The AKT2 potassium channel mediates NaCl induced depolarization in the root of Arabidopsis thaliana

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SHORT COMMUNICATION

The AKT2 potassium channel mediates NaCl induced depolarization in the root of *Arabidopsis thaliana*

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ABSTRACT
Soil salinization is a major cause of plant stress, partly due to the physicochemical similarities between Na^+ and K^+. Na^+ ions compete with K^+ ions for their transport into root cells. However, the point of Na^+ entry remains unidentified. Here, I have applied the Electrical Penetration Graph as a method for whole plant electrophysiology in order to test if (a) root exposure to NaCl induces depolarization waves that propagate from root to shoot via the phloem, and if (b) the electrophysiological effects of root exposure to NaCl require expression of the potassium channels AKT1 and/or AKT2. The data suggest that AKT2 subunit containing K^+ channels mediate NaCl-induced depolarization of root cells, and that this depolarization does not propagate to leaves via the phloem.

Abbreviations: EPG, Electrical Penetration Graph; SE, Sieve element; CC, Companion cell

Land plants are chronically exposed to many environmental variables. Some, like temperature, vary gradually in intensity over circadian and seasonal time scales. Others, like herbivo-rous insects, do not follow predictable temporal patterns. Salinity is a complex environmental factor to which land plants have been exposed for millions of years, and it currently repre-sents a major threat to agriculture.[1] NaCl is a particularly dan-gerous salt; Na^+ competes with K^+ for its transport into root cells, but the ion channel(s) through which it enters remain(s) unidentified". Ion channels that catalyze the influx of K^+ ions into the cytoplasm, known as inward rectifiers, are good candi-dates as points of entry for Na^+ ions. Two important plant inward rectifiers are AKT1 and AKT2. AKT1 is involved in the uptake of K^+ into the root.[2,3] The weak inward rectifier AKT2[4] is expressed in the phloem thorough the entire plant,[4-6] and additionally in mesophyll cells, where it accounts for half of the K^+ permeability.[3] In addition to its effect on root cell physi-ology, soil salinity may be communicated to the shoot via elec-trical waves. In fact, application of salt shocks (100 mM NaCl) to the root of Arabidopsis seedlings was found to induce Ca^{2+} waves that spread to the shoot and involved the participation of endodermal and cortex cells.[7] These results beg the question of whether salt-induced, root-to-shoot electrical signals are propagated via the phloem. Here, I asked whether root expo-sure to NaCl induces electrical waves that are transmitted to the leaves via the phloem, and whether AKT1 and/or AKT2 are involved in the electrophysiological effects of root exposure to NaCl. For comparative purposes, I also examined electro-physiological responses of root exposure to salt in the aquatic fern *Azolla filiculoides*. In this study, I have implemented the Electrical Penetration Graph, or EPG,[8] which measures

potential differences between a root area containing thousands of cells, and a single sieve element/companion cell (SE/CC) complex, usually in a leaf (Fig. 1). Salt was applied during the E2 feeding phase of EPG-recorded aphids, in which the aphid stylet is stably inserted into a SE/CC complex to be used as an intracellular electrode.[8] Fig. 2A–B shows representative depo-larization responses of an Arabidopsis and an Azolla plant to root application of increasing concentrations of NaCl. While 1 mM NaCl induced small depolarizations that spontaneously returned to baseline within 1 or 2 minutes, concentrations of 10 mM or greater induced sustained depolarizations that lasted for several minutes after reaching a steady-state, which were reversible by washout (Fig. 2C). The onset of these depolariza-tions was immediate after salt application - within 1s - and they rose slowly, taking c. 20s to peak. In most cases, a brief hyperpolarization preceded the depolarization onset. Fig. 2D shows average maximum levels of NaCl induced depolariza-tion for *A. thaliana* and *A. filiculoides* as a function of NaCl concentration. Application of 100 mM KCl induced depolari-zations similar to 100 mM NaCl, and were also reversible by washout (Fig. 2E). The root, rather than the SE/CC complex sampled by the aphid stylet, is the most likely origin of these depolarizations, because they started immediately after root salt application. The sustained depolarizations recorded with external coarse electrodes would correspond to the summed electrical activity of thousands of cells within a volume of excit-able tissue,[9] in this case root tissue. Perhaps that the molecular determinant(s) for the salt-induced depolarizations are shared between ancient and modern plants, given the similarities of the responses root NaCl application between Azolla and Arabidopsis.
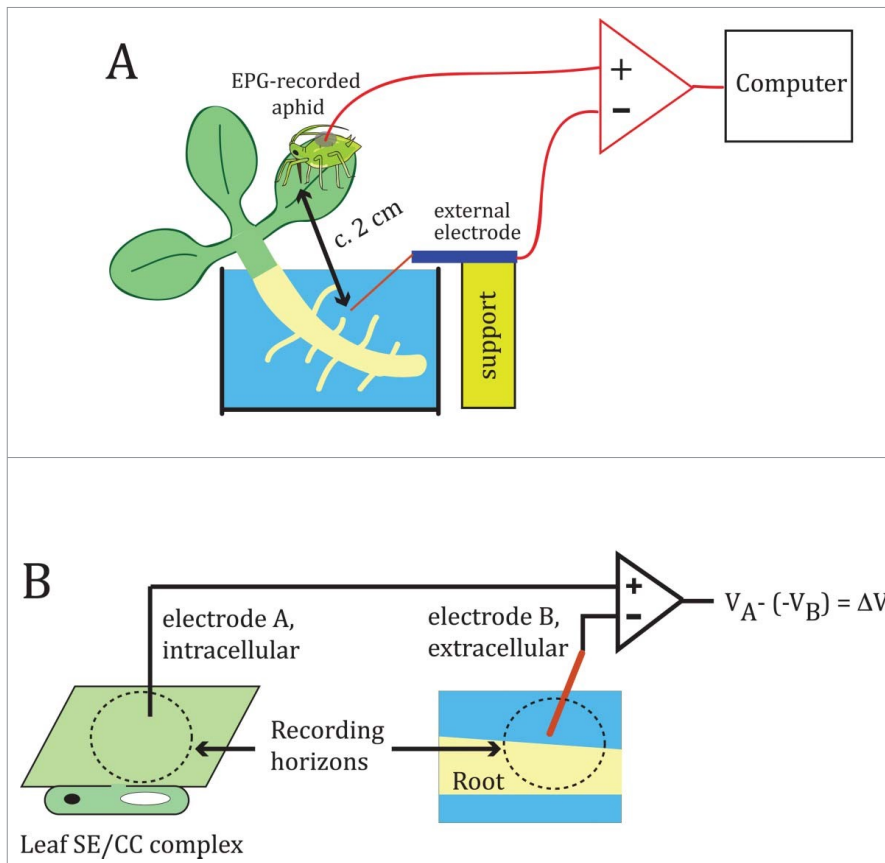
Figure 1. The Electrical Penetration Graph (EPG) rig for recording salt-induced electrical signals in plants. (A) General configuration of the EPG, as set up for this study. The EPG measures the potential difference between a fine intracellular electrode specific for the SE/CC complex (represented by the aphid stylet), and a coarse external copper electrode inserted into the root medium, in this case an aqueous medium. (B) Diagram showing the recording horizons (not to scale) of the intracellular and external EPG electrodes. Since the electrode's recording horizon is positively correlated with the electrode diameter,[16] it is reasonable to assume that the large EPG soil electrode ($\varnothing$ ~1.5 mm) (electrode B) must be able to detect the electrical activity arising from potentially several cell layers of the area(s) of the root in close proximity, whereas the aphid stylet (electrode A), senses the electrical potential within a small area within the SE/CC complex into which it is inserted – through its watery food and salivary canals with diameters of c. 1 and 0.5 mm, respectively. Electrode A feeds into the non-inverting input of the EPG operational amplifier, whereas electrode B feeds into the inverting input. In this manner, the output of the EPG is the difference between the potentials sensed by electrodes A and B, after reversing the sign of the potential recorded by electrode B. A signal can originate from a potential generated by ionic currents within the recording horizon of electrode A, electrode B, or both.

Next, I tested the potential role of two inward K^+ rectifiers, AKT1 and AKT2, as entry points for Na^+ into the root cells. The electrophysiological responses of *akt1* plants to NaCl root application were similar to those of wild type plants. Immediately upon NaCl application, a brief hyperpolarization was followed by a slowly rising depolarization whose maximum values were not statistically different from those of wild type plants (Fig. 3A). In contrast, *akt2* mutant plants did not depolarize in response to NaCl concentrations up to 50 mM, and only minimally in response to 100 mM NaCl. Small and transient hyperpolarizations were, however, present in all of the *akt2* recordings (Fig. 3B). Although the origin of this hyperpolarization is not clear, the fact that it is transient, i.e. returning to the baseline level spontaneously during the presence of NaCl, suggests a biological origin, rather than a polarizing effect of the copper electrode caused by the salt. Since the NaCl solutions were applied manually, the effect of small mechanical disturbances must be taken into consideration. It is thus conceivable that the transient hyperpolarization was caused by mechanically activated ion channels, as is the case with other excitable tissues.[10] Fig. 3C shows averaged maximum depolarization

levels for wild type, *akt1* and *akt2* plants, as a function of NaCl concentration. The average maximal depolarizations induced by 50 or 100 mM NaCl in *akt1* and *akt2* plants were statistically significantly different ($P = 0.0029$ for 50 mM; $P = 0.0001$ for 100 mM).

On the basis of the results presented here, I propose that Na^+ is taken up in root cells via K^+ channels containing AKT2 subunits, and that AKT2 is required for Na^+ uptake. Interestingly Ca^{2+} has a protective effect against salinization,[11] and is known to inhibit inward rectifying K^+ channels.[12,13] It is possible that AKT2 mediates the protective effect of Ca^{2+} against salinity.

Materials and methods

Plants and growth conditions

Wild type *Arabidopsis thaliana L.* Col-0 and homozygous *akt1* and *akt2* Ws-0 plants were used. Arabidopsis plants were grown under long day conditions (16 hrs light, 8 hrs dark; 21 °C) on sand and watered with Hyponex plant food
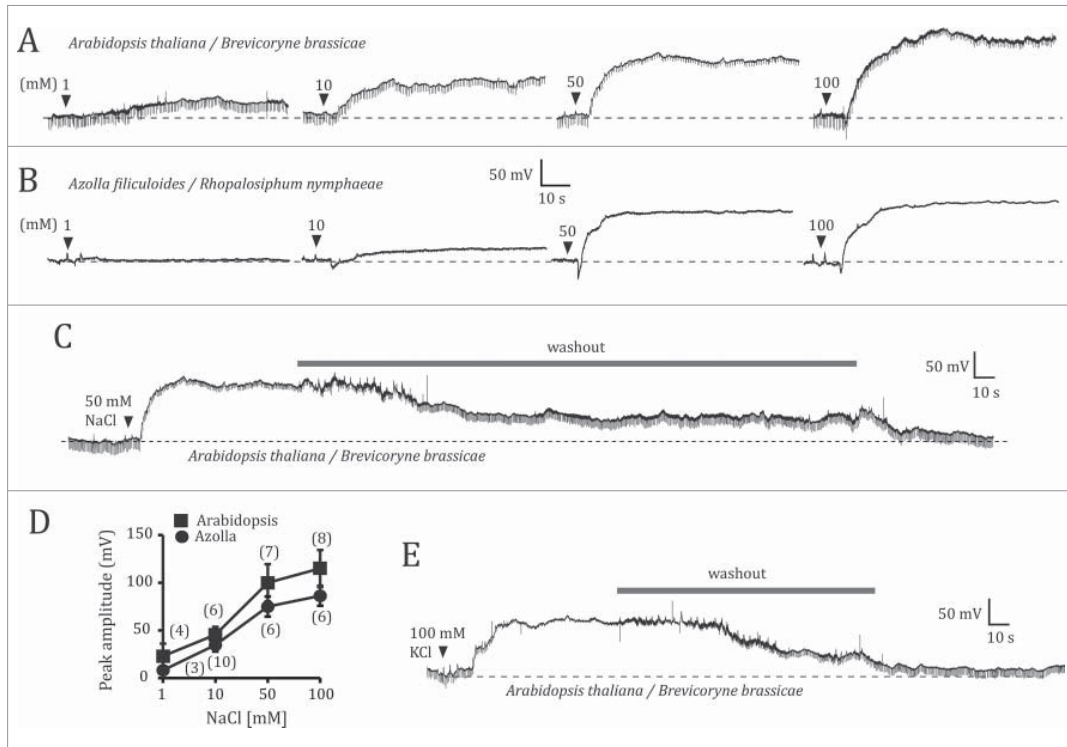
Figure 2. Electrophysiological responses of wild type *Arabidopsis thaliana* and *Azolla filiculoides* to root salt application. (A, B) Electrical potentials generated between the SE/CC-intracellular electrode and the root-external electrode of the EPG, in response to application of increasingly higher concentrations of NaCl (1, 10, 50 and 100 mM) in an *A. thaliana* (A) and in an *A. filiculoides* plant (B). Before applying 10, 50 or 100 mM NaCl, the effects of the previous salt concentration were washed out by replacing the NaCl solution with distilled water, as shown in (C). (D), Comparison between the averaged maximum depolarizations of *A. thaliana* and *A. filiculoides* as a function of NaCl concentration. Data points represent mean ± s.e.m. Numbers in parentheses indicate the number of plants per NaCl concentration. (E), Electrical potential triggered by application of 100 mM KCl to the root of *A. thaliana*, and its reversibility by washout.

solution twice per week, after attaining 2 weeks of age. Before conducting the subsequent the experiments, they were carefully extracted and immersed in distilled water in order to remove all sand. Then they were placed in dishes, with the roots submerged in tap water. The *Azolla filiculoides Lam.* plants were collected from outdoor pools at Plant Research International (Wageningen). They were maintained in a glasshouse at 21⁰C without supplemental lighting in floating culture in a beaker with tap water.

Aphids

Clones of the cabbage aphid *Brevicoryne brassicae L.* were reared on *Brassica oleracea L.* in a glasshouse at Wageningen University (Netherlands) without supplemental lighting, under long day conditions (16 hrs light, 8 hrs dark; 21⁰C). *Rhopalosiphum nymphaeae L.* aphids were collected in a pool in the Wageningen area, and maintained on *Lemna minor L.* in a glasshouse at 21⁰C without supplemental lighting in floating
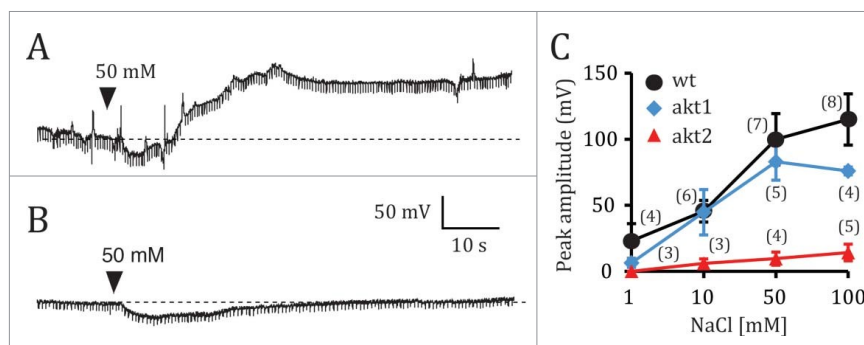

Figure 3. Electrophysiological responses of *akt1* and *akt2* mutants of *Arabidopsis thaliana* to NaCl root application. Electrical potentials induced by 50 mM NaCl to the root of *akt1* (A) and *akt2* (B). (C), Plot of the averaged maximum depolarization levels as a function of NaCl concentration for the wild type, and the *akt1* and *akt2* mutants. Data points represent mean ± s.e.m. Numbers in parentheses indicate the number of plants per NaCl concentration. Only one NaCl concentration per plant was tested. Whenever plants were exposed to several NaCl concentrations, a given NaCl solution was only applied after the effects of the previous NaCl solution had been completely washed out by manual solution exchange.

culture in a beaker with tap water. For EPG recordings, *R. nymphaeae* aphids were transferred from Lemna to Azolla plants on the day of the experiment as I found that no acclimation period was necessary.

Electrophysiology

The Electrical Penetration Graph (EPG) technique was repurposed as a method for recording systemic electrical signals in plants, as described previously.[8] Aphid probing was recorded with a Giga-8 amplifier (8-channel DC system; EPG Systems, Netherlands). The aphid, the plant, and the EPG pre-amplifier were set inside a Faraday cage. Aphids for EPG recording were attached to a gold wire (f 18 mm) by means of a water-based glue droplet containing silver particles, as originally described.[14,15] Signals were acquired at a sampling rate of 100 Hz, digitized with a built-in converter, and analyzed using Stylet+ software (EPG Systems, Netherlands) on a PC. Since the input resistance (Ri) of the DC-EPG amplifier was 1GV, the measured voltage differences were calibrated with a pulse of known voltage (in this case, 50 mV), in order to compensate for the attenuation of the membrane potential changes due to the relatively low Ri. The EPG-recorded aphids were positioned on the midvein at the leaf/petiole junction of leaf 3 or 4 in the case of Arabidopsis, or on a peripheral scale-like leaf in the case of Azolla. The extracellular copper electrode was placed within the aqueous medium surrounding the root.

Data analyses

Statistical analyses were performed with GraphPad software. Data are given as mean ± s.e.m. Statistical differences between means were determined with the Student *t* test for independent samples. The level of statistical significance was set at $P < 0.05$.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

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