Running head:

Root morphology changes upon salt stress

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Capturing Arabidopsis root architecture dynamics with ROOT-FIT reveals diversity in responses to salinity

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Natural variation in Arabidopsis Root System Architecture dynamic response to salt stress can be clustered into four distinct strategies.
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Abstract

The plant root is the first organ to encounter salinity stress, but the effect of salinity on Root System Architecture (RSA) remains elusive. Both reduction in main root elongation and redistribution of the root mass between main and lateral roots are likely to play crucial roles in water-extraction efficiency and ion exclusion. To establish which RSA parameters are responsive to salt stress, we performed a detailed time-course experiment where Arabidopsis seedlings were grown on agar plates under different salt stress conditions. We captured RSA dynamics with quadratic growth functions (ROOT-FIT) and summarized the salt-induced differences in RSA dynamics in three growth parameters: Main Root elongation ($GROWTH_{MR}$), average Lateral Root elongation ($GROWTH_{aLR}$), and increase in number of LR ($INCREASE_{#LR}$). In the Arabidopsis accession Col-0, salt stress affected MR elongation more severely than LR elongation and increase in LRs, leading to a significantly altered RSA. By quantifying RSA dynamics of 31 different Arabidopsis accessions in control and mild salt stress conditions, different strategies for regulation of main and lateral root meristems and root branching were revealed. Different RSA strategies partially correlated with natural variation in abscisic acid (ABA) sensitivity and different Na$^+/K^+$ ratios in shoots of seedlings grown under mild salt stress. Applying ROOT-FIT to describe dynamics of RSA allowed us to uncover the natural diversity in root morphology and cluster it into four response types that otherwise would have been overlooked.
Introduction

Salt stress is known to affect plant growth and productivity as the result of its osmotic and ionic stress components. Osmotic stress imposed by salinity is thought to act in the early stages of the response, by reducing cell expansion in growing tissues and causing stomatal closure to minimize water loss. The build-up of ions in photosynthetic tissues leads to toxicity in the later stages of salinity stress and can be reduced by limiting sodium transport into the shoot tissue and compartmentalization of sodium ions into the root stele and vacuoles (Munns and Tester, 2008). The effect of salt stress on plant development was studied in terms of ion accumulation, plant survival and signalling (Munns et al., 2012; Hasegawa, 2013; Pierik and Testerink, 2014). Most studies focus on traits in the aboveground tissues, since minimizing salt accumulation in leaf tissue is crucial for plant survival and its productivity. This approach has led to the discovery of many genes underlying salinity tolerance (Munns and Tester, 2008; Munns et al., 2012; Hasegawa, 2013; Maathuis, 2013). Another way to estimate salinity stress tolerance is by studying the rate of main root elongation of seedlings transferred to medium supplemented with high salt concentration. This is how Salt Overly Sensitive mutant series were identified, being a classical example of genes involved in salt stress signalling and tolerance (Hasegawa, 2013; Maathuis, 2013). The success of this approach is to be explained by the important role the root plays in salinity tolerance. Roots not only provide anchorage and ensure the water and nutrient uptake, but also act as a sensory system, integrating changes in nutrient availability, water content and salinity to adjust root morphology to exploit available resources to the maximum capacity (Galvan-Ampudia et al., 2013; Gruber et al., 2013). Understanding the significance of environmental modifications of Root System Architecture (RSA) for plant productivity is one of the major challenges of modern agriculture (de Dorlodot et al., 2007; Den Herder et al., 2010).

The RSA of dicotyledonous plants consists of an embryonically derived Main Root (MR) and Lateral Roots (LR) that originate from xylem pole pericycle cells of the main root, or from LRs in case of higher order LRs. Root growth and branching is mainly guided through the antagonistic action of two plant hormones: auxin and cytokinins (Petricka et al., 2012). Under environmental stress conditions, the synthesis of abscisic
acid, ethylene and brassinosteroids is known to be induced and to modulate the growth of MR and LR (Achard et al., 2006; Osmont et al., 2007; Achard and Genschik, 2009; Duan et al., 2013; Geng et al., 2013). In general, lower concentrations of salt were observed to slightly induce MR and LR elongation, while higher concentrations resulted in decreased growth of both MR and LR (Zolla et al., 2010; Wang et al., 2009). The reduction of growth is a result of the inhibition of cell cycle progression and reduction in root apical meristem size (West et al., 2004). However, conflicting results were presented for the effect of salinity on LR density (Wang et al., 2009; Zolla et al., 2010; Galvan-Ampudia and Testerink, 2011). Some studies suggest that mild salinity enhances LR initiation or emergence events, thereby affecting patterning, while others imply salinity to arrest LR development. The origin of those contradictory observations could be due to studying LR initiation and density at single time points rather than observing the dynamics of LR development, as LR formation changes as a function of root growth rate (de Smet et al., 2012). The dynamics of LR growth and development were characterized previously for the MR region formed before the salt stress exposure, identifying the importance of ABA in early growth arrest of post-emerged LR in response to salt stress (Duan et al., 2013). The effect of salt on LR emergence and initiation was found to differ for MR regions formed prior and subsequent to salinity exposure (Duan et al., 2013), consistent with LR patterning being determined at the root tip (Moreno-Risueno et al., 2010). Yet, the effect of salt stress on the reprogramming of entire RSA on a longer timescale remains elusive.

Natural variation in Arabidopsis is a great source for dissecting the genetic components underlying phenotypic diversity (Trontin et al., 2011; Weigel, 2012). Genes underlying phenotypic plasticity of RSA to environmental stimuli, were also found to have high allelic variation leading to differences in root development between different Arabidopsis accessions (Rosas et al., 2013). Supposedly, genes responsible for phenotypic plasticity of the root morphology to different environmental conditions are under strong selection for adaptation to local environments. Various populations of Arabidopsis accessions were used to study natural variation in ion accumulation and salinity tolerance (Rus et al., 2006; Jha et al., 2010; Katori et al., 2010; Roy et al., 2012). Also, a number of studies focusing on the natural variation in RSA have been published, identifying QTLs and allelic variation for genes involved in RSA development under control conditions (Mouchel et al., 2004; Meijón et al., 2013) and nutrient-deficient
conditions (Chevalier et al., 2003; Gujas et al., 2012; Gifford et al., 2013; Kellermeier et al., 2013; Rosas et al., 2013). Exploring natural variation not only expands the knowledge of genes and molecular mechanisms underlying biological processes, but also provides insight in how plants adapt to challenging environmental conditions (Weigel, 2012) and whether the mechanisms are evolutionarily conserved. The early growth arrest of newly emerged LRs upon exposure to salt stress was observed to be conserved among the most commonly used accessions Col-0, Ler and Ws (Duan et al., 2013). By studying salt stress responses of entire RSA and a wider natural variation in root responses to stress, one could identify new morphological traits being under environmental selection and possibly contributing to stress tolerance.

In this work, we not only identify the RSA components responsive to salt stress, but also describe the natural variation in dynamics of salt-induced changes leading to redistribution of root mass and different root morphology. The growth dynamics of Main and Lateral Roots under different salt stress conditions were described by fitting a set of quadratic growth functions (ROOT-FIT) to individual RSA components. Studying salt induced changes in RSA dynamics of 31 Arabidopsis accessions revealed four major strategies conserved among the accessions. Those four strategies were due to differences in salt stress sensitivity of individual RSA components i.e. growth rates of MR, LR and increase in number of emerged L.R. This diversity in root morphology responses caused by salt stress was observed to be partially associated with differences in ABA, but not ethylene sensitivity. Additionally, we observed that a number of accessions exhibiting a relatively strong inhibition of LR elongation showed a smaller increase in the Na+/K+ ratio in shoot tissue after exposure to salt stress. Our results imply that different RSA strategies identified in this study reflect diverse adaptations to different soil conditions and thus might contribute to efficient water extraction and ion compartmentalization in their native environments.

Results

Salt stress decreases MR and LR length but does not affect LR density

Salinity stress is known to affect root growth (West et al., 2004) and to induce the growth arrest of emerged LR (Duan et al., 2013), yet its relative effect on individual RSA
components on a larger timescale is less clear. In order to determine which RSA parameters are most responsive to salt stress treatment, a dose-response experiment was performed on 4 different natural Arabidopsis accessions (Col-0, Bur-0, C24 and Tsu-0), previously described to differ in their salinity tolerance (Jha et al., 2010; Katori et al., 2010; Galpaz and Reymond, 2010). Four days-old seedlings were transferred to media supplemented with different NaCl concentrations (0, 25, 50, 75, 100, 125 and 150 mM NaCl), scanned at 7 days after transfer (Fig. 1A), and 27 RSA traits (Table I) were quantified with EZ Rhizo software (Armengaud et al., 2009). Next to the standard RSA parameters provided by EZ-Rhizo, LR length, average LR length were calculated. In order to distinguish the effect of salt on the LR development between the MR region formed prior and after the start of the salt stress treatment, the formation and length of LRs was studied for those two regions separately (region A and B respectively). Moreover, the ratios between MR length, average LR length and Total Root Size (TRS) were calculated for all conditions studied. For trait descriptions and an overview of salt stress induced changes in individual trait values, see Table I. The Pearson correlation coefficients ($r^2$) between MR length and other RSA parameters studied are listed in Table II.

Interestingly, lower salt concentrations (25 and 50 mM NaCl) slightly increased TRS, MR length and number of LR in Col-0 seedlings, while those traits were significantly reduced in Tsu-0 and Bur-0 and not affected in C24 seedlings (Fig. 1B, C and D). When the average LR length was studied for the region A and B separately, the differences in RSA responses between Bur-0 and Tsu-0 became more apparent. While average LR length of Bur-0 and Tsu-0 seedlings was only slightly reduced or unaffected in the region above the transfer point, average LR length below the transfer point was significantly reduced for both accessions (Fig. 1G and H). Those results indicate that the differences in RSA responses between the accessions at very low salt concentrations are best to be dissected into the regions formed prior and after the start of salt stress exposure.

At higher concentrations all accessions tested showed similar responses for all RSA components and for regions above and below the transfer point (Fig. 1G-H, Fig S1). TRS, MR length, number of LR and LR length were all significantly reduced in four accessions studied (Fig. 1B - E). The average length of LRs formed in regions A and B decreased in all four accessions (Fig. 1G – H), while the number of LRs was observed to
decrease only in region B (Fig. S1). This decrease can be explained by a reduction in MR length, as the density of LRs in neither of the regions was altered compared to control conditions (Fig. S1).

Correlation strength between MR length and both LR length and average LR length was observed to decrease with increasing salt stress concentrations (Table II), suggesting independent regulation of MR and LR growth under salt stress conditions. Increasing salt concentrations had no significant effect on LR density calculated either for MR length, region A, region B or per length of branched zone (Fig. 1 F, Fig. S1, Table I). Furthermore, the number of LRs was observed to strongly correlate with length of the MR among all salt concentrations tested (Table II). Although the experimental set up used here did not allow us to study the very early stages of LR development, our results suggest that the reduction in number of visible LRs in salt stress conditions is mainly due to a decrease in MR length.

RSA growth dynamics is best captured in quadratic growth functions

Since the increasing concentrations of salt were found to affect MR and LR length in four accessions studied, the observed reduction in TRS could be a result of overall deceleration of root development. Decelerated MR growth by itself would have a tremendous impact on the TRS, since the number of emerged LRs depends on MR length (Table II). In order to determine to what extent salt stress decelerates root development and whether changes in root morphology (RSA) are initiated, the dynamics of root development were examined. Four days old Col-0 seedlings were transferred to plates containing 0, 75 or 125 mM NaCl. RSA development was studied from 4 to 10 days after germination in control conditions and up to 12 days after germination for seedlings transferred to 75 or 125 mM NaCl by scanning the plates every other day. RSA phenotypes observed at the different developmental stages (Fig. 2 A) indicated RSA reprogramming by salinity stress, rather than only retardation in root development.

In order to quantify the RSA development, RSA was dissected into three components, MR length, number of LR and average LR length (Fig. 2 B). All three main components of RSA showed reduction in response to salinity exposure (Fig. 2 C, solid lines). Control and salt stress conditions were significantly different from the 6th day after germination for MR length, while number of LR was significantly affected by salt stress from the 8th day after germination. The difference in average LR length between 0
and 125 mM NaCl grown seedlings was also significant from the 6th day after germination, while average LR length of seedlings grown at 75 mM differed significantly from control conditions only 10 days after germination (Fig. 2C). No effect on LRD calculated for entire MR length was observed (Fig. S2). Interestingly, when LRD was calculated for region A and B separately, no differences in LRD were observed for region A, while a mild reduction in LRD was found only at 8 days after germination for region B (Fig. S2).

Next, growth rates of MR and LRs and increase in #LR were described by fitting mathematical functions to the observed growth rates (Fig. 2B). Since the plants were 4 days old when transferred, the initial length of the MR was taken as a starting point (MR\textit{START}). Root growth during the early seedling growth is a vector of increasing meristem growth (Beemster and Baskin, 1998) and expansion, resulting in quadratic growth. By fitting quadratic functions on the individual RSA parameters, the growth rates were estimated, and subsequently used to calculate the increase in MR length, number of LR and average LR length (respectively \textit{GROWTH\textsubscript{MRL}}, \textit{INCREASE\textsubscript{LRL}} and \textit{GROWTH\textsubscript{LRL}}). The functions describing individual RSA parameters were combined to describe cumulative LR length by multiplying the number of LRs with average LR length. TRS was calculated from individual parameters by adding MR length to cumulative LR length (Fig. 2B). The values of individual parameters and TRS as predicted by the quadratic growth models (Fig. 2C dashed lines) were compared with the increase of root size measured (Fig. 2C solid lines) and fit of the model with measured data was examined by calculating the coefficient of determination ($r^2$) (Table S1). Thus, our descriptive model of fitted quadratic growth functions to RSA dynamics, which we called ROOT-FIT, was found to be in good agreement with the experimental data.

Since LR development in the region formed prior to the salt stress exposure could exhibit a different response to salinity than LRs formed after exposure to stress, an alternative model (ROOT-FIT\textsubscript{A/B}) was made (Fig. S2A) where LR length was calculated separately for the region A and B. The average LR growth in the region A was constructed as described above for \textit{GROWTH\textsubscript{LR}} (Fig. S2A - B), while a one-day delay was added to the model describing average LR growth and increase of LR number in the region B (Fig. S2C, E). In this case, the increase in LR number in region A was best described using a square root function rather than a quadratic one as the length of...
region A did not increase and #LRs was observed to reach a saturation point around 6
LR per cm (Fig. S2 D). However, the use of different functions in the root-fit model
hindered comparisons of the relative effect of salt stress on the individual RSA
parameters, despite the good model fit to the observed increase in total LR length (Fig. S
3 F – G) as well as TRS (Fig. S3 H). So, while the model based on the different regions
could be valuable for other applications, for subsequent analyses of RSA remodelling
and natural variation in RSA responses, we used the root-fit model describing the
dynamics of the entire RSA in only 3 parameters, the growth rates of MR, average LR and
the increase in LR number (Fig. 2 B).

Reduction in growth parameters reveals RSA remodelling in response to salt
In order to study the effect of salt stress on individual components of RSA, the growth
rates of MR, number of LRs and average LR were compared between the control and salt
stress conditions. For all RSA parameters used, salt stress decreased the growth rate
significantly (Fig. 3 A). Relative MR growth was inhibited more severely than the
increase in LR number or average LR growth in salt stress conditions. The effect of salt
stress on re-shaping the distribution of total root mass between MR and LRs was further
explored by calculating the ratios between MR and average LR lengths per TRS over the
days (Fig. 3 C - D). In control conditions the portion of TRS consisting of MR decreased
with root development as more LRs emerge and elongate from the constantly elongating
MR (Fig. 3 C). This progress of TRS into mainly LR-derived root mass was delayed in
both salt stress conditions studied (Fig. 3 C). In order to study the transition from MR
derived root mass into RSA composed mainly of LRs, the ratio between average LR
length and TRS was calculated from the growth rates of individual RSA traits (Fig. 3 D).
In control conditions average LR length as a ratio of TRS increases as the first emerged
LRs start to elongate. However, at the 8th day after germination this ratio drops, as more
newly formed LRs start to emerge, reducing the contribution of individual LRs to TRS
(Fig. 3 D). A similar pattern was observed for seedlings grown on media supplemented
with NaCl. Interestingly, the ratio of average LR length per TRS was observed to reach a
higher maximum in both NaCl conditions than in control conditions. Moreover, the
maximum of aLRL / TRS was reached later and the slope after reaching the maximum
was slightly more horizontal (Fig. 3 D). The higher maximum value of aLRL / TRS implies
that LRs of seedlings grown at mild salt stress conditions are relatively longer before the
new LRs appear, when aLRL to TRS ratio start to decrease. The more moderate slope of
the curve after a LRL/TRS maximum in both salt stress conditions would be caused by
the fewer number of new LR emerging, therefore the contribution of earlier formed
(relatively longer) LRs remains relatively high compared to the control conditions. This
shift in the aLRL/TRS ratio, as well as the more severe impact on growth rate of the MR,
compared to average LR and increase in LR (Fig. 3B), indicates that salt stress does
indeed remodel the RSA of Col-0 seedlings. Salt stress does thus not only retards overall
root development, but also re-shapes the root architecture. Our findings imply
differential regulation and/or salt stress sensitivity of MR and LR growth, in agreement
with the decreasing correlation between MR and LR length in response to increasing salt
stress concentrations (Table II).

**Natural variation in RSA strategies reveals trade-off between MR and LR elongation**

To estimate whether the strategy of RSA response to salt stress observed for Col-0
seedlings is conserved among other Arabidopsis accessions, we studied salt-induced
changes in RSA of 32 different accessions (Table S II). These 32 accessions were chosen
based on the available RIL populations that could be used in further research identifying
the genetic components underlying RSA responses to salinity stress. Phenotyping and
quantification of RSA was performed as described previously for Col-0 seedlings and the
dynamics in individual RSA parameters were described with ROOT-FIT for each accession
individually (Table S III). We observed significant natural variation in all parameters
modelled between the accessions in control and salt stress conditions (Fig. S 3, Fig. 4 A -
C). Accession Yo-0 showed high variability in its average LR growth rates at 75 mM NaCl
and was therefore excluded from further analysis. In general, the growth rates of the
individual RSA components were observed to positively correlate between different
conditions studied (Table S IV). The correlation between the GROWTH_{MRL} and INCREASE_{LR}
was found to remain significant in all conditions and all accessions tested, again
suggesting that decrease in visible LR number is mainly due to reduction of MR length
rather than altered LR patterning (Fig. 4 D, E). Interestingly, under salt stress conditions,
the positive $r^2$ between the GROWTH_{MRL} and GROWTH_{LR} changed into a weak, negative
correlation for both salt stress conditions studied (Fig. D, F), suggesting a trade-off
between investing in either MR or average LR elongation (Fig. 4 D).
Four strategies guiding RSA acclimation responses to salinity stress

In order to identify common morphological strategies among the accessions, the growth rates of each accession were normalized for their growth in control conditions (Fig. 5 B). Since we are interested in the re-distribution of the root mass between MR and LR rather than the decrease in TRS per se, the relative effect of salt on growth rates of individual RSA parameters was calculated. Individual growth parameters were normalized for the general growth decrease by calculating growth rates relative to MR growth reduction. Subsequently, accessions were categorized by applying Ward Linkage hierarchical cluster method, as used by Kellermeier (et al., 2013), on relative $GROWTH_{MR}$ and $INCREASE_{MR}$ at 75 mM NaCl scaled on MR growth rates (Fig. 5 A). In this way, four major strategies were identified (Fig. 5 B-C). Accessions within strategy I showed a more severe reduction in MR elongation than in $GROWTH_{MR}$ and $INCREASE_{MR}$. The strategy II category included accessions for which salt reduced the growth of all RSA parameters with similar impact. Accessions belonging to strategy III showed high reduction of LR elongation compared to reduction of $GROWTH_{MR}$, while accessions associated with strategy IV showed not only reduced $GROWTH_{MR}$ but also exhibited highly reduced $INCREASE_{MR}$ (Fig. 5B-C). Both strategies III and IV could be divided in sub-categories with different impact on $GROWTH_{MR}$ (a and b respectively).

To validate the different categories, the values of RSA parameters relative to control conditions at 12 days after germination in accessions belonging to each category were pooled. The identified strategies (Fig. 5 C) could be split in two groups based on reduction in LR number (Fig. 6 A) strategies I, II and III were only mildly affected by salt, while accessions belonging to strategy IV experienced severe reduction in LR number. Moreover, concerning average LR length (Fig. 6 B), accessions could be grouped into ones showing less (I and II) or more (III and IV) pronounced inhibition of LR elongation rates (Fig. 6 B). The reduction in MR and TRS (Fig. 6 A and B) as well as measured shoot size and root / shoot ratio (Fig. 6 C and D) was similar for all strategies defined.

RSA strategies are partially due to differences in ABA sensitivity and correlate with different $Na^+/K^+$ accumulation in shoot

In order to examine whether the different strategies are due to activation of similar signalling pathways, ten Arabidopsis accessions showing different RSA strategies in response to salt stress were selected and studied for their sensitivity to two hormones
involved in root growth reduction upon salt stress, abscisic acid (ABA) and ethylene (ACC) (Achard et al., 2006; Duan et al., 2013). Four-day-old seedlings were transferred to plates supplemented with 1 μM ABA or ACC and the root growth dynamics were described using ROOT-FIT. In general, RSA of seedlings grown on 1 μM ABA were severely reduced in their LR elongation, while 1 μM ACC treatment mainly affected MR elongation (Fig. 6 C). ABA treatment reduced the growth of MR and average LR as well as the increase in LR number to a similar extent for all accessions belonging to strategy I. Strategy II accessions showed diverse patterns of ABA responses. While Cvi-0 and Van-0 showed significant reduction of GROWTH_{aLR} compared to GROWTH_{MR}, ABA treatment of Nd-1 seedlings reduced GROWTH_{MR} more severely than GROWTH_{aLR}. Interestingly, two out of three accessions (An-0 and Br-0) belonging to strategy III showed ABA hypersensitivity in their GROWTH_{aLR} and INCREASE_{#LR}. Strategy IV accessions exhibited no significant differences between GROWTH_{MR} and GROWTH_{aLR} in response to ABA, but showed different responses concerning the INCREASE_{#LR} in comparison to GROWTH_{aLR}. On the contrary, the patterns of RSA reprogramming by ethylene treatment were conserved in all accessions studied (Fig. 6 D). The GROWTH_{MR} was inhibited more severely than GROWTH_{aLR} in response to ACC treatment in all accessions studied. Nevertheless, some natural variation in the growth inhibition by ethylene was observed in GROWTH_{aLR} (Fig. 5 D), with Oy-0 and Ag-0 being least sensitive to ACC inhibition. Yet within this natural variation no patterns explaining different RSA strategies could be observed. The results indicate that the different RSA strategies are likely partially due to natural variation in ABA responsiveness.

To examine whether different RSA strategies would correspond to salt accumulation in the shoot, the Na⁺ and K⁺ contents were measured in root and shoot tissues of 10 accessions grown for 8 days on control plates or plates supplemented with 75 mM NaCl (Fig. S 7 A - B). Salt stress was observed to shift the ratio of Na⁺/K⁺ in both root and shoot tissue (Fig. S 7 B), due to increased accumulation of Na⁺ and slight reduction of K⁺ in both tissues. The Na⁺/K⁺ ratio increased more severely in the shoot than in the root tissue. Interestingly, the relative increase in the Na⁺/K⁺ ratio measured in the shoot (Fig. 6 E, Fig. S 7 C) was found to be the lowest for the three accessions belonging to strategy III and the highest for strategy IV accessions. Thus, RSA strategies seem to be correlated with ion accumulation in shoot tissue, which is a measure of salinity stress tolerance.
Discussion

Salt stress is known to inhibit plant growth by challenging the osmotic and ionic stress-counteracting capacities of the plant. Plant responses to salt stress involve activation of ion pumps to either restrict Na\(^+\) influx into the plant (SOS1) or compartmentalize the excess of Na\(^+\) into tissues (HKT1) or vacuoles (NHX1) (Munns and Tester, 2008). The initial responses to salinity stress are traditionally studied on the roots of very young seedlings. This approach was successful in identification of genes involved in salinity tolerance in forward and reverse genetic approaches. On the other hand, the effect of salinity on more complex root traits besides Main Root (MR) elongation is less clear. Salt was found to induce changes in MR and Lateral Root (LR) length, depending on the nutrient conditions (Wang et al., 2009; Zolla et al., 2010; Duan et al., 2013). Although the dynamics of initial LR growth arrest were studied in great detail by Duan (et al., 2013) no studies on the complex changes in entire RSA in response to salinity stress, and especially, the dynamics of the response on the longer time scale, are available. In this study we describe key RSA parameters responsive to salt stress and reveal natural variation in growth dynamics within those traits.

Four accessions described to differ in the salinity tolerance related traits Na\(^+\) leaf accumulation, survival and germination (Jha et al., 2010; Katori et al., 2010; Galpaz and Reymond, 2010), were tested for differences in their RSA under increasing salt concentrations (Fig. 1). In agreement with the previously published effect of low salinity inducing LR elongation (Zolla et al., 2010), lower concentrations of salt were found to increase the length and the number of LRs. Our results show that this increase is apparent only for Col-0 seedlings. The growth-promoting effect of low salinity could be due to an increase in the osmotic potential of the cells in the elongation zone or enhanced cell cycle activity. The Na\(^+\) ions at this range could be rapidly compartmentalized into the vacuoles without reaching the maximum capacity, thereby increasing the turgor within the cells and stimulating cell elongation. A possible effect of salt stress on meristem activity as well as on cell length in different accessions will be explored in future studies. Interestingly, accessions described to have increased salinity tolerance, Bur-0 and Tsu-0 (Rus et al., 2006; Katori et al., 2010), showed a reduction in average LR length in the root region formed after exposure to salt stress even at the lowest concentrations of salt studied. Those results suggest that efficient inhibition of
root growth, even at low salt stress levels, could be linked with enhanced salt stress
tolerance, similar to drought-induced inhibition of shoot growth (Claeys and Inzé, 2013).

Higher salt concentrations were found to significantly reduce MR length and TRS,
in accordance with earlier observations (Wang et al., 2009; Zolla et al., 2010). However,
no significant effect on LRD was observed at any concentration studied, conflicting with
previous studies where LR density in salt stress conditions was observed to be either
increased, attributed to auxin signalling (Wang et al., 2009) or decreased due to ABA
signalling (Zolla et al., 2010). The earlier conclusions concerning LR density could be
explained by the different nutrient conditions used in different experiments. The
decrease in LRD observed by Zolla (et al., 2010) is consistent with a transient effect of
salt stress on LRD only in the region of the MR that is formed after transfer (Fig. S 2) and
the partial arrest of LR development at stage V-VI under mild salt stress conditions
(McLoughlin et al., 2012). Yet, by examining the density of visible LRs in different salt
stress concentrations (Fig. 1 C) and also LR density dynamics in time in response to
salinity stress (Fig. S 3), on a longer time scale we could not find any effect of salt stress
on emerged LR density. Thus, although the resolution of our phenotyping technique
does not allow distinguishing the pre-branch sites or primordia and possible effects on
these (Moreno-Risueno et al., 2010), we conclude that no significant changes in LRD of
emerged LRs can be observed for Col-0 seedlings in response to salinity.

Recent work by Geng (et al., 2013) and Duan (et al., 2013) are excellent examples
highlighting the importance of studying RSA dynamics. By examining root responses
during the first 24h or 72h of salinity stress respectively, changes in MR growth and LR
emergence at high time- and spatial-resolution were identified and the importance of
tissue specific ABA signalling was revealed. Building further on this work, here we focus
on dynamics of the entire RSA in response to salt stress on a lower spatial resolution but
larger time scale. We were able to summarize RSA dynamics with quadratic growth
functions describing the growth of MR, average LRL and increase in LR number and
combining them into one descriptive model, ROOT-FIT (Fig. 2). By comparing the growth
rates of individual RSA components across different salt stress conditions as well as the
relative effect of salt stress on each component individually (Fig. 3), we identified
functional reprogramming of RSA in response to salt stress. Differential reduction of
GROWTH\textsubscript{MB} \text{ INCREASE}_{LR} \text{ and GROWTH}_{SLR} \text{ in Col-0 seedlings grown on salt containing media}
was observed. $GROWTH_{MR}$ was affected most severely while $INCREASE_{LR}$ and $GROWTH_{aLR}$ showed a less severe reduction by salt stress. The quadratic growth description of the $GROWTH_{MR}$ and $GROWTH_{aLR}$ is in good agreement with the accelerating root elongation described by Beemster and Baskin (1998), ascribed to increasing root apical meristem size. Salt stress reduces MR growth rate by reducing cell cycle activity throughout the root apical meristem (West et al., 2004) and our data collected on a bigger spatial and temporal scale seems to be in good agreement with salt reducing meristem size. However, while the observed growth rates of seedlings transferred to saline media in our conditions still exhibit accelerated root growth, although to a lower extent, the growth rates observed by West (et al., 2004) followed rather a linear growth curve. The quadratic growth observed could be ascribed to the longer time scale used in our experimental set up, as cell cycle activity was shown to recover to near control levels at 72 h after exposure to salt stress (West et al., 2004).

Based on the ROOT-FIT model, our results provide evidence that salt stress does not only retard RSA development, but also re-shapes the root architecture. Interestingly, earlier observations indicated an opposite effect: at 100 mM of NaCl, LR growth was observed to be more severely reduced compared to MR growth (Duan et al., 2013). The possible explanation for this discrepancy is the basic medium, as well as the different time scales used. The severe decrease in LR elongation was observed on 1x MS medium, while at $\frac{1}{2}$ x MS (more similar to our conditions) a less severe inhibition of LR growth was observed (Duan et al., 2013). In addition, Duan (et al., 2013) described LR development for 4 days after salt stress treatment, while the LR development in this study was examined up to 8 days post transfer into saline media. By this time, the LRs were observed to surpass the quiescent stage and root growth was largely recovered. Together, these studies highlight the importance of studying RSA responses on different basic media and different time scales to examine the full range of phenotypic plasticity in environmental stress responses (de Smet et al., 2012; Kellermeyer et al., 2014).

By studying natural variation in RSA dynamics to salinity stress and the correlations between RSA traits, we found that MR growth and increase in LR number are positively correlated in all conditions, again implying little change in LR patterning due to salt stress exposure. Although correlation between growth rates of MR and average LR was not observed to be significant, the value of correlation coefficient ($r^2$)
changed from positive in control conditions to negative in salt stress conditions, suggesting a trade-off between MR and LR elongation after exposure to salt stress. Clustering of relative changes in individual RSA parameters of all studied accessions enabled us to categorize their natural variation into four distinct RSA strategies differing mostly in their relative average LR growth and increase in LR number (Fig. 5). The majority of the accessions clustered in the strategies with heavily reduced LR elongation (III and IV), few accessions did not show any substantial re-modelling of RSA (II) and only couple of accessions showed an RSA response similar to Col-0, with higher reduction in $\text{GROWTH}_{MR}$ than in $\text{GROWTH}_{LR}$ or $\text{INCREASE}_{LR}$ (I).

The difference in MR and LR elongation in Col-0 under salt stress conditions is mainly accomplished by differential ABA sensitivity of LR and MR (Duan et al., 2013). Additionally, growth reduction by salinity has also been ascribed to increased synthesis of ethylene (Achard et al., 2006). The effect of ethylene on RSA development was observed to differ from ABA in terms of relative growth inhibition of MR and LR (Duan et al., 2013). In order to examine whether the differences in RSA responses to salinity stress are due to natural variation in ABA or ethylene sensitivity, the sensitivity to both phytohormones was examined on 10 accessions exhibiting different RSA responses (Fig. 6 C – D). In general, the observed patterns in ABA and ethylene treatment were in general agreement with data previously published (Duan et al., 2013), as ABA mainly inhibited LR elongation and ethylene treatment severely reduced MR growth. The observed variation in RSA responses to salt could be partially explained by their differences in ABA sensitivity, as two accessions showing high reduction in aLRL growth on salt stress were observed to be hypersensitive to ABA with respect to aLRL growth. Additionally, we identified one accession, Nd-1, where ABA treatment inhibited the MR elongation more severely than its aLRL growth. On the other hand, natural variation in ethylene sensitivity was conserved in terms of relative effect on MR and LR elongation, indicating that the cellular synthesis or signalling pathways regulating RSA remodelling though ethylene are more conserved in the accessions studied than those for ABA. The underlying allelic variation will be further explored by forward genetic approaches, focusing on the genetic machinery controlling the difference in ABA sensitivity between MR and LR meristems.
Although the correlation between RSA and salt accumulation or salinity tolerance is underexplored for most plant species, studies in rice (Faiyue et al., 2010a; Faiyue et al., 2010b), suggest an important role for LR formation in those traits. Sodium ions were observed to enter the transpiration stream through the sites of the MR where LRs emerged, suggesting that plants with a reduced LR number would have an advantage in terms of ion exclusion. However, in our experimental set up, the accessions showing a heavy reduction in LR elongation, rather than in LR number increase, showed the smallest increase in Na+/K+ ratio after exposure to salt stress (Fig. 6 E). The discrepancy between our results and observations made in rice (Faiyue et al., 2010a; Faiyue et al., 2010b), could be due to the differential mechanisms involved in interactions between RSA and salinity tolerance in monocot and dicot plant species. Another explanation could be that our experiments were performed in non-transpiring conditions, reducing the transpiration flow, and limiting the Na+ influx into the shoot tissue. Nevertheless, our results imply the importance of LR growth inhibition by salinity stress as described by Duan (et al., 2013), as the accessions with the lowest increase in Na+/K+ ratio were those that showed the most severe inhibition of LR growth. Although this correlation should be examined in different experimental set ups for more accessions, this is the first study presenting a possible correlation between reduced growth of RSA and enhanced salinity stress tolerance.

In conclusion, our work illustrates how the use of a simple mathematical descriptive model, ROOT-FIT, combined with statistical methods, such as Ward linkage clustering, can uncover natural diversity in morphological changes that would normally have been overlooked. Classification into different RSA strategies rather than raw RSA traits themselves could be used for forward and reverse genetic approaches to identify genes underlying alternative RSA responses to salinity, but also to other environmental stress factors. Moreover, the genetic plasticity in the RSA response to salt stress suggests that this trait could be under environmental selection, showing different acclimation strategies that are beneficial in specific natural environments.
**Material & Methods**

**Plant material and growth conditions**

Arabidopsis accessions (Table S II) were obtained from the European Arabidopsis Stock Centre (www.nasc.nl). The 31 accessions were chosen based on availability of RILs (INRA, Versailles). Seeds used for phenotyping of Root System Architecture came from plants propagated together 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 the 3rd week after germination to ensure flowering of all accessions. 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 20ml household bleach and 600uL 40% HCl for 3 hours. The seeds were stratified in 0.1% agar at 4°C in the dark for 48h 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 control medium supplemented with NaCl as indicated per experiment. Each plate contained four seedlings of two genotypes (two seedlings per genotype). Plates were placed in the growth chamber following a random design.

**Dose dependent response of Root System Architecture changes to salt stress**

Plates were scanned 11 days after germination using an Epson Perfection V700 Scanner at 200dpi resolution. Root phenotypes of four different accessions (Col-0, Bur-0, Tsu-0 and C24) on media supplemented with 0, 25, 50, 75, 100, 125 and 150 mM NaCl were quantified using EZ-Rhizo software (Armengaud et al., 2009). In addition to the parameters measured by EZ-Rhizo, the lateral root length and average length of lateral root (lateral root length divided by number of lateral roots) was calculated for each root and LR number, length and average LR length were calculated for region above and below the point of transfer (region A and B respectively) were calculated based on the individual positions of LRs. All data was cleared from outliers. Statistical analysis was performed in SPSS using one-way ANOVA with Tuckey’s post-hoc test for significance.
Descriptive model of salt induced changes in Root System Architecture in Col-0

Plates were scanned 4, 6, 8, 10 and 12 days after germination using an Epson Perfection V700 Scanner at 200 dpi resolution. Root phenotypes on media supplemented with 0, 75 and 125 mM NaCl were quantified using EZ-Rhizo software (Armengaud et al., 2009).

Root phenotypes of 12 days old plants grown in control conditions were not quantified, since the roots were too long and entangled. Data was collected from plants grown in 6 different experiments with 4 individual seedlings per salt stress concentration per experiment (in total n=24). All data was cleared from outliers and statistical analysis was performed with Excel. The average values of Main Root (MR) length, average Lateral Root (LR) length and number of LR at 4, 6, 8, 10 and 12 days after germination were calculated for plants growing on media supplemented with different salt concentrations (n=24).

The change in MR length was described with a quadratic function for all salt concentrations: 

\[ MR = MRL_{\text{START}} + \text{GROWTH}_{\text{MR}} \times t^2, \]

where \( MRL_{\text{START}} \) is the Main Root Length 4 days after germination (same for all concentrations), \( t \) is time in days after transfer and \( \text{GROWTH}_{\text{MR}} \) is a growth rate (cm x day\(^{-2}\)), specific for the growth conditions. The dynamics in average Lateral Root Length and number of Lateral Roots were described with quadratic functions for all salt concentrations: 

\[ \text{noLR} = \text{INCREASE}_{\#LR} \times t^2 \quad \text{and} \quad \text{LR L} = \text{GROWTH}_{\text{LR L}} \times t^2, \]

where \( \text{GROWTH}_{\text{LR L}} \) are growth rates for number of LR (\#LR x day\(^{-2}\)) and average LR length (cm x day\(^{-2}\)) respectively. The growth rates for quadratic growth models were calculated on a square root transformed average values measured in a time course experiment, thereby making the growth a linear function. Linear function was calculated by using a “linest” function in Excel. The calculated values for linear function were subsequently squared to fit the quadratic growth of individual parameters. Total Root Size was calculated from the three values: 

\[ \text{TRS} = MRL + \text{noLR} \times \text{LR L}. \]

We also constructed an alternative model, which used different formulas for calculating LR increase and elongation in the MR portion above and below the point of transfer (Fig. S2). In alternative model, the MR growth was defined as described above. The average LR elongation above the point of transfer was modelled as the dynamics in average LRL described above but fitted on the average LR growth data from the region A. The average LR growth below the transfer point was modelled using the same formula,
although the delay of one day (t-1) was introduced as those LR developed later. The
same was done for increase in LR number below the transfer point. Modelling LR
increase above the transfer point was performed using a square root function, as the
length of the region A did not increase. The growth rates were calculated by squaring the
raw phenotypic data, thereby making the increase in LR in region A a linear function and
fitting a linear growth function using a “linest” function in Excel. Total Root Size was
calculated from the five values: TRS = MRL + noLR_A × aLRL_A + noLR_B × aLRL_B.

Additionally the increase in LR density per entire MR region as well as per region above
and below transfer point (region A and B) was calculated by using a linear growth
function (Figure S 3). The growth rates were calculated by fitting a linear growth
function on the average of LR density values from individual days using a “linest”
function in Excel.

Fit of the descriptive growth models was tested on the average of raw data collected by
calculating the coefficient of determination (r²). The r² for individual RSA parameters
are presented in Table SI.

Describing natural variation in RSA responses to salt and categorizing accessions
into different groups
The seedlings of 31 Arabidopsis accessions (Table S II) were germinated on standard
media and at 4th day after germination transferred to plates supplemented with 0, 75 or
125 mM NaCl. The plates were scanned 4, 6, 8, 10 and 12 days after germination using
an Epson Perfection V700 Scanner at 200dpi resolution. Root phenotypes on media
supplemented with 0, 75 and 125 mM NaCl were quantified using EZ-Rhizo software
(Armeniaud et al., 2009). The descriptive models for each accession were calculated as
described above for Col-0 on data collected from 4 individual seedlings per accession
per condition. The fit of the descriptive models with the data was tested on average
values per RSA trait per condition by calculating the coefficient of determination (r²).

The growth rates calculated were scaled for MR growth, by dividing the relative growth
rates of aLR and number of LR by MR growth rate and used for Hierarchical cluster
analysis performed using XLstat add in for excel by using Ward Linkage clustering, as
described in Kellermeier (et al., 2013).
Examining ABA and ethylene sensitivity of selected accessions with different salt stress induced RSA response types

The seedlings of 10 Arabidopsis accessions exhibiting different RSA responses to salt stress were germinated on standard media and at 4th day after germination transferred to standard plates supplemented with 1 μM Abscisic Acid (ABA), 1 μM 1-aminocyclopropane-1-carboxylic acid (ACC) or to control plates. Root phenotypes on media supplemented with 1 μM ACC and control plates were quantified between 4th and 10th day after germination, while RSA of plants grown on 1 μM ABA was quantified between 4th and 12th day after germination using EZ-Rhizo software (Armengaud et al., 2009). The descriptive models for each accession were calculated as described above for Col-0 on data collected from 16 individual seedlings per accession per condition.

Salt accumulation in root and shoot tissue of accessions with different salt stress induced RSA response types

The seedlings of 10 Arabidopsis accessions exhibiting different RSA responses to salt stress were germinated on standard media and at 4th day after germination transferred to standard plates supplemented with 0 or 75 mM NaCl. After 8 days, ten plants of the same genotype were pooled together. The seedlings were divided into shoot and root, dried and analyzed for Na⁺ and K⁺ concentrations using an atomic absorption spectrometer (AAanalyst 200, PerkinElmer AAS) as described by (Wei et al., 2014). The average of four biological replicas was used for the ion concentration calculations per g of dry weight. All data was cleared from outliers and statistical analysis was performed in Excel and SPSS was used for one-way ANOVA with Tuckey’s post-hoc test for significance.

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The authors would like to thank Joost Keurentjes from Wageningen University for providing seeds of Arabidopsis accessions, and Bondien Dekker and Marthe Noordhoorn Boelen for their practical assistance during their BSc student internship.
References


salt tolerance in seedlings of crop-wild hybrids of lettuce. Molecular Breeding 1–12


Figure legends

Figure 1. Increasing salt concentrations gradually change Root System Architecture.
Four days old seedlings of four Arabidopsis accessions (Col-0, Tsu-0, C24 and Bur-0) were
transferred to media supplemented with different salt concentrations ranging from 0 to 150
mM NaCl. 11 days after germination the RSA of the seedlings grown at different conditions
was quantified using EZ-Rhizo software. (A) Representative pictures of RSA of 11 days old
Col-0 seedlings grown for 7 days on different salt concentrations. The length of MR at the
transfer from standard to treatment media is indicated with red arrowhead. The effect of salt
stress on individual RSA parameters was studied by comparing RSA observed at different
salt stress conditions to control conditions. Increasing concentrations of salt caused dose-
dependent reduction in (B) Total Root Size, (C) MR length, (D) number of emerged LR, (E)
average LR length but did not significantly affect (F) LR density. The average LR length was
also significantly reduced when calculated separately for both regions (G) above and (H)
below the transfer point. Individual points represent the average value of 8 replicates and
error bars represent standard errors. The effect of salt stress was defined as significant when
the RSA trait value in salt stress conditions was tested significantly different from control
conditions with Tuckey’s post-hoc test with significance of 0.05 (*) or 0.01 (**). The significant
differences are indicated with asterisks in colours corresponding to those of the individual
accessions.

Figure 2. Quadratic growth functions used for description of RSA growth dynamics.
Four days old seedlings of Col-0 were transferred to media supplemented with 0, 75 or 125
mM NaCl. (A) RSA of seedlings was quantified every two days between 4 and 12 days after
germination. (B) TRS is defined by the combination of MR length, average LR length and
number of emerged LR. MR length of 4 days old seedlings is the starting point (MR_{START}) and
the growth of the MR was expressed as \text{GROWTH}_{MR} by fitting a quadratic function on the
observed MR growth. A similar approach was used to calculate the increase in number of
emerged LR and average LR length (respectively \text{INCREASE}_{#LR} and \text{GROWTH}_{LRL}). The models
describing individual RSA parameters were combined for describing cumulative LR length by
multiplying number of LR with average LR length. TRS was calculated from individual
parameters by adding MR length to cumulative LR length. (C) The descriptive quadratic
growth models for individual parameters (dashed lines) were compared with the observed
growth rates (solid lines) at growth conditions for Main Root Length (MRL), number of
emerged Lateral Roots (# LR), average LR length (average LRL) and Total Root Size (TRS).
The individual data points for observed growth are representing the average value of 24
replicates. Error bars represent the standard error. The $r^2$ values for goodness of fit are to be
found in Table SII. The effect of salt stress was defined as significant when the RSA trait value in salt stress conditions was tested significantly different from control conditions with Tuckey’s post-hoc test with significance of 0.05 (*) or 0.01 (**). The significant differences are indicated with asterisks in colours corresponding to those of the salt stress treatments.

Figure 3. Salt stress decelerates and modifies root development of Col-0 seedlings. (A) The growth rates of individual RSA parameters were calculated for control conditions as well as two different salt stress conditions. The bars represent the average growth rate calculated from 24 replicates. Error bars represent standard error. The differences between the growth rates at different conditions were tested with one-way ANOVA with Tuckey’s post-hoc test of significance. Different letter codes represent distinct groups with significance level of 0.05. (B) The relative effect of salt stress on growth of individual parameters was calculated. The severity of growth reduction caused by salinity stress could be compared over the individual RSA components. Different letter codes represent distinct groups with significance level of 0.05 as tested with Tukey’s post-hoc test. To identify the changes in root mass distribution between MR and LR, MR and average LR length were calculated as a ratio of TRS for different growth conditions. (C) The ratio between MR length and TRS changed over time as more LRs developed (blue line). This transition was delayed in plants grown at 75 and 125 mM NaCl (red and green line respectively). (D) The ratio of average LR length per TRS was more dynamic, since initially elongating LR, increased the portion of TRS ascribed to individual LR, however when new LR started to emerge, average LR length ratio per TRS decreased (blue line). A similar pattern with a slight shift in time and maximum was observed for 75 mM NaCl (red line). This delay in maximum reach and increase in maximum height was more pronounced for the seedlings grown at 125 mM NaCl (green line). The ratios were calculated using the quadratic model for three different salt stress concentrations.

Figure 4. Natural variation reveals trade-off between salt induced reduction in MR and LR growth rate. 32 different natural Arabidopsis accessions were studied for the salt induced changes in RSA dynamics induced by salt stress. Four days old seedlings were transferred to media supplemented with 0, 75 or 125 mM NaCl and the growth rates of MR, number of LR and average LR were described between 4-10 (control conditions) or 4-12 (salt) days after germination using ROOT-FIT. Natural variation in (A) GROWTHMR, (B) INCREASE\#LR and (C) GROWTHaLR was observed and several accessions were identified as outliers in MR and average LR growth rates. The boxplots represent the median growth rate as observed in 32 accessions studied. 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. (D) To establish whether salt
influenced the correlation between different RSA traits, Pearson correlation coefficients ($r^2$) between the growth rates of individual RSA parameters were determined. Significant correlations are designed * - for 0.05 and ** - for 0.01 significance level. (E) The strong positive correlation between MR growth and increase in emerged LR number remained at 75 mM NaCl. (F) The correlation between the growth of MR and average LR of accessions changed to a negative correlation at 75 mM NaCl, indicating that under salinity stress maintenance of LR growth goes to the expense of MR growth and vice versa. The growth rates per individual accession were calculated based on 4 replicates per condition tested.

Figure 5. Four distinct RSA response strategies to salt stress identified within the collection of natural accessions. Four days old seedlings of 32 different accessions were transferred to media supplemented with 0, 75 or 125 mM NaCl. RSA of seedlings was quantified between the 4th and 12th day after germination. The changes in growth rates of MR, LR and number of LR were described with ROOT-FIT and the growth rates relative to control conditions were calculated, scaled for MR growth reduction and used as an input for hierarchical clustering for discovering the most common and conserved RSA responses among tested accessions. (A) Dendrogram of accessions obtained by hierarchical clustering by Ward Linkage method. (B) Relative growth rates of MR, LR and number of emerged LR of 31 accessions tested. Growth rates were calculated by using quadratic growth models on the data collected from 4 seedlings per accession per treatment. The bars represent the average growth rate relative to control conditions. The error bars represent the standard error. Different letters are used to indicate the significant differences between the relative growth rates of MR, increase in emerged LR number and average LR growth within the accession as tested by post-hoc Tukey’s test with significance levels of 0.05 (C) Pictures of RSA of representative accessions belonging to different RSA strategies grown for 8 days on medium supplemented with 75 mM NaCl.

Figure 6. Different RSA strategies are partially explained by natural variation in ABA sensitivity and correlate with differences in Na$^+$/K$^+$ ratios in the shoot. (A) The relative reduction in #LR and (B) average LR length was calculated for a set of 10 accessions representing different strategies. The reduction was calculated from 12 days old plants based on the quadratic growth model (Fig. 2). The difference in (C) ABA and (D) ACC sensitivity were examined by quantifying the RSA dynamics of seedlings grown between the 4th and 12th day on 1μM ABA supplemented media and between the 4th and 10th day after germination on 1μM ACC supplemented media. The changes in $\text{GROWTH}_{\text{MR}}$, $\text{INCREASE}_{\#\text{LR}}$ and $\text{GROWTH}_{\text{aLR}}$ were described with a quadratic growth model and the growth rates relative to control conditions were calculated for MR growth, number of LR and average LR growth reduction. Different letters are used to indicate the significant differences between the
relative growth rates of MR, increase in emerged LR number and average LR growth within each accession as tested by post-hoc Tukey’s test with significance levels of 0.05 (C) Na⁺ and K⁺ accumulation was measured in 12 days old seedlings grown on 0 or 75 mM NaCl for 8 days. The bars represent the average change in Na⁺/K⁺ ratio compared to control conditions (Fig. S 8) as measured for 4 biological replicas consisting of 10 seedlings pooled together. The error bars represent the standard error. Different letters are used to indicate the significant differences between the accessions as tested by post-hoc Tukey’s test with significance levels of 0.05
Table I. Overview of RSA parameters obtained from EZ-Rhizo and their response to salinity stress. The effect of salt stress on individual RSA parameters was studied by comparing RSA observed at different salt stress conditions to control conditions. The effect of salt stress was defined as significant when the RSA trait value in salt stress conditions was tested significantly different from control conditions with Scheffe’s post-hoc test with significance of 0.05 (*) or 0.01 (**) for at least two different accessions at three salt stress concentrations used.

<table>
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<th>Unit</th>
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<td>MRL</td>
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<tr>
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<td>Main Root Vector Length</td>
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<tr>
<td># LR_B</td>
<td>Number of Lateral Roots in region below transfer point</td>
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<td>n.s.</td>
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Table II. Salt stress weakens the correlation between MR and LR length. Pearson correlation coefficients calculated between MR length and other RSA parameters of 4 days old seedlings transferred for 7 days to different salt concentrations. Pearson correlation coefficients were correlated on average values of 8 replicates for all 4 accessions used with SPSS software. The significant correlations are marked * for significance levels of 0.05 and ** for 0.01.

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Figure 1

(A) NaCl (mM)

(B) TRS

(C) MRL

(D) #LR

(E) average LR length

(F) LRD/MRL

(G) aLRL region A

(H) aLRL region B
Figure 2

A

Days after germination

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B

ROOT-FIT

MR length = MRL_{START} + GROWTH_{MR} \times t^2

Average LR length = GROWTH_{aLR} \times t^2

LR # = INCREASE_{aLR} \times t^2

C

MRL

Average LRL

# LR

TRS

0 mM NaCl data
75 mM NaCl data
125 mM NaCl data
0 mM NaCl model
75 mM NaCl model
125 mM NaCl model
Figure 3

A

GROWTH<sub>MR</sub>

(INCREASE<sub>#LR</sub>)

GROWTH<sub>aLR</sub>

B

75 mM NaCl

C

MR length per TRS

D

Average LR length per TRS

75 mM NaCl

125 mM NaCl