The cyclic nucleotide-gated channel AtCNGC10 transports Ca\(^{2+}\) and Mg\(^{2+}\) in Arabidopsis

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The suppression of the cyclic nucleotide-gated channel (CNGC) AtCNGC10 alters K\(^{+}\) transport in Arabidopsis plants. Other CNGCs have been shown to transport Ca\(^{2+}\), K\(^{+}\), Li\(^{+}\), Cs\(^{+}\) and Rb\(^{+}\) across the plasma membrane when expressed in heterologous systems; however, the ability of the AtCNGC10 channel to transport nutrients other than K\(^{+}\) in plants has not been previously tested. The ion fluxes along different zones of the seedling roots, as estimated by the non-invasive ion-specific microelectrode technique, were significantly different in two AtCNGC10 antisense lines (A2 and A3) in comparison to the wild type (WT). Most notably, the influxes of H\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) in the meristem and distal elongation zones of the antisense A2 and A3 lines were significantly lower than in the WT. The lower Ca\(^{2+}\) influx from the external media corresponded to a lower intracellular Ca\(^{2+}\) activity, which was estimated by fluorescence lifetime imaging measurements (FLIM). On the other hand, the intracellular pH values in the meristem zone of the roots of A2 and A3 seedlings were significantly lower (more acidic) than that of the WT, which might indicate a feedback block of H\(^{+}\) influx into meristematic cells caused by low intracellular pH. Under the control conditions, mature plants from the A2 and A3 lines contained significantly higher K\(^{+}\) and lower Ca\(^{2+}\) and Mg\(^{2+}\) content in the shoots, indicating disturbed long-distance ion transport of these cations, possibly because of changes in xylem loading/retrieval and/or phloem loading. Exposing the plants in the flowering stage to various K\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) concentrations in the solution led to altered K\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) concentrations in the solution led to altered K\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) content in the shoots of A2 and A3 plants in comparison with the WT, suggesting a primary role of AtCNGC10 in Ca\(^{2+}\) (and probably Mg\(^{2+}\)) transport in plants, which in turn regulates K\(^{+}\) transporters’ activities.

Introduction

The Arabidopsis genome contains 20 members of the cyclic nucleotide-gated channel (CNGC) family (Maser et al. 2001, Talke et al. 2003). Despite intensive work on heterologous expression and knockout studies, the function of these channels in plants is still unclear (Chan et al. 2003, Gobert et al. 2006, Kaplan et al. 2007, Leng et al. 1999, Leng et al. 2002, Li et al. 2005). There are indications that CNGCs are involved in plant response...
to pathogens because AtCNGC4 mutants demonstrated loss of hypersensitive response death (Jurkowski et al. 2004). In addition, several CNGCs were shown to be involved in pollen development (Bock et al. 2006), and AtCNGC18 mutant was found to be male sterile (Frietsch et al. 2007).

CNGC channels in plants have been suggested as potential candidates of voltage-independent non-selective cation channels (Demidchik et al. 2002, Kaplan et al. 2007, Very and Sentenac 2002). In addition, some Arabidopsis CNGC mutants demonstrated altered cation content in plants, indicating the involvement of CNGCs in ion uptake and translocation (Chan et al. 2003, Guo et al. 2008, Li et al. 2005). CNGCs might also be involved in the uptake of toxic ions, such as Pb2+ (Sunkar et al. 2000) and Na+, into plant cells (Gobert et al. 2006, Guo et al. 2008, Sunkar et al. 2000).

Although the channels from the Arabidopsis CNGC family have sequence similarity ranging between 55 and 83%, they have different ion specificity. The sequence of the pore filter of plant CNGCs is different from sequences found in animal CNGCs and plant K+ shaker channels, which might make them different in selectivity towards K+, Na2+, Ca2+ and other ions (Kaplan et al. 2007). Electrophysiological studies have revealed that AtCNGC1 and AtCNGC4 can transport cations, such as Na+ and K+ (Balague et al. 2003, Leng et al. 2002). AtCNGC3 is a non-selective ion transporter of Na+ and K+ involved in seed germination (Gobert et al. 2006). In contrast, AtCNGC2 is selective to K+, is permeable to Ca2+ (Hua et al. 2003) and can transport other monovalent cations (Li+, Cs+ and Rb+), but the influx of Na+ is low (Leng et al. 1999, Leng et al. 2002). However, AtCNGC2 has a unique amino acid sequence in the selective filter (Kaplan et al. 2007), which might make it stand alone among other plant CNGCs. Heterologous expression of CNGC18 in Escherichia coli demonstrated time- and concentration-dependent accumulation of Ca2+ (Frietsch et al. 2007). Complementation analysis using a Ca2+-uptake-deficient yeast mutant demonstrated that the AtCNGC11/12 channel is permeable to Ca2+ (Urquhart et al. 2007). AtCNGC10 expression rescued potassium channel mutants of E. coli (LB650) and yeast (CY162) (Li et al. 2005), indicating its potential involvement in K+ transport.

This variation in cation transport among members of the highly conserved CNGC family has caused different phenotypes in CNGC mutants. The AtCNGC1 knockout has improved tolerance to Pb2+ (Sunkar et al. 2000) and contains lower Ca2+ concentrations in the tissues (Ma et al. 2006). AtCNGC3 mutants are sensitive to Na+ toxicity during germination and have altered monovalent cation content (Gobert et al. 2006). Knockout AtCNGC2 plants are hypersensitive to Ca2+ but not to Na+ or K+ (Chan et al. 2003). An analysis of AtCNGC2 mutants revealed the function of this channel in Ca2+-mediated plant development as well as in disease resistance and programmed cell death (Clough et al. 2000). In addition, AtCNGC10 conducts Ca2+ into plant leaf cells and is linked to NO signalling pathway (Ali et al. 2007). Likewise, AtCNGC4 mutants have altered responses to hypersensitive pathogen resistance signalling (Balague et al. 2003) as do AtCNCG11 and AtCNCG12 (Yoshioka et al. 2006). In our previous study, we demonstrated altered K+ and Na+ uptake by AtCNGC10 mutants (Guo et al. 2008) and varied salt tolerance at different developmental stages. In order to elucidate the relationship between the functions of the AtCNGC10 channel and cation uptake, we tested the ability of the AtCNGC10 channel to transport Ca2+, Mg2+ and K+ to the roots and shoots. In this study, we used AtCNGC10 antisense knockdown lines to non-invasively measure ion fluxes along the roots, to assess the intracellular concentrations of Ca2+ and H+ in the root tips using fluorescence lifetime imaging measurement (FLIM) analysis and to estimate the long-distance transport of ions based on changes in the ion content in the shoots and roots under plant exposure to high and low concentrations of Ca2+, Mg2+ and K+.

Materials and methods

Plant material and growth conditions

Wild-type (WT) Arabidopsis plants [Arabidopsis thaliana L. (Heynh)] ecotype Col-0 and AtCNGC10 antisense lines, A2 and A3, in a Columbia background were grown in a temperature-controlled chamber with a cycle of 16 h light (75 µmol m–2 s–1) and 8 h dark at 20 ±1°C. Seeds were surface-sterilised, placed on a thin (1–2 mm) rock-wool base and put into a 1/4 Hoagland nutrient solution (0.25 mM KH2PO4, 1.25 mM KNO3, 1.25 mM Ca(NO3)2, 0.5 mM MgSO4, 5 µM FeEDTA, 11 µM H3BO3, 2.3 µM MnCl2, 0.2 µM ZnCl2, 0.07 µM CuCl2 and 0.03 µM Na2MoO2) that was changed every 3 days. The WT was germinated 10 days earlier than A2 and A3 to synchronise the growth stage for the measurements. Low K+ and high Ca2+ and Mg2+ treatments were applied at the same developmental stage (at the start of the budding stage, which is easy to identify). Instead of having the original K+, Ca2+ and Mg2+ ion concentrations in the control (1/4 Hoagland solution), there was 0.05 mM K+ in the low-K treatment, 5 mM Ca2+ in the high-Ca treatment and 5 mM Mg2+ in the high-Mg treatment.

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For the ion flux, intracellular pH and cytosolic Ca\(^{2+}\) measurements, seeds were surface sterilised and placed into Petri dishes on agar media [basic salt medium (BSM): 1 mM KCl, 0.1 mM CaCl\(_2\) and 0.8% w/v agar]. Petri dishes were placed vertically in a growth chamber (25/20°C, 14/10 h day/night cycle, 120 μmol m\(^{-2}\) s\(^{-1}\)).

**Ion fluxes along the root surface**

Ion fluxes were measured non-invasively using the MIFE\(^\text{®}\) system (University of Tasmania, Hobart, Australia) as described by Newman (2001). The electrodes were pulled from borosilicate glass capillaries (GC150-10, Harvard Apparatus, Kent, UK), dried at 230°C for about 5 h and silanised with tributylchlorosilane (90765, Fluka Chemicals, Castle Hill, NSW, Australia). The tips of the dried and cooled electrode blanks were broken to a diameter of approximately 5 μm and then back-filled with the appropriate solutions. The back-filling solutions were 15 mM NaCl and 40 mM KH\(_2\)PO\(_4\) for the hydrogen electrode, 500 mM KCl for the potassium electrode, 500 mM CaCl\(_2\) for the calcium electrode and 500 mM MgCl\(_2\) for the magnesium electrode. Immediately after back-filling, the electrode apices were front-filled with commercially available ionophore cocktails for measuring hydrogen (#95297, Fluka Chemicals), calcium (#21048, Fluka Chemicals) and magnesium (#63048, Fluka Chemicals). A reference electrode was fabricated in a similar way from a borosilicate glass capillary and filled with 0.1 M KCl in 1% (w/v) agar. Electrodes were calibrated against a range of standards. Electrodes with responses less than 50 mV per decade were discarded.

**FLIM measurements**

**Dye loading**

Both fluorescent dyes 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) acetoxymethyl ester (Molecular Probes, Eugene, OR) and Calcium Green\™ (Ca-G-AM, Molecular Probes) were dissolved in dimethylsulphoxide (DMSO) (Sigma, Castle Hill, Australia) and diluted with a loading solution (0.2 mM CaCl\(_2\) and 50 mM mannitol, pH 4.2) to a final concentration of 20 μM. The final concentration of DMSO in the loading solution was 1% v/v. After a 2-h treatment in the loading solution on ice, plants were placed into the BSM solution for 30 min to recover.

**Measurements**

For two-photon FLIM measurements, a seedling was placed in the chamber on the stage of an inverted confocal microscope (Leica TCS SP2 AOB, Leica Microsystems GmbH, Wetzlar, Germany). Light pulses were generated by a Mai Tai Laser (Spectra Physics, Mountain View, CA). The fluorescence was recorded by photo multipliers, and the FLIM analysis was performed using electronics (SPC-730; Becker & Hickl, Berlin, Germany) and software (SPC.7.22; Becker & Hickl) for time-correlated single-photon counting (O’Connor and Desmond 1984). Lifetime images were analysed using SPCIMAGE Version 2.6 (Becker & Hickl).

For BCECF calibration, we used the median lifetime, assuming a single-exponential decay as described earlier (Babourina and Rengel 2009, Guo et al. 2009).

For the FLIM calibration of cytosolic Ca\(^{2+}\) activity ([Ca\(^{2+}\)\(_\text{cyt}\)], a stock solution of Ca-Green potassium salt was prepared in a 0 mM Ca\(^{2+}\) buffer (Calcium Calibration Kit #2, Molecular Probes). Immediately after preparation, the stock solution was diluted to 2 μM in
different Ca\(^{2+}\) buffers (free Ca\(^{2+}\) concentration ranged from 0 to 1 mM). For calibration purposes, we used the ratio of intensities of the first component and the second component \((\alpha_1/\alpha_2)\), assuming a double-exponential decay for Ca-Green as described earlier (Guo et al. 2009).

The seedlings were attached to the cover glass polarised with a drop of a poly-L-lysine solution (Sigma) for about 30 min before rinsing with de-ionized water. To localize the solution to a certain area, an Aqua-Hold Barrier Pap Pen was used to draw a line along the edges of the cover slip. Each lifetime image of the plant was taken for 5 min. The emission wavelength was 940 nm (preliminary experiment demonstrated that this wavelength minimized contribution from autofluorescence). The internal \([\text{Ca}^{2+}]_{\text{cyt}}\) and pH were calculated according to calibration curves using Data Analysis Software for Fluorescence Lifetime Imaging Microscopy Systems (SPCIMAGE Version 2.6, MP-FLIM and D-FLIM).

**Statistical analysis**

For different treatments or different genotypes, significant differences between means were assessed by MS Excel Software for t-tests and Genstat (8th edition) (VSN International Ltd, Hemel Hempstead, UK) for ANOVA analyses.

**Results**

**Chlorophyll content and ion composition under control conditions**

A2 and A3 plants were smaller than WT in size (Fig. 1A). The average weight of a 5- to 6-week single plant of WT, A2 and A3 was 26.2 ± 3.2, 5.6 ± 0.1 and 8.2 ± 0.9 mg, correspondingly.
Chlorophyll a and b contents were higher in leaves of WT than in leaves from A2 and A3 plants under control conditions, \( \frac{1}{4} \) Hoagland solution (Fig. 1B). Contents of both chlorophyll a and b were significantly lower in A2 plants than in WT, whereas chlorophyll a content in A3 plants was significantly lower only at \( P \leq 0.01 \). The combined content of chlorophyll a and b \((a + b)\) was significantly lower for both antisense lines compared with WT \((P \leq 0.05)\). Although the chlorophyll a/b ratio of WT was higher than in both lines, it was only was significantly lower in A3 at \( P \leq 0.05 \) (Fig. 1B).

**H\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) fluxes along different root zones**

The fluxes of H\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) were measured at three positions (the meristem, distal elongation and mature zones) along the seedling roots of the WT and antisense A2 and A3 AtCNGC10 lines (Fig. 2). In all the genotypes studied, the H\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) fluxes demonstrated the highest positive values (influx) in the meristematic zone compared with the other root zones. However, the A2 and A3 lines had significantly lower H\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) influxes than the WT \((P \leq 0.05)\).

**Intracellular Ca\(^{2+}\) and pH**

The intracellular pH values of the root meristematic cells were more acidic in the A2 and A3 seedlings than the WT \((P \leq 0.05)\). No significant difference among the genotypes was found in the elongation and mature zones (Fig. 3).

The root meristematic cells of the A2 and A3 seedlings had lower [Ca\(^{2+}\)]\(_{cyt}\) values than found for the WT,
whereas the root distal elongation and mature zones of the A2 and A3 lines were not different from the WT (Fig. 4).

**Low-K⁺, high-Ca²⁺ and high-Mg²⁺ treatments**

Under control conditions (1/4 Hoagland solution), the shoots of the AtCNGC10 antisense lines A2 and A3 had significantly lower Ca²⁺ and Mg²⁺ and higher K⁺ content compared with the WT (Fig. 6). Hence, we tested whether altering the K⁺, Ca²⁺ and Mg²⁺ concentrations in the growth media would alter nutrient accumulation in the A2 and A3 lines compared with the WT. Based on the preliminary experiments in which we assessed WT plant growth in response to different external concentrations of K⁺, Mg²⁺ and Ca²⁺ (data not shown), we chose the following treatments: 0.05 mM K⁺ for low-K⁺, 5 mM Ca²⁺ for high-Ca²⁺ and 5 mM Mg²⁺ for high-Mg²⁺ treatments instead of the original K⁺, Ca²⁺ and Mg²⁺ concentrations in the 1/4 Hoagland solution.

All lines studied responded similarly in biomass accumulation when the Ca²⁺ and Mg²⁺ concentrations were higher in the growing solution: the shoot and root biomass increased (Fig. 5A, B). There was a trend in increasing A2 and A3 the shoot biomass under low-K⁺ treatment and the whole-plant biomass of A2 and A3 under high-Mg²⁺ treatment, in comparison with WT (Fig. 5).

There were no significant differences in the root K⁺, Ca²⁺ and Mg²⁺ content between the WT and A2 or A3 lines (Fig. 6B, D, F) under control conditions. In all the treatments, there was a trend of higher K⁺ and lower Ca²⁺ and Mg²⁺ content in the shoots of A2 and A3 plants compared with the WT.

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**Fig. 4.** Intracellular Ca²⁺ concentration in different root zones of 4-day-old seedlings of Arabidopsis WT and AtCNGC10 antisense A2 and A3 lines assessed by FLIM analysis (right panels). Values are means ± SE (n = 5–7). Asterisk indicates significant differences at P ≤ 0.05. The left panels show representative roots of the three Arabidopsis lines with the selected regions of interest: the meristem zone is framed by the red rectangle and the distal elongation zone is framed by the white rectangle. The colour range in the images shown at the bottom of the left panels is based on $\alpha_1/\alpha_2$ ratio measured for Ca-Green assuming a double-exponential decay for lifetime distribution for this dye.
Plants of the two antisense lines had lower shoot and root K\(^+\) concentrations in the low-K\(^+\) treatment and higher shoot Ca\(^{2+}\) and Mg\(^{2+}\) concentrations in the high-Ca\(^{2+}\) and high-Mg\(^{2+}\) treatments when each was compared with its own control treatment (Fig. 6). However, there was a trend of lower shoot Mg\(^{2+}\) concentrations in the high Ca\(^{2+}\) treatment and lower shoot Ca\(^{2+}\) concentrations in the high-Mg\(^{2+}\) treatment compared with their own controls. Therefore, there was interference between Ca\(^{2+}\) and Mg\(^{2+}\) uptake and/or translocation in all three Arabidopsis genotypes.

In the low-K\(^+\) and high-Mg\(^{2+}\) treatments, the Ca\(^{2+}\) concentration in shoots of A2 and A3 plants was significantly lower than that in the WT plants (P ≤ 0.05). Compared with the WT, the shoot Mg\(^{2+}\) concentration was significantly lower in the A3 shoots with high-Ca\(^{2+}\) and high-Mg\(^{2+}\) treatments (P ≤ 0.05) (Fig. 6E).

The root K\(^+\) content were significantly lower in A2 plants than in the WT after low-K\(^+\) treatment (P ≤ 0.01) (Fig. 6B). Compared with the WT, the root Ca\(^{2+}\) content were generally higher in the A2 and A3 plants in all the treatments, with significantly higher values in the A2 and A3 lines under low-K\(^+\) and high-Ca\(^{2+}\) treatments (P ≤ 0.05 for both treatments) and in the A3 line treated with high Mg\(^{2+}\) (P ≤ 0.05). After low-K\(^+\) treatment, Mg\(^{2+}\) content were significantly higher in roots from the A2 and A3 lines compared with the WT (P ≤ 0.01) (Fig. 6F).

**Discussion**

Earlier studies on ion fluxes along the root surface of maize seedlings indicated that in the meristem and distal elongation zones, there were H\(^+\) and Ca\(^{2+}\) influxes (Ryan et al. 1990). To our knowledge, the Mg\(^{2+}\) flux profile along the plant root is reported for the first time in the current study. Our results showed a similar pattern of H\(^+\) and Ca\(^{2+}\) flux distribution for Arabidopsis WT and AtCNGC10 antisense A2 and A3 lines as reported by Ryan et al. (1990) for maize. However, H\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) influxes in the meristem zone of A2 and A3 seedlings (Fig. 2) were significantly smaller than that in the WT. A smaller Ca\(^{2+}\) influx in A2 and A3 seedlings correlated with a lower [Ca\(^{2+}\)]\(_{\text{cyt}}\) in the root meristematic cells compared with the WT (Figs 2 and 4), indicating possible AtCNGC10 involvement in Ca\(^{2+}\) uptake by the root. Furthermore, significantly lower Ca\(^{2+}\) content in the shoots of the A2 and A3 lines compared with the WT under control conditions (Fig. 5 C) might also indicate the lower uptake of these ions by the root and/or their disturbed loading into the xylem and non-vascular cells of leaves, resulting in lower Ca\(^{2+}\) translocation to the shoot. Following the same logic, we can suggest AtCNGC10 involvement in Mg\(^{2+}\) uptake and translocation because of the lower Mg\(^{2+}\) influxes into the roots and lower Mg\(^{2+}\) content in the shoots. However, further intracellular Mg\(^{2+}\) measurements should reinforce this hypothesis.

The opposite relationship between H\(^+\) fluxes across the root surface and intracellular pH (lower H\(^+\) influx and more acidic internal pH compared with the WT) in the root meristematic cells of A2 and A3 seedlings (Figs 2 and 3) could be explained by a negative-feedback mechanism, whereby low intracellular pH hampers H\(^+\) influx. A more acidic intracellular pH in lines is highly unlikely to be regulated by AtCNGC10 directly; however, indirect regulation might be linked to altered K\(^+\) (or Na\(^+\)) transport in these plants.

The most noticeable difference in the ion fluxes and internal ion content found for the meristem zone of A2 and 3 plants can be explained by morphological features of this root zone: cells in this zone contain vacuoles that are smaller in size, and thereby making the contribution of the ion transporters localised in the plasma membrane
Fig. 6. K⁺ (A and B), Ca²⁺ (C and D) and Mg²⁺ (E and F) content in the shoots (A, C and E) and roots (B, D and F) of WT Arabidopsis plants and AtCNGC10 antisense lines (A2 and A3) under different K⁺, Ca²⁺ and Mg²⁺ concentrations in an external solution (instead of K⁺, Ca²⁺ and Mg²⁺ concentrations in 1/4 Hoagland solution, we used 0.05 mM K⁺ for low-K⁺, 5 mM Ca²⁺ for high-Ca²⁺ and 5 mM Mg²⁺ for high-Mg²⁺ treatments). Treatments were applied at the start of budding. Plants were harvested on day 6 after the treatment onset. Values are means ± SE (n = 3). Single and double asterisks indicate significant differences at P ≤ 0.05 and P ≤ 0.01, respectively, when WT plants were compared by a paired t-test with the A2 or A3 plants with the same treatment.

more distinct. In addition, considering our results on ion content of mature plants exposed to different external concentrations of K⁺, Ca²⁺ and Mg²⁺, we might suggest that this transporter is involved in ion translocation, including Ca²⁺, from the roots to the shoots.

The lower chlorophyll content in A2 and A3 lines could be linked to Mg²⁺ deficiency. Recently, it has been shown that the reduction of chlorophyll content in Mg-deficient plants is rather because of a depressive effect of sugars on Cab2 (encoding a chlorophyll a/b protein) expression, rather than a lack of Mg atoms for chelating chlorophyll molecules (Hermans and Verbuggen 2005). Earlier it has been found that A2 and A3 plants have an increased level of starch in leaves (Borsics et al. 2007) similar to findings in Mg-deficient plants (Hermans and Verbuggen 2005).

It is generally difficult to separate primary and secondary effects of the suppressed activity of a single transporter because the transport and accumulation of mineral ions are highly linked with each other (Assunção et al. 2008, Jakobsen 1992). In addition, the transport of all major ions might be duplicated by several transporters, diminishing the contribution of the suppressed transporter. Our findings on the changed ion content in the root and shoot tissues suggest cumulative ion uptake and transport processes in plants, reflecting the suppression of the known AtCNGC10 as well as alteration of activities of other...
transporters. For instance, higher K\textsuperscript{+} concentrations in a tissue might lead to a changed plasma membrane potential (Maathuis and Sanders 1996) that, in turn, might regulate Ca\textsuperscript{2+} and Mg\textsuperscript{2+} transport via voltage-gated channels other than AtCNGC10. On the other hand, lower Ca\textsuperscript{2+} concentrations in a tissue might lead to lower [Ca\textsuperscript{2+}]tot and affect the calmodulin regulation of K\textsuperscript{+} movement (Hua et al. 2003) through AtCNGC10 channels. Therefore, some additional experiments were needed to clarify which of these events, higher Ca\textsuperscript{2+} (Mg\textsuperscript{2+}) or lower K\textsuperscript{+} concentration in shoots, is the primary factor.

To answer the question whether the decreased Ca\textsuperscript{2+} concentrations in the shoots affected the K\textsuperscript{+} content, we tested high-Ca\textsuperscript{2+} and high-Mg\textsuperscript{2+} treatments on AtCNGC10 antisense lines. High Ca\textsuperscript{2+} treatment was supposed to infuse plants with Ca\textsuperscript{2+}, while high Mg\textsuperscript{2+} treatment was designed to reduce Ca\textsuperscript{2+} in plant tissues. As predicted, the exposure of plants to high Ca\textsuperscript{2+} concentrations in the media resulted in a significantly higher content of Ca\textsuperscript{2+} in roots and increased Ca\textsuperscript{2+} content in the shoots of A2 and A3 lines, making the difference in the Ca\textsuperscript{2+} content between the WT, A2 and A3 lines statistically insignificant. At the same time, higher K\textsuperscript{+} content in shoots of antisense lines also became insignificant. High Mg\textsuperscript{2+} treatment, as predicted, decreased the Ca\textsuperscript{2+} content in the shoots in A2 and A3 lines compared with the WT in all the lines studied that kept lower Ca\textsuperscript{2+} content and higher K\textsuperscript{+} content in antisense lines (significantly, in A2). Hence, taking into account the changes in the K\textsuperscript{+} concentrations under high-Ca\textsuperscript{2+} and high-Mg\textsuperscript{2+} treatments, we can hypothesise that Ca\textsuperscript{2+} regulation of K\textsuperscript{+} transport in shoots specifically inhibited K\textsuperscript{+} loading into the phloem, as was suggested earlier (Guo et al. 2008). Further research on Ca\textsuperscript{2+} regulation of K\textsuperscript{+} loading into the xylem in the WT should confirm our hypothesis.

The found effect of external Ca\textsuperscript{2+} and K\textsuperscript{+} on decreasing internal K\textsuperscript{+} content (Fig. 6A, B) might also explain different K\textsuperscript{+} content in tissues of WT, A1 and A3 plants measured in different laboratories (Guo et al. 2008, Li et al. 2005, and the current study). On the other hand, the low K\textsuperscript{+} treatment did not change the lower Ca\textsuperscript{2+} content in the shoots of the A2 and A3 plants, although the K\textsuperscript{+} content in the shoots was lower in all the lines studied, and the difference between the WT and antisense lines became statistically insignificant (Fig. 6A). This observation indicates that K\textsuperscript{+} regulation of Ca\textsuperscript{2+} uptake and further xylem loading is unlikely.

Therefore, we can propose the primary involvement of AtCNGC10 in Ca\textsuperscript{2+} (Mg\textsuperscript{2+}) transport, which, in turn, regulates K\textsuperscript{+} (and Na\textsuperscript{+} under salinity) transport in Arabidopsis plants.

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