Multiple paths lead to salt tolerance - pre-adaptation vs dynamic responses from two closely related extremophytes

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Keywords: Extremophyte, *Schrenkiella parvula*, *Eutrema salsugineum*, omics, convergent response, pre-adaptation, stress-preparedness, dynamic response

Abstract

Salinity stress is an ongoing problem for global crop production. *Schrenkiella parvula* and *Eutrema salsugineum* are salt-tolerant extremophytes closely related to *Arabidopsis thaliana*. We investigated multi-omics salt stress responses of the two extremophytes in comparison to *A. thaliana*. Our results reveal that *S. parvula* limits Na accumulation while *E. salsugineum* shows high tissue tolerance to excess Na. Despite this difference, both extremophytes maintained their nutrient balance, while *A. thaliana* failed to sustain its nutrient content. The root metabolite profiles of the two extremophytes, distinct at control conditions, converged upon prolonged salt stress. This convergence was achieved by a dynamic response in *S. parvula* roots increasing its amino acids and sugars to the constitutively high basal levels observed in *E. salsugineum*. The metabolomic adjustments were strongly supported by the transcriptomic responses in the extremophytes. The predominant transcriptomic signals in all three species were associated with salt stress. However, root architecture modulation mediated by negative regulators of auxin and ABA signaling supported minimally affected root growth unique to each extremophyte during salt treatments. Overall, *E. salsugineum* exhibited more preadapted responses at the metabolome level while *S. parvula* showed predominant pre-adaptation at the transcriptome level to salt stress. Our work shows that while salt

tolerance in these two species shares common features, they substantially differ in pathways leading to convergent adaptive traits.

Introduction

Plants differ greatly in their tolerance to salt stress and there is a metabolic cost for adaptation to salt stress, reflected by differences in growth and yield when grown in high saline soils (Santiago-Rosario et al., 2021; Flowers et al., 2015). Only ~2 % of angiosperms are adapted to grow in high saline environments while the remaining 98 %, including most crop plants, are highly sensitive to salt stress (Flowers and Colmer, 2008; Hajiboland et al., 2018). Previous studies have largely used salt-sensitive model plants or crops to understand genetic mechanisms underlying salt tolerance. Insight gained from examining the molecular processes in salt tolerant extremophytes contrasted to a salt-sensitive model offers a promising prospect to discover tolerance mechanisms that are absent in the salt-sensitive plants and proven to be evolutionary successful against salt stress.

Schrenkiella parvula and *Eutrema salsugineum* (Brassicaceae) are two extremophyte models with foundational genomic resources made available. They are also closely related to *Arabidopsis thaliana* making it feasible to readily deduce orthologous relationships among the three models (Dassanayake et al., 2011; Wu et al., 2012). These extremophytes, currently used as leading models to investigate genetic mechanisms underlying salt stress adaptation (Kazachkova et al., 2018), show salt resilient growth even at salinities reaching seawater strengths (Inan et al., 2004; Orsini et al., 2010; Kazachkova et al., 2018). While *S. parvula* is found near salt lakes in the Irano-Turanian region (Hajiboland et al., 2018; Tug et al., 2019), *E. salsugineum* has a wider distribution from coastal to inland saline fields in the northern temperate to sub-arctic regions including the United States, Canada, Russia, and China (Lee et al., 2016). Despite multiple studies highlighting some of the metabolomic and transcriptomic responses of the extremophytes to salt stress (Gong et al., 2005; Kazachkova et al., 2013; Oh et al., 2014; Lee et al., 2016; Prerostova et al., 2017), molecular phenotypes determining how both extremophytes have convergently achieved salt adapted growth unlike their salt stress-sensitive relative, *A. thaliana* have not been explored.

Excess salt exerts cellular stress as osmotic, oxidative, ionic, and water-deficit stresses (Pantha and Dassanayake, 2020; Zhao et al., 2020; van Zelm et al., 2020). Multiple genetic mechanisms mediated by ABA as well as non-ABA dependent pathways have been shown to modulate salt stress responses in *A. thaliana*, all major crops, and selected halophytes (Takahashi and Shinozaki, 2019; Takahashi et al., 2020; Zhao et al., 2020). These studies collectively support the view that salt stress-adapted plants will show a highly coordinated response to survive salt stress that requires synergistic coordination between root and shoot responses. Despite the complexity of adapting to salt stress at the whole plant level, multiple angiosperm lineages have evolved this complex trait repeatedly (Flowers et al., 2010; Bennett et al., 2013). Therefore, it is one of the main complex traits studied for convergent evolution in plants. Even if a large body of work exist to identify many of the individual cellular, molecular, or physiological responses in selected tissues that contribute to salt stress adaptations, we still have a large gap in understanding how a common set of orthologs from a closely related group of plants selectively modulate salt stress adaptations at a cellular level (Isayenkov and Maathuis, 2019) and which pathways are synergistically used to achieve salt stress adaptation facilitating stress resilient growth in extremophytes.

In this study, we used a combined transcriptomic, ionomic, and metabolomic experimental design to investigate the coordinated cellular responses of *S. parvula*, *E. salsugineum*, and *A. thaliana* to salt treatments in root and shoot tissues. We examined the coordination of multiple genetic pathways used by the three models at different salt stress intensities. We identified preadapted responses mostly at the metabolome level for *E. salsugineum* while *S. parvula* showed more stress-preparedness at the transcriptome level. Additionally, both extremophyte showed molecular phenotypes suggesting induction of complementary cellular processes that used core pathways present in all plants, but with modifications to those in ways that optimized balance between stress tolerance and growth.

Results

Previous studies using either *S. parvula* or *E. salsugineum* in a comparative study with *A. thaliana* have successfully used 150 mM NaCl to elicit salt stress responses in the extremophyte

while avoiding induction of immediate tissue necrosis in *A. thaliana* in the short-term (Kazachkova et al., 2018). We grew *S. parvula*, *E. salsugineum*, and *A. thaliana* hydroponically for 28 days, added NaCl to the growth medium (Figure S1A). *Arabidopsis thaliana* did not show severe stress symptoms until four days of exposure to salt. Therefore, in our comparative system with three species, we decided to capture the short-term effects of salt at 0, 3, 24, and 72 hr durations with 150 mM NaCl to include timepoints that would precede the onset of stress phenotypes. Additionally, 250 mM NaCl was used to further deduce salt stress responses induced in the extremophytes. We used the initial three timepoints to detect immediate responses of the ionome and the transcriptome to salt and used 0 hr and the latter two timepoints to detect the subsequent metabolome level changes (Figure S1B).

High tissue tolerance to Na accumulation vs limiting Na accumulation in tissues

All three species accumulated salt as the duration of the treatment or the concentration of NaCl increased. *Arabidopsis thaliana* accumulated Na in shoots much more than in roots but did not limit Na accumulation in roots compared to *S. parvula* (Figure 1A). *Arabidopsis thaliana* shoots showed a 19-fold increase of Na compared to control within 24 hr. Tissue accumulation of Na was remarkably low in both roots and shoots of *S. parvula* compared to the other two species. Interestingly, *E. salsugineum* allowed high Na accumulation in both roots and shoots compared to *S. parvula* and at levels similar to those observed for *A. thaliana* roots under salt stress (Figure 1A). This suggests that *S. parvula* limited total Na accumulation in tissues while *E. salsugineum* showed high tissue tolerance to Na accumulation, a trend maintained for 250 mM NaCl treatments in extremophytes (Figure 1A). High Na levels interfere with K and other nutrient uptake in plants (Munns and Tester, 2008). We examined whether Na accumulation triggered nutrient imbalance in any of the test species, by quantifying the abundance of 13 plant nutrients during salt stress treatments. The K level significantly dropped within 3 hr in *A. thaliana* roots but did not change in the extremophytes regardless of the duration and intensity of the Na⁺ treatments (Figure 1B). In shoots, we did not observe any significant changes in K levels by salt stress treatments in any of the three species. When we examined the elemental profiles that include all quantified nutrients, we found that shoots exhibited minimal changes in

all three species in response to salt stress (Figure 1C). Intriguingly, *A. thaliana* roots showed significant depletion of 6 out of 13 nutrients under salt treatments whereas both extremophytes showed minimal nutrient disturbances (Figure 1C and Table S1). We then examined nutrient compositions in the control samples (basal levels) to identify if any nutrient was preferentially enriched in any of the species (Figure 1D and Table S1). Notably, Ca and Mg, which are known to aid in selectivity of Non-Selective Cation Channels (NSCCs) for K over Na in plants (Shabala et al., 2006), and B were found at higher basal levels in *S. parvula* roots compared to other species. Similarly, Cu and Co were high in *E. salsugineum* whereas Fe, P, K, and Mo were high in *A. thaliana*.

Primary metabolite pools decreased from high basal levels in *E. salsugineum* but increased from low basal levels in *S. parvula* upon salt treatments

Salt stress requires metabolic adjustments in plants (Pantha and Dassanayake, 2020). Therefore, we examined whether the extremophytes showed similar metabolic adjustment strategies that were distinct from the salt-sensitive *A. thaliana* when treated with salt. We quantified a total of 716 metabolites using GC-MS for each species (see Methods), of which 182 with known specific structures were referred to as “known metabolites” while the remaining were collectively referred to as “unknown metabolites” in this study (Table S2). The overall quantified metabolite pool in roots showed a relatively strong correlation in metabolite abundance between selected pairwise comparisons resulting in two distinct trends (Figure 2A). First, the highest pairwise correlation was detected between the control groups of *S. parvula* and *A. thaliana* which decreased with time as the duration of salt treatment increased (Figure 2A, upper panel). Second, *S. parvula* and *E. salsugineum* profiles were moderately correlated at control conditions, but as both species were treated with salt, the correlation became stronger indicating that the majority of metabolites in the extremophytes adjusted to similar levels in the roots in response to salt treatment. This trend was observed at a shorter duration of salt treatment at 24 hr when the NaCl concentration was higher at 250 mM (Fig. 2A, lower panel). In contrast to roots, the shoot metabolomes of *S. parvula* and *E. salsugineum* remained divergent following salt treatment (Figure S2A).

We also found distinct patterns in the direction of salt responses among species. Under salt treatments, metabolite abundances in both *A. thaliana* and *E. salsugineum* roots largely decreased while *S. parvula* increased metabolite abundances (Figure 2B). This trend for *S. parvula* was not upheld in shoots, where all three species predominantly decreased in metabolite abundances (Figure S2B). We next examined if the dynamically changing metabolites were categorically associated with sugars and amino acids often known for their roles as organic osmolytes (Slama et al., 2015). Figure 2C shows percent abundance among the three species at control levels in roots, for all sugars, amino acids, and their immediate derivatives (see Methods) quantified in our study that showed significant difference at basal level abundance. Even before the salt treatment, *E. salsugineum* had accumulated much higher levels for most of these metabolites than the other species. For instance, the percent abundance of sucrose in *E. salsugineum* accounted for 98.6 % of the combined sucrose abundance in the roots of all three species (Figure 2C) and this was 155- and 131-fold higher than that in *A. thaliana*, and *S. parvula*, respectively (Table S2). Similarly, glucose, raffinose, and fructose sugars and proline were much higher in *E. salsugineum* compared to the other species (Figures 2C and Table S2).

We then tested the possibility of one extremophyte maintaining a higher basal abundance for key metabolites associated with osmoregulation while the other extremophyte actively induced those under salt stress, eventually converging to a metabolic status distinct from that of *A. thaliana* under salt stress, as we observed in Figure 2A. We clustered the profiles of all 146 known metabolites that changed abundance significantly differently at basal level or in at least one stress condition from roots of all three species (Figure 2D). Two readily identifiable clusters emerged (boxes outlined in Figure 2D), composed of metabolites that showed lower abundances in *A. thaliana* and *S. parvula* at the control condition compared to *E. salsugineum*, but increased in abundance exclusively in *S. parvula* upon prolonged salt treatment, to the high level constitutively found in *E. salsugineum*. These clusters were enriched in metabolites known for their role as osmoprotectants or antioxidants (Slama et al., 2015), including sucrose, fructose, glucose, GABA, proline, and dehydroascorbic acid (full list in Table S3). These results indicate a basal level metabolic “preparedness” in *E. salsugineum* roots

compared to active induction of many osmoprotectants and antioxidants in *S. parvula* when responding to salt treatments. On the other hand, *A. thaliana* lacks neither a preadapted- nor dynamic-strategy found in the two extremophytes.

***A. thaliana* showed stronger transcriptomic responses compared to the extremophytes during salt stress**

Previous studies have reported that nearly 20 % of the *A. thaliana* transcriptome responds to salt stress (Gong et al., 2005; Oh et al., 2014). We tested if the transcriptomic salt responses from *A. thaliana* aligned more with either extremophyte or if the extremophytes showed a largely overlapping response that were distinguished from *A. thaliana*. The overall root and shoot transcriptome profiles were grouped into species-tissue clusters (Figures 3A, S3). This dominant species-level distinctions in transcript profiles were further demonstrated with pairwise correlations of entire transcriptome profiles where none of the comparisons showed correlations (Figure S3) unlike the similarities detected in metabolite profiles (Figure 2A and Figure S2A). Among differently regulated genes (DEGs), we observed more transcripts induced, than suppressed, by salt treatments in all three species, and the majority of the DEGs were identified in 1-to-1 ortholog groups (OGs) (see Methods) (Figure 3B, Table S4). *A. thaliana* showed the largest number of DEGs in response to salt stress in both roots and shoots. The *A. thaliana* shoot transcriptome contained more than twice the number of DEGs than in the root transcriptome (Figure 3B), despite fewer changes in the nutrient profile were observed in the shoot than in the root (Figure 1C). Notably, the shoot transcriptome of *A. thaliana* included orders of magnitude more salt-responsive DEGs than those of the extremophytes.

We next examined if transcriptomic changes in cellular homeostasis functions during salt stress observed for *A. thaliana* entailed processes already maintained or induced in the extremophytes. This search was done at two levels. First, we clustered expression of all 1-to-1 orthologs at their basal level (from control samples), filtered the clusters to show expression patterns that differed at least in one species compared to the other two, and identified enriched cellular processes in each cluster (Table 1). Responses associated with abiotic stress were among the top highly representative processes in each of these functional clusters.

Second, we investigated the changes in transcriptomic profiles during salt treatments, by identifying functional clusters enriched among all OGs that included at least one DEG in a species, for roots and shoots separately (Figure 3C and Table S5 and S6). Response to stress formed the largest functional cluster including the most OGs in both roots and shoots (RC1 and SC1 in Figure 3C), which were further sub-clustered to highlight various salt responsive pathways mediated by auxin and ABA regulation and oxidative stress responses (Figure S4). Interestingly, amino acid metabolism (RC2) and sugar metabolism (RC3) formed the next two largest root clusters followed by transmembrane transport (RC4) and ion transport (RC5) (Figure 3C). In shoots, the second and third largest functional clusters (SC2 and SC3 in Figure 3C) suggested cellular processes involved in plant development and growth/cell cycle, in which the majority of the *A. thaliana* orthologs were notably salt-repressed. While the *A. thaliana* orthologs were the most regulated among these OGs, extremophytes responded in similar functional processes associated with abiotic stresses when treated with 250 mM NaCl (Figure S5).

We have identified ortholog clusters showing salt-responsive co-expression across time points within tissues per species. We present the two most dominant co-expression clusters in roots and shoots (Figure 3D and Table S7). In roots, the largest co-expression cluster showed *A. thaliana* orthologs substantially salt-induced compared to a much smaller magnitude of induction seen in the extremophyte orthologs (Figure 3D- left panel). In shoots, the largest co-expression cluster included *A. thaliana* orthologs suppressed in response to salt while expression of extremophyte orthologs were stable (Figure 3D- right panel).

The predominant transcriptomic signature in roots supports divergent auxin-dependent root growth during salt stress

In all three species, response to stress was the most representative function in roots enriched among ortholog sets showing either different basal-level expression (Table 1) or salt-responses (Figure 3C) among species. The most dominant sub-functional clusters/processes was response to hormones in all ortholog sets especially in roots (Table 1, Figure S4A, and Figure S5 A and C). Hormonal regulation plays an important role in salt stress responses (Yu et

al., 2020). Furthermore, our comparative ortholog expression profiling suggested that regulation of hormone signal transduction was distinct among the target species (Figures 4A, S6 and Table S8). We found that genes mediating auxin response via auxin/indole acetic acid (Aux/IAA) repressors (Gallei et al., 2020) and the type 2C protein phosphatases (PP2Cs) that negatively regulate abscisic acid (ABA) signaling (Hauser et al., 2011) showed contrasting salt-responsive expression among the three species, while those of other regulatory phases in auxin and ABA signaling together with other hormone signaling pathways largely conserved (Figures 4A, S6, and Table S8). Notably, none of the *AUX/IAAs* and *PP2Cs* showed any differential expression in response to salt in *S. parvula* while 15 out of total 24 *AUX/IAAs* and *PP2Cs* orthologs in the other two species were significantly induced when treated with salt (Figure 4A). When the basal expression was compared, 11 out of 17 *E. salicigineum* *AUX/IAAs* orthologs and 5 out of 7 *S. parvula* *PP2Cs* orthologs showed higher levels compared to their respective orthologs in the other species (Figure 4A, the bottom panel, and Table S8).

Auxin and ABA suppress root elongation and lateral root initiation during salt stress in *A. thaliana* (Ding and De Smet, 2013; Ding et al., 2015). Therefore, we first curated genes involved in primary root development (GO:0080022) and lateral root development (GO:0048527) and checked for functionally verified phenotypes associated with them that describe root growth in *A. thaliana*. Then, we assigned a binary category of promoted or suppressed root growth to the orthologs based on their basal expression levels in our target species and the genetically verified functions of *A. thaliana* ortholog (see a full list of references used for the selected genotype-phenotype associations in Table S9). We identified 35 single-copy orthologs that showed a distinct expression level in one species compared to the other two species and assigned their binary category on their effects on root growth, given their presumed effect on root phenotypes (Figures 4B). We further mapped them to a lateral root development gene network (modified from Banda et al., 2019; De Rybel et al., 2010 and Figure S7). Among the total of 35, we found 27 orthologs in *S. parvula* to suggest increased primary root elongation or suppressed lateral root initiation compared to the other species, while 23 orthologs in *E. salicigineum* suggested slower primary and lateral root growth (Figure 4B and S7). Contrastingly, *A. thaliana* showed 25 orthologs that supported its fast primary root growth and

increased lateral root number. Notably, the majority (71%) of the 35 orthologs were annotated as auxin-responsive genes (Figure 4B).

In line with the observed difference in expression of orthologs associated with root development (Figure 4B), *S. parvula* and *A. thaliana* seedlings have comparable primary root lengths at control conditions, longer than that in *E. salsugineum* (Figure 4C and D). Moreover, *S. parvula* showed uncompromised primary root growth compared to *E. salsugineum* and *A. thaliana* when treated with salt for an extended time (Figures 4C, D, S8). *S. parvula* and *E. salsugineum* seedlings also had fewer lateral roots compared to *A. thaliana* at control conditions (Figure 4E). However, lateral root growth, assessed using total lateral root number and density during a week-long salt treatment, indicated that *S. parvula* not only sustained uninterrupted root growth but also induced lateral root growth upon salt treatment, in contrast to the responses observed for *A. thaliana* and *E. salsugineum* (Figure 4E, F, and S8).

We aimed to further identify orthologs in *S. parvula* that may support its uninterrupted primary and lateral root initiation during salt stress that distinguishes it from the other two species. Therefore, we checked co-expression clusters that included differentially expressed ortholog groups (OGs) in roots and found two clusters where *S. parvula* showed a different pattern compared to the other two species (Figure 4G). The first cluster showed salt-induced expression uniquely observed for *S. parvula*. This accounted for 11% (294 ortholog groups) of differentially expressed OGs (DEOGs) in roots (Table S10). The second cluster with 113 OGs (5% of all root DEOGs) groups showed induction of genes in response to salt stress in *A. thaliana* and *E. salsugineum* while the orthologs in *S. parvula* did not alter their expression (Figure 4G). Interestingly, within these two clusters, nearly 50% of orthologs did not have a specific GO annotation indicating the level of functional obscurity associated with those genes that uniquely respond in *S. parvula* to salt stress (Table S10).

Salt-responsive transcriptome-metabolome coordination supports cellular protection in extremophytes

In response to salt treatments there were a total of 7852 orthologs identified as differentially expressed genes (DEGs) and a total of 634 metabolites identified as differently

abundant metabolites (DAMs) for the three species in all salt treatments (Figures 2 and 3, Table S2 and 4). The difference in % change in response to salt between DAMs and DEGs was the lowest in *A. thaliana*, while in extremophytes metabolic responses were relatively larger compared to the overall transcriptomic response during salt treatments (Figure 5A and S9A). This indicates that there is more transcriptome level preparedness to respond to salt in the extremophytes than in *A. thaliana*.

The second and third largest functional clusters over-represented among DEOGs in roots were amino acid and sugar metabolism (550 orthologs) in roots (Figure 3C, RC2 and RC3). Additionally, our overall metabolite response indicated a high correlation of metabolite levels between the two extremophytes *S. parvula* and *E. salsugineum* during salt treatments, and the DAMs shared between the extremophytes were enriched in amino acids and sugars (Figure 2). Collectively, these findings led us to examine whether DEGs associated with amino acid and sugar metabolism support a salt-induced metabolic response associated with amino acid and sugars in the extremophytes. In line with the global trend (Figure 5A), we observed fewer DEGs associated with amino acid and sugar metabolism in both extremophyte roots than in *A. thaliana* roots, but more amino acid and sugar metabolites were found as DAMs in extremophytes in roots (Figure 5B).

Given the established role of amino acids and sugars as organic osmoprotectants during salt stress (Slama et al., 2015), we focused on the most highly responsive DAMs that are also osmoprotectants and the key genes that are involved in the metabolism of those metabolite (Figures 5 C and D, and S9A). Proline was one of the most significant DAMs found in roots of both extremophytes that not only had a higher basal level than in *A. thaliana*, but also its abundance further increased with salt treatments concordantly with the transcriptional induction of *pyrroline-5-carboxylate synthetase (P5CS1)* and suppression of *Pro-dehydrogenase (ProDH1)* in extremophytes (Figure 5C and S9B). Furthermore, shikimic acid derived amino acids, tyrosine, and phenylalanine (precursors of phenylpropanoids) showed higher basal levels or significant induction in roots of both extremophytes compared to *A. thaliana* (Figure 5C). Interestingly, genes coding for the enzymes directly involved in the conversion of phosphoenolpyruvate to shikimic acid such as DAHPS1, DAHPS2, DHQS, DHQ, SK1, ESPS, and CS

were not detected as DEGs in any of the three species (Figure S9C). However, *MYB15*, a master regulator of shikimic acid biosynthesis pathway (Chen et al., 2006), was highly induced in all three species (Figure 5C). *Chorismate mutases 1 (CM1)*, coding for the enzyme involved in the first committed step of tyrosine and phenylalanine biosynthesis was salt-induced in *E.*

salsugineum, while it was constitutively expressed at a high level in *S. parvula* (Figure 5C).

E. salsugineum maintained a higher basal abundance than the other two species for multiple sugars in roots used as organic osmolytes in plants, while these metabolites increased their abundances in response to salt in *S. parvula* (Figure 5D). The genes involved in the biosynthesis of these metabolites such as *sucrose synthase 1 (SUS1)*, *cell wall invertase 1 (cwIN1)*, *galactinol synthase 2 (GolS2)*, and *raffinose synthase* were mostly induced under salt or constitutively expressed with high basal levels in the extremophytes (Figure 5D). This concordant alignment of DAMs and DEGs or maintenance of high constitutive abundance of metabolites with high basal expression of genes highlighted for osmoprotectants in the extremophytes was similar to coordination observed between known antioxidants and their associated genes such as the dehydroascorbic acid pathway (Figure S9D).

Coordination between nutrient balance and gene expression associated with ion transport

Two other dominant functional clusters in roots, including 156 OGs, assessed using salt stress responsive ortholog groups identified ion transport and membrane transport as the main processes, (Figure 3C, RC4 and RC5). We also observed extremophyte ionomic profiles that maintained nutrient balance unlike that of *A. thaliana* during salt treatments (Figure 1C).

Hence, we investigated all 148 *A. thaliana* genes encoding transporters based on Araport 11 annotation (Cheng et al., 2017) in the following four categories: (1) aquaporins (*PIPs*, *NIPs*, *TIPs*, and *SIPs*), (2) cation transporters (*CAXs*, *NHXs*, *KEAs*, *CHXs*, *KUPs*, *HKT1*, and *TRH1*), (3) non-selective cation channels (*CNGCs* and *GLRs*), and (4) K channels (*SKOR*, *GORK*, *AKTs* and *KCOs*).

We found 109 corresponding one-to-one ortholog groups (OGs), among which 64 OGs were differentially expressed at basal level in the three species (Table S11). We focused on the OGs encoding transporters and channels that were expressed at significantly different basal levels in both extremophytes compared to *A. thaliana* (Figure 6A). Genes encoding transporters known

to exclude Na from the cell (*SOS1*), transporters involved in Ca uptake and transport (*CNGC1*, *CNGC12*, *CAX1*, and *CAX5*), K channel (*KAT1*), and endosomal K transporter (*KEA5*) that aid in pH and ion homeostasis (Zhu et al., 2018; Sustr et al., 2019) showed higher basal expression in the extremophytes than the *A. thaliana* orthologs, while genes involved in K efflux/Na influx to the cell or water transport such as *TIP1;1*, *PIP2;2*, *SIP1;2*, *SIP2;1*, *KUP5/6/10/11*, and *NHX3* (Figure 6A). *NHX1/2*, the main transporters involved in Na sequestration in the vacuole, showed higher basal level expression in *E. salsugineum* compared to the other two species (Table S11).

We then examined how these transporter classes changed in expression in the three species when treated with salt (Figure 6B and Table S12). Interestingly, the majority of the genes across the four categories were either induced or constant in expression during salt treatments in *S. parvula*, whereas *A. thaliana* and *E. salsugineum* had a mixed response (Figure 6B). Notably, *E. salsugineum* mostly repressed the expression of genes encoding non-selective cation channels (e.g. *GLRs*) in response to all salt treatments (Figure 6B and Table S12). Other observable trends included the induction of *CHX17* (functions in K uptake) identified as the only induced transporter in all three species in our selected set, a 50-fold induction of *CHX2* (involved in Na transport to vacuole) in *A. thaliana* (Table S12), and *GORK* as the only K channel up-regulated in *E. salsugineum* under salt treatment while maintaining high basal level expression in *S. parvula*.

We next expanded the focus on the transporter genes to include copy number variation among species and expression partitioned to paralogs present in each species (Figure 6C). We searched for transporter gene orthologs with increased copy numbers in the extremophytes: *SpNIP6;1/2* in *S. parvula*, *EsCNGC4;1/2* in *E. salsugineum*, *SpKUP9;1/2* and *EsKUP9;1/2* in both extremophytes (Figure 6C, top panels, and Table S13). All extremophyte paralogs either showed differential expression in response to salt or high constitutive expression compared to *A. thaliana* (Figure 6C, bottom panels).

Finally, we examined the expression profiles of all non-Na/K transporters involved in nutrient uptake that were included in an ortholog group in Figure 3C functional clusters RC4 and RC5 (Figure 6D and Table S13). Notably, *S. parvula* induced orthologs encoding a large and diverse set of transporters involved in nutrient uptake upon salt treatments, while *A. thaliana*

and *E. salsugineum* showed salt-induction of a limited group associated with Fe, Zn, and Cu uptake (e.g. *FER1/2/3*, *MTPB1*, *ZF14*, *ZIP2/11*,) (Figure 6D).

Discussion

The direct flow of Na⁺ ions into plant tissue is unavoidable in saline soils. Plants show adaptations at varying degrees to limit accumulation of Na⁺ or mitigate cellular toxicity caused by Na⁺ in tissues when exposed to increasing levels of salt (Flowers and Colmer, 2008; Pantha and Dassanayake, 2020; Santiago-Rosario et al., 2021). In the tolerance spectrum for salt, *S. parvula* and *E. salsugineum* are considered halophytes whereas *A. thaliana* is not (Kazachkova et al., 2018). We show that both extremophytes accumulate less Na at the whole plant level than *A. thaliana* during salt treatments, with *S. parvula* accumulating the lowest levels (Figure 1A), consistent with previous reports (Volkov et al., 2004; Ghars et al., 2008; Oh et al., 2014). Figure 7 summarizes the most notable ionic, metabolomic, transcriptomic, and phenotypic adjustments that distinguish each model plant from the other two when responding to salt treatments.

Upholding nutrient balance is a shared outcome in the extremophytes when responding to increasing external salt levels

When compared to *A. thaliana*, which loses its nutrient balance under salt stress as the Na content increases (Figure 1A-C), the two extremophytes present two distinct paths for regulating Na levels in roots coincident to how each plant achieves nutrient balance as a shared outcome. *S. parvula* regulates Na accumulation to maintain it at levels observed for control conditions, while preventing the loss or maintaining the uptake of K and Ca during salt stress. In contrast, *E. salsugineum* does not show such a restriction to Na accumulation in roots but reaches the same outcome as *S. parvula* for both 150 and 250 mM NaCl treatments at 24 hr preventing a significant drop in its K and Ca contents.

Regulation of Ca levels coordinated with K is a critical requirement for ion homeostasis and excess Na in plant tissues when exposed to salt stress challenges this balance (Flowers and Colmer, 2008; Pantha and Dassanayake, 2020). Ca activates the efflux of Na via SOS2-SOS3

signaling pathway which in turn activates the plasma membrane localized Na-exporter, *SOS1* (Halfter et al., 2000). Additionally, the plasma membrane localized non-selective cation channels (NSCCs) are reportedly a main entry point of Na into root cells and are regulated by Ca (Demidchik and Tester, 2002; Han et al., 2014). Genes encoding for NSCCs are highly suppressed in *E. salsugineum* consistent with previous findings during salt stress (Volkov and Amtmann, 2006) (Figure 6B and 7). However, counterintuitively, these NSCCs were induced or unaltered in *S. parvula* during salt treatments (Figure 6B), while *S. parvula* maintains a relatively smaller Na content than *E. salsugineum* when treated with salt (Figure 1A). The high basal level of Ca in *S. parvula* roots may alleviate Na-induced cellular toxicity by limiting the selectivity of NSCCs to Na (Han et al., 2014) (Figure 1C). This suggests that Ca may play a key role to achieve ion homeostasis in *S. parvula* during salt stress at a much higher level than reported for *A. thaliana* (Choi et al., 2014).

Halophytes are known for their ability to maintain high K/Na ratios in roots when exposed to salt even if the species-dependent ratios can have large differences between halophytes compared to more salt-sensitive species (Flowers and Colmer, 2008; Cuin et al., 2008; Sun et al., 2009; Cheng et al., 2015). Under salt stress, the negative effect of Na-induced membrane depolarization at the root epidermis could be reversed by the activation of K outward rectifying channel, *GORK* by releasing some of the cytosolic K as shown in *A. thaliana* root hairs (Ivashikina et al., 2001; Shabala and Cuin, 2008). *GORK* expression was induced in *E. salsugineum* when exposed to 250 mM NaCl stress and maintained at high basal level in *S. parvula* while it was low at basal level and unaltered in *A. thaliana* roots under salt stress (Table S4 and 12). Therefore, it may help to prevent the membrane from further depolarization in extremophytes at high salinities. However, how the extremophytes regulate their K transporters to allow K uptake and simultaneously prevent excessive leakage of K during salt stress is unclear. The extremophytes provide an alternative co-regulation of Na and K transporters different from *A. thaliana*. One such modified pathway in the extremophytes compared to *A. thaliana* from our comparative analysis point to a possible synergistic activity between *GORK* and *KAT1* (K channels) synchronized with Na exclusion mediated by *SOS1* to regulate cytosolic K levels, pH, and ion homeostasis in the two extremophytes when exposed to

salt. Coordinated expression of *GORK* and *KAT1* is a prevalent pathway mostly reported for guard cells in *A. thaliana* and *KAT1* expression is thought to be highly reduced in roots (Ache et al., 2000; Philippar et al., 2004). In contrast, *KAT1* is highly expressed even at basal expression levels in both extremophytes (Figure 6A). Notably, these K channels are regulated by ABA and auxin and both extremophytes seem to differently regulate these two hormones in conjunction with overall root growth modulation in response to salt differently compared to that in *A. thaliana* roots (Figure 4, S7, and 7). The intracellular control of K transport appears to be uniquely regulated in the extremophytes compared to *A. thaliana*. For example, *KEA5* primarily expressed in the trans-Golgi network (Zhu et al., 2018; Zhang et al., 2020) is expressed at much higher levels in both extremophytes than in *A. thaliana* (Figures 6A and 7). Additionally, *KUP9* which has undergone tandem duplication in both extremophytes, has one paralog in each extremophyte that show much higher basal expression as well as induction following salt treatments, in contrast to lower constitutive expression of the *A. thaliana* ortholog (Figure 6). *KUP9* in *A. thaliana* mediates auxin efflux from endoplasmic reticulum (Zhang et al., 2020). It would be interesting to find out if the *KUP9* duplication in the extremophytes have led to divergent regulation in auxin homeostasis. Future research exploring this aspect may lead to a missing link in how root growth is modulated under salt stress in salt adapted species.

High tissue tolerance to Na is illustrated by high Na accumulation in *E. salsugineum* roots without losing its nutrient balance during salt treatments (Figure 1). This is thought to be primarily facilitated by vacuolar Na-K/H transporters, *NHX1* and *NHX2* (Bassil et al., 2011). *NHX1* and *NHX2* are co-induced with *V-ATPase* and this coordinated transcription concurrent to increasing salt treatments is observed as expected in *E. salsugineum*, but not in the other two models (Table S11 and Figure 7). *S. parvula* may have reduced the need for vacuolar sequestration of Na differently from *E. salsugineum*. If it is achieved by regulating Na entry to the cell via CNGCs or aquaporins as proposed by previous studies conducted on *A. thaliana* (Demidchik et al., 2002; Byrt et al., 2017), transcript level coordination alone is insufficient to deduce the orthologous functions in *S. parvula* as both groups are categorically highly induced under salt treatments instead of being suppressed to reduce Na entry (Figure 6B).

Metabolic preadaptation or a dynamic response equally allow adaptation to salt stress in extremophytes

Sugars, amino acids, or their associated derivatives are used in all plants for osmoregulation during salt stress (Slama et al., 2015). The most striking metabolic feature among the three species in our study is the extraordinary level of sugars and amino acids in *E. salsugineum* even in control conditions compared to the other two species of which many of those metabolites can serve as osmoprotectants or antioxidants (Figure 2C and 7). The metabolite profiles we observed are consistent with earlier studies that examined metabolites in *E. salsugineum* (Gong et al., 2005; Kazachkova et al., 2013; MacLeod et al., 2015; Lee et al., 2016; Eshel et al., 2017; Shamustakimova et al., 2017; Yin et al., 2018; Pinheiro et al., 2019). Previous work had suggested that *E. salsugineum* leaves and seedlings are metabolically preadapted to salt stress (Gong et al., 2005; Kazachkova et al., 2013; Lee et al., 2016). Our results reinforce this view and extend these observations to roots (Figure 2C and 7). Complementary to a dynamic metabolic response, *S. parvula* in comparison to the other two species exhibits a higher transcriptome level preadaptation to salt stress (concordant with its fewer DEGs in both roots and shoots; Figures 3B and 5A). Interestingly, during salt treatments, *S. parvula* which maintained much lower levels of sugars and amino acids comparable to levels seen in *A. thaliana* at control conditions, raised its metabolite pools of amino acids and sugars to the high basal level present in *E. salsugineum* (Figures 2 and 7).

Proline is among the most studied metabolites during salt stress in *E. salsugineum* and generally in extremophytes due to its role as an osmoprotectant and an antioxidant (Taji et al., 2004; Kant et al., 2008; Flowers and Colmer, 2008; Bartels and Dinakar, 2013). While the key proline biosynthesis gene, *P5CS1* was induced in all three species under salt treatments, *Prodehydrogenase (ProDH1)*, encoding an enzyme that degrades proline, was suppressed only in the extremophytes, coincident to a significant boost in proline exclusively in the extremophytes (Figure 5C). The expression of *ProDH1* is known to be suppressed by sucrose (Funck et al., 2010). Our results may suggest a novel regulatory alteration involving high sucrose levels concomitant to the induction of *sucrose synthase (SUS1)* to maintain high proline levels in the extremophytes via sucrose mediated suppression of *ProDH1* (Figure 5D). *A. thaliana SUS1* is

known to be regulated by osmotic stress independent of ABA and is also less expressed in roots compared to other *SUS* members (Déjardin et al., 1999; Bieniawska et al., 2007).

An alternative view to why sugars and amino acids are maintained in *E. salsugineum* at high levels as a preadapted feature to abiotic stress tolerance is proposed based on those metabolites serving as a carbon or nitrogen source to facilitate growth when photosynthesis and nitrogen acquisition may decrease with abiotic stress (Grime and Hunt, 1975; Pinheiro et al., 2019; Yu et al., 2020). *E. salsugineum* roots grow much slower than both *A. thaliana* and *S. parvula* primary roots under control conditions (Figure S8) (Orsini et al., 2010). A high proportional allocation in roots for sugars such as sucrose and raffinose together with amino acids known for nitrogen storage, especially proline is a common trait associated with slow growing plants (Poorter, 1989; Atkinson et al., 2012). This idea aligns better with *E. salsugineum* viewed as a slow growing annual compared to the other two models. Some of these metabolites that are high at basal levels then have the added benefits for serving as osmoprotectants or antioxidants when the plants experience high salinity levels.

Accumulation of sugars is reported as a metabolic trait in response to drought in *A. thaliana* leaves (Sperdouli and Moustakas, 2012). Contradictory to viewing the accumulation of sugars as a growth promoting adaptation or an adaptation to stress tolerance, it has been proposed as an outcome of photosynthates passively accumulating in the absence of active growth under stressed conditions (Martínez-Vilalta et al., 2016; Granda and Camarero, 2017). *Schrenkiella parvula* dynamically accumulates these metabolites during salt treatments without an indication of compromised growth to match the levels maintained in *E. salsugineum* (Figures 4 and 5 and Tran et al., 2021). The source of increased sugars in *S. parvula* is expected to result from starch hydrolysis mediated by ABA upon salt stress (Thalmann et al., 2016). Therefore, at least in the extremophytes, the shared outcome of high sugars and amino acids seems to be an active metabolic state that could be considered as an adaptive feature to cope with salt stress more than a passive outcome of excess photosynthesis under interrupted growth during stress.

Root transcriptional networks contain multiple divergent points between the extremophytes with convergent outcomes for stress-resilient growth

Schrenkiella parvula and *Eutrema salsugineum* have lower lateral root densities compared to *A. thaliana* (Figure 4F). Auxin is among the main hormones that regulate root architecture including lateral root development (Wang et al., 2009). Lateral root initiation is regulated by binding of auxin to its receptor TIR1, resulting in degradation of auxin signaling repressors, the Aux/IAAs (Lakehal et al., 2019). The *TIR* orthologs in both extremophytes are suppressed in control conditions coincident to successive suppression of multiple *Aux/IAAs* and other downstream transcription factors in control or salt treated conditions, a pattern collectively suggesting limited lateral root initiation (Banda et al., 2019) compared to *A. thaliana* (Figure 4B and S7). Both extremophytes either having a slower root growth rate (in *E. salsugineum*) or higher primary root growth rate but with lower lateral root density (in *S. parvula*), which may reduce the total surface contact area with salt by reducing lateral root density. Therefore, the transcriptional network mediated by auxin and other hormones in the extremophytes suggests multiple regulatory points for root growth modulation that could result in different root architecture (Figure S7). The overall transcriptional response promoting root growth is unique to each extremophyte, but supports uninterrupted nutrient acquisition, water transport, and Na⁺ sequestration to synchronize both developmental and metabolic coordination during salt stress differently from *A. thaliana* (Figure 4 and 6).

Molecular phenotypes for transcriptional preadaptation associated with multiple abiotic stresses have been reported for *S. parvula* and *E. salsugineum* separately to highlight genes uniformly expressed before and after stress treatments compared to differential expression of orthologs in a stress sensitive sister species (Gong et al., 2005; Oh et al., 2014; Lee et al., 2016; Simopoulos et al., 2020; Wang et al., 2021; Pantha et al., 2021). Our work indicated that stress-associated functional gene clusters that exhibit transcriptional preadaptation in one extremophyte is in many instances contrasted by a dynamic induced response by the orthologs from the other extremophyte that ultimately match the expression level found in the preadapted species (e.g. Figure 4G). This highlights the diversity of independent and divergent regulation of orthologs even between closely related extremophytes in response to the same treatments given in the same growth conditions but demonstrating different adaptive strategies.

Overall, our study highlights the multiple different combinatorial expression modules that can be examined for stress optimized growth using two extremophytes adapted to grow in high salinities. The hormone-mediated root growth and especially the selective release of repression in auxin and ABA signaling pathways that may lead to different *S. parvula* and *E. salsugineum* root growth strategies absent in *A. thaliana* needs to be further investigated to identify additional regulatory dependencies using target studies in the extremophyte models. Similarly, comparisons from multiple extremophytes can serve as training data to identify compatible regulatory pathways that can coexist but are currently absent in crops. Such pathways need to be investigated for their functionality to metabolic cost to evaluate whether constitutive expression (as pre-adaptive traits) or induced expression (as dynamic responses) offer an optimum strategy depending on a stress condition being constant or intermittent in certain environments. The scarcity of halophytes despite their recurrent evolution implies a high metabolic cost and complex regulation required for salt stress adaptation for coordinated growth from cellular to whole plant level (Flowers and Colmer, 2015). Extremophytes will be a direct resource when modeling stress resilient growth based on which combination of core stress-response pathways are critical to deliver growth and survival during environmental stress (Zandalinas et al., 2021). For sustainable global food security, our crops need to be diversified, less dependent on fresh water and high nutrient soils while being adapted to varying levels of marginal soils with different salinities (Bailey-Serres et al., 2019; Pareek et al., 2020). Therefore, basic research examining genetic regulation underlying stress optimized growth will be a prerequisite when selecting new crops in light of a climate crisis.

Materials and methods

Plant growth and treatments

Schrenkiella parvula (ecotype Lake Tuz, Turkey; Arabidopsis Biological Resource Center/ABRC germplasm CS22663), *Eutrema salsugineum* (ecotype Shandong, China; ABRC germplasm CS22504), and *Arabidopsis thaliana* (ecotype Col-0) seeds were surface-sterilized and stratified at 4 °C for 7 days (for *A. thaliana* and *S. parvula*) or 14 days (for *E. salsugineum*). Stratified seeds were germinated and grown in a hydroponic system as described by Conn et al.,

(2013) for transcriptomic, ionic, and metabolomic experiments (Figure S1B). The plants were grown in aerated 1/5x strength Hoagland's solution in a growth cabinet set to 22-23 °C, photosynthetic photon flux density at 80-120 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and a 12 hr light / 12 hr dark cycle. Fresh Hoagland's solution was replaced every two weeks. Four-week-old plants were randomly placed in 1/5x strength Hoagland's solution with and without NaCl and incubated for indicated duration in each experiment.

Root growth analysis

Five-day old seedlings were transferred to 1/4x MS plates supplied with 150 mM NaCl. Root growth was recorded every two days for one week. Control and treated plates were scanned and analyzed using ImageJ (Schneider et al., 2012) to quantify primary root length and number of lateral roots. Three biological replicates were used with 7 seedlings per replicate from each species.

Elemental analysis

Elemental quantification was conducted for Na, K, Ca, P, S, Mg, Fe, B, Zn, Mn, Mo, Cu, Ni, and Co using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS, Elan 6000 DRC-PerkinElmer SCIEX) at the US Department of Agricultural Research Service at Donald Danforth Plant Science Center. We used dried root and shoot samples from 3-4 biological replicates from each control and treated condition harvested at 3, and 24 hr (Figure S1) processed as described in Baxter et al., (2014). Changes in element contents were calculated as the $\log_2$ fold change of the element level in treated samples over that in control samples and visualized with the pheatmap package in R. Significant differences between treatments within species were determined by one-way ANOVA followed by Tukey post-hoc test using agricolae package in R with an adjusted p -value cutoff of 0.05. Basal level of each element per species was calculated as a % contribution from each species that added to 100% for each element, using the following formula: $X_i / (A_i + S_i + E_i) * 100$ where X_i represents the abundance of element i in *A. thaliana*, *S. parvula* and *E. salicigineum*, respectively. Only elements that showed significant differences in abundance among the three species were visualized on ternary diagrams using ggtern R.

Metabolite analysis

Untargeted high throughput metabolite profiling was conducted using gas chromatography-mass spectrometry (GC-MS) service at the West Coast Metabolomics Center, University of California Davis. Root and shoot samples in 4 biological replicates were harvested at 24, and 72 hr (Figure S1B), flash frozen in liquid N₂, and processed as described in Fiehn (2017) and quantified as described in Pantha et al., (2021).

Pearson correlation coefficients per each test condition were calculated between species using normalized metabolite abundances. Significant differences in metabolite abundances across treatments within species (differently abundant metabolites, DAMs) was determined by one-way ANOVA followed by Tukey post-hoc test using an agricolae package in R with an adjusted *p*-value cutoff of 0.05. Structurally annotated (known) metabolites were further categorized into functional groups according to the refmet database (<https://www.metabolomicsworkbench.org/databases/refmet/index.php>) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Metabolites were clustered into amino acids, sugars, nucleic acids, and other organic acids. Derivatives or precursors of those were included in the same metabolite category if those individual metabolites were found to be within three steps of the main metabolite category identified in a KEGG pathway (Table S2). Basal level of each metabolite per species was calculated as a % contribution from each species that added to 100% for each metabolite similar to the elemental basal level calculation described earlier. Metabolites that showed significant differences in abundance at basal level or under salt treatments at least in one species were used for K-mean clustering (k= 9) (Mannor et al., 2011). The metabolite profiles across samples were done using hierarchical clustering function in pheatmap package.

Orthologous group identification

Ortholog groups were identified using *S. parvula* gene models version 2.2 (<https://phytozome-next.jgi.doe.gov/>); *A. thaliana* gene models version 10 (TAIR10) (<https://www.arabidopsis.org/download/>), and *E. salisugineum* gene models (Wu et al., 2012)

which were updated based on reference-guided transcript assembly using hisat2 (version 2.0.1)-stringtie (version 1.2.1) pipeline (Pertea et al., 2015, 2016) and RNA-seq reads from this study. The updated gene models for *Eutrema salsugineum* and *Schrenkiella parvula* are available at www.lsugenomics.org. The ortholog gene pairs between species were identified by reciprocal blastp with default parameters and an e-value cutoff of 1E-5. The blastp results were filtered using a custom python script to select the best High-scoring Segment Pair (HSPs) to ensure only unique query-subject pairs were retained. Pairs with an alignment coverage smaller than 50% of the query or the subject were removed. The ortholog-pair list included only non-redundant pairs with the highest bit score optimized for highest % coverage, and % identity. Ortholog pairs were additionally filtered to exclude any orthologs that had a more than $\pm 30\%$ length difference in the coding sequence between the two sequences. A total of 16,591 one-to-one ortholog groups were identified among the three species and used for all downstream analyses (Table S3).

Gene expression profiling and analysis

Total RNA was extracted from control and treated samples at 3 and 24 hr with three biological replicates (Figure S1) using Qiagen RNeasy Plant Mini kit, with an on-column DNase treatment. mRNA enriched samples were converted to libraries using True-Seq stranded RNAseq Sample Prep kit (Illumina, San Diego, CA, USA), multiplexed, and sequenced on a HiSeq4000 (Illumina) platform at the Roy K. Carver Biotechnology Center, University of Illinois at Urbana-Champaign. A minimum of >15 million 50-nucleotide single-end reads per sample were sequenced for a total of 138 RNAseq samples.

After quality checks, the reads were mapped to reference transcript model sequences of gene models in each species (described earlier) using Bowtie (Langmead and Slazberg, 2013) with -m 1 --best -n 1 -l 50. A custom python script was used to count uniquely mapped reads for each gene model. Differentially expressed genes (DEGs) across treatments within each species were identified using DESeq2 (Love et al., 2014). Genes with an adjusted p-value ≤ 0.01 were further filtered using the following criteria: 1) $|\log_2 \text{fold change}| \geq 1$ or 2) $|\log_2 \text{fold change}| \geq 0.5$ if normalized mean expression across samples ≥ 100 . Genes that passed these filters were

considered as DEGs. Reads per kilobase per million reads (RPKM) were calculated for each gene from the raw read counts for all samples. These RPKM values were $\log_2$ -transformed and median-normalized when used in PCA-UMAP (McInnes et al., 2018) and for co-expression cluster determinations. Co-expression clusters were done using fuzzy K-mean clustering with a membership cutoff ≥ 0.4 and $\log_2$ fold change clustering. $\log_2$ fold changes were computed for all ortholog groups (OGs) from three species, used to calculate Pearson correlation coefficients when comparing transcriptome profiles between species/samples, and were visualized with ggplot2 in R. Ortholog groups where at least one of the genes in the group was identified as a DEG in any species under any stress condition was considered to be a differentially expressed ortholog group (DEOG).

To compare the basal expression levels of orthologs among species, we generated a raw count matrix based on reads uniquely mapped to coding sequences (CDS) for the 16,591 orthologs from control samples. Orthologs that are differently expressed between any two of the three species were identified using DESeq2 at an adjusted p -value ≤ 0.001 in a pairwise manner. We next assigned the expression of each gene within any given OG as High (H), Medium (M), and Low (L) based on their relative expression level resulting in 12 clusters. The six largest clusters in both roots and shoots highlighted the expression difference in one species compared to the other two species. OGs were assigned to these clusters represented by HLH, LHL, HHL, LLH, LHH, and HLL (expression profiles). Each expression profile was then subjected to functional gene enrichment analysis.

BiNGO (Maere et al., 2005) was used to identify Gene Ontology (GO) terms enriched in selected DEGs and DEOGs from shoots and roots. To reduce the redundancy between enriched GO terms, we further grouped these GO terms into GO clusters using GOMCL (Wang et al., 2020) with settings for -Ct 0.5 -l 1.5 -Sig 0.05 -hm -nw -hgt -d -gosize 3500 -gotype BP. Sub-clustering of selected clusters was performed using GOMCL-sub with the same parameters. OrthNets for selected genes were generated using the CLfinder-OrthNet pipeline with the reciprocal blastp results as input using default OrthNet settings (Oh and Dassanayake, 2019), and visualized in Cytoscape.

Curated gene sets for primary metabolism, hormone signaling, root development, and transporter functions

DEOGs and OGs that showed significant differences at basal level/control condition or under treatments were mapped to the KEGG pathways associated with primary metabolism, hormone regulation, or root development. Log₂ fold changes were computed for all selected OGs and visualized with heatmap in R. Genes involved in root development were identified from Gene Ontology annotations, GO:0048527 for lateral root development and GO:0080022 for primary root development. Genes coding for transport functions associated with K/Na transport were mined from Araport 11. To assess the correlation between changes at the transcriptomic level and the metabolic level, we calculated the percentage of DEGs and differently abundant metabolites (DAMs) in each species at early (3 hr for transcriptome and 24 hr for metabolome) and late (24 hr for transcriptome and 72 hr for metabolome) response to salt. Percent DEGs was calculated by dividing the number of DEGs by the total number of expressed genes (RPKM $\geq$ 1) in each species. Similarly, the percent DAMs was calculated by dividing the number of DAMs by the total number of quantified metabolites within a species. We extracted DEGs and DAMs related to amino acid and sugar metabolism based on GO (GO:0006520 for amino acid metabolism and GO:0005975 for carbohydrate metabolism). The count of DEGs and DAMs were separately normalized to the total number of genes and metabolites in each of the two categories.

Data availability

All Illumina sequence data are deposited at National Center for Biotechnology Information BioProject PRJNA63667. Mapped RNAseq data can be browsed using genome browsers created for *Schrenkiella parvula* and *Eutrema salsugineum* at www.lisugenomics.org.

Acknowledgement

This work was supported by the US National Science Foundation IOS-EDGE 1923589/2019610, NSF-MCB-1616827, US Department of Energy BER-DE-SC0020358, Next-Generation BioGreen21 Program of Republic of Korea (PJ01317301) awards. Graduate students K.T., G.W. were supported by an Economic Development Assistantship from LSU. We thank the

LSU High Performance Computing facility for providing computational resources; undergraduate students Jordan Vivien, Christine Tran, Ashley Doan, and Jason Garcia at LSU for assisting with plant growth and phenotyping; Drs. Alvaro Hernandez and John Cheeseman at University of Illinois for providing assistance with Illumina sequencing.

Author contribution: K.T. prepared plant samples and conducted data analyses; K.T., G.W., D-H.O., J.L., A.S., and M.D. contributed to data interpretation. K.T. and M.D. wrote the manuscript with input from all co-authors who revised and approved the final manuscript. M.D. conceptualized and supervised the overall project.

Main figure captions

Figure 1: Sodium accumulation and its effect on nutrient balance in the extremophytes compared to *A. thaliana*. [A] Na^+ and [B] K^+ content in roots and shoots. Data are mean $\pm$ SD ($n \geq 4$, at least 4 plants per replicate). Asterisks indicate significant differences ($p \leq 0.05$) between the treated samples and their respective control samples, determined by Student's t-test. Open circles indicate the biological replicates. [C] Macro- and micro-nutrient fold changes between treated and its respective control sample. [D] Percent abundance of nutrients that were significantly different at basal levels among the three species. The three axes of the ternary plots are marked with *A. thaliana*, *S. parvula*, and *E. salsugineum*. The gridlines in species designated colors point to the relevant % abundance of the element in each axis. Significant difference in elemental abundance was determined by one-way ANOVA with post-hoc Tukey's test at $p \leq 0.05$.

Figure 2: Overall metabolic preparedness and adjustments in the roots of the extremophytes compared to *A. thaliana*. [A] Pairwise correlation of 716 quantified metabolites (182 known, and 534 unknown) across all conditions and species. Correlation calculated using Pearson correlation coefficients. [B] Total number of metabolites that significantly changed in abundance in each species compared to its respective control sample. Known metabolite numbers given in parenthesis. [C] Basal level metabolite abundances in all three species for sugars, amino acids, and their derivatives. [D] Hierarchical clustering of known metabolites

shown in [C]. Blue box indicates clusters of interest. Treatment concentrations are 150 and 250 mM NaCl; Treatment durations are 24 and 72 hr. Significant differences were determined by one-way ANOVA with post-hoc Tukey's test at $p \leq 0.05$ ($n = 4$, at least 4 plants per replicate).

Figure 3: Transcriptomic overview *S. parvula*, *E. salsugineum*, and *A. thaliana* in response to salt. [A] Overall transcriptome clustering using one-to-one ortholog groups (OGs) in shoots and roots from all replicates in all conditions. [B] Number of differently expressed genes (DEGs) in response to salt treatments. [C] Functionally enriched processes clustered among OGs that included a DEG from at least one species in roots and shoots. Functional annotations were based on GO annotations assigned to OGs. The bar graphs assigned to each cluster represent percent allocation of induced and suppressed DEGs with the number of genes that did not significantly change in each species given in the white space between induced (red) and suppressed (blue) bars. [D] Dominant gene expression clusters of orthologs across time points and species from roots and shoots that included an *A. thaliana* ortholog that was either induced or suppressed in response to salt. Differentially expressed genes identified at $p\text{-adj} \leq 0.01$. $N = 3$ (at least 4 plants per replicate).

Figure 4: Root growth responses in line with gene expression mediated by auxin and ABA in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema salsugineum* when treated with salt. [A] Expression responses of auxin/indole acetic acid (Aux/IAA) repressors and type 2C protein phosphatases (PP2Cs) that regulate auxin and ABA signaling. [B] Curated genes from lateral root development (GO:0048527) and primary root development (GO:0080022) used to infer root growth phenotypes based on functional genetic studies in *A. thaliana*. Orthologs in the three species are indicated as high (yellow), low (blue), and indistinguishable levels between species (no-color) assigned to relative basal expression levels and their effect inferred with arrows (up – promote growth and down – suppress growth). Genes associated with Auxin, ABA, and other hormones were labeled in pink, gold, and black respectively. [C] Root growth assessment of 12-day-old seedlings in response to 150 mM NaCl treated for 7 days. Quantification of [D] primary root growth, [E] number of lateral roots, and [F] lateral root density. Data given as mean $\pm$ SD ($n = 3$, at least 7 plants per replicate). Open circles indicate the measurements from each plant. Asterisks indicate significant differences ($p \leq 0.05$) between treated samples and their

respective control samples, determined by one-way ANOVA with post-hoc Tukey's test. [G]
Gene co-expression clusters of differentially expressed orthologs that showed similar
expression pattern in *A. thaliana* and *E. salsugineum* roots compared to a different pattern
observed for *S. parvula* under salt stress.

Figure 5: Coordination between differently expressed genes and differently abundant
metabolites during responses to salt in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema*
salsugineum. [A] The difference in percent change in total differentially expressed genes (DEGs)
and total differently abundant metabolites (DAMs) in response to salt treatments. [B] % DEGs
and % DAMs involved in the metabolism of amino acids, sugars, and their immediate
derivatives (GO:0006520 and GO:0005975). [C and D] Selected pathways in amino acids and
sugar metabolism. Line graphs represent normalized Log2 relative metabolite abundance.
Boxplots represent normalized expression values. Center line shows median; box indicates
interquartile range (IQR); notch is for $1.58 \times \text{IQR}/\sqrt{n}$; whiskers show $1.5 \times \text{IQR}$. Asterisks
indicate DEGs and DAMs assigned using 3-4 biological replicates. Early (E) and late (L) responses
for transcripts refer to 3 and 24 hr. E and L responses for metabolites refer to 24 and 72 hr.
Metabolites are shown in the backbone of the pathway while genes encoding for key
enzymes/transcription factors are placed beside arrows. Black labels in pathway maps indicate
quantified metabolites and genes while grey labels indicate metabolites not quantified.

Figure 6: Basal and differential expression associated with salt and nutrient transport in
Arabidopsis thaliana, *Schrenkiella parvula*, and *Eutrema salsugineum*. [A] Basal level expression
given as fold changes between species. The dashed lines indicate 2-fold differences to highlight
genes either highly suppressed or highly expressed in the extremophytes in roots at control
conditions. [B] Aggregated expression profiles of transporters/channels associated with Na^+/K^+
transport activity during salt treatments. [C] Selected cation transporters that showed higher
gene copy number in extremophytes in ortholog groups and their expression during salt
treatments. Nodes of each ortholog network is assigned with species colors connected by edges
based on the relationship properties of the homologs. Edges show relationships for co-linear
reciprocal orthologs (cl-rc), co-linear unidirectional orthologs (cl-uni), transposed -unidirectional
duplicates (tr-uni), or tandem duplicated-unidirectional paralogs (td-uni). Asterisks indicate

differentially expressed genes identified at $*p\text{-adj} \leq 0.05$ and $**p\text{-adj} \leq 0.01$. N= 3 (at least 4 plants per replicate). [D] Genes associated with nutrient transport identified in Figure 3C.

Figure 7: Summary of ionomic, metabolomic, transcriptomic, and phenotypic adjustments and preadaptation trends to salt stress in *Arabidopsis thaliana*, *Schrenkiella parvula* and *Eutrema salsugineum* roots. Top 9 metabolites that increased abundance in *S. parvula* were Fru, fructose; Asn, asparagine, Raf, raffinose, DHA, dehydroascorbic acid; Pro, proline; Shkm, shikimic acid; Suc, sucrose, GABA, gamma-aminobutyric acid; Gln, glutamine.

Orthologs in the three species are indicated as high (yellow), low (blue) assigned to relative basal expression levels. Blue and red indicate the induction and suppression gene expression, respectively. KEA5, K⁺ efflux antiporter 5; KUP9, K⁺ uptake permease 9; KAT1, K⁺ channel in *Arabidopsis thaliana* 1; GLRs, glutamate-like receptors; NHX1/2, Na⁺/H⁺ exchanger1/2; V-ATPases, vacuoles-ATPases; SOS1, salt overly sensitive 1; GORK, gated outwardly-rectifying K⁺ channel.

Main table caption

Table 1: Table 1: Enriched cellular processes of ortholog groups at basal expression level clustered in shoots and roots of *S. parvula* (purple), *E. salsugineum*, (red) and *A. thaliana* (green).

Supplement figure and table captions

Figure S1: Figure S1: Effect of salt stress on phenotype of *Schrenkiella parvula* (Sp), *Eutrema salsugineum* (Es) and *Arabidopsis thaliana* (At). [A] Four-week-old hydroponic grown plants were treated with indicated salt concentrations. [B] Experimental design and sample scheme. There were at least 4 replicates used for ionomic and metabolomic profiling, and 3 replicates for transcriptomic profiling with at least 4 plants per replicate.

Figure S2: Overall shoot metabolite responses in the extremophytes compared to *A. thaliana*. [A] Pairwise correlation of 725 quantified metabolites across all conditions and species. Correlation calculated using Pearson correlation coefficients. [B] Total number of metabolites that significantly changed in abundance in each species compared to its respective control

sample. Known metabolite numbers given in parenthesis. [C] Basal level metabolite abundances in all three species for sugars, amino acids, and their derivatives. Significant differences were determined by one-way ANOVA with post-hoc Tukey's test at $p \leq 0.05$ ($n = 4$, at least 4 plants per replicate).

Figure S3: Transcriptomic profiles of *S. parvula*, *E. salsugineum*, and *A. thaliana* were diverged in response at basal level and under salt stress. [A] roots, [B] shoots. Correlation was calculated using log2 fold change of treated over control samples from differentially expressed ortholog pairs which included DEGs from at least one condition in one species using Pearson correlation coefficients. *S. parvula* (purple), *E. salsugineum* (red) and *A. thaliana* (green).

Figure S4: Sub-clustering of dominant cluster - Response to stresses. Overlap and similarity between sub-clusters determined using GO-MCL-sub and cumulative number of ortholog groups (OGs) in [A and B] roots and [C and D] shoots sub-clusters.

Figure S5: Species dependent responses to salt treatments in *Schrenkiella parvula* and *Eutrema salsugineum*. Differently expressed genes (DEGs) in *S. parvula* [A] root and [B] shoot and *E. salsugineum* [C] root and [D] shoot in response to 150 and 250 mM NaCl stress at 3 and 24 hr of treatment. Yellow highlighted data points in the Upset plots for each panel show DEGs that were uniquely expressed at 250 mM salt treatments. Functionally enriched processes for these selected DEGs are shown in the horizontal bar graphs given as insets outlined in yellow for each panel. Differentially expressed genes identified at $p\text{-adj} \leq 0.01$. $N = 3$ (at least 4 plants per replicate).

Figure S6: Hormonal signaling pathways in response to salt treatment in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema salsugineum*. Expression responses of genes associated with hormonal signaling pathways obtained from KEGGs. Note that only genes that were significantly different at either basal level condition or under salt treatments were shown. Orthologs in the three species are indicated as high (yellow), low (blue), and indistinguishable levels between species (no-color) assigned to relative basal expression levels.

Figure S7: Lateral root development gene network adopted from Banda et al., 2019 and Rybel et al., 2010. The expression levels of orthologs were annotated as high (H), medium (M), or low (L) relative to each other at basal levels in each ortholog group. Specific paths in the network in

which *S. parvula* and *E. salsugineum* consistently regulated to inhibit lateral root formation were highlighted in ribbons across the network in species-specific colors.

Figure S8: Root growth of *E. salsugineum*, *S. parvula* and *A. thaliana* before and after salt treatments. *S. parvula* (Sp) promoted primary root growth and reduced lateral root formation while *E. salsugineum* (Es) showed slower primary root growth with reduced lateral root number compared to *A. thaliana* (At) roots that showed severe root growth inhibition under salt stress. Five-day-old seedlings were treated for 11 days on plates supplemented with 150 mM NaCl.

Figure S9: Metabolite abundance and expression of associated genes encoding the enzymes in shikimic acid and ascorbic acid pathways. [A] percent change in total differentially expressed genes (DEGs) and total differently abundant metabolites (DAMs) in response to salt treatments. [B] pathway involved in proline metabolism. [C] pathway involved in the conversion from phosphoenolpyruvate to chorismate. [D] pathway involved in ascorbic acid metabolism. Line graphs represent normalized Log2 relative metabolite abundance. Boxplots represent normalized expression values. Center line shows median; box indicates interquartile range (IQR); notch is for $1.58 \times \text{IQR}/\sqrt{n}$; whiskers show $1.5 \times \text{IQR}$. Asterisks indicate DEGs (Differently expressed genes) and DAMs (Differently abundant metabolites). Early (E) and late (L) responses for transcripts refer to 3 and 24 hr. E and L responses for metabolites refer to 24 and 72 hr. Metabolites are shown in the backbone of the pathway while genes encoding for key enzymes/transcription factors are placed beside arrows. Black labels in pathway maps indicate quantified metabolites and genes while grey labels indicate metabolites not shown.

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Figure S1: Effect of salt stress on phenotype of *Schrenkiella parvula* (Sp), *Eutrema salsugineum* (Es) and *Arabidopsis thaliana* (At). [A] Four-week-old hydroponic grown plants were treated with indicated salt concentrations. [B] Experimental design and sample scheme. There were at least 4 replicates used for ionomic and metabolomic profiling, and 3 replicates for transcriptomic profiling with at least 4 plants per replicate.

Figure 1: Sodium accumulation and its effect on nutrient balance in the extremophytes compared to *A. thaliana*. [A] Na⁺ and [B] K⁺ content in roots and shoots. Data are mean $\pm$ SD (n $\geq$ 4, at least 4 plants per replicate). Asterisks indicate significant differences ($p \leq 0.05$) between the treated samples and their respective control samples, determined by Student's t-test. Open circles indicate the biological replicates. [C] Macro- and micro-nutrient fold changes between treated and its respective control sample. [D] Percent abundance of nutrients that were significantly different at basal levels among the three species. The three axes of the ternary plots are marked with *A. thaliana*, *S. parvula*, and *E. salsugineum*. The gridlines in species designated colors point to the relevant % abundance of the element in each axis. Significant difference in elemental abundance was determined by one-way ANOVA with post-hoc Tukey's test at $p \leq 0.05$.

Figure 3: Transcriptomic overview *S. parvula*, *E. salsugineum*, and *A. thaliana* in response to salt. [A] Overall transcriptome clustering using one-to-one ortholog groups (OGs) in shoots and roots from all replicates in all conditions. [B] Number of differentially expressed genes (DEGs) in response to salt treatments. [C] Functionally enriched processes clustered among OGs that included a DEG from at least one species in roots and shoots. Functional annotations were based on GO annotations assigned to OGs. The bar graphs assigned to each cluster represent percent allocation of induced and suppressed DEGs with the number of genes that did not significantly change in each species given in the white space between induced (red) and suppressed (blue) bars. [D] Dominant gene expression clusters of orthologs across time points and species from roots and shoots that included an *A. thaliana* ortholog that was either induced or suppressed in response to salt. Differentially expressed genes identified at $p\text{-adj} \leq 0.01$. N= 3 (at least 4 plants per replicate).

Table 1: Enriched cellular processes of ortholog groups at basal expression level clustered in shoots and roots of *S. parvula* (purple), *E. salsgineum*, (red) and *A. thaliana* (green).

Patterns	Clstr	# GO	# genes	Cumulative genes %	Functional category
<p>2159 OGs (Sh:1215 - Rt:1351)</p>	1	101	703	51.4%	Hormone and abiotic/biotic stimuli signaling
	2	58	458	70.2%	Ion and vesicular transport
	3	65	417	77.6%	Organ development
	4	26	337	82.5%	Transcriptional control of organ development
<p>2042 OGs (Sh:1141 - Rt:1241)</p>	1	74	708	63.8%	Hormone and abiotic stimuli regulation
	2	83	497	88.1%	RNA and amino acid processes
	3	53	437	99.4%	Organelle biogenesis
	4	6	23	99.8%	Phospholipid metabolism
<p>1898 OGs (Sh:1134 - Rt:1084)</p>	1	160	776	67.4%	Hormone and abiotic stimuli regulation
	2	100	520	88.9%	RNA and amino acid processes
	3	29	376	99%	Ion and vesicular transport
	4	4	112	99.7%	Homeostasis processes
<p>1879 OGs (Sh:1098 - Rt:1050)</p>	1	32	606	52.8%	Organ development
	2	58	519	74.0%	Hormone and abiotic/biotic stimuli signaling
	3	21	324	80.6%	Transcriptional regulation of development
	4	11	291	88.7%	Lipid metabolism
<p>2086 OGs (Sh:1199 - Rt:1239)</p>	1	44	671	65.8%	RNA metabolism and gene expression
	2	11	331	82.9%	Shoot and flower development
	3	19	268	91.5%	Cell cycle regulation
	4	4	159	95.5%	Abiotic stimuli responses
<p>2225 OGs (Sh:1321 - Rt:1263)</p>	1	106	775	55.3%	Hormone and abiotic/biotic stimuli signaling
	2	92	572	72.6%	Organ development and reproduction
	3	74	398	84.4%	Amino acid & lipid metabolism
	4	43	348	92.1%	Ion and vesicular transport

Figure S3: Transcriptomic profiles of *S. parvula*, *E. salsgineum*, and *A. thaliana* were diverged in response at basal level and under salt stress. [A] roots, [B] shoots. Correlation was calculated using log₂ fold change of treated over control samples from differentially expressed ortholog pairs which included DEGs from at least one condition in one species using Pearson correlation coefficients. *S. parvula* (purple), *E. salsgineum* (red) and *A. thaliana* (green).

Figure S4: Sub-clustering of dominant cluster - Response to stresses. Overlap and similarity between sub-clusters determined using GO-MCL-sub and cumulative number of ortholog groups (OGs) in [A and B] roots and [C and D] shoots sub-clusters.

Figure S5: Species dependent responses to salt treatments in *Schrenkiella parvula* and *Eutrema salsugineum*. Differentially expressed genes (DEGs) in *S. parvula* [A] root and [B] shoot and *E. salsugineum* [C] root and [D] shoot in response to 150 and 250 mM NaCl stress at 3 and 24 hr of treatment. Yellow highlighted data points in the Upset plots for each panel show DEGs that were uniquely expressed at 250 mM salt treatments. Functionally enriched processes for these selected DEGs are shown in the horizontal bar graphs given as insets outlined in yellow for each panel. Differentially expressed genes identified at $p\text{-adj} \leq 0.01$. N = 3 (at least 4 plants per replicate).

Figure 4: Root growth responses in line with gene expression mediated by auxin and ABA in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema salsugineum* when treated with salt. [A] Expression responses of auxin/indole acetic acid (Aux/IAA) repressors and type 2C protein phosphatases (PP2Cs) that regulate auxin and ABA signaling. [B] Curated genes from lateral root development (GO:0048527) and primary root development (GO:0080022) used to infer root growth phenotypes based on functional genetic studies in *A. thaliana*. Orthologs in the three species are indicated as high (yellow), low (blue), and indistinguishable levels between species (no-color) assigned to relative basal expression levels and their effect inferred with arrows (up – promote growth and down – suppress growth). Genes associated with Auxin, ABA, and other hormones were labeled in pink, gold, and black respectively. [C] Root growth assessment of 12-day-old seedlings in response to 150 mM NaCl treated for 7 days. Quantification of [D] primary root growth, [E] number of lateral roots, and [F] lateral root density. Data given as mean $\pm$ SD ($n = 3$, at least 7 plants per replicate). Open circles indicate the measurements from each plant. Asterisks indicate significant differences ($p \leq 0.05$) between treated samples and their respective control samples, determined by one-way ANOVA with post-hoc Tukey's test. [G] Gene co-expression clusters of differentially expressed orthologs that showed similar expression pattern in *A. thaliana* and *E. salsugineum* roots compared to a different pattern observed for *S. parvula* under salt stress.

Figure S6: Hormonal signaling pathways in response to salt treatment in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema salsugineum*. Expression responses of genes associated with hormonal signaling pathways obtained from KEGGs. Note that only genes that were significantly different at either basal level condition or under salt treatments were shown. Orthologs in the three species are indicated as high (yellow), low (blue), and indistinguishable levels between species (no-color) assigned to relative basal expression levels

Figure S7: Lateral root development gene network adopted from Banda et al., 2019 and Rybel et al., 2010. The expression levels of orthologs were annotated as high (H), medium (M), or low (L) relative to each other at basal levels in each ortholog group. Specific paths in the network in which *S. parvula* and *E. salsugineum* consistently regulated to inhibit lateral root formation were highlighted in ribbons across the network in species-specific colors.

Figure S8: Root growth of *E. salsugineum*, *S. parvula* and *A. thaliana* before and after salt treatments. *S. parvula* (Sp) promoted primary root growth and reduced lateral root formation while *E. salsugineum* (Es) showed slower primary root growth with reduced lateral root number compared to *A. thaliana* (At) roots that showed severe root growth inhibition under salt stress. Five-day-old seedlings were treated for 11 days on plates supplemented with 150 mM NaCl.

Figure 5: Coordination between differentially expressed genes and differentially abundant metabolites during responses to salt in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema salsugineum*. [A] The difference in percent change in total differentially expressed genes (DEGs) and total differentially abundant metabolites (DAMs) in response to salt treatments. [B] % DEGs and % DAMs involved in the metabolism of amino acids, sugars, and their immediate derivatives (GO:0006520 and GO:0005975). [C and D] Selected pathways in amino acids and sugar metabolism. Line graphs represent normalized Log2 relative metabolite abundance. Boxplots represent normalized expression values. Center line shows median; box indicates interquartile range (IQR); notch is for $1.58 \times \text{IQR}/\sqrt{n}$; whiskers show $1.5 \times \text{IQR}$. Asterisks indicate DEGs and DAMs assigned using 3-4 biological replicates. Early (E) and late (L) responses for transcripts refer to 3 and 24 hr. E and L responses for metabolites refer to 24 and 72 hr. Metabolites are shown in the backbone of the pathway while genes encoding for key enzymes/transcription factors are placed beside arrows. Black labels in pathway maps indicate quantified metabolites and genes while grey labels indicate metabolites not quantified.

Figure S9: Metabolite abundance and expression of associated genes encoding the enzymes in shikimic acid and ascorbic acid pathways. [A] percent change in total differentially expressed genes (DEGs) and total differently abundant metabolites (DAMs) in response to salt treatments. [B] pathway involved in proline metabolism. [C] pathway involved in the conversion from phosphoenolpyruvate to chorismate. [D] pathway involved in ascorbic acid metabolism. Line graphs represent normalized Log2 relative metabolite abundance. Boxplots represent normalized expression values. Center line shows median; box indicates interquartile range (IQR); notch is for $1.58 \times \text{IQR}/\sqrt{n}$; whiskers show $1.5 \times \text{IQR}$. Asterisks indicate DEGs (Differently expressed genes) and DAMs (Differently abundant metabolites). Early (E) and late (L) responses for transcripts refer to 3 and 24 hr. E and L responses for metabolites refer to 24 and 72 hr. Metabolites are shown in the backbone of the pathway while genes encoding for key enzymes/transcription factors are placed beside arrows. Black labels in pathway maps indicate quantified metabolites and genes while grey labels indicate metabolites not shown.

Figure 6: Basal and differential expression associated with salt and nutrient transport in *Arabidopsis thaliana*, *Schrenkiella parvula*, and *Eutrema salsugineum*. [A] Basal level expression given as fold changes between species. The dashed lines indicate 2-fold differences to highlight genes either highly suppressed or highly expressed in the extremophytes in roots at control conditions. [B] Aggregated expression profiles of transporters/channels associated with Na⁺/K⁺ transport activity during salt treatments. [C] Selected cation transporters that showed higher gene copy number in extremophytes in ortholog groups and their expression during salt treatments. Nodes of each ortholog network is assigned with species colors connected by edges based on the relationship properties of the homologs. Edges show relationships for co-linear reciprocal orthologs (cl-rc), co-linear unidirectional orthologs (cl-uni), transposed -unidirectional duplicates (tr-uni), or tandem duplicated-unidirectional paralogs (td-uni). Asterisks indicate differentially expressed genes identified at *p-adj ≤ 0.05 and **p-adj ≤ 0.01. N = 3 (at least 4 plants per replicate). [D] Genes associated with nutrient transport identified in Figure 3C.

Figure 7: Summary of ionomic, metabolomic, transcriptomic, and phenotypic adjustments and preadaptation trends to salt stress in *Arabidopsis thaliana*, *Schrenkiella parvula* and *Eutrema salsugineum* roots. Top 9 metabolites that increased abundance in *S. parvula* were Fru, fructose; Asn, asparagine; Raf, raffinose; DHA, dehydroascorbic acid; Pro, proline; Shkm, shikimic acid; Suc, sucrose; GABA, gamma-aminobutyric acid; Gln, glutamine. Orthologs in the three species are indicated as high (yellow), low (blue) assigned to relative basal expression levels. Blue and red indicate the induction and suppression gene expression, respectively. KEA5, K⁺ efflux antiporter 5; KUP9, K⁺ uptake permease 9; KAT1, K⁺ channel in *Arabidopsis thaliana* 1; GLRs, glutamate-like receptors; NHX1/2, Na⁺/H⁺ exchanger1/2; V-ATPases, vacuoles-ATPases; SOS1, salt overly sensitive 1; GORK, gated outwardly-rectifying K⁺ channel.