Chapter 6

Natural variation in Arabidopsis root plasticity reveals a role for HKT1-mediated ion transport in lateral root formation

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Abstract

Increased soil salinity reduces plant growth and yield. Although elongation of the main root is often used as a proxy for salt stress tolerance, the complex changes in root system architecture (RSA) are less well studied. Screening the HapMap population of Arabidopsis accessions for their RSA responses in control and two salt stress conditions followed by Genome Wide Association Study, revealed 100 bona fide candidate loci. The majority of the associations were identified with ratios between individual RSA parameters rather than the absolute size of RSA components. Genes underlying 71% of identified loci exhibited altered expression in response to salt stress in earlier studies. Natural variation in expression of Arabidillo-2 and HKT1 was linked to altered development of Lateral Roots (LR) and these associations were confirmed by phenotyping of mutant lines. Overexpression of HKT1 in the root stele reduced emergence and elongation of LR under saline conditions. The results presented here show that individual aspects of salinity stress can be dissected by focusing on specific RSA components such as LR emergence and elongation. Further validation of allelic variation underlying RSA plasticity will provide more insight in the molecular mechanisms leading to morphological adaptations and enhanced salt stress tolerance.

Introduction

The potential of a plant to thrive and survive in extreme climates largely depends on its genetic background. The first complete plant genome sequence provided insight in plant-specific gene families functioning in stress and development (Arabidopsis Genome Initiative, 2000). More recently, genetic variation was observed in hundreds of genes from different Arabidopsis accessions (Clark et al., 2007; Ossowski et al., 2008), highlighting the importance to study genetic diversity beyond the reference genotype Col-0. The value of genetic diversity was already recognized at the beginning of XXth century by Nikolaj Vavilov, who initiated a collection of different landraces and wild relatives of economically important crops (Vavilov, 1926). Today, many genome-sequencing projects explore the genetic variation across different accessions of various plant species from Arabidopsis to rice. Arabidopsis, commonly growing in the climate chambers of the laboratories around the globe, has a natural distribution across the Northern hemisphere. Wide natural distribution can result in adaptation to various local conditions present at the site of origin, which can be of great value for scientific and breeding purposes. With the available genomic data, the molecular players and allelic variation responsible for adaptation to harsh environmental conditions can be identified. Natural variation in Arabidopsis was previously used for successful identification of allelic variation in candidate genes involved in flowering and ion accumulation by means of GWAS (Atwell et al., 2010) or QTL mapping (Roy et al., 2013).

Salt stress is one of the major threats for modern agriculture, as it affects 20% of the cultivated area worldwide and half of the irrigated farmlands (FAO, 2012). The
studies of natural variation are of added value in the context of salinity tolerance, since plants can adopt multiple strategies to increase salt stress tolerance, such as altering their growth rate, compartmentalization of sodium ions or production of osmolytes (Munns and Tester, 2008). One of the most robust and relevant traits when screening for salinity tolerance is accumulation of Na\(^+\) in shoot tissue. The build up of Na\(^+\) ions in leaf tissue strongly correlates with decreased photosynthesis, reduced growth rate and yield (Munns and Tester, 2008). Allelic variation in HKT1 and CIPK13 was previously established to play a major role in Na\(^+\) exclusion from the shoot (Rus et al., 2006; Baxter et al., 2010; Munns et al., 2012; Roy et al., 2013). Another widely used phenotype is the elongation of the Main Root (MR) in salt stress conditions. This approach was frequently used in EMS mutagenesis screens and led to discovery of the Salt Overly Sensitive mutant series (Wu et al., 1996; Ding and Zhu, 1997; Liu and Zhu, 1997). As the root is the first plant organ exposed to salinity stress, it plays important role in salt sensing (Galvan-Ampudia et al., 2013) and signal transduction to the shoot tissue (Choi et al., 2014).

How salinity affects root growth in the context of the complexity of the entire root system is only starting to be uncovered (Duan et al., 2013; Geng et al., 2013; Julkowska et al., 2014). Roots are highly flexible organs and Root System Architecture (RSA) depends on the integration of developmental and environmental signals. RSA is composed of an embryo-derived Main Root (MR), and Lateral Roots (LRs), which develop from xylem pole pericycle cells in the MR. As the root grows, auxin signaling at the root tip undergoes rhythmic fluctuations. The maximal activation of auxin signaling leads to the formation of founder cells, which are spread regularly along the MR and form the starting point for LR development (Moreno-Risueno et al., 2010). Unlike other cells in the pericycle, founder cells undergo further cell division and differentiation, resulting in LR primordium development (Petricka et al., 2012). Cells flanking the active LR primordium are deflated facilitating the emergence of the LR (Vermeer et al., 2014). Salt stress exposure reduces the cell cycle activity at the root meristems, resulting in growth arrest (West et al., 2004). Quiescence of LRs is initiated by endodermal ABA signaling (Duan et al., 2013) and activation of ABA signaling plays an important role in transcriptional regulation of genes involved in initiation of quiescence as well as growth recovery (Geng et al., 2013). In the long term, salt stress was observed to reduce MR growth more severely than LR elongation in the Arabidopsis accession Col-0. However, this pattern was not conserved among other Arabidopsis accessions (Julkowska et al., 2014). Since RSA changes are not only important for anchorage, but also for efficient water extraction and ion exclusion (Faiyue et al., 2010), the changes in RSA are likely to contribute to salinity tolerance. The natural variation observed in earlier studies on RSA responses to salinity (Julkowska et al., 2014) indicates substantial natural variation that can be further exploited for candidate gene identification by means of GWAS.

In this study we screened the HapMap population of 347 Arabidopsis accessions for natural variation in RSA in control and two salt stress conditions (75 and 125 mM NaCl). Natural variation in salt induced changes in RSA was associated with 100 putative loci. Three candidate loci linked to average LR length (aLRL) and the ratio between aLRL and MR were selected for further validation, as these RSA traits
correspond to different strategies identified in an earlier study (Julkowska et al., 2014). Among the identified loci, we found a SNP in the proximity of *Arabidillo-2*, a gene involved in auxin independent LR development. Additionally, high expression of the sodium transporter *HKT1* in the root stele resulted in inhibited LR development under salt stress conditions, which effect was observed to be partially due to K⁺ depletion. Our results imply that the allelic variation is better explored by studying complex RSA traits, leading to identification of novel candidate genes.

**Results**

**Natural variation in RSA remodeling by salt stress**

Salt stress is known to reduce plant growth as well as to remodel RSA (Julkowska et al., 2014). In order to examine the natural variation in RSA development under salt stress conditions, the HapMap population, consisting of 347 Arabidopsis accessions, was screened (Fig. S1, Table S1). Four days old seedlings were transferred to media containing 0, 75 or 125 mM NaCl and the RSA of 8 and 12 days old seedlings was quantified with EZ-Rhizo software (Armengaud et al., 2009) in control and salt stress conditions respectively (Fig. S1). Col-0 was used as the reference accession across individual experimental batches, indicating high reproducibility between the experiments (Fig. S1). Natural variation within the HapMap population was quantified and presented using notched box-plots. Considerable natural variation in Total Root Size (TRS), Main Root Length (MRL) and average Lateral Root Length (aLRL) was observed in all conditions studied (Fig. 1A). In majority of RSA traits the range of natural variation was larger for plants growing at 75 mM NaCl, while it was comparable between control and 125 mM NaCl conditions (Fig. 1A, Fig. S2). The accessions identified as outliers varied among the traits and conditions (Fig. 1A, Fig. S2). In order to examine whether the differences in RSA observed under control conditions would play a role in salt stress induced RSA responses, the accession-specific correlations of traits between control and salt stress conditions were examined (Table 1). All RSA traits studied except the Main Root Vector Angle (MRVA), exhibited significant (p-value < 0.01) correlations between the control and salt stress conditions, albeit the correlation strength differed between individual RSA traits. In order to investigate to what extent the salt stress results in remodeling of RSA, the correlations between individual RSA traits per condition were studied (Fig. 1B, Table S2). The correlation between average LR length and MR length was very strong at control conditions, but decreased in both salt stress conditions. A similar observation was made for the correlation between length of the branched zone and average LR length. Those results imply that although natural variation in RSA development under saline conditions strongly depends on the developmental differences between the accessions, exposure to salt stress induces RSA remodeling, as the correlations between individual RSA parameters are decreasing in plants grown under salt stress conditions.

Principal Component Analysis was performed in order to obtain a low dimensional summary of the data by extracting 3 individual Principal Components
(PCs), explaining 41, 18.8 and 11.2% of the observed variance in respectively PC1, PC2 and PC3 for the entire population (Table S3). PC values for individual conditions clustered into distinct populations (Fig. S3 A). The patterns in response to salt stress of individual PCs were observed to vary between accessions (Fig. S3 B). PC analysis of RSA development implied that salt stress significantly remolds RSA, as the salt-induced changes are evident even when complex RSA traits are reduced to three dimensions in form of PCs.
Table 1. **Overview of 17 Root System Architecture traits measured.** The means were calculated across all 347 accessions used for this study for all conditions studied. Pearson correlation coefficients ($r^2$) are shown for correlations between control and both salt stress conditions, calculated from average values for each accession. Significant correlations are designed * - for 0.05 and ** - for 0.01 significance level.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Description</th>
<th>Unit</th>
<th>0mM NaCl</th>
<th>Median</th>
<th>SD</th>
<th>75mM NaCl</th>
<th>Median</th>
<th>SD</th>
<th>125mM NaCl</th>
<th>Median</th>
<th>SD</th>
<th>Correlation with control conditions</th>
<th>75mM NaCl</th>
<th>125mM NaCl</th>
</tr>
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<tr>
<td>TRS</td>
<td>Total Root Size</td>
<td>cm</td>
<td></td>
<td>5.471</td>
<td>1.794</td>
<td>9.077</td>
<td>3.973</td>
<td></td>
<td>2.983</td>
<td>1.250</td>
<td></td>
<td>0.61**</td>
<td>0.529**</td>
<td></td>
</tr>
<tr>
<td>MRL</td>
<td>Main Root Length</td>
<td>cm</td>
<td></td>
<td>3.969</td>
<td>0.779</td>
<td>4.689</td>
<td>0.963</td>
<td></td>
<td>2.100</td>
<td>0.597</td>
<td></td>
<td>0.527**</td>
<td>0.466**</td>
<td></td>
</tr>
<tr>
<td>MRVL</td>
<td>Main Root Vector Length</td>
<td>cm</td>
<td></td>
<td>3.506</td>
<td>0.836</td>
<td>4.244</td>
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<td></td>
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<td>0.584</td>
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<td>Depth</td>
<td>cm</td>
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<td>0.693</td>
<td>4.042</td>
<td>0.865</td>
<td></td>
<td>1.768</td>
<td>0.492</td>
<td></td>
<td>0.447**</td>
<td>0.374**</td>
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<tr>
<td>MRVA</td>
<td>Main Root Vector Angle</td>
<td>°</td>
<td></td>
<td>11.007</td>
<td>9.690</td>
<td>-3.905</td>
<td>9.073</td>
<td></td>
<td>-0.426</td>
<td>8.858</td>
<td></td>
<td>-0.039</td>
<td>0.002</td>
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<tr>
<td>Straightness</td>
<td>MRL / MRVL Ratio</td>
<td></td>
<td></td>
<td>0.884</td>
<td>0.026</td>
<td>0.876</td>
<td>0.030</td>
<td></td>
<td>0.872</td>
<td>0.040</td>
<td></td>
<td>0.224**</td>
<td>0.184**</td>
<td></td>
</tr>
<tr>
<td>noLR / MR</td>
<td>Number of Lateral Roots per Main Root</td>
<td># LR</td>
<td></td>
<td>8.000</td>
<td>3.792</td>
<td>18.000</td>
<td>7.832</td>
<td></td>
<td>7.000</td>
<td>4.302</td>
<td></td>
<td>0.456**</td>
<td>0.453**</td>
<td></td>
</tr>
<tr>
<td>LRD / MR</td>
<td>Lateral Root Density per MR cm</td>
<td>#LR / cm</td>
<td></td>
<td>2.117</td>
<td>0.733</td>
<td>3.889</td>
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<td>3.303</td>
<td>1.299</td>
<td></td>
<td>0.443**</td>
<td>0.363**</td>
<td></td>
</tr>
<tr>
<td>LRD / BZ</td>
<td>Lateral Root Density per Branched Zone cm</td>
<td># LR / cm</td>
<td></td>
<td>6.266</td>
<td>1.826</td>
<td>6.685</td>
<td>2.031</td>
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<td>6.412</td>
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<td>LRL</td>
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<td></td>
<td>0.831</td>
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<td>0.599**</td>
<td>0.443**</td>
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<td>aLRL</td>
<td>Average Lateral Root Length</td>
<td>cm</td>
<td></td>
<td>0.173</td>
<td>0.112</td>
<td>0.235</td>
<td>0.220</td>
<td></td>
<td>0.104</td>
<td>0.137</td>
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<td>0.575**</td>
<td>0.322**</td>
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<tr>
<td>Apical</td>
<td>Length of Apical Zone</td>
<td>cm</td>
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<td>2.457</td>
<td>0.740</td>
<td>1.831</td>
<td>0.589</td>
<td></td>
<td>0.735</td>
<td>0.402</td>
<td></td>
<td>0.347**</td>
<td>0.186**</td>
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</tr>
<tr>
<td>Basal</td>
<td>Length of Basal Zone</td>
<td>cm</td>
<td></td>
<td>0.154</td>
<td>0.177</td>
<td>0.133</td>
<td>0.212</td>
<td></td>
<td>0.148</td>
<td>0.188</td>
<td></td>
<td>0.289**</td>
<td>0.227**</td>
<td></td>
</tr>
<tr>
<td>Branched</td>
<td>Length of Branched Zone</td>
<td>cm</td>
<td></td>
<td>1.348</td>
<td>0.617</td>
<td>2.807</td>
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<td></td>
<td>0.512**</td>
<td>0.477**</td>
<td></td>
</tr>
<tr>
<td>MRL / TRS</td>
<td>Main Root Length per Total Root Size</td>
<td>Ratio</td>
<td></td>
<td>0.734</td>
<td>0.146</td>
<td>0.525</td>
<td>0.188</td>
<td></td>
<td>0.742</td>
<td>0.178</td>
<td></td>
<td>0.62**</td>
<td>0.462**</td>
<td></td>
</tr>
<tr>
<td>LRL / MRL</td>
<td>Lateral Root Length per Main Root Length</td>
<td>Ratio</td>
<td></td>
<td>0.363</td>
<td>0.343</td>
<td>0.904</td>
<td>0.754</td>
<td></td>
<td>0.347</td>
<td>0.476</td>
<td></td>
<td>0.559**</td>
<td>0.367**</td>
<td></td>
</tr>
<tr>
<td>aLRL / MRL</td>
<td>Average Lateral Root Length per Main Root Length</td>
<td>Ratio</td>
<td></td>
<td>0.044</td>
<td>0.062</td>
<td>0.050</td>
<td>0.117</td>
<td></td>
<td>0.048</td>
<td>0.112</td>
<td></td>
<td>0.33**</td>
<td>0.144**</td>
<td></td>
</tr>
</tbody>
</table>

**Candidate gene discovery by means of Genome Wide Association Study**

The natural variation in 17 RSA traits and three PCs was used as an input for GWAS using the scan_GLS algorithm (Kruijer et al., 2014), with implemented correction for population structure (Cao et al., 2011). The phenotypic data was associated with a collection of 214 051 SNP markers (Atwell et al., 2010) and the associations above -10log(P-value) threshold determined with Gao-correction (Gao, 2011) were selected. The heritability of RSA traits varied between 0.09 for LRD / BZ at 75 mM NaCl and 0.840 for MRVA at control conditions (Table S4). RSA traits with heritability below 0.2 were excluded from further analysis. In total, GWAS identified 150, 132 and 254 significantly

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Table 2. Overview of number of significant associations identified. The associations identified with 17 RSA traits for all conditions used in this study with –log10(p-value) > 5.6. Ordering of traits is based on the number of associations identified at 75 mM NaCl

<table>
<thead>
<tr>
<th>Trait</th>
<th>0 mM NaCl</th>
<th>75 mM NaCl</th>
<th>125 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>aLRL / MRL</td>
<td>58</td>
<td>53</td>
<td>134</td>
</tr>
<tr>
<td>Basal</td>
<td>2</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>PC3</td>
<td>9</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Straightness</td>
<td>0</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>aLRL</td>
<td>3</td>
<td>7</td>
<td>49</td>
</tr>
<tr>
<td>Depth</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>MRL</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>LRL</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>MRVL</td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MRVA</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>noLR / MR</td>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td>LRL / MRL</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>PC2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TRS</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>4</td>
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<tr>
<td>Apical</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Branched</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MRL / TRS</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PC1</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LRD / BZ</td>
<td>48</td>
<td>n/a</td>
<td>3</td>
</tr>
</tbody>
</table>

The associations identified with 17 RSA traits for all conditions used in this study with –log10(p-value) > 5.6. Ordering of traits is based on the number of associations identified at 75 mM NaCl.

The candidate loci associated with RSA phenotypes in saline conditions associated SNPs for control, 75 and 125 mM NaCl conditions respectively (Table 2). The RSA traits associated with most loci were aLRL, aLRL / MRL and Straightness.

Since GWAS is prone to identification of false positive association, the identified loci underwent further selection. In order to group associations dependent on their locations, all identified SNPs were assigned to the closest Arabidopsis gene. The robustness of associations was determined based on different GWAS settings (RSA trait values calculated on average vs. individual replica per accession), association strength above the Bonferroni threshold (Johnson et al., 2010) and Minor Allele Frequency of the SNP marker (Fig. 2 A). A list of candidate genes was established based on the above-mentioned criteria for each condition (Tables S5, S6 and S7 for 0, 75 and 125 mM NaCl respectively). Next, overlap of identified associations across different conditions was examined (Fig. 2 B). Only one locus (At5g53290) was associated with RSA development in control and 125 mM NaCl conditions. At control conditions the associations was found with LRD / BZ, while at 125 mM the locus was associated with aLRL, aLRL / MRL and PC3. Therefore, At5g53290 could play a role in generic RSA development under control and salt stress conditions. Five candidate genes were identified to associate with RSA traits at both 75 and 125 mM NaCl. The overlap in associations found in two salt stress conditions suggests a robust role of underlying genes in RSA development under salt stress conditions. Since most of the significant associations were identified with aLRL, aLRL / MRL or the PC corresponding with those traits (PC3), the associations found with those RSA traits were explored in more detail for both salt stress conditions studied (Fig. 2 C). At 75 mM NaCl, 18 candidate genes in total were identified with at least one of those traits and one gene (At4G10310) that was associated with both aLRL and aLRL / MRL and PC3. Therefore, At4G10310 could play a role in generic RSA development under salt stress conditions. At 125 mM NaCl 58 genes were associated with at least one of the selected phenotypes, while 9 genes were associated with both aLRL and aLRL / MRL, 2 neighboring genes associated with aLRL / MRL and PC3 and 5 genes associated with all of the phenotypes of interest.

The candidate loci associated with RSA phenotypes in saline conditions...
Figure 2. **Selection of robust GWAS associations.** Genome Wide Association Study was performed on 17 RSA traits measured on 347 Arabidopsis accessions in three conditions (0, 75 and 125 mM NaCl). (A) Associations identified in GWAS performed using individual values per accessions (green circles), genotypic means (purple circles) and Minor Allele Frequency of 1% (yellow circles) or $-\log_{10}(p$-value threshold above strong correction for multiple testing (pink circles) were identified as robust associations and were considered for further selection (see Tables S5, S6 and S7 for associations found with RSA traits of seedlings grown at control, 75 and 125 mM NaCl conditions respectively). (B) The selected associations from different conditions were examined for overlapping correlations between 0, 75 and 125 mM NaCl (blue, orange and red circles respectively). (C) The associations for most interesting RSA traits, average LR length (aLRL), ratio between aLRL and Main Root Length (aLRL/MRL) and Principal Component 3 (PC3) were investigated in more detail for 75 and 125 mM NaCl conditions (orange and red circle respectively). The genes listed in individual sections are the genes directly underlying identified SNPs and the extent of Linkage Disequilibrium is not accounted for. (Tables S6 and S7) were examined for alteration in cell type specific expression in response to salt (Dinneny et al., 2008). The genes directly underlying the associated SNP as well as the neighboring genes were screened for at least two-fold transcriptional changes in response to salt (Supplemental table 2 in Dinneny et al., 2008). From 100 loci associated with RSA development under salt stress conditions, the expression of 10 genes, directly underlying the associations was altered by salt stress treatment (Table S8). In addition, expression of 61 genes proximal to underlying candidate genes was also
Table 3. List of putative candidate genes identified with GWAS. The putative candidate genes chosen for validation by study of expression and T-DNA insertion lines phenotyping based on the –log10(p-value) threshold, Minor Allele Frequency (MAF) of the SNP associated with the RSA phenotype, number of SNPs associated with single locus and association when using Multiple Testing Correction (MTC). The list represent the genes associated with SNPs identified by means of GWAS as well as genes in the Linkage Disequilibrium region around the selected SNPs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Position</th>
<th>RSA trait</th>
<th>[NaCl] (mM)</th>
<th>-log10(p-value)</th>
<th>MAF</th>
<th>MTC</th>
<th>Gene function</th>
</tr>
</thead>
<tbody>
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<td>AT1G29880</td>
<td>1</td>
<td>10459869</td>
<td>aLRL / MRL</td>
<td>125</td>
<td>11.239</td>
<td>0.01</td>
<td>+</td>
<td>glycyl-tRNA synthetase</td>
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<td></td>
<td></td>
<td>125</td>
<td>8.430</td>
<td>0.01</td>
<td>+</td>
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<td>10463585</td>
<td>aLRL / MRL</td>
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<td>5.998</td>
<td>0.01</td>
<td>-</td>
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<td>75</td>
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<tr>
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<td>75</td>
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<td>HOMEBOX-LEUCINE ZIPPER PROTEIN 3 - Transcription Factor involved in control of shoot apical meristem</td>
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significantly altered in response to salt (Table S8). This overlap between the candidate loci associated with salt induced changes in RSA and cell-type specific alterations in transcription in response to salt implies the validity of GWAS approach in identification of salt responsive candidate genes and allelic variation therein.

Although the associations identified with GWAS were found across multiple conditions and RSA traits and were correlated with the transcriptional changes observed in other studies, further validation of the candidate gene function is necessary. Three candidate loci (Table 3) found across different conditions (AT1G29890) and associated with multiple individual RSA traits at 75 mM NaCl (AT3G60370 and AT4G10310) were selected for further analysis. Since the associations could be also due to neighboring genes in Linkage Disequilibrium (LD) with the identified SNPs, the selection of candidate genes was extended depending on the LD in the region (Kooke, 2015).
The associations were validated with available mutant lines and the extent of natural variation in transcription levels of candidate genes was examined.

**Allelic variation in RWA4 / Ven3 underlie the natural variation in root development**

Five SNPs associating with aLRL / MRL at 75 and PC2 and PC3 at 125 mM NaCl were localized in the coding regions of the adjacent genes At1g29880, encoding *glycyl-tRNA synthetase* and At1g29890, encoding *Reduced Wall Acetylation 4 (RWA4)*, involved in acetylation of xyloglucans during secondary cell wall synthesis. The identified SNPs were found to be in LD with the SNPs in neighboring At1g29900 encoding *Venosa-3 (Ven-3)*, involved in biosynthesis of arginine. Natural variation in expression of the candidate genes was studied in root and shoot tissue of five days old seedlings, transferred to control (C) or 75 mM NaCl (S) for 24 hours (Fig. 3 A – B). The expression of At1g29880 was reduced by twofold in root and shoot tissue by salt stress treatment in all accessions studied (Fig. 3 A), except in the Cvi (Fig. 3 B), which showed low expression of At1g29880 across all conditions studied. The expression of RWA4 (At1g29890) was, in general, highest in the root under control conditions (Fig. 3 A), with some accessions (Co-4 and Si-0) showing higher expression in the shoot tissue at control conditions (Fig. 3 B). The expression of RWA4 was largely reduced by salinity stress in root (Jl-3, DraiV6-1, Hs-0 and MIB-22) or shoot tissue (MNF-Che-2, Can-0, Lov-5 and CUR-3) (Fig. 3 A - B). The expression of Ven-3 (At1g29900) exhibited high natural variation in the root tissue at control conditions and expression was severely reduced when seedlings were treated with mild salt stress (Fig. 3 A). Only expression of Ven-3 in root under control conditions exhibited significant correlations with MRL in control conditions ($r^2 = 0.396$, Figure S4 A, Table S13). In order to validate the role of candidate genes in RSA development in control and salt stress conditions, the available T-DNA insertion lines were examined. Only one T-DNA insertion line in the promoter region of Ven-3 (SALK_047105) (Fig. 3 C) could be validated as homozygous. T-DNA insertion lines of other two candidate genes were either not available (At1g29880) or could not be properly genotyped (At1g29890). The ven3 mutant line showed reduced LRL development under control conditions (Fig. 3 D, Fig. S4 B). Seedlings of ven-3 did not show any significant alteration in RSA development at 75 mM NaCl, when compared to Col-0 seedlings (Fig. 3 D, Fig. S4 B), suggesting a role for Ven-3 in root growth under control conditions.

The allelic variation in the region spanning the candidate genes that are in LD with the identified SNPs was explored by sequencing the entire locus in accessions with different expression levels of candidate genes under control conditions, as the natural variation observed therein was larger than under salt stress conditions. Based on sequence similarity, a Maximum Parsimony (MP) tree was calculated (Tamura et al., 2011) (Fig. 3 E). The promoter regions were analyzed for alterations in predicted cis-regulatory elements, while polymorphisms in gene coding regions were studied for non-synonymous changes in amino acid sequence (Fig. 3 F, Tables S15 and S16 respectively). Although five SNPs were identified in the promoter region of Ven-3,
Figure 3. Phenotypic variation in aLRL / MRL at salt stress corresponds to Ven-3 and RWA4. (A) Natural variation in the expression of genes underlying the associated locus was studied in root and shoot tissue in 5 days old seedlings treated with mock (C) or 75 mM NaCl (S) for 24 h. The box plots represent the median and extent of natural variation as observed for population of 48 accessions. (B) The natural variation in expression of individual candidate genes was explored in further detail with heat maps normalized per individual gene. The red indicate high and blue low relative expression as observed for 3 biological replicas. (C) The positions of GWAS
associated SNPs in the locus are indicated with dashed lines. The position of available T-DNA insertion line, ven3 (SALK_047105), used for mutant phenotyping study is indicated with triangular insertion. (D) 4 days old seedlings of Col-0 and ven-3 were transferred to 0 and 75 mM NaCl and the RSA of 8 ad 12 days old seedlings respectively was quantified. Bar-plots represent the average length as observed in 16 replicates and error bars represent SE. Significant differences between the lines were calculated using one-way ANOVA with Tukey’s post-hoc test. * and **- represents the significance of 0.05 and 0.01 respectively. (E) The locus underlying the associated SNPs was sequenced in seven accessions showing varying levels of transcription in candidate genes. The sequences were used for calculating maximum parsimony. (F) The polymorphisms leading to non-synonymous changes in amino acid sequence or alterations in cis-regulatory elements in promoter region are highlighted in red. The accessions carrying the identified polymorphisms are listed above individual SNPs. The sequences of cis-regulatory elements identified are highlighted in blue. The full list of polymorphisms identified can be found in Table S15 and S16 for polymorphisms identified in promoter and exon regions respectively.

each leading to significant changes in predicted cis-regulatory elements (Table S15), none of these were shared between the accessions with similar Ven-3 expression levels. Additional to 3 SNPs in the promoter region of Wt-5, exhibiting low Ven-3 expression in the root, a deletion of 9 nucleotides in the 5’ UTR region was found. The majority of the polymorphisms were found in the promoter and coding regions of At1g29880 and At1g29890. Three accessions, Db-0, DraIV6-1 and Jl-3, carry an insertion of one nucleotide in the 7th exon of RWA4, leading to a frame shift mutation and premature termination of translation.

Allelic variation resulting in a premature STOP codon in RWA4 suggests that the association identified by GWAS could be due to natural variation in the RWA4 gene-coding region. On the other hand, the results obtained from the ven-3 mutant, show that Ven-3 is involved in maintenance of lateral root growth under control conditions. Although the role of RWA4 in root development is still to be confirmed, our analysis reduces the candidate genes underlying the association found at this locus to RWA4 and Ven-3.

Arabidillo-2 enhances RSA development in control and salt stress conditions

The SNP corresponding to At3g60370, encoding an immunophilin protein (FKBP20-2), was found to be associated with aLRL / MRL and PC3 at 75 mM NaCl. As the LD at this specific locus extended from At3g60260 up to At3g60380 gene, the list of the candidate genes was extended from At3g60350 to At3g60380. Natural variation in expression of candidate genes was studied in root and shoot tissue of five days old seedlings grown at control (C) or 75 mM NaCl (S) media for 24 hours (Fig. 4 A). At3g60360, encoding U3 small nucleolar RNA-associated protein 11 (UTP11), exhibited the highest expression in the root under control and salt stress conditions (Fig. 4 A-B), while At3g60370 (FKBP20-2) was expressed predominantly in the shoot tissue at both conditions in all accessions (Fig. 4 A-B). The expression of At3g60380, encoding an unknown protein, was predominant in the shoot tissue at control conditions, with only one accession (Jl-3) showing higher expression in the root tissue (Fig. 4 B). Salt treatment strongly reduced the expression of At3g60380, encoding an unknown protein, in roots and shoots of all accessions. Expression of At3g60350, encoding an Arm-repeat protein Arabidillo-2, was relatively high in root and shoot tissues at control conditions, and was reduced after exposure to salt stress. Interestingly, Arabidillo-2 expression was below the detection
Figure 4. Expression of Arabidillo-2 corresponds to LRL at control and salt stress conditions. (A) Genes in LD with associated SNP were examined for the natural variation in their expression in root and shoot tissue in 5 days old seedlings treated with mock (C) or 75 mM NaCl (S) for 24 h. The box plots represent the median and extent of natural variation as observed for population of 48 accessions. (B) The natural variation in expression of individual candidate genes was explored in further detail with heat maps normalized per individual gene. The red boxes indicate high and blue low relative expression as observed for 3 biological replicas. (C) The positions of GWAS associated SNPs in the locus are indicated with dashed lines. The position of available T-DNA insertion lines, ara2-1 (SALK_084537), ara2-2 (SAIL_162_B11), unknwn1-1 (SALK_064996) and unknwn1-2 (SALK_030527)), used for mutant phenotyping study is indicated with triangular insertions. (D) 4 days old seedlings of Col-0 and T-DNA lines were transferred to 0 and 75 mM NaCl and the RSA of 8 ad 12 days old seedlings respectively was quantified. The bar-plots represent the average TRS as observed in 16 replicates and error bars...
level in one accession (UKSE06-272), implying UKSE06-272 to be a natural knockout mutant of Arabidillo-2 (Fig. 4 B).

In order to further examine the role of putative candidate genes in RSA development, the phenotypes of available T-DNA insertion lines were studied (Fig. 4 C and D). No significant differences in RSA phenotypes were observed in T-DNA insertion lines located in the exon of At3g60380 (unknwn1-1 and unknwn1-2). Two T-DNA insertion lines in Arabidillo-2 showed either higher (ara2-1, SALK_084537) or lower (ara2-2, SAIL_162_B11) expression of Arabidillo-2 compared to Col-0 (Fig. S5 A). The TRS of ara2 mutant lines correlated with Arabidillo-2 expression, with ara2-1 developing slightly larger and ara2-2 significantly smaller RSA in both control and salt stress conditions (Fig. 4 D, Fig. S5 A).

Accessions showing differential levels of Arabidillo-2 expression were explored for a possible correlation between Arabidillo-2 expression and RSA traits (Fig. 4 E). Additional RSA phenotyping of seven accessions revealed a significant correlation at salt stress conditions between LRL and Arabidillo-2 expression. Moreover, the accession identified as a natural Arabidillo-2 knockout (UKSE06-272) was observed to develop shorter LRL under salt stress conditions (Fig. 4 E). The allelic variation in the locus was further examined by sequencing the region spanning FKB20-2 and Arabidillo-2 in selected accessions. The promoter regions were analyzed for alterations in predicted cis-regulatory elements, while gene-coding regions were examined for non-synonymous changes in amino acid sequence (Figure 4 F, Tables S17 and S18 respectively). Four non-synonymous mutations in the exons of Arabidillo-2 were identified (V103A, T237A, S734A and E913G). In other candidate genes, a single nucleotide insertion was observed in the 5th exon of At3g60360, leading to a frame shift mutation. The polymorphisms in the promoter region specific to UKSE06-272, a natural knockout of Arabidillo-2, were explored. Four UKSE06-272-specific polymorphisms were identified (P2, P3, P5 and P7), of which three (P3, P5 and P7) lead to 4 additional cis-elements and disturbed 6 cis-regulatory elements present in Col-0. Although polymorphisms P3 and P7 were shared with Ha-0 and Van-0 accessions P5, a single nucleotide substitution, was specific to UKSE06-272. Surprisingly, this substitution results in a loss of the E-box / ABRE motif and a hypothetical MYC-recognition site, and addition of a rolD motif (Table S17). In combination with other polymorphisms shared with other accessions, this small change in the promoter sequence could underlie decreased expression of Arabidillo-2 in UKSE06-272.
High HKT1 expression reduces LR development in saline conditions

The candidate locus that was identified with aLRL and aLRL / MRL at 75 mM NaCl was located in the promoter region of At4g10310, a gene coding for *Arabidopsis High Affinity K*⁺ *transporter 1* (AtHKT1). The extent of natural variation in HKT1 expression was observed to be the highest in the root tissue of seedlings grown at control conditions (Fig. 5 A), with Gr-5, Hs-0, N4, Ga-2 and Jl-3 showing the highest expression and LDV-58, CUR-3 and Tsu-0 the lowest. Expression of HKT1 was reduced by salt stress exposure in almost all accessions, except in Hs-0. Mutant lines with reduced HKT1 expression (Fig. S6 A) did not exhibit any differences in RSA development from Col-0 in control or salt stress conditions (Fig. 5 B - C, Fig. S6 B). However, mutant lines with enhanced root stellar expression of HKT1 in Col-0 and C24 backgrounds (Moller et al., 2009) developed less and shorter LR under salt stress conditions compared to the

**Figure 5. High HKT1 expression reduces LR development in salt stress conditions.** (A) Natural variation in the HKT1 expression was studied in root and shoot tissue in 5 days old seedlings treated with mock (C) or 75 mM NaCl (S) for 24 h. The box plots represent the median and extent of natural variation as observed for population of 48 accessions. (B) The SNP identified by means of GWAS in the promoter region is indicated with dashed line and locations of T-DNA insertion lines, hkt1-1 (GK-386D05) and hkt1-2 (GK-795G10), tested for RSA development are indicated with triangular insertions. (C) T-DNA insertion lines with reduced HKT1 expression as well as (D) UAS-HKT1 lines with enhanced HKT1 expression were examined for salt induced changes in RSA. 4 days old seedlings of Col-0 and T-DNA lines were transferred to 0 and 75 mM NaCl and the RSA of 8 ad 12 days old seedlings respectively was quantified. The bar-plots represent the average trail value as observed in 16 replicates and error bars represent SE. The phenotypes of T-DNA insertion lines were tested for significant differences, calculated using one-way ANOVA with Tukey’s post-hoc test. Different letters are used to indicate the significant differences between the genotypes per condition as calculated using one-way ANOVA with Tukey’s post-hoc test with significance levels of 0.05. (E) Pictures of representative 12 days old seedlings of UAS-HKT1 lines grown at 75 mM NaCl.
respective background lines (Fig. 5 D - E). Additionally, enhanced HKT1 expression in Col-0 background (E2586 UAS-HKT1) caused a severe reduction in MR growth under saline conditions (Fig. 5 D - E). When UAS-HKT1 lines were grown on media supplemented with 150 mM mannitol, no differences between the background lines and lines overexpressing HKT1 could be observed (Fig. S7 A). These observations indicate that the reduced LR number and length phenotype of UAS-HKT1 lines is due to ionic stress rather than the osmotic component of salinity.

As LR patterning is determined at the root tip (Moreno-Risueno et al., 2010), the effect of salt on LR patterning and emergence is different for the MR regions formed

Figure 6. Reduction in LR development above and below the transfer point is due to sodium toxicity and potassium starvation respectively in HKT1 root stellar overexpression lines. As the patterning of LRs is determined at the root tip, the effect of enhanced HKT1 expression on LR patterning and development of established LR primodia was tested by examining the LR development in the MR fragment formed above (fragment A) and below (fragment B) transfer point. In UAS-HKT1 lines with enhanced HKT1 expression in root stellar cells (A) number of emerged LR as well as (B) aLRL was reduced in both fragments by salinity stress compared to respective background lines. In order to dissect the effect of sodium toxicity and potassium starvation on RSA development, the media were supplemented with additional potassium. While (C) the number of emerged LR was rescued by potassium supplementation only above transfer points (D) aLRL was complemented above the transfer point for both lines. The bar-plots represent the average value as observed over 16 replicates and error bars represent SE. Different letters are used to indicate the significant differences between the genotypes per condition as tested by one-way ANOVA with post-hoc Tukey’s test with significance levels of 0.05.
prior and after the transfer to salt stress conditions (Duan et al., 2013). In order to examine at which stage enhanced HKT1 expression inhibits LR development, the LRs above and below the transfer point were analyzed separately (fragment A and B of the MR, respectively, **Fig. 6 A - B**). The reduction in LR number in fragment A was less severe than in fragment B (**Fig. 6 A**), while the decrease in aLRL was observed to be similar for both fragments (**Fig. 6 B**). As salt stress results in potassium starvation, the effect of additional K\(^+\) on UAS-HKT1 lines phenotype was examined. Although potassium supplementation did not cause any significant differences between the mutant lines under control conditions, supplementing salt-containing media with additional K\(^+\) rescued LR emergence in the MR fragment formed prior to salt stress exposure, but did not affect LR emergence in fragment B (**Fig. 6 C**). Additional K\(^+\) rescued LR elongation in UAS-HKT1 lines in fragment A under salt stress conditions (**Fig. 6 D**). The reduction of MR length in Col-0 UAS-HKT1 line was not affected by additional potassium (**Fig. S7 B**). Those results illustrate that potassium starvation affect LR development above and below transfer point. Enhanced HKT1 expression promotes sodium ion accumulation in root tissue, resulting in reduced LR development, which cannot be rescued by additional K\(^+\).

UAS-HKT1 lines were previously described to exhibit enhanced salt stress tolerance (Moller et al., 2009). However, in the experimental set up used by Moller (et al., 2009), plants were grown in transpiring conditions and salt stress was applied 3 weeks after germination. In order to examine the relationship between the altered RSA in UAS-HKT1 lines and salt stress tolerance, UAS-HKT1 and the background lines (J2731 and E2586)

**Figure 7.** The effect of enhanced HKT1 expression on salinity tolerance depends on developmental stage and genetic background. (A) E2586, E2586 UAS-HKT1, J2731 and J2731 UAS-HKT1 lines were germinated under short day conditions and treated from above with 75 mM NaCl one, two or three weeks after germination. The pictures of six weeks old plants with the distribution of the genotypes per pots indicated in the upper right panel. (B) Fresh-weight of the rosette was determined 6 weeks after germination. The bar-plots represent average fresh weight observed in 15 replicates and error bars represent Standard Error. Different letters are used to indicate the significant differences between the genotypes and conditions as tested with one-way ANOVA with post-hoc Tukey’s test with significance levels of 0.05
were examined for salt stress tolerance in transpiring conditions. Seedlings were
germinated in pots and stressed 1, 2 or 3 weeks after germination with 75 mM NaCl
applied to the soil from above (Fig. 7 A). UAS-HKT1 line in Col-0 background (E2586
UAS-HKT1) was observed to develop smaller rosettes than its background line (E2586)
when plants were stressed one week after germination (Fig. 7 B). This difference
between the E2586 and E2586 UAS-HKT1 lines was less pronounced when plants
were treated 2 weeks after germination or later. UAS-HKT1 line in C24 background
developed larger rosettes than the background line (J2731) independent of the timing of
stress treatment. Interestingly, the rosettes of J2731 plants grown in control conditions
showed no significant difference with plants stressed for 2 or 3 weeks with salt stress
(Fig. 7 B). Those results suggest that C24 is less responsive to growth reduction by
salinity stress than Col-0 and that enhanced HKT1 expression is detrimental for rosette
development in Col-0 background when salt stress is applied at the early stage of plant
development. Therefore, the correlation between high HKT1 expression and salinity
tolerance is dependent on the background and the developmental stage at which plants
are exposed to salinity stress.

In order to examine the allelic variation corresponding to the initial association
with aLRL and aLRL / MRL under salt stress conditions, the correlation between HKT1
expression and aLRL in salt stress conditions was explored in further detail (Fig. 8
A, Table S13). Although no overall correlation was observed, lines with low HKT1
expression exhibited a wide range of aLRL phenotypes under saline conditions, while
lines with strong HKT1 alleles, like Hs-0, Gr-5, N4 and Jl-3, developed short aLRL at
saline conditions. The allelic variation in the locus was further examined by sequencing
the promoter and coding region of HKT1 gene in selected accessions. A maximum
Parsimony (MP) tree was calculated (Tamura et al., 2011), based on sequence similarity
and omitting the insertion / deletion polymorphisms (Fig. 8 B). Only two accessions
showing high HKT1 expression, Gr-5 and N4, clustered into a group in the MP analysis.
The regions encoding the two first exons of HKT1 were found to carry 11 polymorphisms,
which lead to 5 non-synonymous changes in the amino acid sequence (Fig. 8 C, Table
S20), however none were located in the trans-membrane domain. The first intron of
HKT1 contained a deletion of 400 base pairs in all accessions except in Col-0 and WI-0
(Fig. 8 D). In total, more than 90 polymorphisms were found in the promoter region and
the consequence of those SNPs for the predicted cis-regulatory elements was examined
(Higo et al., 1999) (Table S19). In total 98 Col-0 specific and 98 non-Col-0 specific
cis-regulatory elements were found. 33 cis-regulatory elements shared between two
accessions, N4 and Gr-5, carrying a strong HKT1 allele were identified (Fig. 8 B). The
location of the promoter elements specific for Gr-5 and N4 was spread between -1330
and -3530 base pairs from the HKT1 START codon. Although the promoter region of
HKT1 shows low conservation, the shared cis-regulatory elements between two strong
HKT1 alleles in N4 and Gr-5 accessions suggest that the allelic variation is involved in
distal regulation of HKT1 expression.
Discussion

Root elongation in saline conditions is broadly used as an indicator for salinity tolerance. Salt stress not only reduces the development of roots, but also causes reprogramming and re-distribution of the root mass between MR and LRs (Julkowska et al., 2014). In this study, 347 Arabidopsis accessions were screened for RSA changes in control and salt stress conditions. The assay we employed for studying RSA proved to be reproducible both technically and biologically (Fig. S1), providing reliable quantitative parameters for root development under different conditions. Quantification of RSA in 17 traits (Table 1) revealed natural variation present within each parameter (Fig. 1 A, Fig S 2). Analysis of the correlations for individual RSA traits between control and salt stress conditions (Table 1) revealed a significant correlation between the RSA development in control and salt stress conditions. Those results suggest that natural variation in RSA development observed in control conditions is an important factor when seedlings are grown under saline stress. Observed differences in correlation strength within (Table 1) and between (Fig. 1 B, Table S2) individual RSA traits in control and

Figure 8. Allelic variation underlying enhanced HKT1 expression present in the distal promoter region. (A) The correlation between natural variation in HKT1 expression and aLRL at salt stress conditions was not significant, yet a clear trend could be observed as the accessions with high HKT1 expression consequently developed short aLRL. (B) The HKT1 promoter and coding region up to 3 kbp from START codon was sequenced in seven accessions. The sequences were used for calculating maximum parsimony (MP) tree and analyzed for the presence of cis-regulatory elements in the promoter region. The number of cis-acting elements shared between the accessions clustered into distinct groups based on MP is shown in the Venn-diagram overlapping the MP tree. (C) Number of polymorphisms leading to non-synonymous changes in amino acid sequence or alterations in cis-regulatory elements in promoter region is highlighted in red. The accessions with identified polymorphisms are listed above individual SNPs. The sequences of cis-regulatory elements identified on leading DNA strand specific to N4 and Gr-5 accessions are highlighted in blue. The SNP identified with GWAS is indicated with dashed line. The full list of polymorphisms identified can be found in Table S19 and S20 for polymorphisms identified in exon and promoter regions respectively.
saline conditions, suggests that there is significant natural variation in relative salinity sensitivity and confirms different RSA strategies identified between Arabidopsis accessions (Julkowska et al., 2014).

The GWAS analysis performed on the collected data revealed a great number of associations with individual RSA traits in the two salt stress conditions (Table 2). Traits commonly used as salt stress tolerance indicators such as MRL or TRS yielded limited number of associations, while RSA traits corresponding to the largest number of associations were LRD / BZ and aLRL / MRL (Table 2). The number of identified associations should be taken with caution, as GWAS is known to be prone to false positive associations (Mitchell-Olds, 2010). Those results highlight the potential of GWAS in identification of novel genes involved in root development under control and saline condition through studying complex RSA traits. The number of false positive associations was further reduced by implementation of population structure (Cao et al., 2011), adjusting the –log10(p-value) threshold and by applying a correction for multiple testing (Duggal et al., 2008; Gao, 2011). Next to these classical criteria, we took advantage of the complexity of the associations observed in different RSA traits and/or at different saline conditions studied (Fig. 2 B - C).

In total, this analysis yielded a selection of 100 putative candidate loci associated with RSA phenotypes at 75 and 125 mM NaCl. Unlike other forward genetic screens, which used root growth as a readout for salinity tolerance, we did not identify any associations with Salt Overly Sensitive (SOS) genes (Wu et al., 1996; Ariga et al., 2013). Although mutations in those genes are known to cause severe phenotypes in RSA under salt stress conditions, low allelic variation at those loci could prevent their identification by screening natural accessions. From the 100 selected loci, expression of 71 genes was previously found to be altered in cell type specific salt stress responses of root transcriptome (Dinneny et al., 2008). This selection contained novel putative players in salt stress-induced RSA changes, such as SnRK2.7, EIN2 and CYP79B2. This overlap between identified loci and cell-type specific expression suggests association with genuine candidates in RSA development under salt stress conditions. Remaining candidates showing no alterations in transcription levels in response to salinity stress could either be involved in plant development or their function could be regulated on a post-transcriptional level. In any case, further validation of candidate gene function and the allelic variation therein is necessary before conclusions can be drawn about functional importance of candidates in the process of salt-induced RSA plasticity.

For a selected set of candidates, we assessed the natural variation in expression and allelic variation affecting the gene function, and studied knockout mutant phenotypes in the Col-0 background. Based on association strength and repeated association with multiple RSA parameters, three loci were selected for further validation (Table 3). Multiple SNPs clustered in the coding region of At1g29890, encoding Reduced Wall Acetylation 4 (RWA4). RWA4 encodes a protein that functions in acetylation of xylan, one of the major components of secondary cell walls, expressed in secondary xylem and interfascicular fiber cells (Lee et al., 2011). However, role of RWA4 involvement in RSA could be studied since the T-DNA insertion were not available or could not be confirmed as homozygous. However, T-DNA insertion line in neighboring Ven3
(At1g29900) exhibited a significant reduction in LR development at control conditions (Fig. 3 D). *Ven3* encodes an enzyme involved in arginine synthesis (Mollá-Morales et al., 2010). Arginine is crucial for plant development and acts as precursor for the polyamines, involved in salinity tolerance (Urano et al., 2004). Both candidate genes, *RWA4* and *Ven3*, could potentially be involved in the phenotypic variation associated with RSA development in salt stress conditions.

Since the SNPs identified with GWAS usually do not alter transcription or gene function by themselves but are rather linked to the causal mutation, the region in Linkage Disequilibrium with the identified SNP needs to be considered for possible candidate genes. The LD for the SNP identified in the coding region of At3g60370 extended over the *Arabidillo-2* gene (At3g60350), encoding a protein containing an F-box domain, Leucin rich-repeats and an Arm-repeat domain, previously shown to be involved in LR branching (Coates et al., 2006). A T-DNA insertion line with reduced *Arabidillo-2* expression (*ara2-2*) (Fig. S5 A) exhibited smaller TRS in both control and salt stress conditions (Fig. 4 D). By studying natural variation in expression of *Arabidillo-2*, a natural knockout of *Arabidillo-2*, UKSE06-272, was identified (Fig. 4 B), which exhibited reduced LR development under salt stress conditions (Fig. 4 E). Sequencing of the locus indicated two UKSE06-272 specific SNPs in the Arabidillo-2 promoter that were predicted to alter several cis-regulatory elements (Table S16). The protein coding part of *Arabidillo-2* was observed to contain only one Ler specific polymorphism (S734A) found in the last Armadillo domain, important for interactions with other proteins (Gibbs et al., 2014). The identified polymorphisms in the promoter region of a natural *Arabidillo-2* knockout suggest that the cis-acting elements disturbed by a single SNP are important for *Arabidillo-2* transcription. The role of *Arabidillo-2* in shaping the RSA during salt stress can be further explored by allelic complementation and identification of transcription factors binding to the cis-regulatory elements.

A SNP in the promoter of *HKT1* was associated with aLRL and aLRL / MRL at 75 mM NaCl. Associations with *HKT1* have previously been identified in many studies focusing on natural variation in sodium accumulation in the leaf tissue (Rus et al., 2006; Baxter et al., 2010; Munns et al., 2012). *HKT1* was so far not acknowledged for a role in RSA development, although it has previously been identified as complementing the SOS1 phenotype in root growth (Rus et al., 2001). T-DNA insertion lines with reduced *HKT1* expression did not develop significantly altered RSA under any condition studied (Fig. 5 C, Fig. S6), and accessions exhibiting low *HKT1* expressions showed great diversity in RSA phenotypes (Fig. 8 A). However, enhanced *HKT1* expression in UAS-*HKT1* lines (Fig. 5 D), as well as high *HKT1* expression in several natural accessions (Fig. 8 A), resulted in a severe decrease in LR development in salt stress conditions. The allelic variation responsible for high HKT1 expression was determined to be widespread across the distal promoter-region (Fig. 8 C) with 33 predicted cis-regulatory elements shared between accessions with strong *HKT1* alleles (Fig. 8 B). The 33 predicted cis-regulatory elements did not overlap with either the minimal promoter of *HKT1* (Maser et al., 2002), or ABI4 binding sites (Shkolnik-Inbar et al., 2013). Further validation of the role of allelic variation underlying enhanced *HKT1* expression will require identification of transcription factors and their binding sites enhancing *HKT1* expression.
Lines with root stele specific overexpression of HKT1 (UAS-HKT1) are an
elegant tool for tissue specific manipulation of sodium accumulation (Moller et al.,
2009; Plett et al., 2010; Hill et al., 2013). Although salinity tolerance of a C24 J2731
UAS-HKT1 line was reported to be enhanced when 3 weeks old plants were exposed
to salinity (Moller et al., 2009), enhanced HKT1 expression was observed to reduce
RSA development when 4 days old seedlings were exposed to salt. An age dependent
effect of HKT1 overexpression on salinity tolerance was further demonstrated for the
Col-0 E2586 UAS-HKT1 line (Fig. 7), and could be explained by differences in
sodium-storage capacity of 4 days old roots versus 3 weeks old roots. J2731 UAS-
HKT1, in agreement with previously published data (Moller et al., 2009) exhibited
increased salinity tolerance independent of the developmental stage at the application
of salt stress (Fig. 7). Interestingly, the J2731 line did not exhibit salt-induced rosette
growth inhibition as severe as the E2586 line, suggesting that rosette growth of the C24
background is less sensitive to salt stress than the Col-0 background.

UAS-HKT1 lines were further used for examining the effect of different aspects
of salinity stress on RSA development. The reduction in LR development is ascribed to
high cytosolic sodium levels and activation of ABA signaling, leading to LR quiescence
(Duan et al., 2013) and remodeling of auxin distribution (Galvan-Ampudia and
Testerink, 2011). Interestingly, when seedlings were grown with additional potassium
supplement, LR elongation and LR emergence in the root fragment above the transfer
point were restored in both UAS-HKT1 lines (Fig. 6 C - D), while the LR development
below the transfer point remained repressed (Fig. 6 C). Those results suggest that the
development and/or emergence or LR primodia is highly sensitive to Na⁺ toxicity, while
LR elongation or release of ABA-dependent LR quiescence (Duan et al., 2013) seems to
be inhibited by K⁺ starvation and disturbed Na⁺/K⁺ ratio. Interestingly, LR emergence is
not reduced at mild salt stress (25 - 50 mM NaCl), while a reduction in LR elongation
is observed (Zolla et al., 2010), implying that the effects of K⁺ starvation are visible
at conditions that do not induce Na⁺ toxicity. Those findings suggest that RSA shows
distinct responses to different aspects of salinity stress, such as Na⁺ toxicity and Na⁺/K⁺
balance. Further validation of the candidates associated with aLRL and other aspects of
RSA development in saline conditions should provide more insight on other aspects of
salinity stress and subsequent changes in different RSA parameters.

Material & Methods

Plant material and growth conditions
Arabidopsis accessions belonging to HapMap collection were obtained from the European Arabidopsis Stock
Centre (www.nasc.nl). The HapMap population consisting of 360 accessions was propagated under long day
conditions (21°C, 70% humidity, 16/8h light/dark cycle), with 8 weeks long vernalization (between 4 and
8°C, 70% humidity, 16/8h light/dark cycle) starting at 3rd week after germination to ensure flowering of all
the accessions. The accessions that failed to germinate or flower were excluded from the screen resulting in
347 accessions in total (Table S1). Seeds used for the experiments were between 2 months and 1 year old.

Seeds were surface sterilized in a desiccator of 1.6L volume using 20 ml household bleach and 600
μL 40% HCl for 3 hours and were put in the laminar flow for 1.5 hours to remove toxic vapors. The seeds were
stratified in 0.1% agar at 4°C in the dark for 72 h and sown on square petri dishes containing 50ml of control
growth medium consisting of ½ Murashi-Skoog, 0.5% sucrose, 0.1% M.E.S. Monohydrate and 1% Daishin
agar, pH 5.8 (KOH), dried for 1 h in a laminar flow. Plates were placed vertically under the angle of 70°. Seeds were germinated under long day conditions (21°C, 70% humidity, 16/8h light/dark cycle). Four days old seedlings were transferred to square petri dishes containing basic medium supplemented with 0, 75 or 125 mM NaCl. Each plate contained four seedlings of two genotypes (two seedlings per genotype). Plates were placed in the growth chamber following a random design. The plates were scanned with Epson perfection V700 scanner at 200 dpi every other day until 8th day post transfer. The 8 days old seedlings grown in 0 mM NaCl were used for phenotyping of RSA in control conditions while for both salt stress conditions (75 and 125 mM NaCl) phenotypes of 12 days old seedlings were scored. The pictures were analyzed with EZ-Rhizo software (Armengaud et al., 2009). The entire population of 360 accessions was screened over six individual experiments with Col-0 as internal reference (Fig. S1). The reproducibility of the RSA phenotypes of Col-0 was high despite the low number of biological replicates (n=4) used.

Analysis of natural variation in RSA phenotypes
The collected data on RSA phenotypes was cleared of outliers and the correlations between the individual traits in control and salt stress conditions were studied using SPSS (Table 1). The correlations among different RSA traits at different conditions are presented in Table S2. The natural variation in the studied population was further explored by using average values per accessions as an input for notched boxplots (Spitzer et al., 2014) (Figure 1 A, Figure S2). In order to reduce the number of parameters Principal Component Analysis was performed. The raw data was first normalized per trait by distracting the average value of each RSA trait from the individual data points and subsequently dividing the individual data points by the variance observed in individual phenotypes. By doing so the relative importance of the different traits and variance was normalized. The Principal Components were calculated using Mat Lab. The importance of individual RSA traits for each PC is presented in Table S2. Individual PC’s as well as the raw phenotypic data were used as input for Genome Wide Association Study.

GWAS
The RSA phenotypes were linked to published genomic data on accessions from a 250k SNP chip with average SNP density of one SNP in 500 bp (Atwell et al., 2010). We performed different GWAS using entire SNP data set as well as excluding the SNPs with minor allele frequency of 0.01, 0.05 and 0.10 in order to avoid misleading associations. The associations between each SNP and individual RSA phenotypes were tested using a scan_GLS algorithm (Kruijer et al., 2014), which is based on EMMA-X. The method implements two corrections for multiple testing in order to minimize the false discovery rates. In first, stricter method, the threshold is determined by \(-\log_{10}(\alpha \times p\text{-value})\), where \(\alpha\) is significance level of either 0.01 or 0.05 (referred in the scan_GLS.r script as BT=1). The other method uses the number of markers (p) replaced by the number of effective tests approach, as in (Gao, 2011) (referred in the scan_GLS.r script as BT=4). Both of the methods were applied on individual phenotypic values per accession (n=4) and genomic means per accession with \(\alpha\) of 0.01 and 0.05 and MAF of 0.00, 0.01, 0.05 and 0.10.

To prioritize genomic regions a list of genes associated with the significant p-value was generated for mapping with individual values and genomic means. The list of genes was subsequently divided using metrics considering the minor allele frequency at which the association was found, significance of the association and the correction for multiple testing. The locus was considered to be associated with RSA when it was found associating in GWAS with average and individual values and at least one other criterion (MAF ≥ 0.01 and / or correction for multiple testing). The full lists of the associations found in control, 75 mM and 125 mM NaCl conditions are to be found in Tables S5, S6 and S7. The candidates from all conditions studied were compared and the associations overlapping between different conditions were identified. Moreover the phenotypes that gave clear differences between different RSA strategies (Julkowska et al., 2014), such as average LR length (aLRL) and average LR length as a ratio of MR (aLRL / MRL) were studied in greater detail in both salt stress concentrations. The final selection of the putative candidates was made based on the number of significantly associated SNPs within one locus (within 10kb interval from each other), \(-\log_{10}(p\text{-value})\) score (Tables S5, S6 and S7) and the trait heritability (Table S4). The putative candidate list was extended to neighboring genes depending on the genomic region being in LD with the SNPs found significantly associated (Table S9)
T-DNA insertion lines genotyping and phenotyping

The lines were ordered from the European Arabidopsis stock center (nasc.org.uk). The full list of t-DNA insertion lines used is to be found in Table S10. The T-DNA insertion lines were genotyped by extracting DNA from leaf material grounded in liquid nitrogen using 10% Chelex (Bio-rad) in MilliQ followed by 15 minutes incubation at 95°C and 15 minute centrifugation at maximum speed in the standard tabletop centrifuge. The supernatant was used as an input for the PCR reaction. The primers used for t-DNA insertion lines identification are listed in Table S10. The RSA phenotypes of T-DNA insertion lines were studied as described above for the phenotyping of Arabidopsis accessions (n=16).

The expression levels of gene of interest in T-DNA insertion lines were studied by qPCR analysis. The RNA was extracted from the whole seedlings grown on agar plate for 12 days in control conditions with TRI-reagents (Sigma Aldrich) with additional chloroform cleaning step, followed by TURBO DNase treatment (Ambion). The RNA was checked for the integrity on 2% agarose gel. The cDNA was synthesized from 1μg total RNA using reverse transcriptase (Fermentas), diluted to approximately 10ng/μl and used for qPCR reaction using Eva-Green kit (Solis Biodyne), Applied Biosystems sds7500 machine using three biological replicates and two technical replicates. The expression was normalized using AT1G13320 transcript levels. The expression levels of putative candidate genes were calculated by ΔCt = 2- (Ct-value target) ⁄ 2- (Ct-value reference). The sequences of the primers used are listed in Table S12.

Expression analysis

48 Arabidopsis accessions were selected for studying the natural variation in the expression of candidate genes. The list of accessions used for expression study can be found in Table S11. 4 days old seedlings of 48 different accessions were transferred to plates supplemented with 0 or 75 mM NaCl. After 24 h the seedlings were harvested, snap-frozen in liquid nitrogen and divided into root and shoot fraction. RNA extraction was performed with TRI-reagents (Sigma) with additional chloroform cleaning step, followed by TURBO DNase treatment (Ambion). The RNA was checked for the integrity on 2% agarose gel. The cDNA was synthesized from 1μg total RNA using reverse transcriptase (Fermentas). The cDNA was diluted to approximately 10ng/μl. The diluted cDNA was used in a specific target amplification (STA) reaction and subjected to PCR on a Biomark genetic analysis system on a 96 x 96 Dynamic array, according to manufacturers instruction (Fluidigm). The primer sequence for qPCR was designed focusing on the 3’ end and trying to avoid the sites with high natural variation in the sequence as found out in 1001 sequence browser. The sequences of the primers used are to be found in Table S12. The transcript levels of At1G07920, At1G13320, At3g04120, At5G12240 and At5G46630 were used for normalization of expression.

The Ct-values ≥ 30 were set to 30 in order to reduce the background noise. The expression levels of target genes were calculated by ΔCt = 2- (Ct-value target) ⁄ 2- (Ct-value reference). In order to normalize the expression for all five reference genes used, geometrical mean was calculated from delta-Ct normalized expression for each reference gene. The outliers were removed and the average expression per accession, tissue and conditions were calculated for each putative candidate gene.

In order to study the differences in tissue specific expression and salt responsiveness of the transcripts, the relative expression was calculated by defining a natural logarithm for ratios of expression values in root vs. shoot expression in control conditions (root vs. shoot); root in salt stress vs. control conditions (root salt vs. control) and shoot in salt stress vs. control conditions (shoot salt vs. control). The correlations between the raw expression data and RSA phenotypes observed in the earlier experiments were performed using SPSS. The values of Pearson correlation coefficients of RSA traits and expression levels are to be found in Table S13.

Sequencing the loci and parsimony calculations

The sequencing of three loci was performed on seven accessions for each locus. The accessions were chosen based on their RSA phenotype and relative expression of candidate genes. The primers used for each locus are listed in Table S14. The sequences of individual accessions were aligned using MegAlign software and aligned to each other in MegAlign ClustalW algorithm. The sequences of locus AT1G29870 - AT1G29900, AT3G60350 - AT3G60380 and AT4G10310 are presented in .pdf files available on-line. The sequences encoding exons are highlighted in yellow, introns in purple and UTRs in red. The identified polymorphisms were investigated by examining the patterns in the promoter region using the web-based database for Plant Cis-acting Regulatory DNA Elements (PLACE) (Higo et al., 1999), by examining the presence / absence
of cis-regulatory element in the sequence flanking the polymorphism. Translating exon sequences into the protein sequences in JalView was performed to examine the non-synonymous changes in amino acid sequence. The polymorphisms located in intron region were examined whether they interfere with the splicing acceptor, donor or branching site. The polymorphisms found outside of those areas were assumed to have no major effect. The polymorphisms identified in promoter and exon regions were encoded with P-numbers and in exons with E-numbers for individual locus separately and are listed in Tables S15 – S20.

The evolutionary history was inferred for each locus separately using the Maximum Parsimony method. The most parsimonious tree with length is shown. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 0 in which the initial trees were obtained by the random addition of sequences (10 replicates). The MP trees are drawn to scale; with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. The analysis involved 7 nucleotide sequences from different Arabidopsis accessions. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

**Salinity tolerance assessment in transpiring conditions**

The seed of UAS-HKT1 and the background lines to be tested were stratified for 48h at 4°C and sown in ground. Seeds of different lines were put in one pot (four plants per pot) and were germinated under short day conditions (21°C, 70% humidity, 11/13h light/dark cycle). After one, two or three weeks the seedlings were treated with 75 mM NaCl applied from above every second day for 6 weeks. After 7 weeks of growth the fresh weight of the rosette was measured. The statistical analysis was performed in Excel and SPSS was used for one-way ANOVA with Scheffe’s post-hoc test for significance.

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**References**


Supplemental Material

(A) The effect of salt stress on RSA was examined by stratifying the seeds of Arabidopsis accessions for 3 days at 4°C and subsequently growing them for 4 days at long day conditions. Seedlings were transferred to basic media supplemented with 0, 75 or 125 mM NaCl. The plates were scanned at 200 dpi every second day after transfer up till 12 days after germination. The Root System Architecture (RSA) was quantified 8 and 12 days after germination for seedlings grown in control conditions and both salt stress conditions respectively.

(B) Overview of RSA parameters obtained from EZ-Rhizo. Additionally LR length, average LR length as well as the ratios between MR, average LR length and TRS were calculated. All RSA traits are listed in Table 1. (C) Col-0 was used as an internal reference for all six experiments over which the RSA phenotyping of 347 accessions was performed. The phenotypes of Col-0 lines in different experiments were tested for significant differences using one-way ANOVA with Tukey’s post-hoc test. * - represents the significance of 0.05, ** - sig. of 0.01.

Figure S1. Experimental set up and reference for screening natural variation in RSA responses to salt stress. (A) The effect of salt stress on RSA was examined by stratifying the seeds of Arabidopsis accessions for 3 days at 4°C and subsequently growing them for 4 days at long day conditions. Seedlings were transferred to basic media supplemented with 0, 75 or 125 mM NaCl. The plates were scanned at 200 dpi every second day after transfer up till 12 days after germination. The Root System Architecture (RSA) was quantified 8 and 12 days after germination for seedlings grown in control conditions and both salt stress conditions respectively. (B) Overview of RSA parameters obtained from EZ-Rhizo. Additionally LR length, average LR length as well as the ratios between MR, average LR length and TRS were calculated. All RSA traits are listed in Table 1. (C) Col-0 was used as an internal reference for all six experiments over which the RSA phenotyping of 347 accessions was performed. The phenotypes of MR length, average LR length, number of LR, Total Root Size and LR density were calculated for four replicates per experiment per condition. The error bars represent standard error. Different shades of grey represent different experiments. The phenotypes of Col-0 lines in different experiments were tested for significant differences using one-way ANOVA with Tukey’s post-hoc test. * - represents the significance of 0.05, ** - sig. of 0.01.
Figure S2. Natural variation in all RSA phenotypes studied. Natural variation in different RSA traits was observed for Hap Map population and several accessions were determined as outliers in each trait studied. The boxplots represent the median length as observed in 342 accessions studied at 0, 75 and 125 mM NaCl (white, light grey and dark grey box plots respectively). The whiskers extend to data points that are less than 1.5x from interquartile range (IQR) away from 1st and 3rd quartile. Notches represent 1.58 x IQR / sqrt (n) and give 95% confidence that two medians differ.
The natural variation in three PCs observed for Hap Map population was observed and several accessions were determined as outliers. The boxplots represent the median length as observed in 342 accessions studied at 0, 75 and 125 mM NaCl (white, light grey and dark grey box plots respectively). The whiskers extend to data points that are less than 1.5x from interquartile range (IQR) away from 1st and 3rd quartile. Notches represent 1.58 x IQR / sqrt (n) and give 95% confidence that two medians differ.
Figure S4. Natural variation in Ven3 expression correlates with MRL at control conditions and RSA phenotypes of Ven3 t-DNA insertion lines. (A) The natural variation in expression of Ven3 was observed to significantly correlate with the MR length at control conditions. The individual points represent relative expression observed in 3 biological replicates and MRL observed over 4 replicates per accession. (B) The Lateral Root Length, average LR Length and Lateral Root Density of Col-0 and one T-DNA insertion line in the promoter of Ven3 was studied at control (light bars) and 75 mM NaCl (dark bars). The bar-plots represent the average length / density as observed in 16 replicates. The error bars represent SE. The phenotypes of T-DNA insertion line were tested for significant differences from Col-0, which were calculated using one-way ANOVA with Tukey’s post-hoc test. * - represents the significance of 0.05, ** - sig. of 0.01.
Figure S5. Arabidillo-2 expression is correlated with enhanced MR and average LR growth under salt stress and control conditions. (A) The expression of Arabidillo-2 in Col-0 and two T-DNA insertion lines ara2-1 (SALK_084537) and ara2-2 (SAIL_162_B11). The expression was normalized for the transcription levels of At1g13320 with Ct-delta method. The bars represent the average expression as observed over 3 replicas. The error bars represent the standard error. (B) Main Root Length, Lateral Root Density and average LR Length of Col-0 and two T-DNA insertion lines was studied at control (light bars) and 75 mM NaCl (dark bars). The bar-plots represent the average length / density as observed in 16 replicates. The error bars represent SE. The phenotypes of both T-DNA insertion lines were tested for significant differences from Col-0, which were calculated using one-way ANOVA with Tukey’s post-hoc test. * - represents the significance of 0.05, ** - sig. of 0.01.

Figure S6. Natural variation in HKT1 expression among root and shoot tissue in control and salt stress conditions. (A) The expression of HKT1 in Col-0 and two T-DNA insertion lines hkt1-1 (GK-386D05) and hkt1-2 (GK-795G10). The expression was normalized for the transcription levels of At1g13320 with Ct-delta method. The bars represent the average expression as observed over 3 replicas. The error bars represent the standard error. (B) The Total Root Size, Lateral Root Density and MR Length of Col-0 and two T-DNA insertion lines was studied at control (light bars) 75 mM (dark grey bars) and 125 mM NaCl (black bars). The bar-plots represent the average length / density as observed in 16 replicates. The error bars represent SE. The phenotypes of both T-DNA insertion lines were tested for significant differences from Col-0, which were calculated using one-way ANOVA with Tukey’s post-hoc test. * - represents the significance of 0.05, ** - sig. of 0.01.
Figure S7. RSA phenotype of UAS-HKT1 lines is partially rescued by addition of K+ at 75 mM NaCl. Lines overexpressing HKT1 in root pericycle in Col-0 (E2586) and C24 (J2731) backgrounds were studied for their RSA phenotype in (A) control (light grey bars) and osmotic stress condition (150 mM mannitol) (dark grey bars). (B) The effect of K+ supplementation on RSA responses of UAS-HKT1 lines was studied by supplementing 30 mM KCl to standard growth media (grey bars), 75 mM NaCl (dark grey bars) or exposing the seedlings to 75 mM KCl (light grey bars). The bar-plots represent the average length / density as observed in 16 replicates. The error bars represent SE. Different letters are used to indicate the significant differences between the genotypes per condition as calculated using one-way ANOVA with Tukey’s post-hoc test with significance levels of 0.05.
Supplemental Material Available on-line

Sequence At1g29870 - At1g29900.pdf The sequence of the locus spanning from 5’ UTR of At1g29880 to 5’ UTR of At1g29900 of Col-0, Db-0, DraIV6-1, Jl-3, an-0, Wi-0 and Wt-5. The exons are highlighted with yellow, introns with purple, UTR’s with red and STOP and START codons with blue.

Sequence At3g60350 - At3g60380.pdf The sequence of the locus spanning from 3’ UTR of At3g60350 to 1 kbp from 5’ UTR of of Col-0, Co-4, Ha-0, Bro1-6, UKSE06-272 and Van-0. The exons are highlighted with yellow, introns with purple, UTR’s with red and STOP and START codons with blue.

Sequence At4g10310.pdf The sequence of the locus spanning from 4 kbp from At4g10310 START codon to the second intron of At3g10310 of Col-0, Gr-5, Jl-3, Lov-5, N4, Tscha-1 and Wi-0. The exons are highlighted with yellow, introns with purple, UTR’s with red and STOP and START codons with blue.

Table S1. List of Arabidopsis accessions used for RSA phenotyping at control and salt stress conditions.

Table S2. Correlations between RSA traits in different growth conditions. Pearson correlation coefficients \( r^2 \) are shown for correlations between different RSA traits in all conditions, calculated from average values for each accession on each condition. Significant correlations are designed * - for 0.05 and ** - for 0.01 significance level.

Table S3. The scores of individual RSA traits for three Principal Components used for GWAS.

Table S4. Heritability for individual RSA traits calculated on individual values with MAF of 0%

Table S5. List of significant associations with RSA traits at 0 mM NaCl

Table S6. List of significant associations with RSA traits at 75 mM NaCl

Table S7. List of significant associations with RSA traits at 125 mM NaCl

Table S8. List of the candidate genes identified with RSA traits at 75 or 125 mM NaCl with significant alterations in the cell type specific expression in response to salt (Dinneny et al., 2008).

Table S9. The overview of Linkage Disequilibrium calculated for SNPs associated with 7 candidate loci (Table 3)

Table S10. Overview of t-DNA insertion lines studied including the primers used for genotyping t-DNA insertion lines.

Table S11. The list of accessions studied for natural variation in candidate gene expression. The accessions were chosen based on their haplotype as determined from 250.000 SNP dataset and their RSA phenotypes in mild (75 mM NaCl) salt stress conditions. The haplotype similarity to Col-0 is represented as a ratio of SNPs in haplotype that are Col-0 like (0) or non-Col-0 like (1).

Table S12. List of primers used for expression study of candidate genes

Table S13. Correlation between RSA traits and expression levels of candidate genes in 48 different Arabidopsis accessions. Pearson correlations \( r^2 \) are shown for correlations between the gene expression in control conditions or ratio between root and shoot specific expression and RSA phenotypes observed for 8 days old seedlings grown at control conditions. The gene expression in salt stress conditions or ratio between control and salt induced expression in root or shoot tissue was examined for correlation with the RSA phenotypes observed at 12 days old seedlings grown at 75 mM NaCl for 8 days. The correlations were calculated from average values for each accession on each condition. Significant correlations are designed * - for 0.05 and ** - for 0.01 significance level.

Table S14. List of primers used for sequencing the loci of putative candidate genes

Table S15. The list of polymorphisms found in the promoter regions of At1g29880, At1g29890 and At1g29900 and predicted changes in cis-regulatory elements as analyzed with PLACE (Higo et al., 1999)
Table S16. The list of polymorphisms found in the exon regions of At1g29880, At1g29890 and At1g29900.

Table S17. The list of polymorphisms found in the promoter regions of At3g60350, At3g60360, At3g60370 and At3g60380 and predicted changes in cis-regulatory elements as analyzed with PLACE (Higo et al., 1999)

Table S18. The list of polymorphisms found in the exon regions of At3g60350, At3g60360, At3g60370 and At3g60380.

Table S19. The list of polymorphisms found in the promoter region of At4g10310 and predicted changes in cis-regulatory elements as analyzed with PLACE (Higo et al., 1999)

Table S20. The list of polymorphisms found in the exon regions of At3g10310.