

REVIEW



## Genetic mechanisms of salt stress responses in halophytes

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### ABSTRACT

Abiotic stress is a major threat to plant growth and development, resulting in extensive crop loss worldwide. Plants react to abiotic stresses through physiological, biochemical, molecular, and genetic adaptations that promote survival. Exploring the molecular mechanisms involved in abiotic stress responses across various plant species is essential for improving crop yields in unfavorable environments. Halophytes are characterized as plants that survive to reproduce in soils containing high salt concentrations, and thus act as an ideal model to comprehend complicated genetic and physiological mechanisms of salinity stress tolerance. Plant ecologists classify halophytes into three main groups: euhalophytes, recretahalophytes, and pseudo-halophytes. Recent genetic and molecular research has showed complicated regulatory networks by which halophytes coordinate stress adaptation and tolerance. Furthermore, investigation of natural variations in these stress responses has supplied new perspectives on the evolution of mechanisms that regulate tolerance and adaptation. This review discusses the current understanding of the genetic mechanisms that contribute to salt-stress tolerance among different classes of halophytes.

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### KEYWORDS

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### Introduction

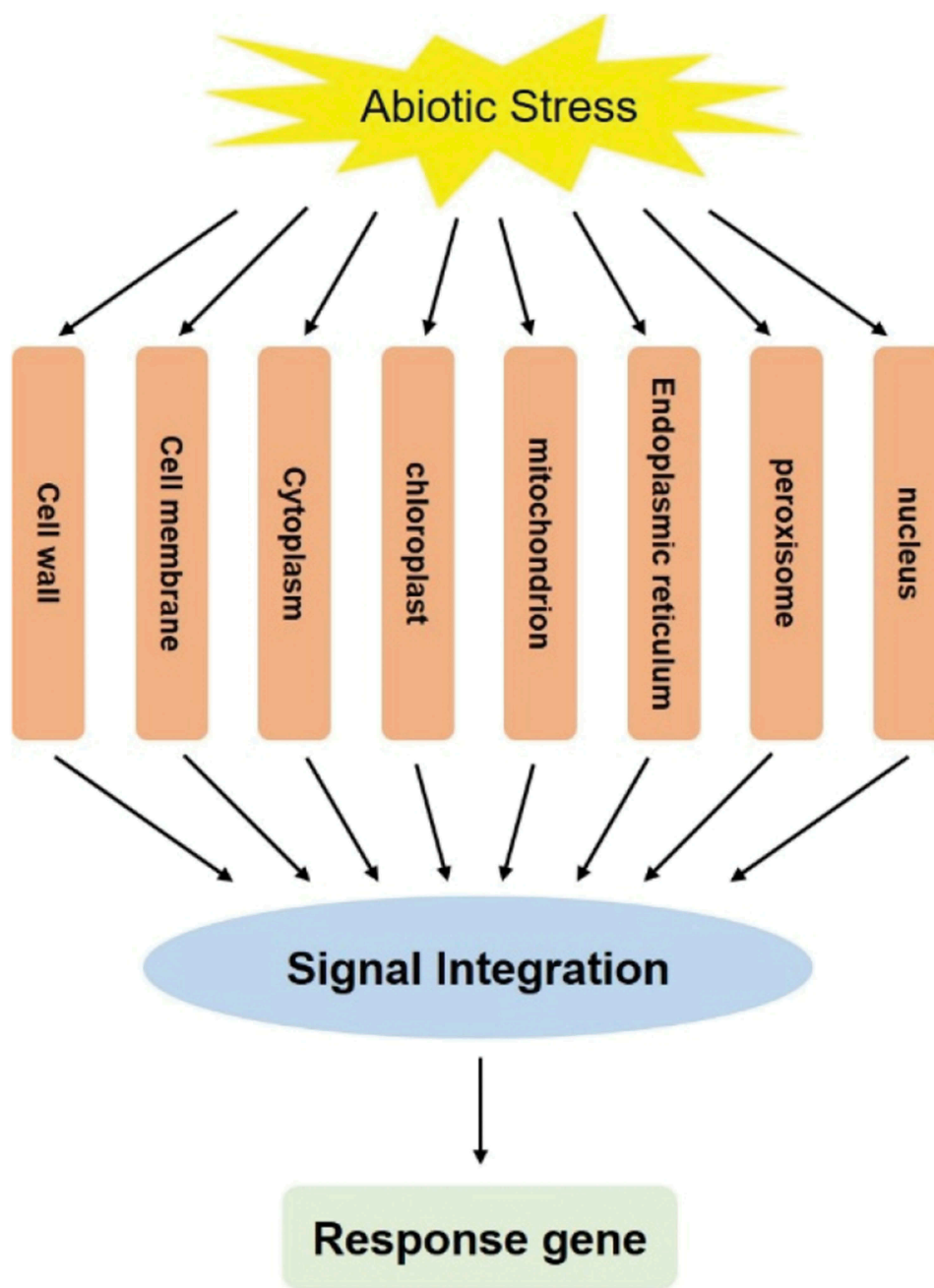
Abiotic stress is characterized as the adverse effects of non-living factors on living organisms.<sup>1,2</sup> Plants must survive under ever-changing environmental conditions characterized by variations in a lot of abiotic stresses, such as salt,<sup>3,4</sup> cold,<sup>5–7</sup> flooding,<sup>8</sup> and drought.<sup>9–11</sup> In some environmental niches, plants must respond to multiple abiotic stresses.<sup>12–14</sup> As a result, they have evolved mechanisms to respond to abiotic stress, including changes in growth and development resulting from altered metabolism, as well as modifications in morphology (Figure 1). Among abiotic stress, soil salinity has become a serious problem in many parts of the world, especially in arid and semi-arid areas.<sup>15,16</sup> As reported, over 800 million ha of land worldwide is salt-affected, which accounts for about 6% of the world's total land area.<sup>17,18</sup> Soil salinity has an enormous effect on the agricultural yield all over the world, for it affects the establishment, development, and growth of plants, which finally lead to crop yield loss.<sup>19–21</sup>

Salt stress decreases the yield of a number of crops, including wheat,<sup>22–24</sup> maize,<sup>25–27</sup> and rice,<sup>28,29</sup> presenting a challenge for feeding the increasing global population.<sup>30</sup> To elucidate mechanisms that contribute to stress adaptation and tolerance, it becomes more and more useful to characterize the physiological and genomic characteristics of individual crop plants that thrive in unfavorable environments.<sup>31,32</sup> Although many researchers have performed a number of relevant studies in *Arabidopsis* and other crop model systems,<sup>33,34</sup> halophytes could offer valuable resources to help us uncover the mystery of salt-related signaling pathway.<sup>35,36</sup> The study of

how halophytes tolerate salt could offer biologists' clues to increase salt tolerance in conventional crops.

Halophytes could not only grow well in environments where the salt concentration is around 200 mM NaCl or more, but survive in other harsh environments, including drought, cold or flooding.<sup>37</sup> Therefore, they act as an ideal model to understand complicated genetic and physiological mechanisms of abiotic stress tolerance. Plant ecologists classify halophytes into three main groups: euhalophytes, recretahalophytes, and pseudo-halophytes.<sup>38,39</sup> Euhalophytes can dilute salt within their stems or leaves and have a strong ability to tolerate salt.<sup>40,41</sup> Recretahalophytes can secrete salt from their leaves, and grows widely around the world, inland saline lands and inhabiting seawater.<sup>42,43</sup> Salt glands and bladders are salt-secreting structures, which can directly secrete ions out of the plants. Pseudo-halophytes could not only hold up ions in roots but also minimize its transport to the shoot parts, so as to protect itself from metabolic tissue.<sup>44,45</sup>

*Suaeda salsa* and *Salicornia europaea* are typical euhalophytes, which can dilute salt within their stems or leaves and have a strong ability to tolerate salt. *S. salsa* could survive well in 200mM NaCl concentration,<sup>46,47</sup> and also grow in the environments where salt stress and drought stress coexist. *S. europaea* have much broader salinity tolerance. Researchers have performed a lot of studies in *S. salsa* and *S. europaea*, and cloned a series of genes involved in abiotic tolerance and tested their functions. These studies have indicated that euhalophytes play an essential role in agricultural production.<sup>48,49</sup> Recretahalophytes possess unique salt-secreting structures,



**Figure 1.** Model of dispersed stress sensing by organelles.

namely salt glands and bladders, which can directly secrete these ions out of the plants.<sup>50</sup> Therefore, salt bladders and salt glands play essential roles for the recretohalophytes to secrete excess salt.<sup>51</sup> *Limonium bicolor* is a good example of recretohalophyte, which can desalinate saline-alkali soil and preserve high rates of photosynthesis under the treatment of 200–300 mM NaCl.<sup>52</sup> *Reaumuria trigyna* is an endangered recretohalophyte and has salt excretion glands to respond to salt stress.<sup>53</sup> Pseudo-halophytes could intercept ions in roots and minimize its transport to the shoot parts. *Eutrema salsugineum* (previously *Thellungiella salsuginea*, salt cress) is a representative pseudo-halophyte, which could not only survive well under more than 300 mM NaCl but grow well in cold regions.<sup>54,55</sup> Different categories of halophytes have

distinct abilities to address high salt concentrations or other stresses, finally finish their life cycle.

Halophytes have evolved diverse responses to environmental variations.<sup>56,57</sup> Natural variation studies have provided novel views for the evolutionary processes that shape stress responses, also showed previously undiscovered loci related to these mechanisms.<sup>58–61</sup> Studies are performed on natural variation in stress response traits, providing essential sources of genetic variation.<sup>62–64</sup> These important sources can help us understand the coordinated regulation of stress responses and play a role in improving agronomic crops.<sup>65</sup> In plants, it becomes a complex process to understand and identify the crucial regulatory factors that are related to multiple stress responses. However, it is essential to identify traits that can

protect plants from environmental stresses, and genomic studies across a wide variety of species are needed to address this aim.<sup>66</sup> Advances in functional genomics will help us identify and understand potential stress-tolerant traits.<sup>67–71</sup> Therefore, this review focuses on the role of functional genomics and system biology approaches in discovering the mechanisms that underlie stress-tolerance traits in halophytes.

### The mechanism of euhalophytes in salt stress

*S. salsa*, a typical euhalophyte, is a good example to understand the molecular mechanisms in abiotic stress. *S. salsa* is an annual herbaceous C-3 euhalophyte that belongs to the Chenopodiaceae family,<sup>72,73</sup> a group of plants that produce dimorphic seeds on the same plant under standard conditions.<sup>74–76</sup> Recent studies have shown that NaCl concentration plays a key role in seed vitality in *S. salsa* by regulating the levels of stored compounds, such as proteins, starch, and fatty acids.<sup>77,78</sup> *S. salsa* could tolerate high salt concentration and offers good sources for salt-related genes.<sup>79</sup> A series of salt-tolerant genes from *S. salsa* have been cloned and their functions were tested. One important feature of halophytes is highly efficient vacuolar sequestration of cytotoxic Na<sup>+</sup>, which requires that Na<sup>+</sup> could be pumped into the vacuole against the electrochemical gradient and that Na<sup>+</sup> in the vacuole could be prevented from leaking back into the cytosol. Na<sup>+</sup>/H<sup>+</sup> antiporters make a contribution to the vacuolar compartmentalization of Na<sup>+</sup>.<sup>80,81</sup> One study showed that the expression of *SsNHX1* was up-regulated in *S. salsa* leaves under 500 mM NaCl treatment. The product of *SsNHX1* might be an Na<sup>+</sup>/H<sup>+</sup> antiporter, which plays essential roles in *S. salsa* salt tolerance. Treatment of *S. salsa* with increasing concentrations of NaCl increased the activity of the plasma membrane (PM) H<sup>+</sup>-ATPase to promote salt tolerance.<sup>82</sup> Later study illustrated that this increase in PM H<sup>+</sup>-ATPase activity attribute to both an increase in PM H<sup>+</sup>-ATPase protein levels and transcriptional levels. Also, Na<sup>+</sup> efflux across the plasma membrane is due to Salt-Overly-Sensitive1(*SOS1*) Na<sup>+</sup>/H<sup>+</sup> antiporter, and researchers have demonstrated that *SsSOS1* may be involved in Na<sup>+</sup> efflux both in leaves and in roots. Besides these genes, the expression of *SsHKT1* increases under salt stress, suggesting that *SsHKT1* is essential for K<sup>+</sup> uptake under high salinity.<sup>83</sup> Further research confirmed that transgenic *Arabidopsis*, which *SsHKT1* was overexpressed, revealed increased salt tolerance. Ca<sup>2+</sup> plays an essential role in maintaining K<sup>+</sup> and Na<sup>+</sup> homeostasis under salt stress, while *SsCAX1* is important to lower cytosolic Ca<sup>2+</sup> burst under salt stress.<sup>84,85</sup> Overexpression S-adenosylmethionine synthetase gene from *S. salsa* could promote salt tolerance in tobacco, suggesting that this pathway also contributes to salt tolerance.<sup>86</sup>

*S. salsa* could also grow in saline inland soils of arid zone, characterized by both high salinity and drought. Compared to salt stress, drought problem is much more pervasive and negatively affects crop yields seriously. Dehydration-responsive element-binding (DREB) transcription factor (TF) is involved in abiotic stress tolerance in plants. Researchers have cloned *DREB* from succulent halophyte *S. salsa* (*SsDREB*) and tested its functions in tobacco.

Transgenic tobacco plants transformed with *SsDREB* showed improved drought and salt tolerance, compared with wild-type controls.<sup>87</sup> Also, researchers have cloned a vacuolar H<sup>+</sup>-pyrophosphatase gene from the halophyte *S. salsa* (*SsVP*) and identified its function in *Arabidopsis*. Transgenic *Arabidopsis* plants transformed with *SsVP* showed higher V-ATPase and the V-PPase activities, and increased drought tolerance in comparison with wild-type plants.<sup>88</sup> Another study showed that transgenic *Arabidopsis*, transformed with *ssNHX1*, grew well under 200mM NaCl treatment and drought stress.<sup>89</sup> Later study demonstrated that transgenic plants showed higher photosynthesis activity and reduced toxic effects of Na<sup>+</sup> accumulation in the cytosol. These studies testified euhalophytes *S. salsa* have evolved many mechanisms to drought stress.

Almost all the halophytes must face the challenge of osmotic adjustment in reaction to lower external water supply, including organic and inorganic solutes.<sup>90</sup> Glycinebetaine and proline are related to osmotic adjustment in certain halophytes. The concentration of glycinebetaine exceeded 25 mM kg<sup>-1</sup> of fresh weight in *S. salsa* under 400 mM NaCl treatment, which was deduced to be important in *S. salsa* osmotic adjustment. While the contribution rate of proline to osmotic potential was <0.5% in *S. salsa* under salt stress, therefore the cytoplasmic glycinebetaine might act a more essential role in osmotic adjustment compared with proline.<sup>91</sup> Researchers have discovered two genes related to glycinebetaine synthesis, including glycinebetaine aldehyde dehydrogenase (*BADH*) and choline monooxygenase (*CMO*), which catalyze glycinebetaine synthesis.<sup>92</sup> Researchers have cloned *SsCMO* and *SsBADH* from *S. salsa* and identified their functions. Transgenic tobacco overexpressing *SsCMO* showed higher tolerance to salt stress. Furthermore, researchers have cloned several important genes involved in this function in *S. salsa*, of which *SsINPS* is related to myoinositol synthesis and *SsP5CS* is related to proline synthesis. In addition, *S. salsa* expressed more *SsP5CS* and *SsINPS* under salt treatment.

Halophytes are famous for their ability to endure and quench toxic reactive oxygen species (ROS), for they possess powerful antioxidant system.<sup>93,94</sup> Researchers have detected Mn-SOD and several isoforms of CuZn-SOD and Fe-SOD in *S. salsa* leaf extracts, also cloned several genes related to oxidative stress tolerance in *S. salsa*, including *Ss.sAPX*, *SsCAT2*, *SsPrxQ*, *SsGST*, *SsCAT1*, *SsAPX*, and *SsTypA1*.<sup>95,96</sup> Later study displayed that transgenic *Arabidopsis* plants overexpressing *Ss.sAPX* improved the germination rate, cotyledon growth, survival rate, and salt tolerance. In addition, transgenic plants showed higher total chlorophyll content, longer roots, higher total APX activity and less cell membrane damage than wild-type. These results demonstrated that *Ss.sAPX* may be essential to protect higher plants against salt-induced oxidative stress.<sup>97,98</sup>

*S. europaea* is another annual succulent euhalophytes and belongs to Chenopodiaceae family. Under seawater irrigation, *S. europaea* seeds have 28% oil content and 30.2% protein content, also unsaturated fatty acid account for high level in the seed oil, which makes *S. europaea* act as a potential competitive oilseed crop.<sup>99</sup> Recent studies have shown that salinity treatment increased significantly the contents of

proline, reducing saccharide, soluble saccharide and oligosaccharide in *S. europaea*. And MDA contents increased in *S. europaea* under high salt concentrations exceeding 300mM. Also, proteins' and polysaccharides' contents reduced under salt stress in *S. europaea*. In addition, salinity treatment decreased the  $K^+$  contents as well as carotenoids and chlorophylls in seedlings of *S. europaea*. *S. europaea* could tolerate high salt concentration and offers good sources for salt-related genes.<sup>100</sup> Researchers have cloned phytoene synthase from *S. europaea* (*SePSY*) and tested its function in *Arabidopsis*. The study showed that *SePSY* overexpression enhanced the growth of the transgenic line. Under 100mM NaCl treatment, transgenic *Arabidopsis* displayed higher photosystem II activity and photosynthesis rate, as well as SOD and POD activity, and showed lower MDA and  $H_2O_2$  contents in comparison with non-transformed line.<sup>101</sup> This study demonstrated that the *SePSY* gene could enhance salt tolerance in *Arabidopsis*. Another study showed choline monooxygenase from *S. europaea* (*SeCMO*) could increase salt tolerance. CMO catalyzes the synthesis of Glycinebetaine, which is an osmoprotectant accumulated by stresses in plants. *SeCMO* transgenic tobacco could survive under 300mM NaCl treatment and displayed vigorous conditions in comparison with the wild-type control. Also, transgenic tobacco showed higher betaine accumulation and chlorophyll content, lower electrical conductivity. These studies demonstrate that *SeCMO* could effectively improve salt-tolerance in tobacco.<sup>102</sup> Another study showed that researchers have cloned  $\beta$ -lycopene cyclase gene from *S. europaea* (*SeLCY*) and tested its functions. *SeLCY* transgenic *Arabidopsis* showed increased oxidative stress tolerance and salt-stress tolerance. Also, transgenic *Arabidopsis* grew better, displayed higher photosystem activity and less  $H_2O_2$  accumulation, and retained higher carotenoid contents. All these studies demonstrated that euhalophytes *S. europaea* have evolved complex mechanism to resist salt stress.

### The mechanism of recretahalophytes in salt stress

Recretahalophytes, typical halophytes, can excrete excessive salt to the environment through epidermal salt bladders or

salt glands (Figure 2).<sup>103,104</sup> Therefore, salt bladders and salt glands play essential roles for the recretahalophytes to secrete excess salt.<sup>105</sup> *L. bicolor*, which possess salt glands that secrete excess salt, is extensively studied to understand the high molecular mechanism of recretahalophytes.<sup>106,107</sup> The *L. bicolor* salt gland is comprised of 16 cells and is regulated by levels of specific cations.<sup>108</sup> The divalent cation  $Ca^{2+}$  has been demonstrated to function crucially in plant growth, development, and salt tolerance. And increases in  $Ca^{2+}$  levels not only markedly enhance its development, but also promote salt-secretion rates in *L. bicolor* leaves.<sup>109</sup> In addition, under conditions of elevated salinity,  $K^+$  accumulation in salt gland cells may play a part in salt secretion in *L. bicolor*.<sup>110</sup> Despite the important roles of  $Ca^{2+}$  and  $K^+$  levels, environmental scanning electron microscopy has shown that the chemical composition of secretions from the secretory pores is primarily composed of NaCl.

Many studies are performed to screen candidate genes involved in salt stress in *L. bicolor*. Researchers used the fluorescence method to screen ~10,000 seedlings in which seeds were gamma-irradiated, and obtained 15 mutants with increased salt gland density and 4 mutants with reduced salt gland density.<sup>111</sup> These two group mutants will be helpful to isolate genes related to salt secretion and salt gland development in *L. bicolor*. Also, high-throughput RNA-sequencing analysis was performed to screen genes related to salt secretion in *L. bicolor*. This study showed that 2040 genes were differentially expressed among 27311 total genes of database, of which 1260 genes were down-regulated and 744 were up-regulated with the NaCl versus the control treatment. Further analysis showed that 102 of these genes might be related to salt secretion, including *NHX* genes, vesicle-associated membrane protein (*VAMP*) and so on.<sup>112</sup> This study identifies the candidate genes that are related to salt secretion in the *L. bicolor* salt glands.<sup>113</sup>

Apart from salt stress, *L. bicolor* could also survive in a wide range of harsh environments, implying that it has evolved physiological and molecular systems to adjust to harmful stress conditions. Plant glutathione S-transferases (GSTs) play essential roles in protecting plants against diverse abiotic and biotic stresses. A novel GST gene was cloned from

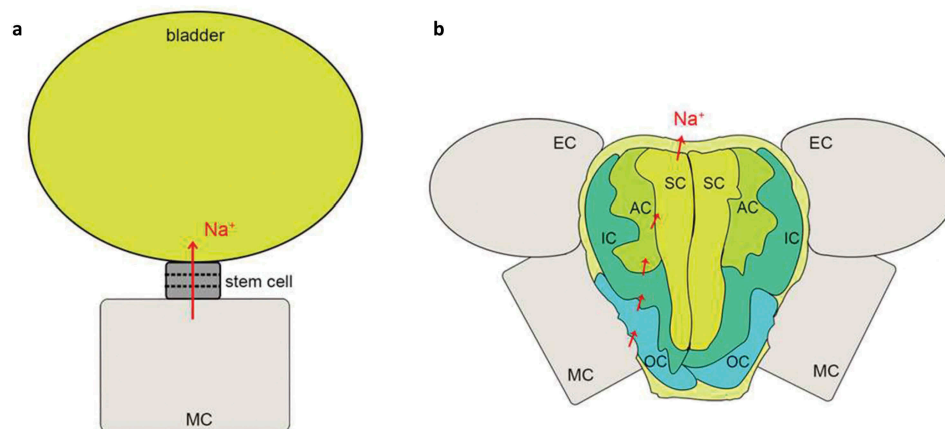


Figure 2. The structure and  $Na^+$  secretion pathway of a salt bladder (a) and a salt gland (b)(Yuan et al., 2016).

*L. bicolor* (*LbGST1*), and its functions were tested in tobacco. Transgenic tobacco plants, which *LbGST1* is overexpressed, showed both glutathione peroxidase and GST activities. Furthermore, catalase, peroxidase (POD) and superoxide dismutase showed higher activities in transgenic plants than WT plants, especially under salt treatment.<sup>114</sup> Similarly, transgenic plants showed higher levels of proline than WT plants under NaCl treatment. In addition, transgenic plants showed lower Na<sup>+</sup> content than WT plants under these stress conditions. Another study showed that transgenic yeast harboring the *LbGST1* showed elevated tolerance to drought and freezing compared with the control transformants. These studies suggest that *LbGST1* might be involved in many physiological pathways that can improve stress resistance in plants. Also, researchers have cloned a novel dehydration-responsive element-binding (DREB) gene from *L. bicolor* (*LbDREB*) and characterized its function in copper stress. The study showed that transgenic tobacco plants transformed with *LbDREB* revealed higher contents of soluble protein and proline, and higher ratio of K<sup>+</sup> to Na<sup>+</sup> under the treatment of CuSO<sub>4</sub>.<sup>113</sup> In addition, some genes involved in stress are up-regulated in *LbDREB* transgenic plants, including late embryogenesis abundant (LEA), PODs, Cu/Zn SOD, and lipid transfer proteins (LTP). This study demonstrates that *LbDREB* can strengthen copper stress tolerance by up-regulating a series of genes involved in stress, consequently regulating stress tolerance-related physiological processes in plants.

*R. trigyna*, an endangered dicotyledonous shrub, is another recretohalophyte belonging to Tamaricaceae family. It grows in a salinized desert in Inner Mongolia, China, which is characterized by high salinity, low temperature, and hyper-drought conditions.<sup>115</sup> Therefore, exploring the molecular mechanisms employed by *R. trigyna* will offer us valuable resources in salt-related signaling pathway. Many researches are performed to study the mechanisms in response to salt stress in *R. trigyna*. Researchers have isolated Na<sup>+</sup>/H<sup>+</sup> antiporter from *R. trigyna* (*RtNHX1*) and tested its function in *Arabidopsis*. Transgenic *Arabidopsis*, which *RtNHX1* was overexpressed, exhibited enhanced seed germination rate, root elongation, biomass accumulation, and chlorophyll content in comparison with control lines. Further study showed that *RtNHX1* transgenic *Arabidopsis* showed increased activities of POD and CAT, RWC, and proline content. Also, the leaves of transgenic *Arabidopsis* accumulated more K<sup>+</sup> and less Na<sup>+</sup>, and lower ratio of Na<sup>+</sup>/K<sup>+</sup>. In addition, *RtNHX1* transgenic yeast vacuole showed increased accumulation of Na<sup>+</sup> and K<sup>+</sup> and decreased Na<sup>+</sup>/K<sup>+</sup> ratio. These studies revealed that *RtNHX1* acts as an antiporter which sequesters Na<sup>+</sup> and K<sup>+</sup> in the vacuole, and could resist salt tolerance.<sup>116</sup> Another study showed that a high-affinity potassium transporter gene was isolated from *R. trigyna* (*RtHKT1*), which was up-regulated under high Na<sup>+</sup> or low K<sup>+</sup> treatment in *R. trigyna*. Transgenic *Arabidopsis* enhanced the accumulation of K<sup>+</sup>, prevented the transport of Na<sup>+</sup> from roots to shoots, and increased biomass under salt stress in comparison with wild control. This study suggests that *RtHKT1* could increase salt tolerance by maintaining the homeostasis of Na<sup>+</sup>/K<sup>+</sup>.<sup>115</sup> Recent research showed that salt stress could activate the flavonoid biosynthesis pathway, while flavanone-

3-hydroxylase (F3H) is involved in this process. The study showed that the transcription level of *RtF3H1* and *RtF3H2* was increased in *R. trigyna* under salt stress. Also, transgenic *RtF3H1* or *RtF3H2* *Escherichia coli* lines showed a higher survival rate than the control lines under salt stress. Another study showed that the Group II WRKY transcription factor was isolated from *R. trigyna* (*RtWRKY23*), which was induced by salt treatment. *RtWRKY23* transgenic *Arabidopsis* revealed increased chlorophyll content, fresh weight, and root length. Further study showed that *RtWRKY23* transgenic *Arabidopsis* had a higher content of proline and activity of peroxidase, and lower H<sub>2</sub>O<sub>2</sub> and MDA contents in comparison with wild-type plants under salt stress.<sup>117</sup> All these studies demonstrated that recretohalophyte *R. trigyna* evolved many mechanisms in response to salt stress.

### The mechanism of pseudo-halophytes in salt stress

Pseudo-halophytes could not only tolerate strong salinity but escape from it by locating the active part of the root system in less saline soil levels.<sup>50,118</sup> *E. salsugineum* is a representative pseudo-halophyte and could survive well under more than 300 mM NaCl.<sup>119</sup> According to high sequence similarity between *E. salsugineum* and *Arabidopsis*, researchers have cloned several *E. salsugineum* genes involved in salt stress and characterized its functions, including *TsVP* which encodes vacuolar pyrophosphatase, *ThCBL9* which encodes a calcineurin-B-like protein, *ThHSC70* which encodes a heat-shock protein, *ThCYP1* which encodes a cyclophilin, *ThZF1* which encodes a Cys-2/His-2-type transcription factor, *TsnsLTP4* which encodes nonspecific lipid transfer proteins, *TsGOLS2* which encodes galactinol synthase, *TsLEA1* and so on.<sup>120–124</sup> *TsVP*-transformed transgenic cotton and maize showed improved salt and drought tolerance, which is related to the higher activity of vacuolar H<sup>+</sup>-PPase. Also, the leaves of *TsVP*-transformed tobacco accumulated more Na<sup>+</sup>, owing to efficient vacuolar Na<sup>+</sup> compartmentalization. Overexpression of *ThCBL9* enhanced its tolerance to salt and osmotic stress in *A. thaliana*, and overexpression of *ThHSC70* in *A. thaliana* improved tolerance to chilling and high temperature. *ThCYP1*-transformed fission yeast and tobacco cells showed increased salt tolerance, indicating that *ThCYP1* might mediate the correct folding of certain stress-related proteins. Also, ectopic expression of *ThZF1* in *Arabidopsis* mutant *azf2* showed that *ThZF1* might function similarly as *Arabidopsis* *AZF2* in regulating downstream gene expression and plant development. Overexpression of *TsGOLS2*, a galactinol synthase, in *Arabidopsis thaliana* increased its tolerance to high salinity and osmotic stresses.<sup>122</sup> In addition, *TsLEA1* maintained salt tolerance in yeast as well as in plants, suggesting that *TsLEA1* may be involved in protection plant and yeast cells under stress conditions. Apart from salt-related genes, *E. salsugineum* small RNA libraries were constructed and sequenced, and the study showed that *T. salsugineum* diverse set of miRNAs could respond to salt stress and act an important role in response to salt stress.<sup>125</sup> Clearly, more researches are needed to perform to identify the detailed molecular mechanisms of *E. salsugineum* in abiotic stress.

*E. salsugineum* commonly grows in extreme environments, characterized by both high salinity and low temperature.<sup>125</sup> Freezing is another major environmental stress, which seriously affects plant growth and development, contributing to the decrease of crop yield and quality.<sup>126</sup> The *TsFtsH8* gene is related to cold tolerance in *E. salsugineum*, and *TsFtsH8-RNAi* lines exhibit severe chlorophyll decomposition and organelle deterioration, also reveal the reductions of the rates of open photosystem II reaction centers.<sup>127</sup> Five aquaporin genes involved in cold stress have been identified through RNA-sequencing experiments, including three *TIPs*, one *PIPs*, and one *NIPs*. In addition, in response to low temperatures, expression of DREB1/CBF-cold signaling pathway genes are altered, including *COR47*, *ICE1*, and *CBF1* (Figure 3). KEGG

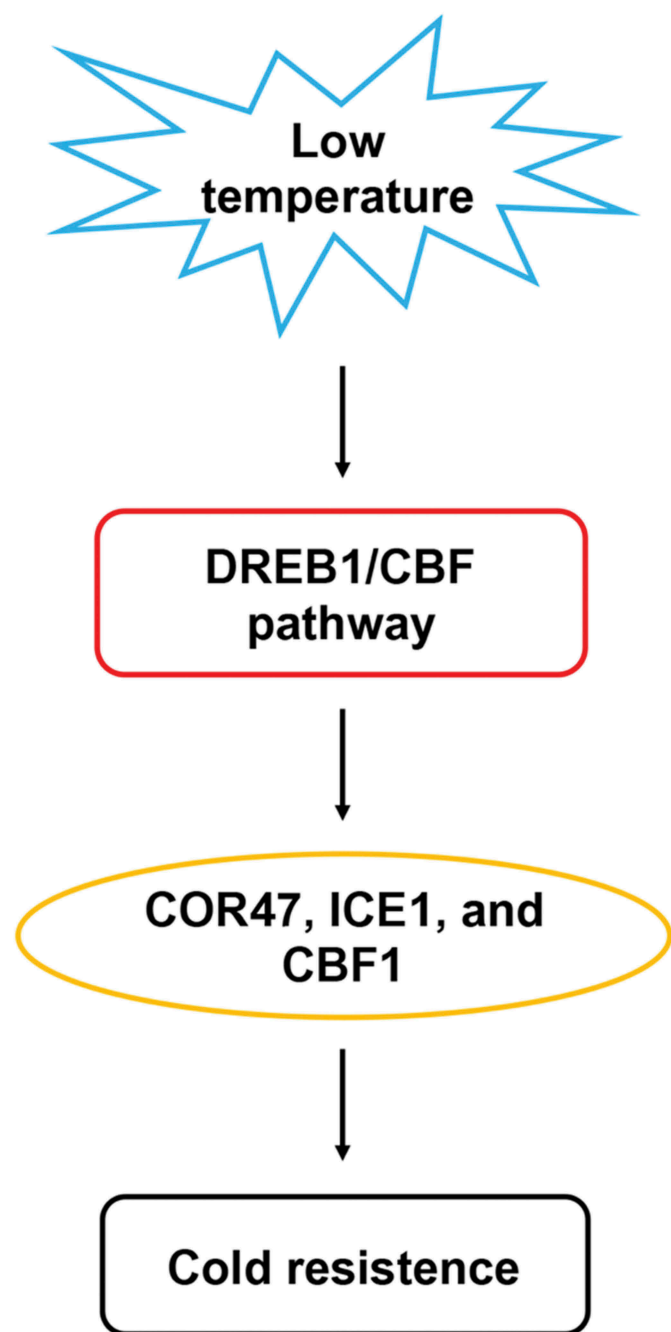


Figure 3. Regulation network model of DREB1/CBF pathway.

pathway analysis was performed, showing that cold-regulated genes were also involved in photosynthesis, circadian rhythm, metabolism, and transcriptional regulation.<sup>126</sup>

Aquaporin (AQP) membrane channels play essential roles in high salt tolerance and drought tolerance in *E. salsugineum*. Researchers have cloned a tonoplast AQP gene (*TsTIP1;2*) from the *E. salsugineum* and identified its functions. *TsTIP1;2*-transformed *Arabidopsis* showed strikingly strengthened tolerance to oxidative, drought, and salt stresses. And *TsTIP1;2*-expressed in *Xenopus* oocytes revealed water channel activity. Also, *TsTIP1;2* could conduct H<sub>2</sub>O<sub>2</sub> molecules into yeast cells under oxidative stress.<sup>44</sup> This study showed that *TsTIP1;2* played multifunctional roles in the survival of *E. salsugineum*.

### Future perspective

Herein, we have described the complicated regulatory processes that coordinate tolerance and responses to abiotic stresses in halophytes. We anticipate several major future areas of active investigation in plant responses to abiotic stresses. First, emerging sequencing techniques and platforms will help researchers characterize individual regulatory components in detail.<sup>128–131</sup> Second, genomic-scale experimental data, coupled with computational biology modeling, will serve as clues to discover stress response regulatory genes.<sup>132–135</sup> In future, we will see a much clearer picture of the abiotic stress signaling pathway among various species.

### Conflict of interest

Authors state no conflict of interest.

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