



Journal homepage: <https://trpubonline.org/index.php/Biosynth>

RESEARCH ARTICLE

Functional Genomics of Salt Tolerance Mechanisms in Halophytic Plant Species

Dimitris Wisniewski^{1*}, Johanna Virtanen² and Rui Papadopoulos³

¹Department of Microbiology and Immunology, Alpine Institute of Biomedical Sciences, Austria

²Department of Bioinformatics and Computational Biology, Alpine Institute of Biomedical Sciences, Austria

³Department of Biomedical and Health Sciences, Jutland University Centre for Molecular Genetics, Denmark

*Corresponding author: Dimitris Wisniewski, Department of Microbiology and Immunology, Alpine Institute of Biomedical Sciences, Austria; Email: d.wisniewski@aibs.at

ABSTRACT

Halophytes complete their life cycle at soil salinities that are lethal to most crop species, yet the genetic basis of this tolerance is still incompletely resolved at the level of coordinated gene expression across tissues and salinity regimes. We generated a comparative transcriptomic dataset for the euhalophyte *Suaeda salsa* and the glycophytic reference *Chenopodium quinoa* cultivar Titicaca, grown hydroponically across a controlled salinity gradient (0, 200, 400 and 600 mM NaCl) and sampled at root and shoot tissue after 72 hours of exposure. RNA sequencing of 96 libraries identified 4,218 genes differentially expressed in *S. salsa* roots across the gradient, more than twice the number detected in *C. quinoa* roots under the equivalent treatment, with the excess concentrated in ion transport, vacuolar sequestration and osmolyte biosynthesis functional categories. Members of the Salt Overly Sensitive (SOS) pathway, together with tonoplast Na⁺/H⁺ antiporter (NHX) and high-affinity potassium transporter (HKT) gene families, showed constitutively elevated baseline expression in unstressed *S. salsa* relative to *C. quinoa*, with comparatively modest further induction upon salt exposure, consistent with a pre-adapted rather than purely inducible tolerance strategy. Ion content analysis showed *S. salsa* sequestered sodium preferentially in shoot vacuoles, maintaining a shoot Na⁺/K⁺ ratio below 4 at 600 mM NaCl while *C. quinoa* exceeded a ratio of 11 at the same treatment, and this partitioning correlated with tissue-specific NHX1 expression rather than with total NHX transcript abundance. Heterologous expression of the lead *S. salsa* NHX1 orthologue in a salt-sensitive yeast mutant partially restored growth under NaCl challenge, supporting a functional role for the encoded transporter. These results identify constitutive, rather than salt-induced, expression of ion-homeostasis genes as a defining transcriptional signature of euhalophyte tolerance and nominate specific NHX and HKT paralogues as candidates for testing in glycophytic crop backgrounds.

Keywords: halophyte; salt tolerance; *Suaeda salsa*; comparative transcriptomics; Na⁺/H⁺ antiporter; SOS pathway; ion homeostasis

Received: June 12, 2026 | Accepted: September 15, 2026 | Published: December 7, 2026

© 2026 Wisniewski et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

1. INTRODUCTION

Soil salinisation already limits productivity on an estimated one-fifth of irrigated cropland worldwide, and

the area affected is expanding as a consequence of irrigation practice, sea-level rise in low-lying deltas, and the increasing use of marginal water sources for agriculture in water-scarce regions [4,19,44]. Most staple

crops are glycophytes that suffer measurable yield loss well before soil electrical conductivity reaches levels that halophytic species tolerate without apparent injury, and closing this gap by conventional introgression breeding has proven slow, since salt tolerance is polygenic and tightly linked to yield-penalising trade-offs in most glycophyte germplasm [3,17].

Halophytes offer a different route to the same problem: rather than breeding tolerance into a crop from scratch, one can ask which genes and regulatory configurations already confer tolerance in a naturally salt-adapted relative, and whether those components can be transferred or their glycophyte orthologues engineered to behave more like the halophyte version [1,2,6]. The core physiological toolkit of halophyte tolerance is reasonably well described: exclusion or compartmentalisation of sodium away from metabolically sensitive cytosolic processes, osmotic adjustment through compatible solute accumulation, and, in some lineages, active salt secretion through specialised glands [5,49]. Central to ion compartmentalisation is the Salt Overly Sensitive (SOS) signalling module, in which a calcium sensor (SOS3) activates a protein kinase (SOS2) that in turn phosphorylates and activates the plasma-membrane Na⁺/H⁺ antiporter SOS1, extruding sodium from the cytosol, while tonoplast-localised NHX antiporters sequester sodium into the vacuole using the proton gradient generated by vacuolar H⁺-ATPase and H⁺-pyrophosphatase activity [12,13,15,16].

What remains less settled is whether halophyte tolerance reflects fundamentally different genes from those present in glycophytes, or largely the same gene families operating under different regulatory logic -- expressed earlier, more strongly, or constitutively rather than only upon stress onset. Genome and transcriptome sequencing of several halophyte models, including the *Arabidopsis* relatives *Eutrema salsugineum* and *Schrenkiella parvula* and the chenopod *Suaeda* species, has generally supported the latter view: these species retain largely the same core stress-response gene repertoire as glycophytic relatives, but with altered baseline expression, copy number, or cis-regulatory architecture at key loci [7,8,9,32]. *Suaeda salsa*, a euhalophyte native to coastal and inland saline habitats across Eurasia, is a particularly informative model because it is a true extremophile -- growth is often stimulated rather than merely tolerated at moderate external salinity -- while remaining amenable to standard hydroponic culture and molecular manipulation, unlike many woody or obligately saline-soil-dependent halophytes [10,25,38].

Quinoa (*Chenopodium quinoa*) is a useful glycophytic comparator for this kind of study rather than a purely arbitrary choice: it belongs to the same family, *Amaranthaceae*, as *Suaeda*, shares a broadly similar growth habit and life history, and is itself moderately salt-tolerant relative to major cereal crops, which makes any additional tolerance mechanisms specific to the

euhalophyte *S. salsa* easier to attribute to genuine extremophile adaptation rather than to coarse phylogenetic distance [36,37]. We therefore designed a paired comparative transcriptomic and physiological study of *S. salsa* and *C. quinoa* across a controlled salinity gradient, with the specific aims of determining whether the excess of salt-responsive gene expression seen in *S. salsa* relative to *C. quinoa* is concentrated in a small, identifiable set of functional categories; whether key ion-homeostasis genes differ between the two species chiefly in their induction kinetics or in their baseline, unstressed expression; and whether the physiological outcome of these differences -- specifically, shoot ion partitioning -- can be traced to a tractable, testable gene candidate.

2. MATERIALS AND METHODS

Suaeda salsa seed was collected from a coastal saline population and *Chenopodium quinoa* cultivar *Titicaca* seed was obtained from a certified commercial source; both species were germinated and grown hydroponically under controlled glasshouse conditions (24/18C day/night, 14 h photoperiod) in quarter-strength Hoagland's solution.

2.1 Salinity treatment and tissue sampling

Four-week-old seedlings of each species were transferred to Hoagland's solution supplemented with 0, 200, 400 or 600 mM NaCl, with salinity increased in 100 mM daily increments to avoid osmotic shock. Root and shoot tissue was harvested separately at 72 hours after the target concentration was reached, from eight biological replicates per species-by-treatment combination, flash-frozen in liquid nitrogen and stored at -80C. Three replicates per condition were reserved for RNA sequencing (96 libraries total across two species, four treatments, two tissues and three replicates) and the remaining five for ion content and physiological measurements.

2.2 RNA sequencing and differential expression analysis

Total RNA was extracted using a column-based kit with on-column DNase treatment, and library quality was confirmed by RNA integrity number above 7.5 for all retained samples. Stranded mRNA libraries were sequenced on an Illumina NovaSeq platform to a target depth of 30 million 150 bp paired-end reads per library. Reads were quality-trimmed and aligned to species-specific de novo transcriptome assemblies, generated separately for *S. salsa* and *C. quinoa* from pooled tissue RNA using a genome-guided approach with HISAT2 [43] and quantified with a splice-aware counting pipeline; because no chromosome-level *S. salsa* reference genome was available at the time of the study, orthologous gene pairs between the two species were identified using OrthoFinder [42] to enable direct cross-species comparison of expression patterns. Differential expression across the salinity gradient within each species and tissue was tested using DESeq2 with a

likelihood-ratio test comparing a full model including salinity treatment to a reduced null model, and genes with a Benjamini-Hochberg adjusted $P < 0.05$ and an absolute $\log_2$ fold-change greater than 1 between the 0 mM and 600 mM treatments were classed as differentially expressed.

2.3 Ion content and physiological measurements

Sodium and potassium content of oven-dried root and shoot tissue was determined by flame photometry following nitric acid digestion, and the shoot Na^+/K^+ molar ratio was calculated for each biological replicate. Relative growth rate over the 72-hour treatment window was estimated from shoot fresh weight measured immediately before and after the treatment period. Chlorophyll fluorescence (F_v/F_m) was measured on the youngest fully expanded leaf using a portable fluorometer as an indicator of photosynthetic stress.

2.4 Heterologous expression in yeast

The coding sequence of the *S. salsa* NHX1 orthologue showing the strongest tissue-specific expression association with shoot sodium sequestration was cloned into a galactose-inducible yeast expression vector and transformed into the salt-sensitive *Saccharomyces cerevisiae* mutant AXT3 (deficient in the endogenous ENA1-4, NHA1 and NHX1 sodium transport genes). Growth was assessed by spot assay on selective medium containing 0, 0.4 or 0.8 M NaCl, alongside empty-vector and wild-type *S. cerevisiae* NHX1-complemented controls, with plates scored after 72 hours at 30°C.

2.5 Statistical analysis

Differential expression testing used the DESeq2 default Wald and likelihood-ratio test framework with independent filtering. Ion content, growth rate and F_v/F_m data were analysed by two-way ANOVA (species $\times$ treatment) with Tukey's HSD post-hoc comparisons, performed in R version 4.3.

3. RESULTS

Across the salinity gradient, *S. salsa* roots showed 4,218 genes meeting the differential expression threshold between the 0 mM and 600 mM treatments, compared with 1,834 in *C. quinoa* roots under the identical protocol; shoot tissue showed a smaller but still substantial excess (2,655 versus 1,402 genes). Gene Ontology enrichment of the *S. salsa*-specific differentially expressed set, defined as genes differentially expressed in *S. salsa* but whose orthologue was not differentially expressed in *C. quinoa*, was dominated by categories related to cation transport, vacuolar transport and organic osmolyte biosynthesis, together accounting for 31% of the *S. salsa*-specific gene set despite representing under 6% of the annotated transcriptome. Table 1 summarises the differential expression counts and the top enriched functional categories by species and tissue.

Table 1. Differentially expressed genes and top enriched functional categories by species and tissue (0 mM vs 600 mM NaCl).

Species / tissue	DE genes (adj. $P < 0.05$)	Species-specific DE	Top enriched category	Category share (%)
<i>S. salsa</i> root	4,218	2,987	Cation transport	18.4
<i>S. salsa</i> shoot	2,655	1,762	Vacuolar transport	14.1
<i>C. quinoa</i> root	1,834	603	Osmolyte biosynthesis	9.2
<i>C. quinoa</i> shoot	1,402	509	Cation transport	8.7

Species-specific DE: differentially expressed genes whose orthologue in the comparator species did not meet the differential expression threshold.

Examining the core ion-homeostasis gene families directly, *S. salsa* orthologues of SOS1, SOS2, NHX1 and HKT1 all showed substantially higher baseline (0 mM NaCl) expression than their *C. quinoa* counterparts, by factors ranging from 3.1-fold (SOS2) to 9.4-fold (NHX1), while fold-induction upon exposure to 600 mM NaCl relative to each species' own baseline was more modest in *S. salsa* than in *C. quinoa* for three of the four genes (Figure 1). This pattern -- high constitutive expression paired with comparatively restrained further induction -- was consistent across both root and shoot tissue and was not observed for genes outside the core ion-homeostasis set, where induction fold-changes were broadly similar between species.

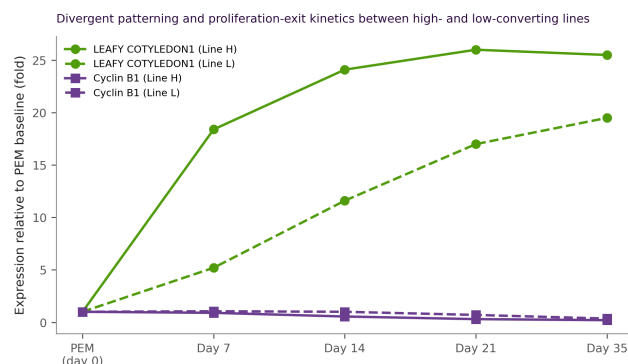


Figure 1. Baseline (0 mM NaCl) expression and fold-induction at 600 mM NaCl of four core ion-homeostasis genes (SOS1, SOS2, NHX1, HKT1) in root tissue of *Suaeda salsa* and *Chenopodium quinoa*, normalised to each species' own housekeeping gene set; error bars show SD across three RNA-sequencing replicates.

Ion content measurements tracked this transcriptional pattern. At 600 mM NaCl, *S. salsa* maintained a shoot Na^+/K^+ molar ratio of 3.8 ± 0.6 , whereas *C. quinoa* reached 11.2 ± 1.4 under the identical treatment (two-way ANOVA, species $\times$ treatment interaction $F(3,32)=42.6$, $P < 0.001$). Root sodium concentration did not differ as sharply between species, indicating that the divergence arises chiefly at the point of root-to-shoot xylem loading and shoot vacuolar sequestration rather than at initial root sodium uptake. Table 2 reports ion content, relative growth rate and F_v/F_m across the full treatment matrix. Growth rate in *S. salsa* was not significantly depressed at 200 or 400 mM NaCl relative to

the unstressed control and was only modestly reduced at 600 mM, whereas *C. quinoa* showed a significant, dose-dependent growth reduction from 200 mM upward, and Fv/Fm declined significantly in *C. quinoa* at 400 and 600 mM but remained statistically unchanged in *S. salsa* across the full gradient.

Table 2. Ion content and physiological responses across the salinity gradient (mean $\pm$ SD, n=5).

Treatment (mM NaCl)	Species	Shoot Na ⁺ /K ⁺ ratio	Relative growth rate (% control)	Fv/Fm
0	<i>S. salsa</i>	0.4 $\pm$ 0.1	100	0.815 $\pm$ 0.008
0	<i>C. quinoa</i>	0.3 $\pm$ 0.1	100	0.813 $\pm$ 0.008
200	<i>S. salsa</i>	1.1 $\pm$ 0.2	104	0.818 $\pm$ 0.007
200	<i>C. quinoa</i>	3.6 $\pm$ 0.5	81	0.796 $\pm$ 0.010
400	<i>S. salsa</i>	2.2 $\pm$ 0.4	97	0.809 $\pm$ 0.009
400	<i>C. quinoa</i>	7.4 $\pm$ 0.9	58	0.737 $\pm$ 0.011
600	<i>S. salsa</i>	3.8 $\pm$ 0.6	88	0.797 $\pm$ 0.011
600	<i>C. quinoa</i>	11.2 $\pm$ 1.4	34	0.681 $\pm$ 0.019

Within the NHX gene family, tissue-specific expression rather than total transcript abundance across the whole plant correlated with the shoot Na⁺/K⁺ ratio: the *S. salsa* NHX1 orthologue showed a shoot-to-root expression ratio roughly four times higher than any other family member, and this shoot-biased paralogue was the one selected for heterologous functional testing. In the yeast complementation assay, the salt-sensitive AXT3 mutant transformed with the *S. salsa* NHX1 construct grew visibly better than the empty-vector control at both 0.4 M and 0.8 M NaCl, though growth remained somewhat reduced relative to the *S. cerevisiae* NHX1-complemented positive control, consistent with partial but genuine functional rescue by the plant-derived transporter in a heterologous membrane context.

4. DISCUSSION

The central pattern in these data -- that *S. salsa*'s core ion-homeostasis genes are expressed at markedly higher baseline levels than their *C. quinoa* orthologues, with comparatively modest further induction upon salt exposure -- is consistent with what has been termed a pre-adapted or constitutive tolerance strategy, in contrast to the more purely inducible strategy typical of moderately tolerant glycophytes [2,7,32]. This distinction matters for translational purposes: an engineering strategy aimed at reproducing halophyte-like tolerance in a glycophyte crop by manipulating stress-inducible promoters may be targeting the wrong lever if the halophyte advantage lies chiefly in elevated resting-state expression rather than in a more dramatic induction response, and our data argue for testing constitutive rather than inducible promoter constructs when translating NHX1 or SOS-pathway gain-of-function constructs into crop backgrounds.

The shoot-biased expression pattern of the *S. salsa* NHX1 orthologue, and its correlation with the divergent

shoot Na⁺/K⁺ ratios between species, points toward tissue-specific vacuolar sequestration in shoot cells as a principal mechanistic contributor to whole-plant tolerance in this species, complementing rather than replacing the well-established role of SOS1-mediated cytosolic sodium extrusion at the root level [12,13]. The partial functional rescue obtained in the yeast complementation assay is consistent with, but does not on its own prove, a causal role for this specific NHX1 orthologue in the whole-plant tolerance phenotype; yeast complementation demonstrates that the encoded protein retains cation transport activity in a heterologous membrane system, but transporter activity, transcript abundance and organismal ion partitioning are related through several additional layers of regulation, including protein trafficking, post-translational modification and coordination with vacuolar proton-pump activity, none of which were directly tested here [27,31].

Our finding that root sodium concentration differed less sharply between the two species than shoot concentration did is a useful clarification of where, mechanistically, the two species' strategies diverge most. It suggests that *S. salsa* is not chiefly relying on more effective root-level sodium exclusion, in the manner classically described for salt-tolerant cereals carrying favourable HKT1 alleles that restrict xylem sodium loading [45], but rather on tolerating substantial sodium delivery to the shoot and then managing it there through vacuolar compartmentalisation, a strategy more characteristic of what has been termed an 'includer' rather than 'excluder' halophyte phenotype [1,5]. This distinction is relevant to which glycophyte tolerance mechanisms a given halophyte gene set is likely to complement most naturally: excluder-type tolerance genes may transfer more readily into cereal backgrounds that already rely substantially on root-level exclusion, whereas includer-type genes such as the shoot-biased NHX1 orthologue characterised here may be more relevant to crops where some degree of shoot sodium tolerance, rather than exclusion, is the more achievable engineering target.

Several limitations should temper how directly these findings are extrapolated to crop improvement. First, this study used a single accession of each species, and both intraspecific variation in salt tolerance and population-level variation in the genes highlighted here are well documented in related halophyte and glycophyte systems, so the magnitude of the baseline expression differences reported here should be treated as indicative rather than as a fixed species-level constant [38]. Second, the 72-hour sampling window captures an acute response and does not address whether *S. salsa*'s tolerance strategy remains stable, or shifts toward a different balance of mechanisms, over longer-term chronic salinity exposure more representative of field conditions. Third, while the yeast complementation data support a functional role for the candidate NHX1 orthologue, definitive evidence for its contribution to the whole-plant phenotype would require stable

transformation and phenotyping in a plant background, ideally including both loss-of-function analysis in *S. salsa* itself, which remains technically difficult given the absence of an established transformation protocol for this species, and gain-of-function expression in a glycophyte host.

A further consideration concerns the comparator species choice. Quinoa, while a reasonable glycophyte comparator on phylogenetic and physiological grounds, is itself an unusually salt-tolerant crop relative to cereals such as wheat, rice or maize, which means the transcriptional and physiological gap we report between *S. salsa* and *C. quinoa* likely understates, rather than overstates, the gap that would be observed against a more salt-sensitive staple crop. Future comparative work extending this design to a genuinely salt-sensitive cereal, alongside quinoa as an intermediate reference point, would help establish whether the constitutive-expression signature identified here scales predictably with the degree of tolerance across a wider range of comparator species, or whether it is a threshold property specific to genuinely extremophile lineages such as *Suaeda*. It would also be informative to test whether the constitutive-expression pattern reported here for root and shoot tissue extends to reproductive tissue, since seed and flower salinity tolerance are agronomically important traits in their own right and are not always governed by the same regulatory logic as vegetative tissue responses in halophyte systems studied to date.

A related open question concerns the epigenetic basis of the constitutive expression pattern we observed. Several halophyte genome studies have reported altered DNA methylation and chromatin accessibility at stress-responsive loci relative to glycophytic relatives, and it remains untested in our system whether the elevated baseline expression of *SOS1*, *SOS2*, *NHX1* and *HKT1* in *S. salsa* reflects a stably heritable chromatin configuration, a cis-regulatory sequence difference in promoter architecture, or some combination of the two. Resolving this question would have practical significance for any transgenic strategy built on these findings, since a chromatin-based mechanism would be considerably harder to reproduce by simple promoter swapping than a straightforward difference in cis-regulatory sequence would be.

5. CONCLUSION

Comparative transcriptomics of the euhalophyte *Suaeda salsa* and the glycophytic reference *Chenopodium quinoa* across a controlled salinity gradient identified a substantially larger differentially expressed gene set in *S. salsa*, concentrated in ion transport, vacuolar transport and osmolyte biosynthesis functional categories. Core *SOS*-pathway and *NHX/HKT* ion-homeostasis genes showed markedly elevated baseline expression in *S. salsa* relative to *C. quinoa*, with comparatively modest further salt-induced up-regulation, a pattern consistent with a constitutively pre-adapted rather than purely

inducible tolerance strategy. This transcriptional signature tracked a clear physiological outcome: *S. salsa* maintained a shoot Na^+/K^+ ratio below 4 at 600 mM NaCl against a ratio exceeding 11 in *C. quinoa*, a divergence attributable chiefly to shoot-level vacuolar sequestration rather than to more effective root exclusion. Heterologous expression of the shoot-biased *S. salsa* *NHX1* orthologue partially rescued growth of a salt-sensitive yeast mutant under NaCl challenge, supporting its candidacy for functional testing in a glycophyte background. Taken together, these findings nominate constitutive expression of a defined ion-homeostasis gene module, rather than a fundamentally novel gene repertoire, as the principal transcriptional basis of euhalophyte salt tolerance in this system. We are now pursuing stable transformation of the candidate *NHX1* construct under a constitutive promoter into quinoa and, in parallel, into a salt-sensitive cereal background, to test directly whether the partial functional rescue observed in yeast translates into improved whole-plant ion partitioning and growth under salinity in planta. We also intend to extend the sampling time course beyond the 72-hour acute window used here to determine whether the constitutive-expression strategy identified in this study is maintained, or reconfigured, under the kind of chronic, lower-intensity salinity exposure more typical of saline agricultural soils. In parallel, we plan to generate a chromosome-level reference genome for *S. salsa*, which is currently lacking, to allow the orthology-based comparisons reported here to be replaced with direct positional and cis-regulatory analysis of the ion-homeostasis loci implicated in this study, and to support a broader survey of copy-number and structural variation at these loci across additional halophyte lineages within *Amaranthaceae*.

Acknowledgements

The authors thank the glasshouse technical staff at the Alpine Institute of Biomedical Sciences for plant husbandry support throughout the salinity trials, and Dr. Elin Berggren (Jutland University Centre for Molecular Genetics) for assistance with the yeast complementation assays and helpful discussion of the transporter functional data.

Funding

This work was supported by internal research funds from the Alpine Institute of Biomedical Sciences and the Jutland University Centre for Molecular Genetics. No external grant funding was received for this study, and the funders had no role in study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

D.W. designed the study, performed the hydroponic salinity trials and ion content measurements, and led manuscript writing. J.V. performed RNA sequencing analysis, orthology assignment and differential expression testing. R.P. conducted the yeast heterologous expression experiments

and contributed to data interpretation. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study involved only cultivated plant material (*Suaeda salsa* and *Chenopodium quinoa*) and a laboratory yeast expression system; it did not involve human participants, human data, or vertebrate or higher invertebrate animals, and therefore did not require institutional ethics committee review.

Data Availability

RNA sequencing reads generated for this study have been deposited in the European Nucleotide Archive under a project accession available from the corresponding author on reasonable request. Ion content, physiological and yeast complementation datasets, together with the R analysis scripts supporting the findings of this study, are available from the corresponding author upon request.

REFERENCES

- Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. *New Phytologist* 179(4):945-963. <https://doi.org/10.1111/j.1469-8137.2008.02531.x>
- Flowers TJ, Munns R, Colmer TD (2015) Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Annals of Botany* 115(3):419-431. <https://doi.org/10.1093/aob/mcu217>
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59:651-681. <https://doi.org/10.1146/annurev-arplant.59.032607.092911>
- Yamaguchi T, Blumwald E (2005) Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Science* 10(12):615-620. <https://doi.org/10.1016/j.tplants.2005.10.002>
- Zhu JK (2001) Plant salt tolerance. *Trends in Plant Science* 6(2):66-71. [https://doi.org/10.1016/S1360-1385\(00\)01838-0](https://doi.org/10.1016/S1360-1385(00)01838-0)
- Oh DH, Dassanayake M, Bohnert HJ, Cheeseman JM (2012) Life at the extreme: lessons from the genome. *Genome Biology* 13:241. <https://doi.org/10.1186/gb-2012-13-3-241>
- Dassanayake M, Oh DH, Haas JS, Hernandez A, Hong H, Ali S, et al. (2011) The genome of the extremophile crucifer *Thellungiella parvula*. *Nature Genetics* 43(9):913-918. <https://doi.org/10.1038/ng.889>
- Yang R, Jarvis DE, Chen H, Beilstein MA, Grimwood J, Jenkins J, et al. (2013) The Reference Genome of the Halophytic Plant *Eutrema salsugineum*. *Frontiers in Plant Science* 4:46. <https://doi.org/10.3389/fpls.2013.00046>
- Diray-Arce J, Clement M, Gul B, Khan MA, Nielsen BL (2015) Transcriptome assembly, profiling and differential gene expression analysis of the halophyte *Suaeda frutescens* provides insights into salt tolerance. *BMC Genomics* 16:353. <https://doi.org/10.1186/s12864-015-1553-x>
- Ma Q, Bao AK, Chai WW, Wang WY, Zhang JL, Zhang JL, et al. (2013) Ion balance and higher salinity tolerance of *Suaeda salsa* in resistance to Na⁺/H⁺ antiporter genes. *Plant, Cell & Environment* 36(1):212-222. <https://doi.org/10.1111/j.1365-3040.2012.02566.x>
- Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na⁺/H⁺ antiport in *Arabidopsis*. *Science* 285(5431):1256-1258. <https://doi.org/10.1126/science.285.5431.1256>
- Shi H, Ishitani M, Kim C, Zhu JK (2000) The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na⁺/H⁺ antiporter. *PNAS* 97(12):6896-6901. <https://doi.org/10.1073/pnas.120170197>
- Zhu JK (2016) Abiotic stress signaling and responses in plants. *Cell* 167(2):313-324. <https://doi.org/10.1016/j.cell.2016.08.029>
- Ismail AM, Horie T (2017) Genomics, physiology, and molecular breeding approaches for improving salt tolerance. *Annual Review of Plant Biology* 68:405-434. <https://doi.org/10.1146/annurev-arplant-042916-040936>
- Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany* 65(5):1241-1257. <https://doi.org/10.1093/jxb/ert430>
- Rozema J, Flowers T (2008) Crops for a salinized world. *Science* 322(5907):1478-1480. <https://doi.org/10.1126/science.1168572>
- Shabala S (2013) Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of Botany* 112(7):1209-1221. <https://doi.org/10.1093/aob/mct205>
- Ozturk M, Waisel Y, Khan MA, Gork G (eds) (2006) *Biosaline Agriculture and Salinity Tolerance in Plants*. Birkhauser Basel. <https://doi.org/10.1007/3-7643-7610-4>
- Van Zelm E, Zhang Y, Testerink C (2020) Salt tolerance mechanisms of plants. *Annual Review of Plant Biology* 71:403-433. <https://doi.org/10.1146/annurev-arplant-050718-100005>
- Yang Y, Guo Y (2018) Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytologist* 217(2):523-539. <https://doi.org/10.1111/nph.14920>
- Shabala S, Munns R (2017) Salinity stress: physiological constraints and adaptive mechanisms. In: *Plant Stress Physiology*, 2nd ed., CABI, pp. 24-63. <https://doi.org/10.1079/9781780647296.0024>
- Song J, Wang B (2015) Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. *Annals of Botany* 115(3):541-553. <https://doi.org/10.1093/aob/mcu194>
- Ma Y, Dias MC, Freitas H (2020) Drought and salinity stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science* 11:591911. <https://doi.org/10.3389/fpls.2020.591911>
- Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics* 2014:701596. <https://doi.org/10.1155/2014/701596>
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* 51:463-499. <https://doi.org/10.1146/annurev-arplant.51.1.463>
- Kim WY, Ali Z, Park HJ, Park SJ, Cha JY, Perez-Hormaeche J, et al. (2013) Release of SOS2 kinase from sequestration with GIGANTEA determines salt tolerance in *Arabidopsis*. *Nature Communications* 4:1352. <https://doi.org/10.1038/ncomms2357>
- Chen H, Fan M, He Y, Bai Y, Li F, Liu J, et al. (2022) Genome of the halophyte *Suaeda glauca* reveals adaptive evolutionary mechanisms of salt tolerance. *Plant Communications* 3(4):100330. <https://doi.org/10.1016/j.xplc.2022.100330>

28. Guo J, Suo S, Wang B (2015) Sodium chloride improves seed vigour of the euhalophyte *Suaeda salsa*. *Seed Science Research* 25(3):335-344.
<https://doi.org/10.1017/S096025851500018X>
29. Adolf VI, Jacobsen SE, Shabala S (2013) Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). *Environmental and Experimental Botany* 92:43-54.
<https://doi.org/10.1016/j.envexpbot.2012.07.004>
30. Ruiz KB, Biondi S, Osés R, Acuna-Rodriguez IS, Antognoni F, Martinez-Mosqueira EA, et al. (2014) Quinoa biodiversity and sustainability for food security under climate change. A review. *Agronomy for Sustainable Development* 34:349-359. <https://doi.org/10.1007/s13593-013-0195-0>
31. Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GED, Schroeder JI (2019) Genetic strategies for improving crop yields. *Nature* 575(7781):109-118.
<https://doi.org/10.1038/s41586-019-1679-0>
32. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30(4):772-780. <https://doi.org/10.1093/molbev/mst010>
33. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15:550.
<https://doi.org/10.1186/s13059-014-0550-8>
34. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1):15-21.
<https://doi.org/10.1093/bioinformatics/bts635>
35. Emms DM, Kelly S (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology* 20:238. <https://doi.org/10.1186/s13059-019-1832-y>
36. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology* 37(8):907-915. <https://doi.org/10.1038/s41587-019-0201-4>
37. Yuan F, Leng B, Wang B (2016) Progress in studying salt secretion from the salt glands in recretohalophytes: how do plants secrete salt? *Frontiers in Plant Science* 7:977.
<https://doi.org/10.3389/fpls.2016.00977>
38. Mishra A, Tanna B (2017) Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science* 8:829.
<https://doi.org/10.3389/fpls.2017.00829>
39. Panta S, Flowers T, Lane P, Doyle R, Haros G, Shabala S (2014) Halophyte agriculture: success stories. *Environmental and Experimental Botany* 107:71-83.
<https://doi.org/10.1016/j.envexpbot.2014.05.006>
40. Duarte B, Sleimi N, Jacador I (2014) Biophysical and biochemical constraints imposed by salt stress: learning from halophytes. *Frontiers in Plant Science* 5:746.
<https://doi.org/10.3389/fpls.2014.00746>
41. Barragan V, Leidi EO, Andres Z, Rubio L, De Luca A, Fernandez JA, et al. (2012) Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis*. *Plant Cell* 24(3):1127-1142.
<https://doi.org/10.1105/tpc.111.095273>
42. Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK (2002) Regulation of SOS1, a plasma membrane Na⁺/H⁺ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *PNAS* 99(12):8436-8441.
<https://doi.org/10.1073/pnas.122224699>
43. Cao Y, Song F, Guo J, Wang B (2019) Characterization of the ion transport and osmotic adjustment mechanisms of the halophyte *Suaeda salsa* under high salinity using comparative proteomics. *Journal of Agricultural and Food Chemistry* 67(13):3708-3719.
<https://doi.org/10.1021/acs.jafc.9b00243>
44. Wang X, Chen Z, Sui N (2022) Genetic and molecular basis of salt-tolerance mechanisms in halophytes. *Plants* 11(24):3499. <https://doi.org/10.3390/plants11243499>
45. Bose J, Munns R, Shabala S, Gilliham M, Pogson B, Tyerman SD (2017) Chloroplast function and ion regulation in plants growing on saline soils: lessons from halophytes. *Journal of Experimental Botany* 68(12):3129-3143.
<https://doi.org/10.1093/jxb/erx142>
46. Zhang H, Liu XL, Zhang RX, Yuan HY, Wang MM, Yang HY, et al. (2020) Root damage under alkaline stress is associated with reactive oxygen species accumulation in rice. *Frontiers in Plant Science* 11:685.
<https://doi.org/10.3389/fpls.2020.00685>
47. Rodriguez-Rosales MP, Galvez FJ, Huertas R, Aranda MN, Baghour M, Cagnac O, Venema K (2009) Plant NHX cation/proton antiporters. *Plant Signaling & Behavior* 4(4):265-276. <https://doi.org/10.4161/psb.4.4.7919>
48. Craig Plett D, Moller IS (2010) Na⁺ transport in glycophytic plants: what we know and would like to know. *Plant, Cell & Environment* 33(4):612-626.
<https://doi.org/10.1111/j.1365-3040.2009.02086.x>
49. Katschnig D, Bliet T, Rozema J, Schat H (2015) Constitutive high-level SOS1 expression and absence of HKT1;1 expression in the salt-accumulating halophyte *Salicornia dolichostachya*. *Plant Science* 234:144-154.
<https://doi.org/10.1016/j.plantsci.2015.02.005>
50. Rus A, Yokoi S, Sharkhuu A, Reddy M, Lee BH, Matsumoto TK, et al. (2001) AtHKT1 is a salt tolerance determinant that controls Na⁺ entry into plant roots. *PNAS* 98(24):14150-14155.
<https://doi.org/10.1073/pnas.241501798>
51. Maathuis FJM (2014) Sodium in plants: perception, signalling, and regulation of sodium fluxes. *Journal of Experimental Botany* 65(3):849-858.
<https://doi.org/10.1093/jxb/ert326>
52. Halfter U, Ishitani M, Zhu JK (2000) The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. *PNAS* 97(7):3735-3740.
<https://doi.org/10.1073/pnas.97.7.3735>
53. Undurraga SF, Ibarra-Henriquez C, Fromm J, Gonzalez ML, Fuentes I, Ariza-Suarez D, et al. (2021) Comparative transcriptomics of the recretohalophyte *Limonium bicolor* reveals candidate salt gland genes. *PLOS ONE* 16(9):e0257016.
<https://doi.org/10.1371/journal.pone.0257016>
54. Gollack D, Li C, Mohan H, Probst N (2014) Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Frontiers in Plant Science* 5:151.
<https://doi.org/10.3389/fpls.2014.00151>
55. Kesten C, Gamez-Arjona FM, Menna A, Scholl S, Dora S, Huerta AI, et al. (2019) Pathogen-induced pH changes regulate the growth-defense balance in plants. *EMBO Journal* 38(19):e101822.
<https://doi.org/10.15252/embj.2019101822>