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Under salt stress guard cells rewire ion transport and abscisic acid signaling

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Summary

- Soil salinity is an increasingly global problem which hampers plant growth and crop yield. Plant productivity depends on optimal water-use efficiency and photosynthetic capacity balanced by stomatal conductance. Whether and how stomatal behavior contributes to salt sensitivity or tolerance is currently unknown. This work identifies guard cell-specific signaling networks exerted by a salt-sensitive and salt-tolerant plant under ionic and osmotic stress conditions accompanied by increasing NaCl loads.
- We challenged soil-grown *Arabidopsis thaliana* and *Thellungiella salsuginea* plants with short- and long-term salinity stress and monitored genome-wide gene expression and signals of guard cells that determine their function.
- *Arabidopsis* plants suffered from both salt regimes and showed reduced stomatal conductance while *Thellungiella* displayed no obvious stress symptoms. The salt-dependent gene expression changes of guard cells supported the ability of the halophyte to maintain high potassium to sodium ratios and to attenuate the abscisic acid (ABA) signaling pathway which the glycophyte kept activated despite fading ABA concentrations.
- Our study shows that salinity stress and even the different tolerances are manifested on a single cell level. Halophytic guard cells are less sensitive than glycophytic guard cells, providing opportunities to manipulate stomatal behavior and improve plant productivity.

Introduction

Guard cells play an important role in balancing CO_2 assimilation and water loss in plants (Hetherington & Woodward, 2003). Saline soil has a great impact on stomatal conductance and thereby biomass production (Lawson & Vialet-Chabrand, 2019). Root-to-shoot translocation of NaCl salt depends on stomata apertures defining the whole-plant transpiration rate (Kerstiens *et al.*, 2002; Chaves *et al.*, 2009; Hedrich & Shabala, 2018). Increasing salt concentrations in soil lower the water potential similar to drought, compromising water uptake by the root (Munns, 2002) and causing an imbalance in ion homeostasis (Muchate *et al.*, 2016).

To avert drought and salt stress disturbances, abscisic acid (ABA) production is increased and ABA signaling enhanced (Golldack *et al.*, 2014). Guard cells sense changes in relative air humidity directly in contrast to water potential changes sensed by the root which addresses ABA allocation to the stomata (Ko & Helariutta, 2017). *Arabidopsis thaliana* guard cells express the entire ABA biosynthesis pathway, enabling them to produce the stress hormone autonomously (Bauer *et al.*, 2013). The core ABA signaling pathway functioning in stomata regulation comprises the PYR/PYL/RCAR ABA receptors, PP2C phosphatases, SnRK2 kinase OST1, as well as SLAC1 and QUAC1 type anion channels (Geiger *et al.*, 2009, 2011; Lee *et al.*, 2009, 2011; Imes *et al.*, 2013; Hedrich & Geiger, 2017). When ABA binds to the PYR/PYL/RCAR class of ABA receptors (Ma *et al.*, 2009; Park *et al.*, 2009; Nishimura *et al.*, 2010) and activates the downstream signaling cascade, it causes closing of stomata even during the day and terminates water loss by transpiration (Julkowska & Testerink, 2015). The ABA receptor is also known to address the coreceptor phosphatase ABI1 and the calcium (Ca^{2+})-

independent kinase OST1. There is also evidence for Ca^{2+}-dependent signaling in guard cells involving the kinases CPKs or the CBL/CIPK complexes (Levchenko *et al.*, 2005; Maierhofer *et al.*, 2014; Edel & Kudla, 2016; Huang *et al.*, 2019). Both branches of the ABA signaling pathway activate the S-type anion channel SLAC1, the master regulator of stomatal closure.

In addition to osmotic stress, NaCl causes an imbalance in cellular ion homeostasis associated with an altered potassium (K^+) to sodium (Na^+) ratio (Isayenkov & Maathuis, 2019). Maintenance of the cytosolic K^+ : Na^+ ratios under saline conditions is crucial for proper plant growth and development (Munns & Tester, 2008; Maathuis, 2014). Genetic-based mechanisms and compatible solutes are known to keep the Na^+ concentration in the cytoplasm of leaf cells low and protect against toxic Na^+ effects (Carillo *et al.*, 2011). Sodium detoxification involves the Salt Overly Sensitive (SOS) signaling pathway which consists of the Ca^{2+} sensor SOS3/CBL4, the SnRK3 kinase SOS2/CIPK24 and the Na^+/proton (H^+) antiporter SOS1/NHX7 (Ji *et al.*, 2013). This Na^+/H^+ antiporter maintains K^+ homeostasis by removing Na^+ from the cytosol before the alkali cation reaches toxic values. The chloride anion (Cl^-) of NaCl has been considered as a toxic component too. When roots are challenged by Cl^- loads, ABA concentrations in leaf cells and the apoplastic space surrounding them increases, followed by a drop in stomata conductance (Geilfus *et al.*, 2015; Li *et al.*, 2017; Franco-Navarro *et al.*, 2019).

Plants vary notably in their tolerance to saline environments. Salt stress tolerance can be ascribed to the ability of a plant to maintain photosynthetic activity that depends on leaf carbon dioxide (CO_2) gas intake via the stomata (Gong *et al.*, 2005; Flowers & Colmer, 2015). It is not surprising, therefore, that the salt-tolerant species *Thellungiella*, but not the salt-sensitive *Arabidopsis*, maintains CO_2 assimilation rates under salinity stress (James *et al.*, 2008; Stepien & Johnson, 2009).

Stomatal conductance is regulated by the turgor pressure of guard cells, which is powered by intake and release of K^+ salts. The key elements for guard cell swelling (opening) are ATP-driven H^+ pumps, voltage (hyperpolarization)-activated K^+-selective ion channels (Szyroki *et al.*, 2001; Ivashikina *et al.*, 2005), H^+-driven Cl^- and nitrate carriers as well as production of the organic anion malate (Hedrich, 2012). Guard cells deflate in the depolarized state when anions and K^+ are released accompanied by osmotic water efflux. Depending on ion availability, guard cells seem to use either K^+ or Na^+ to drive stomatal movements (Robinson *et al.*, 1997; Rienmüller *et al.*, 2010, Yu and Assmann, 2016).

To draw a comprehensive picture on the functioning of stomata under short-term and long-term salinity stress we compared the molecular genetic and physiological responses of guard cells of the glycophyte *Arabidopsis* and halophyte *Thellungiella*. *Arabidopsis* guard cells showed a stronger response to both salt regimes than *Thellungiella*. This resulted in rewiring ion transport, reducing stomatal conductance, higher ABA concentrations and upregulation of the genes of the complete ABA signaling pathway. In contrast to the glycophyte, guard cells of the salt-insensitive halophyte *Thellungiella* did not display pronounced responses to salinity stress.

Materials and Methods

Plant species, growth conditions and salt treatments

Arabidopsis thaliana (cv Columbia-0) and its close relative *Thellungiella salsuginea* (= *Eutrema salsugineum* cv Shandong) were grown in pots (diameter 7 cm, volume 200 ml) filled with 91 ± 7 g soil substrate (Einheitserde Typ P; Patzer Erden GmbH, Sinntal-Altengronau, Germany). Plants were illuminated for 12 h (10:00 to 22:00 h) with a light intensity of 80–110 µmol s^{-1} m^{-2} using fluorescent lamps (L58W/77 Fluora; Osram, Munich, Germany) at 22°C in the light and 16°C in the dark and 60% relative air humidity. Two different salt treatment protocols, a short- and a long-term one, were applied to 4- to 5-wk-old *Arabidopsis* and 7-wk-old *Thellungiella* plants (Supporting Information Fig. S1), soaking the soil pots in salt solutions each time for 2 h. In the long-term salt stress experiment one group of plants was treated three times with 200 mM NaCl solutions (lss) – on days 1 and 2, on days 7 and 8, and on days 13 and 14 (termed 3× 200 mM) – and, in parallel, the second group of plants received incrementally increasing NaCl concentrations (ssu) of 75, 150, and 200 mM NaCl (termed 3× salt). In the short-term salt stress (sss) experiment, plants were treated with 200 mM NaCl on days 13 and 14 (termed 1× salt) while the control group received tap water (termed (–)salt). All samples were collected at the end of the salt experiments on day 15 to ensure that plants have the same plant age and developmental stage.

Guard cell extraction

For guard cell extraction the blender method was used as described by Bauer *et al.* (2013) and Jalakas *et al.* (2017). Mature leaves without petioles and midribs from two to three plants were used for one guard cell sample, collected in 1.5 ml tubes, and immediately frozen in liquid nitrogen until further processing.

Ion concentrations in dry soil, soil solution, and leaves

Potassium and sodium Dried soil samples were used for ion extraction as described by Jenway (Bibby Scientific; Keison Products, Chelmsford, UK) protocols – P05-002A for Na^+ and P05-001A for K^+ – by avoiding contaminations with roots. Soil solutions were obtained by filtering the soil from day 15 of the salt experiments using a syringe (3 ml) with a filter attached (Filtropur S, 45 µm; Sarstedt, Nümbrecht, Germany). Leaves were washed thoroughly in ultrapure water and dried at 65°C for 1 wk. Ion extraction was performed using 0.5 M HNO_3 according to Munns *et al.* (2010). A flame photometer (PFP7; Jenway) was used for quantification according to the manufacturer's protocol. Standard solutions (1, 0.2, 0.1, 0.05, 0.02 mM) were prepared from NaCl and KCl salts (AppliChem GmbH, Darmstadt, Germany).

Chloride Dried soil substrate samples (3.5–5 g) were cooked in distilled H_2O for 5 min (ratio of soil to distilled H_2O was 1 mg : 250 µl) and after cooling on ice centrifuged for 5 min at 19 705 ***g***. The filtrated supernatant was used for Cl^- determination by silver ion titration (Mohr method; Sheen & Kahler, 1938) and a Chloride Meter (6610; Eppendorf AG, Hamburg, Germany). For this, 200 µl filtrate was mixed with 1 ml gelatin indicator solution (Biorapid GmbH, Freiburg, Germany) and 15 ml acid buffer (6.4 ml l^{-1} 65% nitric acid and 57.6 ml l^{-1} 100% acetic). For Cl^- determination in leaves, 1 mg dried leaf samples were cooked in 250 µl digestion solution (digestion solution: two parts 69% + one part 30% H_2O_2) at 190°C for 25 min in a microwave. The Cl^- concentrations were quantified from filtrated sampled photometrically (Photometer PCP6121; Eppendorf AG) using the ferricyanide method (Munns *et al.*, 2010).

Ion composition in guard cells

The elements (Na, K and Cl) were determined in guard cells of the plants from the salt stress experiments exposed to light by using energy-dispersive X-ray analysis (EDXA). Intact leaves were collected, washed quickly in ultrapure water and shock-frozen in slush ethanol (Rondeau, 1966). The frozen leaves were freeze-dried using a lyophilizer (Alpha 1-2 LDplus; Martin Christ, Osterode am Harz, Germany) for 48 h at −48°C and 1.4 Pa atmospheric pressure. Freeze-dried samples were analyzed by EDXA for quantification of elements as described by Fromm *et al.* (1987). The data represent % atomic of each element relative to all elements measured.

Leaf surface area, water content, osmotic potential and pigments

Each of the parameters was obtained from plant rosette leaves at the end of the salt stress experiment on day 15 (Fig. S1).

For leaf surface area determination whole plant rosettes were scanned (Sharp MX M362N scanner) and the areas were calculated using the image processing program ImageJ (imagej.nih.gov/ij/).

Plant water content was determined from whole plant rosettes. Plants were weighed immediately after harvest (FW) and DWs of the samples were recorded after drying at 37°C for 2–3 wk. The relative plant water content (%) was calculated as follows: % water content = ((FW − DW)/FW) × 100.

The osmotic potential was determined of leaves washed in ultrapure water and freeze-dried for 48 h at −48°C and 0.014 mbar. Powder of the freeze-dried material was suspended in equal volumes of ultrapure water followed by centrifugation at 13 148 ***g*** (miniSpin Plus; Eppendorf) for 10 min. Osmolarity was measured of the supernatants using an osmometer (Vapro Model 5520; Kreienbaum Wissenschaftliche Meßsysteme eK, Langenfeld, Germany). Osmolarity was converted to osmotic potential as follows: $\Psi s\ [MPa] = C_i RT$ (Ψs, osmotic potential; C_i, osmolarity; R, gas constant; T, temperature (in K)).

Plant pigments (Chl*a*,*b*, carotenoids and anthocyanins) were quantified from freeze-dried leaf samples using an UV-VIS spectrophotometer (U-1500; Hitachi, Tokyo, Japan). Chlorophyll and carotenoids were extracted and quantified as described previously (Misyura *et al.*, 2013). Anthocyanin extraction was performed using 1% acidic methanol as described in Heijde *et al.* (2013) and MacLeod *et al.* (2015). Anthocyanins were quantified from the equation, $A_{530} - 0.33 \times A_{657}$ (where A = absorption value).

Stomatal apertures and stomatal density

Stomatal apertures were determined from images taken from the abaxial side of intact leaves attached to the plant using a microscope (Axioskop 2 MOT Plus; Zeiss). For stomata size measurements the pictures were processed in ImageJ (imagej.nih.gov/ij).

Stomatal density (number of stomata mm^{-2}) was calculated from imprints of thin transparent films of nail polish (DW 150; Jade, France) applied onto the adaxial and abaxial leaf surfaces. To count the number of stomata, microscopic images (Axioskop 2, MOT Plus; Zeiss) were taken from the transparent films after removal from the leaf surface and analyzed using ImageJ.

Gas exchange, CO_2 assimilation rates, and intrinsic water use efficiency

Gas exchange measurements were performed according to a protocol by Mueller *et al.*, (2017) with whole rosettes of intact plants. Soil transpiration was avoided by tightly covering the soil surface with plastic foil. Illumination was provided at a photon flux density of 125 µE. Plant rosettes were scanned at the end of the measurements, and H_2O transpiration rates (mmol m^{-2} s^{-1}) as well as CO_2 assimilations rates (µmol m^{-2} s^{-1}) were calculated (Ball, 1987).

Plant intrinsic water-use efficiency (WUEi) was calculated by dividing transpiration rates vs CO_2 assimilation rates as described by Polley (2002) and Tambussi *et al.*, (2007).

Abscisic acid quantification

Abscisic acid was quantified from leaves, guard cell preparations (see earlier), cell debris and washing solutions by using LC-MS. Cell debris was obtained by centrifugation of the washing solution at 4°C and 1593 ***g*** (Avanti J-20 XP; Beckman Coulter GmbH, Krefeld, Germany). The supernatant and pellet were frozen at −80°C followed by freeze-drying (Alpha 1-2 LDplus; Martin Christ). For ABA quantitation the material was frozen in liquid nitrogen and ground into a fine powder. Extraction and chromatographic separation were carried out as described in Stingl *et al.* (2013), with 5 ng of 6H2-ABA as internal standard, using a Waters Acquity I-Class ultrahigh-performance liquid chromatography system (Milford, MA, USA) coupled to an AB Sciex 6500+ Qtrap tandem mass spectrometer (AB Sciex; Framingham, MA, USA), operated in the negative electrospray ionization mode (ESI). Analyses were performed using the following source conditions: ion spray voltage was −4000 V, curtain gas was kept at 207 kPa, nebulizer gas (GS1) and drying gas (GS2)

were adjusted to 345 and 552 kPa, respectively, at 600°C. Detection of ABA was carried out by multiple reaction monitoring (MRM) with a dwell time of 25 ms and the CAD gas was set to 62 kPa (ABA: *m/z* 263 → 153; declustering potential, DP, −40 V; collision energy, CE, −14 V; collision cell exit potential, CXP, −9 V; $^{13}C_2$-ABA: 265 → 153; DP, −45 V; CE, −14 V; CXP, −9 V; 6H2-ABA: 269 → 159; DP, −25 V; CE, −14 V; CXP, −11 V). Data analysis was performed using ANALYST software 1.6 from AB Sciex.

In a separate experiment, guard cells were isolated in the presence of labeled ABA. For that, a known concentration (10 ng g^{-1} FW) of the stable ABA isotope $^{13}C_2$-ABA (S-(+)-ABA$^{13}C_2$; Qmx Laboratories Ltd, Essex, UK) was added to *Arabidopsis* leaf samples before guard cell extraction using the blender method (see earlier).

Guard cell transcriptome analysis

For transcriptome analysis of *Thellungiella* guard cells, microarray analysis was performed at Oak Labs (www.oak-labs.com/) using a 60 mer oligonucleotide probe array (44 K, design number 031554; Agilent, Santa Clara, CA, USA) generated by Lee *et al.* (2013). *Arabidopsis* guard cell transcriptome analysis was conducted at the Department of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nuernberg using the Agilent GeneChip® array V4. Total RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol from salt- (1× and 3× salt) and nonsalt-treated plants kept in the light for 4 h after the beginning of the photoperiod (10:00–12:00 h). Four guard cell samples of each treatment were prepared and, hence, a total 12 samples for *Arabidopsis* and 12 of *Thellungiella* were used for microarray analysis.

Data preprocessing was performed using the BIOCONDUCTOR software (Huber *et al.*, 2015) with the statistical programming environment R (R Core Team, 2011). Normalization has been performed using negative control probes and quantile normalization using negative and positive control probes as implemented in the 'neqc' function (Shi *et al.*, 2010) of the LIMMA package (Ritchie *et al.*, 2015). Differential expression of genes for all stimuli was calculated using the moderated *t*-statistic approach as implemented in the R-package LIMMA, which has been specifically developed for the analysis of small sample size experiments. The *P*-values of all results were corrected for multiple testing using the false discovery rate (FDR) (Benjamini & Hochberg, 1995). Transcripts showing a $\log_2$-fold-change ≥ 1 or ≤ −1 with a $P \leq 0.05$ between salt treatment (1× or 3× salt) and control (no salt) were accepted as significantly changed. *Arabidopsis* and *Thellungiella* genes were annotated according to the database Mercator4 v.2.0x (https://plabipd.de/portal/mercator4).

To validate the microarray results, quantitative polymerase chain reaction (qPCR) was carried out using gene-specific primers of selected genes (Table S1) and a Mastercycler RealPlex (Eppendorf) according to the protocol Boehm *et al.* (2018). Pearson's correlation coefficient of qPCR and microarray results was calculated to determine the association between both datasets. Analysis was performed using R v.3.5.3 (R Core Team, 2019).

Correspondence analysis was performed as implemented in the VEGAN package (Oksanen *et al.*, 2020).

Gene ontology (GO) term annotation of the *T. salsuginea* dataset was performed using TRINOTATE (Bryant *et al.*, 2017). GO term enrichments were calculated with ONTOLOGIZER v.2.1 (Bauer *et al.*, 2008) using the parent–child method as described by Grossmann *et al.* (2007) and Benjamini–Hochberg correction for multiple testing.

Results

To compare the impact of salt stress on guard cell performance, we established a protocol simulating environmental-like challenges of plants with soil salinity (Fig. S1). We chose to compare the glycophyte *Arabidopsis thaliana* (cv Columbia-0) and its halophytic Brassicaceae relative *Thellungiella salsuginea* (cv Shandong). Plants growing in soil were treated either with 200 mM NaCl (1× salt) for a 2 d period to apply a short-term salt stress (sss), or with three successive salt applications of increasing NaCl concentrations (3× salt: 75 + 150 + 200 mM) each for 2 d followed by a 4 d interruption (salt steps up, ssu), and with three successive applications of the same high NaCl concentration (3× 200 mM) to apply long-term salt stress (lss).

Soil salt exposure of *Arabidopsis* plants resulted in retarded growth (Fig. 1a) and the development of stress symptoms such as reduced Chl*a* and carotenoid concentrations as well as accumulation of anthocyanin (Fig. 2a,b). The lss with 3× 200 mM salt finally caused death of the entire *Arabidopsis* population (Fig. 1b). Under identical conditions, however, *Thellungiella* plants showed no obvious stress symptoms, flowered and set seeds.

Under saline conditions *Arabidopsis* K^+ intake is compromised

Arabidopsis survived and multiplied when salt concentrations were increasing stepwise (ssu, 3× salt), although the Na^+ concentrations did not differ much between the ssu (3× salt) and lss (3× 200 mM) salt experiments in the soil matrix and soil solution (Fig. S2a,b). This indicates that the salt administration protocol very much matters. Under the same salt-feeding regimes (1× salt, 3× salt, 3× 200 mM) much higher K^+ concentrations remained in the soil solutions of the pots containing *Arabidopsis* plants compared to *Thellungiella* (Fig. S2c,d). This finding suggests that under salt stress *Arabidopsis* K^+ uptake is compromised when compared with the halophyte *Thellungiella*.

As *Arabidopsis* does not survive the 3× 200 mM NaCl applications in the long run (Fig. 1b), we performed all further studies with plants that were either conditioned by sss (1× salt) or ssu (3× salt). Under these salt treatments the K^+ concentrations decreased in *Arabidopsis* leaves, while they increased in *Thellungiella* leaves (Fig. 2d). In contrast to the similar Na^+ concentrations in both leaf types, the Cl^- concentrations were higher in *Arabidopsis* than in *Thellungiella*, indicating that the halophyte better controls Na^+ intake and resists Cl^- entering the leaf.

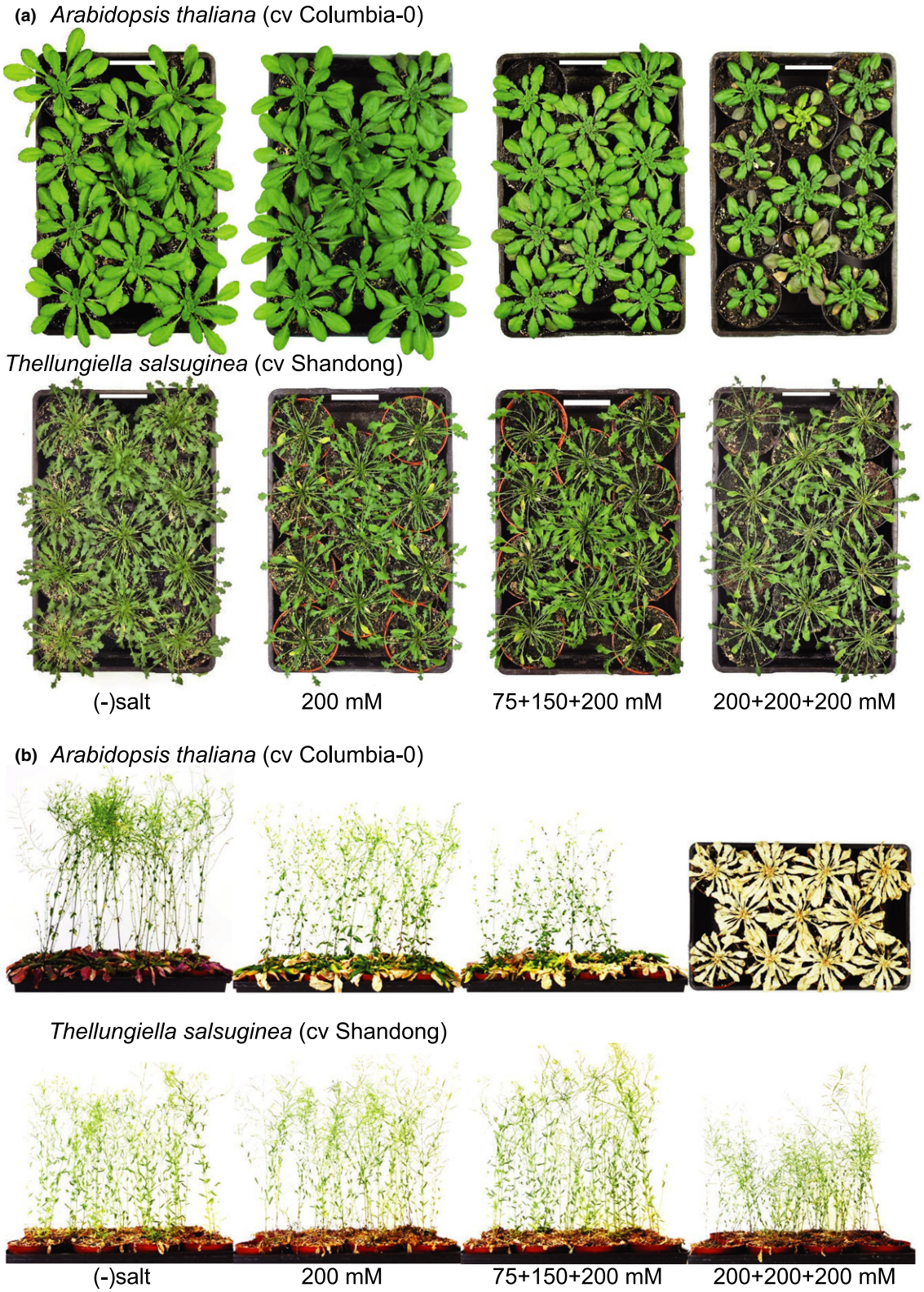


Fig. 1 Phenotypes of *Arabidopsis thaliana* (cv Col-0) and *Thellungiella salsuginea* (cv Shandong) cultivated on nonsalt- and salt-treated soil. (a, b) Representative images of plants at the time point of harvest on day 15 (a) and at the end of their reproductive growth phase (b). Plants grown in soil pots received: one-time 200 mM NaCl; successively increasing NaCl concentrations (75 + 150 + 200 mM); or three times the same high NaCl concentrations 200 + 200 + 200 mM. The control plants ((–)salt) were treated with tap water in parallel. Bars, 5 cm.

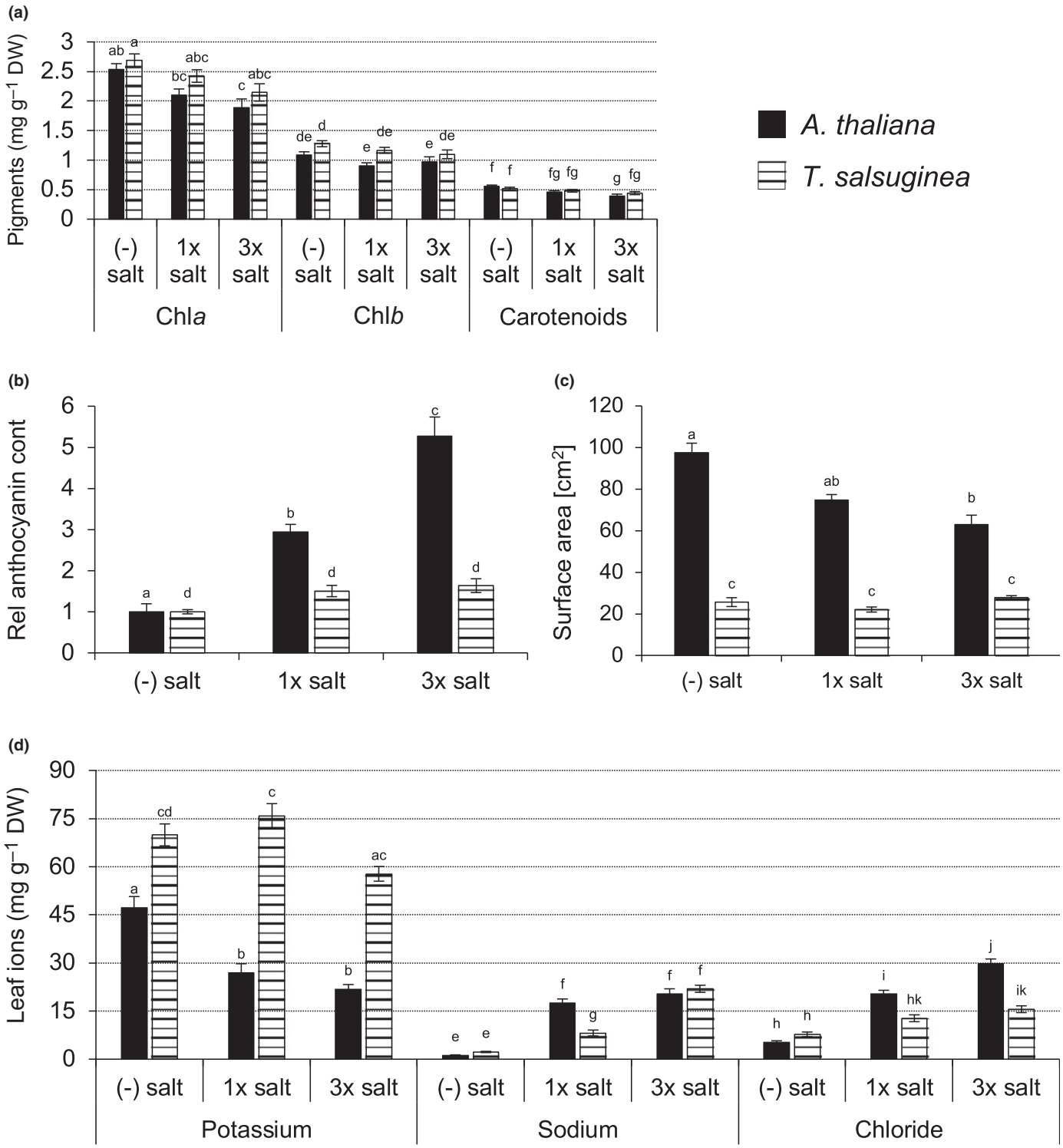
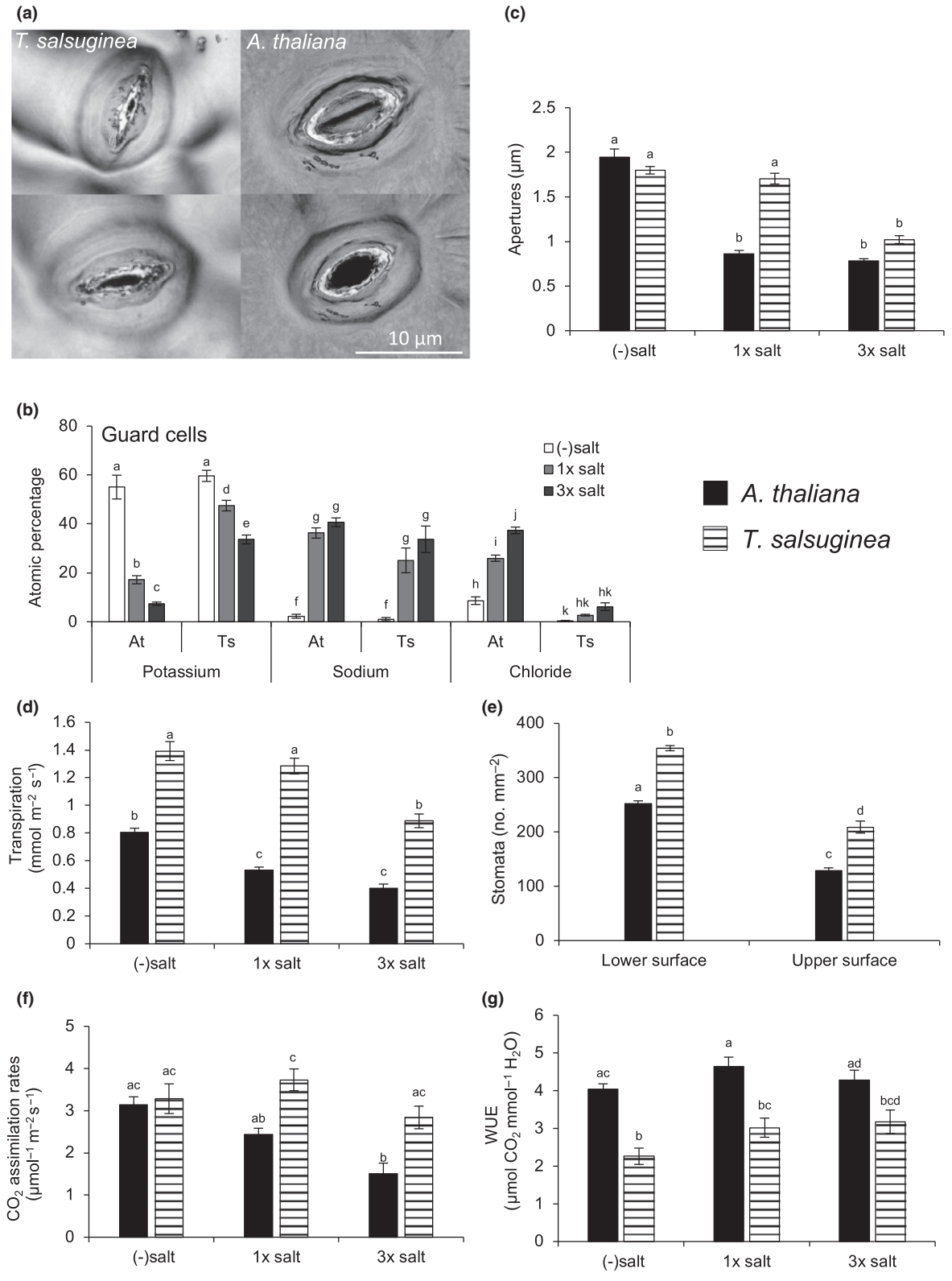


Fig. 2 Salt-dependent symptoms of rosette leaves from *Arabidopsis thaliana* and *Thellungiella salsuginea* plants grown on saline and nonsaline soil. (a) Concentrations of pigments; (b) anthocyanin contents of salt-treated relative to tap water ((–)salt)-treated plants; (c) surface areas of whole plant rosettes; and (d) leaf ion concentrations. Soil pots with plants were treated with 1× salt (200 mM NaCl on days 13 and 14), 3× salt (75 mM NaCl on days 1 and 2, 150 mM NaCl on days 7 and 8, and 200 mM NaCl on days 13 and 14) or tap water ((–)salt). Data represent means of eight to 19 samples per treatment (± SE) of three independent experiments. Statistical analysis was conducted by two-way ANOVA followed by Bonferroni *post hoc* test for multiple testing correction. Different letters indicate significant differences according to $P \leq 0.05$.



Guard cells respond to soil salinity

The observed physiological differences at the plant and leaf levels between the salt-sensitive *Arabidopsis* and salt-tolerant *Thellungiella* trigger questions about whether and how stomatal guard cells of both species function under salt stress (Fig. 3a). Do guard cells of the glycophyte and halophyte take advantage or suffer from the extra Na^+ and Cl^- loads? Using EDXA, we found that both guard cell types contained similar relative concentrations of Na^+ under the two saline conditions (Fig. 3b); however, K^+

Fig. 3 Guard cell responses of *Arabidopsis thaliana* and *Thellungiella salsuginea* to saline soil. (a) Examples of closed (upper row) and open (lower row) stomata on the upper leaf surfaces under control conditions taken with a laser scanning microscope (VK-X1000; Keyence, Neu-Isenburg, Germany). (b) Percentage of potassium, sodium, and chloride atoms relative to all atoms measured in guard cells of *Arabidopsis* (At) and *Thellungiella* (Ts) determined by energy-dispersive X-ray analysis (EDXA). Mean values (± SE) of six to 13 guard cells per treatment are presented. (c) Stomatal apertures of intact rosette leaves attached to plants. Mean values (± SE) of 50–140 stomata per treatment are shown. (d) Steady-state transpiration rates of whole *Arabidopsis* and *Thellungiella* rosettes after 50 min. Data represent the means of eight to 19 samples (± SE) per treatment of three independent experiments. (e) Stomatal density on the upper (adaxial) and lower (abaxial) surfaces of *Arabidopsis* and *Thellungiella* rosette leaves. Mean values (± SE) of 4 to 5 plants per treatment are shown. (f) CO_2 assimilation rates of whole rosettes at 125 μmol m^{-2} s^{-1} light intensity and ambient CO_2 concentrations. (g) Intrinsic water-use efficiency (WUE_i) of whole rosettes. Data represent means of eight to19 samples (± SE) per treatment. Plants grown in soil pots were treated with 1× salt (200 mM NaCl on days 13 and 14), 3× salt (200 mM NaCl solutions on days 1 and 2, days 7 and 8, and days 13 and 14) or tap water ((–)salt). Statistical analysis was performed by two-way ANOVA followed by Bonferroni *post hoc* test for multiple testing correction. Different letters indicate significant differences based on $P \leq 0.05$.

decreased much more in *Arabidopsis* than in *Thellungiella*. The two Brassicaceae species also differed with respect to Cl^- uptake. While *Arabidopsis* guard cells took up large quantities of the halide, their counterparts of *Thellungiella* did not. This behavior is in line with the hypothesis that the halophyte can tightly control the root-to-shoot translocation of the halide while *Arabidopsis* is unable to do so (Fig. 2d). Thus, *Thellungiella* guard cells seem to suppress Cl^- intake.

Salt stress affects ABA concentrations in guard cells and stomatal conductance

The stress hormone ABA rises in plants when water availability is limited. With increasing salt loads in the soil, the relative water contents of *Arabidopsis* and *Thellungiella* leaves decreased (Fig. 4a). The osmotic potentials of the leaf sap of *Arabidopsis* and *Thellungiella* were similar under nonsaline conditions but dropped in *Arabidopsis* leaves after sss (Fig. 4b). By contrast, *Thellungiella* plants were not affected by sss and the osmotic potential only dropped in response to ssu. The difference between the glycophyte and halophyte in water content and osmotic potential was mirrored by the ABA concentrations (Fig. 4c). The ABA concentration rose strongly in *Arabidopsis* leaves after sss but leveled off again after ssu treatment. The ABA concentration changes in *Thellungiella* leaves were much less pronounced. This behavior indicates that short-term salt application influences the water and ABA status of *Thellungiella* to a lesser degree than *Arabidopsis*.

What about ABA in guard cells? To analyze the guard cell ABA concentration, we used MS. Owing to technical limitations, so far only turgor-less protoplasts, but not intact, turgescent guard cells, have been accessed for their ABA concentration (Weiler *et al.*, 1982; Lahr & Raschke, 1988). We used the 'Blender' method with excised leaves to separate the vital small-sized guard cells from the large common epidermal cells that are mechanically destroyed by shearing stress (cf. Raschke & Hedrich, 1989; Bauer *et al.*, 2013). To verify that the ABA we obtained with our guard cell preparation was of guard cell origin rather than a contamination from the ABA adhering to epidermal fragments of mesophyll cells, we added a stable ABA isotope as an internal standard. The isotope $^{13}C_2$-ABA (S-(+)-ABA$^{13}C_2$ (Qmx Laboratories Ltd) was added to *Arabidopsis* leaf samples before guard cell preparation. The ABA isotope was only recovered from the washing solution (WS) but not from cell debris (CD) of destroyed leaf cells or from guard cells (GC; Fig. 5a). This finding shows that foreign ABA adhering to the epidermal cell walls gets efficiently removed by the several washing steps of the 'Blender' method, while the guard cell fraction enriches. Thus, the ABA we measured in our guard cell preparations represents the quantity of the guard cell intrinsic stress hormone.

In guard cells of *Arabidopsis*, sss treatment caused a clear increase in ABA concentrations which was less pronounced in *Thellungiella* guard cells (Fig. 5b). The ABA concentrations in guard cells of both plant species returned, however, to the pre-stimulus value after prolonged salt treatment. The gain-of-function mutant MYB60p-ABA3ABA confirmed that sss treatment (1× salt) causes a rise in ABA in guard cells. This *Arabidopsis* plant expresses a functional ABA3 protein under the control of the guard cell-specific promotor MYB60 in the *aba3-1* mutant background (Bauer *et al.*, 2013). One salt episode caused a two-fold increase in the guard cell-specific ABA concentrations of the MYB60p-ABA3 plants, but not in the loss-of-function mutant *aba3-1* (Fig. 5c). This indicates that a rise in ABA could trigger guard cell processes required to detoxify sudden salt loads, but not for ssu management.

As osmotic changes in guard cell feedback on stomatal apertures (Fig. 3c), we used an infrared gas analyzer (IRGA) system and compared *Arabidopsis* and *Thellungiella* stomatal conductance as functions of the soil salt management. Facing a sss event, *Arabidopsis* reduced its transpiration level, but it was not further lowered under additional salt exposure (ssu) with increasing salt concentrations (Fig. 3d). By contrast, transpiration of *Thellungiella* did not respond to the initial salt challenge but only to the prolonged salt treatment. The difference in transpiration between the halophyte and glycophyte derives from the higher stomata density of *Thellungiella* as compared with *Arabidopsis* (Fig. 3e).

A reduced stomatal aperture limits water loss and the uptake of CO_2 at the same time. The relative rations of water loss and CO_2 gain is defined as water-use efficiency (WUE_i). In IRGA measurements under control conditions similar CO_2 assimilation rates were found for both species (Fig. 3f). Following an increase in soil salinity, CO_2 intake in *Arabidopsis* decreased continuously, while in *Thellungiella* the carbon gain remained at a higher level. WUE_i of *Arabidopsis* leaves was higher than that of *Thellungiella*

under all saline conditions and approached WUE_i of *Thellungiella* only after prolonged salt stress (Fig. 3g). As a result, biomass production of *Thellungiella* was lower than that of *Arabidopsis* under normal growth conditions and it hardly dropped under saline conditions (Fig. 2c).

Soil salinity alters the transcriptomic landscape of guard cells

To gain insights into the molecular mechanisms underlying salt-mediated guard cell responses, we monitored transcriptional changes in the stomatal motor cells on a genome-wide level (Fig. S3). Plants were conditioned by 1× salt and 3× salt treatments (Fig. S1) or grown under control conditions before total RNA was extracted from guard cell fractions obtained with the 'Blender' method and analyzed with microarrays (Agilent). Correspondence analysis revealed that the salt treatments (1× salt, 3× salt) separated the data points of *Arabidopsis* (CA1, 42.2%; Fig. 6a) and *Thellungiella* guard cells (CA1, 51.46%; Fig. 6b) from control ((–)salt) conditions. Furthermore, the data points for *Arabidopsis* guard cells also clustered according to the strength of the salt treatments, 1× salt and 3× salt (CA2, 19.5%), while no separation of data points from the two salt treatments was observed for *Thellungiella* (CA2, 14.68%). This indicates that genes of *Arabidopsis* guard cells are more sensitive than those of *Thellungiella* to the intensity of the salt management.

When the differentially expressed genes (DEGs) were assigned to GO terms (Fig. 7), the overall number of significantly ($P \leq 0.05$) enriched GO terms was lower for *Thellungiella* than for *Arabidopsis* guard cells under both saline conditions. Furthermore, nine GO terms enriched in upregulated genes of *Arabidopsis* were enriched in downregulated genes of *Thellungiella* when plants were challenged with sss (marked by asterisks in Fig. 7). These contrasting gene expression profiles indicate that guard cells from the two Brassicaceae species react differently to salinity.

Guard cells rewire ion transport under salt stress

The GO terms 'membrane' and 'plasma membrane' were among the nine with oppositely regulated guard cell DEGs between *Arabidopsis* and *Thellungiella*. Which genes do we find in these GO terms and what does that tell us? Against our expectations, the classical salt stress-responsive genes involved in Na^+ uptake (HAK5, HKTs), Na^+ release (SOS1), vacuolar sequestration of Na^+ (NHXs, Table S2), and their regulatory factors (SOS2, MOCA1) did not respond to salt stress in *Arabidopsis* as well as *Thellungiella* guard cells. Apparently, salt stress does not address the SOS pathway in guard cells, in contrast to its impact on seedlings (Ji *et al.*, 2013).

The ability of a cell to maintain a high K^+ : Na^+ ratio has been discussed as a major mechanism for establishing salt tolerance (Carillo *et al.*, 2011). Proton ATPases generate the proton-motive force required for K^+ uptake. We found the expression of the P-type ATPase genes, PAA2 and AHA7 (Hoffmann *et al.*, 2019), induced under 1× salt and 3× salt, respectively, in *Arabidopsis* guard cells but not in *Thellungiella* (Table S3). Nevertheless, sss (1× salt) caused upregulation of *Thellungiella* guard cell genes involved in K^+ transport (Table S2). Among them we found the K^+ channel AKT2/3 and the K^+ carriers TPK2, KUP7 and KEA5 (Wang & Wu, 2013), with the latter one engaged with chloroplast function (Bose *et al.*, 2017). Thus, *Thellungiella* equips the guard cells with a K^+ transporter repertoire which retains high K^+ concentrations under salt stress. The dramatic loss of K^+ in *Arabidopsis* guard cells was accompanied by an upregulation of the K^+ efflux channel gene GORK (Ache *et al.*, 2000) and the regulator of inward-rectifying K^+ channels AtKC1 (Reintanz *et al.*, 2002). It is worth noting that the guard cell-specific K^+ influx channel gene KAT2 was found downregulated, which together with upregulation of GORK may explain why *Arabidopsis* guard cells cannot maintain high K^+ concentrations under saline conditions.

The cation channel CNGC12 was strongly salt-induced in guard cells of *Arabidopsis* (Table S2) and may be involved in the generation of salt-dependent Ca^{2+} signals (Dietrich *et al.*, 2020). It is very likely that under salt stress Ca^{2+} entry via CNGC12 activates S-type anion channels in guard cells, which will cause stomatal closure. This chain of events would explain why salt stress has a much stronger impact on the stomatal conductance of *Arabidopsis* as compared with *Thellungiella* (Fig. 3d).

Elevated salt concentrations cause osmotic stress which is sensed by the hyperosmolality-gated Ca^{2+}-permeable channels OSCA1 (Yuan *et al.*, 2014) and OSCAs, which are known to modulate stomatal movement (Thor *et al.*, 2020). The K^+ loss of salt-treated *Arabidopsis* guard cells and reduced stomatal opening (Fig. 3b) coincided with the downregulation of OSCA1.2 and OSCA1.6, as well as of the ortholog OSCA2.1 (Table S2). In response to sss (1× salt), *Thellungiella* upregulates OSCA2.1 expression and resisted stomata closure.

Salt-based soil salinity comes with excessive amounts of plant-available Cl^- (Fig. S2e). Under sss conditions *Thellungiella* guard cells reduced the expression of the anion channel and transporter genes CLCA, CLCB, MSL5, NRT2.2 and Fabaceae N70 but induced the guard cell-specific anion release channel SLAC1 (Vahisalu *et al.*, 2008; Geiger *et al.*, 2009; Table S4). These changes may explain why the halophyte is able to exclude excessive Cl^- intake into guard cells (Fig. 3b). In contrast to *Thellungiella*, *Arabidopsis* guard cells accumulated Cl^-. In line with the increasing Cl^- concentrations, guard cells strongly upregulated several anion channel/transporter genes (CLCA, SLAH2, SLAH3, MLS4, four NPF/NTRs, two Fabaceae-type N70s) under both saline conditions (Table S4). However, several of these genes are known to function in NO_3^- rather than Cl^- transport.

Salt strengthens *Arabidopsis* but not *Thellungiella* guard cell ABA signaling

The glycophyte's guard cells increased ABA concentrations upon sss treatment but not under ssu, while the halophyte's guard cells did not change their ABA concentration prominently (Fig. 5b). *Arabidopsis* and *Thellungiella* guard cell genes involved in ABA signaling responded differently as well (Table S5). *Thellungiella*

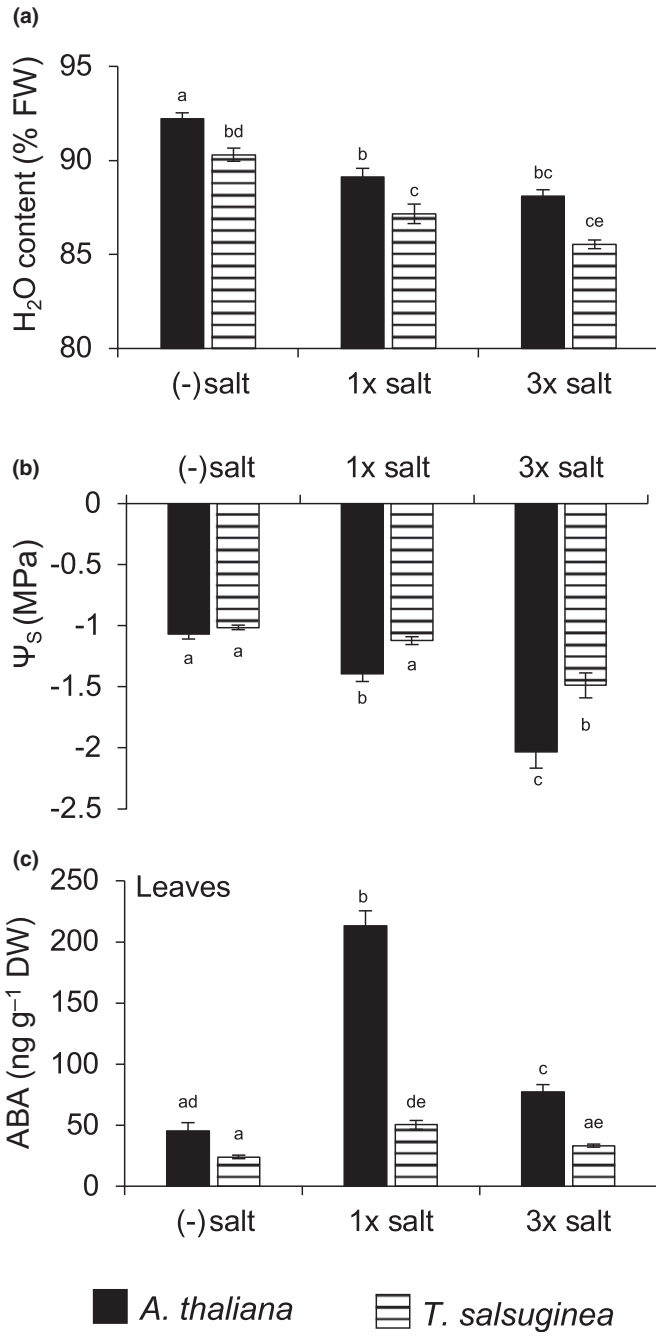


Fig. 4 Water relations of *Arabidopsis thaliana* and *Thellungiella salsuginea* rosette leaves under NaCl stress. Relative water content (a), osmotic potential (Ψ_S) (b), and abscisic acid (ABA) concentrations (c) of fresh rosette leaves. Plants in soil pots were treated with 1× salt (200 mM NaCl on days 13 and 14), 3× salt (200 mM NaCl solutions on days 1 and 2, days 7 and 8, and days 13 and 14) or tap water ((–)salt). Data represent means of eight to 19 samples per treatment (± SE) of three independent experiments. Statistical analysis was performed by two-way ANOVA followed by Bonferroni *post hoc* test for multiple testing correction. Different letters indicate significant differences according to $P \leq 0.05$.

guard cells strongly reduced expression of the ABA receptor genes PYL1, PYL4, PYL5 and PYL6 under both salt treatments. This finding suggests repression of ABA sensing in *Thellungiella* guard cells despite induction of the SNRK2.9 kinase and phosphatase

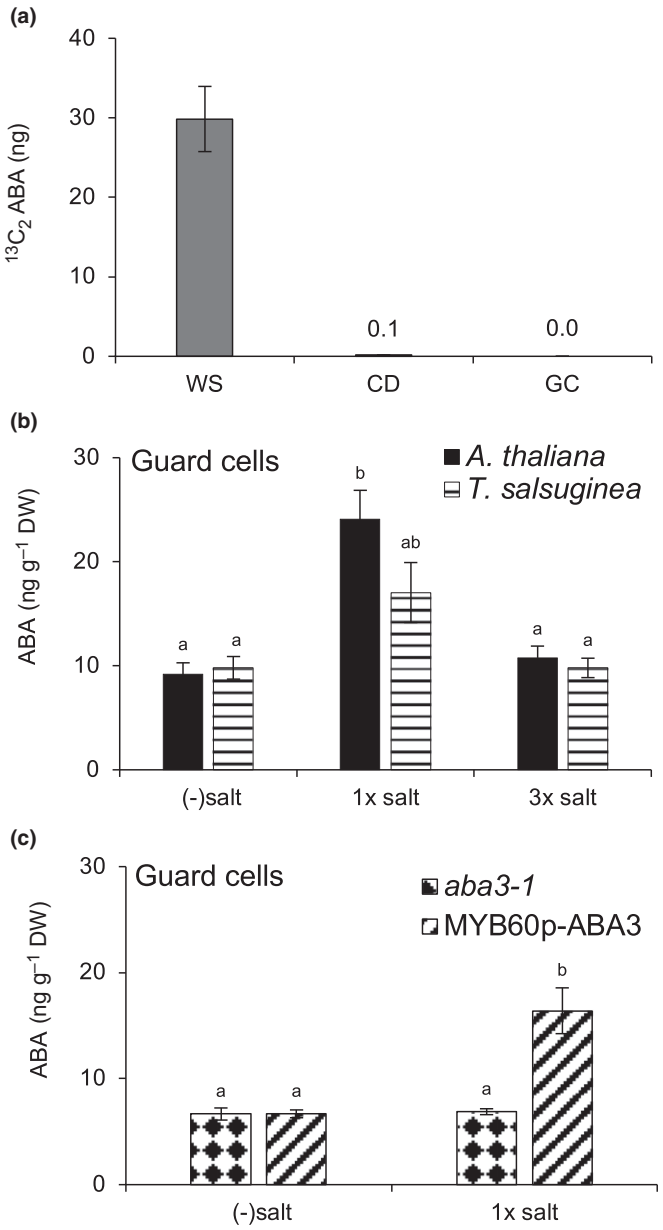


Fig. 5 Abscisic acid (ABA) concentrations in guard cells of *Arabidopsis thaliana* and *Thellungiella salsuginea* plants grown on saline and nonsaline soil. (a) Amount of ABA isotope (S-(+)-ABA$^{13}C_2$; Qmx Laboratories Ltd, Thaxted, UK) externally added to the leaf samples before guard cell preparation and recovered from the washing solution (WS), cell debris (CD) and guard cells (GC) of *Arabidopsis*. (b) ABA concentrations in guard cells of *Arabidopsis* and *Thellungiella* rosette leaves after cultivation of plants on nonsaline ((-) salt) or saline soil (1× salt: 200 mM NaCl on days 13 and 14; 3× salt: 75 mM NaCl on days 1 and 2, 150 mM NaCl on days 7 and 8, and 200 mM NaCl on days 13 and 14). Mean values of eight to 10 guard cell preparations (± SE) per treatment of three independent experiments are displayed. (c) ABA concentrations in guard cells of ABA mutant plants (*aba3-1*) and *aba3-1* mutant plants complemented with the wild-type *ABA3* coding sequence expressed under control of the guard cell-specific promoter MYB60 (MYB60p-ABA3) after one-time treatment with (1× salt) or without ((–)salt) salt. Shown are mean values of four to six samples per treatment (± SE). Statistical analysis was performed using two-way ANOVA followed by Bonferroni *post hoc* test for multiple testing correction. Different letters indicate significant differences according to $P \leq 0.05$.

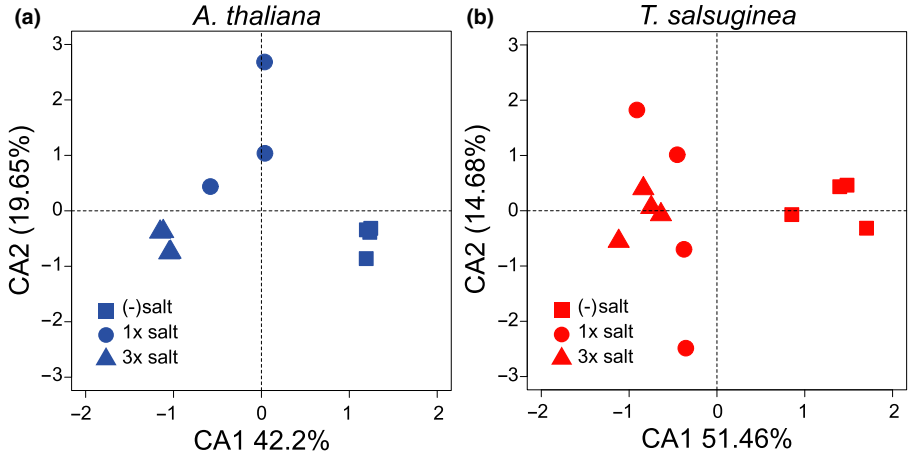


Fig. 6 Transcriptome differences of guard cells from *Arabidopsis thaliana* and *Thellungiella salsuginea plants* grown on saline and nonsaline soil. (a, b) Correspondence analysis with normalized signal intensities of the microarray data (Agilent) hybridized with *Arabidopsis* (a) and of *Thellungiella* guard cells (b). Plants were cultivated in the abscense ((-) salt) and presence of salt (1× salt: 200 mM NaCl on days 13 and 14; 3× salt: 75 mM NaCl on days 1 and 2, 150 mM on days 7 and 8, and 200 mM on days 13 and 14). Four guard cell RNA samples per treatment (one less for *Arabidopsis* 1× salt) were used for transcriptome analysis (see also Supporting Information Fig. S3).

HAI gene expression under ssu conditions. *Arabidopsis* guard cells instead upregulated the complete ABA signaling pathway even more strongly under long-term salt stress with low ABA concentrations. This involves the ABA receptors PYL5 and PYL6, the PP2CA phosphatases AHG1, HAI1 and HAI3, the Ca^{2+}-independent kinase SNRK2.9, and Ca^{2+}-dependent CBL-interacting kinases of the SNRK3/CIPK family as well as the positive signaling regulator CAR6. Thus, salt-stressed *Arabidopsis* guard cells induce a molecular machinery most likely to feed forward ABA sensing, signaling and stomatal closure.

Discussion

A principal salt tolerance feature is preventing excessive accumulation of Na^+ within the shoot via control of the transpiration rates. Earlier studies have shown that the halophyte *Aster tripolium* but not the related glycophyte *Aster amellus* strongly prevents translocation of Na^+ to the shoot and induces partial stomatal closure only when Na^+ exceeds a certain threshold in the apoplast surrounding guard cells (Kerstiens *et al.*, 2002). In agreement with this study, we found that the halophyte *Thellungiella* closes stomata partially only under prolonged growth on saline soil (1× salt) when leaves accumulated more than twice (2.7-fold) as much Na^+ as under short-term salt treatment conditions (3× salt). The leaf Na^+ concentrations of the glycophyte *Arabidopsis* was high under both short- and long-term salt applications. This finding supports the notion that the glycophyte cannot prevent Na^+ translocation to the shoot as effectively as the halophyte.

Salt stress compromises K^+ and anion transport of the glycophyte's guard cell

Under our saline growth conditions guard cells of the salt-tolerant *Thellungiella* and the salt-sensitive *Arabidopsis* plants both accumulated Na^+ to a similar extent, whereas the K^+ concentrations remained high in *Thellungiella* guard cells only. The SOS pathway (SOS1, SOS2) maintaining ion homeostasis does not respond to soilborne salinity stress in both guard cell types. Nevertheless, *Thellungiella* guard cells are known to be equipped with the molecular tools maintaining the K^+ : Na^+ balance under salt stress (Wu *et al.*, 2018; Zelm et al., 2020). This was also the case under our salt stress conditions, as *Thellungiella* guard cells activate expression of genes for K^+ uptake (AKT2/3, KUP7) and vacuolar Na^+ sequestration (NHX6). From salt-tolerant accessions of *Arabidopsis* it is known that they upregulate K^+ transporter genes while Na^+-related transporter genes do not change their expression (Sun *et al.*, 2015). Guard cells of the salt-sensitive *Arabidopsis* cv Col-0 we investigated did not make use of this salt tolerance mechanism but increased expression of the K^+ release channel GORK. This finding is supported by a study on guard cells of the glycophyte *Vicea faba* which also cannot maintain a high K^+ : Na^+ ratio under salt stress (Franzisky *et al.*, 2020).

Patch-clamp studies on guard cells of the halophyte *A. tripolium* and the nonhalophyte *A. amellus* species have shown that both guard cell types are equipped with a similar array of cation channels, but their activities are differentially regulated by Na^+ in the two species, ultimately leading to a differential stomatal response (Véry *et al.*, 1998). Sodium inhibits the inward rectifying K^+ conductance and increases the outward-rectifying K^+ conductance of halophytic guard cells, thereby reducing stomatal apertures. The differential stomatal responses of *Arabidopsis* and *Thellungiella* we observed under sss (but not ssu) conditions may be controlled by NaCl-dependent upregulation of guard cell genes involved in K^+ transport. Salt stress upregulates the K^+ outward rectifier GORK in *Arabidopsis* guard cells. By contrast, *Thellungiella* guard cells induced the K^+ uptake channel/transporter genes AKT2/3/KUP7 under saline conditions. This difference in gene expression pattern between the halophytic and

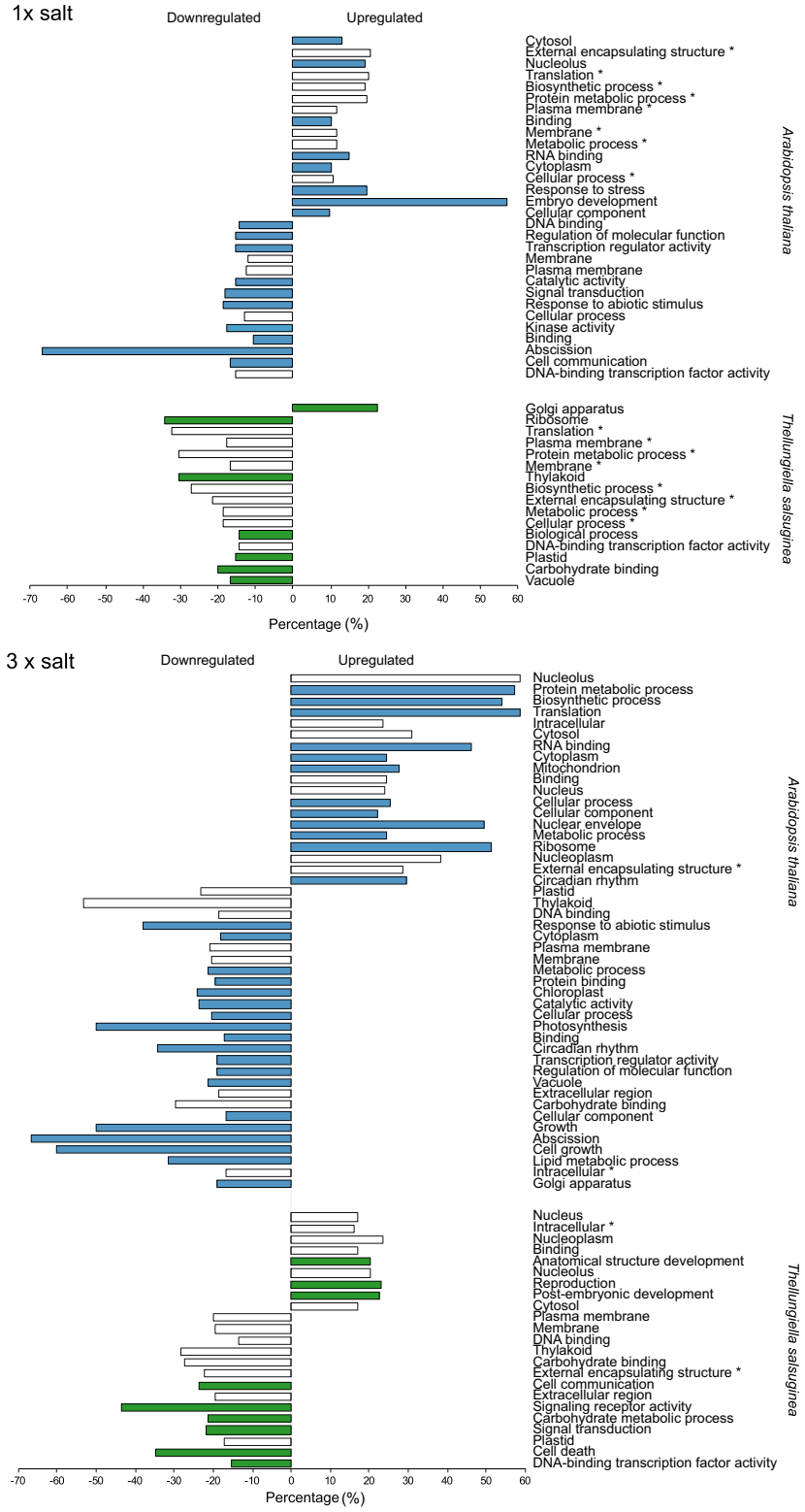


Fig. 7 Gene ontology (GO) terms significantly enriched in differentially expressed genes (DEGs) of *Arabidopsis thaliana* and *Thellungiella salsuginea* guard cells under salt stress. GO slim enrichment analysis with differentially expressed guard cell genes ($P \geq 0.05$; DEGs). DEGs were calculated for salt (1× salt (200 mM NaCl on days 13 and 14) and 3× salt (200 mM NaCl solutions on days 1 and 2, days 7 and 8, and days 13 and 14)) vs nonsalt samples ((–)salt). Shown are solely GO terms (adjusted $P \leq 0.05$) with a relative enrichment either in upregulated (positive values) or downregulated (negative values) DEGs. The GO terms are ranked from the highest (top) to the lowest (bottom) significance level. Blue columns are specific for *Arabidopsis*, green for *Thellungiella* and white columns indicate GO terms enriched in DEGs of both guard cell types. Asterisks (*) mark the GO terms enriched in upregulated *Arabidopsis* DEGs but downregulated in *Thellungiella* DEGs and vice versa.

glycophytic guard cell type is confirmed by electrophysiological studies of the salt-tolerant *Lamprothamnium papulosum* and the salt-sensitive *Chara corallina*. The salt tolerant *Lamprothamnium* activates H^+ pumps upon salt exposure which keep K^+ flowing into the cell (Beilby & Shepherd, 2001). By contrast, the salt-sensitive Chara removes K^+ through outward-rectifying K^+ channels from the cell as a result of the lack of H^+ pump activity (Shepherd *et al.*, 2008).

Our studies also show that the halophytic guard cell type excludes Cl^-, while those of the glycophyte accumulate Cl^- and lose K^+. This is reflected by the *Arabidopsis* guard cell's transcriptome, in which the expression of both K^+ efflux channel/transporter and anion transporter genes was elevated. Chloride ions neutralize the positive charges of K^+ and Na^+ and also play a role in regulating osmotic potential and cell turgor (Wege *et al.*, 2017; Geilfus, 2018; Franco-Navarro *et al.*, 2019). *Arabidopsis* guard cells seem to compensate the increase in positive charges by an increase in Cl^- other than *Thellungiella*. The *Arabidopsis* cation : Cl^- ratios were two-fold (sss) and 1.3-fold (ssu), whereas much higher ratios of 27-fold (sss) and 11-fold (ssu) were found for *Thellungiella* guard cells. This implies that Cl^- is not required to compensate Na^+ uptake in *Thellungiella* guard cells as they resist Cl^- intake by downregulation of anion uptake transporter genes and favor expression of the anion release channel SLAC1. The guard cells' expression pattern of genes involved in NO_3^- transport or organic acid biosynthesis does not support the possibility to replace Cl^- by NO_3^- or an organic anion. Which counter anions are used by *Thellungiella* guard cells to balance positive charges is something that needs to be explored in future studies.

Long-term salt stress alters guard cell ABA sensitivity

Stomatal closure is initiated by binding of ABA to the PYR/PYL/RCAR receptors (Ma *et al.*, 2009; Park *et al.*, 2009; Nishimura *et al.*, 2010; Julkowska & Testerink, 2015; Fernando & Schroeder, 2016; Cotelle & Leonhardt, 2019). The binding of ABA to SnRK2 receptors activates kinases and finally the anion efflux channel SLAC1 in guard cells which results in stomatal closure (Hedrich & Shabala, 2018). For *Arabidopsis* guard cells it has been shown that they are able to autonomously produce ABA under dry air conditions by inducing gene expression of the ABA biosynthesis pathway (Bauer *et al.*, 2013). Our guard cell-specific gene expression data suggest that under salinity stress *Arabidopsis* guard cells do not activate endogenous ABA production (Table S5). The reason for this may be that saline soil is sensed by roots and the root triggers ABA production and translocation to the shoot (Jia *et al.*, 2002; Ko & Helariutta, 2017). Interestingly, this is not the case in *Arabidopsis* and *Thellungiella* under ssu. Although, under ssu, ABA concentrations in guard cells remain low, stomatal conductance was found to be reduced. This may imply that long-term salt exposure sensitizes guard cells to read even fading ABA concentrations as closing signals. This assumption is supported by an upregulation of several ABA-responsive marker genes (e.g. KIN2, COR15A, LEA4-5, HB7, HB12, RD29A, RAB18, P5CS, ADH1; Hoth *et al.*, 2002).

The core ABA signaling pathway comprises the PYR/PYL/RCAR ABA receptors, PP2Cs phosphatases, and different kinase families (Roelfsema *et al.*, 2012). Of the latter, both the Ca^{2+}-independent SnRK2.6 kinase OST1 and the calcium-dependent kinase CIPK23 can activate the *Arabidopsis* S-type anion channels SLAC1 and SLAH3 to induce stomatal closure. The ABI phosphatases counteract the ABA-mediated stomatal closure (Maierhofer *et al.*, 2014; Hedrich & Geiger, 2017). Recent studies have shown that the ABA receptor genes expressed in *Arabidopsis* guard cells respond to environmental changes in a stimulus-dependent manner (Dittrich *et al.*, 2019). Upon dry air, transcription of PYR1, PYL2, PYL4, PYL5 and PYL8 is downregulated. Instead, the reduced stomatal conductance of salt-treated *Arabidopsis* plants was accompanied by upregulation of the ABA receptors PYL5 and PYL6, the kinases SnRK2.9, CBL4/SOS3, and several CIPK/SnRK3 members, the phosphatases AGH1, HAI1 and HAI3, as well as the anion channels SLAH2 and SLAH3 even under low ABA concentrations (ssu treatment) in guard cells. Future studies have to demonstrate how these *Arabidopsis* genes increase sensitivity of guard cells to ABA and command stomatal movement.

In *Thellungiella* guard cells PYL5 and PYL6 as well as PYL1 and PYL4 responded to ssu conditions, however, with downregulation of gene transcription. Nevertheless, the downstream ABA signaling components, the Ca^{2+}-independent kinase SnRK2.9 and the Ca^{2+}-dependent kinase CPK32, as well as the phosphates HAI1, were upregulated. It has to be shown how halophytic guard cells sense ABA to mediate stomatal movement under salt stress. Taken together, salinity stress adds new factors to the diversity of ABA signaling components, which differ between glycophytic and halophytic guard cell types. Thus, our study provides novel unexploited targets involved in stomatal functions under changing environmental conditions. Future work should focus on manipulation of these targets to improve stomatal behavior and plant productivity.

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Author contributions

RD, RH and AHA planned and designed the research and wrote the manuscript; SMK, HMM, PA, BMW, M Knoblauch, JF, M Krischke, MJM and C-MG performed the experiments and analyzed the data. TM, MD and MF analyzed large-scale datasets.

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Data availability

The data that support the findings of this study are openly available at Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/): reference numbers are GSE169230 for *A. thaliana* microarrays and GSE169311 for *T. salsuginea* microarrays.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Setup of the salt treatment experiments with *Arabidopsis thaliana* (cv Columbia-0) and *Thellungiella salsuginea* (cv Shandong).

Fig. S2 Ion concentrations of the soil matrix and soil solution in pots containing *Arabidopsis thaliana* and *Thellungiella salsuginea* at the end of the salt experiments.

Fig. S3 Verification of microarray-based differential gene expression analysis of *Arabidopsis thaliana* and *Thellungiella salsuginea* guard cells by quantitative real-time PCR.

Table S1 List of primers.

Table S2 Salt-dependent differentially expressed genes (DEGs) of *Arabidopsis thaliana* and *Thellungiella salsuginea* guard cells involved in cation transport.

Table S3 Salt-dependent differentially expressed genes (DEGs) of *Arabidopsis thaliana* and *Thellungiella salsuginea* guard cells encoding adenosine-tri-phosphatases (ATPases) and pyrophosphatases (PPases).

Table S4 Salt-dependent differentially expressed genes (DEGs) of *Arabidopsis thaliana* and *Thellugiella salsuginea* guard cells involved in anion transport.

Table S5 Salt-dependent differentially expressed genes (DEGs) of *Arabidopsis thaliana* and *Thelungiella salsuginea* guard cells involved in abscisic acid (ABA) metabolism, signaling and transport.

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