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Early Zn\(^{2+}\)-induced effects on membrane potential account for primary heavy metal susceptibility in tolerant and sensitive Arabidopsis species

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• Background and Aims Uptake of heavy metals by plant root cells depends on electro-physiological parameters of the plasma membrane. In this study, responses of the plasma membrane in root cells were analysed where early reactions to the metal ion-induced stress are localized. Three different Arabidopsis species with diverse strategies of their adaptation to heavy metals were compared: sensitive Arabidopsis thaliana and tolerant A. halleri and A. arenosa.

• Methods Plants of A. thaliana Col-0 ecotype and plants of A. arenosa and A. halleri originating from natural metallicolous populations were exposed to high concentrations of Zn\(^{2+}\). Plants were tested for root growth rate, cellular tolerance, plant morphology and cell death in the root apex. In addition, the membrane potential (\(E_\text{m}\)) of mature cortical root cells and changes in the pH of the liquid culture media were measured.

• Key Results Primary roots of A. halleri and A. arenosa plants grew significantly better at increased Zn\(^{2+}\) concentrations than A. thaliana plants. Elevated Zn\(^{2+}\) concentrations in the culture medium induced rapid changes in \(E_\text{m}\). The reaction was species-specific and concentration-dependent. Arabidopsis halleri revealed the highest insensitivity of the plasma membrane and the highest survival rate under prolonged treatment with extra-high concentrations. Plants were able to effectively adjust the pH in the control, but much less at Zn\(^{2+}\)-induced lower pH.

• Conclusions The results indicate a similar mode of early reaction to Zn\(^{2+}\), but with different extent in tolerant and sensitive species of Arabidopsis. The sensitivity of A. thaliana and A. arenosa was demonstrated. Plasma membrane depolarization was lowest in the hyperaccumulator A. halleri and highest in A. thaliana. This indicates that rapid membrane voltage changes are an excellent tool to monitor the effects of heavy metals.

Key words: Arabidopsis arenosa, Arabidopsis halleri, Arabidopsis thaliana, heavy metal tolerance, membrane potential, pH, root growth, zinc, Zn\(^{2+}\).

INTRODUCTION

Zinc is an essential micronutrient required for optimal plant growth and development. In plant cells Zn\(^{2+}\) is involved in the regulation of many metabolic processes, and also plays important roles in structure and functionality of transcription factors, enzymes and membranes (Rout and Das, 2003; Broadley et al., 2007). Due to serious problems with extensive metal pollution and the wide distribution of heavy metal-contaminated areas, study and understanding of the mechanisms of Zn\(^{2+}\) uptake, transport, accumulation and detoxification by plants is one of the basic needs in the effort to minimize the negative effects of Zn\(^{2+}\) toxicity in plants.

Chemical and physiological conditions in the rhizosphere influence considerably the availability of Zn\(^{2+}\) to plants. Thus, soil pH has a significant effect on Zn\(^{2+}\) accessibility for plants (McBride et al., 1997; Broadley et al., 2007). Zn\(^{2+}\) is usually more available for plant roots in slightly acidic conditions while solubility and uptake of Zn\(^{2+}\) decrease with increasing soil pH (McGrath et al., 1988). In addition, soil organic matter and available phosphorus and iron may interact by complexing with Zn\(^{2+}\) and thus affect its availability and uptake. Consequently, Zn\(^{2+}\) toxicity will normally be related to acidic soils with pH lower than 4.5. At low soil pH, not only Zn\(^{2+}\) but also other divalent metals are highly available as free ions in the soil solution (Jeffery and Uren, 1983; Msaky and Calvet, 1990). At pH < 5.5, Zn\(^{2+}\) uptake and plant growth per se are usually affected. Hence in a certain pH range (4.0–4.6), toxicity effects on plant growth were related to pH rather than to Zn\(^{2+}\) (Chairidchai and Ritchie, 1993). Taking into account the importance of soil pH for Zn\(^{2+}\) in plant nutrition, it is important that plants can increase the pH of the rhizosphere, thereby precipitating and complexing such metals. Together with excreting anions such as phosphate and organic anions, this is one of the mechanisms preventing excess metal from entering the plant and lowering its toxicity (Reichman, 2002).
As for several other heavy metals, high concentrations of available Zn\(^{2+}\) in the soil or root medium are a serious stress factor for plants. The extent of metal tolerance varies among plant species. *Arabidopsis thaliana* normally does not grow in metal-rich soils, does not tolerate or accumulate Zn\(^{2+}\) and only basic cellular tolerance to Zn\(^{2+}\) of this species has been described (Bert et al., 2000; Cho et al., 2003; Becher et al., 2004; Arrivault et al., 2006). *Arabidopsis arenosa* is a tolerant species with the capacity to accumulate Zn\(^{2+}\) or to exclude it, depending on the actual Zn\(^{2+}\) concentration in the soil (Claus and Koch, 2006; Przedpelska and Wierzbicka, 2007; Banásová et al., 2012). *Arabidopsis halleri* is an example of Zn\(^{2+}\)- and Cd\(^{2+}\)-hyperaccumulating plants, which are capable of concentrating great amounts of metal ions in their leaves without showing toxicity symptoms (Bert et al., 2000; Dräger et al., 2004; Elbaz et al., 2006; Meyer et al., 2010). Hyperaccumulators are generally characterized by tolerance to heavy metals, but particularly by sequestration of heavy metals in their shoots at high quantity, exceeding conventional threshold levels (Baker, 1981). When plants of *A. thaliana* and *A. halleri* were treated in parallel with 300 \(\mu M\) ZnSO\(_4\) for 4 d, the Zn\(^{2+}\) content in the roots was equal in the two species, but up to five-fold higher in the shoots of *A. halleri* than in *A. thaliana* (Becher et al., 2004).

Plants may tolerate high levels of Zn\(^{2+}\) in the rhizosphere by applying several different Zn\(^{2+}\)-eliminating mechanisms. Evidence for metal ion exclusion, their detoxification in the cytoplasm by chelation or for effects of phytochelatins were obtained for several Zn\(^{2+}\)-tolerant species (Van de Mortel et al., 2008; Yadav, 2010; Seth et al., 2012). A common strategy known from hyperaccumulating species is attenuation of heavy metal ions in the apoplasm and/or compartmentalization and sequestration in vacuoles (reviewed by Krämer et al., 2007). Compartmentation of metals within the cell is a necessary and effective way for their separation from active cellular metabolic components and for keeping the cytoplasmic Zn\(^{2+}\) concentration as low as necessary (Krämer et al., 2007). The mechanism of compartmentation comprises regulation of both the metal influx and an effective metal efflux system, responsible for translocation of metal ions from cell to cell towards the destination tissue or organ and final sequestration in the vacuoles. This system is responsible for metal ion homeostasis in plants and, by such processes, the vacuoles become the main storage place for inactivation of heavy metals (Mendoza-Cózatl et al., 2011; Rascio and Navari-Izzo, 2011). Maintenance of metal homeostasis in cells is achieved by activity of specific transporters and metal pumps. These are membrane proteins, belonging to different plant metal transporter families, coordinating equilibrium among apoplasmic metal levels, its transmembrane gradient, intracellular metal influx and efflux away from the cytoplasm. They control metal ion transport across the plasma membrane, the tonoplast and other endomembranes (Krämer et al., 2007; Maestri et al., 2010). Activity and expression of these proteins are enhanced in tolerant and hyperaccumulating plant species. There are numerous reports on Zn\(^{2+}\)-responsive genes and proteins known to be deregulated upon metal treatment based on transcriptomic and proteomic analyses (Becher et al., 2004; Weber et al., 2004; Fukao et al., 2009; Farinati et al., 2009; reviewed by Broadley et al., 2007). This analysis is the prime source for elucidating fundamental regulatory and effective components establishing the basis of plant metal tolerance.

Metal sequestration in plant vacuoles might be the preferred way in some plant species to achieve a metal tolerance status. However, significant proportions of metal ions may accumulate at the cell wall – plasma membrane interface. Accumulation of Zn\(^{2+}\) in the cell wall of the root rhizodermis of *A. halleri* plants in hydroponic culture has been described. Here, the precipitation of Zn\(^{2+}\) phosphates substantially prevented entry of Zn\(^{2+}\) to the cell and diminished intracellular Zn\(^{2+}\) accumulation (Käpper et al., 2000). Because the plasma membrane (PM) of root cells is the first barrier for heavy metals entering the symplasm, any interaction of ions with the PM including their negative and even toxic effects should be studied at the level of the PM first. Metal ion toxicity on the PM may be expressed as disturbed membrane functionality, and may stimulate lipid peroxidation and subsequent ion imbalance in the cytoplasm due to loss of highly mobile ions, such as K\(^{+}\). Such disturbances of PM functions inducing a misbalanced ion influx/efflux ratio cause changes in membrane potential (\(E_M\)) and increase plant water losses (Chaoui et al., 1997; Madhava Rao and Sresty, 2000; Llamas et al., 2008). Effects of divalent metal cations on \(E_M\) differ, mainly according to available heavy metal concentration, to its affinity status and mode of action. For metal ions such as Cd\(^{2+}\) and Zn\(^{2+}\) it has been shown that they induce serious and continuous membrane depolarization in root cells (Aidid and Okamoto, 1992; Kennedy and Gonsalves, 1987). However, assessments of PM-originating reactions based on changes in \(E_M\) comparing Zn\(^{2+}\)-tolerant and Zn\(^{2+}\)-sensitive species of *Arabidopsis*, as well as Zn\(^{2+}\)-induced changes in the pH of culture medium have not been performed.

The fact that *A. thaliana* is sensitive to Zn\(^{2+}\) whereas *A. halleri* and *A. arenosa* are tolerant provides ideal opportunities for comparative analysis of the mechanisms setting diverse strategies of their adaptation to heavy metals. We characterized responses to different Zn\(^{2+}\) concentrations based on determination of root growth, index of tolerance and cell viability responses with implications for electrophysiological parameters in three different *Arabidopsis* species under high and toxic concentrations of Zn\(^{2+}\) in standardized culture conditions. Based on the measured parameters, including changes in root \(E_M\), we confirmed the sensitivity of *A. thaliana* and a high tolerance of *A. halleri* and *A. arenosa*. At all concentrations of Zn\(^{2+}\) tested, *A. halleri* showed the highest PM insensitivity and the highest survival capacity under the prolonged influence of extra-high concentrations of Zn\(^{2+}\). The results indicate a similar mode of early reactions to Zn\(^{2+}\), but with distinctly different magnitude in tolerant and sensitive species of *Arabidopsis*.

**MATERIALS AND METHODS**

**Cultivation of plants**

Seedlings of *Arabidopsis* species were cultivated *in vitro* on modified MS medium supplemented with different concentrations of Zn\(^{2+}\) in the form of ZnSO\(_4\) or ZnCl\(_2\). A concentration...
of 10 μM Zn$^{2+}$ was used as control, while high and toxic concentrations of Zn$^{2+}$ tested ranged from 100 μM to 10 mM. Plants of Arabidopsis arenosa [Cardaminopsis arenosa (L.) Hayek] subsp. borbasii and Arabidopsis halleri [Cardaminopsis halleri (L.) Hayek] subsp. tatrica originating from natural metallicolous populations in Slovakia were described and characterized by Staňová et al. (2010, 2012). Arabidopsis arenosa subsp. borbasii was collected at Štiavnické Bane, Terézia (48°27′04″N, 18°52′27″E; 840 m asl), and of A. halleri subsp. tatrica at Krompachy (48°55′04″–07″N, 20°53′24″–39″E; 420 m asl). Seeds of these plants collected in the field at the end of vegetative seasons 2009–2011 were used for experiments and the results were compared with control A. thaliana Col wild-type plants. For determination of root growth rate and root morphology seeds were germinated and plants grown on solidified culture media with modified MS salts, 1 % sucrose, pH 5.5 using 0.1 M HCl. The length of primary roots of plants grown on Phytagar-solidified media was measured in seedlings 6 d after germination (DAG). The index of tolerance was calculated as a ratio of primary seminal root length of plants grown in elevated concentrations of Zn$^{2+}$ to primary seminal root length of plants grown in control condition (Przedpelska and Wierzbicka, 2007). The test of cellular tolerance was performed on surface-parallel sections from mature leaves of plants originating from natural field conditions. The sections were placed into graded concentrations of ZnSO$_4$. After 48 h cell viability was tested using cell plasmolysis ability by adding of 10 μM sucrose to the sample. For determination of physiological responses, seedlings of 2–3 DAG were transferred to modified liquid MS medium on microscope slides (Ovečka et al., 2005). Root growth rate and changes in pH of the liquid culture medium were determined in 24-h culture periods for control, 100 μM, 1 mM and 2 mM concentrations of ZnSO$_4$. Parameters of root growth were documented with an Olympus BX61 light microscope (Tokyo, Japan) and quantified using Cell F image analysis software (Olympus Soft Imaging Solution, Germany).

Electrophysiological measurements

Measurements of $E_M$ were performed on mature cortical root cells of intact plants using standard microelectrode techniques as described in detail by Pavlovkin et al. (1993). After rinsing the roots with 0.5 mM CaSO$_4$, intact seedlings from in vitro culture were mounted in a 4-mL volume plexiglass chamber and were constantly perfused (10 mL min$^{-1}$) with bathing solution containing 0.1 mM KCl, 0.1 mM CaCl$_2$ and 0.1 mM CaSO$_4$ adjusted to pH 5.5 using 0.1 M HCl. Experimental Zn$^{2+}$-containing solutions consisted of 0.1 mM KCl, 0.1 mM CaCl$_2$ and 0.1–10 mM ZnCl$_2$. The presence of Zn$^{2+}$ as a dichloride salt in the perfusion solution induced immediate membrane changes of the root cells. Both Zn$^{2+}$ and Cl$^-$ ions contribute to membrane depolarization, as previously described for Ni$^{2+}$-induced effects on rice root cells (Llamas et al., 2008). Therefore, to separate and reset the effect of Cl$^-$, subsequent experiments were performed by pretreatment of plants with bathing solution in which total Zn$^{2+}$ was replaced by Ca$^{2+}$ at the same concentration, added in the form of CaCl$_2$ in the permanent presence of 0.1 mM CaSO$_4$, to ensure protection of membrane integrity. The $E_M$ of both control and Zn$^{2+}$-treated roots were then measured using micropipettes filled with 3 mM KCl. Tip diameter was 0.5 μm with a tip potential from −5 to −15 mV. A micromanipulator was used to insert micropipettes into a single cortical cell of the mature portion of the root. Insertion of the microelectrode was performed under microscope control. $E_M$ changes induced by Zn$^{2+}$ were measured continuously during the whole experiment (40–60 min) at 22 °C. Experiments on the long-term influence of extra-high Zn$^{2+}$ concentrations were performed with 5 mM ZnCl$_2$ in the medium. Plants were transferred from sterile solid control culture medium to sterile filter paper soaked with liquid medium containing 5 mM ZnCl$_2$. $E_M$ and $E_D$ (diffusion potential) changes in Zn$^{2+}$-treated plants were compared with those of control plants after 24 and 48 h of treatment. Fusicoxcin, a plasma membrane H$^+$-ATPase stimulator, was used to monitor the functionality of the plasma membrane H$^+$-pump (Marrè, 1979), at a final concentration of 30 μM in 0.1 % ethanol. To establish anoxic conditions for determination of $E_D$ values, the perfusion solution was saturated with N$_2$ gas by flushing. Flow of the perfusion solution through the measuring chamber at 10 mL min$^{-1}$ was sufficient to establish and to maintain anoxia (Pavlovkin et al., 1986).

Plant development and cell viability test

Control plants germinating and growing on control solid culture medium were passed 4–6 DAG to Petri dishes, where filter paper was soaked with liquid culture medium containing 0.1 mM KCl and 5 mM ZnCl$_2$. Seedlings in the control culture were cultivated in 0.1 mM KCl and 5 mM CaCl$_2$. pH was adjusted to 5.5 using 0.1 M HCl. Dishes 10 cm in diameter supplemented with 5 mL of the liquid culture medium were kept at an inclined position (at an angle less than 45°) to allow the filter paper to stay sufficiently wet and to keep the excess medium at the bottom of the plate. After 4 d under the controlled conditions (16 h light/25 °C and 8 h darkness/22 °C regime) plants were photographed documented for shoot and root morphology development. Resistance of root cells to this high Zn$^{2+}$ concentration was tested using the propidium iodide cell viability test with propidium iodide concentration of 2 μg ml$^{-1}$ in the control and/or Zn$^{2+}$-containing solution. Samples were examined with an Olympus FV1000 confocal microscope (Olympus, Japan) with excitation laser line 543 nm, and BA560–660 barrier emission filter.

Statistics

Experiments were performed in triplicate with $n = 10$ plants (in root growth rate and pH change experiments), $n = 6$ (in cell viability tests) or $n = 3$ plants with 12–25 measured cells per plant per treatment (in electrophysiological experiments). Statistical analyses of the data were carried out by two-way ANOVA test and Bonferroni post-test. Means were separated by the least significant difference test at $P < 0.05$. Means are presented ± s.d.
RESULTS

Root growth

The length of primary roots and morphology of the root system were used as test parameters for determination of the $\text{Zn}^{2+}$ sensitivity in three different Arabidopsis species. Growth efficiency of seedlings on solidified culture media with different concentrations of $\text{Zn}^{2+}$ reflected differences in their $\text{Zn}^{2+}$ tolerance (Fig. 1). A higher $\text{Zn}^{2+}$ concentration (1 mM $\text{ZnSO}_4$) in solid culture medium significantly diminished primary root growth of *A. thaliana* and induced enhanced formation of lateral roots (compare Fig. 1A and B). There was no apparent change, or only slight change, in root length in *A. arenosa* and *A. halleri* (Fig. 1A, B). At high $\text{Zn}^{2+}$ concentration (5 mM $\text{ZnSO}_4$), significant root growth and development of seedlings was recorded in the tolerant species *A. arenosa* and *A. halleri* (Fig. 1C), in contrast to *A. thaliana* plants. After appearance of the primary root and first lateral roots, elongation gradually declined and these roots did not survive through the experimental period (Fig. 1C). Although the length of primary roots of *A. halleri* plants was diminished at 5 mM $\text{ZnSO}_4$ (Fig. 1C), this species grew significantly better than *A. arenosa*, where only short primary roots and partially swollen lateral roots appeared (Fig. 1C). Arabidopsis *halleri* showed a similar phenotype only at extra-high $\text{Zn}^{2+}$ concentration in solid culture medium (10 mM $\text{ZnSO}_4$, Fig. 1D), which decreased the growth of *A. arenosa* seedlings to a minimum (Fig. 1D) and completely halted any development of *A. thaliana* (Fig. 1D). Thus, root growth analysis revealed a $\text{Zn}^{2+}$ tolerance range, apparently increasing from *A. thaliana* to *A. arenosa* to *A. halleri*.

This evidence is supported by two additional parameters. The index of tolerance quantified at 1 mM $\text{ZnSO}_4$ was 95 and 99 % for *A. halleri* and *A. arenosa*, respectively, compared with only 80 % for *A. thaliana* (Table 1). At high $\text{Zn}^{2+}$ concentration (5 mM $\text{ZnSO}_4$), the index of tolerance decreased for *A. arenosa* to a level comparable with *A. thaliana* (35 and 32 %, respectively), but remained higher for *A. halleri* (47 %, Table 1). The index of tolerance at 10 mM $\text{ZnSO}_4$ clearly revealed species-specific tolerance for *A. halleri* and *A. arenosa* (20 and 6 %, respectively) and sensitivity for *A. thaliana* (1 %, Table 1). Cellular tolerance data also revealed clear differences, where leaf cells of *A. halleri* and *A. arenosa* survived a 10-fold higher $\text{Zn}^{2+}$ concentration in comparison with *A. thaliana* cells (Table 1). Together, the data from testing of plants originating from natural metallocoious populations (*A. arenosa* and *A. halleri*) and control *A. thaliana* Col ecotype plants indicated intolerance of

![Fig. 1. Growth of seedlings of three Arabidopsis species on solidified culture media with different $\text{Zn}^{2+}$ concentrations. Seedlings of *A. thaliana*, *A. arenosa* and *A. halleri* grown on control medium (A), medium with 1 mM $\text{ZnSO}_4$ (B), 5 mM $\text{ZnSO}_4$ (C) and 10 mM $\text{ZnSO}_4$ (D) for 6 d. High $\text{Zn}^{2+}$ concentrations (1 and 5 mM $\text{ZnSO}_4$) significantly inhibited root growth in *A. thaliana* (B, C), less in *A. arenosa* (C) and much less in *A. halleri* (B, C). Extra-high $\text{Zn}^{2+}$ concentration (10 mM $\text{ZnSO}_4$) inhibited root growth of *A. arenosa* (D), stopped development of *A. thaliana* completely (D), but significant root growth of *A. halleri* was still recorded (D). Representative plants of at least three independent experiments are shown. Scale bar = 5 mm; 1 mm for inset in (D). *A. thaliana.*](http://aob.oxfordjournals.org/ by guest on May 2, 2016)

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<th>Table 1. Index of tolerance and cellular tolerance test of <em>A. thaliana</em>, <em>A. arenosa</em> and <em>A. halleri</em> plants</th>
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<td><strong>Index of tolerance (%) at different concentrations of $\text{ZnSO}_4$</strong></td>
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The index of tolerance was calculated as a ratio of primary seminal root length of plants 6 d after germination on solid culture medium with different concentrations of $\text{ZnSO}_4$ to primary seminal root length of similar plants in control condition. The highest index of tolerance represents the highest level of tolerance. Low levels of the index of tolerance indicate more severe inhibition of root growth rate due to sensitivity of plants to high concentrations of $\text{Zn}^{2+}$. A value of 100 % indicates no change of root growth rate at high concentrations of $\text{Zn}^{2+}$. Lower values indicate root growth inhibition and higher values indicate root growth stimulation by $\text{Zn}^{2+}$. The test of cellular tolerance was performed on surface-parallel sections from mature leaves, placed into graded concentrations of $\text{ZnSO}_4$ and scored for viability based on cell plasmolysis ability after 48 h.
A. thaliana and tolerance of A. arenosa and A. halleri to stress-inducing Zn$^{2+}$ concentrations.

Zn$^{2+}$-dependent changes in membrane potential ($E_M$)

The presence of high, toxic concentrations of Zn$^{2+}$ in the culture medium provoke defence and adaptation responses of plants. The first organ facing elevated Zn$^{2+}$ concentrations is the root. Higher Zn$^{2+}$ concentrations in the liquid medium and its perfusion to experimental plants allowed us to record measurable electrophysiological parameters of individual root cortical cells in short-term experiments. We recorded concentration-dependent changes in $E_M$. Effects of Zn$^{2+}$ were monitored during continuous exchange of the perfusion solution, stepwise with control culture medium applied first, followed immediately by culture medium with high Zn$^{2+}$ concentration. For adequate balancing of high concentrations of Zn and Cl, the resting potential was first measured at corresponding concentrations of CaCl$_2$, which were then replaced by the same concentrations of ZnCl$_2$, with permanent presence of 0-1 mM CaSO$_4$. Stable $E_M$ values of root cortical cells in control culture medium before Zn$^{2+}$ application indicated standard behaviour of all plasma membrane-resident processes (Figs 2 and 3). After treatment with different Zn$^{2+}$ concentrations, $E_M$ in mature root cortical cells of A. thaliana, A. arenosa and A. halleri showed dynamic changes. In A. thaliana root cells, immediately after adding 1 mM ZnCl$_2$ to the culture medium, $E_M$ transiently hyperpolarized by Δ$E$ = 14 ± 1-46 mV for 1–2 min (Figs 2–4), as was also the case with 0-1 and 0-5 mM ZnCl$_2$. The extent of initial hyperpolarization was much less in 2 mM and disappeared completely in 5 mM ZnCl$_2$ (Figs 2 and 3). In tolerant A. arenosa and A. halleri, the reaction to Zn$^{2+}$ in the medium always began with transient hyperpolarization, as was the case also at extra-high Zn$^{2+}$ content (Figs 3 and 4). In addition to its constitutive presence, the magnitude and duration of hyperpolarization was not the same in the two tolerant species. Hyperpolarization in A. halleri was much greater and longer than in A. arenosa (Figs 3 and 4). Interestingly, at 0-5 mM ZnCl$_2$ the transient hyperpolarization was the preferred reaction of A. halleri mature root cortical cells (Fig. 3).

After transient hyperpolarization the PM depolarized. The magnitude and duration of depolarization depended on Zn$^{2+}$ concentration and plant species. At all concentrations tested, the magnitude of the depolarization was higher in the sensitive A. thaliana than in the tolerant A. arenosa and A. halleri (Figs 3 and 4). In A. thaliana root cells considerable PM depolarization was recorded even at 0-1 mM ZnCl$_2$: 0-5 mM ZnCl$_2$ resulted in a similar but stronger depolarization curve. Although it appears to be an unspecific electrical reaction, as the threshold concentration of Zn$^{2+}$ in the culture medium inducing a depolarization of the cell membranes in roots was 0-5 mM in A. arenosa and 1 mM in A. halleri (Fig. 3). Full repolarization of cell membranes within the experimental period occurred at 0-5, 1 and 2 mM ZnCl$_2$ in A. arenosa root cells (Fig. 3). Because A. thaliana plants did not survive the treatment with 10 mM ZnCl$_2$ in the perfusion medium (Figs 3, 4), experiments with prolonged influence of extra-high Zn$^{2+}$ concentration was performed with 5 mM ZnCl$_2$ with all species. Plants were transferred from sterile solid control culture medium to sterile filter paper soaked with liquid medium containing 5 mM ZnCl$_2$ and changes of $E_M$ and $E_D$ (diffusion potential) were measured after 24 and 48 h treatment. $E_M$ in root cortical cells of A. thaliana was lowered after 24 h from −107 ± 8 to −93 ± 8 mV and even more after 48 h from −105 ± 8-6 to −88 ± 7 mV (Fig. 5). A large number of root cortical cells lost the capacity of the PM to maintain its active component of $E_M$, and only values lower than $E_D$ were registered [85 % (n = 66) and 89 % (n = 97)] of measured cells after 24 h and 48 h of treatment, respectively.

$E_M$ of A. arenosa, as a tolerant species, decreased less after 24 h (from −108 ± 7 to −100 ± 8 mV) than A. thaliana, but the drop particularly after 48 h of treatment from −105 ± 8 to −94 ± 5 mV was apparent and significant (Fig. 5). $E_M$ in root cortical cells of A. halleri was highly resistant with only a small decrease from −109 ± 6 to −106 ± 7-6 mV after 24 h and from −106 ± 7 to −103 ± 9-8 mV after 48 h (Fig. 5). A similar tendency was recorded also in gradual changes of $E_D$ among A. thaliana, A. arenosa and A. halleri plants after 24 and 48 h: a strong decrease occurred in A. thaliana, a moderate decrease in A. arenosa and only a small decrease in A. halleri (Fig. 5). When the hyperpolarizing compound fusicoccin (FC) was added to roots treated for 48 h with 5 mM ZnCl$_2$, a rapid increase of $E_M$ was observed. This increase started within 1 min and reached a maximum value of −111 mV in A. thaliana and −122 mV in A. halleri (Supplementary Data Fig. S1). These values did not agree with those usually observed in FC-treated control root cells (approx. −140 mV, data not shown). Although both plant species showed changes of $E_M$ of similar magnitude (24 and 28 mV for A. thaliana and A. halleri, respectively) the final value of $E_M$ of −111 mV in A. thaliana was lower than that of −122 mV in tolerant species A. halleri (Supplementary Data Fig. S1).

Morphology of seedlings influenced by prolonged Zn$^{2+}$ application

Together with physiological changes reflected by $E_M$ changes, the development and morphology of plants and the range of cell death in the root apex were documented. Plants cultivated for 4 d on sterile filter paper soaked with liquid culture medium containing 5 mM ZnCl$_2$ showed different symptoms of excess Zn$^{2+}$. Seedlings of the sensitive species A. thaliana were seriously damaged and did not survive. The shoots were yellow or transparent (Fig. 6A) and the roots showed no branching and stopped growth soon after transfer from control conditions (Fig. 6B). Tolerant plants of A. arenosa and A. halleri survived with less serious defects. Arabidopsis arenosa had red-to-yellow leaf coloration (Fig. 6A) and extensively branched roots (Fig. 6B). Although showing these symptoms, A. arenosa survived this treatment. However, the integrity of the aerial part and the structure of the root system under prolonged influence of this
extra-high Zn\(^{2+}\) concentration were better preserved in A. halleri (Fig. 6). Well-developed leaves were green (Fig. 6A) and roots contained numerous laterals (Fig. 6B). The cell viability test in roots revealed massive cell death in the root tip of A. thaliana, while only some individual cells or groups of cells died in the root tips of A. arenosa and A. halleri (Fig. 7). The number of dead cells was lower in A. halleri roots than in A. arenosa roots (Fig. 7B). The first signs of cell death in A. thaliana roots were detected just 24 h after transferring the plants to the medium containing 5 mM ZnCl\(_2\) (data not shown).

**Zn\(^{2+}\)-induced pH changes in liquid culture medium**

The relationship between high Zn\(^{2+}\) concentrations, growth rate of the primary root and properties of the medium was tested in 24-h experiments in liquid culture supplemented with three different concentrations of Zn\(^{2+}\). Seedlings were placed into micro-chambers made from microscopic slides and coverslips (Ovečka et al., 2005) and cultivated in liquid culture medium supplemented with 0·1, 1 or 2 mM ZnSO\(_4\). Root growth rate of seedlings and changes in pH of the liquid culture medium were determined in a 24-h culture period.
[ZnCl₂]

0.5 mM

A. thaliana

+Zn –119 mV

–114 mV

–115 mV

A. arenosa

+Zn –120 mV

–119 mV

–115 mV

A. halleri

+Zn –124 mV

–120 mV

–118 mV

1 mM

+Zn –113 mV

–107 mV

–91 mV

10 mV

10 min

2 mM

+Zn –107 mV

–96 mV

–81 mV

5 mM

+Zn –105 mV

–97 mV

–86 mV

10 mM

+Zn –120 mV

–87 mV

–83 mV

Fig. 3. Comparison of dynamic changes of $E_M$ in mature root cortical cells of *A. thaliana*, *A. arenosa* and *A. halleri* upon treatment with different concentrations of ZnCl₂ in culture medium. In comparison with *A. thaliana* root cortical cells, the reaction of *A. arenosa* and *A. halleri* root cortical cells to any concentration of Zn²⁺ in the medium always started with a short transient hyperpolarization period. The magnitude and duration of the subsequent depolarization were dependent on the Zn²⁺ concentration and plant species. There was no change of $E_M$ recorded in tolerant *A. arenosa* and *A. halleri* species below 0.5 mM ZnCl₂, and no reaction of root cells of *A. thaliana* to extra-high concentration of 10 mM ZnCl₂ was recorded. The time point of perfusion application of the Zn²⁺-containing medium is indicated by arrowheads. Representative curves of individual cells ($n = 12–25$) are shown.
Seedlings were 2–3 DAG old at the time of transfer to liquid culture and only primary roots were developed. The growth rate of the primary root was influenced differently according to Zn$^{2+}$ concentrations. ZnSO$_4$ at 1 mM caused a lower primary root length in all three Arabidopsis species. However, only 7 and 13 % less length were recorded in A. halleri and A. arenosa, respectively, while the difference was 26 % in A. thaliana (Fig. 8A). Root growth inhibition was much more apparent in the presence of 2 mM ZnSO$_4$, where both A. thaliana and A. arenosa roots grew only up to 55 and 57 % of the control, respectively (representing 45 and 43 % inhibition, respectively; Supplementary Data Fig. S2A). Seedlings of A. halleri were most resistant to this concentration and inhibition of their root growth rate was only 26 % (Supplementary Data Fig. S2A). Interestingly, the medium containing 0-1 mM ZnSO$_4$ did not inhibit root growth rate, but root growth rate was even slightly stimulated. Roots of A. thaliana were 18 % and A. arenosa 12 % longer after 24 h of 0-1 mM ZnSO$_4$ treatment and although not significant, A. halleri expressed a slight increase in root growth rate (4 %; Supplementary Data Fig. S2A).

Addition of ZnSO$_4$ induced pH changes in the medium depending on the Zn$^{2+}$ concentrations. We analysed the pH of the control medium with and without plants, as well as ZnSO$_4$-enriched medium with and without plants at the beginning (0 h) and at the end of the experiment (24 h later) (Fig. 8B–D). The pH of the control culture medium without plants was more or less constant. Plants growing in the control medium (ten plants in 25 ml medium per repetition) changed the pH to higher values. The original pH 5.5 rose in all control culture media with plants and resulted in pH 5.73–5.86 in all variations of the experiment (Fig. 8B–D, Supplementary Data Fig. S2B). The situation was different in culture media with added Zn$^{2+}$. For all concentrations of ZnSO$_4$ tested (0-1, 1 and 2 mM), the pH 5.5 adjusted at the beginning dropped after Zn$^{2+}$ addition at time 0 h and before transfer of the experimental plants (Fig. 8B–D, Supplementary Data Fig. S2B). Twenty-four hours after adding ZnSO$_4$ to the culture medium without plants, pH reached 5.23–5.38 with 0-1 mM, 5.04–5.10 with 1 mM and 4.79–4.99 with 2 mM ZnSO$_4$ (Fig. 8B–D, Supplementary Data Fig. S2B). Plants present in the culture media containing different concentrations of ZnSO$_4$ for 24 h effectively adjusted the pH of the medium only at 0-1 mM ZnSO$_4$. In media with high concentrations of ZnSO$_4$ and plants present in the medium, pH still tended to increase, but the shift was minimal (Fig. 8B–D, Supplementary Data Fig. S2B).

Together, the trend of pH changes was similar in all species when influenced by the same ZnSO$_4$ concentration. There were only slight differences in the extent of the pH change (ΔpH) at the lowest concentration. To ensure that pH of the culture medium is excluded as a primary explanation for the electrophysiological changes recorded here, a negative control experiment was performed. The state of $E_M$ after exchanging the media of different pH would discriminate between unspecific effects induced by the pH change itself and specific effects induced by Zn$^{2+}$. Perfusion with culture medium of pH 6.5 immediately followed by culture medium of pH 5.5 induced only a slight decrease of $E_M$ (Supplementary Data Fig. S3A), but not with the curve shape typical for Zn$^{2+}$ application (Figs 2 and 3). In addition, such root cells pretreated with culture medium of changed pH value responded to added ZnSO$_4$ with the typical hyperpolarization and subsequent depolarization (Supplementary Data Fig. S3B). This typical membrane reaction was Zn$^{2+}$ concentration- and species-dependent, as documented by application of 1 mM ZnSO$_4$ to A. arenosa (Supplementary Data Fig. S3B) and A. thaliana (Supplementary Data Fig. S3C) root cortical cells.

**DISCUSSION**

Uptake of heavy metals by plants from the soil in its complexity depends in many aspects on the activity and regulation of PM-located processes. Structural, physical and chemical properties of the PM itself as well as any effects of metal ions at the cell surfaces in general have an impact on transport processes. Here we show a time course of rapid and short-term changes of the PM immediately after the root cells were exposed to elevated concentrations of Zn$^{2+}$ in the culture medium. Zn$^{2+}$ in different concentrations induced rapid membrane changes shown as initial hyperpolarization and subsequent depolarization in mature root cortical cells. The magnitude of the two alterations in $E_M$ was concentration-dependent. Membranes in the depolarized state were able partly to repolarize in the presence of the same Zn$^{2+}$ concentration. The ability to repolarize the PM spontaneously after being strongly depolarized seems to be a non-specific electrical reaction that indicates Zn$^{2+}$-induced changes in the charge at the cytoplasmic face.

**Fig. 4.** Mean values (± s.d.) of (A) membrane hyperpolarization and (B) depolarization in cortical root cells of A. thaliana, A. arenosa and A. halleri upon treatment with different concentrations of ZnCl$_2$ in the culture medium. Different letters indicate a significant difference at $P<0.05$. 

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of the PM. PM depolarization can generally be induced by transmembrane movement of different ions, and thus by influx of H\(^+\), K\(^+\) or Ca\(^{2+}\) and by efflux of Cl\(^-\) or K\(^+\) or as a consequence of a decrease in H\(^+\)-ATPase activity. If the changes in \(E_M\) are induced by a certain stimulus, it may be considered as a primary signal leading to activation of a relevant intercellular signalling cascade. For different biotic and abiotic stresses, changes in \(E_M\) and related alterations in ion fluxes at the PM are usually regarded as the earliest cellular responses (Ebel and Mithöfer, 1998).

In agreement with the effects of Zn\(^{2+}\) on total health and growth of the plants and together with the fact that the magnitude of the recorded changes was concentration-dependent in all three species of *Arabidopsis* tested, a valuable result of this comparative analysis is that the reaction of root cells to high Zn\(^{2+}\) concentration was clearly species-specific.
A typical pattern of initial hyperpolarization and subsequent depolarization of $E_M$ shows a similar course in all three studied Arabidopsis species at micro- and millimolar Zn$^{2+}$ concentrations. But at highest Zn$^{2+}$ concentrations, a lack of hyperpolarization and the magnitude of depolarization discriminated between Arabidopsis species. The rate of hyperpolarization in A. thaliana, lasting 1–2 min and not exceeding 3 mV at concentrations up to 1 mM ZnCl$_2$, was clearly different from that in A. halleri, where the hyperpolarization was five- to six-fold higher (Figs 3 and 4). Arabidopsis arenosa root cells reacted to Zn$^{2+}$ by immediate hyperpolarization of a similar magnitude as that of A. thaliana. However, in contrast to A. thaliana cells, this appeared as the primary reaction at all Zn$^{2+}$ concentrations, even with extra-high Zn$^{2+}$. This was also found for A. halleri root cells, although the hyperpolarization was stronger than that of A. arenosa (Fig. 4). Conversely, the ability to hyperpolarize A. thaliana cells was considerably lower at 2 mM and was completely absent at 5 mM ZnCl$_2$. Another difference between A. halleri and A. thaliana root cells was that hyperpolarization phases lasted considerably longer in A. halleri, and also were longer than in A. arenosa. The magnitude and duration of depolarization were also concentration-dependent and closely related to the plant species. As with the hyperpolarization phase of the reaction, the magnitude of the depolarization was highest in A. thaliana and lowest in A. halleri.

This biphasic reaction of Arabidopsis root cells to Zn$^{2+}$, and particularly of the initial hyperpolarization, could be explained by both a dependence of PM-$E_M$ on the ion concentration and transport velocity across the PM and species-specific tolerance as main factors. We suggest that upon addition of a Zn$^{2+}$ salt to the perfusion solution in $E_M$ experiments, Zn$^{2+}$ may at first replace H$^+$ at the apoplastic side of the PM, causing a short hyperpolarization, which is soon counteracted by the Zn$^{2+}$-induced PM depolarization at the cytoplasmic side upon Zn$^{2+}$ influx. This effect was particularly evident in A. halleri at 1 mM Zn$^{2+}$, where the hyperpolarization was most conspicuous because the intracellular depolarizing Zn$^{2+}$ was rapidly detoxified, probably removed by complexing.

These differences in $E_M$ were fully consistent with Zn$^{2+}$ sensitivity levels of the three different Arabidopsis species recorded in the root growth rate analysis (Figs 1 and 8A), and in principle also with the index of tolerance and in cellular tolerance (Table 1). This was further supported by higher protection of whole plants of A. halleri and A. arenosa as well as by greater cell viability in roots under exposure to high Zn$^{2+}$ concentration for several days. Together, these data underlined the intolerance of A. thaliana and tolerance of A. arenosa and A. halleri to high Zn$^{2+}$ concentrations. The immediate responses of Arabidopsis plants are apparently expressed in developmental changes of the whole roots that are visible at a later stage, reflecting long-term responses. As an example, root thickness and root tissue volume were significantly lower in the sensitive A. thaliana than in the tolerant A. halleri and A. arenosa. Interestingly, root thickness in the...
tolerant species increased under high Zn\(^{2+}\) concentrations. Thus, the rate of root growth and quantitative root anatomy may exhibit morphological traits contributing to heavy metal tolerance of *Arabidopsis* species (Štaňová *et al.*, 2012).

Root cells treated with heavy metal ions normally express changes at the PM that can be recorded as changes of *E_M*, which may also indicate, in addition to ion uptake into the cell, alterations in membrane permeability. PM depolarization, however, could be just a transient change, without alteration of membrane permeability. Although this process is highly concentration-dependent, no permanent effects on PM permeability always must occur. For example, treatment of rice plants with 0.5 mM Ni\(^{2+}\) induced a concentration-dependent transient PM depolarization in root cells, but the activity of the PM H\(^{+}\)-ATPase was not inhibited by the nickel ions in short-term experiments (up to 8 h). Spontaneous repolarization was observed still in the presence of nickel ions, but if the treatment lasted for several days, a drastic loss of K\(^+\) was recorded (Llamas *et al.*, 2008). PM depolarization, however, may be a consequence of inhibitory effects on PM H\(^{+}\)-ATPase activity, particularly at higher concentrations of heavy metal ions, as was suggested for cadmium in *Nitellopsis obtusa* cells (Kurtyka *et al.*, 2011a). Cd\(^{2+}\) was found to be a critical factor for successful hyperpolarization induced by FC. In maize coleoptile segments, hyperpolarization was not affected at 100 \(\mu\)M, but was suppressed at 1000 \(\mu\)M Cd\(^{2+}\) (Kurtyka *et al.*, 2011b). When we applied FC to root cells of *Arabidopsis* species treated for 48 h with 5 mM ZnCl\(_2\), a rapid increase of *E_M* was observed (Supplementary Data Fig. S1). This clearly indicates that Zn\(^{2+}\) at high concentrations did not inhibit plasma membrane H\(^{+}\)-ATPase to any considerable extent in mid- and long-term experiments with an excess of Zn\(^{2+}\).

It has been reported and well documented that the activities of enzymes located in the cell wall, within the plasma membrane and in the cytoplasm are modified during local changes in ion concentrations (Davies, 2004). Thus, the propagation of electrical signals in plants mediated by ion channels, their generation upon certain stimuli and related physiological consequences have been the focus of many studies. The data from such studies highlight the importance of pH changes.

Liquid media used for determination of plant root growth rate in 24-h experiments were prepared on the basis of MS salts. They contained 0.01 mM ZnSO\(_4\) in the control and the original pH was adjusted to 5.5, as optimal for root growth. In variants with increasing Zn\(^{2+}\) concentration, the initial pH gradually decreased upon Zn\(^{2+}\) addition. The pH shift was dependent on the ZnSO\(_4\) concentration, decreasing from pH 5.23 in media with 0.1 mM to pH 4.8 with 2 mM ZnSO\(_4\). With the control medium showing constant pH during the whole experiment, these basic settings of the medium allowed us to determine pH changes due to buffering activity of the plants. Control plants increased the pH from the original value of 5.5 to 5.73–5.86 within 24 h. Plants growing in the culture media with 0.1 mM ZnSO\(_4\) raised the pH by 0.2–0.4 units. In media with high concentrations of ZnSO\(_4\), the differences
in pH were minimal (ΔpH 0.04–0.07 for 1 mM and 0.06–0.09 for 2 mM ZnSO₄). Interestingly, no clear differences were observed in the trend of pH changes between Arabidopsis species when treated with the same ZnSO₄ concentration. Under normal circumstances when plants are cultivated in culture media at slightly acidic pH, an alkalinizing activity of root cells is found that works normally. In the pH range of 3.7–5 in the culture MS medium, seedlings of A. thaliana were able to restore the pH to values reaching 6 within 3 d. This ability is not affected to any great extent or prevented by treatment of plants with sodium orthovanadate, an inhibitor of membrane ATPases (Loyola-Vargas et al., 2007). This clearly indicates that inhibition of H⁺-ATPase in the plasma membrane did not prevent ‘buffering’ activities of root cells and hence the pump itself was not indispensable for fine and local pH tuning in the close rhizosphere.

Closely connected to the ability of plants to modulate the pH of the soil solution, the form of N supply in the rhizosphere is known to play a central role. The mechanism of nitrate uptake has been demonstrated to be one of H⁺-nitrate co-transport (Ullrich, 1987) at a 2:1 stoichiometry between H⁺ and nitrate co-transport. The remaining cation concentration can explain the alkalinization of the medium during nitrate uptake (Mistrik and Ullrich, 1996). In general, the uptake of NH₄⁺ cations results in soil acidification. Thus, the toxicity of certain elements may also be related to nitrogen-related changes in soil pH (reviewed by Miller and Cramer, 2004). Uptake of NO₃⁻ is closely associated with the activity of proton-pumping ATPase (H⁺-ATPase) that maintains the electrochemical potential gradient across the PM to drive the co-transport (McClure et al., 1990). However, the role of manipulated, and therefore also plant-induced, pH contradicts the pH hypothesis, because plants of Deschampsia caespitosa grown in solutions with high NO₃⁻ supply did not show tolerance to Zn²⁺ different from plants grown in solutions with high NH₄⁺ supply (Smirnoff and Stewart, 1987).

Root cells of Arabidopsis species growing in media with high Zn²⁺ concentrations were faced with unfavourable external pH forcing them to activate their pH-equilibrating capacity, because it is known that Zn²⁺ added in the form of ZnSO₄ normally induces soil acidification due to the remaining anions (White et al., 1979a, b). Although not generalizable, there is evidence supporting active equilibration of rhizosphere pH by plant roots to avoid or diminish metal toxicity symptoms. Exposure of A. thaliana to aluminium, which is known to induce developmentally dependent specific reactions of root cells (Illeš et al., 2006), was accompanied by specific changes of pH in the rhizosphere solution. A close connection between aluminium toxicity and low pH has been described extensively. While the pH of the root solution of wild-type plants remained unchanged during the experiment, the Al-tolerant mutants caused an increase of the solution pH from 4.3 to 4.5. Interestingly, this pH increase supported root growth, while buffering the solution prevented the Al tolerance behaviour of the mutant connected with increases in rhizosphere pH (Degenhardt et al., 1998).

Acidification of the root rhizosphere can also influence other physiological parameters of the root cells. In addition to transport of ions and nutrients, the water permeability of the PM has also been shown to be lowered by acidification of the
medium (Amodeo et al., 2002; Gerbeau et al., 2002; Tournaire-Roux et al., 2003). Short-term regulation of water transport of Beta vulgaris PM vesicles was extremely sensitive to low pH. Acidification at the cytoplasmic face of the membrane was responsible for shut-down of the aquaporins. High sensitivity of the PM water transport to pH and Ca\(^{2+}\) concentration changes on the cytoplasmic face of the membrane expressed by closure of aquaporins establishes membrane charges as one of the signalling mechanisms (Alleva et al., 2006).

Low pH of the rhizosphere, in addition to enhancing the heavy metal toxicity in the soil, affects root growth of the plants (Koyama et al., 1995, 2001; Lazof and Holland, 1999; Kidd and Proctor, 2001). Tolerance of plants to low pH, however, has only rarely been studied in detail. Examination of A. thaliana for its rhizosphere alkalinizing capacity, intracellular K\(^+\) concentration and changes in PM-E\(_{\text{M}}\) revealed that the low pH treatment depolarized the PM in both the distal elongation zone of the root and mature root zone. In unbuffered experiments, wild-type plants increased the pH of the medium from 4.2 to approx. 5.2 within 12 h and to approx. 5.7 within 24 h of treatment. In all genotypes tested, the low pH treatment induced an increased H\(^+\) influx in both distal elongation and mature root zones (Bose et al., 2010). This emphasizes an alkalinization capacity of Arabidopsis roots and their ability to grow under low pH. Making the rhizosphere less acidic is closely linked to the lowering sensitivity of plants to heavy metals, which in a certain range can be assessed as a primary non-specific but direct action of plant root cells to prevent excessive intoxication by reactive heavy metal ions. Arabidopsis mutants altered in aluminium resistance provide an example: more resistant plants versus less resistant plants may differ in the ability to actively equilibrate rhizosphere pH. Based on these data, one of the main aims of our study was to test an ability of A. thaliana, A. halleri and A. arenosa plants to resist and actively respond to changes of external pH and if this activity is related to their Zn\(^{2+}\)-related stress responses. The differences in E\(_{\text{M}}\) observed between sensitive A. thaliana and highly tolerant A. halleri and A. arenosa might be explained by a Zn\(^{2+}\)-detoxifying mechanism in the cells. Zn\(^{2+}\) depolarized the inner PM surface upon influx, most strongly in A. thaliana, a plant which cannot detoxify entering Zn\(^{2+}\) and thus suffering from metabolic inhibition, although less Zn\(^{2+}\) is taken up. Also in A. halleri, Zn\(^{2+}\) induced rapid PM depolarization, but the detoxifying property immediately diminished the concentration of entered Zn\(^{2+}\), resulting in reduced PM depolarization. This hypothesis and the present data may help to better characterize the complex process of heavy metal stress tolerance, which is primarily species-based, but also modulated by physiological reactions in root cells. Properties of the PM and regulation of trans-membrane ion gradients are integral parts of the heavy metal stress-relieving strategy. Further study should reveal the significance of the PM electrical and molecular properties in plant heavy metal tolerance. The results presented here indicate that monitoring of rapid membrane voltage changes may be an excellent tool to monitor heavy metal effects on plants. With this method, plants can be rapidly, easily and efficiently checked for their sensitivity, heavy metal-detoxifying properties and velocity of the detoxifying mechanisms.

Conclusions

We characterized physiological responses to high and toxic Zn\(^{2+}\) concentrations in culture among three Arabidopsis species with diverse strategies of their adaptation to heavy metals. The level of metal tolerance in different plant species may also include basal defence mechanisms, such as transmembrane ion fluxes. All measured parameters, including root E\(_{\text{M}}\), underlined the sensitivity of A. thaliana and high tolerance of A. halleri and A. arenosa. The set of anti-stress reactions presented here may represent the group of first, non-specific defence reactions, commonly activated in all plants, although with distinct magnitude and effectiveness in tolerant and sensitive genotypes or species.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: reaction of depolarized root PM to 30 \(\mu\)M FC in root cortical cells of A. thaliana and A. halleri treated with 5 mM ZnCl\(_2\) for 48 h. Figure S2: effect of Zn\(^{2+}\) on root growth and pH changes in liquid culture medium. Figure S3: E\(_{\text{M}}\) changes for different pH values of the culture medium.

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LITERATURE CITED


