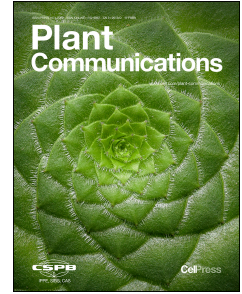# Journal Pre-proof

Chasing the Mechanisms of Ecologically Adaptive Salinity Tolerance

Silvia Busoms, Sina Fischer, Levi Yant

Please cite this article as: Busoms, S., Fischer, S., Yant, L., Chasing the Mechanisms of Ecologically Adaptive Salinity Tolerance, *PLANT COMMUNICATIONS* (2023), doi: https://doi.org/10.1016/j.xplc.2023.100571.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Chasing the Mechanisms of Ecologically Adaptive Salinity Tolerance

Silvia Busoms[1,#], Sina Fischer[2,3,#], and Levi Yant[2,4]

[1]Plant Physiology Laboratory, Bioscience Faculty, Universitat Autònoma de Barcelona, Bellaterra, Barcelona E-08193, Spain.

[2]Future Food Beacon of Excellence, University of Nottingham, Nottingham, NG7 2RD, UK.

[3]School of Biosciences, University of Nottingham, Nottingham, NG7 2RD, UK.

[4]School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK.

Running title: Evolved Mechanisms of Salinity Tolerance

Contact: *Levi Yant levi.yant@nottingham.ac.uk*

# contributed equally

Abstract:
Plants adapted to challenging environments offer fascinating models of evolutionary change. Importantly, they also give important information to meet our pressing need to develop resilient, low-input crops. With mounting environmental fluctuation – including temperature, rainfall, soil salinity and degradation – this is more urgent than ever. Happily, solutions are hiding in plain sight: the adaptive mechanisms from natural adapted populations, once understood, can then be leveraged. Much recent insight has come from the study of salinity, a widespread factor limiting productivity, with estimates of 20% of all cultivated lands affected. This is an expanding problem, given increasing climate volatility, rising sea levels and increasing irrigation. We therefore highlight recent benchmark studies of ecologically adaptive salt tolerance in plants, assessing macro- and microevolutionary mechanisms, and the recently recognised role of ploidy and the microbiome on salinity adaptation. We synthesise insight specifically on naturally evolved adaptive salt-tolerance mechanisms, as these works move substantially beyond traditional mutant or knockout studies, to show how evolution can nimbly 'tweak' plant physiology to optimise function. We then point to future directions to advance this field, that intersects evolutionary biology, abiotic-stress tolerance, breeding and molecular plant physiology.

Keywords:
Adaptation, salinity, polyploidy, microbiome, evolution, ecology

Short Summary:
Adaptive natural responses to saline soils serve as powerful examples of evolutionary change and allow inference for rational crop development. Mechanistic insight into these evolved responses has increased dramatically, with notable progress in our understanding of the effects of polyploidy and the microbiome. Here we synthesise this work, highlighting benchmark studies deconstructing mechanisms of adaptation using genomic, functional, and ecological approaches.

Widespread, but Costly, and Transitory? The Evolution of Salinity Tolerance

While sodium is an essential plant nutrient, high concentrations of Na^+ ions severely inhibit growth (Bernstein, 1975; Greenway and Munns, 1980). This effect, termed salinity stress, is linked to water uptake challenges (Reina-Sánchez *et al.*, 2005), impaired metabolic processes (Che-Othman *et al.*, 2017), and decreased photosynthesis (Ashraf & Harris, 2013). Plants can employ diverse strategies to mitigate these impacts, with the result that rapid adaptive evolution is seen in many taxa, mediating varying degrees of tolerance. At the high end, the term halophyte is reserved for lineages endemic to salty habitats, specifically growing in salinities greater than 200 mM NaCl (Flowers & Colmer, 2008).

Salinity tolerance can arise rapidly and can vary dramatically between species (Flowers *et al.*, 2010). This rapid evolution has been linked to dynamic environmental conditions which serve as drivers of plant adaptation to salinity and other soil-related (edaphic) stressors (Cheeseman, 2015). In some families, however, salinity tolerance evolved early and has been broadly retained. For example, in Chenopodiaceae, adaptations such as succulence and other physiological mechanisms are derived from C_3 lineages, and have been conserved mainly in the evolved C_4 salt-tolerant species (Kadereit *et al.*, 2012). However, in other groups, with grasses as a prime example, there have been many independent origins of salinity tolerance, most of which are recent and result in only one or a few salinity-tolerant species each (Bennett *et al.*, 2013; Moray *et al.*, 2015). In most orders that contain halophytes, these comprise 1% or less of lineages, indicating a secondary evolution of the derived trait (Flowers *et al.*, 2010). Thus, there is now general agreement that the most parsimonious scenario is that halophytes more commonly evolve independently in taxonomically diverse lineages (Bromham, 2015). For instance, the distribution of salt glands in over 50 plant species in several different families, indicates that this innovation evolves repeatedly in species adapted to saline environments, not only to avoid Na^+ and/or Cl^- toxicity but also to regulate Ca^{2+} concentrations in the aerial tissues (Dassanayake & Larkin, 2017; Caperta *et al.*, 2020). This raises the question: what underlies such convergence? There are good examples of what precedes it. For example, preadaptation to high salinity can be seen across the plant kingdom, with the required physiological or anatomical changes building rapidly on precursor traits acquired earlier (Moray *et al.*, 2015). For example, grasses with C_4 photosynthesis have a greater rate to gain and

2

81 lose tolerance (Bennett *et al.*, 2013), possibly because C$_4$ increases water-use
82 efficiency, limiting water stress and reducing ion uptake (Bromham & Bennett, 2014).
83 Morphological specializations such as vivipary and aerial roots have also been seen
84 as facilitating adaptation to harsh coastal environments in mangrove species (Shi *et*
85 *al.*, 2005). Therefore, it is important to not underestimate these latent traits that do not
86 fit into the classical physiological mechanisms of salinity tolerance, because they can
87 facilitate novel adaptations in plants evolving in saline environments.
88
89 The fact that salinity tolerance does tend to occur recently at the 'tips' of phylogenies,
90 rather than the bases, suggests some inherent cost, which may lead to reversion or
91 eventual extinction (Bromham *et al.*, 2020). This may also be linked to biogeography:
92 although in some saline regions, such as along coasts, salinity can persist for long
93 periods; in others salinity can vary over small spatial scales or shift at the population
94 level seasonally (e.g. Busoms *et al.*, 2018). If lineages are rapidly responding to
95 fluctuating salinity (with high transition rates), this could partly explain why we infer
96 mostly shallow gains of salinity tolerance that give rise to only one or a few extant
97 halophytes (Bromham, 2015). Another explanation for why there are so many small
98 clades of halophytes is that salinity tolerance may be costly (Munns *et al.*, 2020) and
99 thus difficult to maintain. For example, high phenotypic plasticity or capability could
100 enable some lineages to transition into harsh novel habitats over evolutionarily short
101 time scales (Edwards & Donoghue, 2013). However, maintaining salinity tolerance
102 requires plants to produce osmolytes or investment in ROS scavenging and
103 antioxidant production. Key enzymes in the detoxification of ROS are encoded by the
104 RBOH genes. A recent review traced the evolution of salinity tolerance through
105 changes in RBOH genes and showed a reduction in the number of isoforms to
106 correlate with increases in salinity tolerance. Additionally, it showed that rather than
107 forge new proteins, salt-tolerant plants modified RBOH protein phosphorylation sites
108 which allows for improved activation of RBOH proteins (Liu *et al.*, 2020b). This
109 impressive efficiency contrasts with the general view that high physiological costs
110 lead to increased extinction rates in halophytes, or high reversal rates of lineages that
111 invest less in tolerance mechanisms have a strong competitive advantage. Such a
112 view has been put forward to help explain why individuals from the same species
113 adapted to coastal conditions perform more poorly in inland sites where conditions
114 are usually more favourable to the species as a whole (e.g. Nagy **&** Rice, 1997).

115

Evolutionary Dynamics of Adaptive Salinity Tolerance

116

117 Ecological specialization occurs primarily through local adaptation (VanWallendael *et*
118 *al.*, 2019), a process often required for successful establishment of populations in
119 challenging new habitats. In this scenario, reproductive assurance (the ability to
120 reproduce in small and/or isolated populations), and some prevention of gene flow from
121 less fit relatives, are crucial. In an early work, Lowry and Willis showed that
122 chromosomal inversions in *Mimulus* species contribute to reproductive isolation
123 barriers between coastal and inland ecotypes of this species (Lowry & Willis, 2010).
124 For the newly adapted population, a reproductive assurance can be gained by a
125 transition to selfing during this time (Wright *et al.*, 2013). However, outcrossers,
126 especially obligate outcrossers, have high genetic variability which of course facilitates
127 adaptive evolution. Other phenological changes, particularly a shift in flowering time,
128 also lead to reproductive isolation (McNeilly & Antonovics, 1968), boosting the
129 likelihood that young adapted lineages may avoid influx of maladaptive genotypes from
130 neighbours.

131

132 Halophyte species have evolved a range of adaptations to tolerate high concentrations
133 of salts and colonize harsh environments (see Flowers & Colmer, 2015 for an excellent
134 discussion). Thus, they can be a powerful genetic resource for biosaline agriculture.
135 However, a lack of genomic information and low genetic similarity with major crops
136 have compelled a focus on generic physiological mechanisms or particular gene
137 variants that might be introduced in salt-sensitive species (Shabala et al., 2013;
138 Abobatta et al., 2020). Despite a strong focus of modern research yielding advances
139 on our understanding of adaptive mechanisms of halophytes (reviewed recently in
140 Rahman et al., 2021), the molecular mechanisms of whole plant adaptive responses
141 to salinity are still unclear. A reason for this is that salinity tolerance in halophyte
142 species is by definition constitutive to the entire species: thus, intraspecies variation is
143 scant in halophytes, hindering e.g. genome-wide association studies in discovering
144 novel allelic candidates. That is why choosing non-halophyte species with contrasting
145 within-species phenotypes in salinity tolerance is a particularly good approach for
146 uncovering the mechanisms of ecologically adaptive salinity tolerance.

147

148 To date, local adaptation to high salinity has been typically associated with oligogenic

149 architectures. In contrast to polygenic changes, which are defined by consisting of

150 many genes with small effects, "oligogenic" indicates the involvement of few major

151 effect loci, with single alleles explaining up to 10% of the observed variation (Bell,

152 2009). For salinity tolerance this often involves mutations of ion transporters and

153 pumps (Volkov, 2015), either in their coding regions or mutations with effects on

154 expression. It is thought that the type of genetic architecture (e.g. either oligogenic or

155 polygenic) may be dependent on the type of environment and therefore the type of

156 selection in a particular context (Whiteman, 2022). Accordingly, it is important to note

157 that despite our ability to explain large parts of this adaptive variation, in the cases

158 where we have been able to find a major effect locus underlying adaptation to high

159 salinity, such as the *HIGH AFFINITY POTASSIUM TRANSPORTER* (*HKT1*) in *A.*

160 *thaliana* (An *et al.*, 2017), still the majority of the variation is left unexplained and is

161 likely due to the effects of many other genes.

162

163 Single-locus control of complex traits that do not obey a simple Mendelian inheritance

164 pattern is uncommon, but blocks of linked genes, such as those associated with some

165 types of structural genomic variation (SV, genomic variants > 50bp), are rapidly

166 emerging as important in species subjected to environmental pressures (Zhang et al.,

167 2021). For example, haplotype blocks associated with seed size, flowering time and

168 soil fertility in dune-adapted sunflower species were found to be highly divergent and

169 associated with structural variants (Todesco *et al.*, 2020). Also, natural variation (InDel)

170 in the promoter of Gs*ERD15B* found in wild soybean affects the expression of this gene

171 and others related to salinity tolerance mechanisms (Jin *et al.*, 2021). Linkage among

172 such variants may then be advantageous in loci under positive selection because it

173 can allow the rapid, joint recruitment of multiple genes. However, under directional

174 selection, local adaptation may also be based on successive recruitment of alleles at

175 different loci, a process made possible by reduced gene flow (Llaurens *et al.*, 2017).

176 We do not yet have a good concept for how salt stress generally acts on recruitment

177 of new "tolerance loci" and further research should explore these concepts to shed

178 more light here.

179

180 Contrary to traits under selection, where new adaptive combinations may rapidly

181 replace ancestral ones, in traits under balancing selection, several alternative

182 combinations may be maintained at relatively high frequencies, providing ample
183 opportunity for recombination to adjust phenotypes by generating diverse
184 combinations of polymorphisms (Delph & Kelly, 2014). Here, also HKT1 provides a
185 clear example where we can see balancing selection in the context of adaptive
186 evolution to increased salinity (below; Busoms *et al.*, 2018).

187

188   **Box 1. Salinity Adaptation-Relevant Evolutionary Terminology.**

189 Genetic diversity depends in part on ***de novo* mutations** entering a population and their effect on fitness.
190 Most novel mutations are thought to be either deleterious or neutral, but occasionally they can be
191 beneficial (e.g. Jin et al., 2021).
192 **Directional selection** can cause advantageous alleles to become more frequent in a population, driven
193 by a selective advantage. Directional selection often reduces the diversity of alleles around a causative
194 locus, and therefore, at least briefly, the genetic variation in a population, in the form of bottlenecks. But
195 this reduction in local genomic diversity is of course beneficial when it leads to **local adaptation**. Here,
196 for example, when salinity acts as an agent of directional selection favouring alleles that allow plant
197 survival in coastal habitats (e.g. Busoms *et al.*, 2015).
198 **Purifying selection** is a prevalent form of natural selection that constantly removes deleterious
199 mutations. However, purifying selection is weak enough for some mutations to be able to establish
200 themselves in the population if purifying selection is of the same order or lower than genetic drift. Where
201 purifying selection is weak, **standing variation** is increased, providing a substrate upon which selection
202 may act (e.g. Wang *et al.*, 2021).
203 **Genetic drift** is the change in allele frequencies that occurs mainly in smaller (or inbred) populations
204 due to the random sampling of alleles. Genetic drift can be distinguished from selection because the
205 entire genome is generally affected, not only a single locus. It is worth noting in respect to salinity
206 adaptation, it is very likely that isolated populations suffer genetic drift, which counter the maintenance
207 of adaptation to salinity, with little alternative but to migrate inland or go extinct (e.g. Prinz *et al.*, 2013).
208 **Migration** is a counteracting force to genetic drift. By mixing alleles among populations, migration
209 distributes and homogenizes genetic variation across species ranges, countering strong directional
210 selection and bottlenecks. Migration can also contribute to **negative frequency-dependent selection**,
211 favouring rare immigrants over locally adapted plants (e.g. Posavi *et al.*, 2014).
212 **Balancing selection** occurs when multiple alleles are maintained in a population, which can result in
213 their preservation over long periods. Such selection occurs in intermediate salinity sites or fluctuating
214 environments, and it allows two or more allelic groups to be maintained in a population, in many cases
215 reproductively isolated, at the same site (e.g. Busoms *et al.*, 2018).

216

217 It is now clear that even the frequency of *de novo* mutation varies considerably across
218 the genome (Lynch *et al.*, 2016; Monroe *et al.*, 2022), with mutation bias (Martincorena
219 & Luscombe, 2013) holding broad consequences for our consideration of the
220 mechanisms of evolutionary change. In fact, this mutation bias can interact with salinity
221 specifically: in controlled conditions, Jiang *et al.* (2014) found that even in short-term
222 mutation accumulation experiments of less than a dozen generations, *A. thaliana*
223 subjected to salinity stress accumulated twice as many mutations, and that these
224 mutations actually exhibit a distinctive spectrum. In particular, they accumulated
225 around 45% more differentially methylated cytosine positions at CG sites (CG-DMPs)

than controls; and stress-associated CG-DMPs arose more frequently in genic rather than in non-genic regions of the genome. Further, Lu *et al.* (2021) concluded that heat stress over multiple generations accelerated mutation accumulation in intergenic regions, coding regions, and transposable elements, as well as non-synonymous mutations in functional genes. These results suggest that commonly encountered environmental stresses can accelerate the accumulation of mutations, and change the profiles of novel variants.

Importantly, work to date has focused on SNPs rather than SV; even so, some of the clearest cases of adaptive evolution to edaphic stressors are SV, such as HMA4, HKT1 and MOT1 (Hanikenne *et al.*, 2013; Busoms *et al.*, 2018, 2021). For example, in the case of HMA4, a gene triplication set the stage for positive selection at the promoter region of this gene that results in elevated levels of gene product, improving heavy metal tolerance (Hanikenne *et al.*, 2013). Different structural variants of HKT1 were associated with habitats close to the sea, and thus salt (Busoms *et al.*, 2018), and deletions and duplications around the MOT1 gene have been associated with biomass and fitness changes under salinity stress (Busoms *et al.*, 2021). Accordingly, we predict that the currently estimated impact of SV is greatly underestimated, and this will change once efficient population-level SV assessment is broadly applied. This time is not far off: pangenome approaches have recently made great advances with the improvements in both sequencing technologies (Campoy *et al.*, 2020; Della Coletta *et al.*, 2021; Meier *et al.*, 2021) and approaches for the construction of graph-based multiple reference frameworks to incorporate SV diversity into references themselves (Garrison *et al.*, 2018; Sirén *et al.*, 2021). Such approaches use multiple, high-quality reference assemblies in a single graph-based representation, allowing efficient representation of SV across many genomes. To these genome graphs, alignment of large panels of sequenced populations provide information about allele frequencies of SV in populations (Bayer *et al.*, 2020). Approaches such as these have already been useful to study general evolutionary processes (Qin *et al.*, 2021) and are illuminating the hitherto dark zone of SV in many plant systems (Zhou *et al.*, 2019, 2022; Song *et al.*, 2020; Alonge *et al.*, 2020; Liu *et al.*, 2020a; Cai *et al.*, 2021; Hämälä *et al.*, 2021).

Benchmark Studies Defining Mechanisms of Salinity Adaptation

7

259 The matching of an organism's genome to the environment optimises fitness to local
260 habitat. Such genomic adaptation is driven by selective pressures acting at discrete
261 geographic locations over dynamic timescales, and is governed by a set of rules that
262 allow life to optimise exploitation of a highly heterogeneous world. Both Wallace and
263 Darwin recognized this, with Wallace noting "nothing can be more abrupt than the
264 change often due to diversity of soil, a sharp line dividing a pine or heather-clad moor
265 from calcareous hills" (Brady *et al.*, 2005). Though over a century has passed since
266 these observations, a systematic understanding of the molecular mechanistic basis of
267 genomic reconstruction across species still eludes us.

268

269 In part, this is due to pervasive confounding effects of demography on studies of
270 adaptive variation. Substantial recent progress has been made by high-density
271 sampling at a local scale, largely controlling for the effects of demography. This retains
272 contrasting phenotypes to as small a geographical region (and thus genetic dispersion)
273 as possible (for a description of what makes 'benchmark study', see box 2). For
274 example, in a study of fine-scale local adaptation of *A. thaliana* plants in the Iberian
275 Peninsula, a clear signal of salinity adaptation emerged over a distance of only 30km,
276 as shown in reciprocal transplant experiments (Busoms *et al.*, 2015). In this study both
277 reciprocal transplant experiments done in several years (as well as common garden
278 experiments) confirmed local adaptation to coastal and later also to inland soils (Terés
279 et al., 2019). However, it is worth noting that such an experiment does not constitute
280 formal proof for adaptation to high salinity, as inland and coastal soils vary for additional
281 physiochemical properties. Thus, to isolate the specific impact of Na⁺, salinity stress
282 experiments were performed, in both soil and hydroponics. These revealed that plants
283 from coastal habitats have increased tolerance specifically to elevated NaCl,
284 establishing that elevated salinity in coastal soils is a key selective agent driving local
285 adaptation (Busoms *et al.*, 2015).

286

287 In coastal areas salinity challenges come in two major physical modalities:
288 aboveground due to salt spray and belowground due to soil salinity (Du & Hesp, 2020).
289 Popovic & Lowry (2020) implemented a manipulative reciprocal transplant of *Mimulus*
290 *guttatus* in coastal and inland sites excluding aboveground stressors. They found that
291 inland plants cultivated in the coast but protected with enclosures exhibited the same
292 fitness than in inland sites, proving the importance of salt spray effects. This suggest

293   that in this system most of the salt enters the aerial organs of plants due to long-term
294   exposure to salt spray (Lowry et al., 2009). Once the salt has entered the leaf tissue,
295   most of it is translocated to the tips of leaves, accumulated or compartmentalized there,
296   loaded to the phloem, or secreted using the same mechanisms employed to remove
297   an excess of salt translocated from the roots (Tester & Davenport, 2003). Tolerance to
298   salt spray increases with the growth of vegetation because well-developed cuticles
299   prevent salt penetration. The exception is that reproductive organs are usually much
300   more sensitive to salt spray than plant leaves (Griffiths et al., 2006) and therefore
301   escape strategies can be essential. Additionally, it is important to note that various
302   coastal species have evolved particular traits to avoid salt stray injury (see Maun,
303   2009), including morphological and hormone signalling changes affecting the growth
304   habit. For example, the coastal short ecotype of *Setaria viridis* exhibits higher salt spray
305   tolerance than the coastal tall ecotypes because the compact stature offers major
306   protection to the strong winds from the open sea (Itoh, 2021). Relating adaptive
307   changes in stature to a basis in hormone regulation, Wilkinson et al. (2019) showed
308   that differences in the auxin pathway contributed to the repeated evolution of erect and
309   prostrate forms of *Senecio lautus* along the Australian coast.

310

311   We speculate that whole-plant changes in structure, habit, and physiology require the
312   modification of multiple loci; what about single major effect natural changes?
313   Worldwide, natural alleles of the HKT1 gene are the single greatest component
314   explaining variation in leaf Na^+ accumulation in A. *thaliana* (Baxter *et al.*, 2010). HKT1
315   is a Na^+ transporter that functions to recycle Na^+ out of the xylem and restricts Na^+
316   transport to the shoot (Horie *et al.*, 2009). Indeed, an *HKT1;1* variant that is only weakly
317   expressed in roots and associated with elevated leaf Na^+ is enriched in coastal regions,
318   including from the coastal region in the Iberian Peninsula (Baxter *et al.*, 2010). The
319   coastal allele of *HKT1;1* was shown to have enhanced shoot expression, which
320   protects the inflorescence from excessive Na^+ accumulation (An *et al.*, 2017), further
321   suggesting mechanistic roles in coastal adaptation. But the *HKT1;1* story is not so
322   simple as a binary phenotype. Extending this thread, a quantitative response was
323   established, with the coastal allele of *HKT1;1* being in fact maladaptive to the highest
324   soil Na^+ concentrations found directly along the coastline. Instead, this adaptive coastal
325   allele occurs only in plants ~500 – 1,500m from the sea, where soil salinity is
326   intermediate and strongly influenced over short timescales by rainfall levels. Further,

327 at these locations this allele is under dynamic year-to-year fluctuating selection due to
328 oscillating soil salinity driven by annual variation in rainfall (Busoms *et al.*, 2018).
329

330 Moving beyond *A. thaliana*, *HKT1* has also been shown to explain interspecific
331 variation in Na$^+$ acquisition in crops, and to alter yield under Na$^+$ stress (Kotula *et al.*,
332 2020). In particular, studies of Na$^+$ content and tolerance in barley (Hazzouri *et al.*,
333 2018; van Bezouw *et al.*, 2019), rice (Zhang *et al.*, 2018, 2019), and wheat (Byrt *et al.*,
334 2007) point to *HKT1* as a broadly flexible gene modulating salinity-related phenotypes
335 across both monocots and dicots. However, it has not yet been studied how extensively
336 variation in this locus serves a natural, adaptive evolutionary function, as it does in *A.*
337 *thaliana*, because all the crops noted above were strongly subject to artificial selection.
338

339 Fascinatingly, a clear parallel to the *HKT1;1* story emerged in the same fine-scale
340 'natural laboratory' in the Iberian Peninsula. But in this case the locus primarily
341 controlled molybdenum accumulation, with additional pleiotropic effects on copper and
342 sodium. There, naturally evolved variants of the molybdenum transporter *MOT1* were
343 analogously associated with coastal adaptation (Busoms *et al.*, 2021). In a worldwide
344 sampling, natural variation at *MOT1* explains a high proportion of the global, species-
345 wide variation in leaf molybdenum in *A. thaliana* (Forsberg *et al.*, 2015). Also, strikingly
346 similar to the case of *HKT1;1*, a natural deletion in the promoter of the *MOT1*
347 transporter leads to low expression of the allele (Baxter *et al.*, 2008), a weak allele of
348 *MOT1*, which appears to mediate adaptation to coastal habitats (Busoms *et al.*, 2021).
349 Here, also the low-expressing allele was only found within <3km of the coast, and
350 reciprocal transplants demonstrated enhanced fitness specific to the coast.
351 Mechanistically, the *MOT1* variant harbouring this SV, a promoter deletion, appears to
352 be part of a complex crosstalk between Mo, Cu, and Na$^+$. This results in enhanced Cu
353 uptake, and improved formation of Moco - an essential co-factor in ABA biosynthesis
354 that promotes ABA production - helping reduce Na$^+$ accumulation. It is worth noting,
355 however, that both examples are not completely similar. The variant of the *HKT1;1*
356 allele is likely using Na$^+$ as a cheap osmoticum to enable plants to maintain water and
357 ion transport at elevated levels of soil sodium (Munns & Tester, 2008). In contrast, the
358 variation observed in *MOT1* leads to an indirect adaptation to soil salinity through ABA
359 signalling, promoting Na$^+$ efflux and water uptake as observed in other species
360 (e.g.Kong *et al.*, 2016).

361

362 Further molecular insight into mechanisms of adaptive salinity tolerance has often
363 come from genomic association studies. A particularly well-studied case concerns the
364 distribution of *Mimulus guttatus* along the west coast of the USA (Lowry *et al.*, 2008).
365 In a reciprocal transplant experiment, the authors compared coastal and inland
366 individuals of *M. guttatus*, which have a strongly differentiated population structure.
367 They showed that local plants consistently outperformed foreign plants in survival, as
368 well as the fitness proxies number of flowers and growth. They related at least part of
369 this effect to sea spray by showing high damage in inland plants, relative to more
370 tolerant coastal plants. A follow-up study described the genome-wide differentiation
371 between the coastal and inland plants (Gould *et al.*, 2017), highlighting differentiation
372 for two large SV: chromosomal inversions. In these regions shielded from
373 recombination the frequency of non-synonymous changes was elevated, and the
374 authors suggested plausible candidate genes that may underlie the observed adaptive
375 differences. Though this has not been shown in follow up studies as none of the
376 candidate alleles has been functionally confirmed, it implies that the SV in this case
377 may underpin salinity tolerance. As this study focussed on speciation, the authors do
378 not draw any conclusion about the mechanism of salinity tolerance per se, but they do
379 point to salt and drought stress response genes, gibberellic acid signalling and
380 developmental genes as possible candidates as mediating local adaptation.

381

382 In the context of very high-salt endemics, traits related to higher tolerance were the
383 focus of a study of halophytes growing along a natural gradient of salinity (Howard,
384 2010; Rouger & Jump, 2015). There, *Haloxylon aphyllum* populations showed varying
385 morphological and physiological adaptations in different genotypes, which all indicated
386 salinity tolerance. For example, the authors detected high levels of K^+ under all levels
387 of salinity stress in plants which were able to maintain a steady growth under increasing
388 salinity. They also showed evidence that higher proline levels were beneficial at the
389 highest salinities. These different adaptations were interpreted to underlie contrasting
390 mechanisms of salt tolerance (Shuyskaya *et al.*, 2014). Here, interestingly similar to
391 the *HKT1* scenario, the authors found the greatest genetic diversity at intermediate Na^+
392 levels (Shuyskaya *et al.*, 2012), indicating the ability to select genes useful for
393 performance on either higher or lower levels of salinity.

394

395 Candidate genes underlying adaptation to coastal environments have been identified
396 in various studies, but interestingly, high salinity is not always the factor best linked to
397 the adaptations. Other traits are sometimes better correlated with occurrence in high
398 saline areas. Studies on these could illuminate different, important selection pressures
399 related to adaptation to high salt areas, where the mechanism of apparent salinity
400 tolerance is more related to mitigating deleterious effects of the environment in general.
401 For example, coastal areas are often unsurprisingly less arid habitats than inland, and
402 coastal *M. guttatus* have larger leaves, more branches, greater overall size and flower
403 later (Lowry *et al.*, 2008). This syndrome is broadly related to marine habitats, as it
404 corresponds to a higher photosynthetic rate which comes at the cost of high water loss,
405 which is of course detrimental when water is less available (Stebbins, 1952; Hayford
406 *et al.*, 2022). Indeed, three of the adaptive candidate genes detected by (Gould *et al.*,
407 2017), ent-Kaurene oxidase (*KO*), AGAMOUS-like 8 (*AGL8*) and auxin response factor
408 8 (*ARF8*), co-localize with QTLs for flowering and developmental traits (Hall *et al.*,
409 2006). *KO* is involved in gibberellic acid (GA) biosynthesis (Helliwell *et al.*, 1999), *AGL8*
410 is expressed in shoot meristems and is, together with other factors, responsible for the
411 initiation of flowering (Hempel *et al.*, 1997), and *ARF8* promotes jasmonic acid (JA)
412 production and is, together with *ARF6*, essential for flower maturation (Nagpal *et al.*,
413 2005). Further candidates also relate to GA metabolism, flowering and auxin, as well
414 as brassinosteroid signalling and ABA synthesis (Gould *et al.*, 2017). ABA, as
415 previously mentioned, is a phytohormone to signal stress and in the case of salt, it
416 promotes Na^+ efflux and water uptake (Kong *et al.*, 2016). The signalling hormone has
417 been shown to act through Late Embryogenesis Abundant (LEA) proteins which are
418 upregulated by ABA and whose high abundance leads to salinity tolerance (Dalal *et
419 al.*, 2009). Alternatively, in an ABA independent mechanism, dehydration-responsive
420 element binding (DREB) genes are known to regulate many downstream targets during
421 salt stress (Yan *et al.*, 2014), but no evolutionary signal for selection has yet been
422 found for DREBs. Neither has such a role been found for other signalling compounds
423 such as nitric oxide (NO) or small molecules like polyamines. These have been shown
424 to also protect against salinity. Polyamines are required for Ca signalling which is
425 important in reducing salt toxicity symptoms (Yamaguchi *et al.*, 2006). Interacting with
426 polyamines is NO which is required for post-translational modifications on proteins and
427 subsequent changes in enzymatic activities and gene expression changes which have
428 been correlated with salt stress responses (Napieraj *et al.*, 2020). The phytohormone

12

429 GA, with its impact on plant morphological parameters, is able to promote growth under

430 Na stress (Wen *et al.*, 2010).

431

432 Genes like *ARF8* and genes related to GA signalling are all likely connected to other

433 phenotypic adaptations to coastal areas, such as early flowering (*ARF8* and *KO*) and

434 changes in morphology (GA). However, evidence for divergence in the genome of

435 coastal and inland *M. guttatus* was also detected for genes involved in ion

436 homeostasis. Such genes, or their promotors, were in the top 1% of the most

437 differentiated genes between coastal and inland plants of *M. guttatus*. Among them

438 *SALT OVERLY SENSITIVE 1* (*SOS1)* and *SOS3*, two members of the well described

439 SOS pathway (Quintero *et al.*, 2002) for Na^+ tolerance. Additionally, divergence for

440 *HKT1* alleles was also detected (Gould *et al.*, 2017).

441

442 **Box 2. Benchmark Approaches to Define Local Adaptation**

443 Where tested, the sum output of ecological and genetic factors, **local adaptation**, has been broadly

444 observed. For example, a meta-analysis of 32 species showed that local plants outperform foreign plants

445 in 71% of cases (Leimu and Fischer, 2008). Clear divergent selection was observed in a more stringent

446 comparison between both environments in 45% of cases. This effect is best observed in large

447 populations, suggesting that smaller populations lack sufficient genetic diversity for rapid adaptation.

448 However, what is a sufficient definition for local adaptation? Only where local plants outperform foreign

449 plants in both habitats under investigation, can we specify local adaptation of both genotypes. If one

450 plant outperforms another in both habitats, however, true local adaptation cannot be inferred. This is

451 further supported by Nuismer & Gandon (2008) who show by modelling that only reciprocal transplant

452 experiments are capable of measuring local adaptation. They attribute this to the properties of common

453 garden experiments to measure only spatial covariance between genotype frequencies. Reciprocal

454 transplant experiments on the other hand incorporate spatial variability in the ecological environment as

455 a further term.

456

457 Local adaptation to salinity stress is often studied in reciprocal transplant experiments in coastal and

458 inland habitats. Growth and fitness are then compared to assess relative performance of all plants.

459 Growth is thereby an indirect measure. Ecologically relevant fitness captures the ability of an individual

460 to transmit their genotype to following generations; by estimating the number of fertile progenies an

461 individual can produce in prevailing conditions. Common garden experiments are often used, frequently

462 in addition to reciprocal transplants, to infer the presence of locally adaptive evolutionary change. In a

463 common garden experiment, plants of differing origins are grown at a single site. These can be in

464 controlled environments, such as greenhouses or growth chambers, or in the field. Unlike in a reciprocal

465 transplant experiment, the effect of the environmental variation on fitness is not assessed, unless

466 multiple gardens are used. In each case the impact of genetic variation on phenotype can then be

467 estimated. An overview of published plant reciprocal transplant and common garden experiments is

468 given in **Table 1**.

469

470 Further convincing evidence of population-level, within-species salinity adaptation has

471 been seen in *Medicago truncatula* sampled across a salinity gradient in Tunisia

472 (Friesen *et al.*, 2014). Populations originating from saline sites proved to be locally

13

473   adapted, displaying higher biomass in high salinity common gardens as well as in
474   reciprocal transplant experiments. The authors showed that traits like increased leaf
475   water content, and early germination and flowering, are favoured in populations from
476   saline soils. Integrating genome scans with ecological experiments and selection
477   analysis, 16 genomic regions and 198 candidate genes were linked to the soil of origin,
478   and therefore potentially underpinning local adaptation to high saline soil. Among these
479   candidates there are ABA and JA regulators, as well as a gene involved in trehalose
480   metabolism that could function in osmotic protection. Importantly, the researchers also
481   discovered a *CIPK* gene, orthologous to *CIPK21*, as well as Ca^{2+} signalling candidates
482   such as Calcium Protein Kinases. This supports the interesting idea that Ca^{2+} signalling
483   may be adaptively tuned. Given the central role of Ca^{2+} in stress signalling, the idea
484   that adaptive modulation of Ca^{2+} transport may act as a more global molecular rheostat
485   in stress signalling was speculated by Arnold *et al*. (2016), who observed convergence
486   on multi-hazard – and severely Ca^{2+}-challenged environments – of serpentine sites.
487   This idea was later supported by the discovery of remarkably specific, convergent *de*
488   *novo* substitutions in the selectivity gate of the central Ca transporter and stress
489   signalling hub TWO PORE CHANNEL 1 only on serpentine sites (Konečná *et al*.,
490   2020), despite strict conservation at that residue across plant diversity (and indeed
491   homologs in other kingdoms).

492

493   The above studies provide generally clear evidence for the mechanisms (both
494   evolutionary and molecular) underlying adaptation to salinity and related ionomic
495   challenges. However, most often information on the mechanisms for underlying
496   adaptive traits is still missing, especially in less established model systems. This is a
497   real shortcoming, since other wild species than e.g. *A. thaliana* harbour the greatest
498   potential for understanding salinity adaptation. This can be seen in a variety of
499   ecological studies. For example, *Hydrocotyle bonariensis* showed local adaptation as
500   defined by Leimu and Fischer (2008) between high dune areas, further away from the
501   water edge and with generally dryer conditions with less vegetation, and low dune
502   areas, which are often flooded (Knight & Miller, 2004). The species had been shown
503   to occur in heterogeneous environments including steep soil saline gradients from
504   0.5% to 16% (Evans & Whitney, 1992). This interesting work did not fully dissect the
505   basis of local adaptation, but given the salinity gradient naturally present within the
506   environment, it is likely that each local population is adapted to the soil salinity level.

507 The same is true for the relatively salt tolerant *Triplasis purpurea*, which provides an
508 interesting counterexample. There, different populations were subjected to varying
509 degrees of sea salt spray, but did not differ in traits such as tiller number and biomass
510 (Cheplick & White, 2002). In contrast, the authors found a phylogenetic family effect
511 for most traits they measured, which indicated a genetic relatedness. Consequently,
512 instead of showing selection at a particular locus, this indicates plants were only
513 recently derived from a common ancestor. This means that plants are not yet adapted
514 to high salinity but rather respond differently to salinity stress based on different allele
515 combinations inherited by their parents. Such patterns are less likely due to local
516 selection but rather demographic history and they hold the opportunity for rapid
517 adaptations.

518

519 As mentioned above, local adaptation is often required when migrants experience a
520 new or challenging habitat. Invasive species have a knack for this and consequently,
521 we find clear examples of local adaptation to salinity among them. On the other hand,
522 invasive species can exhibit obvious adaptive traits, generalist strategies, and/or
523 plasticity. This then raises the question: what might be common evolutionary or
524 molecular mechanisms to be shared by salt tolerant invasive species? Such
525 integrative, comparative studies are generally very rare, but one work compared two
526 invasives: *Phragmites australis* (which is invading North American salt marshes that
527 are normally the home of *Sporobolus alterniflora*), and *Sporobolus* spp. (derived from
528 *S. alterniflora*, which is invasive in European marshes home to *P. australis*), in terms
529 of salinity tolerance (Vasquez *et al.*, 2006). In common garden experiments, *S.
530 alterniflora* produced much more biomass at higher NaCl than *P. australis*. In contrast,
531 at low NaCl, *P. australis* had relatively more rhizomes than *S. alterniflora*, indicating
532 potentially higher rates of vegetative reproduction in low saline environments. North
533 America's salt marshes are experiencing a reduction in their salinity, potentially
534 favouring *P. australis* and allowing it to become invasive. Further examples of invasive
535 salt tolerant species include *Spartina alterniflora*, a perennial grass native to North
536 America but invasive in South China. Here, *S. alterniflora* is disrupting mangrove
537 ecosystems due to its high salinity tolerance, which is connected to increased
538 production and signalling through hydrogen sulphate. This mitigates damage from
539 reactive oxygen species and helps to maintain Na^+/K^+ homeostasis (Li *et al.*, 2020).
540 Similar mechanisms were also part of the tolerance strategy of *Acacia longifolia*, an

541 invasive species in Portuguese sand dunes, which copes better with Na⁺ stress

542 through higher K^+/Na^+ ratio and higher ROS scavenging capacity (Morais *et al.*, 2012).

543 Many more examples exist (Rouifed *et al.*, 2012; Gonzalez-Mateu *et al.*, 2020);

544 however in most studies, mechanistic insights into the Na⁺ tolerance of invasives is still

545 missing and no broad scale comparisons have been performed.

546

547 Extreme salinity may even enable invasion, as is currently occurring in *Cochlearia*

548 *danica,* a recently-formed allohexaploid*.* This species, an Atlantic coastal halophyte, is

549 spreading exceptionally rapidly along major motorways across Europe, triggered by

550 the widespread use of salt-based road de-icing since the 1970's. The mechanism of

551 their extreme salt-tolerance is unknown, nor do we yet know for certain if salt-tolerance

552 in this case means sodium tolerance specifically. However, it has been shown that *C.*

553 *danica* seeds can germinate at very high sodium concentrations (Pegtel, 1999),

554 allowing the rapid invasion of competitor-sparce habitats (Fekete *et al.*, 2018).

555

Thus-far Discovered Mechanisms

556

557 Taking the work discussed above as a whole, the primary molecular mechanisms for

558 salinity tolerance can be grouped into three non-exclusive categories - osmotic stress

559 tolerance, ion exclusion, and tissue tolerance - and all have been excellently described,

560 especially in mutant and crop studies (e.g. Munns and Tester, 2008; Almeida *et al.*,

561 2017). Explicit discussion of evolutionary mechanisms is more difficult to find.

562 Additionally, the genomic basis of these mechanisms has been mainly studied in model

563 plants like *A. thaliana*. Luckily, despite being considered a glycophyte, there are wild

564 populations of *A. thaliana* with contrasting salinity tolerance phenotypes. This fact has

565 allowed the discovery of natural variants such as *HKT1* (Baxter *et al.*, 2010) though

566 genome-wide association analysis (GWA). In the past decade, GWA and QTL studies

567 have enabled progress in the identification of major-effect genes controlling salt

568 tolerance (Wani *et al.*, 2020; Li, 2020). As a fascinating example of evolutionary

569 convergence, rice *SKC1* (Ren *et al.*, 2005), wheat *Kna1* (Munns *et al.*, 2012)*, Nax1*

570 (Byrt *et al.*, 2007) and *Nax2* (Huang *et al.*, 2006)*,* and maize *ZmNC1* (Zhang *et al.*,

571 2018) salt-tolerant QTLs are all based on *HKT1* homolog-mediated mechanisms.

572 Additionally, tomato *SlHAK20* (Wang *et al.*, 2020) and maize *XmHAK4* (Zhang *et al.*,

573 2019) are members of the HAK/KUP/KT Na⁺-selective ion transporters that promote

574 shoot Na⁺ exclusion and confer salinity tolerance.

575

576 Early work on the SALT OVERLY SENSITIVE (SOS) pathway pioneered the molecular

577 understanding of salinity tolerance in *Arabidopsis thaliana* (Wu et al., 1996). The SOS

578 pathway is broadly essential for salinity tolerance, conserved functionally across dicots

579 and monocots. Strikingly, however evidence natural adaptive genetic variation in SOS

580 genes is minimal. For example, large-scale GWAS in *A. thaliana* (Baxter et al 2010;

581 Almira et al., 2023), rice (Lv et al., 2022), maize (Luo et al., 2019), and barley (Tu et

582 al., 2021) have not detected putatively adaptive variation in SOS genes. This stands

583 in contrast to HKT1, which exhibited repeated adaptive variation to natural salinity

584 challenge (Rus et al., 2006; Baxter et al., 2010; Zhang et al., 2018, Busoms et al.,

585 2018). While the degree of adaptive flexibility at HKT is much greater, we note that in

586 association with domestication deleterious hypomorphic or loss of function SOS alleles

587 have been observed, for example during the domestication of Tomato (Wang et al.,

588 2021) and Maize (Zhou et al., 2022). Interestingly, the well-characterised adaptive

589 'weak allele' of HKT1 shares with these SOS alleles low expression, but crucially in

590 HKT1 this low expression has been associated with adaptive value against elevated

591 salinity in nature, while in SOS this has not been observed.

592

593 GWA studies have also been integrated with mutant analysis, expression networks

594 and other 'omic' techniques to identify promising genes. For example, Tu *et al.* (2021)

595 identified 39 salt-responding genes in barley, including the salt signalling genes *CYPs*,

596 *LRR-KISS* and *CML*, integrating GWA and RNA-Seq analysis. However, given

597 limitations in power, all current approaches are biased toward discovering the largest

598 effect loci, and thus relatively oligogenic architectures. This is of course a bane across

599 studies of adaptation, but is slowly being overcome by novel approaches and

600 increased power in e.g. very large-scale association studies. Such studies typically

601 provide a much more locally refined picture of genetic variation and therefore enable

602 more meaningful genotype environment or subpopulation correlations. They also

603 increasingly include complementary data types and analysis such as the prediction of

604 tertiary protein structures, network analysis or interactomes (e.g. Wu et al., 2021).

605 Increasing application of such analyses to non-standard models will provide greater

606 insight into a broader array of adaptive mechanisms.

607

608 **A Rare, Salient Role for Salt Adaptation in Polyploids?**

609   The product of whole-genome duplication (WGD), polyploidy occurs prevalently across
610   the plant kingdom (Cui *et al.*, 2006; Wood *et al.*, 2009; Alix *et al.*, 2017), and leads to
611   instant speciation. The immediate physiological effects of WGD, however, are
612   notoriously idiosyncratic (Yant & Bomblies, 2015; Doyle & Coate, 2019; Bomblies,
613   2020). Most obviously, WGD instantly allows for doubled mutational targets, freeing up
614   genetic material for novel innovations. However, given time, polyploids eventually
615   revert back to diploidy. But before that occurs, they typically accumulate mutations
616   resulting in adaptive phenotypes, and subsequently often niche shifts/expansions,
617   along with sometimes increased colonization potentials.

618

619   While we strongly underscore that every polyploidy event generates variable
620   phenotypes, there appears to be a tendency for neopolyploids to exhibit some fairly
621   common, ecologically relevant benefits (reviewed recently by Baduel *et al.*, 2018;
622   Bomblies, 2020). Increased salinity tolerance is perhaps the clearest among these.
623   This was best shown in a panel of neo-tetraploid *A. thaliana* lines that were in all
624   respects isogenic to their diploid counterparts, except for their lab-induced polyploidy.
625   These early generation of autotetraploids exhibited higher seed production and
626   survival under Na^+ stress than their isogenic diploid sisters (Chao *et al.*, 2013)This
627   effect was concomitant with increased shoot K^+ concentrations and an improved
628   K^+/Na^+ ratio under Na^+ stress. This effect was also shown in an established polyploid
629   *A. thaliana* accession. Maintaining a balanced K^+/Na^+ ratio is important for Na^+
630   stressed plants, because increased Na^+ concentrations in root and shoot cells can
631   displace other ions, most notably K^+, from binding sites and inhibit cellular functions
632   (Nitsos & Evans, 1969). We speculate that this immediately altered intracellular
633   ionomic environment in young polyploids may act as an evolutionary spandrel, later
634   serving as a trait that is then selected on when the nascent polyploid encounters novel
635   environmental challenges. Supporting the argument that K^+/Na^+ homeostasis is
636   important for polyploids to develop Na^+ tolerance, is that an improved K^+/Na^+ ratio also
637   coincides with better Na^+ tolerance in other systems such as through mycorrhizal
638   colonization of *Acacia nilotica* with *Glomus fasciculatum* (Giri *et al.*, 2007).
639   Interestingly, improved growth and decreased Na^+ concentrations under salinity stress
640   was also observed in neo-tetraploid rice (Tu *et al.*, 2014; Wang *et al.*, 2021), where the
641   authors conclude that neo-tetraploids are better able to cope with the Na^+ stress due
642   to their increased vigor, and activated jasmonic acid controlled stress response.

643   Further, the diploids *Brassica oleracea*, *B. campestris* and *B. nigra* are less salinity
644   tolerant than their amphidiploid (contains diploid sets of chromosomes from each
645   parent) offspring *B. napus*, *B. carinata* and *B. juncea* (Ashraf *et al.*, 2001). The
646   amphidiploids also accumulate higher concentrations of K^+ under salinity stress.
647   Moreover, tetraploid citrange also showed less leaf damage and defoliation after
648   salinity treatment (Ruiz *et al.*, 2016).

649

650   It is clear that ploidy increase can bring amplified salinity tolerance (Gerstein *et al.*,
651   2006; Saleh *et al.*, 2008). However, we do not yet understand the molecular
652   mechanisms underpinning this. Genetic analysis of neo-tetraploid mutants indicates
653   that increased shoot K^+ concentrations are regulated through a gene network that is
654   comprised of hubs of endodermal and cell wall modification genes (Fischer *et al.*,
655   2021). Population genomic analysis of polyploid, salt tolerant *Cochlearia* populations
656   revealed selective sweeps for the orthologues of *SOS1* and HKT1 in the autotetraploid
657   relative to inland diploids (Bray *et al.*, 2020). This Na^+/H^+ transporter is relevant for Na^+
658   tolerance in *A. thaliana* (Quintero *et al.*, 2002, 2011), and was also found to affect shoot
659   potassium concentration in neo-tetraploid *A. thaliana* (Fischer *et al.*, 2022). Bray *et al.*
660   (2020) also elaborated that very similar processes (relevant to salinity: ion
661   homeostasis), but not orthologous genes were under selection after whole genome
662   duplication in *Arabidopsis arenosa* (Yant *et al.*, 2013) and *Cardamine amara*
663   (Bohutínská *et al.*, 2021). These examples point to common, shared mechanisms—
664   with ion homeostasis prominent among them—underpinning adaptation to the
665   transformed intracellular WGD state. Indeed, tetraploid *A. arenosa* populations have
666   been found on highly diverse soils, including mines and serpentines, if not explicitly
667   saline environments. That said, dedicated studies failed to detect niche differentiation
668   between diploid and tetraploid *A. arenosa*, although one showed niche expansion for
669   the tetraploids (Molina-Henao & Hopkins, 2019; Morgan *et al.*, 2020). Common garden
670   experiments utilizing diverse cytotypes of many populations to capture variation, and
671   natural soil with contrasting elemental profiles, will establish the impact of genotype
672   and cytotypes on growth and the plant ionome. Reciprocal transplant experiments
673   between sites with contrasting soil physiochemical properties together with cytology
674   and genomic techniques will allow us to assess adaptive responses and study the
675   molecular mechanisms behind the improved salinity tolerance of polyploids.

676

677 **A little help from friends**

678 Complex interactions, which evolved between plants and associated microbiomes, are

679 now well recognized as key determinants of plant health (Berendsen *et al.*, 2012). The

680 microbiome works with plants in obtaining nutrients, protecting against infections, and

681 enduring stresses. An array of recent studies highlight the importance of microbial

682 communication with the plant, proposing mechanisms based on plant-microbe

683 associations that accentuate plant defence (Petrić *et al.*, 2022). Location, soil

684 properties and plant genotype have a significant effect on microbial communities (see

685 Morales Moreira *et al.*, 2021). Different microbial compartments (bulk soil, rhizosphere,

686 and rhizoplane) also harbour contrasting microbial compositions due to the distance to

687 the host root (e.g. Edwards *et al.*, 2015). The soil microbiome is directly impacted by

688 environmental fluctuations, while rhizosphere microbiomes are influenced also

689 indirectly by host responses (Trivedi *et al.*, 2022). Endophytes are likely less impacted

690 by environmental fluctuation, as they occupy relatively more stable internal plant tissue

691 environments, and they are typically more host-specific.

692

693 Saline soils are unique ecological niches inhabited by extremophilic microorganisms

694 with specific adaptation strategies. For some years now, dedicated studies have aimed

695 for the isolation and characterization of plant endophytes living in saline and other

696 extreme environments (Otlewska *et al.*, 2020). Around 350 species of the more than

697 1200 halophytes catalogued in the 'eHALOPH database' are recorded as having

698 associated microorganisms and mycorrhizal status (Santos *et al.*, 2016). These

699 represent a severely under-exploited reservoir for potential treatments against abiotic

700 stresses impacting agriculture, including extreme temperatures, drought, salinity, or

701 heavy metal stress (see Kumar and Verma, 2018). This halophytic root microbiome

702 can positively influence the host through several routes: providing nutrients or

703 favouring nutrient acquisition; modulating phytohormone levels; regulating antioxidant

704 responses, synthesizing exopolysaccharides (EPS); maintaining plant defence against

705 biotic stress; accumulating organic solutes such as proline and betaine, and increasing

706 soil aggregation (Akyol *et al.*, 2020).

707

708 **Box 3. Microbiome reciprocal transplants**
709

710 To understand evolved, adaptive soil-plant–microbiome associations, field transplant experiments that
711 match the host and microbiome in an ecological setting are essential (Reed & Martiny, 2007). However,
712 to assess the impact on plant performance of a particular microbiome, controlled environment reciprocal

713 transplants using sterilized soil have been productive (Figure 2; e.g. Smith *et al.*, 2018; Van Nuland *et*
714 *al.*, 2019). Although the composition of the microbiome cannot be easily manipulated in the field,
715 applying these approaches can begin to tease apart the effects of the microbial composition of
716 microbiomes from environmental parameters, and at the same time, allow the study of a single stressor
717 such as soil salinity.

718

719 Plant-growth-promoting rhizobacteria (PGPR) and mycorrhizae have now well under-

720 stood root colonizing capacities and some have can alleviate the inhibitory impact of

721 salinity on plant growth (Zheng *et al.*, 2021; Evelin *et al.*, 2019). For example, Yasmin

722 *et al.*, (2020) found that *Pseudomonas pseudoalcaligenes* and *Bacillus subtilis*

723 significantly improved the growth of soybean under salinity stress through the impact

724 on a series of physiological regulatory processes mainly related to the activation of

725 antioxidant defence system in order to reduced ROS overproduction. Parvin *et al.*

726 (2020) concluded that specific arbuscular mycorrhizal fungi can promote salt tolerance

727 and productivity in rice, likely by improving photosynthetic efficiency and K^+/Na^+ ratio,

728 and restricting Na^+ uptake and translocation. However, to date these mechanisms

729 have only been documented in a few cases, and their distribution in the whole microbial

730 community of salinity-tolerant plants remains to be defined.

731

732 Core microbiomes are shared features of microbial communities that, because of their

733 conservation, are inferred to have importance for host fitness, and therefore promise

734 the potential to rationally manage plant microbiomes towards specific outcomes (Toju

735 *et al.*, 2018). Excellent earlier reviews have discussed a wide range of plant beneficial

736 traits provided by diverse microbial groups under stress conditions (Friesen *et al.*,

737 2011; de Zelicourt *et al.*, 2013; Tkacz & Poole, 2015; Qin *et al.*, 2016). In Table 2 we

738 give an updated overview of relevant studies that have characterized saline-associated

739 core microbiomes.

740

741 From this body of work, we suggest that *Proteobacteria & Firmicutes* and *Ascomycota*

742 *& Glomeromycota* appear to be the most abundant and non-species-specific bacterial

743 and fungal taxa present in plant microbiomes from saline environments (Table 2).

744 Representatives from both phyla could mitigate salt stress by direct mechanisms

745 involved in protecting the plants (ACC deaminase, EPS, phytohormone production) or

746 by indirect mechanisms based on modification of the plant metabolome. Of late, there

747 has been a growth in studies correlating metabolomic and transcriptomic data to

748 understand the crosstalk between plants and microorganisms (e.g. Wu *et al.*, 2020;

749 Salas-González *et al.*, 2021; Rane *et al.*, 2022). However, there is little information on
750 expression changes in response to fluctuating abiotic stresses in the plant-microbiome
751 defined transcriptome. Dedicated metagenomic studies over time in natural conditions
752 are required to fully understand these interactions. Together, this work will offer plant
753 breeders the power to select the best cultivar-inoculum pairs, in order to optimise
754 resilience and yield of crops in the face of increasing climate volatility.

755

756 **A View Toward Future Progress**

757 Here we have focused on fascinating recent empirical examples of plant adaptation to
758 extreme environments, highlighting both evolutionary and molecular mechanisms. We
759 emphasized benchmark studies of ecologically adaptive salt tolerance in plants,
760 highlighting the now quite clear interplay between salinity adaptation and both
761 increased ploidy and the microbiome.

762

763 The rapid development of genomics based on both large scale and long read data to
764 test evolutionary hypotheses is increasingly providing nucleotide-level resolution of the
765 molecular mechanisms of adaptive evolution. This holds even for complex hazards and
766 highly polygenic polyploid adaptation events (Konečná *et al.*, 2021), long restricted
767 largely to theoretical work (Haldane, 1930; Barton, 2022). As evolutionary genomics
768 using very high-density data (thousands of complete genomes in single datasets to
769 powerfully target candidate adaptive mechanisms) is increasingly combined with
770 detailed assessments of adaptive phenotypes, we will rapidly identify adaptive
771 mechanisms across plant diversity. A good choice for studies of molecular
772 convergence in salt-adapted species would be the sequencing of diverse species that
773 otherwise share the same niche and the same selective pressures (including the same
774 host range), as has been attempted with various woody plants at the land-sea interface
775 (He *et al.*, 2020). Given also the pervasive role of structural genomic variation
776 underlying adaptive evolution to edaphic stressors (most often through expression
777 changes of transporters, see Baxter *et al.*, 2008; Hanikenne *et al.*, 2013; Busoms *et*
778 *al.*, 2018, 2021), we also underscore that long read-based graphical pangenomics will
779 play a key role in detecting these SV in future studies of salinity tolerance. Already
780 these studies are commonplace in major crops (Zhou *et al.*, 2019, 2022; Song *et al.*,
781 2020; Alonge *et al.*, 2020; Liu *et al.*, 2020a; Cai *et al.*, 2021; Hämälä *et al.*, 2021), and

782 we foresee their application to studies of natural adaptive variation in the very near

783 future.

784

785 But the best future studies will not just employ high throughput long read pangenomics

786 to probe the genomic basis of adaptation. They will naturally be explicitly

787 interdisciplinary, combining innovations in functional phenomics, imaging, ionomics,

788 and remote sensing with genomics. For example, to capture the finest-scale variability

789 in phenotypic data of an entire region in high density and high throughput, the use of

790 automated drones capable of operating over extended time periods and over large

791 areas, along with sensor loggers to monitor air humidity and soil moisture (Zribi *et al.*,

792 2012). Broader adoption of such tools will greatly enhance our ability to understand

793 and correlate environmental variation, which for soil parameters can shift within only a

794 few meters, to genetic variation even within one site.

795

796 Such high-resolution studies have so far not been possible due to expenses in

797 sequencing technologies and limitations in capturing environmental parameters,

798 especially over time. It is now also obvious that microbiome characterization is required

799 to ascertain soil health (Wilhelm *et al.*, 2022), yielding datasets in which researchers

800 can study microclimate associations with phenotypes, and to resolve the influence of

801 individual abiotic components much more precisely. Also, of great importance in such

802 projects will be the use of machine learning algorithms, employed to handle large

803 multidimensional genomic and phenotypic datasets (Lürig *et al.*, 2021), through which

804 predictions of gene-to-phenotype relationships will be greatly enhanced (Cheng *et al.*,

805 2021; Jammer et al., 2022). All these innovations, the studies in natural conditions, and

806 the integration of 'omic' techniques considering not only the plants but also the

807 microorganisms who cohabit with them, will give a much clearer view of the fascinating

808 and diverse natural mechanisms of salinity tolerance available in our ecosystems, thus

809 allowing their adoption for the improvement of crops and our understanding of the

810 fundamental mechanisms of evolutionary change.

811

23

815

Author contributions

817   S.B., S.F., and L.Y. wrote the manuscript together.

818

Acknowledgments

822

References

Abobatta WF. 2020. Plant responses and tolerance to extreme salinity: Learning from halophyte tolerance to extreme salinity. *Salt and Drought Stress Tolerance in Plants: Signaling Networks and Adaptive Mechanisms,* 177-210.

Akyol TY, Sato S, Turkan I. **2020**. Deploying root microbiome of halophytes to improve salinity tolerance of crops. *Plant Biotechnology Reports* **14**: 143–150.

Alix K, Gérard PR, Schwarzacher T, Heslop-Harrison JS (Pat). **2017**. Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. *Annals of Botany* **120**: 183–194.

Almira Casellas MJ, Pérez-Martín L, Busoms S, Boesten R, Llugany M, Aarts MG, Poschenrieder C. 2023. A genome-wide association study identifies novel players in Na and Fe homeostasis in *Arabidopsis thaliana* under alkaline-salinity stress. *The Plant Journal* **113**: 225-245.

Alonge M, Wang X, Benoit M, Soyk S, Pereira L, Zhang L, Suresh H, Ramakrishnan S, Maumus F, Ciren D, *et al.* 2020. Major Impacts of Widespread Structural Variation on Gene Expression and Crop Improvement in Tomato. *Cell* **182**: 145-161.e23.

An D, Chen J-G, Gao Y-Q, Li X, Chao Z-F, Chen Z-R, Li Q-Q, Han M-L, Wang Y-L, Wang Y-F, *et al.* 2017. AtHKT1 drives adaptation of Arabidopsis thaliana to salinity by reducing floral sodium content. *PLOS Genetics* **13**: e1007086.

Ansari MI. **2018**. Plant microbiome and its functional mechanism in response to environmental stress. *International Journal of Green Pharmacy (IJGP)* **12**.

Ashraf M, Harris PJC. **2013**. Photosynthesis under stressful environments: An overview. *Photosynthetica* **51**: 163–190.

Ashraf M, Nazir N, McNeilly T. **2001**. Comparative salt tolerance of amphidiploid and diploid Brassica species. *Plant Science* **160**: 683–689.

Baduel P, Bray S, Vallejo-Marin M, Kolář F, Yant L. **2018**. The "Polyploid Hop": Shifting Challenges and Opportunities Over the Evolutionary Lifespan of Genome Duplications. *Frontiers in Ecology and Evolution* **6**.

Barton NH. **2022**. The "New Synthesis". *Proceedings of the National Academy of Sciences* **119**: e2122147119.

Baxter I, Brazelton JN, Yu D, Huang YS, Lahner B, Yakubova E, Li Y, Bergelson J, Borevitz JO, Nordborg M, *et al.* 2010. A Coastal Cline in Sodium Accumulation in Arabidopsis thaliana Is Driven by Natural Variation of the Sodium Transporter AtHKT1;1. *PLoS Genet* **6**: e1001193.

Baxter I, Muthukumar B, Park HC, Buchner P, Lahner B, Danku J, Zhao K, Lee J, Hawkesford MJ, Guerinot ML, *et al.* 2008. Variation in Molybdenum Content Across Broadly Distributed Populations of Arabidopsis thaliana Is Controlled by a Mitochondrial Molybdenum Transporter (MOT1). *PLoS Genet* **4**: e1000004.

862 **Bayer PE, Golicz AA, Scheben A, Batley J, Edwards D**. **2020**. Plant pan-genomes are the new
863 reference. *Nature Plants* **6**: 914–920.

864 **Bell G**. **2009**. The Oligogenic View of Adaptation. *Cold Spring Harbor Symposia on*
865 *Quantitative Biology* **74**: 139–144.

866 **Bennett TH, Flowers TJ, Bromham L**. **2013**. Repeated evolution of salt-tolerance in grasses.
867 *Biology Letters* **9**: 20130029.

868 **Berendsen RL, Pieterse CMJ, Bakker PAHM**. **2012**. The rhizosphere microbiome and plant
869 health. *Trends in Plant Science* **17**: 478–486.

870 **Bernstein L**. **1975**. Effects of Salinity and Sodicity on Plant Growth. *Annual Review of*
871 *Phytopathology* **13**: 295–312.

872 **van Bezouw RFHM, Janssen EM, Ashrafuzzaman M, Ghahramanzadeh R, Kilian B, Graner A,**
873 **Visser RGF, van der Linden CG**. **2019**. Shoot sodium exclusion in salt stressed barley
874 (Hordeum vulgare L.) is determined by allele specific increased expression of HKT1;5. *Journal*
875 *of Plant Physiology* **241**: 153029.

876 **Bohutínská M, Alston M, Monnahan P, Mandáková T, Bray S, Paajanen P, Kolář F, Yant L**.
877 **2021**. Novelty and convergence in adaptation to whole genome duplication (M Purugganan,
878 Ed.). *Molecular Biology and Evolution*: msab096.

879 **Bomblies K**. **2020**. When everything changes at once: finding a new normal after genome
880 duplication. *Proceedings of the Royal Society B: Biological Sciences* **287**: 20202154.

881 **Brady KU, Kruckeberg AR, Bradshaw HD**. **2005**. Evolutionary Ecology of Plant Adaptation to
882 Serpentine Soils. *Annual Review of Ecology, Evolution, and Systematics* **36**: 243–266.

883 **Bray SM, Wolf EM, Zhou M, Busoms S, Bohutínská M, Paajanen P, Monnahan P, Koch J,**
884 **Fischer S, Koch MA,** *et al.* **2020**. Convergence and novelty in adaptation to whole genome
885 duplication in three independent polyploids. *bioRxiv*: 2020.03.31.017939.

886 **Bromham L**. **2015**. Macroevolutionary patterns of salt tolerance in angiosperms. *Annals of*
887 *Botany* **115**: 333–341.

888 **Bromham L, Bennett TH**. **2014**. Salt tolerance evolves more frequently in C4 grass lineages.
889 *Journal of Evolutionary Biology* **27**: 653–659.

890 **Bromham L, Hua X, Cardillo M**. **2020**. Macroevolutionary and macroecological approaches
891 to understanding the evolution of stress tolerance in plants. *Plant, Cell & Environment* **43**:
892 2832–2846.

893 **Busoms S, Paajanen P, Marburger S, Bray S, Huang X-Y, Poschenrieder C, Yant L, Salt DE**.
894 **2018**. Fluctuating selection on migrant adaptive sodium transporter alleles in coastal
895 Arabidopsis thaliana. *Proceedings of the National Academy of Sciences*: 201816964.

896 **Busoms S, Teres J, Huang X-Y, Bomblies K, Danku J, Douglas A, Weigel D, Poschenrieder C,**
897 **Salt DE**. **2015**. Salinity Is an Agent of Divergent Selection Driving Local Adaptation of
898 Arabidopsis to Coastal Habitats. *Plant Physiology* **168**: 915–929.

899 **Busoms S, Terés J, Yant L, Poschenrieder C, Salt DE. 2021**. Adaptation to coastal soils
900 through pleiotropic boosting of ion and stress hormone concentrations in wild Arabidopsis
901 thaliana. *New Phytologist* **232**: 208–220.

902 **Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M, Munns R**.
903 **2007**. HKT1;5-Like Cation Transporters Linked to Na+ Exclusion Loci in Wheat, Nax2 and
904 Kna1. *Plant Physiology* **143**: 1918–1928.

905 **Cai X, Chang L, Zhang T, Chen H, Zhang L, Lin R, Liang J, Wu J, Freeling M, Wang X. 2021**.
906 Impacts of allopolyploidization and structural variation on intraspecific diversification in
907 Brassica rapa. *Genome Biology* **22**: 166.

908 **Campoy JA, Sun H, Goel M, Jiao W-B, Folz-Donahue K, Wang N, Rubio M, Liu C, Kukat C,**
909 **Ruiz D,** *et al.* **2020**. Gamete binning: chromosome-level and haplotype-resolved genome
910 assembly enabled by high-throughput single-cell sequencing of gamete genomes. *Genome*
911 *Biology* **21**: 306.

912 **Caperta AD, Róis AS, Teixeira G, Garcia-Caparros P, Flowers TJ. 2020**. Secretory structures in
913 plants: Lessons from the Plumbaginaceae on their origin, evolution and roles in stress
914 tolerance. *Plant, Cell & Environment* **43**: 2912–2931.

915 **Chao D-Y, Dilkes B, Luo H, Douglas A, Yakubova E, Lahner B, Salt DE. 2013**. Polyploids
916 Exhibit Higher Potassium Uptake and Salinity Tolerance in Arabidopsis. *Science* **341**: 658–
917 659.

918 **Cheeseman JM. 2015**. The evolution of halophytes, glycophytes and crops, and its
919 implications for food security under saline conditions. *New Phytologist* **206**: 557–570.

920 **Cheng C-Y, Li Y, Varala K, Bubert J, Huang J, Kim GJ, Halim J, Arp J, Shih H-JS, Levinson G,** *et*
921 *al.* **2021**. Evolutionarily informed machine learning enhances the power of predictive gene-
922 to-phenotype relationships. *Nature Communications* **12**: 5627.

923 **Che-Othman MH, Millar AH, Taylor NL. 2017**. Connecting salt stress signalling pathways with
924 salinity-induced changes in mitochondrial metabolic processes in C3 plants. *Plant, Cell &*
925 *Environment* **40**: 2875–2905.

926 **Cheplick GP, White TP. 2002**. Saltwater spray as an agent of natural selection: no evidence
927 of local adaptation within a coastal population of Triplasis purpurea (Poaceae). *American*
928 *Journal of Botany* **89**: 623–631.

929 **Cui L, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, Doyle JJ, Soltis PS, Carlson JE,**
930 **Arumuganathan K, Barakat A,** *et al.* **2006**. Widespread genome duplications throughout the
931 history of flowering plants. *Genome Research* **16**: 738–749.

932 **Dalal M, Tayal D, Chinnusamy V, Bansal KC. 2009**. Abiotic stress and ABA-inducible Group 4
933 LEA from Brassica napus plays a key role in salt and drought tolerance. *Journal of*
934 *Biotechnology* **139**: 137–145.

935 **Dassanayake M, Larkin JC. 2017**. Making Plants Break a Sweat: the Structure, Function, and
936 Evolution of Plant Salt Glands. *Frontiers in Plant Science* **08**.

937 **Della Coletta R, Qiu Y, Ou S, Hufford MB, Hirsch CN**. **2021**. How the pan-genome is changing
938 crop genomics and improvement. *Genome Biology* **22**: 3.

939 **Delph LF, Kelly JK**. **2014**. On the importance of balancing selection in plants. *New Phytologist*
940 **201**: 45–56.

941 **DeRose-Wilson L, Gaut BS**. **2011**. Mapping Salinity Tolerance during Arabidopsis thaliana
942 Germination and Seedling Growth. *PLOS ONE* **6**: e22832.

943 **Doyle JJ, Coate JE**. **2019**. Polyploidy, the Nucleotype, and Novelty: The Impact of Genome
944 Doubling on the Biology of the Cell. *International Journal of Plant Sciences* **180**: 1–52.

945 **Du J, Hesp PA. 2020**. Salt spray distribution and its impact on vegetation zonation on coastal
946 dunes: a review. *Estuaries and Coasts*, **43**: 1885-1907.
947

948 **Edwards EJ, Donoghue MJ**. **2013**. Is it easy to move and easy to evolve? Evolutionary
949 accessibility and adaptation. *Journal of Experimental Botany* **64**: 4047–4052.

950 **Evans JP, Whitney S**. **1992**. Clonal Integration Across a Salt Gradient by a Nonhalophyte,
951 Hydrocotyle Bonariensis (apiaceae). *American Journal of Botany* **79**: 1344–1347.

952 **Fekete R, Mesterházy A, Valkó O, Molnár V. A**. **2018**. A hitchhiker from the beach: the
953 spread of the maritime halophyte Cochlearia danica along salted continental roads. *PRESLIA*
954 **90**: 23–37.

955 **Fischer S, Flis P, Zhao F-J, Salt DE**. **2021**. *Transcriptional networks underpinning ploidy*
956 *related increased leaf potassium in neo-tetraploids*. Plant Biology.

957 **Flowers TJ, Colmer TD. 2015** Plant salt tolerance: Adaptations in halophytes. *Annals of botany*
958 **115**: 327–331.
959

960 **Flowers TJ, Galal HK, Bromham L, Flowers TJ, Galal HK, Bromham L**. **2010**. Evolution of
961 halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology* **37**:
962 604–612.

963 **Forsberg SKG, Andreatta ME, Huang X-Y, Danku J, Salt DE, Carlborg Ö**. **2015**. The Multi-
964 allelic Genetic Architecture of a Variance-Heterogeneity Locus for Molybdenum
965 Concentration in Leaves Acts as a Source of Unexplained Additive Genetic Variance. *PLoS*
966 *Genet* **11**: e1005648.

967 **Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E**. **2011**.
968 Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and*
969 *Systematics* **42**: 23–46.

970 **Friesen ML, von Wettberg EJ, Badri M, Moriuchi KS, Barhoumi F, Chang PL, Cuellar-Ortiz S,**
971 **Cordeiro MA, Vu WT, Arraouadi S, *et al.* 2014**. The ecological genomic basis of salinity
972 adaptation in Tunisian Medicago truncatula. *BMC Genomics* **15**: 1160.

973 **Garrison E, Sirén J, Novak AM, Hickey G, Eizenga JM, Dawson ET, Jones W, Garg S, Markello**
974 **C, Lin MF, *et al.* 2018**. Variation graph toolkit improves read mapping by representing
975 genetic variation in the reference. *Nature Biotechnology* **36**: 875–879.

976 **Gerstein AC, Chun H-JE, Grant A, Otto SP. 2006**. Genomic Convergence toward Diploidy in
977 Saccharomyces cerevisiae. *PLOS Genetics* **2**: e145.

978 **Giri B, Kapoor R, Mukerji KG. 2007**. Improved Tolerance of Acacia nilotica to Salt Stress by
979 Arbuscular Mycorrhiza, Glomus fasciculatum may be Partly Related to Elevated K/Na Ratios
980 in Root and Shoot Tissues. *Microbial Ecology* **54**: 753–760.

981 **Gonzalez Mateu M, Baldwin AH, Maul JE, Yarwood SA. 2020**. Dark septate endophyte
982 improves salt tolerance of native and invasive lineages of Phragmites australis. *The ISME*
983 *Journal* **14**: 1943–1954.

984 **Gould BA, Chen Y, Lowry DB. 2017**. Pooled ecotype sequencing reveals candidate genetic
985 mechanisms for adaptive differentiation and reproductive isolation. *Molecular Ecology* **26**:
986 163–177.

987 **Griffiths ME. 2006.** Salt spray and edaphic factors maintain dwarf stature and community
988 composition in coastal sandplain heathlands. *Plant Ecology* **186**: 69–86.
989
990 **Haldane JBS. 1930**. Theoretical genetics of autopolyploids. *Journal of Genetics* **22**: 359–372.

991 **Hall MC, Basten CJ, Willis JH. 2006**. Pleiotropic Quantitative Trait Loci Contribute to
992 Population Divergence in Traits Associated With Life-History Variation in Mimulus guttatus.
993 *Genetics* **172**: 1829–1844.

994 **Hämälä T, Wafula EK, Guiltinan MJ, Ralph PE, dePamphilis CW, Tiffin P. 2021**. Genomic
995 structural variants constrain and facilitate adaptation in natural populations of Theobroma
996 cacao, the chocolate tree. *Proceedings of the National Academy of Sciences* **118**:
997 e2102914118.

998 **Hanikenne M, Kroymann J, Trampczynska A, Bernal M, Motte P, Clemens S, Krämer U**.
999 **2013**. Hard Selective Sweep and Ectopic Gene Conversion in a Gene Cluster Affording
1000 Environmental Adaptation. *PLOS Genetics* **9**: e1003707.

1001 **Hayford RK, Serba DD, Xie S, Ayyappan V, Thimmapuram J, Saha MC, Wu CH, Kalavacharla**
1002 **VK. 2022**. Global analysis of switchgrass (Panicum virgatum L.) transcriptomes in response to
1003 interactive effects of drought and heat stresses. *BMC Plant Biology* **22**: 107.

1004 **Hazzouri KM, Khraiwesh B, Amiri KMA, Pauli D, Blake T, Shahid M, Mullath SK, Nelson D,**
1005 **Mansour AL, Salehi-Ashtiani K, *et al.* 2018**. Mapping of HKT1;5 Gene in Barley Using GWAS
1006 Approach and Its Implication in Salt Tolerance Mechanism. *Frontiers in Plant Science* **9**.

1007 **Helliwell CA, Poole A, James Peacock W, Dennis ES. 1999**. Arabidopsis ent-Kaurene Oxidase
1008 Catalyzes Three Steps of Gibberellin Biosynthesis. *Plant Physiology* **119**: 507–510.

1009 **Hempel FD, Weigel D, Mandel MA, Ditta G, Zambryski PC, Feldman LJ, Yanofsky MF**. **1997**.
1010 Floral determination and expression of floral regulatory genes in Arabidopsis. *Development*
1011 **124**: 3845–3853.

1012 **Horie T, Hauser F, Schroeder JI**. **2009**. HKT transporter-mediated salinity resistance
1013 mechanisms in Arabidopsis and monocot crop plants. *Trends in plant science* **14**: 660–668.

1014 **Howard RJ**. **2010**. Intraspecific Variation in Growth of Marsh Macrophytes in Response to
1015 Salinity and Soil Type: Implications for Wetland Restoration. *Estuaries and Coasts* **33**: 127–
1016 138.

1017 **Huang S, Spielmeyer W, Lagudah ES, James RA, Platten JD, Dennis ES, Munns R**. **2006**. A
1018 Sodium Transporter (HKT7) Is a Candidate for Nax1, a Gene for Salt Tolerance in Durum
1019 Wheat. *Plant Physiology* **142**: 1718–1727.

1020 **Itoh M**. **2021**. Phenotypic variation and adaptation in morphology and salt spray tolerance in
1021 coastal and inland populations of *Setaria viridis* in central Japan. *Weed Research* **61**: 199-209.
1022
1023 **Jiang C, Mithani A, Belfield EJ, Mott R, Hurst LD, Harberd NP**. **2014**. Environmentally
1024 responsive genome-wide accumulation of de novo Arabidopsis thaliana mutations and
1025 epimutations. *Genome Research* **24**: 1821–1829.

1026 **Jin T, Sun Y, Shan Z, He J, Wang N, Gai J, Li Y**. **2021**. Natural variation in the promoter of
1027 GsERD15B affects salt tolerance in soybean. *Plant Biotechnology Journal* **19**: 1155–1169.

1028 **Knight TM, Miller TE**. **2004**. Local adaptation within a population of Hydrocotyle bonariensis.
1029 *Evolutionary Ecology Research* **6**: 103–114.

1030 **Konečná V, Bray S, Vlček J, Bohutínská M, Požárová D, Choudhury RR, Bollmann-Giolai A,**
1031 **Flis P, Salt DE, Parisod C, *et al.***. **2021**. Parallel adaptation in autopolyploid Arabidopsis
1032 arenosa is dominated by repeated recruitment of shared alleles. *Nature Communications* **12**:
1033 4979.

1034 **Kong X, Luo Z, Dong H, Eneji AE, Li W**. **2016**. H2O2 and ABA signaling are responsible for the
1035 increased Na+ efflux and water uptake in Gossypium hirsutum L. roots in the non-saline side
1036 under non-uniform root zone salinity. *Journal of Experimental Botany* **67**: 2247–2261.

1037 **Kotula L, Garcia Caparros P, Zörb C, Colmer TD, Flowers TJ**. **2020**. Improving crop salt
1038 tolerance using transgenic approaches: An update and physiological analysis. *Plant, Cell &*
1039 *Environment* **43**: 2932–2956.

1040 **Leimu R, Fischer M**. **2008**. A Meta-Analysis of Local Adaptation in Plants. *PLOS ONE* **3**: e4010.

1041 **Li B**. **2020**. Identification of Genes Conferring Plant Salt Tolerance using GWAS: Current
1042 Success and Perspectives. *Plant and Cell Physiology* **61**: 1419–1426.

1043 **Li J, Yu Z, Choo S, Zhao J, Wang Z, Xie R**. **2020**. Chemico-Proteomics Reveal the
1044 Enhancement of Salt Tolerance in an Invasive Plant Species via H2S Signaling. *ACS Omega* **5**:
1045 14575–14585.

1046 **Liu Y, Du H, Li P, Shen Y, Peng H, Liu S, Zhou G-A, Zhang H, Liu Z, Shi M, *et al.* 2020a**. Pan-
1047 Genome of Wild and Cultivated Soybeans. *Cell* **182**: 162-176.e13.

1048 **Liu M, Yu H, Ouyang B, Shi C, Demidchik V, Hao Z, Yu M, Shabala S. 2020b**. NADPH oxidases
1049 and the evolution of plant salinity tolerance. *Plant, Cell & Environment* **43**: 2957–2968.

1050 **Llaurens V, Whibley A, Joron M. 2017**. Genetic architecture and balancing selection: the life
1051 and death of differentiated variants. *Molecular Ecology* **26**: 2430–2448.

1052 **Lowry DB, Hall MC, Salt DE, Willis JH. 2009.** Genetic and physiological basis of adaptive salt
1053 tolerance divergence between coastal and inland *Mimulus guttatus*. *New Phytologist* 183:
1054 776-788.
1055

1056 **Lowry DB, Rockwood RC, Willis JH. 2008**. Ecological Reproductive Isolation of Coast and
1057 Inland Races of Mimulus Guttatus. *Evolution* **62**: 2196–2214.

1058 **Lowry DB, Willis JH. 2010**. A Widespread Chromosomal Inversion Polymorphism Contributes
1059 to a Major Life-History Transition, Local Adaptation, and Reproductive Isolation. *PLOS*
1060 *Biology* **8**: e1000500.

1061 **Lu Z, Cui J, Wang L, Teng N, Zhang S, Lam H-M, Zhu Y, Xiao S, Ke W, Lin J, *et al.* 2021**.
1062 Genome-wide DNA mutations in Arabidopsis plants after multigenerational exposure to high
1063 temperatures. *Genome Biology* **22**: 160.

1064 **Luo X, Wang B, Gao S, Zhang F, Terzaghi W, Dai M. 2019**. Genome-wide association study
1065 dissects the genetic bases of salt tolerance in maize seedlings. *Journal of integrative plant*
1066 *biology* **61**: 658-674.
1067

1068 **Lürig MD, Donoughe S, Svensson EI, Porto A, Tsuboi M. 2021**. Computer Vision, Machine
1069 Learning, and the Promise of Phenomics in Ecology and Evolutionary Biology. *Frontiers in*
1070 *Ecology and Evolution* **9**.

1071 **Lv Y, Ma J, Wei H, Xiao F, Wang Y, Jahan N, Hazman M, Qian Q, Shang L, Guo L. 2022**.
1072 Combining GWAS, genome-wide domestication and a transcriptomic analysis reveals the loci
1073 and natural alleles of salt tolerance in rice (*Oryza sativa* l.). *Frontiers in Plant Science* **13**.
1074

1075 **Lynch M, Ackerman MS, Gout J-F, Long H, Sung W, Thomas WK, Foster PL. 2016**. Genetic
1076 drift, selection and the evolution of the mutation rate. *Nature Reviews Genetics* **17**: 704–
1077 714.

1078 **Maun MA. 2009**. Salt spray and soil salinity. In *The Biology of Coastal Sand Dunes*. Oxford
1079 University Press.
1080

1081 **McNeilly T, Antonovics J. 1968**. Evolution in closely adjacent plant populations IV. Barriers to
1082 gene flow. *Heredity* **23**: 205–218.

1083 **Meier JI, Salazar PA, Kučka M, Davies RW, Dréau A, Aldás I, Box Power O, Nadeau NJ,**
1084 **Bridle JR, Rolian C, *et al.* 2021**. Haplotype tagging reveals parallel formation of hybrid races
1085 in two butterfly species. *Proceedings of the National Academy of Sciences* **118**:
1086 e2015005118.

1087 **Molina-Henao YF, Hopkins R**. **2019**. Autopolyploid lineage shows climatic niche expansion
1088 but not divergence in Arabidopsis arenosa. *American Journal of Botany* **106**: 61–70.

1089 **Monroe JG, Srikant T, Carbonell-Bejerano P, Becker C, Lensink M, Exposito-Alonso M, Klein**
1090 **M, Hildebrandt J, Neumann M, Kliebenstein D, *et al.* 2022**. Mutation bias reflects natural
1091 selection in Arabidopsis thaliana. *Nature* **602**: 101–105.

1092 **Morais MC, Panuccio MR, Muscolo A, Freitas H**. **2012**. Salt tolerance traits increase the
1093 invasive success of Acacia longifolia in Portuguese coastal dunes. *Plant Physiology and*
1094 *Biochemistry* **55**: 60–65.

1095 **Morales Moreira ZP, Helgason BL, Germida JJ**. **2021**. Crop, genotype, and field
1096 environmental conditions shape bacterial and fungal seed epiphytic microbiomes. *Canadian*
1097 *Journal of Microbiology* **67**: 161–173.

1098 **Moray C, Hua X, Bromham L**. **2015**. Salt tolerance is evolutionarily labile in a diverse set of
1099 angiosperm families. *BMC Evolutionary Biology* **15**: 90.

1100 **Morgan EJ, Čertner M, Lučanová M, Kubíková K, Marhold K, Kolář F**. **2020**. Niche similarity
1101 in diploid-autotetraploid contact zones of Arabidopsis arenosa across spatial scales.
1102 *American Journal of Botany* **107**: 1375–1388.

1103 **Munns R, Day DA, Fricke W, Watt M, Arsova B, Barkla BJ, Bose J, Byrt CS, Chen Z-H, Foster**
1104 **KJ, *et al.* 2020**. Energy costs of salt tolerance in crop plants. *New Phytologist* **225**: 1072–
1105 1090.

1106 **Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman SD,**
1107 **Tester M, *et al.* 2012**. Wheat grain yield on saline soils is improved by an ancestral Na+
1108 transporter gene. *Nature Biotechnology* **30**: 360–364.

1109 **Munns R, Tester M**. **2008**. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*
1110 **59**: 651–681.

1111 **Nagpal P, Ellis CM, Weber H, Ploense SE, Barkawi LS, Guilfoyle TJ, Hagen G, Alonso JM,**
1112 **Cohen JD, Farmer EE, *et al.* 2005**. Auxin response factors ARF6 and ARF8 promote jasmonic
1113 acid production and flower maturation. *Development* **132**: 4107–4118.

1114 **Nagy ES, Rice KJ**. **1997**. Local Adaptation in Two Subspecies of an Annual Plant: Implications
1115 for Migration and Gene Flow. *Evolution* **51**: 1079–1089.

1116 **Napieraj N, Reda M, Janicka M, Napieraj N, Reda M, Janicka M**. **2020**. The role of NO in
1117 plant response to salt stress: interactions with polyamines. *Functional Plant Biology* **47**: 865–
1118 879.

1119 **Nitsos RE, Evans HJ**. **1969**. Effects of Univalent Cations on the Activity of Particulate Starch
1120 Synthetase 1. *Plant Physiology* **44**: 1260–1266.

1121 **Otlewska A, Migliore M, Dybka-Stępień K, Manfredini A, Struszczyk-Świta K, Napoli R,**
1122 **Białkowska A, Canfora L, Pinzari F**. **2020**. When Salt Meddles Between Plant, Soil, and
1123 Microorganisms. *Frontiers in Plant Science* **11**.

Pegtel DM. **1999**. Effect of ploidy level on fruit morphology, seed germination and juvenile growth in scurvy grass (Cochlearia officinalis L. s.l., Brassicaceae). *Plant Species Biology* **14**: 201–215.

Petrić I, Šamec D, Karalija E, Salopek-Sondi B. **2022**. Beneficial Microbes and Molecules for Mitigation of Soil Salinity in Brassica Species: A Review. *Soil Systems* **6**: 18.

Popovic D, Lowry DB. 2020. Contrasting environmental factors drive local adaptation at opposite ends of an environmental gradient in the yellow monkeyflower (*Mimulus guttatus*). *American Journal of Botany* **107**: 298-307*.*

Qin Y, Druzhinina IS, Pan X, Yuan Z. **2016**. Microbially Mediated Plant Salt Tolerance and Microbiome-based Solutions for Saline Agriculture. *Biotechnology Advances* **34**: 1245–1259.

Qin P, Lu H, Du H, Wang H, Chen W, Chen Z, He Q, Ou S, Zhang H, Li X, *et al.* 2021. Pan-genome analysis of 33 genetically diverse rice accessions reveals hidden genomic variations. *Cell* **184**: 3542-3558.e16.

Quintero FJ, Martinez-Atienza J, Villalta I, Jiang X, Kim W-Y, Ali Z, Fujii H, Mendoza I, Yun D-J, Zhu J-K, *et al.* 2011. Activation of the plasma membrane Na/H antiporter Salt-Overly-Sensitive 1 (SOS1) by phosphorylation of an auto-inhibitory C-terminal domain. *Proceedings of the National Academy of Sciences* **108**: 2611–2616.

Quintero FJ, Ohta M, Shi H, Zhu J-K, Pardo JM. **2002**. Reconstitution in yeast of the Arabidopsis SOS signaling pathway for Na+ homeostasis. *Proceedings of the National Academy of Sciences* **99**: 9061–9066.

Rahman MM, Mostofa MG, Keya SS, Siddiqui MN, Ansary MMU, Das AK, Rahman MA, Tran LSP. 2021. Adaptive mechanisms of halophytes and their potential in improving salinity tolerance in plants. *International Journal of Molecular Sciences*, **22**: 10733.

Reina-Sánchez A, Romero-Aranda R, Cuartero J. **2005**. Plant water uptake and water use efficiency of greenhouse tomato cultivars irrigated with saline water. *Agricultural Water Management* **78**: 54–66.

Ren Z-H, Gao J-P, Li L-G, Cai X-L, Huang W, Chao D-Y, Zhu M-Z, Wang Z-Y, Luan S, Lin H-X. **2005**. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics* **37**: 1141–1146.

Rouger R, Jump AS. **2015**. Fine-scale spatial genetic structure across a strong environmental gradient in the saltmarsh plant Puccinellia maritima. *Evolutionary Ecology* **29**: 609–623.

Rouifed S, Byczek C, Laffray D, Piola F. **2012**. Invasive Knotweeds are Highly Tolerant to Salt Stress. *Environmental Management* **50**: 1027–1034.

Ruiz M, Quiñones A, Martínez-Cuenca MR, Aleza P, Morillon R, Navarro L, Primo-Millo E, Martínez-Alcántara B. **2016**. Tetraploidy enhances the ability to exclude chloride from leaves in carrizo citrange seedlings. *Journal of Plant Physiology* **205**: 1–10.

Saleh B, Allario T, Dambier D, Ollitrault P, Morillon R. **2008**. Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. *Comptes Rendus Biologies* **331**: 703–710.

Shabala S. 2013. Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of botany* **112**: 1209-1221.

Shi S, Huang Y, Zeng K, Tan F, He H, Huang J, Fu Y. **2005**. Molecular phylogenetic analysis of mangroves: independent evolutionary origins of vivipary and salt secretion. *Molecular Phylogenetics and Evolution* **34**: 159–166.

Shuyskaya EV, Gismatullina LG, Toderich KH, Voronin PYu, Soldatova NV. **2012**. Genetic differentiation of black saxaul, Haloxylon aphyllum (Chenopodiaceae), along a soil salinity gradient in the Kyzylkum Desert. *Russian Journal of Ecology* **43**: 302–306.

Shuyskaya EV, Li EV, Rahmankulova ZF, Kuznetsova NA, Toderich KN, Voronin PYu. **2014**. Morphophysiological adaptation aspects of different Haloxylon aphyllum (Chenopodiaceae) genotypes along a salinity gradient. *Russian Journal of Ecology* **45**: 181–187.

Sirén J, Monlong J, Chang X, Novak AM, Eizenga JM, Markello C, Sibbesen JA, Hickey G, Chang P-C, Carroll A, *et al.* 2021. Pangenomics enables genotyping of known structural variants in 5202 diverse genomes. *Science* **374**: abg8871.

Song J-M, Guan Z, Hu J, Guo C, Yang Z, Wang S, Liu D, Wang B, Lu S, Zhou R, *et al.* 2020. Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of Brassica napus. *Nature Plants* **6**: 34–45.

Stebbins G Ledyard. **1952**. Aridity as a Stimulus to Plant Evolution. *The American Naturalist* **86**: 33–44.

Tester M, Davenport R. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Annals of botany* **91**: 503-527.

Tkacz A, Poole P. **2015**. Role of root microbiota in plant productivity. *Journal of Experimental Botany* **66**: 2167–2175.

Todesco M, Owens GL, Bercovich N, Légaré J-S, Soudi S, Burge DO, Huang K, Ostevik KL, Drummond EBM, Imerovski I, *et al.* 2020. Massive haplotypes underlie ecotypic differentiation in sunflowers. *Nature* **584**: 602–607.

Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito K, Fukuda S, Ushio M, Nakaoka S, Onoda Y, *et al.* 2018. Core microbiomes for sustainable agroecosystems. *Nature Plants* **4**: 247–257.

Trivedi P, Batista BD, Bazany KE, Singh BK. **2022**. Plant–microbiome interactions under a changing world: responses, consequences and perspectives. *New Phytologist* **n/a**.

Tu Y, Fu L, Wang F, Wu D, Shen Q, Zhang G. **2021**. GWAS and transcriptomic integrating analysis reveals key salt-responding genes controlling Na+ content in barley roots. *Plant Physiology and Biochemistry* **167**: 596–606.

Tu Y, Jiang A, Gan L, Hossain M, Zhang J, Peng B, Xiong Y, Song Z, Cai D, Xu W, *et al.* 2014. Genome duplication improves rice root resistance to salt stress. *Rice* **7**: 15.

VanWallendael A, Soltani A, Emery NC, Peixoto MM, Olsen J, Lowry DB. **2019**. A Molecular View of Plant Local Adaptation: Incorporating Stress-Response Networks. *Annual Review of Plant Biology* **70**: 559–583.

Vasquez EA, Glenn EP, Guntenspergen GR, Brown JJ, Nelson SG. **2006**. Salt tolerance and osmotic adjustment of Spartina alterniflora (Poaceae) and the invasive M haplotype of Phragmites australis (Poaceae) along a salinity gradient. *American Journal of Botany* **93**: 1784–1790.

Wang L, Cao S, Wang P, Lu K, Song Q, Zhao F-J, Chen ZJ. **2021**. DNA hypomethylation in tetraploid rice potentiates stress-responsive gene expression for salt tolerance. *Proceedings of the National Academy of Sciences* **118**.

Wang Z, Hong Y, Zhu G, Li Y, Niu Q, Yao J, Hua K, Bai J, Zhu Y, Shi H, *et al.* 2020. Loss of salt tolerance during tomato domestication conferred by variation in a Na^+/K^+ transporter. *The EMBO Journal* **39**.

Wani SH, Kumar V, Khare T, Guddimalli R, Parveda M, Solymosi K, Suprasanna P, Kavi Kishor PB. **2020**. Engineering salinity tolerance in plants: progress and prospects. *Planta* **251**: 76.

Wen F, Zhang Z, Bai T, Xu Q, Pan Y. **2010**. Proteomics reveals the effects of gibberellic acid (GA3) on salt-stressed rice (Oryza sativa L.) shoots. *Plant Science* **178**: 170–175.

Whiteman NK. **2022**. Evolution in small steps and giant leaps. *Evolution* **76**: 67–77.

Wilkinson MC, Roda F, Walter GM, James ME, Nipper R, Walsh J, Liu H, Beveridge CB, Ortiz-Barrientos D. 2019. Divergence in hormone signalling links local adaptation and hybrid failure. *BioRxiv:* 845354.

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. **2009**. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences* **106**: 13875–13879.

Wright SI, Kalisz S, Slotte T. **2013**. Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society B: Biological Sciences* **280**: 20130133.

Wu SJ, Ding L, Zhu JK. 1996. SOS1, a genetic locus essential for salt tolerance and potassium acquisition. *The Plant Cell* **8**: 617-627.

Xu S, Hu B, He Z, Ma F, Feng J, Shen W, Yang J. **2011**. Enhancement of Salinity Tolerance during Rice Seed Germination by Presoaking with Hemoglobin. *International Journal of Molecular Sciences* **12**: 2488–2501.

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Miyazaki A, Takahashi T, Michael A, Kusano T. 2006. The polyamine spermine protects against high salt stress in Arabidopsis thaliana. *FEBS Letters* **580**: 6783–6788.

Yan H, Jia H, Chen X, Hao L, An H, Guo X. 2014. The Cotton WRKY Transcription Factor GhWRKY17 Functions in Drought and Salt Stress in Transgenic Nicotiana benthamiana Through ABA Signaling and the Modulation of Reactive Oxygen Species Production. *Plant and Cell Physiology* **55**: 2060–2076.

Yant L, Bomblies K. 2015. Genome management and mismanagement—cell-level opportunities and challenges of whole-genome duplication. *Genes & Development* **29**: 2405–2419.

Yant L, Hollister JD, Wright KM, Arnold BJ, Higgins JD, Franklin FCH, Bomblies K. 2013. Meiotic Adaptation to Genome Duplication in Arabidopsis arenosa. *Current Biology* **23**: 2151–2156.

Yasmin H, Naeem S, Bakhtawar M, Jabeen Z, Nosheen A, Naz R, Keyani R, Mumtaz S, Hassan MN. 2020. Halotolerant rhizobacteria Pseudomonas pseudoalcaligenes and Bacillus subtilis mediate systemic tolerance in hydroponically grown soybean (Glycine max L.) against salinity stress. *PLOS ONE* **15**: e0231348.

de Zelicourt A, Al-Yousif M, Hirt H. 2013. Rhizosphere Microbes as Essential Partners for Plant Stress Tolerance. *Molecular Plant* **6**: 242–245.

Zhang M, Cao Y, Wang Z, Wang Z, Shi J, Liang X, Song W, Chen Q, Lai J, Jiang C. 2018. A retrotransposon in an HKT1 family sodium transporter causes variation of leaf Na+ exclusion and salt tolerance in maize. *New Phytologist* **217**: 1161–1176.

Zhang M, Liang X, Wang L, Cao Y, Song W, Shi J, Lai J, Jiang C. 2019. A HAK family Na+ transporter confers natural variation of salt tolerance in maize. *Nature Plants* **5**: 1297–1308.

Zheng Y, Xu Z, Liu H, Liu Y, Zhou Y, Meng C, Ma S, Xie Z, Li Y, Zhang C-S. 2021. Patterns in the Microbial Community of Salt-Tolerant Plants and the Functional Genes Associated with Salt Stress Alleviation. *Microbiology Spectrum* **9**: e00767-21.

Zhou X, Li J, Wang Y, Liang X, Zhang M, Lu M, Guo Y, Qin F, Jiang C. 2022. The classical SOS pathway confers natural variation of salt tolerance in maize. *New Phytologist* **236**: 479-494.

Zhou Y, Minio A, Massonnet M, Solares E, Lv Y, Beridze T, Cantu D, Gaut BS. 2019. The population genetics of structural variants in grapevine domestication. *Nature Plants* **5**: 965–979.

Zhou Y, Zhang Z, Bao Z, Li H, Lyu Y, Zan Y, Wu Y, Cheng L, Fang Y, Wu K, *et al.* 2022. Graph pangenome captures missing heritability and empowers tomato breeding. *Nature* **606**: 527–534.

1277 **Figure Legends**

1278

1279 **Figure 1. Schematic of mechanisms of adaptive salt-tolerance**. Consider the

1280 landscape above. Seawater provides a source of sodium ions; wind carries sea spray

1281 inland, creating a gradient of soil salinity. Ancestral populations of wild plant species

1282 originated inland. In this population we observe standing variation which is affected by

1283 *de novo* mutation and purifying selection, removing any alleles that come with a fitness

1284 disadvantage, or in other words, which are maladaptive (e.g. *AtHKT1^{HLS}*). Plant

1285 populations then by chance migrate to the seaside, possibly due to seeds being carried

1286 by humans or other animals. This derived population will represent a subset of the

1287 standing variation observed in the ancestral population. If it carries some of the rarer

1288 alleles, which are under purifying selection further inland, due to the high cost

1289 associated with them, these alleles could now be under positive selection if they are

1290 adaptive in the new location. These alleles would become fixed in this new habitat (e.g.

1291 *GsERD15B^{Ins}*). Under this scenario the effective population size decreases, the

1292 phenotype becomes much more constant, and plasticity is reduced. Other realistic

1293 scenarios include migrants harbouring these alleles at a much higher frequency

1294 representing stepping stones in that direction. In these migrants, balancing selection

1295 maintains a relatively high frequency of an allele. This could reflect the allele being

1296 required at certain times in the year or in certain challenging but regular events (e.g.

1297 mixed population of *AtHKT1^{HLS} and AtHKT1^{LLS}*).

1298

1299

1300 **Figure 2. Experimental setup for a microbiome reciprocal transplant.** Salt-

1301 adapted and salt-sensitive plants cultivated in sterile saline or sterile non-saline soil

1302 will be non-inoculated (N/I), inoculated with their own microbiome (saline microbiome

1303 (S-M) or non-saline microbiome (NS-M), or inoculated with the opposite microbiome,

1304 in each of the four scenarios.

Tables

1305

1306

1307 Table 1. An overview of published plant reciprocal transplant and common garden experiments

Species	Type of Experiment	Years	Environment	Medium	factor	Evidence for local adaptation	Candiate loci	Author
A. thaliana	Reciprocal Transplant	2 years	in field	in situ soil	shade	no	no	Callahan & Pigliucci, 2002
	Common garden	1 year	controlled environment	potting mix				
A. thaliana	Reciprocal Transplant	1 year	in field	in situ soil	dune vs. Inland	local over foreing	no	Arany et al., 2009
A. thaliana	Common garden	1 year	in field	in situ soil	latitude, oceanic VS continental	alleles with lower fitness had greater climate specialization: specialized alleles for special climate: local adaptation	LAC1, AT1G18130, CHR8, AT2G18780, PHYB, delta-TIP, NDF4, TRZ4, AT3G16270, SAG21, AT4G02370, PARP1	Fournier-Level et al., 2011
A. thaliana	Reciprocal Transplant	3 years	in field	in situ soil	north Swede vs. south Italy	local over foreing	15 QTLs	Ågren & Schemske, 2012
A. thaliana	Reciprocal Transplant	2 years	in field	in situ soil	soil salinity	local over foreing	*HKT1*	Busoms et al., 2015
A. thaliana	Response to Na treatment	2 years	controlled environment	ex situ soil	NaCl	not studied	15 genes, AT4g08850, MUSTANG1, AT1G25370	Julkowska et al., 2016
A. thaliana	Reciprocal Transplant	2 years	in field	in situ soil	soil salinity	local over foreing	*HKT1*	Busoms et al., 2018
A. thaliana	Reciprocal Transplant	2 years	in filed	in situ soil	Coastal Vs Inland	local over foreing	*MOT1*	Busoms et al., 2021
	Reciprocal Transplant	2 years	controlled environment	ex situ soil	NaCl			
A. thaliana	Common garden	several	in field	in situ soil	latitude, oceanic VS continental	not studied	Flowering time control for FRI, GIS5, PKT4 and RDO5	Fournier-Level et al., 2022
Avicennia schaueriana	Common garden	1 year	controlled environment	sand: in situ soil	latitud, water deficit and solar radiation	local over foreing	Loci associated with photosynthesis, anthocyanin accumulation, responses to osmotic and hypoxia	Cruz et al., 2019
Borrichia frutescens	Common garden	1 year	controlled environment	sterilized sand: organic medium	NaCl	no	no	Richards et al., 2010
Camissoniopsis cheiranthifolia	Reciprocal Transplant	1 year	in field	in situ soil	Latitud, coastal	no	no	Samis et al., 2016.
Gilia capitata	Reciprocal Transplant	3 years	in field	in situ soil	Coastal Vs Inland	local over foreing	no	Nagy and Rice, 1997
Hydrocotyle bonariensis	Reciprocal Transplant	1 year	in field	in situ soil	hight and low dune with salt gradient	local over foreing if local vegiation was maintained	no	Knight & Miller, 2004

Medicago truncatula	Reciprocal Transplant	1 year	in field	in situ soil	soil salinity	local over foreing	*CIPK21* ortholouge; trehalose-6-phosphate phostatase, regulators of ABA and JA, CPK ortholouge	Friesen et al., 2014
	Common garden	1 year	controlled environment	in situ soil				
	Common garden	1 year	controlled environment	sterile sand				
Mimulus guttatus	Reciprocal Transplant	1 year, 4 locations	in field	in situ soil	Coastal Vs Inland	local over foreing	no	Lowry *et al.*, 2008
	Common garden	1 year	controlled environment	potting mix	NaCl			
Mimulus guttatus	Manipulative reciprocal transplant	1 year	in field	in situ soil	Coastal Vs Inland	costal ecotype outperformed inland ecotype	no	Popovic & Lowry, 2020
Oryza coarctata	Response to Na treatment	2 years	controlled environment	unknown soil with saline river water	NaCl	not studied	no	Bal and Dutt, 1986
Phragmites australis, Spartina alterniflora	Common garden	1 year	controlled environment	mixture of peat and in situ soil	NaCl	foreing over local in a changing habitat	no	Vasquez *et al.*, 2006
Porteresia coarctata	Response to Na treatment	1 year	controlled environment	potting mix	NaCl	not studied	no	Flowers *et al.*, 1990
Triplasis purpurea	Common garden	1 year	controlled environment	sterile sand: in situ soil	sea salt spray	no	no	Cheplick & White, 2002
Zea maise	Common garden	1 year	controlled environment	potting mix	NaCl	not studied	*HKT1, HAK4*	Zhang *et al*., 2018 and 2019
32 plant species	Reciprocal Transplant	varying	varying	varying	various	local over foreing	no	Leimu & Fischer, 2008

1308
1309

1310 Table 2. Studies that have characterized saline-associated core microbiomes

Environmental origin	Plant Species	Microbiome type	Sampling	Comparison strategy	Most abundant taxa	Tested for salinity tolerance in host or non-host species:	Reference
Coastal habitats (high salinity) of Taiwan	*Miscanthus sinensis*	Rhizosphere and endosphere compartments	20 samples: 5 sites, 2 specimens, 2 compatments	Dominant bacteria across all samples	Endophytic *Agrobacterium*, *Amycolatopsis* (with ACC deaminase enzyme) and denitrifying bacteria.		Huang et al., 2020
Coastal cliffs in the North Atlantic coast of Spain	*Festuca rubra pruinosa*	Endophytic mycobiota of roots	105 samples: around 20 samples from 5 sites	Dominant endophytic fungi across all samples	*Fusarium, Diaporthe,* Helotiales, *Drechslera, Slopeiomyces,* and *Penicillium*		Pereira et al., 2019
Coastal habitats (eastern China)	*Suaeda salsa*	Bulk soil and root endosphere	18 samples: 3 sites , 3 speciemens, 2 fractions	Dominant bacteria and fungi across all samples	*Proteobacteria (α and γ), Microbulbifer, Pelagibius, Halomonas, Marinoscill um, Fulvivirga, Haloferula, Pelagicoccus, Marinobacter*	Cucumber, Rice	Yuan et al., 2016
Coastal habitats of San Juan Island Archipelago (WA)	*Leymus mollis*	Fungal endophytes	200 plants collected from several beach habitats in 2 different years	Isolation of culturable fungal endophytes	*Fusarium culmorum*	Tomato, Rice	Rodriguez et al., 2008
Coastal salt marshes on Dauphin Island (Alabama)	*Spartina alterniflora & Juncus roemerianus*	Rhizosphere microbiome	194 samples: 2 plant species, 5 replicates, from 4/ 2015 to 10/2016	Core microbiome of both species in different samples from the same salt marsh	*Anaerolineaceae; Planctomycetes, Proteobacteria (α and δ), Chloroflexi*		Mason et al., 2021
Coastal salt marshes in Southern Spain	*Arthrocnemum macrostachyum*	Bulk soil	8 samples: 2 locations, 2 replicates, 2 seasons	Dominant bacteria	*Proteobacteria, Actinobacteria, Bacteroidetes, Ge mmatimonadetes, Chloroflexi, Firmicutes, Acidoba cteria*		Camacho-Sanchez et al., 2020
Coastal salt marshes of Jiangsu Province (China)	*Limonium sinense*	Bacterial communities of the roots, leaves, rhizosphere and bulk soils	12 samples: 1 site, 3 specimens, 4 fractions	Dominant bacteria across all samples	*Actinobacteria (Glutamicibacter, Streptomyces, Isoptericola); Firmicutes (Bacillus, Lysinibacillus, Staphylococcus); Proteobacteria (Pseudomonas, Serratia, Klebsiella, Neorhizobium)*	*Glutamicibacter halophytocola* strain KLBMP 5180 *tested in L. sinense under 250 mM NaCl*	Qin et al., 2018
Coastal saline fields of west Bengal (India)	*Oryza sativa*	Root endophytic bacteria	6 agroecological regions, 3 sites, 3 specimens	Dominant endophyti bacteria across samples from the Coastal Saline Zone	*Firmicutes* and *Proteobacteria*		Kunda et al., 2021
Hypersaline ecosystems of southern Tunisia	*Salicornia spp.*	Rhizosphere and bulk soil	18 samples: 3 sites, 3 specimens, 2 fractions	Dominant bacteria across all samples	*Halomonas*		Mapelli et al., 2013
Saline habitats of northeastern Pakistan	*Suaeda fruticosa*	Rhizosphere and phytoplane		Halotolerant bacteria	*Gracilibacillus, Staphylococcus, Virgibacillus, Salinicoccus, Bacillus, Zhihengliuella, Brevibacterium, Oceanobacillus, Exiguobacterium, Pseudomonas, Arthrobacter, and Halomonas*	*Staphylococcus jettensis F-11, Zhihengliuella flava F-9, Bacillus megaterium F-58, S. jettensis F-11 and S. arlettae F-71 tested in Zea mays under 200 mM NaCl*	Aslam & Ali, 2018

Location	Plant	Microbiome/Compartment	Samples	Focus	Main findings	Other	Reference
Saline site - Salt Mine (Khewra, Pakistan)	*Salsola stocksii & Atriplex amnicola*	Rhizospheric soil		*Bacillus*-derived bacterial (halophilic, alkaliphilic, and mesophilic)	*Bacillus, Halobacillus, Virgibacillus, Brevibacillus, Paenibacillus, Tumebacillus, and Lysinibacillus*		Mukhtar et al., 2018
Saline sites (Anthropogenic and naturally) of Central Poland	*Salicornia europaea*	Endophytes of roots and shoots	36 samples: 2 sites, 2 seasons, 3 plots, 3 replicates	Dominant endophytes across all samples	*Proteobacteria* and *Bacteroidetes* dominated bacterial assemblages, and *Ascomycetes* were the most frequent fungi. A root core microbiome of the genus *Marinimicrobium* was identified.		Furtado et al., 2019
Saline sites of central Argentina	Chenopodiaceae (*Allenrolfea patagonica, Atriplex argentina, Heterostachys ritteriana* and *Suaeda divaricta*)	Arbuscular mycorrhizal fungi (AMF) of rhyzospheric soil and roots	40 samples: 2 sites, 5 depth intervals, 4 species	AMF diversity	19 morphologicaly distinctive AMFs (more present: *Glomus magnicaule, Septoglomus aff. constrictum, G. brohultti,* and *Septoglomus aff.*)		Becerra et al., 2014
Salterns of Secovlje (Slovenia)	12 halophytic plants	Arbuscular mycorrhizal fungi and/or dark septate endophytes of rhyzospheric soil and roots	8 sites, 12 species, different number of individuals	AMF and DSE identification and colonization levels	Co-ocurrance: Glomus sp. and Diversispora sp. clades		Sonjaket al., 2009
Experimental field station at Shenyang Agricultural University (China)–Soil adjusted to 2.5 g (NaCl) kg⁻¹ to mimic a moderate soil salinity level	*Sorghum bicolour, Arachis hypogaea,* and intercropping system	Peanut rhizosphere (IP), sorghum rhizosphere (IS), and interspecific interaction zone (II)	18 soil samples: 3 sites, 3 replicates, 2 years	Core microbiome of both species in the three zones	Dominant bacterial phyla: *Proteobacteria, Bacteroidota,* and *Acidobacteriota* // Dominant fungal phyla: Ascomycota, Basidiomycota, and Mucoromycota		Shi et al., 2021
Experimental field station of Shihezi University (China)	*Leymus mollis* (dunegrass)	Arbuscular mycorrhizal fulgal		G. mosseae isolate from saline soil *vs* non-saline soil	*Glomus mosseae*	Cotton	Tian et al., 2004
Experimental field station - Saline soil from the Shandong Academy of Agricultural Sciences (China)	*Glycine soja, Sesbania cannabina* and nonlegume *Sorghum bicolor*	Bulk soil, rhizosphere, and nodule microbiome	36 samples: 3 plant species, 3 speciemens, 4 compartments	Core microbiome in the 4 compartements of two legumes and dominant bacteria in the nonlegume	Dominant bacteria belonged to *Proteobacteria* and *Ensifer* for legumes and *Bacillus* for *S. bicolor*		Zheng et al., 2020 & 2021
Deserts and dry lands of Mexico and southern California	Cultivated and native *Agave spp.*	Rhizosphere, phyllosphere, leaf and root endosphere, proximal and distal soil	252 samples: 72 from *A. tequilana*, 72 from *A. salmiana* and 108 from *A. desert*	Core microbiome of 3 Agave species from different locations	Increased abundance of *Proteobacteria* and decreased presence of *Acidobacteria* // Dominated by members of Ascomycota		Coleman-Derr et al. 2016

1311

41

Saline conditions

Non-Saline conditions

Salt-sensitive plants

N/I
NS-M
(Own)
S-M

N/I
S-M
NS-M
(Own)

Salt-adapted plants

N/I
NS-M
S-M
(Own)

N/I
S-M
(Own)
NS-M