang et al. (2011)). Indeed, it has been known for almost 100 years that root tissues are negatively charged (Devaux 1916), and it was proposed at least 40 years ago that this negative charge could possibly explain observed plant-ion interactions (for example, see van Hai and Laudelout (1971)). However, the absence of a readily available, fully parameterized electrostatic model for the computation of ψ_{PM} (and, consequently, $\{I^Z\}_{PM}$) has hindered efforts to examine these interactions. The recent development of such models (Kinraide and Wang 2010; Yermiyahu et al. 1997b) provides an opportunity to investigate the role of ψ_{PM} in plant-ion interactions.

The above introduction omits any discussion of cell wall (CW) influences upon the electrical properties of the PM. However, the PMs of root cells are not exposed directly (or exclusively) to the rooting medium, rather, they are exposed, in part, to the Donnan phase of the CW, which is in turn exposed to the rooting medium. Hence, the composition of the rooting medium influences both ψ_{PM} and the potential of the CW Donnan phase (ψ_{CW}) (see Kinraide (2004) for a comprehensive discussion). However, the presence of the CW has relatively

small effect on $\{I^Z\}_{PM}$, and ion concentrations at the PM surface modelled with and without the CW are highly correlated (Kinraide 2004). Thus, for simplicity, ion activities at the PM surface can be computed as though the CW were absent, which is indeed the case for the cells of many organisms.

The aim of the present study is to present a WEB-accessible computer program that uses a fully parameterized electrostatic model to calculate ψ for plant root cell PMs and CWs based upon the composition of the rooting medium. From this, values for $\{I^Z\}_{PM}$ and $\{I^Z\}_{CW}$ can be determined. In particular, we hope to increase the ease with which these calculations can be conducted in order to promote their use amongst fellow scientists as well as industrial and governmental technologists and regulators. In addition to electrostatic models, the computer program incorporates speciation capabilities so that preliminary speciation by dedicated speciation programs is not required for many applications.

Using this computer program, we present examples of how plant-ion interactions can be interpreted using electrostatic theory. We have chosen to focus on plants in the present study, but the computer program developed here can be applied to PMs of other organisms (Kinraide 2006; Wang et al. 2013).

Materials and methods

A computer program (SGCS, Speciation Gouy Chapman Stern) was written to enable users to perform readily electrostatic calculations for plants growing in commonly encountered solutions. The values for ψ_{PM}, $\{I^Z\}_{PM}$, and other quantities may then be used examine plant-ion interactions. The program is available from the authors or from

www.uq.edu.au/agriculture/sgcs/. Although the program has been substantially verified in terms of agreement between computed and measured values (Kinraide 2004; Kinraide and Wang 2010), we intend to revise and upgrade the program as needed. We invite users to make recommendations.

Microsoft Visual Basic, within Microsoft Visual Studio 2010, was used for programming. The SGCS program runs on both 32-bit and 64-bit operating systems with Microsoft Windows XP or later software. The code is written in three major sections; the first section performs the solution speciation, the second performs the electrostatic calculations, and the third provides the linkages between the graphical user interface (GUI) and the mathematical calculations for speciation and electrostatics.

Speciation

This SGCS computer program does not provide comprehensive speciation capabilities (as do GEOCHEM or PhreeqcI), but rather it provides speciation for the most commonly encountered ions and some of their complexes. Cations in the speciation calculations include Al^{3+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, H^{+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, and Zn^{2+}, while the anions include AsO_4^{3-}, Cl^{-}, CO_3^{2-}, NO_3^{-}, OH^{-}, PO_4^{3-}, and SO_4^{2-}. A full list of included species/complexes can be found within the computer program, but the list includes the complexes Al-OH, Al-SO_4, Al-PO_4, Ca-CO_3, Ca-SO_4, Cd-Cl, Cd-CO_3, Cd-SO_4, Cu-CO_3, Cu-OH, Cu-SO_4, Mg-CO_3, Mg-PO_4, Mg-SO_4, Mn-CO_3, Mn-SO_4, Ni-CO_3, Ni-SO_4, Zn-CO_3, and Zn-SO_4. Activity coefficients are calculated using the extended Debye-Hückle equation (equation 2.11 of Lindsay (1979)) with simplified values for the effective size ($10^8 d_i$) of hydrated ions: $d_i = 3$

for monovalent ions, $d_i = 6$ for divalent ions, and $d_i = 9$ for trivalent ions. When necessary, all speciation constants are easily accessed and modified by the user from within the GUI.

Electrostatic theory at the PM surface

The electrostatic theory used in the development of the computer program can be expressed in a few equations. Here we provide a brief synopsis of the theory as presented in detail by Yermiyahu and Kinraide (2005) and Kinraide and Wang (2010). The PM is modelled as though it were composed of negatively charged (R^-) and neutral (P^0) sites to which ions (I^Z) may bind to form species RI^{Z-1} and PI^Z. R_T is the sum of all R sites whether free or binding an ion ($R_T = R^- + RI^{Z-1}$), and P_T is the sum of all P sites whether free or binding an ion ($P_T = P^0 + PI^Z$). Equations 1 and 2 express the binding reactions.

$$R^- + I^Z \rightleftharpoons RI^{Z-1} \qquad \text{Eq. 1}$$

$$P^0 + I^Z \rightleftharpoons PI^Z \qquad \text{Eq. 2}$$

Binding constants may be expressed as

$$K_{R,I} = [RI^{Z-1}]/([R^-][I^Z]_{PM}) \qquad \text{Eq. 3}$$

$$K_{P,I} = [PI^Z]/([P^0][I^Z]_{PM}) \qquad \text{Eq. 4}$$

$[R^-]$, $[P^0]$, $[RI^{Z-1}]$, and $[PI^Z]$ denote membrane surface densities expressed in units mol m^{-2}. $[I^Z]_{PM}$ denotes the concentration of the free ion at the PM surface expressed in M units. Its value is computed from ion concentration in the rooting medium ($[I^Z]_b$) by a Boltzman equation.

$$[I^Z]_{PM} = [I^Z]_b \exp[-Z_i F \psi_{PM}/(RT)] \quad \text{Eq. 5}$$

F, R, and T are the Faraday constant, the gas constant, and the temperature, respectively; RT/F = 25.7 mV at 25°C, so $-Z_i F \psi_{PM}/(RT) = -Z_i \psi_{PM}/25.7$ at 25°C for ψ_{PM} expressed in mV.

Equation 5 cannot be solved without a value for ψ_{PM} which appears also in the Gouy-Chapman equation for surface charge density (σ, in units Coluombs per m^2 (C m^{-2})).

$$\sigma^2 = 2\varepsilon_r \varepsilon_0 RT \Sigma_i [I^Z]_b (\exp[-Z_i F \psi_{PM}/(RT)] - 1) \quad \text{Eq. 6}$$

$2\varepsilon_r \varepsilon_0 RT$ = 0.00345 at 25°C for concentrations expressed in M (ε_r is the dielectric constant for water and ε_0 is the permittivity of a vacuum). σ is also equal to the sums of all PM surface species (in units mol m^{-2}) times the charge of each species times F (in units C mol^{-1}).

$$\sigma = \{-[R^-] + \Sigma_i (Z_i - 1)[RI^{Z-1}] + \Sigma_i Z_i [PI^Z]\} F \quad \text{Eq. 7}$$

Values for ψ_{PM} are computed by incremental and progressive assignment of trial values to it in Equations 5 and 6 until values for σ in Equations 6 and 7 converge. σ is the contingent surface charge density and is dependent upon the bathing medium and the ion binding expressed in Equations 1 and 2. The intrinsic surface charge density (σ_0) is the total charge density in the absence of binding ($\sigma_0 = FR_T$). To compute the variables in Equation 7, the constants σ_0, $K_{R,I}$, $K_{P,I}$, R_T, and P_T must be known. Values for them for plants have been estimated in previous studies (summarized in Kinraide and Wang (2010)). Finally, values for

185 $\{I^Z\}_{PM}$ is computed from ion activity in the rooting medium ($\{I^Z\}_b$) by the Nernst variation of
186 the Boltzman equation (Equation 5).
187
188 $$\{I^Z\}_{PM} = \{I^Z\}_b \exp[-Z_i F \psi_{PM}/(RT)] \quad \text{Eq. 8}$$
189
190 *Electrostatic theory in the Donnan phase*
191 For plants, the program also permits calculation of ψ in the Donnan space (also known as free
192 space) of the CWs (ψ_{CW}). The CW was modelled initially as a Donnan phase composed of
193 immobile negatively charged (R_{CW}^-) and neutral (P_{CW}^0) sites to which ions can bind.
194 However, the experimental data indicate that H^+ binds to R_{CW}^- strongly and that all other
195 binding is relatively very weak and makes no significant contribution to ψ_{CW} (Kinraide 2004;
196 Shomer et al. 2003). Computed values for ψ_{CW} correspond well to values obtained by
197 implantations of microelectrodes into CWs and ζ-potential measurements of CW fragments
198 (Shomer et al. 2003). Ion activities in the Donnan space of CWs are calculated with a Nernst
199 equation, $\{I^Z\}_{CW} = \{I^Z\}_b \exp[-Z_i F \psi_{CW}/(RT)]$ (Nobel 2009).
200
201 **Results**
202 *Use of the computer program*
203 The graphical user interface (GUI) allows for the input and output of data as well as
204 customization of some of the model parameters. All speciation constants and binding
205 constants (for ion binding to PM sites) are easily modified from within the GUI. Similarly,
206 values for R_T and P_T (μmol m^{-2}) can be modified within the GUI.
207

Data can be inputted into the SGCS program by two general methods, ‘manual input’ or ‘batch input’. For manual input, data for each solution must be manually entered into the program and run individually, with the results displayed within the program window. For batch input, data for multiple solutions can be entered into a CSV (comma-separated values) file which is then imported into the program. Then, calculated values (e.g., bulk concentrations, bulk activities, and PM surface activities for free ions and the most common species) are written to an output CSV file designated by the user. The CSV file must have a defined structure and layout (as expected by the program) and templates are provided.

For either of these input methods (manual or batch), the program can be run in two modes – ‘speciation’ or ‘manual speciation’. For ‘speciation’, the program runs both the speciation and electrostatic components. Hence, in ‘speciation’ mode, the total elemental concentrations are inputted and speciation calculations are performed prior to the electrostatic calculations being conducted. For ‘manual speciation’, the speciation component of the program is by-passed, and the user must enter the concentrations of each individual ionic species manually (as determined in another chemical speciation program such as PhreeqcI or GEOCHEM). These values are then used directly for electrostatic calculations.

The SGCS program conducts speciation calculations for only certain elements (see above). However, in order to increase the versatility of the program, it is possible to input concentration values for additional ions without speciation calculations for those ions being performed by the SGCS program, but these added ions are still used in the electrostatic calculations. In particular, concentrations can be inputted for Ag^{+}, Ba^{2+}, Co^{2+}, Cs^{+}, Fe^{2+}, Fe^{3+}, Ga^{3+}, Gd^{3+}, Hg^{2+}, In^{3+}, La^{3+}, Pb^{2+}, Sc^{3+}, Sr^{2+}, and Tl^{+}. For example, if the user wishes to add

10 µM Ag_2SO_4 to 1 mM $CaCl_2$ at pH 5.0, the concentration of free bulk-phase Ag^+ inputted should be 9.8 µM (due to the formation of 0.2 µM $AgSO_4^-$) as determined by independent speciation. These free ion concentrations (e.g., 9.8 µM Ag^+) are then used in electrostatic calculations with their appropriate binding constants available in Kinraide (2009).

If desired, the user can balance cation/anion charge in the solutions using Cl^-. In these instances Cl^- will be added to, or removed from, the solution to ensure the solution remains balanced (although charge cannot be balanced if the total Cl^- required is < 0). If the user does not want the charge to be balanced using Cl^-, the program will not run if the 'charge error' is > 30%, where error = 100(positive – negative)/(positive + negative). The user is also able to equilibrate the solutions with atmospheric CO_2 (H_2CO_3) partial pressure if desired.

Accuracy of the computer program

Firstly, we checked the accuracy of the speciation calculations by comparing to those using PhreeqcI 2.17 and the MINTEQ database. The relevant constants of the MINTEQ database were modified to be the same as those in the SGCS database. Three solutions were used to compare the two programs, with input concentrations in µM units: Solution 1 = 5 $Al_2(SO_4)_3$, 1000 $CaSO_4$, 10 $CuSO_4$, and sufficient H_2SO_4 to reduce the pH to 4.5; Solution 2 = 375 $CaCl_2$, 125 $Ca(H_2PO_4)_2$, 500 $CaSO_4$, 5 $CdCl_2$, 10 $ZnCl_2$, and sufficient HCl to reduce pH to 5.5; Solution 3 = 660 $CaCl_2$, 90 $CaCO_3$, 250 $CaSO_4$, 5 $CdCl_2$, and 10 $ZnCl_2$. Values calculated using the SGCS program corresponded well to PhreeqcI values, although slight differences were observed with some CO_3-species (such as $CaHCO_3^+$, Table 1).

Secondly, and importantly, values for ψ_{PM} calculated using the electrostatic model outlined here (Eq. 1 to Eq. 8) have been shown to correspond well to surface electrical potentials measured by electrophoresis (to obtain zeta (ζ) potentials) or the surface attraction of ionic dyes (Kinraide and Wang 2010; Wang et al. 2013; Wang et al. 2008).

Discussion

The SGCS computer program allows the calculation of ion activities at the outer surface of the PM. ψ_{PM} is typically negative relative to the bulk medium. It therefore increases the PM-surface activity of cations and reduces the PM-surface activity of anions. The extent to which ions are attracted to or repelled from the PM surface depends also upon the charge of the ion (see the Equations 5 and 8). For example, when ψ_{PM} = –59.2 mV, monovalent cations will be concentrated 10-fold relative to the bulk solution, divalent cations will be concentrated 100-fold, and trivalent cations will be concentrated 1,000-fold. In contrast, anions will be depleted 10-, 100-, or 1,000-fold. Depolarization (decrease in negativity) of the PM caused by the addition of salts to the bulk solution (Fig. 1a) will reduce the concentration of cations at the PM surface but will increase the concentration of anions (Fig. 1b). Thus, an increase in the cation concentration in the bulk solution typically decreases the uptake and toxicity of other cations. This 'competition' among cations based upon global (whole surface) electrostatic interactions is different in principle from competition based upon site-specific interactions among ions as described by the biotic ligand model (BLM) (Kinraide 2006). Furthermore, interactions between cations and anions (e.g., enhancement by cations of anion uptake and toxicity) can be understood by electrostatic principles but not by site-specific competitions.

Here, using the SGCS computer program, we present examples of how activities of ions at the outer PM surface can be used to interpret plant-ion interactions. Although we have focused on plant-ion interactions, this electrostatic theory can be used to examine interactions in other species, including bacteria and animals (Kinraide 2006). The results below are presented in two parts, the first examining the uptake of essential nutrient ions in the deficient to adequate supply range, and the second examining the toxicity (and alleviation of toxicity) of essential and non-essential ions.

Mineral nutrition of essential cations and anions

Whilst plants are selective and active in their uptake of nutrients (and different conditions influence the selectivity of this uptake), we suggest that the uptake of nutrient ions is influenced by the activity of the ion at the outer surface of the root cell PM, which may differ greatly from its activity in the bulk-phase rooting medium.

Two cationic nutrients are examined here, the first being Zn. Chaudhry & Loneragan (1972a; b) investigated the effect of Ca, Mg, Ba, Sr, and H on the absorption of Zn by wheat roots at concentrations relevant for Zn nutrition (0.1 to 10 µM). The authors observed that at a constant bulk concentration of Zn, increases in Ca, Mg, Ba, Sr, or H reduced the uptake of Zn (Fig. 2a). For example, with 1 µM Zn in the bulk solution, a decrease in pH from 7 to 3, with 350 µM basal Ca, reduced Zn uptake from 430 to 3.5 ng g^{-1} d^{-1}. Given that the concentration of Zn in solution was constant, these changes in Zn uptake induced by H cannot be explained by changes in $\{Zn^{2+}\}_b$ (Fig. 2a). However, the increase in H^+ from 0.1 to 1000 µM activity was computed to reduce the negativity of ψ_{PM} from –51 to +33 mV thereby reducing $\{Zn^{2+}\}_{PM}$ from 45 to 0.06 µM. Therefore, we suggest that these changes in Zn uptake resulted

302 from changes in the negativity of ψ_{PM} which in turn influenced $\{Zn^{2+}\}_{PM}$. Indeed, across all
303 cations, when Zn uptake was related to $\{Zn^{2+}\}_{PM}$ rather than $\{Zn^{2+}\}_{b}$, the r^2 value improved
304 from 0.604 to 0.925 (Fig. 2).
305
306 Calcium was the second cationic nutrient examined. It is often reported that plant growth in
307 saline conditions is reduced because cationic salts (typically Na or Mg) compete with Ca for
308 uptake and induce Ca deficiency (Grattan and Grieve 1992; 1999). We contend that the
309 changes in Ca nutrition do not result from site-specific competition, but rather, from changes
310 in $\{Ca^{2+}\}_{PM}$ resulting from variations in ψ_{PM}. For example, Carter et al. (1979) examined the
311 influence of cations (Mg, Na, and K) on Ca deficiency for the growth of barley (*Hordeum*
312 *vulgare* L.) shoots and roots in nutrient solutions simulating saline soil solutions of Canada.
313 No significant relationship was found between dry mass and $\{Ca^{2+}\}_{b}$ (r^2 = 0.442, Fig. 3a). In
314 contrast, the data demonstrate that changes in the solution composition influenced ψ_{PM} and
315 that Ca availability was related to $\{Ca^{2+}\}_{PM}$ (r^2 = 0.910, Fig. 3b). Thus Ca deficiency in saline
316 soils does not result from direct competition between Ca and other cations, but rather, from a
317 non-specific, cation-induced reduction in $\{Ca^{2+}\}_{PM}$ to deficiency levels (see also Kopittke et
318 al. (2011a)).
319
320 The SGCS model was also used to investigate the role of ψ_{PM} for anionic nutrients. In contrast
321 to cationic nutrients, a reduction in the negativity of ψ_{PM} (caused by an increase in the
322 concentration of cations) is expected to increase the activity of the anionic nutrient at the PM
323 surface and hence increase uptake (Fig. 1). However, our analyses here were hindered by a
324 lack of suitable published data for nutrient anions (to be suitable, a study must examine
325 uptake across an adequate range of solution compositions). In one study, Franklin (1969)

studied uptake of P in excised roots of barley and found that the addition of various metals influenced the uptake of P (ranging from 5 to 18 μg g^{-1}). In solutions with a constant P concentration of 20 μM, the order of effectiveness upon uptake (least to greatest) was K ~ Li ~ Na < Ba ~ Sr ~ Mg ~ Ca < Fe(II) < Fe(III) ~ Al. Noticeably, this effect on uptake was the opposite to that observed for cationic nutrients (Fig. 2 and Fig. 3), *viz*. an increase in the concentration of (depolarizing) cations increased P uptake but decreased Zn and Ca uptake. However, the results of Franklin (1969) cannot be re-analysed precisely; solution pH was not stated and hence the distribution among P-species (for example, HPO_4^{2-} and $H_2PO_4^-$) cannot be calculated nor can ψ_{PM} be calculated (for some of the cations, solution pH likely varied widely among treatments). Regardless, given that the total P concentration remained constant in the bulk solution, it is unlikely that these changes in uptake can be explained by changes in bulk activities of P species.

Significantly, the sequence of metals enhancing P uptake (K to Al above) corresponds to the effectiveness with which these metals depolarize the PM (Kinraide 2009). P uptake increased with the strength of metal binding to hard ligands such as the carboxylic acid groups of the PM (Fig. 4). Thus P uptake was lowest in solutions containing cations that depolarize the PM the least and highest in the solutions containing cations that depolarize the PM the most. These results hold some significance for acidic soils that may be rich in Al and poor in available P.

Toxicity of essential and non-essential cations and anions

We also used the SGCS model to investigate the phytotoxicity of essential and non-essential ions. For cationic toxicants (such as Cu, Ni, or Cd), toxicity is commonly understood

350 according to the biotic ligand model (BLM) which postulates that the adverse effect of a
351 toxicant is determined by the degree of its binding to a site (the 'biotic ligand') whose
352 occupancy by the toxicant leads to the toxicity (Paquin et al. 2002). A further postulate of the
353 BLM is that the beneficial effects of ameliorating cations (such as Ca, Mg, or H) arise from
354 competition between the ameliorant and the toxicant at the biotic ligand (Di Toro et al. 2001;
355 Paquin et al. 2002; Slaveykova and Wilkinson 2005). In contrast, we postulate that the
356 principle ameliorative effect of cations such as Ca, Mg, or H can be explained, usually, by this
357 sequence: 1. Ameliorative cations reduce the negativity of ψ_{PM}. 2. The reduced negativity of
358 ψ_{PM} reduces the PM-surface activity of the cationic toxicant. 3. The reduced PM-surface
359 activity of the toxicant reduces its binding to the biotic ligand, if indeed such a ligand plays a
360 role. In some cases one or more of the steps in the sequence may not be significant. An
361 ameliorative cation may be effective at such low concentrations that neither the PM-surface
362 negativity nor the PM-surface activity of the toxicant are significantly reduced (see below).
363
364 We reanalysed the data of Wu & Hendershot (2010) who examined the influence of H and Ca
365 on the toxicity of Ni to roots of pea (*Pisum sativum* L.). The authors reported that the addition
366 of either H or Ca typically decreased the toxicity of the Ni (Fig. 5a). For example, in solutions
367 with 10 µM Ni and 2 mM Ca, relative root elongation improved from 51 % with 1 µM H^+ to
368 130 % with 100 µM H^+ (Fig. 5a). Indeed, across all treatments, the toxicity of Ni was only
369 poorly correlated with $\{Ni^{2+}\}_b$ (r^2 = 0.580, Fig. 5a). In contrast, the toxicity of Ni was more
370 closely correlated to $\{Ni^{2+}\}_{PM}$ (r^2 = 0.761, Fig. 5b), which decreased with the addition of
371 cations (Ca^{2+} or H^+).
372

A second cationic toxicant was examined; in saline systems, the excess salt may lead to specific-ion toxicities (Munns 2002). We reanalysed the data of Davenport et al. (1997) who examined the effect of Ca (0.06 to 3.1 mM) on the uptake of Na from saline solutions containing 5 to 150 mM Na. As expected, the authors observed that the addition of Ca reduced uptake of Na. For example, in solutions containing 110 mM $\{Na^+\}_b$, an increase in Ca from 0.06 to 3.1 mM decreased Na uptake from 20 to 10 μmol g^{-1} root h^{-1} (Fig. 6b). Indeed, across all treatments, the relationship between Na uptake and $\{Na^+\}_b$ was comparatively poor (r^2 = 0.686, Fig. 6a) – the authors stating that this Ca-induced reduction in uptake was possibly due to the binding of Ca to the cation channels through which Na uptake was occurring. While Ca blockade of Na-conducting channels may indeed be a factor in Na uptake and toxicity, the Ca-induced reduction in $\{Na^+\}_{PM}$ is surely important; r^2 = 0.925 (Fig. 6b) for $\{Na^+\}_{PM}$ *vs.* Na uptake. Therefore, this ameliorative effect of Ca was in part non-specific, with the addition of Ca decreasing the negativity of ψ_{PM} and hence decreasing uptake by lowering the activity of Na at the PM surface. Other examples regarding the importance of electrostatic interactions in regulating the toxicity of cations (including Cu, Pb, and Mn) are given in Kinraide (2006), Kinraide & Wang (2010), and Kopittke et al. (2011b).

Finally, we examined the exacerbation of anion toxicity and the enhancement of uptake caused by an increase in the concentration of cations as reported for selenate, SeO_4^{2-} (Kinraide 2003a). These observations cannot be explained by the usual hypotheses such as channel blockade. As found for anionic nutrients (see earlier), comparatively few data are available for examining the effect of cations on the uptake of anionic toxicants. In a study with wheat, Wang et al. (2011) found that the addition of Ca, Mg, or H slightly increased both the toxicity and uptake of arsenate (AsO_4^-) (data not presented). This is in contrast to the

effects upon cationic toxicants such as Ni and Na (see above) where an increase in the concentration of cations decreased toxicity and uptake. However, relating As uptake to $\{H_2AsO_4^-\}_{PM}$ rather than $\{H_2AsO_4^-\}_b$ did not improve the r^2 value (r^2 = 0.803 versus 0.839). This demonstrates that whilst ψ_{PM}-controlled PM-surface activities are important in regulating plant-ion interactions across a range of conditions, other factors influenced by ψ_{PM} are important also. Kinraide (2001) and Wang et al. (2011) provide evidence that the surface-to-surface transmembrane potential difference ($E_{m,surf}$) is also influential as it provides the 'driving force' for ion transport across the PM, but $E_{m,surf}$ becomes more negative as ψ_{PM} becomes less negative. Indeed, inclusion of an additional term to account for $E_{m,surf}$ in the regression analyses for arsenate uptake improved the r^2 value to 0.922 (data not presented).

The preceding discussion, examining several cationic and anionic nutrients and toxicants, highlights the importance of electrostatic properties of the PM in determining the bioavailability of ions. We suggest that the electrostatic properties of the root PM have a non-specific effect on plant-ion interactions whereby an increase in the concentration of cations decreases the negativity of ψ_{PM} (Fig. 1a) thereby decreasing the activity of cations at the PM surface but increasing the activity of anions (Fig. 1b). We propose that many of the ameliorative effects typically ascribed to 'competition' can be explained by electrostatic effects. We do not, however, suggest that site-specific competition does not ever occur – certainly there are examples where we would assert that competition does indeed directly reduce toxicity of trace metals. For example, Zn toxicity was alleviated in wheat and radish (*Raphanus sativus* L.) by 1 to 5 µM Mg, concentrations too low to affect $\{Zn^{2+}\}_b$ or $\{Zn^{2+}\}_{PM}$ (Zn^{2+} and Mg^{2+} have similar ionic radii) (Pedler et al. 2004). Similarly, 50 µM Mg has been reported to alleviate Al toxicity in soybean, concentrations again too low to affect $\{Al^{3+}\}_b$ or

$\{Al^{3+}\}_{PM}$ (Silva et al. 2001). In a final example, 1 μM Cu (c. 5 μM $\{Cu^{2+}\}_{PM}$) at pH 5.6 strongly inhibits reproduction in free-living rhizobia, but each 0.1 unit reduction in the pH of the culture medium causes a ten-fold increase in the reproduction (Kinraide and Sweeney 2003). These small reductions in pH cause the negativity of ψ_{PM} to decline by only 0.3 mV and the $\{Cu^{2+}\}_{PM}$ to decline by only 0.1 μM. In such cases the ameliorative cation may act principally as a direct competitor with the toxicant at a biotic ligand, and global electrical effects may play no important role, but in our experience global electrical effects appear to play the greater role, especially in the case of cationic enhancement of anion toxicity where site-specific competition would appear to be unlikely. Finally, we have not considered here how different plant species respond to variations in $\{I^Z\}_{PM}$, although certainly such differences exist (for a detailed discussion, see Kinraide and Wang (2010)). For example, in Fig. 3 it can be seen that Ca deficiency reduces the growth of barley by 10 % when $\{Ca^{2+}\}_{PM}$ < ca. 4 mM. However, other species have different thresholds: growth of cowpea roots was decreased at ≤ 1.6 mM (Kopittke et al. 2011a), wheat roots at ≤ 0.76 mM (Kinraide 1999), pea (*Pisum sativum* L.) roots at ≤ 6 mM (Wang et al. 2010), and melon (*Cucumis melo* L.) roots at ≤ 15 mM (Yermiyahu et al. 1997a). Such variations need to be taken into account.

We hope that the WEB-accessible SGCS computer program described here will increase the ease with which electrostatic calculations can be performed, thereby allowing further investigation of the role of electrostatics in plant-ion interactions across a wide range of situations.

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Table 1. Comparison of speciation calculations performed either with the SGCS program or with PhreeqcI 2.17 using the Minteq database modified so that the selected constants equalled those in the default SGCS database. Data in the table are presented as ionic activities (µM). Three solutions were examined, with input concentrations of (µM): Solution 1 = 5 $Al_2(SO_4)_3$, 1000 $CaSO_4$, 10 $CuSO_4$, and sufficient H_2SO_4 to reduce the pH to 4.5; Solution 2 = 375 $CaCl_2$, 125 $Ca(H_2PO_4)_2$, 500 $CaSO_4$, 5 $CdCl_2$, 10 $ZnCl_2$, and sufficient HCl to reduce pH to pH 5.5; Solution 3 = 660 $CaCl_2$, 90 $CaCO_3$, 250 $CaSO_4$, 5 $CdCl_2$, and 10 $ZnCl_2$. Only selected ionic species are shown.

	Solution 1		**Solution 2**		**Solution 3**	
	SGCS	**PhreeqcI**	**SGCS**	**PhreeqcI**	**SGCS**	**PhreeqcI**
Al^{3+}	2.9	2.9	0	0	0	0
$AlOH^{2+}$	0.9	0.9	0	0	0	0
$Al(OH)_2^{+}$	0.2	0.2	0	0	0	0
$AlSO_4^{+}$	3.2	3.2	0	0	0	0
Ca^{2+}	700	700	740	740	770	770
$CaHCO_3^{+}$	0	0	0	0	1.8	1.4
$CaHPO_4^{0}$	0	0	1.5	1.8	0	0
$CaH_2PO_4^{+}$	0	0	4.2	4.2	0	0
$CaSO_4^{0}$	101	100	53	52	27	27
Cd^{2+}	0	0	3.4	3.4	3.4	3.3
$CdCl^{+}$	0	0	0.3	0.3	0.4	0.4
$CdSO_4^{0}$	0	0	0.3	0.3	0.2	0.2
Cl^{-}	0	0	770	780	1400	1400
Cu^{2+}	6.9	6.8	0	0	0	0
$CuSO_4^{0}$	1.1	1.1	0	0	0	0
H^{+}	34	34	3.4	3.4	0.033	0.033
HPO_4^{2-}	0	0	3.8	4.5	0	0
$H_2PO_4^{-}$	0	0	220	220	0	0
SO_4^{2-}	930	930	450	450	220	220
Zn^{2+}	0	0	7.4	7.4	7.2	7.0
$ZnHCO_3^{+}$	0	0	0	0	0.2	0.1
$ZnCO_3^{0}$	0	0	0	0	0.4	0.3
$ZnSO_4^{0}$	0	0	0.6	0.5	0.3	0.3

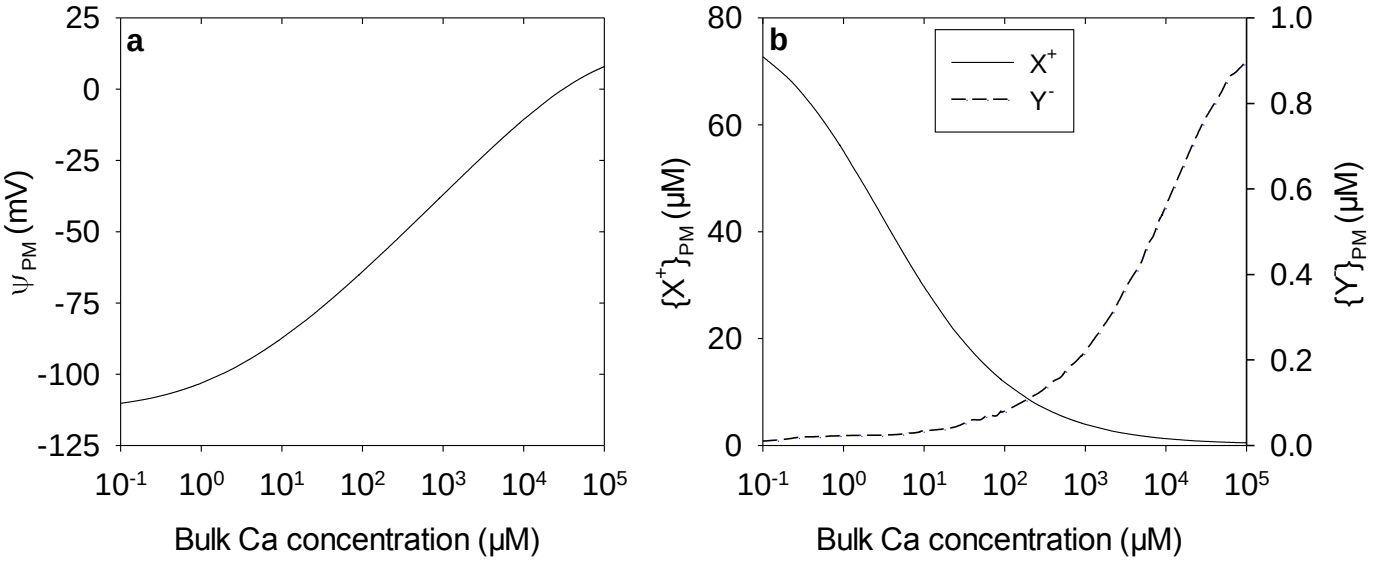

Fig. 1 (a) Electrical potential at the outer surface of the root PM (ψ_{PM}) exposed to $CaCl_2$ solutions at pH 5.6. (b) Activities of a monovalent cation (X^+) and a monovalent anion (Y^-) at the outer surface of the PM, when the concentrations of those ions in the bulk solution are 1 μM. Note that, compared to the bulk solution, negative values for ψ_{PM} increase the activity X^+ at the PM surface ($\{X^+\}_{PM}$) but decrease the activity of Y^- at the PM surface ($\{Y^-\}_{PM}$)

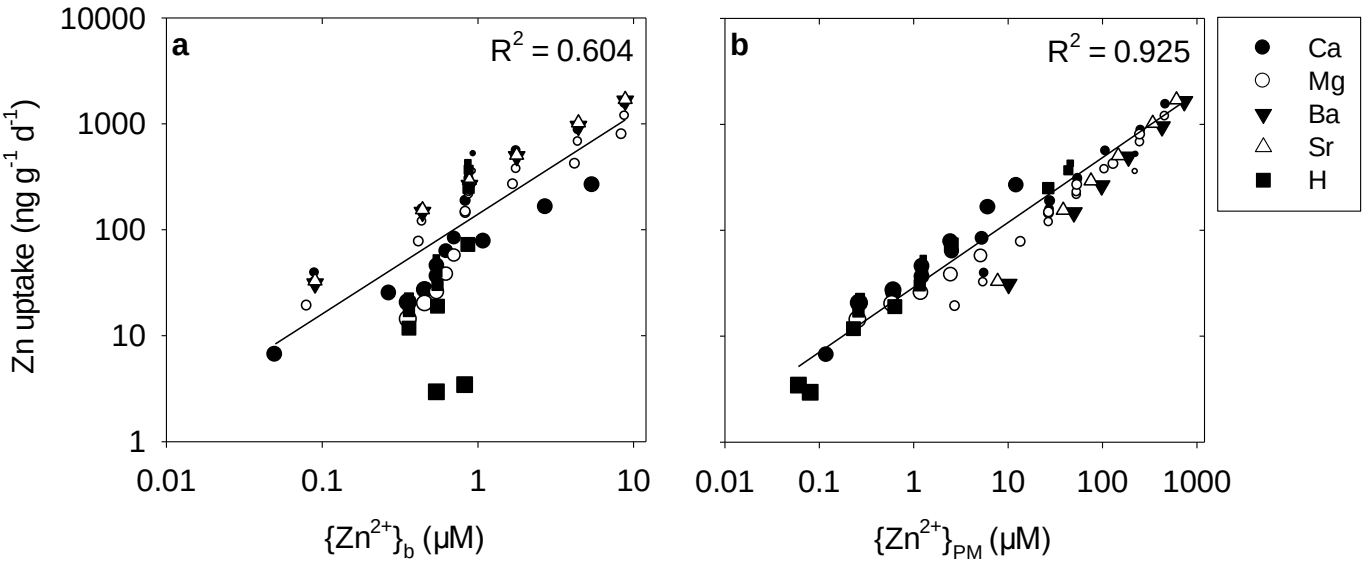

Fig. 2 Effect of Ca, Mg, Ba, Sr, and H (pH) on the absorption of Zn by wheat seedlings related to the activity of Zn^{2+} in the bulk solution (a) or at the outer surface of the root PM (b). Data were taken from Fig. 4 of Chaudhry & Loneragan (1972b) for H and from Table 1 and Fig. 2 of Chaudhry & Loneragan (1972a) for the other cations (data are excluded from the highest Ca and Mg treatments due to deleterious osmotic effects). Other than for the H^+-treatments, a constant pH of 5.6 was assumed. The size of the symbols is proportional to the concentration of the cation

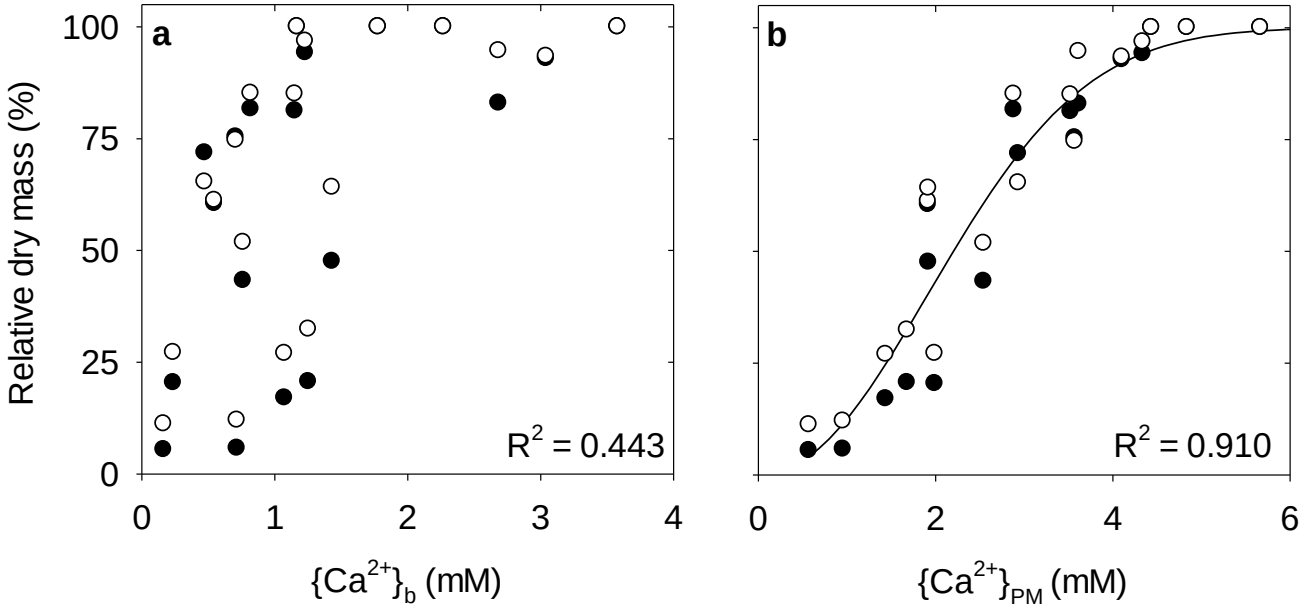

Fig. 3 The relative dry mass of shoots (●) and roots (○) of barley (*Hordeum vulgare* L.) grown in nutrient solutions for three to four weeks as related to the activity of Ca^{2+} in the bulk solution (a), or at the outer surface of the root PM (b). The solutions were at pH 5.5 and contained 0.5 to 10 mM Ca, 1.5 to 90 mM Na, 0.8 to 6.0 mM K, and 0.25 to 15.5 mM Mg. Data were taken from Experiments 1 to 4 of Carter et al. (1979)

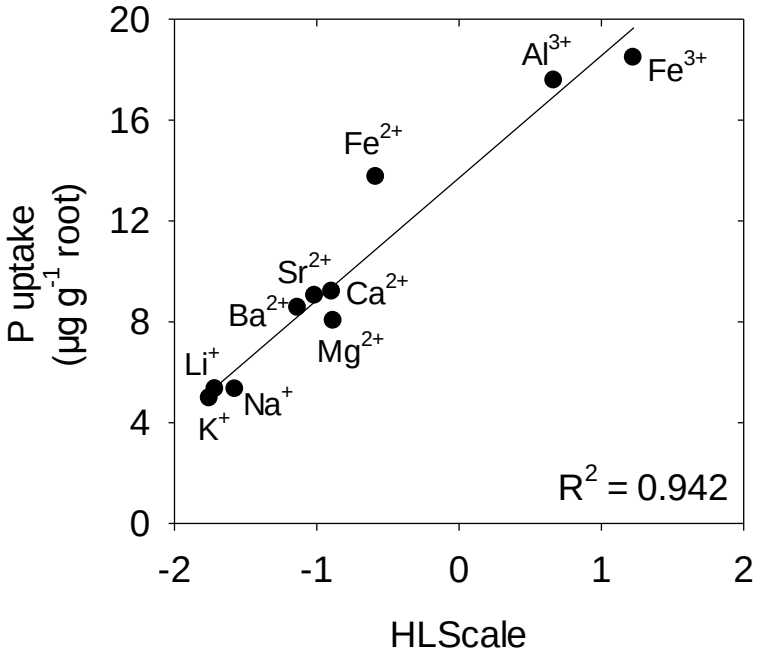

Fig. 4 The uptake of P by excised roots of barley (*Hordeum vulgare* L.) related to the Hard Ligand Scale (HLScale) for ten metals. The HLScale is a logarithmic scale of binding strength between metals and hard ligands such as the carboxylic acid groups of the PM. Thus the HLScale is a measure of the effectiveness with which cations reduce the surface negativity of the PM (Kinraide 2009). Data for P uptake are taken from Franklin (1969)

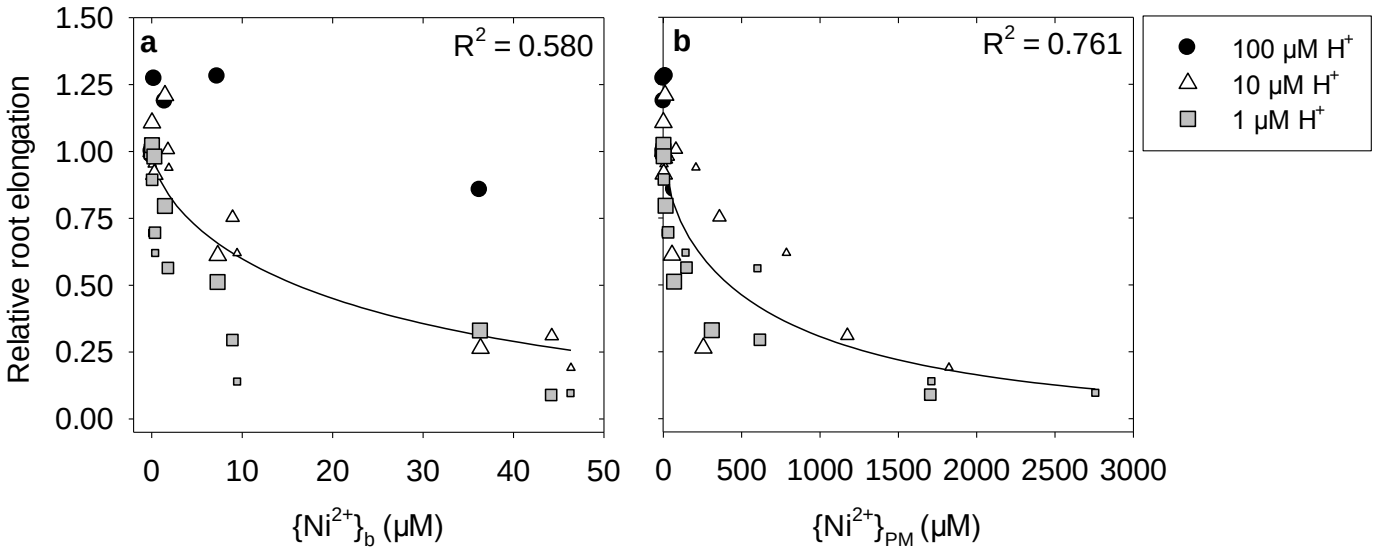

Fig. 5 Growth of pea roots in solutions with toxic levels of Ni related to the activity of Ni^{2+} in the bulk solution (a) or at the outer surface of the root PM (b). Solution pH values were 4, 5, or 6 with Ca concentration at 0.04 mM (small symbols), 0.2 mM (medium symbols), or 2 mM (large symbols). Data were taken from Table 1 of Wu and Hendershot (2010) with the pH 4 treatments at 0.04 and 0.2 mM Ca excluded due to poor root growth (> 80 % reduction when compared to other treatments)

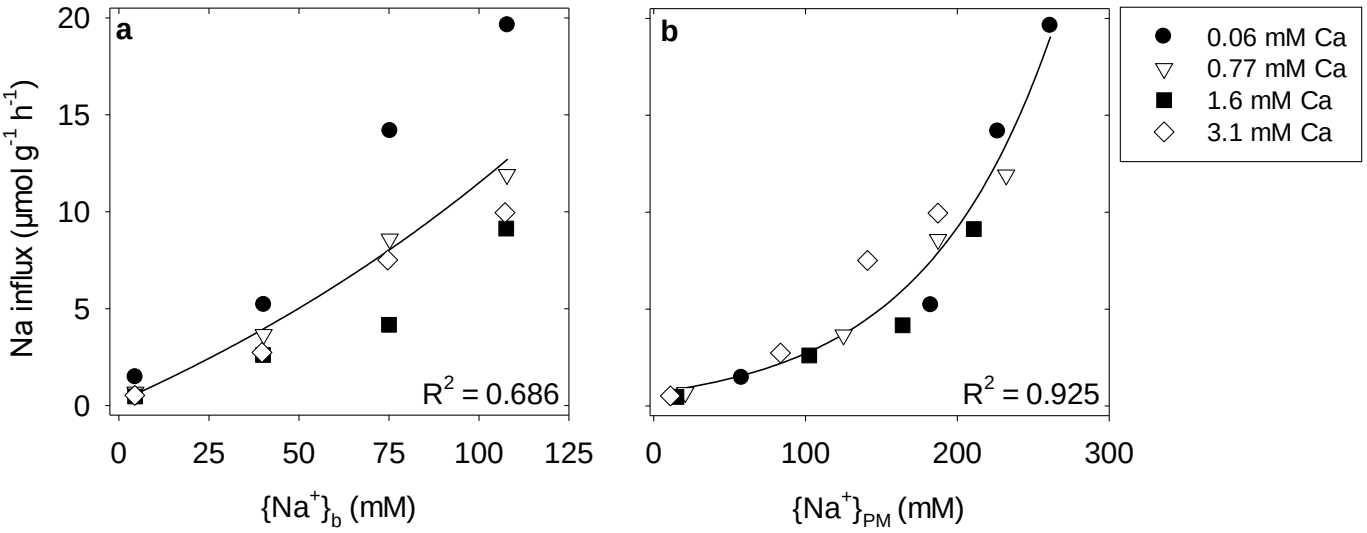

Fig. 6 Effect of Ca on the short-term influx of Na in roots of *Triticum aestivum* L. cv Kharchia, related to the activity of Na^+ in the bulk solution (a) or at the outer surface of the root PM (b). Data were taken from Fig. 2 of Davenport et al. (1997), with all solutions at pH 6.5