



Adaptative Mechanisms of Halophytic *Eutrema salsugineum* Encountering Saline Environment

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Salt cress (*Eutrema salsugineum*), an *Arabidopsis*-related halophyte, can naturally adapt to various harsh climates and soil conditions; thus, it is considered a desirable model plant for deciphering mechanisms of salt and other abiotic stresses. Accumulating evidence has revealed that compared with *Arabidopsis*, salt cress possesses stomata that close more tightly and more succulent leaves during extreme salt stress, a noticeably higher level of proline, inositols, sugars, and organic acids, as well as stress-associated transcripts in unstressed plants, and they are induced rapidly under stress. In this review, we systematically summarize the research on the morphology, physiology, genome, gene expression and regulation, and protein and metabolite profile of salt cress under salt stress. We emphasize the latest advances in research on the genome adaptive evolution encountering saline environments, and epigenetic regulation, and discuss the mechanisms underlying salt tolerance in salt cress. Finally, we discuss the existing questions and opportunities for future research in halophytic *Eutrema*. Together, the review fosters a better understanding of the mechanism of plant salt tolerance and provides a reference for the research and utilization of *Eutrema* as a model extremophile in the future. Furthermore, the prospects for salt cress applied to explore the mechanism of salt tolerance provide a theoretical basis to develop new strategies for agricultural biotechnology.

Keywords: *Arabidopsis* relative model system, salt cress, salt stress tolerance, saline adaptation, antioxidant system, osmo-adaptation, ion homeostasis, gene expression

INTRODUCTION

Soil salinity is one of the major environmental stress factors that restrict the functioning of plants in natural ecosystems, and greatly reduce crop yields which subsequently leads to a threat to food security (Zhu, 2002; Vera-Estrella et al., 2005; Zhao et al., 2020). As we know, the enormous value will come from a better understanding of the mechanisms through which plant tolerance of abiotic stresses is achieved (Zhao et al., 2020). Most studies on salt stress response mechanisms of plants have been conducted using the glycophyte model *Arabidopsis thaliana* and some important clues were gained (Wu et al., 2012). However, *Arabidopsis* has a relatively low capacity to survive salt stress, thus it may lack the protective mechanisms required for growing in harsh environments (Lee et al., 2013). Therefore, searching for the novel stress tolerance determinants including novel genes

and novel stress tolerance mechanisms has led to increasing interest in the plants native to growing in extreme environments called “extremophytes” (John and Spangenberg, 2005; Amtmann, 2009; Gechev et al., 2012; Bressan et al., 2013; Oh et al., 2013; Cheeseman, 2015; Flowers et al., 2015; Eshel et al., 2017).

Halophytes can naturally thrive under high salinity conditions, such as in marshlands, swamps, and intertidal estuarine areas, and their tolerance to salt stress may occur through various evolutionary and molecular mechanisms, which can provide crucial insights into the underlying mechanisms that endow tolerance of plants to salt stress (Flowers et al., 1986; Bressan et al., 2001; Zhu, 2002; Amtmann, 2009; Rajalakshmi and Parida, 2012; Wu et al., 2012). The crucifer *Eutrema salsugineum* (salt cress) formerly classified as *Thellungiella salsuginea* or *Thellungiella halophila* is a close relative of *Arabidopsis*. *Eutrema salsugineum* can naturally grow well in extreme environments and displays exceptionally high resistance to salt as well as cold, drought, and oxidative stresses, but its genetic make-up, morphology, and development are similar to the glycophyte *Arabidopsis* (Bressan et al., 2001; Inan et al., 2004; Taji et al., 2004; Volkov et al., 2004; Wang et al., 2004; Gong et al., 2005; Wong et al., 2006; Amtmann, 2009; Orsini et al., 2010; Wu et al., 2012; Koch and German, 2013; **Figure 1**). Furthermore, salt cress is an ideal model organism due to its short life cycle, self-fertility, and being genetically transformable, and has a relatively small genome (241 Mb) with approximately twice the genome size of *Arabidopsis* (Inan et al., 2004; Wu et al., 2012). Importantly, salt cress has the availability of extensive ecotypes exhibiting a range of stress responses to adapt to different environmental stresses (Inan et al., 2004; Wong et al., 2005; Li et al., 2006; Amtmann, 2009; Hou and Bartels, 2015). These advantages characteristics make the halophytic *Eutrema* species amenable to the functional genomics approaches designed to identify the novel genes and new molecular mechanisms involved in stress resistance (Lugan et al., 2010).

Over the past few years, this salt cress species has come to the fore in the research into abiotic stress resistance and is considered a plant model of stress resistance well comparable with the *Arabidopsis* genome (Bressan et al., 2001; Inan et al., 2004; Amtmann et al., 2005; Amtmann, 2009). Firstly, the genome of salt cress provides a resource for identifying naturally occurring genetic alterations which could contribute to the adaptation of the halophyte to soil salinity, and that might be bioengineered in related crop species (Yang et al., 2013). The characterization of transcripts with unknown functions will possibly reveal the novel defense mechanisms in this halophyte (Rajalakshmi and Parida, 2012). Secondly, comparative analyses of the genome structures, protein-coding genes, microRNAs, stress-related pathways, and proteomic and metabolic profiles between *Eutrema* and *Arabidopsis* suggest that adaptation of the halophyte to environmental salt stress may occur *via* a global network adjustment of multiple regulatory mechanisms (Yang et al., 2013). Furthermore, the research of *Eutrema* is supported by growing technical resources including the physiological and molecular protocols, ecotype collections, and comparison of genome, transcriptome, and metabolome. Hence, recent scientific advances make exploration of the mechanisms

of environmental adaptation in plants more feasible which could result in a larger implication and gains in genetic improvement of crops.

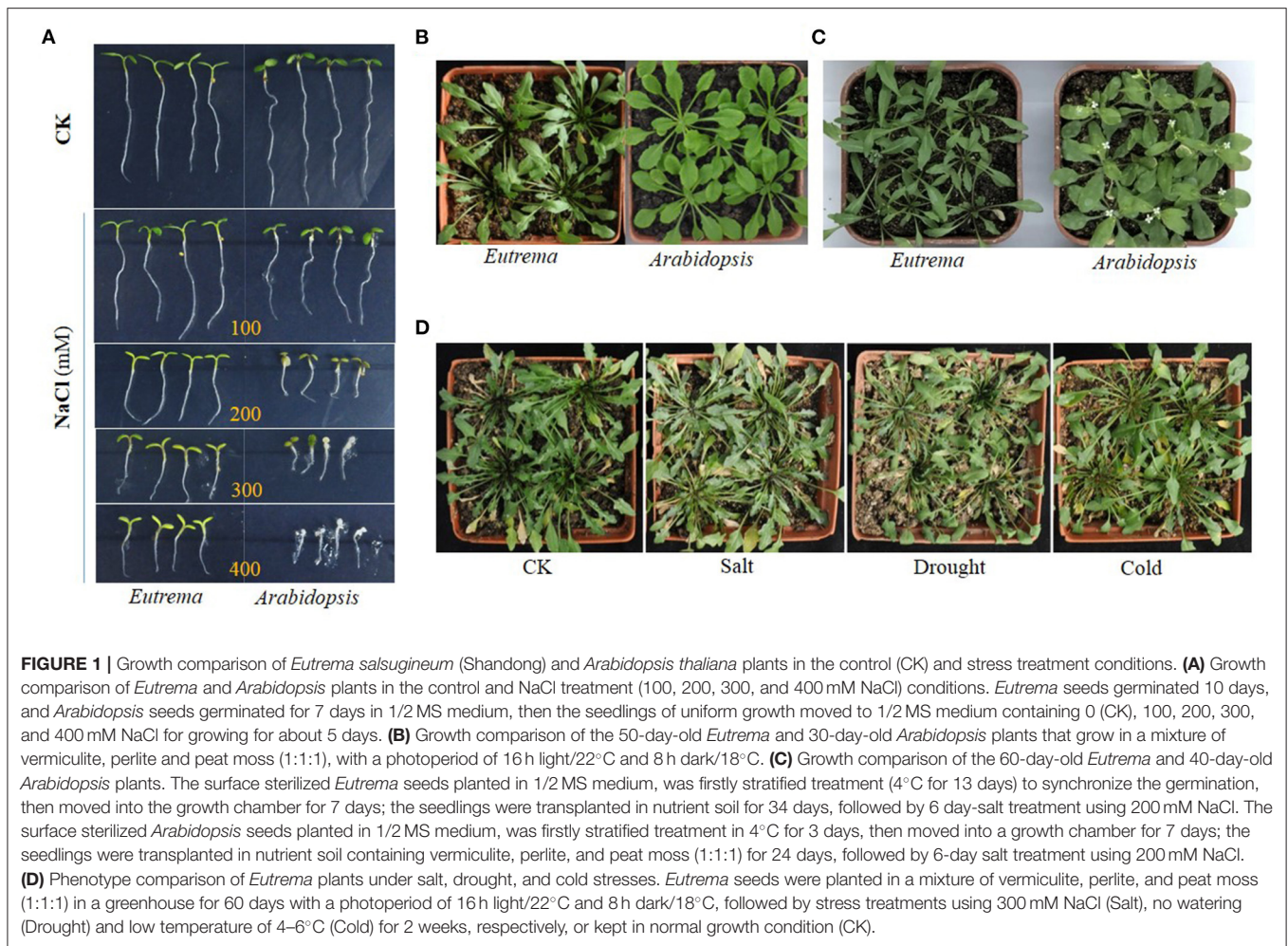
In this review, we systematically summarize the recent research advances in understanding morphology, physiology and biochemistry, expression and expression regulation of genes, and evaluating its usefulness as a model for research into plant stress tolerance, as well as emphasizing the possible molecular mechanisms underlying adaptation of the halophytic *Eutrema* to saline environments. This can provide a comprehensive insight to understand the mechanisms conferring a high level of salt stress tolerance in the halophyte.

GENOME EVOLUTION ADAPTED TO SALINE ENVIRONMENT

High levels of stress tolerance in plants are the result of evolutionary adaptation, thus the genotypic selection of plants for adaptation to various environments plays an important role in agriculture and ecology (Boyer, 1982; Janz et al., 2010). Due to the natural adaptations of *E. salsugineum* to various harsh climates and soil conditions, it is considered as an important model for deciphering the mechanisms of salt and other abiotic stress tolerance of plants (Gong et al., 2005; Griffith et al., 2007; Lamdan et al., 2012; Wang et al., 2021).

Sixteen accessions of halophytic *E. salsugineum* species including previously called *T. salsuginea*, *T. halophila*, and *T. botschantzevii* were used to investigate their natural variation in salinity tolerance and found that these accessions could survive up to 700 mM NaCl in hydroponic culture, however, their relative salt tolerance has a considerable variation (Lee et al., 2016). Using 90 resequencing whole genomes data from 90 individuals in 21 natural populations, the analysis of *Eutrema* species across central Asia to North America revealed that the selection signals for genes were related to the adaptation of salt and other abiotic tolerance, at the species level (Wang et al., 2021).

Gene duplication, a major source of genetic diversity that drives the adaptive evolution of plants (Ohno, 1970; Kondrashov, 2012; Monihan et al., 2019). Wu et al. (2012) demonstrated that, although the gene spaces display an extensive colinearity between *E. salsugineum* and *A. thaliana*, the salt stress-related gene families expanded in *E. salsugineum* genome (Wu et al., 2012). For instance, 21 transcription factor (TF) families expanded in the *E. salsugineum* genome, compared to *A. thaliana*. Among these, the *AtNFXL1* gene encoding NF-X1 type zinc finger proteins was suggested to play a regulatory role in protecting photosynthesis, and be required for the growth of *Arabidopsis* plants under salt stress. The members of *NF-X1*, *HSF*, *Trihelix*, and *GRAS* TF families involved in abiotic stress response, were also expanded in numbers in salt cress (**Table 1**). The expansions of these gene family members are suggested to be associated with the adaptation of salt cress to harsh environments, due to the fact that their orthologous gene members in *Arabidopsis* and other plants have been documented to be associated with stress resistance (Lisso et al., 2006; Ogawa et al., 2007; Xie et al., 2009; Wu et al., 2012). These expanded family members possibly endow



E. salsugineum with more flexibility in response to salinity stress (Wu et al., 2012). Additionally, the RAV gene family reported to respond to high salt and cold stresses, had been expanded from six members in *A. thaliana* to nine in *E. salsugineum* genome (Fowler et al., 2005; Sohn et al., 2006; Wu et al., 2012; Table 1). However, the *AtRAV1* gene overexpressed plants showed strong growth retardation. And comparing to the wild type, a more inhibition of seed germination in salt conditions, and the transgenic plants exhibited higher transpirational water loss in drought conditions (Fu et al., 2014). Given the expressions of RAVs in *Arabidopsis* were reduced by salt and dryness, it is inferred that adaption of reduced *AtRAV1* expression is involved in the adaption of salinity environment in *Arabidopsis* (Fu et al., 2014). Moreover, the transcripts of *EsRAVs* in the group A (A-*EsRAVs*) from *E. salsugineum* seedling exhibited a moderate decline gradually by salt treatment, and stronger inhibition of seed germination and seedling root elongation occurred in the 35S:A-*EsRAV* transgenic plants in presence of NaCl, suggesting the roles of 35S:A-*EsRAVs* in negatively controlling plant growth (Yang et al., 2016; Table 1).

LEA proteins can be involved in the “molecular shield function” to protect enzymes from induced aggregation when

plants encounter stress in their environments (Goyal et al., 2005). In *E. salsugineum*, LEA genes were found to be higher copy numbers than their orthologous genes in the *Arabidopsis* genome, which is in agreement with a higher frequency of gene duplications observed in the *E. salsugineum* and *E. parvula* genomes (Table 1). This indicated that, since the last whole genome duplication event or after tandem duplications leading to neofunctionalization, the two *Eutrema* species (*E. salsugineum* and *E. parvula*) retained a higher fraction of duplicated genes, which is regarded as an important adaptive strategy for salt stress to survive in habitats with more extreme environmental conditions than *Arabidopsis* (Blanc and Wolfe, 2004a,b; Maere et al., 2005; Dassanayake et al., 2011; Wu et al., 2012; Lee et al., 2013; Oh et al., 2013).

The salt overly sensitive (SOS) pathway functions in preventing the toxic accumulation of sodium in the cytosol when *A. thaliana* are grown in salt-affected soils. In this pathway, *Arabidopsis* AtCBL10 calcium sensor interacts with the AtSOS2 kinase to activate the AtSOS1 plasma membrane sodium/proton exchanger, which initiates the transport of sodium out of the cell (Shi et al., 2000; Qiu et al., 2002, 2003; Quintero et al., 2002). In *E. salsugineum*, the *CBL10* gene had been duplicated into

TABLE 1 | The expanded genes or gene family members involved in response to salt stress in *Eutrema* compared with *Arabidopsis*.

Gene family	Number of genes or gene family members		<i>Eutrema</i>		<i>Arabidopsis</i>		Function of genes	References
	<i>Eutrema</i>	<i>Arabidopsis</i>	Gene name	Gene ID	Gene name	Gene ID		
CBL10	2	1	EsCBL10a	Thhalv10026019m	AtCBL10	AT4G33000	Calcium-mediated signaling capacity; Salt tolerance	Monihan et al., 2019
HKT1	3	1	EsCBL10b	Thhalv10028908m	AtHKT1	AT4G10310	Na ⁺ /K ⁺ co-transporter	Ali et al., 2012 Ali et al., 2013
			EsHKT1;1	Thhalv10028767m				
			EsHKT1;2	Thhalv10028594m				
LEA	42	40	EsHKT1;3	Thhalv10028595m	AtEM1	AT3G51810	Salt and drought tolerance	Xiang and Man, 2018
			EsEM1	Thhalv10010923m				
			EsLEA1	Thhalv10014897m				
NFXL	3	2	EsNFXL1	Thhalv10012642m	AtNFXL1	AT1G10170	Salt tolerance	Lisso et al., 2006
HSF	28	24	EsHSF2	Thhalv10002333m	AtHSF2	AT2G26150	Salt and osmotic stress tolerance; callus growth	Ogawa et al., 2007
RAV	9	6	EsRAV1	Thhalv0010019m	AtRAV1/EDF4	AT1G13260	<i>Arabidopsis</i> RAVs are negative regulators of growth and salt stress; <i>Eutrema A</i> -EsRAV inhibits seed germination and seedling root elongation; Pepper CaRAV1 is involved in bacterial infection resistance and osmotic tolerance	Sohn et al., 2006
			EsRAV2	Thhalv0019566m	AtRAV2/EDF2	AT1G68840		
			EsRAV3	Thhalv0004508m	AtRAV3/EDF3	AT3G25730		
			EsRAV4	Thhalv0007983m	AtRAV4/EDF1	AT1G25560		
			EsRAV5	Thhalv0012152m	AtRAV5	AT1G51120		
			EsRAV6	Thhalv0012161m	AtRAV6	AT1G50680		
			EsRAV7	Thhalv0012356m				
			EsRAV8	Thhalv0012377m				
			EsRAV9	No found				
Trihelix	31	29	EsGT2	Thhalv10018336m	AtGT2	AT1G76890	Salt, freezings and drought tolerance	Xie et al., 2009; Fang et al., 2010
GRAS	40	33	EsCBF1	Thhalv10027405m	AtCBF1	AT4G25490	Freezing tolerance; growth-repressing	Achard et al., 2008

EsCBL10a and *EsCBL10b* (Table 1), and the down-regulation of either of the two *EsCBL10* genes decreased the growth of plants in the presence of salt, indicating that both genes function in the response of plants to salinity stress (Monihan et al., 2019). Further, *EsCBL10b* was demonstrated to have an enhanced ability to activate the SOS pathway, while the role of *EsCBL10a* is in an alternative pathway with different functions from *AtCBL10* or *EsCBL10b*. Taken together, the duplication of *Eutrema EsCBL10* obviously increased calcium-mediated signaling capacity and conferred an enhanced salt tolerance when compared with salt-sensitive *Arabidopsis* (Monihan et al., 2019).

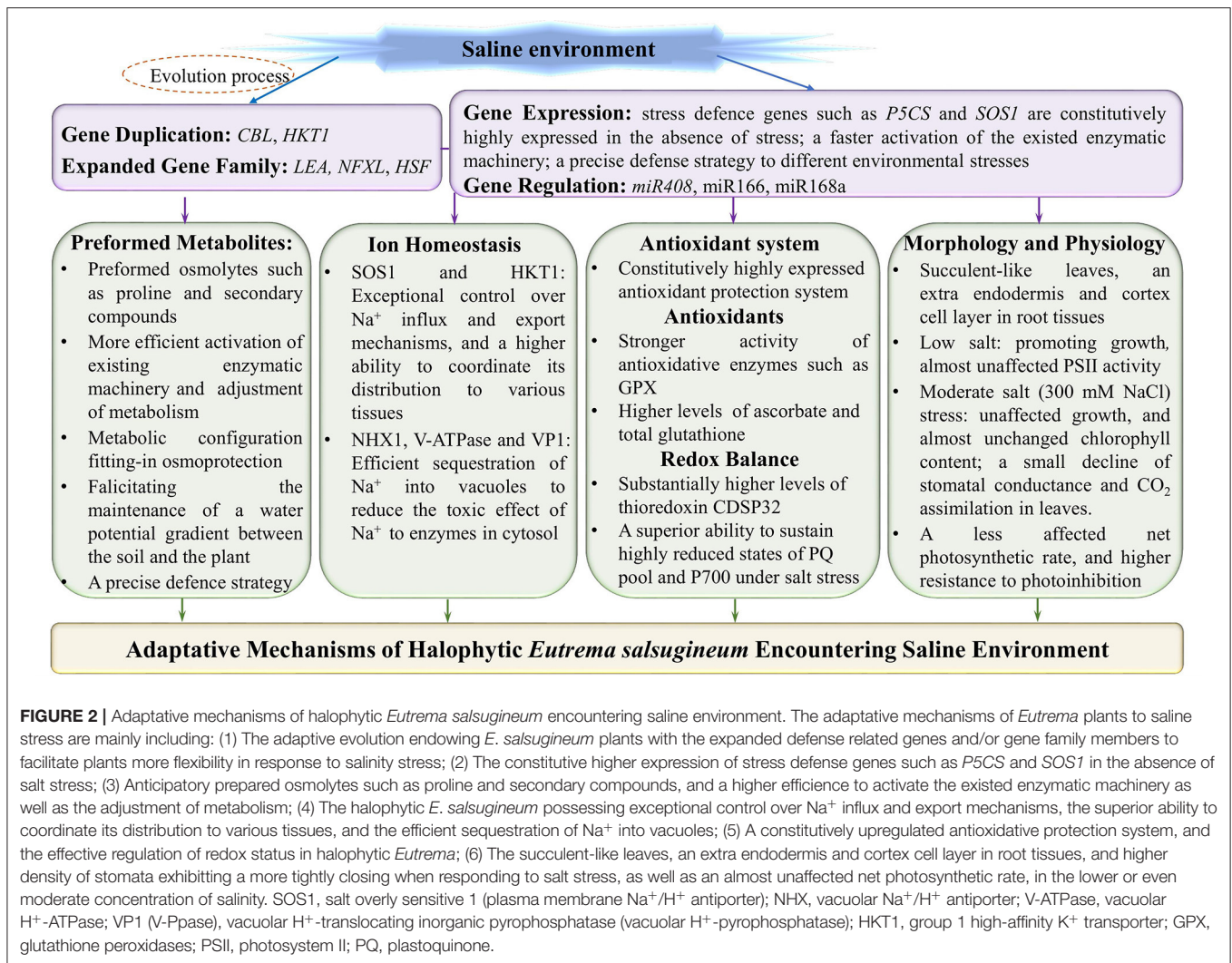
Altogether, the maintenance of duplicated gene pairs in the genome of plants is indicative of an adaptive benefit conferred by the paralogous genes (Hahn, 2009; Kondrashov, 2012; Monihan et al., 2019). Undoubtedly, genetic variation and natural selection are fostering stress-related genes and gene interaction networks

in the whole genome, which will prompt the local adaptation and differentiation of *Eutrema* plants to extreme salt stress in a non-random way.

MORPHOLOGICAL CHARACTERISTICS AND PHYSIOLOGICAL RESPONSES

The exposure of plants to high salt stress leads to the damage of cell structure, and disorder of many physiological functions (Sairam and Tyagi, 2004). As a consequence, this will eventually result in photosynthesis inhibition and metabolic impairment to further impaired growth and fertility, and early senescence of plants (Larcher, 2003).

Salt cress can natively grow in extreme salinity environments, and reproduce after exposure to 500 mM NaCl (Inan et al.,



2004). Under treatments of 100 and 200 mM NaCl, an increased growth was observed in *E. salsugineum* accompanied by almost unchanged fresh and dry weights (Figures 1, 2), whereas a significant growth reduction in *Arabidopsis*. In a moderate salinity of 300 mM NaCl, *E. salsugineum* plants grew rapidly accompanied by almost unaffected chlorophyll content, a small decline of stomatal conductance and CO_2 assimilation in leaves, indicating the photosynthetic apparatus was little influenced by this salt concentration (Sui and Han, 2014; Figure 2). However, the growth of *Arabidopsis* was remarkably decreased by the 100 and 200 mM NaCl treatments, which was associated with strong suppression in both leaf initiation and leaf expansion (M'rah et al., 2007; Sui and Han, 2014). Compared to *Arabidopsis*, *E. salsugineum* leaves are more succulent-like, have a second layer of palisade mesophyll cells, and frequently fall off during extreme salt stress, while root tissues develop an extra endodermis and cortex cell layer (Inan et al., 2004; Figure 2). Meanwhile, the stomata of *E. salsugineum* leaf surface are present at a higher density and less open than that in *Arabidopsis*, and exhibit a more tightly closing when responding to salt stress (Inan et al.,

2004; Figure 2). Therefore, when compared with *Arabidopsis*, *E. salsugineum* can maintain a high water uptake and ion transport (Volkov et al., 2004; M'rah et al., 2006, 2007; Amtmann, 2009).

Photosynthesis is a primary process that is influenced by salinity stress, that mainly attributed to a decreased CO_2 availability caused by diffusion limitations via the stomata and the mesophyll, or a generated secondary oxidative stress, which will seriously affect leaf photosynthetic machinery (Lawlor and Cornic, 2002; Flexas et al., 2004, 2007; Munns et al., 2006; Chaves et al., 2009). In leaves of *Arabidopsis*, the photosynthetic activity was suppressed after 15 days of salt treatment with higher than 50 mM NaCl, which could be explained by the stomata closure and dramatic shrinkage of the RubisCO pool. By contrast, *E. salsugineum* leaves still remained at 50% of the stomatal conductance of the control, and a negligible influence of RubisCO quantity, when exposed to the same salt-treated conditions (M'rah et al., 2007).

Chlorophyll (Chl) and PSII play a key role in the response of leaf photosynthesis to environmental stresses (Baker, 1991). When plants are subjected to 100 or 200 mM NaCl treatments,

there are no remarkable changes in the chlorophyll content and Chl a/b ratio, as well as almost do not affect the PSII activity in *E. salsugineum* leaf (Figure 2), whereas a progressive decline exists in *Arabidopsis* leaf, which suggests this halophyte has effective mechanisms to protect photosystem against salinity (Sui and Han, 2014). In addition, *E. salsugineum* was found to have an increased chlorophyll a/b ratio accompanied by a higher effective photochemical quantum yield (Y_{II}) value and lower non-photochemical quenching (NPQ) value, and a more active photosystem I (PSI) (Wiczarz et al., 2015).

All in all, these studies clearly demonstrate that, compared to *A. thaliana*, the *E. salsugineum* displays a relatively stable stomatal conductance and CO₂ assimilation, higher chlorophyll content, Chl a/b ratio, and higher resistance to photoinhibition, as well as almost unaffected net photosynthetic rate and Fv/Fm (maximal photochemical efficiency of PSII; Sui and Han, 2014; Wiczarz et al., 2015; Figure 2), thus protecting photosystem activities to maintain normal growth of salt cress, in the lower or even moderate concentration of salinity.

MAINTAIN ION HOMEOSTASIS UNDER SALINE ENVIRONMENT

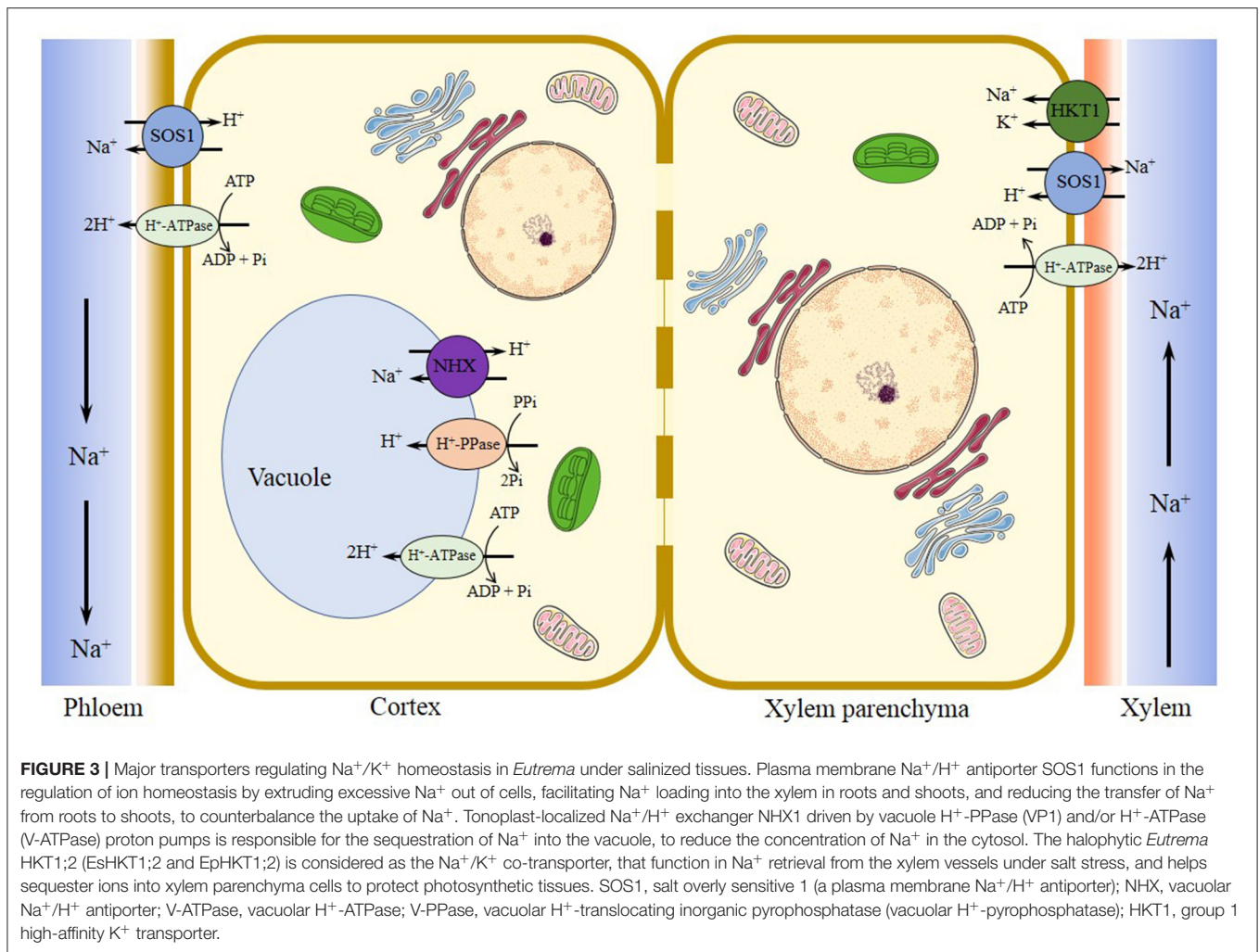
To survive salt stress, plants must maintain a balance between sodium (Na⁺) and potassium (K⁺) ions, thus the homeostasis of Na⁺ and K⁺ is considered the most important mechanism to reducing NaCl stress in higher plants (Zhao et al., 2020). Halophytes can activate the protective mechanisms to prevent high Na⁺ accumulation in the cytosol and to maintain photosynthesis (Niewiadomska and Wiczarz, 2015; Bose et al., 2017; Kazachkova et al., 2018; Grigore, 2019).

The plasma membrane (PM) Na⁺/H⁺ antiporter (SOS1, Salt Oerly Sensitive 1) is involved in the transport of sodium ions across the plasma membrane and retrieves Na⁺ ions from the xylem sap, which is essential for Na⁺ and K⁺ homeostasis (Qiu et al., 2002; Shi et al., 2002). Taji et al. (2004) found, in *E. salsugineum*, a constitutively high expressed *EsSOS1* RNA in the absence of stress, and an increased *EsSOS1* expression observed in the PM of salt-treated roots and leaves (Taji et al., 2004; Vera-Estrella et al., 2005; Figures 2, 3). Likewise, Kant et al. (2006) demonstrated that *E. salsugineum* SOS1 transcript was strongly induced by salt in the shoots, and a constitutively high expression existed in roots in unstressed salt cress, which was consistent with a lower salt-induced Na⁺ concentration in xylem sap of *E. salsugineum* than that in *Arabidopsis* (Kant et al., 2006). Further, ectopic expression of *EsSOS1* from *E. salsugineum* suppressed the salt-sensitive phenotype of *Saccharomyces cerevisiae* strains lacking Na⁺ efflux transporters (SOS1), and enhanced the salt tolerance of wild-type *Arabidopsis* (Oh et al., 2009). *E. salsugineum* SOS1-RNA interference (RNAi) significantly induced reduction of *SOS1* gene, which led to faster leaf senescence and severe shoot water loss, accompanied by fragmentation of vacuoles, inhibition of endocytosis, and apoplastic Na⁺ influx into the stele and shoot (Oh et al., 2009). Another study also demonstrated that *EsSOS1* conferred greater salt tolerance than *AtSOS1* when expressed

in a salt-sensitive strain of *S. cerevisiae*, and suggested that the salt tolerance of *E. salsugineum* may at least in part be derived from SOS1-mediated Na⁺ extrusion (Jarvis et al., 2014; Figures 2, 3). Therefore, it is suggested that *EsSOS1* activity limits Na⁺ accumulation and affects the distribution of Na⁺, and acts as an important tolerance determinant in shaping the halophytic character of the *E. salsugineum* species (Oh et al., 2009; Figures 2, 3).

The compartmentalization of Na⁺ into vacuole can alleviate the toxicity of Na⁺ and Cl⁻ in the cytosol and enhance vacuolar osmoregulatory capacity, which could confer salt tolerance of plants (Gaxiola et al., 2001). The vacuolar ATPase (V-ATPase) and pyrophosphatase-dependent H⁺-pyrophosphatase (VP1) as the proton pumps function in establishing and maintaining the electrochemical proton gradient across the tonoplast (Kirsch et al., 1996; Maeshima, 2000). The Na⁺ sequestration process is mediated by the vacuolar Na⁺/H⁺ antiporter (NHX) that is driven by V-ATPase and VP1 (Apse et al., 2003; Bhaskaran and Savithramma, 2011). When *E. salsugineum* plants were treated with NaCl, a salt-induced increase in the *EsNHX1* protein expression from PM fractions of roots, and a similar increased expression of the vacuolar H⁺-translocating ATPase E subunits (VHA-E) in leaf tissues were detected (Vera-Estrella et al., 2005; Kant et al., 2006). Taken together, salt stress enhanced H⁺-transport and hydrolytic activity of *E. salsugineum* tonoplast and plasma membrane H⁺-ATPases, and also greatly stimulated the activity of tonoplast Na⁺/H⁺ exchange in *E. salsugineum* leaves and roots (Vera-Estrella et al., 2005; Figures 2, 3).

High-affinity potassium transporters (HKTs) play a key role in reducing Na⁺ toxicity through K⁺ uptake (Rus et al., 2004). The study found that *EsHKT1;2* mRNA is dramatically induced, whereas *AtHKT1* is downregulated upon salt stress (Ali et al., 2013; Figures 2, 3). And *EsHKT1;2*-RNAi lines display a severe potassium deficiency, and are sensitive to high Na⁺ (Ali et al., 2013). *E. salsugineum* *EsHKT1;2* ectopically expressed in yeast mutants lacking Na⁺ or K⁺ transporters showed a strong K⁺ transporter activity, and selectivity for K⁺ over Na⁺, suggesting *EsHKT1;2* act as maintenance of K⁺ uptake under salt stress that supporting the halophytic lifestyle (Ali et al., 2012, 2013; Figures 2, 3). Further, the plants overexpressing *Eutrema* *EsHKT1;2* in *Arabidopsis* *athkt1* mutant was proved to be less sensitive to Na⁺ as well as less K⁺ deficiency, compared to the plants overexpressing *AtHKT1* gene in *athkt1* (Ali et al., 2012). Importantly, the K⁺ specificity of *EsHKT1;2* protein is based on amino acid differences in the pore of the transporter protein relative to *AtHKT1* (Ali et al., 2013). In addition, the transcript level of *EpHKT1;2* from halophytic *E. parvula* (formerly known as *Thellungiella parvula*) increased rapidly in response to high salinity, and the yeast cells expressing *EpHKT1;2* could tolerate high concentrations of NaCl (Ali et al., 2018). Moreover, *Arabidopsis* plants (*Col-gl*) overexpressing *EpHKT1;2* gene accumulated less Na⁺ and more K⁺, and displayed significantly higher tolerance to salt stress when compared to those overexpressing *EpHKT1;1* or *AtHKT1*. Thus, *EpHKT1;2* is suggested to mediate tolerance to Na⁺ ion toxicity in *E. parvula*, and is a major contributor to its halophytic nature (Ali et al., 2018; Figure 3).



Besides, the major site of Na^+ accumulation occurs in old leaves, followed by young leaves and taproots, and the least accumulation occurred in the lateral roots of *E. salsugineum* plants under salt stress (Vera-Estrella et al., 2005). Notably, although most genes in *E. salsugineum* exhibit ~90% identity with *Arabidopsis* genes, the size of *E. salsugineum* transport genes population is ~1.5 times that compared of the corresponding *Arabidopsis* genes (Taji et al., 2008). This suggests that the transporter encoding genes could be remarkably different from their co-orthologs in *Arabidopsis*, and regulate a unique ion transportation system in this halophyte (Taji et al., 2008).

Taken together, these results revealed *E. salsugineum* as a halophyte is able to distribute and store Na^+ by very strict control of ion movement across both the tonoplast (TP) and plasma membrane (PM) (Vera-Estrella et al., 2005; Figures 2, 3). Correspondingly, *Eutrema* accumulated lower levels of Na^+ and Cl^- than *Arabidopsis*, and its rosette leaves displayed more efficient protective mechanisms against Na^+ metabolic toxicity when plants were exposed to higher salinity stress (M'rah et al., 2007). These works of key Na^+ -transport mechanisms provide some detailed mechanisms analysis underlying salinity tolerance

in this halophytic *E. salsugineum*, an *Arabidopsis* relative model system (Vera-Estrella et al., 2005; Figures 2, 3).

ANTIOXIDANTS, REDOX BALANCE, AND SALT STRESS TOLERANCE

A common reaction of plants to salt-triggered osmotic stress and ionic imbalance is to increase the levels of reactive oxygen species (ROS; Bose et al., 2014; Kumar et al., 2017). The ROS species of superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\bullet), and singlet oxygen ($^1\text{O}_2$) are generated constantly as an unavoidable consequence of aerobic metabolism (Møller et al., 2007). The primarily negative effects of ROS molecules on the cells include lipid peroxidation, protein denaturation, and DNA damage (Møller et al., 2007). On the other hand, ROS as universal signaling metabolites has been perceived to regulate plant growth and development, as well as defense against biotic and abiotic stresses (Foyer et al., 2017; Czarnocka and Karpiński, 2018; Fichman and Mittler, 2020). Usually, low levels of H_2O_2 could modulate many biological and physiological roles, whereas a high

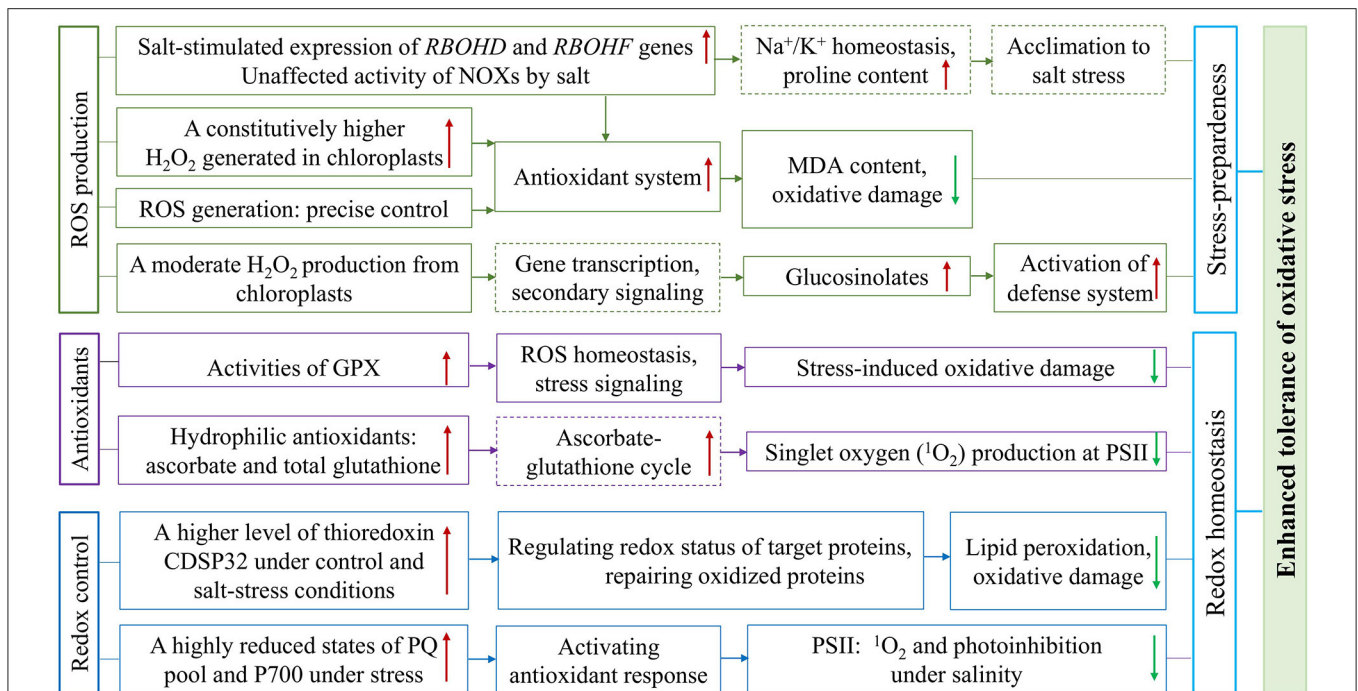


FIGURE 4 | Overview of the constitutively higher expressed antioxidative protection system and effective regulation of redox status in halophytic *Eutrema*. The plasma membrane ROS produced through NADPH oxidases (NOXs), the important components of signal transduction, encoded by *RBOHD* and *RBOHF* genes, is associated with the regulation of Na^+/K^+ homeostasis and increased accumulation of proline involved in salt tolerance. The enhanced antioxidant tolerance of *Eutrema* is associated with: (1) A constitutively enhanced H_2O_2 generated to keep the antioxidant system up-regulated to preadapted plant to salinity stress, as well as a chloroplastic production of H_2O_2 inducing the accumulation of glucosinolates to activate the stress defense system, (2) Antioxidative enzymes such as GPX, and non-enzymatic antioxidants such as ascorbate and glutathione facilitating ROS homeostasis and alleviating damage of singlet oxygen ($^1\text{O}_2$) to PSII under salt stress, (3) A higher expression level of thioredoxin CDSP32 regulating redox status of target proteins and highly reduced states of PQ pool and P700 facilitating redox homeostasis and lower photoinhibition under salt stress. NOXs, NADPH oxidases; GPX, glutathione peroxidases; PSII, photosystem II; PQ, plastoquinone.

concentration of H_2O_2 will cause damage to cellular structures resulting in severe injury to plants (Ozyigit et al., 2016). Thereby, the salt tolerance of halophytes seems to be associated with their ability to control the redox balance (Ozgun et al., 2013; Bose et al., 2014; Surówka et al., 2019; Pilarska et al., 2021; **Figures 2, 4**).

The H_2O_2 checked in *A. thaliana* and *E. salsugineum* plants treated with H_2O and the salt solution containing 150 and 300 mM NaCl revealed that, in the chloroplasts of salt cress mesophyll cells, electron-dense perhydroxide cerium precipitates were detectable in control, and more pronounced visible dark spots found after salt stress (Pilarska et al., 2016). In contrast, the chloroplasts in *A. thaliana* leaves displayed no perhydroxide deposits in the control condition, while salt stress associated with H_2O_2 accumulation was mainly visible at the edges of grana in thylakoids (Pilarska et al., 2016). This result suggested that a constitutively enhanced H_2O_2 generated in chloroplasts may be a crucial component of stress-preparedness of this halophyte (Pilarska et al., 2016; **Figures 2, 4**). Similarly, increased content of H_2O_2 in leaves was documented in *E. salsugineum* than in *A. thaliana*. Meantime, the salinity-induced thylakoid swelling was detected only in *Arabidopsis* chloroplasts, while no signs of such destruction occurred in *E. salsugineum* chloroplasts (Pilarska et al., 2016). However, a very low level of MDA was

detected, in spite of the higher ROS availability in *E. salsugineum* leaves, indicating a low extent of oxidative damage to membrane lipids (Pilarska et al., 2016). Thus, it is suggested that enhanced leakage of H_2O_2 from plastids keeps the antioxidant system up-regulated to make plants preadapted to the salinity stress (Pilarska et al., 2016; **Figures 2, 4**). The effect of H_2O_2 signaling not only depends on its type, but also on the site of its origins such as chloroplasts, mitochondria, and peroxisomes in the cell (Sewelam et al., 2014; Pilarska et al., 2021). The comparison of *A. thaliana* and *E. salsugineum* demonstrated that the latter was capable of enhancing the production of H_2O_2 under control and stress conditions (Wiczarz et al., 2015; Pilarska et al., 2016, 2021; **Figures 2, 4**). These studies indicate a different regulation of ROS formation in the two relative species, and the precise control of ROS generation may contribute to plant stress responses (Pilarska et al., 2021).

In the plasma membrane, ROS are synthesized by NADPH oxidases (NOXs), termed respiratory burst oxidase homologs (RBOHs; Kaur et al., 2018), and NOXs is considered an important component of signal transduction under salt stress (Pilarska et al., 2021). *Arabidopsis* NOX genes, *RBOHD* and *RBOHF*, are the main genes encoding NADPH oxidases associated with acclimation to salinity (Ma et al., 2012; Pilarska et al., 2021).

After *E. salsugineum* was exposed to 5 days of salinity, a salt-stimulated expression in *RBOHD* and *RBOHF* genes in leaf tissues was revealed (Figures 2, 4), while their orthologous genes expression in *Arabidopsis* decreased (Pilarska et al., 2021). The ROS produced by *RBOHD* and *RBOHF* is associated with the regulation of Na^+/K^+ homeostasis under salinity (Chung et al., 2008; Ma et al., 2012), as well as the increased the accumulation of proline involved in salt tolerance (Ben Rejeb et al., 2015; Pilarska et al., 2021; Figure 4). Moreover, the different salt-induced expression patterns of *RBOHD* and *RBOHF* genes between the *E. salsugineum* and *A. thaliana* point to, in leaves of the halophytic *Eutrema*, the two *NOX* genes (*RBOHD* and *RBOHF*) be involved in the late acclimation response to ionic and osmotic stress (Pilarska et al., 2021; Figure 4). Further, the total activity of *NOXs* in *E. salsugineum* was demonstrated to be unaffected by severe salt stress (Figure 4), whereas a declined activity was seen in *A. thaliana* (Pilarska et al., 2021). And the prolonged mild salinity did not lead to obvious changes in the activity of *NOXs* in leaves, indicating that the acclimation to salinity in the halophytic *E. salsugineum* was in connection with the maintenance of the basal activity of *NOXs* (Pilarska et al., 2021; Figure 4). This opinion is in agreement with the study from Srivastava et al. (2015), that the total *NOXs* activity in the halophyte *Sesuvium portulacastrum* was unaffected, whereas a significantly decreased *NOXs* activity in the glycophyte *Brassica juncea* (Srivastava et al., 2015).

Antioxidative enzymes play an important role in scavenging toxic radicals in the different organelles of plants such as chloroplasts, cytosol, mitochondria, and peroxisomes, particularly in environmental stress such as salinity (Rajalakshmi and Parida, 2012). In *E. salsugineum*, the enzymatic antioxidant systems could contribute to its extremely high level of tolerance to salt, drought, cold, and oxidative stresses (Gao et al., 2014). Glutathione peroxidases (*GPXs*) catalyze the reduction of H_2O_2 and organic hydroperoxides into the water and correspondingly alcohols, using reduced glutathione (*GSH*). Plant *GPXs* play essential roles that are not only involved in ROS homeostasis, stress signaling, and protecting plants from stress-induced oxidative damage to membrane and protein, but also in plant growth and development (Gao et al., 2014; Ozyigit et al., 2016). The gene and protein expression profiles of eight *GPXs* identified in *E. salsugineum* displayed that *GPX5*, *GPX7*, and *GPX8* genes were upregulated in both leaves and roots upon salt stress (Figures 2, 4), while *GPX1*, *GPX2*, and *GPX3* were only upregulated in roots under salt stress; *GPX1*, *GPX3*, *GPX4*, *GPX7* genes in leaves and almost all *GPX* genes in roots were upregulated under osmotic stress (Gao et al., 2014). An enhanced *GPX* activity (Figures 2, 4), but decreased activity of ascorbate peroxidase (*APX*) were revealed in *Eutrema* plants (Pilarska et al., 2016). These studies documented that, the different members of the *GPX* gene family were modulated in specific environmental stresses and/or in different tissues, indicating the important roles of *EsGPXs* in salt and osmotic stress response of *Eutrema* plants (Gao et al., 2014).

Thioredoxin is a small oxidoreductase functioning as a hydrogen donor of target proteins and is considered a specific marker for assessment of oxidative stress (Holmgren, 1985;

M'rah et al., 2007). Thioredoxin CDSP32 participating in the repair of oxidized proteins during environmental constraints is regarded as a critical component of the defense system against lipid peroxidation and oxidative damage (Rey et al., 2005; Dos Santos and Rey, 2006; M'rah et al., 2007). It was reported that potato (*Solanum tuberosum*) CDSP32 exhibited a substantial accumulation in chloroplast stroma and preserved chloroplastic structures against oxidative injury under drought and oxidative stresses (Broin et al., 2000). In *E. salsugineum*, thioredoxin CDSP32 was found to be higher in abundance than that in *A. thaliana* under control and salt-stress conditions (Figures 2, 4), and strongly diminished in salt-treated *Arabidopsis* plants (M'rah et al., 2007). Furthermore, the magnitude of electrolyte leakage and level of lipid peroxidation were modest in *E. salsugineum*, and very remarkable in *Arabidopsis* (M'rah et al., 2007).

In addition, a significantly increased hydrophilic antioxidants (ascorbate and total glutathione), but no changed lipophilic antioxidants existed in the halophytic *E. salsugineum* when exposed to 300 mM *NaCl* (Figures 2, 4). In contrast, the glycophyte *Arabidopsis* showed a decreased ascorbate content but increased lipophilic antioxidants (α -tocopherol, plastochromanol, and hydroxy-plastochromanol) due to 150 mM *NaCl* (Wiciarz et al., 2018). The redox states of plastoquinone (*PQ*) and *P700* were differently regulated by salinity between the halophyte *E. salsugineum* and glycophyte *A. thaliana*, which was documented by effectively avoiding harmful singlet oxygen ($^1\text{O}_2$) in the PSII of *Eutrema* (Figures 2, 4), whereas an increased oxidation extent was observed in *Arabidopsis* (Wiciarz et al., 2018). Moreover, *E. salsugineum* has a very efficient antioxidant protection in which glucosinolates may play a specific role (Pilarska et al., 2016; Figure 4).

To sum up, these researches proposed that the salt tolerance of halophytic *Eutrema* species is associated with constitutively expressed protection systems against oxidative stress such as *GPX* and thioredoxin CDSP32, as well as the ability to sustain a highly reduced state of *PQ* pool and *P700*, to alleviate the damage of PSII under salt stress (Figures 2, 4).

PREFORMED METABOLITES AND OSMOTIC ADAPTATION

Accumulation of osmoprotectants is one of the defense mechanisms for plants to cope with abiotic stresses (Rajalakshmi and Parida, 2012; Zhao et al., 2020). Several studies have shown that plant metabolism is strongly impacted by salt stress (Flowers and Colmer, 2008; Bartels and Dinakar, 2013; Lee et al., 2016). The metabolites positively correlating with salt stress are reported to participate in the underlying mechanisms including cell-wall remodeling (hydroxyproline), osmoprotectant and storage (proline and sucrose), and photorespiration (glycine and serine; Lukan et al., 2010). The *E. salsugineum* had much higher levels of most metabolites than *A. thaliana* under the salt or osmotic stress treatment (Figure 2), despite the fact that the

same metabolic pathways were regulated by salt stress in both species (Lugan et al., 2010). Importantly, significant differences observed in *E. salsugineum* and *A. thaliana* were also associated with the physicochemical properties of their metabolomes, such as water solubility and polarity (Lugan et al., 2010). Comprehensive quantification of organic and mineral solutes documented relative stability of the total solute content in the two species under various treatment conditions (Lugan et al., 2010). But *E. salsugineum* could cope with osmotic stress by tolerating dehydration via the metabolic configuration to lend itself to osmoprotectant rather than osmo-adjustment strategy (Lugan et al., 2010; **Figure 2**). Moreover, the leaves of *E. salsugineum* have a constitutively lower water content than in *A. thaliana*, and have the ability to lose more water without losing turgor, which could contribute to the maintenance of a water potential gradient between the soil and the plant (Lugan et al., 2010; **Figure 2**). Taken together, salt stress markedly decreased the osmotic potential of shoots, increased total solute levels to sustain a water potential gradient with the environment (**Figure 2**).

Furthermore, several studies supported the constitutively higher levels of proline, inositols, sugars, and organic acids in *E. salsugineum*, which were interpreted as metabolic stress-anticipation (Gong et al., 2005; Lee et al., 2016; **Figure 2**). The salt responses of most metabolites in *E. salsugineum* Yukon took place at 200 mM NaCl, and a few additional changes were observed between 200 and 500 mM (Lee et al., 2016).

The compatible osmolyte proline's accumulation is an important marker of salt tolerance in plants (Verbruggen and Hermans, 2008). Salt cress accumulated a markedly higher amount of proline than that of *Arabidopsis* under normal growth conditions, thus suggesting that the characteristic of extreme tolerance of salt cress to high salinity is due in part to the overaccumulation of proline under unstressed conditions (Taji et al., 2004; **Figure 2**). Likewise, Kant et al. (2006) demonstrated that *E. salsugineum* contained higher unstressed levels of proline than *Arabidopsis*, while under salt stress, *Eutrema* accumulated more proline mainly in shoots (Kant et al., 2006). The evaluation of salinity stress tolerance of eight different *Eutrema* accessions revealed that Tuva and Buriatia classified as salt susceptible ecotypes treated at 500 mM NaCl had seven-fold higher proline than that in Altai 1 and Altai 2 identified as salt tolerant ecotypes when treated with a similar concentration of NaCl (Gandour et al., 2019). Another study revealed that phenotypic and metabolic adaptive plasticity observed in *E. salsugineum* in different growth conditions could be an inherent trait endowing the flexibility of the halophyte that is required for an extremophile lifestyle (Guevara et al., 2012; Kazachkova et al., 2013).

In summary, these presented data support the markedly higher basal metabolite levels (proline and secondary compounds) in *E. salsugineum* than *Arabidopsis* in normal growth conditions (**Figure 2**), which is an important characteristic of salt tolerance of salt cress plants. The metabolic pre-adaptation of this halophyte could in part be attributed to the decreased osmotic potential in shoots and increased total solute levels, as well as the physicochemical properties of the metabolites such as water solubility and polarity (**Figure 2**).

GENE EXPRESSION AND REGULATION

Salt cress plants can grow in high salinity coastal areas, even in a 500 mM NaCl medium, but they do not have salt glands or other significant morphological alterations either before or after salt adaptation (Inan et al., 2004; Taji et al., 2004). Thus, the salt-tolerant halophyte *E. salsugineum* and salt-sensitive glycophyte *A. thaliana* are considered to use common mechanisms to cope with salinity, and the differences in salt tolerance between the two species can be mainly attributed to the changes in the regulation of a basic set of salt tolerance genes (Kant et al., 2006).

The gene expression profiles in *E. salsugineum* (Shandong ecotype) using an *Arabidopsis* full-length cDNA microarray revealed that, in contrast to *Arabidopsis*, only a few genes were induced by 250 mM NaCl stress, and many known stress-inducible genes such as *Fe-SOD*, *P5CS*, *PDF1.2*, *AtNCED*, *P-protein*, *b-glucosidase*, and *SOS1* were expressed at high levels in salt cress even in the absence of stress (Taji et al., 2004; **Figure 2**). The higher expression of the *P5CS* gene, encoding a key enzyme of proline biosynthesis, is in accordance with a much higher level of accumulated proline in salt cress than did *Arabidopsis* under normal growing conditions (Taji et al., 2004). Likewise, a comparison of transcript profiles between *Arabidopsis* at 150 mM NaCl and *Eutrema* (Shandong) exposed to 250 mM NaCl, using a microarray platform and *Arabidopsis* homologous gene probes, demonstrated that 40% shared and 60% distinguished regulated genes existed in the halophyte and the glycophyte (Gong et al., 2005). The combined transcript and metabolite analyses notably pointed toward a stress-anticipatory preparedness in *E. salsugineum* (Gong et al., 2005). In comparison with a great activation of transcription detected in *A. thaliana*, only a slight change in gene expression was found in *E. salsugineum* after stress treatment (Taji et al., 2004; Wong et al., 2006). Collectively, the analysis of expression profiling suggested that the stress-inducible signaling pathways are constitutive and active in salt cress even in the absence of stress (**Figure 2**), which is considered an important strategy for the extreme salt tolerance of this halophyte (Taji et al., 2004; Gong et al., 2005).

Also, comparative proteomics of salt responses between *A. thaliana* and *E. salsugineum* revealed more changes in protein abundance in *Arabidopsis* than in *Eutrema* (Pang et al., 2010). This indicates that the adjustment of metabolism and activation of the already existing enzymatic machinery could serve as a faster and more efficient strategy to cope with salt stress than the synthesis of new proteins (Pilarska et al., 2016; **Figure 2**). Furthermore, *E. salsugineum* exposed to 300 mM NaCl was used to investigate global transcriptional changes, and genes of several pathways mainly involved in lignin biosynthesis and accumulations of soluble sugars, as well as genes in autophagy and peroxisome pathways, were significantly enriched in the halophyte, suggesting these upregulated genes could contribute to salt tolerance of the halophytic *Eutrema* (Li et al., 2021).

In addition, the cDNA microarray containing 3,628 stress-induced *E. salsugineum* (Yukon ecotype) unique sequences were used to investigate the gene expression-profiling between Yukon and *Arabidopsis* subjected to saline, simulated drought, and cold stress as well as recovery from water deficits. The result revealed

that, among 154 differentially regulated transcripts obtained in all studied conditions, only six genes responded to all three stresses of salinity, drought, and cold (Wong et al., 2006). The low overlapping genes indicated an extensive divergence that exists in the responses of *Eutrema* Yukon to different stresses (Wong et al., 2006). There were relatively few transcripts responding to high salinity in this halophytic *E. salsugineum*, in contrast with *A. thaliana* (Wong et al., 2006). Intriguingly, in addition to activating the expression of some well-known stress-responsive genes, a large number of biotic stress-related genes under salinity and drought treatments were found to be down-regulated, suggesting a precise defense strategy to different environmental stresses in *E. salsugineum* (Figure 2), thereby maximizing its survival potential *via* conserving energy and resources (Wong et al., 2006).

MicroRNAs (miRNAs) are known to regulate gene expression by cleavage of complementary target mRNA, repression of translation of target mRNA, and transcriptional silencing of target mRNA (Sunkar and Zhu, 2004; Mittal et al., 2016). Solexa sequencing platform together with microarray and qRT-PCR was used to identify salt-stress responsive miRNAs in *E. salsugineum*. The differential response of the conserved high abundance miR166f, miR168a, miR408, and miR408-5p to salt stress suggests that they may play a key role in salt tolerance of *Eutrema* (Zhang et al., 2013; Figure 2). In agreement with this result, miR166, miR168a, and miR408 were all previously consistently demonstrated to be responsive to stress conditions, including salt and drought stress (Liu et al., 2008; Kong et al., 2010; Trindade et al., 2010; Zhou et al., 2010; Li et al., 2012; Figure 2). Another research on the large-scale characterization of *E. salsugineum* miRNAs and analysis of potential targets demonstrated that 11 conserved miRNA families including miR160, miR161, miR162, miR164, miR171, miR319, miR390, and miR827, and four novel miRNAs displayed a significant response to 300 mM NaCl stress (Wu et al., 2016). Importantly, *E. salsugineum* miR408 was strongly induced by 200 mM NaCl treatment, and *Arabidopsis* miR408 was induced by cold and mannitol, but not by salt (Liu et al., 2008; Zhang et al., 2013). The higher expression of miR408 leads to improved tolerance to salinity, cold and oxidative stress in *Arabidopsis* (Ma et al., 2015). Therefore, it is suggested that miR408 could play a critical role in the salt tolerance of *E. salsugineum* (Figure 2).

Long non-coding RNAs (lncRNAs) are implicated in the regulation of gene expression at the level of chromatin modification, transcription, and post-transcriptional processing, and are suggested to play an important role in a wide range of biological processes, such as genomic imprinting, chromatin remodeling, transcriptional activation, transcriptional interference, and cell cycle (Mercer et al., 2009; Sun et al., 2018). Recently, lncRNAs were proposed to play an important role in response to abiotic and biotic stress including a significant role in plant salt stress response (Gai et al., 2018). The leaf transcriptomes of Yukon and Shandong *E. salsugineum* plants subjected to a progressive, two-stage drought treatment were analyzed, and 1,007 lncRNAs were detected. Among them, 8% were only expressed in stress-treated plants, which suggested a

documented association between lncRNA expression and stress (Simopoulos et al., 2020).

In conclusion, these analyses of gene expression profiles based on the microarray platform and transcriptome in *E. salsugineum* provide useful global insight for elucidating the underlying salt tolerance mechanisms that distinguish it from *Arabidopsis* and help our more in-depth analyzing the extreme stress tolerance in this halophyte species. The investigation of the patterns of miRNA accumulation and target gene analysis revealed the possible roles of miRNAs in the adaptive response of *E. salsugineum* to salt stress (Figure 2), which enables us to access the salt tolerance mechanism of this halophyte from the perspective of small RNA regulation. However, research on the role of lncRNAs in salt resistance of plants is in its infancy, and the function and specific mechanism of action of the lncRNAs in salt tolerance of salt cress await to be studied and explored.

CANDIDATE GENES FOR SALT TOLERANCE

Halophytes have the potential to serve as a rich repository for genes to study abiotic stress mechanisms in plants (Rajalakshmi and Parida, 2012). Based on the various plant defense mechanisms adopted by halophytes, many groups have identified some genes functioning in tolerance to salinity *via* expressing them in transgenic systems (Rajalakshmi and Parida, 2012). In *E. salsugineum*, some important candidate genes for salt tolerance have been identified.

Aquaporins Genes AQPs

The exposure of plants to abiotic stresses challenges the plant water status and triggers highly specific hydraulic responses. Aquaporins (AQPs), a large family of channel proteins (Maurel et al., 2009; Wang et al., 2014), could selectively transport water molecules across the cell membrane that function in the plant water balance (Martre et al., 2002; Li et al., 2011, 2014; Qin et al., 2019). Plant AQP genes respond to diverse abiotic stresses such as salinity and drought (Alexandersson et al., 2005; Guo et al., 2006), and play important functions in the responses to environmental stimuli (Maurel et al., 2008; Aroca et al., 2012; Wang et al., 2014).

In *Arabidopsis*, the PIPs (plasma membrane intrinsic proteins) aquaporins were well-characterized to be involved in the regulation of root hydraulic conductivity (L_{pr}) (Javot et al., 2003; Tournaire-Roux et al., 2003). It has been reported that *Arabidopsis* PIP2;1 and PIP2;2 contribute to approximately 40% of the root hydraulic conductivity (Sutka et al., 2011; Péret et al., 2012), and the modification and localization of PIP aquaporins and root hydraulic conductivity could be regulated by the salt stress (Boursiac et al., 2008; Prak et al., 2008; Martinière et al., 2012; Qin et al., 2019). In *E. salsugineum* (*T. halophila*) seedlings, expression of the *EsPIP1* gene in the shoot and root tissues were induced by high salinity. Overexpression of the *EsPIP1* gene in rice markedly enhanced the tolerance of transgenic plants to salt stress *via* regulating the osmotic potential, accumulation of organic small molecules substances, and the ratio of K^+/Na^+

in the plant cells (Qiang et al., 2015). Moreover, EsPIP1 protein was shown to specifically interact with EsPIP2 and a non-specific lipid-transfer protein 2, suggesting that *E. salsugineum* EsPIP1 probably plays key roles in the response to multiple external stimuli, and participates in various physiological processes when plants subjected to salt stress (Qiang et al., 2015). Several *E. salsugineum* PIPs were observed to have a more stable expression in the roots compared with that in the shoots under salt stress conditions, and this specific expression pattern of PIP aquaporins is suggested to improve their ability to maintain a higher level of water transport activity in the roots, and decreasing water transpiration in the shoots (Qin et al., 2019).

The tonoplast intrinsic protein gene *EsTIP1;2* from the halophyte *E. salsugineum* was found to have water channel activity when expressed in *Xenopus* oocytes. And this gene was also able to conduct H₂O₂ into yeast cells in response to oxidative stress, and the inhibited growth of *EsTIP1;2* transgenic yeast cells were suggested as a result of H₂O₂ influx (Wang et al., 2014). In plants, ectopic overexpression of *E. salsugineum EsTIP1;2* in *A. thaliana* plants revealed an enhanced oxidative stress tolerance of plants after methyl viologen (MV) treatment, accompanied by a decrease of H₂O₂ level in the leaf cells (Wang et al., 2014). Meanwhile, *EsTIP1;2* could facilitate the entry of Na⁺ ions into plant cell vacuoles under high salinity conditions, through an indirect process (Wang et al., 2014). In all, the tonoplast AQP gene *EsTIP1;2*, was induced by multiple external stimuli, and a significantly elevated tolerance to salt, drought, and oxidative stress was observed in *EsTIP1;2* transgenic *Arabidopsis* plants (Wang et al., 2014). This suggested that *EsTIP1;2* could act as a multifunctional contributor to the survival of *Eutrema* plants in highly stressful habitats *via* mediating the conduction of both H₂O and H₂O₂ across membranes.

To sum up, the *E. salsugineum* aquaporin gene *EsPIP1;4* (*ThPIP1*) or *EsTIP1;2* (*TsTIP1;2*) overexpressed in heterologous species displayed an enhanced plant abiotic stress tolerance in transgenic plants (Wang et al., 2014; Qiang et al., 2015). These data enhance our understanding of the water balance regulation mechanisms involved in plant water status and root hydraulic conductivity, as well as the responses of aquaporin families to salt stress, in this halophyte.

Late Embryogenesis Abundant Genes (LEAs)

Late embryogenesis abundant (LEA) proteins described as multi-functional stress proteins that play active and significant roles in the stress resistance of plants *via* their function in defencing against dehydration caused by drought and salt (Close, 1996; Tunnacliffe and Wise, 2007). When plants are exposed to salinity, drought, or cold stresses, LEA2 protein members typically accumulated in dehydrating plant seeds and/or in tissues (Veeranagamallaiah et al., 2011), while dehydrins (DHNs) referred to as RAB proteins could be induced in vegetative tissues (Hundertmark and Hinch, 2008; Zhang et al., 2020). The expression of HVA1, a LEA3 protein from barley (*Hordeum vulgare* L.) conferred tolerance of transgenic rice plants to salt stress (Xu et al., 1996). Lee et al. (2013) found that,

in *E. salsugineum*, 52 LEA protein sequences represented a high sequence similarity with the orthologs in *Arabidopsis* (Lee et al., 2013). Furthermore, 64 *E. salsugineum* LEA proteins were identified to be classified into eight families, based on transcriptome sequencing, as well as the domain structures, numbers, and composition of conserved repeat motifs in LEAs. The remarkably induced expression of *EsLEA1*, *EsLEA4*, *EsLEA6*, and *EsDehydrin* genes was revealed in leaves and/or roots, which implies that the expression of LEA proteins could be one of the important strategies for *E. salsugineum* to deal with salt stress (Li et al., 2021).

Eutrema salsugineum (*Eutrema halophilum*) *EsEm1* (*EhEm1*) is a hydrophilic protein and contains three different 20-mer conserved motifs of N-domain, M-domain, and C-domain. *EsEm1* gene was revealed to be categorized into group 1 LEA proteins, and its expression was dramatically induced by salt, dehydration, abscisic acid (ABA), and cold stresses in young seedlings (Xiang and Man, 2018; **Table 1**). Overexpression of the *EhEm1* gene in rice demonstrated an enhanced tolerance to salt and drought stresses in transgenic plants, which was based on the better germination performances, higher survival rates, more accumulation of proline and increased peroxidase activity, as well as the reduced chlorophyll damage and less accumulation of malondialdehyde (Xiang and Man, 2018). And the *EsEm1* gene overexpressed in rice led to an upregulated transcript levels of several stress-responsive genes of *OsCDPK6*, *OsCDPK9*, *OsCDPK13*, and *rab16a* under both salt and drought conditions. In addition, *EsEm1* protein could effectively prevent the lactate dehydrogenase (LDH) enzyme from inactivation caused by salt and desiccation (Xiang and Man, 2018). Therefore, it is suggested that the *EsEm1* gene was associated with an enhanced salt and drought tolerance in transgenic rice, possibly through its contribution to the stabilization of LDH activity, and the up-regulation of *OsCDPK* genes (Xiang and Man, 2018). In addition, the LEA protein gene *EsLEA1* (*TsLEA1*, EU365627) was characterized as a salt induced gene, and it could improve the survival of salt-sensitive yeast cells, as well as the salt tolerance of transgenic *Arabidopsis* plants when ectopically expressed (Zhang et al., 2012; **Table 1**). Taken together, it was suggested that the increased expression of LEA protein families acts as protects plants from dehydrating damage, which probably is one of the outstanding mechanisms for adaptation of salt cress to the salt environment.

CONCLUSIONS AND PERSPECTIVES

Salt cress (*E. salsugineum* or *E. parvula*), a close relative of the model plant *A. thaliana*, occurs and thrives in harsh environments, thus a number of factors contribute to its salt stress tolerance. First, the duplicated gene pairs, particularly the expansion of important resistance genes such as ion transport-related genes in the genome of *Eutrema* may be considered an important adaptive benefit; the generated sub-functionalization and neo-functionalization of duplicated genes could be factors for salt tolerance of salt cress (Hahn, 2009; Kondrashov, 2012; Monihan et al., 2019; **Figure 2**). Second, the transcriptional and

metabolic comparisons of salt tolerant *Eutrema* and susceptible *Arabidopsis* pointed to a stress-anticipatory preparedness with constitutively high expression of the defense genes and relatively few salt-responsive genes detected, accompanied by preformed osmolyte of proline and secondary compounds, and lending metabolite configuration to fit osmoprotectant. And a precise defense strategy facilitates to conservation of energy that makes this halophyte to adapt various extreme environments (Figure 2). Third, *E. salsugineum* emerging as the halophyte, a major advantage appears to be their exceptional control over Na⁺ influx together with export mechanisms, the ability to coordinate its distribution to various tissues, and efficient sequestration of Na⁺ into vacuoles (Figure 2). Fourthly, the combined numerous studies revealed that the key components of underlying mechanisms in salt tolerance of the halophytic *Eutrema*, which is involved in a stronger antioxidative enzymatic system and substantially higher levels of thioredoxin, as well as the superior ability to sustain a highly reduced states of plastoquinone (PQ) pool and P700 under salt stress; the constitutively expressed protection systems against oxidative stress will facilitate to maintain a relatively stable stomatal conductance and CO₂ assimilation, and enhanced chlorophyll content to keep almost unaffected net photosynthetic rate (Figure 2). Finally, the stress-related genes such as *EsSOS1*, *EsEm1*, and *EsTIP1;2* that are expressed in transgenic systems led to enhanced tolerance to salinity stress, which indicates they play critical roles in the salt tolerance of *E. salsugineum*.

Tremendous genetic diversity that exists in the halophytic *Eutrema* can be exploited (Koorneef et al., 2004; Gong et al., 2005; Bohnert et al., 2006). And the comparative and functional genomics from this halophyte species have provided important lessons on stress tolerance. Also, overexpression of some abiotic stress-responsive genes from this halophyte into the transgenic model plants and crops has been achieved limited success. However, due to the fact that abiotic stress tolerance cannot be manipulated by a single quantitative trait locus (QTL), and genes could be a part of several pathways at different weights, thus the

analysis of a vast of data is needed to explore the complicated regulating network in plants (Rajalakshmi and Parida, 2012). Considering the multigenic nature of abiotic stress tolerance, researchers are working toward regulating networks with more than one kind of gene implicated in stress tolerance, to improve the efficacies of transgenic systems (Rajalakshmi and Parida, 2012).

Going forward, these genetic variations of *E. salsugineum* are providing valuable resources for further deciphering mechanisms underlying the stress tolerance and local adaptations of this halophytic species (Wang et al., 2021). Moreover, *Eutrema* plants undoubtedly have the potential to serve as a rich repository for genes, and the QTL-based approaches in halophytes may be utilized in breeding for abiotic stress tolerance (Rajalakshmi and Parida, 2012). Herein, we provide the recent scientific advance underlying the halophytic *Eutrema* that makes exploration of these fundamental adaptive mechanisms of salt stress more feasible, and to gain opportunities for improving the production of crops in unfavorable salt environments.

AUTHOR CONTRIBUTIONS

QZ conceived the manuscript. QZ and YanZ wrote the manuscript with contributions from CL, CD, HZ, YaoZ, and ZM. CL and CD performed the collection of documents and arrangement of data. All authors have read and approved the final manuscript.

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REFERENCES

- Achard, P., Gong, F., Cheminant, S., Alioua, M., Hedden, P., and Genschik, P. (2008). The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. *Plant Cell* 20, 2117–2129. doi: 10.1105/tpc.108.058941
- Alexanderson, E., Fraysse, L., Sjövall-Larsen, S., Gustavsson, S., Fellert, M., Karlsson, M., et al. (2005). Whole gene family expression and drought stress regulation of aquaporins. *Plant Mol. Biol.* 59, 469–484. doi: 10.1007/s11103-005-0352-1
- Ali, A., Cheol Park, H., Aman, R., Ali, Z., and Yun, D.-J. (2013). Role of HKT1 in *Thellungiella salsuginea*, a model extremophile plant. *Plant Signal. Behav.* 8:e25196. doi: 10.4161/psb.25196
- Ali, A., Khan, I. U., Jan, M., Khan, H. A., Hussain, S., Nisar, M., et al. (2018). The high-affinity potassium transporter *EphKT1; 2* from the extremophile *Eutrema parvula* mediates salt tolerance. *Front. Plant Sci.* 9:1108. doi: 10.3389/fpls.2018.01108
- Ali, Z., Park, H. C., Ali, A., Oh, D.-H., Aman, R., Kropornicka, A., et al. (2012). *TsHKT1; 2*, a HKT1 homolog from the extremophile *Arabidopsis* relative *Thellungiella salsuginea*, shows K⁺ specificity in the presence of NaCl. *Plant Physiol.* 158, 1463–1474. doi: 10.1104/pp.111.193110
- Amtmann, A. (2009). Learning from evolution: *thellungiella* generates new knowledge on essential and critical components of abiotic stress tolerance in plants. *Mol. Plant.* 2, 3–12. doi: 10.1093/mp/ssn094
- Amtmann, A., Bohnert, H. J., and Bressan, R. A. (2005). Abiotic stress and plant genome evolution. Search for new models. *Plant Physiol.* 138, 127–130. doi: 10.1104/pp.105.059972
- Apse, M. P., Sottosanto, J. B., and Blumwald, E. (2003). Vacuolar cation/H⁺ exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of *AtNHX1*, the *Arabidopsis* vacuolar Na⁺/H⁺ antiporter. *Plant J.* 36, 229–239. doi: 10.1046/j.1365-313X.2003.01871.x
- Aroca, R., Porcel, R., and Ruiz-Lozano, J. M. (2012). Regulation of root water uptake under abiotic stress conditions. *J. Exp. Bot.* 63, 43–57. doi: 10.1093/jxb/err266
- Baker, N. R. (1991). A possible role for photosystem II in environmental perturbations of photosynthesis. *Physiol. Plant.* 81, 563–570. doi: 10.1111/j.1399-3054.1991.tb05101.x

- Bartels, D., and Dinakar, C. (2013). Balancing salinity stress responses in halophytes and non-halophytes: a comparison between *Thellungiella* and *Arabidopsis thaliana*. *Funct. Plant Biol.* 40, 819–831. doi: 10.1071/FP12299
- Ben Rejeb, K., Lefebvre-De Vos, D., Le Disquet, I., Leprince, A. S., Bordenave, M., Maldiney, R., et al. (2015). Hydrogen peroxide produced by NADPH oxidases increases proline accumulation during salt or mannitol stress in *Arabidopsis thaliana*. *New Phytol.* 208, 1138–1148. doi: 10.1111/nph.13550
- Bhaskaran, S., and Savithamma, D. (2011). Co-expression of *Pennisetum glaucum* vacuolar Na⁺/H⁺ antiporter and *Arabidopsis* H⁺-pyrophosphatase enhances salt tolerance in transgenic tomato. *J. Exp. Bot.* 62, 5561–5570. doi: 10.1093/jxb/err237
- Blanc, G., and Wolfe, K. H. (2004a). Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. *Plant Cell* 16, 1679–1691. doi: 10.1105/tpc.021410
- Blanc, G., and Wolfe, K. H. (2004b). Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. *Plant Cell* 16, 1667–1678. doi: 10.1105/tpc.021345
- Bohnert, H. J., Gong, Q., Li, P., and Ma, S. (2006). Unraveling abiotic stress tolerance mechanisms-getting genomics going. *Curr. Opin. Plant Biol.* 9, 180–188. doi: 10.1016/j.pbi.2006.01.003
- Bose, J., Munns, R., Shabala, S., Gilliam, M., Pogson, B., and Tyerman, S. D. (2017). Chloroplast function and ion regulation in plants growing on saline soils: lessons from halophytes. *J. Exp. Bot.* 68, 3129–3143. doi: 10.1093/jxb/erx142
- Bose, J., Rodrigo-Moreno, A., and Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* 65, 1241–1257. doi: 10.1093/jxb/ert430
- Boursiac, Y., Boudet, J., Postaire, O., Luu, D. T., Tournaire-Roux, C., and Maurel, C. (2008). Stimulus-induced downregulation of root water transport involves reactive oxygen species-activated cell signalling and plasma membrane intrinsic protein internalization. *Plant J.* 56, 207–218. doi: 10.1111/j.1365-313X.2008.03594.x
- Boyer, J. S. (1982). Plant productivity and environment. *Science* 218, 443–448. doi: 10.1126/science.218.4571.443
- Bressan, R. A., Park, H. C., Orsini, F., Oh, D.-h., Dassanayake, M., Inan, G., et al. (2013). Biotechnology for mechanisms that counteract salt stress in extremophile species: a genome-based view. *Plant Biotechnol. Rev.* 7, 27–37. doi: 10.1007/s11816-012-0249-9
- Bressan, R. A., Zhang, C., Zhang, H., Hasegawa, P. M., Bohnert, H. J., and Zhu, J.-K. (2001). Learning from the *Arabidopsis* experience. The next gene search paradigm. *Plant Physiol.* 127, 1354–1360. doi: 10.1104/pp.010752
- Broin, M., Cuiñé, S., Peltier, G., and Rey, P. (2000). Involvement of CDSP 32, a drought-induced thioredoxin, in the response to oxidative stress in potato plants. *FEBS Lett.* 467, 245–248. doi: 10.1016/S0014-5793(00)01165-0
- Chaves, M. M., Flexas, J., and Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 103, 551–560. doi: 10.1093/aob/mcn125
- Cheeseman, J. M. (2015). The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytol.* 206, 557–570. doi: 10.1111/nph.13217
- Chung, J. S., Zhu, J. K., Bressan, R. A., Hasegawa, P. M., and Shi, H. (2008). Reactive oxygen species mediate Na⁺-induced SOS1 mRNA stability in *Arabidopsis*. *Plant J.* 53, 554–565. doi: 10.1111/j.1365-313X.2007.03364.x
- Close, T. J. (1996). Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiol. Plant* 97, 795–803. doi: 10.1111/j.1399-3054.1996.tb00546.x
- Czarnocka, W., and Karpiński, S. (2018). Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to environmental stresses. *Free Radic. Biol. Med.* 122, 4–20. doi: 10.1016/j.freeradbiomed.2018.01.011
- Dassanayake, M., Oh, D.-H., Haas, J. S., Hernandez, A., Hong, H., Ali, S., et al. (2011). The genome of the extremophile crucifer *Thellungiella parvula*. *Nat. Genet.* 43, 913–918. doi: 10.1038/ng.889
- Dos Santos, C. V., and Rey, P. (2006). Plant thioredoxins are key actors in the oxidative stress response. *Trends Plant Sci.* 11, 329–334. doi: 10.1016/j.tplants.2006.05.005
- Eshel, G., Shaked, R., Kazachkova, Y., Khan, A., Eppel, A., Cisneros, A., et al. (2017). *Anastatica hierochuntica*, an *Arabidopsis* desert relative, is tolerant to multiple abiotic stresses and exhibits species-specific and common stress tolerance strategies with its halophytic relative, *Eutrema (Thellungiella) salsugineum*. *Front. Plant Sci.* 7:1992. doi: 10.3389/fpls.2016.01992
- Fang, Y., Xie, K., Hou, X., Hu, H., and Xiong, L. (2010). Systematic analysis of GT factor family of rice reveals a novel subfamily involved in stress responses. *Mol. Genet. Genomics.* 283, 157–169. doi: 10.1007/s00438-009-0507-x
- Fichman, Y., and Mittler, R. (2020). Rapid systemic signaling during abiotic and biotic stresses: is the ROS wave master of all trades? *Plant J.* 102, 887–896. doi: 10.1111/tpj.14685
- Flexas, J., Bota, J., Loreto, F., Cornic, G., and Sharkey, T. (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* 6, 269–279. doi: 10.1055/s-2004-820867
- Flexas, J., DIAZ-ESPEJO, A., Galmes, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbo, M. (2007). Rapid variations of mesophyll conductance in response to changes in CO₂ concentration around leaves. *Plant Cell Environ.* 30, 1284–1298. doi: 10.1111/j.1365-3040.2007.01700.x
- Flowers, T., Hajibagheri, M., and Clipson, N. (1986). Halophytes. *Q. Rev. Biol.* 61, 313–337. doi: 10.1086/415032
- Flowers, T. J., and Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytol.* 945–963. doi: 10.1111/j.1469-8137.2008.02531.x
- Flowers, T. J., Munns, R., and Colmer, T. D. (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot.* 115, 419–431. doi: 10.1093/aob/mcu217
- Fowler, S. G., Cook, D., and Thomashow, M. F. (2005). Low temperature induction of *Arabidopsis* CBF1, 2, and 3 is gated by the circadian clock. *Plant Physiol.* 137, 961–968. doi: 10.1104/pp.104.058354
- Foyer, C. H., Ruban, A. V., and Noctor, G. (2017). Viewing oxidative stress through the lens of oxidative signalling rather than damage. *Biochem. J.* 474, 877–883. doi: 10.1042/BCJ20160814
- Fu, M., Kang, H. K., Son, S.-H., Kim, S.-K., and Nam, K. H. (2014). A subset of *Arabidopsis* RAV transcription factors modulates drought and salt stress responses independent of ABA. *Plant Cell Physiol.* 55, 1892–1904. doi: 10.1093/pcp/pcu118
- Gai, Y.-P., Yuan, S.-S., Zhao, Y.-N., Zhao, H.-N., Zhang, H.-L., and Ji, X.-L. (2018). A novel lncRNA, MuLnc1, associated with environmental stress in Mulberry (*Morus multicaulis*). *Front. Plant Sci.* 9:669. doi: 10.3389/fpls.2018.00669
- Gandour, M., Gharred, J., Taamalli, W., and Abdelly, C. (2019). Comparison of salinity tolerance in geographically diverse collections of *Thellungiella* accessions. *Russ. J. Ecol.* 50, 249–255. doi: 10.1134/S1067413619030020
- Gao, F., Chen, J., Ma, T., Li, H., Wang, N., Li, Z., et al. (2014). The glutathione peroxidase gene family in *Thellungiella salsuginea*: genome-wide identification, classification, and gene and protein expression analysis under stress conditions. *Int. J. Mol. Sci.* 15, 3319–3335. doi: 10.3390/ijms15023319
- Gaxiola, R. A., Li, J., Undurraga, S., Dang, L. M., Allen, G. J., Alper, S. L., et al. (2001). Drought- and salt-tolerant plants result from overexpression of the AVP1 H⁺-pump. *Proc. Natl. Acad. Sci. U.S.A.* 98, 11444–11449. doi: 10.1073/pnas.191389398
- Gechev, T. S., Dinakar, C., Benina, M., Toneva, V., and Bartels, D. (2012). Molecular mechanisms of desiccation tolerance in resurrection plants. *Cell. Mol. Life Sci.* 69, 3175–3186. doi: 10.1007/s00018-012-1088-0
- Gong, Q., Li, P., Ma, S., Indu Rupassara, S., and Bohnert, H. J. (2005). Salinity stress adaptation competence in the extremophile *Thellungiella halophila* in comparison with its relative *Arabidopsis thaliana*. *Plant J.* 44, 826–839. doi: 10.1111/j.1365-313X.2005.02587.x
- Goyal, K., Walton, L. J., and Tunnacliffe, A. (2005). LEA proteins prevent protein aggregation due to water stress. *Biochem. J.* 388, 151–157. doi: 10.1042/BJ20041931
- Griffith, M., Timonin, M., Wong, A. C., Gray, G. R., Akhter, S. R., Saldanha, M., et al. (2007). *Thellungiella*: an *Arabidopsis*-related model plant adapted to cold temperatures. *Plant Cell Environ.* 30, 529–538. doi: 10.1111/j.1365-3040.2007.01653.x
- Grigore, M.-N. (2019). “Defining halophytes-A conceptual and historical approach in an ecological frame,” in *Halophytes and Climate Change: Adaptive Mechanisms and Potential Uses*, eds M. Hasanuzzaman, S. Shabala, and M. Fujita (Wallingford: CABI), 3–18. doi: 10.1079/9781786394330.0003
- Guevara, D. R., Champigny, M. J., Tattersall, A., Dedrick, J., Wong, C. E., Li, Y., et al. (2012). Transcriptomic and metabolomic analysis of Yukon *Thellungiella*

- plants grown in cabinets and their natural habitat show phenotypic plasticity. *BMC Plant Biol.* 12:175. doi: 10.1186/1471-2229-12-175
- Guo, L., Wang, Z. Y., Lin, H., Cui, W. E., Chen, J., Liu, M., et al. (2006). Expression and functional analysis of the rice plasma-membrane intrinsic protein gene family. *Cell Res.* 16, 277–286. doi: 10.1038/sj.cr.7310035
- Hahn, M. W. (2009). Distinguishing among evolutionary models for the maintenance of gene duplicates. *J. Hered.* 100, 605–617. doi: 10.1093/jhered/esp047
- Holmgren, A. (1985). Thioredoxin. *Annu. Rev. Plant Biol.* 54, 237–271. doi: 10.1146/annurev.bi.54.070185.001321
- Hou, Q., and Bartels, D. (2015). Comparative study of the aldehyde dehydrogenase (ALDH) gene superfamily in the glycophyte *Arabidopsis thaliana* and *Eutrema halophytes*. *Ann. Bot.* 115, 465–479. doi: 10.1093/aob/mcu152
- Hundertmark, M., and Hincha, D. K. (2008). LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. *BMC Genomics* 9:188. doi: 10.1186/1471-2164-9-118
- Inan, G., Zhang, Q., Li, P., Wang, Z., Cao, Z., Zhang, H., et al. (2004). Salt cress. A halophyte and cryophyte *Arabidopsis* relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. *Plant Physiol.* 135, 1718–1737. doi: 10.1104/pp.104.041723
- Janz, D., Behnke, K., Schnitzler, J.-P., Kanawati, B., Schmitt-Kopplin, P., and Polle, A. (2010). Pathway analysis of the transcriptome and metabolome of salt sensitive and tolerant poplar species reveals evolutionary adaptation of stress tolerance mechanisms. *BMC Plant Biol.* 10:150. doi: 10.1186/1471-2229-10-150
- Jarvis, D. E., Ryu, C.-H., Beilstein, M. A., and Schumaker, K. S. (2014). Distinct roles for SOS1 in the convergent evolution of salt tolerance in *Eutrema salsugineum* and *Schrenkiella parvula*. *Mol. Biol. Evol.* 31, 2094–2107. doi: 10.1093/molbev/msu152
- Javot, H., Lavergeat, V., Santoni, V., Martin-Laurent, F., Güçlü, J., Vinh, J., et al. (2003). Role of a single aquaporin isoform in root water uptake. *Plant Cell.* 15, 509–522. doi: 10.1105/tpc.008888
- John, U. P., and Spangenberg, G. C. (2005). Xenogenomics: genomic bioprospecting in indigenous and exotic plants through EST discovery, cDNA microarray-based expression profiling and functional genomics. *Comp. Funct. Genomics* 6, 230–235. doi: 10.1002/cfg.475
- Kant, S., Kant, P., Raveh, E., and Barak, S. (2006). Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na⁺ uptake in *T. halophila*. *Plant Cell Environ.* 29, 1220–1234. doi: 10.1111/j.1365-3040.2006.01502.x
- Kaur, G., Guruprasad, K., Temple, B. R., Shirvanyants, D. G., Dokholyan, N. V., and Pati, P. K. (2018). Structural complexity and functional diversity of plant NADPH oxidases. *Amino Acids* 50, 79–94. doi: 10.1007/s00726-017-2491-5
- Kazachkova, Y., Batushansky, A., Cisneros, A., Tel-Zur, N., Fait, A., and Barak, S. (2013). Growth platform-dependent and-independent phenotypic and metabolic responses of *Arabidopsis* and its halophytic relative, *Eutrema salsugineum*, to salt stress. *Plant Physiol.* 162, 1583–1598. doi: 10.1104/pp.113.217844
- Kazachkova, Y., Eshel, G., Pantha, P., Cheeseman, J. M., Dassanayake, M., and Barak, S. (2018). Halophytism: what have we learnt from *Arabidopsis thaliana* relative model systems? *Plant Physiol.* 178, 972–988. doi: 10.1104/pp.18.00863
- Kirsch, M., Zhigang, A., Viereck, R., Löw, R., and Rausch, T. (1996). Salt stress induces an increased expression of V-type H⁺-ATPase in mature sugar beet leaves. *Plant Mol. Biol.* 32, 543–547. doi: 10.1007/BF00019107
- Koch, M. A., and German, D. A. (2013). Taxonomy and systematics are key to biological information: *Arabidopsis*, *Eutrema* (*Thellungiella*), *Noccaea* and *Schrenkiella* (*Brassicaceae*) as examples. *Front. Plant Sci.* 4:267. doi: 10.3389/fpls.2013.00267
- Kondrashov, F. A. (2012). Gene duplication as a mechanism of genomic adaptation to a changing environment. *Proc. R. Soc. B* 279, 5048–5057. doi: 10.1098/rspb.2012.1108
- Kong, Y., Elling, A. A., Chen, B., and Deng, X. (2010). Differential expression of microRNAs in maize inbred and hybrid lines during salt and drought stress. *Am. J. Plant Physiol.* 1:69. doi: 10.4236/ajps.2010.12009
- Koornneef, M., Alonso-Blanco, C., and Vreugdenhil, D. (2004). Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu. Rev. Plant Biol.* 55, 141–172. doi: 10.1146/annurev.arplant.55.031903.141605
- Kumar, V., Khare, T., Sharma, M., and Wani, S. H. (2017). “ROS-induced signaling and gene expression in crops under salinity stress,” in *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation Under Abiotic Stress*, eds M. I. R. Khan and N. A. Khan (Singapore: Springer), 159–184. doi: 10.1007/978-981-10-5254-5_7
- Lamdan, N. L., Attia, Z., Moran, N., and Moshelion, M. (2012). The *Arabidopsis*-related halophyte *Thellungiella halophila*: boron tolerance via boron complexation with metabolites? *Plant Cell Environ.* 35, 735–746. doi: 10.1111/j.1365-3040.2011.02447.x
- Larcher, W. (2003). *Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups*, 4th Edn. New York, NY: Springer.
- Lawlor, D. W., and Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* 25, 275–294. doi: 10.1046/j.0016-8025.2001.00814.x
- Lee, Y. P., Funk, C., Erban, A., Kopka, J., Köhl, K. I., Zuther, E., et al. (2016). Salt stress responses in a geographically diverse collection of *Eutrema/Thellungiella* spp. accessions. *Funct. Plant Biol.* 43, 590–606. doi: 10.1071/FP15285
- Lee, Y. P., Giorgi, F. M., Lohse, M., Kvederavicute, K., Klages, S., Usadel, B., et al. (2013). Transcriptome sequencing and microarray design for functional genomics in the extremophile *Arabidopsis* relative *Thellungiella salsuginea* (*Eutrema salsugineum*). *BMC Genomics* 14:793. doi: 10.1186/1471-2164-14-793
- Li, C., Qi, Y., Zhao, C., Wang, X., and Zhang, Q. (2021). Transcriptome profiling of the salt stress response in the leaves and roots of halophytic *Eutrema salsugineum*. *Front. Genet.* 12:770742. doi: 10.3389/fgene.2021.770742
- Li, G., Santoni, V., and Maurel, C. (2014). Plant aquaporins: roles in plant physiology. *Biochim. Biophys. Acta Gen. Subj.* 1840, 1574–1582. doi: 10.1016/j.bbagen.2013.11.004
- Li, P., Mane, S. P., Sioson, A. A., Robinet, C. V., Heath, L. S., Bohnert, H. J., et al. (2006). Effects of chronic ozone exposure on gene expression in *Arabidopsis thaliana* ecotypes and in *Thellungiella halophila*. *Plant Cell Environ.* 29, 854–868. doi: 10.1111/j.1365-3040.2005.01465.x
- Li, W., Cui, X., Meng, Z., Huang, X., Xie, Q., Wu, H., et al. (2012). Transcriptional regulation of *Arabidopsis* MIR168a and argonaute1 homeostasis in abscisic acid and abiotic stress responses. *Plant Physiol.* 158, 1279–1292. doi: 10.1104/pp.111.188789
- Li, X., Wang, X., Yang, Y., Li, R., He, Q., Fang, X., et al. (2011). Single-molecule analysis of PIP2; 1 dynamics and partitioning reveals multiple modes of *Arabidopsis* plasma membrane aquaporin regulation. *Plant Cell* 23, 3780–3797. doi: 10.1105/tpc.111.091454
- Lisso, J., Altmann, T., and Müssig, C. (2006). The AtNFXL1 gene encodes a NF-X1 type zinc finger protein required for growth under salt stress. *FEBS Lett.* 580, 4851–4856. doi: 10.1016/j.febslet.2006.07.079
- Liu, H.-H., Tian, X., Li, Y.-J., Wu, C.-A., and Zheng, C.-C. (2008). Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. *RNA* 14, 836–843. doi: 10.1261/rna.895308
- Lugan, R., Niogret, M. F., Lepout, L., Guégan, J. P., Larher, F. R., Savouré, A., et al. (2010). Metabolome and water homeostasis analysis of *Thellungiella salsuginea* suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. *Plant J.* 64, 215–229. doi: 10.1111/j.1365-313X.2010.04323.x
- Ma, C., Burd, S., and Lers, A. (2015). miR408 is involved in abiotic stress responses in *Arabidopsis*. *Plant J.* 84, 169–187. doi: 10.1111/tpj.12999
- Ma, L., Zhang, H., Sun, L., Jiao, Y., Zhang, G., Miao, C., et al. (2012). NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na⁺/K⁺ homeostasis in *Arabidopsis* under salt stress. *J. Exp. Bot.* 63, 305–317. doi: 10.1093/jxb/err280
- Maere, S., De Bodt, S., Raes, J., Casneuf, T., Van Montagu, M., Kuiper, M., et al. (2005). Modeling gene and genome duplications in eukaryotes. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5454–5459. doi: 10.1073/pnas.0501102102
- Maeshima, M. (2000). Vacuolar H⁺-pyrophosphatase. *Biochim. Biophys. Acta Biomembr.* 1465, 37–51. doi: 10.1016/S0005-2736(00)00130-9
- Martinière, A., Li, X., Runions, J., Lin, J., Maurel, C., and Luu, D.-T. (2012). Salt stress triggers enhanced cycling of *Arabidopsis* root plasma-membrane aquaporins. *Plant Signal. Behav.* 7, 529–532. doi: 10.4161/psb.19350
- Martre, P., Morillon, R., Barriau, F., North, G. B., Nobel, P. S., and Chrispeels, M. J. (2002). Plasma membrane aquaporins play a significant role during recovery from water deficit. *Plant Physiol.* 130, 2101–2110. doi: 10.1104/pp.009019

- Maurel, C., Santoni, V., Luu, D.-T., Wudick, M. M., and Verdoucq, L. (2009). The cellular dynamics of plant aquaporin expression and functions. *Curr. Opin. Plant Biol.* 12, 690–698. doi: 10.1016/j.pbi.2009.09.002
- Maurel, C., Verdoucq, L., Luu, D.-T., and Santoni, V. (2008). Plant aquaporins: membrane channels with multiple integrated functions. *Annu. Rev. Plant Biol.* 59, 595–624. doi: 10.1146/annurev.arplant.59.032607.092734
- Mercer, T. R., Dinger, M. E., and Mattick, J. S. (2009). Long non-coding RNAs: insights into functions. *Nat. Rev. Genet.* 10, 155–159. doi: 10.1038/nrg2521
- Mittal, D., Sharma, N., Sharma, V., Sopory, S., and Sanan-Mishra, N. (2016). Role of microRNAs in rice plant under salt stress. *Ann. Appl. Biol.* 168, 2–18. doi: 10.1111/aab.12241
- Møller, I. M., Jensen, P. E., and Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* 58, 459–481. doi: 10.1146/annurev.arplant.58.032806.103946
- Monihan, S. M., Ryu, C.-H., Magness, C. A., and Schumaker, K. S. (2019). Linking duplication of a calcium sensor to salt tolerance in *Eutrema salsugineum*. *Plant Physiol.* 179, 1176–1192. doi: 10.1104/pp.18.01400
- M'rah, S., Ouerghi, Z., Berthomieu, C., Havaux, M., Jungas, C., Hajji, M., et al. (2006). Effects of NaCl on the growth, ion accumulation and photosynthetic parameters of *Thellungiella halophila*. *J. Plant Physiol.* 163, 1022–1031. doi: 10.1016/j.jplph.2005.07.015
- M'rah, S., Ouerghi, Z., Eymery, F., Rey, P., Hajji, M., Grignon, C., et al. (2007). Efficiency of biochemical protection against toxic effects of accumulated salt differentiates *Thellungiella halophila* from *Arabidopsis thaliana*. *J. Plant Physiol.* 164, 375–384. doi: 10.1016/j.jplph.2006.07.013
- Munns, R., James, R. A., and Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57, 1025–1043. doi: 10.1093/jxb/erj100
- Niewiadomska, E., and Wicziarz, M. (2015). “Adaptations of chloroplastic metabolism in halophytic plants,” in *Progress in Botany*, Vol. 76, eds U. Lüttge and W. Beyschlag (Cham: Springer International Publishing), 177–193. doi: 10.1007/978-3-319-08807-5_7
- Ogawa, D., Yamaguchi, K., and Nishiuchi, T. (2007). High-level overexpression of the *Arabidopsis* HsfA2 gene confers not only increased thermotolerance but also salt/osmotic stress tolerance and enhanced callus growth. *J. Exp. Bot.* 58, 3373–3383. doi: 10.1093/jxb/erm184
- Oh, D.-H., Dassanayake, M., Bohnert, H. J., and Cheeseman, J. M. (2013). Life at the extreme: lessons from the genome. *Genome Biol.* 13, 1–9. doi: 10.1186/gb-2012-13-3-241
- Oh, D.-H., Leidi, E., Zhang, Q., Hwang, S.-M., Li, Y., Quintero, F. J., et al. (2009). Loss of halophytism by interference with SOS1 expression. *Plant Physiol.* 151, 210–222. doi: 10.1104/pp.109.137802
- Ohno, S. (1970). *Evolution by Gene Duplication*. New York, NY: Springer-Verlag.
- Orsini, F., D'Urzo, M. P., Inan, G., Serra, S., Oh, D.-H., Mickelbart, M. V., et al. (2010). A comparative study of salt tolerance parameters in 11 wild relatives of *Arabidopsis thaliana*. *J. Exp. Bot.* 61, 3787–3798. doi: 10.1093/jxb/erq188
- Ozgun, R., Uzilday, B., Sekmen, A. H., and Turkan, I. (2013). Reactive oxygen species regulation and antioxidant defence in halophytes. *Funct. Plant Biol.* 40, 832–847. doi: 10.1071/FP12389
- Ozyigit, I. I., Filiz, E., Vatansver, R., Kurtoglu, K. Y., and Koc, I., Öztürk, M.X., et al. (2016). Identification and comparative analysis of H₂O₂-scavenging enzymes (ascorbate peroxidase and glutathione peroxidase) in selected plants employing bioinformatics approaches. *Front. Plant Sci.* 7:301. doi: 10.3389/fpls.2016.00301
- Pang, Q., Chen, S., Dai, S., Chen, Y., Wang, Y., and Yan, X. (2010). Comparative proteomics of salt tolerance in *Arabidopsis thaliana* and *Thellungiella halophila*. *J. Proteome Res.* 9, 2584–2599. doi: 10.1021/pr100034f
- Péret, B., Li, G., Zhao, J., Band, L. R., Voß, U., Postaire, O., et al. (2012). Auxin regulates aquaporin function to facilitate lateral root emergence. *Nat. Cell Biol.* 14, 991–998. doi: 10.1038/ncb2573
- Pilarska, M., Bartels, D., and Niewiadomska, E. (2021). Differential regulation of NAPDH oxidases in salt-tolerant *Eutrema salsugineum* and salt-sensitive *Arabidopsis thaliana*. *Int. J. Mol. Sci.* 22, 10341. doi: 10.3390/ijms221910341
- Pilarska, M., Wicziarz, M., Jajić, I., Koziaradzka-Kiszkurmo, M., Dobrev, P., Vanková, R., et al. (2016). A different pattern of production and scavenging of reactive oxygen species in halophytic *Eutrema salsugineum* (*Thellungiella salsuginea*) plants in comparison to *Arabidopsis thaliana* and its relation to salt stress signaling. *Front. Plant Sci.* 7:1179. doi: 10.3389/fpls.2016.01179
- Prak, S., Hem, S., Boudet, J., Viennois, G., Sommerer, N., Rossignol, M., et al. (2008). Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2; 1 in response to salt stress. *Mol. Cell Proteomics* 7, 1019–1030. doi: 10.1074/mcp.M700566-MCP200
- Qiang, X.-J., Yu, G. H., Jiang, L. L., Sun, L. L., Zhang, S. H., Li, W., et al. (2015). *Thellungiella halophila* ThPIP1 gene enhances the tolerance of the transgenic rice to salt stress. *J. Integr. Agric.* 14, 1911–1922. doi: 10.1016/S2095-3119(15)61045-0
- Qin, S., Liu, Y., Han, Y., Xu, G., Wan, S., Cui, F., et al. (2019). Aquaporins and their function in root water transport under salt stress conditions in *Eutrema salsugineum*. *Plant Sci.* 287:110199. doi: 10.1016/j.plantsci.2019.110199
- Qiu, Q.-S., Barkla, B. J., Vera-Estrella, R., Zhu, J.-K., and Schumaker, K. S. (2003). Na⁺/H⁺ exchange activity in the plasma membrane of *Arabidopsis*. *Plant Physiol.* 132, 1041–1052. doi: 10.1104/pp.102.010421
- Qiu, Q.-S., Guo, Y., Dietrich, M. A., Schumaker, K. S., and Zhu, J.-K. (2002). Regulation of SOS1, a plasma membrane Na⁺/H⁺ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8436–8441. doi: 10.1073/pnas.122224699
- Quintero, F. J., Ohta, M., Shi, H., Zhu, J.-K., and Pardo, J. M. (2002). Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na⁺ homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9061–9066. doi: 10.1073/pnas.132092099
- Rajalakshmi, S., and Parida, A. (2012). Halophytes as a source of genes for abiotic stress tolerance. *J. Plant Biochem. Biotechnol.* 21, 63–67. doi: 10.1007/s13562-012-0146-x
- Rey, P., Cuiné, S., Eymery, F., Garin, J., Court, M., Jacquot, J. P., et al. (2005). Analysis of the proteins targeted by CDSP32, a plastidic thioredoxin participating in oxidative stress responses. *Plant J.* 41, 31–42. doi: 10.1111/j.1365-313X.2004.02271.x
- Rus, A., Lee, B. H., Munoz-Mayor, A., Sharkhuu, A., Miura, K., Zhu, J.-K., et al. (2004). ATHKT1 facilitates Na⁺ homeostasis and K⁺ nutrition in planta. *Plant Physiol.* 136, 2500–2511. doi: 10.1104/pp.104.042234
- Sairam, R., and Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.* 86, 407–421. doi: 10.1007/1-4020-4225-6
- Sewelam, N., Jaspert, N., Van Der Kelen, K., Tognetti, V. B., Schmitz, J., Frerigmann, H., et al. (2014). Spatial H₂O₂ signaling specificity: H₂O₂ from chloroplasts and peroxisomes modulates the plant transcriptome differentially. *Mol. Plant* 7, 1191–1210. doi: 10.1093/mp/ssu070
- Shi, H., Ishitani, M., Kim, C., and Zhu, J.-K. (2000). The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na⁺/H⁺ antiporter. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6896–6901. doi: 10.1073/pnas.120170197
- Shi, H., Quintero, F. J., Pardo, J. M., and Zhu, J.-K. (2002). The putative plasma membrane Na⁺/H⁺ antiporter SOS1 controls long-distance Na⁺ transport in plants. *Plant Cell* 14, 465–477. doi: 10.1105/tpc.010371
- Simopoulos, C., MacLeod, M. J., Irani, S., Sung, W. W., Champigny, M. J., Summers, P. S., et al. (2020). Coding and long non-coding RNAs provide evidence of distinct transcriptional reprogramming for two ecotypes of the extremophile plant *Eutrema salsugineum* undergoing water deficit stress. *BMC Genomics* 21:396. doi: 10.1186/s12864-020-06793-7
- Sohn, K. H., Lee, S. C., Jung, H. W., Hong, J. K., and Hwang, B. K. (2006). Expression and functional roles of the pepper pathogen-induced transcription factor RAV1 in bacterial disease resistance, and drought and salt stress tolerance. *Plant Mol. Biol.* 61, 897–915. doi: 10.1007/s11103-006-0057-0
- Srivastava, A. K., Srivastava, S., Lokhande, V. H., D'Souza, S. F., and Suprasanna, P. (2015). Salt stress reveals differential antioxidant and energetics responses in glycophyte (*Brassica juncea* L.) and halophyte (*Sesuvium portulacastrum* L.). *Front. Environ. Sci.* 3:19. doi: 10.3389/fenvs.2015.00019
- Sui, N., and Han, G. (2014). Salt-induced photoinhibition of PSII is alleviated in halophyte *Thellungiella halophila* by increases of unsaturated fatty acids in membrane lipids. *Acta Physiol. Plant* 36, 983–992. doi: 10.1007/s11738-013-1477-5
- Sun, X., Zheng, H., and Sui, N. (2018). Regulation mechanism of long non-coding RNA in plant response to stress. *Biochem. Biophys. Res. Commun.* 503, 402–407. doi: 10.1016/j.bbrc.2018.07.072
- Sunkar, R., and Zhu, J.-K. (2004). Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *Plant Cell* 16, 2001–2019. doi: 10.1105/tpc.104.022830

- Surówka, E., Latowski, D., Libik-Konieczny, M., and Miszański, Z. (2019). "ROS signalling, and antioxidant defence network in halophytes," in *Halophytes and Climate Change: Adaptive Mechanisms and Potential Uses*, eds M. Hasanuzzaman, S. Shabala, and M. Fujita (Boston, MA: CABI), 179–195. doi: 10.1079/9781786394330.0179
- Sutka, M., Li, G., Boudet, J., Boursiac, Y., Dumas, P., and Maurel, C. (2011). Natural variation of root hydraulics in *Arabidopsis* grown in normal and salt-stressed conditions. *Plant Physiol.* 155, 1264–1276. doi: 10.1104/pp.110.163113
- Taji, T., Sakurai, T., Mochida, K., Ishiwata, A., Kurotani, A., Totoki, Y., et al. (2008). Large-scale collection and annotation of full-length enriched cDNAs from a model halophyte, *Thellungiella halophila*. *BMC Plant Biol.* 8:115. doi: 10.1186/1471-2229-8-115
- Taji, T., Seki, M., Satou, M., Sakurai, T., Kobayashi, M., Ishiyama, K., et al. (2004). Comparative genomics in salt tolerance between *Arabidopsis* and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. *Plant Physiol.* 135, 1697–1709. doi: 10.1104/pp.104.039909
- Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.-T., et al. (2003). Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* 425, 393–397. doi: 10.1038/nature01853
- Trindade, L., Capitão, C., Dalmay, T., Fevêreiro, M. P., and Santos, D. M. (2010). miR398 and miR408 are up-regulated in response to water deficit in *Medicago truncatula*. *Planta* 231, 705–716. doi: 10.1007/s00425-009-1078-0
- Tunnacliffe, A., and Wise, M. J. (2007). The continuing conundrum of the LEA proteins. *Naturwissenschaften* 94, 791–812. doi: 10.1007/s00114-007-0254-y
- Veeranagamallaiah, G., Prasanthi, J., Reddy, K. E., Pandurangiah, M., Babu, O. S., and Sudhakar, C. (2011). Group 1 and 2 LEA protein expression correlates with a decrease in water stress induced protein aggregation in horsegram during germination and seedling growth. *J. Plant Physiol.* 168, 671–677. doi: 10.1016/j.jplph.2010.09.007
- Vera-Estrella, R., Barkla, B. J., García-Ramírez, L., and Pantoja, O. (2005). Salt stress in *Thellungiella halophila* activates Na⁺ transport mechanisms required for salinity tolerance. *Plant Physiol.* 139, 1507–1517. doi: 10.1104/pp.105.067850
- Verbruggen, N., and Hermans, C. (2008). Proline accumulation in plants: a review. *Amino Acids* 35, 753–759. doi: 10.1007/s00726-008-0061-6
- Volkov, V., Wang, B., Dominy, P., Fricke, W., and Amtmann, A. (2004). *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, possesses effective mechanisms to discriminate between potassium and sodium. *Plant Cell Environ.* 27, 1–14. doi: 10.1046/j.0016-8025.2003.01116.x
- Wang, L.-L., Chen, A.-P., Zhong, N.-Q., Liu, N., Wu, X.-M., Wang, F., et al. (2014). The *Thellungiella salsuginea* tonoplast aquaporin TsTIP1; 2 functions in protection against multiple abiotic stresses. *Plant Cell Physiol.* 55, 148–161. doi: 10.1093/pcp/pct166
- Wang, X., Rao, H., Ma, J., Chen, X., Li, G., and Zhao, G. (2021). Genomic Variation Landscape of the Model Salt Cress *Eutrema salsugineum*. *Front. Plant Sci.* 12:700161. doi: 10.3389/fpls.2021.700161
- Wang, Z.-L., Li, P. H., Fredricksen, M., Gong, Z. Z., Kim, C., Zhang, C., et al. (2004). Expressed sequence tags from *Thellungiella halophila*, a new model to study plant salt-tolerance. *Plant Sci.* 166, 609–616. doi: 10.1016/j.plantsci.2003.10.030
- Wiczarz, M., Gubernator, B., Kruk, J., and Niewiadomska, E. (2015). Enhanced chloroplastic generation of H₂O₂ in stress-resistant *Thellungiella salsuginea* in comparison to *Arabidopsis thaliana*. *Physiol. Plant* 153, 467–476. doi: 10.1111/ppl.12248
- Wiczarz, M., Niewiadomska, E., and Kruk, J. (2018). Effects of salt stress on low molecular antioxidants and redox state of plastoquinone and P700 in *Arabidopsis thaliana* (glycophyte) and *Eutrema salsugineum* (halophyte). *Photosynthetica* 56, 811–819. doi: 10.1007/s11099-017-0733-0
- Wong, C., Li, Y., Whitty, B., Diaz-Camino, C., Akhter, S., Brandle, J., et al. (2005). Expressed sequence tags from the Yukon ecotype of *Thellungiella* reveal that gene expression in response to cold, drought and salinity shows little overlap. *Plant Mol. Biol.* 58, 561–574. doi: 10.1007/s11103-005-6163-6
- Wong, C. E., Li, Y., Labbe, A., Guevara, D., Nuin, P., Whitty, B., et al. (2006). Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in *Thellungiella*, a close relative of *Arabidopsis*. *Plant Physiol.* 140, 1437–1450. doi: 10.1104/pp.105.070508
- Wu, H.-J., Zhang, Z., Wang, J.-Y., Oh, D.-H., Dassanayake, M., Liu, B., et al. (2012). Insights into salt tolerance from the genome of *Thellungiella salsuginea*. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12219–12224. doi: 10.1073/pnas.1209954109
- Wu, Y., Guo, J., Cai, Y., Gong, X., Xiong, X., Qi, W., et al. (2016). Genome-wide identification and characterization of *Eutrema salsugineum* microRNAs for salt tolerance. *Physiol. Plant* 157, 453–468. doi: 10.1111/ppl.12419
- Xiang, D., and Man, L. (2018). EhEm1, a novel Em-like protein from *Eutrema halophilum*, confers tolerance to salt and drought stresses in rice. *Mol. Breed.* 38, 1–15. doi: 10.1007/s11032-017-0750-5
- Xie, Z.-M., Zou, H.-F., Lei, G., Wei, W., Zhou, Q.-Y., Niu, C.-F., et al. (2009). Soybean Trihelix transcription factors GmGT-2A and GmGT-2B improve plant tolerance to abiotic stresses in transgenic *Arabidopsis*. *PLoS ONE* 4:e6898. doi: 10.1371/journal.pone.0006898
- Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.-H. D., and Wu, R. (1996). Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol.* 110, 249–257. doi: 10.1104/pp.110.1.249
- Yang, R., Jarvis, D. J., Chen, H., Beilstein, M., Grimwood, J., Jenkins, J., et al. (2013). The reference genome of the halophytic plant *Eutrema salsugineum*. *Front. Plant Sci.* 4:46. doi: 10.3389/fpls.2013.00046
- Yang, S., Luo, C., Song, Y., and Wang, J. (2016). Two groups of *Thellungiella salsuginea* RAVs exhibit distinct responses and sensitivity to salt and ABA in transgenic *Arabidopsis*. *PLoS ONE* 11:e0153517. doi: 10.1371/journal.pone.0153517
- Zhang, H. F., Liu, S. Y., Ma, J. H., Wang, X. K., Haq, S. U., Meng, Y. C., et al. (2020). CaDHN4, a salt and cold stress-responsive dehydrin gene from pepper decreases abscisic acid sensitivity in *Arabidopsis*. *Int. J. Mol. Sci.* 21:26. doi: 10.3390/ijms21010026
- Zhang, Q., Zhao, C., Li, M., Sun, W., Liu, Y., Xia, H., et al. (2013). Genome-wide identification of *Thellungiella salsuginea* microRNAs with putative roles in the salt stress response. *BMC Plant Biol.* 13:180. doi: 10.1186/1471-2229-13-180
- Zhang, Y., Li, Y., Lai, J., Zhang, H., Liu, Y., Liang, L., et al. (2012). Ectopic expression of a LEA protein gene TsLEA1 from *Thellungiella salsuginea* confers salt-tolerance in yeast and *Arabidopsis*. *Mol. Biol. Rep.* 39, 4627–4633. doi: 10.1007/s11033-011-1254-8
- Zhao, C., Zhang, H., Song, C., Zhu, J.-K., and Shabala, S. (2020). Mechanisms of plant responses and adaptation to soil salinity. *Innovation* 1:100017. doi: 10.1016/j.xinn.2020.100017
- Zhou, L., Liu, Y., Liu, Z., Kong, D., Duan, M., and Luo, L. (2010). Genome-wide identification and analysis of drought-responsive microRNAs in *Oryza sativa*. *J. Exp. Bot.* 61, 4157–4168. doi: 10.1093/jxb/erq237
- Zhu, J.-K. (2002). Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* 53, 247–273. doi: 10.1146/annurev.arplant.53.091401.143329

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