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# Journal Pre-proof

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1 **To exclude or to accumulate? Revealing the role of the sodium HKT1;5 transporter in**  
2 **plant adaptive responses to varying soil salinity**

3

4 **Running Title: Sodium transporter *HKT1;5* function *vis-a-vis* soil salinity**

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## 26 Abstract

27 Arid/semi-arid and coastal agricultural areas of the world are especially vulnerable to climate  
28 change-driven soil salinity. Salinity tolerance in plants is a complex trait, with salinity  
29 negatively affecting crop yield. Plants adopt a range of mechanisms to combat salinity, with  
30 many transporter genes being implicated in Na<sup>+</sup>-partitioning processes. Within these, the high-  
31 affinity K<sup>+</sup> (HKT) family of transporters play a critical role in K<sup>+</sup> and Na<sup>+</sup> homeostasis in plants.  
32 Among HKT transporters, Type I transporters are Na<sup>+</sup>-specific. While *Arabidopsis* has only  
33 one Na<sup>+</sup>-specific HKT (*AtHKT1;1*), cereal crops have a multiplicity of Type I and II HKT  
34 transporters. *AtHKT1;1* (*Arabidopsis thaliana*) and HKT1;5 (cereal crops) 'exclude' Na<sup>+</sup> from  
35 the xylem into xylem parenchyma in the root, reducing shoot Na<sup>+</sup> and hence, confer sodium  
36 tolerance. However, more recent data from *Arabidopsis* and crop species show that  
37 *AtHKT1;1/HKT1;5* alleles have a strong genetic association with 'shoot sodium accumulation'  
38 and concomitant salt tolerance. The review tries to resolve these two seemingly contradictory  
39 effects of *AtHKT1;1/HKT1;5* operation (shoot exclusion vs shoot accumulation), both  
40 conferring salinity tolerance and suggests that contrasting phenotypes are attributable to either  
41 hyper-functional or weak *AtHKT1;1/HKT1;5* alleles/haplotypes and are under strong selection  
42 by soil salinity levels. It also suggests that opposite balancing mechanisms involving xylem  
43 ion loading in these contrasting phenotypes exist that involve transporters such as SOS1 and  
44 CCC. While HKT1;5 is a crucial but not sole determinant of salinity tolerance, investigation  
45 of the adaptive benefit(s) conferred by naturally occurring intermediate *HKT1;5* alleles will be  
46 important under a climate change scenario.

## 47 1. Introduction

48 Changes in climatic patterns worldwide are increasingly affecting agricultural areas. Due to a  
49 combination of factors, arid, semi-arid and coastal agricultural areas of the world are especially  
50 vulnerable to climate change impacts on soil salinity. In arid and semi-arid regions, soil salinity

51 and irrigation management go hand-in-hand because salinity control is generally a consequence  
52 of leaching (Corwin, 2021). In coastal regions, sea water penetration into coastal estuaries and  
53 groundwater aquifers causes salt accumulation in the root zone. The relationship between  
54 salinity and crop yield is well established and crop yield decreases linearly beyond a specific  
55 soil salinity threshold that is different for each crop or genotype, with yield losses also differing  
56 in a crop or genotype-specific manner (Maas and Hoffman, 1977).

57 The canonical view is that plants respond to salinity in a biphasic manner, with an  
58 immediate, ‘osmotic response’ to salinity stress (following perception) involving stomatal  
59 closure and inhibition of shoot growth that precedes  $\text{Na}^+$  accumulation in the plant. In the  
60 subsequent ‘ionic response’, growth inhibition occurs due to  $\text{Na}^+$  accumulation. However,  
61 more recent data suggests this view is somewhat oversimplified. In addition to sensing  
62 monovalent cations, plants have specific mechanisms to sense  $\text{Na}^+$  (e.g., root halotropism) that  
63 are activated rapidly upon exposure to salt, triggering downstream  $\text{Ca}^{2+}$  spikes as well as the  
64 *Salt Overly Sensitive* (SOS) pathway (Galvan-Ampudia et al., 2013; Lamers et al., 2020; van  
65 Zelm et al., 2020). Toxicity of  $\text{Na}^+$  ions in plants during the longer term ‘ionic phase’ is  
66 resolved by a combination of avoidance and tolerance strategies, involving a range of  
67 transporters expressed at the plasma membrane and/or tonoplast as well as hormone mediated  
68 transcriptional networks governing their expression (Almeida et al., 2017; van Zelm et al.,  
69 2020). When present in high quantities in the root zone,  $\text{Na}^+$  is taken up mainly by non-selective  
70 cation channels (NSCCs) in roots (Demidchik and Tester, 2002) that are unable to discriminate  
71 between potassium and sodium as both have almost similar hydrated ionic radii (Benito and  
72 Gonzalez-Guerrero, 2014). Increased uptake of  $\text{Na}^+$  disrupts  $\text{K}^+$  homeostasis through multiple  
73 mechanisms (Shabala et al., 2016; Rubio et al., 2020; Zhao et al., 2020). While  $\text{Na}^+$  is not an  
74 essential mineral for plant growth (except in halophytes; Kronzucker and Britto, 2011; Shabala,  
75 2013; Maathuis, 2014; Flowers and Colmer, 2015; Wu et al., 2018),  $\text{K}^+$  is an essential

76 macronutrient for plant growth and is crucial for a range of metabolic processes (enzyme  
77 activation, osmotic adjustment, turgor generation, cell expansion, regulation of membrane  
78 electric potential, and pH homeostasis; Jegadeeson et al., 2019). While there is wide variation  
79 in soil  $K^+$  concentrations (0.01-20 mM), plant cells maintain a relatively constant concentration  
80 of 80-100 mM in the cytoplasm (Rodríguez-Navarro, 2000), accumulating large amounts of  
81  $K^+$  in vacuoles that maintains cell turgor, driving cell expansion and growth. Hence,  $K^+$  is the  
82 most abundant cation in plant cells, comprising up to 10% of plant dry weight (White and  
83 Karley, 2010). Increased  $Na^+$  uptake under salinity reduces  $K^+$  content in the cytosol of plant  
84 cells (Wu et al., 2018; Rubio et al., 2020), with major consequences for efficient metabolic  
85 functioning.

86 Tolerance to salinity is a complex trait and plants adopt a range of mechanisms that  
87 contribute to salinity tolerance (tissue tolerance, sodium exclusion, osmotic stress tolerance,  
88 tissue-specific sodium sequestration), with many transporter genes being implicated in  $Na^+$   
89 partitioning processes within plants (Maathuis et al., 2014; Zhao et al., 2020). Availability of  
90 high throughput genomic sequencing resources coupled with Genome Wide Association  
91 Studies (GWAS) make it now possible to look at single nucleotide polymorphisms (SNPs) in  
92 transporter genes (promoter, coding, non-coding) and delineate specific haplotypes that are  
93 associated with salinity tolerance (Li, 2020). This review examines available data regarding  
94 natural variation in a specific subtype of Type I HKT sodium transporter genes in model plants  
95 and crop species in relation to salinity tolerance. Thus, both *AtHKT1;1* (the only HKT present  
96 in *Arabidopsis*) and *HKT1;5* transporters in cereals ‘exclude’  $Na^+$  from the xylem into the  
97 xylem parenchyma in the root, reduce  $Na^+$  flow to the shoot and hence, confer sodium  
98 tolerance. However, more recent data from *Arabidopsis* and crop species show that *AtHKT1;1*  
99 or *HKT1;5* alleles also have a strong genetic association with ‘shoot sodium accumulation’ and  
100 concomitant salt tolerance The review tries to resolve these two seemingly contradictory effects

101 of AtHKT1;1/HKT1;5 function (shoot exclusion vs shoot accumulation), both conferring  
102 salinity tolerance and suggests that contrasting phenotypes are attributable to hyper-functional  
103 or weak *AtHKT1/HKT1;5* alleles/haplotypes, strongly selected by soil salinity. We conclude  
104 that the spectrum of natural haplotypic variation seen in Type I HKT1;5, when viewed in the  
105 context of naturally varying soil salinity, may identify alleles that could be used to develop  
106 stress-resilient crops to account for future climate change.

## 107 **2. The Trk/Ktr/HKT family of transporters**

108 The Trk/Ktr/HKT family of transporters comprises of H<sup>+</sup>-K<sup>+</sup> and Na<sup>+</sup>-K<sup>+</sup> symporters found in  
109 bacteria, fungi and plants or Na<sup>+</sup> uniporters (found only in plants; Corratgé-Faillie et al., 2010).  
110 This family of transporters has evolved from being involved originally only in K<sup>+</sup> transport in  
111 bacteria, fungi and lower plants to contribute subsequently also to xylem sap Na<sup>+</sup>-desalination  
112 and therefore Na<sup>+</sup> detoxification in higher plants (Durrell et al., 1999). Irrespective of their ion  
113 specificity, bacterial Trk and Ktr ion-conducting subunits and fungal/plant Trk/HKTs show a  
114 conserved structural organization: four consecutively arranged transmembrane-pore-  
115 transmembrane (MPM) domains in which the pore domains come together to form the ion  
116 permeation pathway (Durrell and Guy, 1999; Durrell et al., 1999). Within each pore domain,  
117 specific residues called ‘selectivity filters’ (SF) control cation specificity at the binding site. In  
118 plants, HKT transporters are classified into two sub-types based on gene organization, protein  
119 structure and ion specificity as: sodium uniporters (Type I) and sodium/potassium symporters  
120 (Type II). In Type I HKTs, a serine residue is present in the first SF while the remaining three  
121 amino acid residues are glycine (SF2-4: S-G-G-G; Fig. 1A). In addition, amino acid residues  
122 surrounding the selectivity filters within each pore domain are also conserved in Type I HKTs  
123 (Fig. 1B). Type II HKTs show a ‘G’ at all positions in the four selectivity filters (SF: G-G-G-  
124 G motif); however, exceptions to this rule have been reported, implicating additional residues  
125 in conferring cation specificity in plants (Ali et al., 2016).

126 While only one *HKT* gene occurs in *Arabidopsis* (sodium specific *AtHKT1;1*; Uozumi  
127 et al., 2000; Rus et al., 2001; Berthomieu et al., 2003; Rus et al., 2004; Sunarpi et al., 2005;  
128 Baek et al., 2011; An et al., 2017; Wang et al., 2018a), other species (regardless of dicots or  
129 monocots) show a multiplicity of *HKT* genes (Liu et al., 2001; Horie et al., 2009; Asins et al.,  
130 2013; Waters et al., 2013; Almeida et al., 2014; Véry et al., 2014). Within the sodium specific  
131 Type I HKTs, *HKT1;5* has been associated with its ability to reduce shoot  $\text{Na}^+$  load in cereal  
132 crops (Ren et al., 2005; Munns et al., 2012; Byrt et al., 2014; Yang et al., 2014; Jiang et al.,  
133 2018). The ability of *Arabidopsis* and most cereal species (e.g., wheat, rice, barley) to maintain  
134 low  $\text{Na}^+$  concentrations in leaves correlates with improved growth under saline conditions and  
135 is termed shoot or leaf ‘sodium exclusion’ (Berthomieu et al., 2003; Ren et al., 2005; Byrt et  
136 al., 2007; Munns et al., 2012). The subsequent section examines how tissue specificity of  
137 expression of type I HKTs and/or allelic variation contributes to salinity tolerance in plants.

### 138 **3. Root expression of *AtHKT1;1* and *HKT1;5* in cereal crops is associated with salinity** 139 **tolerance**

140 With the exception of *OsHKT1;3* that localizes to the Golgi apparatus (Rosas-Santiago et al.,  
141 2015) all Type I HKTs localize to the plasma membrane (Ren et al., 2005; Byrt et al., 2007;  
142 Munns et al., 2012; Suzuki et al., 2016). The only HKT transporter found in the glycophytic  
143 species *Arabidopsis* is *AtHKT1;1*. *AtHKT1;1* is expressed in the vasculature of the whole  
144 plant (Mäser et al., 2002; Berthomieu et al., 2003), in particular in root in xylem parenchyma  
145 cells (Sunarpi et al., 2005; Fig. 1B). *AtHKT1;1* mutants (Col-0 ecotype) have a higher xylem  
146 sap  $\text{Na}^+$  concentration than wild type plants (Sunarpi et al., 2005), with  $^{22}\text{Na}^+$  quantitative  
147 flux analysis revealing that *AtHKT1;1* controls the rate of  $\text{Na}^+$  transport from root to shoot  
148 by the retrieval of  $\text{Na}^+$  from the xylem sap in roots (Davenport et al., 2007). Vasculature-  
149 specific expression of *AtHKT1;1* is associated with increased salinity tolerance, originating  
150 from reduced shoot  $\text{Na}^+$  accumulation (Berthomieu et al., 2003; Rus et al., 2004; Sunarpi et



151 al., 2005; Davenport et al., 2007) while constitutive expression of *AtHKT1;1* renders  
152 *Arabidopsis* sensitive to salt stress (50-100 mM; Møller et al., 2009). This suggests that tissue  
153 specific expression (in particular in root vasculature) of *AtHKT1;1* is essential to impart  
154 salinity tolerance. In rice, *OsHKT1;5* is orthologous to *AtHKT1;1* (along with *OsHKT1;4*;  
155 Suzuki et al., 2016; Khan et al., 2020) and was first identified as the quantitative trait locus  
156 *SKC1* (Lin et al., 2004), coding for a transporter that unloads Na<sup>+</sup> from the root xylem  
157 (*OsHKT1;5*; Ren et al., 2005). *OsHKT1;5(SKC1)* promoter directed GUS activity is detected  
158 in the vasculature of nodes, internodes, leaf-sheath nodes, roots and leaves. More recently,  
159 Kobayashi et al. (2017) have shown that loss-of-function of *OsHKT1;5* in salt-stressed rice  
160 roots triggers massive Na<sup>+</sup> accumulation in shoots. Immunostaining indicates that *OsHKT1;5*  
161 localizes in cells adjacent to the xylem in roots. Further, higher expression of *OsHKT1;5*  
162 correlates with higher salt tolerance in a number of rice landraces (Cotsaftis et al., 2012). In  
163 bread wheat, *TaHKT1;5-D* is expressed in root tissues but not in leaves (Byrt et al., 2007).  
164 Within roots, *TaHKT1;5-D* expression localizes to the xylem parenchyma and pericycle cells  
165 adjacent to xylem cells in the stele (Byrt et al., 2014). Introgressed *Nax2* locus from durum  
166 wheat into bread wheat also confers salinity tolerance due to xylem parenchyma and  
167 pericycle specific *TmHKT1;5-A* expression in the root stele. *HvHKT1;5* from barley localizes  
168 to the xylem parenchyma and endodermis (Houston et al., 2020). Also, *HvHKT1;5* is highly  
169 expressed in roots compared to shoots, with expression levels correlating inversely with Na<sup>+</sup>  
170 content in barley accessions (van Bezouw et al., 2019).

171 Independent lines of evidence suggest that the expression in root vasculature of  
172 *AtHKT1;1* in *Arabidopsis* or *HKT1;5* in rice is crucial to salinity tolerance. First, increased  
173 expression of *AtHKT1;1* specifically in roots of *Arabidopsis* or rice imparts salinity tolerance  
174 (Møller et al., 2009; Plett et al., 2010). Second, knockout of expression of *OsHKT1;5* (which  
175 is almost root-specific) leads to salt sensitivity (Kobayashi et al., 2017). Third, a

176 transcriptional complex of three proteins (OsSUVH7, OsMYB106 and OsBag4) acts in  
177 concert to upregulate expression of *OsHKT1;5* in roots under salinity (Wang et al., 2020).  
178 Disrupting OsSUVH7 or OsMYB106 binding sites in the *OsHKT1;5* promoter or knocking  
179 out *OsSUVH7*, *OsMYB106* or *OsBag4* reduces *OsHKT1;5* expression in roots and decreases  
180 salinity tolerance of rice. Based on *in situ* expression data for *HKT1;5* in cereals as well as  
181 experimental evidence mentioned above, it appears that root expression of plasma membrane  
182 localized *HKT1;5* under salinity, essentially at the xylem:xylem parenchyma interface, has  
183 evolved as a mechanism to confer shoot sodium exclusion (Fig. 2).

184 It must also be mentioned, that, in addition to a role in mediating xylem Na<sup>+</sup>  
185 exclusion, in *Arabidopsis* and rice, phloem associated roles in salt tolerance have been  
186 evidenced for AtHKT1;1 and proposed for OsHKT1;5. In the former, AtHKT1;1 was shown  
187 to be involved in recirculation of Na<sup>+</sup> to roots *via* phloem (Berthomieu et al., 2003). In the  
188 latter, phloem parenchyma based Na<sup>+</sup> exclusion in diffuse vascular bundles of basal nodes  
189 has been proposed to limit Na<sup>+</sup> transport to younger rice leaves (Kobayashi et al., 2017). The  
190 role of phloem-associated expression of AtHKT1;1 or OsHKT1;5 and its contribution to  
191 salinity tolerance requires further investigation.

#### 192 **4. Allelic variation in HKT1;5 structure in cereal crops influences Na<sup>+</sup> transport and is** 193 **an additional determinant of salinity tolerance**

194 In addition to expression level variation seen in *Arabidopsis*, rice and barley, SNPs involving  
195 non-synonymous substitutions have been demonstrated to influence Na<sup>+</sup> transport capacity  
196 (affinity, conductance) of HKT1;5 transporters in cereal crops that affect salt tolerance. A  
197 50% higher conductance of OsHKT1;5 from the salt tolerant rice landrace Nona Bokra has  
198 been reported when compared with the same transporter from the salt sensitive rice landrace  
199 Koshihikari that differ at four amino acid residues (Nona Bokra vs Koshihikari: A140P,  
200 H184R, D332H and V395L; Ren et al., 2005). Of these four residues in OsHKT1;5, 395L in

201 OsHKT1;5 (Koshihikari), by virtue of having a larger side chain positioned near the pore  
202 entrance, offers steric hindrance and is hypothesized to confer lower conductance (Cotsaftis  
203 et al., 2012). Valine also appears to be present in TaHKT1;5D, TmHKT1;5A, barley and  
204 maize HKT1;5 sequences while aspartate (D332), oriented at the intracellular interface in  
205 OsHKT1;5, is also conserved in TmHKT1;5A and TaHKT1;5D (Su et al., 2015; Shohan et  
206 al., 2019). While the association of valine vs leucine in tolerant vs sensitive rice landraces  
207 strengthens the importance of this amino acid residue change with regard to salinity  
208 tolerance, it must be noted that there is currently no direct experimental data available (e.g.,  
209 site directed mutagenesis coupled with transport assays) to verify this conclusively.

210 Crystal structures of distantly related  $K^+$  transporters TrkH from *Vibrio*  
211 *parahaemolyticus* (Cao et al., 2011) and KtrAB from *Bacillus subtilis* have been used as  
212 templates to model plant HKT structures (Cotsaftis et al., 2012; Xu et al., 2018;  
213 Somasundaram et al., 2020). Though bacterial potassium transporters show low identity to  
214 plant HKT sequences, the MPM domains are sufficiently conserved to model plant HKT  
215 structures including rice, wheat and barley HKT1;5s (Fig. 1B). Using 3D modeling tools  
216 (e.g., Modeller) and using bacterial  $K^+$  transporters as templates, six (of 27) amino acid  
217 residue differences between TmHKT1;5A and TaHKT1;5D are predicted to be significant,  
218 accounting for affinity and conductance differences in *Xenopus* transport assays (Xu et al.,  
219 2018). Among the six residues, D471 and D474 from TmHKT1;5-A occur very close to each  
220 other and form a part of an  $\alpha$ -helix which directly links to one of the loops forming the  
221 selectivity filter. The former has no counterpart in TaHKT1;5D while the latter is substituted  
222 by G473. Reciprocal double mutations increase  $Na^+$  affinity of TmHKT1;5A and reduce  $Na^+$   
223 affinity of TaHKT1;5D to similar levels. 3D modeling of plant HKT structures suggests, that  
224 in most cases, these amino acid residue changes occur in extracellular or intracellular loop  
225 regions connecting the MPM domains. Some of these amino acid residue differences

226 potentially impact Na<sup>+</sup> affinity or conductance (Ren et al., 2005; Cotsaftis et al., 2012;  
227 Somasundaram et al., 2020). More recently however, in both wheat and barley HKT1;5,  
228 single amino acid variants that destabilize a membrane helix have been identified (Borjigin  
229 et al., 2020; Houston et al., 2020; Wege et al., 2021), the impact of which will be discussed  
230 in the subsequent section.

## 231 **5. ‘Hyper-functional versus weak’ HKT1;5 alleles/haplotypes in relation to salinity** 232 **tolerance**

233 The currently accepted notion of ‘shoot exclusion sodium’ and hence salinity tolerance,  
234 conferred by *AtHKT1;1/HKT1;5* thus requires (i) strong salinity induced root specific  
235 expression, and/or (ii) alterations in HKT structure (due to non-synonymous substitutions)  
236 that contribute to its increased Na<sup>+</sup> affinity and/or conductance. This is referred to henceforth  
237 as a ‘hyper-functional allele’. However, the relationship between a ‘hyper-functional’  
238 HKT1;5 allele and salinity tolerance appears to be undergoing a shift that is driven by data  
239 obtained from GWAS studies in *Arabidopsis* and barley as well as comparative analysis of  
240 *HKT1;5* function in salt tolerant and sensitive species of cereals and is summarized below.

241 GWAS data for *Arabidopsis* shows that *AtHKT1;1* controls natural variation  
242 in leaf sodium content in *A. thaliana* populations worldwide (Baxter et al., 2010; Busoms et  
243 al., 2018). ‘Hyper-functional’ *AtHKT1;1* alleles confer high root expression and low  
244 leaf/shoot sodium accumulation (*AtHKT1;1<sup>LLS</sup>*; LLS: Low Leaf Sodium; Col-0 ecotype;  
245 Busoms et al., 2018). ‘Weak’ *AtHKT1;1* alleles, on the other hand, have highly reduced root  
246 expression, leading to Na<sup>+</sup> accumulation in the leaf/shoot (*AtHKT1;1<sup>HLS</sup>*; HLS: High Leaf  
247 Sodium; Rus et al., 2006; Busoms et al., 2018). ‘Weak’ *AtHKT1;1* alleles are found in Ts-1  
248 and Tsu-1 accessions of *A. thaliana*; these accessions lack a distal enhancer element seen in  
249 the Col-0 *AtHKT1;1* promoter that confers high root specific expression (Baek et al., 2011).  
250 The ‘weak’ *AtHKT1;1<sup>HLS</sup>* allele in Ts-1 and Tsu-1 is associated high stem specific expression,

251 conferring an adaptive benefit in limiting Na<sup>+</sup> flow to flowers compared to the ‘strong’  
252 *AtHKT1;1<sup>LLS</sup>* allele seen in Col-0 (An et al., 2017). This, in turn, leads to increased  
253 reproductive fitness in the *Arabidopsis* ecotypes under salinity (increased silique number in  
254 Tsu-1 compared to Col-0; An et al., 2017). The contrasting phenotypes conferred by the  
255 *AtHKT1;1<sup>HLS</sup>* and *AtHKT1;1<sup>LLS</sup>* alleles determines their distribution in coastal demes  
256 (Busoms et al., 2018). Accessions with the *AtHKT1;1<sup>HLS</sup>* should be expected to predominate  
257 at the coast (0-500 m from the sea; high salinity) while *AtHKT1;1<sup>LLS</sup>* should be expected to  
258 occur in more inland sites. However, the reverse is seen. Accessions with the *AtHKT1;1<sup>HLS</sup>*  
259 allele occur only in an intermediate zone (500-1500 m from the coast, with 50-150 mg/g of  
260 soil Na<sup>+</sup>) along with *AtHKT1;1<sup>LLS</sup>* accessions. On the other hand, *AtHKT1;1<sup>LLS</sup>* accessions  
261 only occur in coastal (0-500 m) and inland regions (Busoms et al., 2018). *AtHKT1;1<sup>LLS</sup>*  
262 predominate at the coast (where salinity is highest). These accessions do not accumulate  
263 sodium in the shoot due to high root expression of *AtHKT1;1* under salinity that leads to  
264 efficient shoot sodium exclusion and can thus complete their life cycle albeit, with reduced  
265 reproductive fitness in high soil salinity. *AtHKT1;1<sup>HLS</sup>* cannot predominate in the coast with  
266 elevated soil Na<sup>+</sup> (greater than 150 mg/g), as low root expression of *AtHKT1;1* would  
267 elevate leaf/shoot Na<sup>+</sup> to highly toxic levels and the adaptive benefit conferred by the  
268 *AtHKT1;1<sup>HLS</sup>* allele by excluding Na<sup>+</sup> from reproductive tissues would be lost. Within the  
269 intermediate salinity zone, the presence as well as maintenance of both accessions has been  
270 attributed to local heterogeneities in soil Na<sup>+</sup> content brought about by seasonal rainfall  
271 controlling the direction of selection.

272         Among cereal crops, barley is one of the most salt-tolerant species, and is able to  
273 maintain shoot growth in highly saline soils (Munns and Tester, 2008), with some genotypes  
274 showing less than 50% yield reduction under conditions of extreme salinity (e.g., 300 mM  
275 NaCl; Chen et al., 2007). Under non saline conditions, barley HvHKT1;5 Haplotype 3

276 (*HvHKT1;5<sub>Hap3</sub>*) is associated with higher grain and leaf Na<sup>+</sup> contents relative to haplotypes  
277 *HvHKT1;5<sub>Hap1</sub>* and *HvHKT1;5<sub>Hap2</sub>* (Houston et al., 2020). *HvHKT1;5* encoded by  
278 *HvHKT1;5<sub>Hap3</sub>*, shows a L189P amino-acid residue substitution in a transmembrane region,  
279 leading to “aberrant” localization (not at the plasma membrane) as well as loss of Na<sup>+</sup>  
280 transport capacity in *Xenopus* oocytes and is thus a non-functional allele with regard to Na<sup>+</sup>  
281 retrieval from xylem sap. This *HvHKT1;5<sub>Hap3</sub>* allele thus confers elevated Na<sup>+</sup> in shoots and  
282 grain. In contrast, *HvHKT1;5* encoded by *HvHKT1;5<sub>Hap1</sub>* and *HvHKT1;5<sub>Hap2</sub>* are functional  
283 at the plasma membrane and confer more efficient shoot exclusion of sodium relative to  
284 *HvHKT1;5<sub>Hap3</sub>*. However, under low and moderate soil Na<sup>+</sup>, genotypes containing  
285 *HvHKT1;5<sub>Hap3</sub>* accumulate high concentrations of Na<sup>+</sup> but exhibit no evidence of toxicity.  
286 Further, the frequency of *HvHKT1;5<sub>Hap3</sub>* is significantly enriched in modern day genotypes  
287 (35%), developed by breeders but is highly reduced in older landraces (4%), suggesting it  
288 has been selected for directionally and has an important role in modern day varieties. The  
289 higher Na<sup>+</sup> content in *HvHKT1;5<sub>HAP3</sub>* has been suggested to function as an osmolyte or  
290 replace metabolic function of K<sup>+</sup> in potassium deficient agricultural soils (Houston et al.,  
291 2020). Osmotic adjustment is critical for maintenance of cell turgor and plant growth under  
292 saline conditions, and reliance on organic osmolytes to achieve this comes with a very  
293 significant carbon cost (Munns et al., 2020). Under salinity, K<sup>+</sup> uptake is reduced in plants  
294 (Rubio et al., 2020). Under these circumstances, reliance on Na<sup>+</sup> as a cheap osmoticum gives  
295 plants a competitive advantage (Shabala et al., 2020). An identical amino-acid residue  
296 substitution (L190P) occurs in *TaHKT1;5D* in a saline tolerant bread wheat genotype that  
297 shows 4-6 fold higher Na<sup>+</sup> content in the sheath and fourth leaf blade. The *TaHKT1;5D*  
298 L190P mutant also shows significantly reduced Na<sup>+</sup> conductance in *Xenopus* oocyte transport  
299 assays relative to other tolerant wheat genotypes with a functional *TaHKT1;5D* (Borjigin et

300 al., 2020; Houston et al., 2020), suggesting shoot accumulation of sodium, is an important  
301 mechanism in conferring salinity tolerance in some rather salt tolerant species.

302 Under saline conditions, the “functional” allele *HvHKT1;5<sub>Hap1</sub>* confers reduced grain  
303 and leaf Na<sup>+</sup> accumulation relative to the “mis-functional” *HvHKT1;5<sub>Hap3</sub>* allele. This  
304 suggests that the basic module conferring shoot Na<sup>+</sup> exclusion under salinity (due to  
305 expression at the plasma membrane of *HvHKT1;5*) is functional in barley also. The presence  
306 of the “functional” (*HvHKT1;5<sub>HAP1</sub>*) as well as “mis-functional” (*HvHKT1;5<sub>HAP3</sub>*) alleles at  
307 equal frequencies in the analyzed barley population, suggests that both alleles may confer a  
308 specific competitive advantage under different circumstances (Houston et al., 2020). The  
309 “functional” (*HvHKT1;5<sub>HAP1</sub>*) would be important under salinity to confer shoot exclusion  
310 of sodium while the “mis-functional” (*HvHKT1;5<sub>HAP3</sub>*) would confer an adaptive benefit to  
311 accessions that have obligate requirement for sodium and also possess associated tissue  
312 tolerance mechanisms to counteract the effect of elevated sodium. It may also be that barley  
313 has more of an obligate requirement for Na<sup>+</sup> for growth, given its high salinity tolerance  
314 relative to other cereals. Disentangling ion exclusion from tissue tolerance effects would  
315 require generating *HKT1;5* knockouts in barley. Transgenic seedlings of RNAi-based  
316 *HvHKT1;5* knockouts var. Golden Promise (*HvHKT1;5<sub>Hap1</sub>*) show salinity tolerance when  
317 subjected to 100-200 mM NaCl for two-three weeks (Huang et al., 2019). Surprisingly,  
318 *HvHKT1;5* RNAi knockouts also showed reduced Na<sup>+</sup> in the xylem sap of seedlings treated  
319 with 100 mM Na<sup>+</sup> for four days. The diametrically opposite observation (GWAS versus  
320 RNAi based knockout) regarding *HvHKT1;5* function in barley and barley salt tolerance  
321 may be due to stage specific differences in responses to salinity in barley or differences in  
322 the kinetics of xylem Na<sup>+</sup> loading under salinity. The last point is discussed in some detail in  
323 section 7.

324 In cultivated Asian rice, *Oryza sativa* L., seven major and three minor alleles of  
325 *OsHKT1;5* have been identified in *O. sativa* landraces, with the ‘Aromatic’ allele showing a  
326 very strong correlation with high shoot Na<sup>+</sup> exclusion (Platten et al., 2013). Allelic strength  
327 to confer shoot Na<sup>+</sup> exclusion in *O. sativa* is as follows: Aromatic > Aus ≥ Hasawi > Daw ≅  
328 Agami ≅ IR29 ≥ Japonica. The ‘Aromatic’ allele group confers highest Na<sup>+</sup> exclusion and  
329 includes rice landraces Pokkali and Nona Bokra that have been used in breeding programs  
330 for rice salinity tolerance leading to the identification of QTLs, *saltol* and *SKC1* loci  
331 respectively (Ren et al., 2005; Thomson et al., 2010). The ‘Japonica’ allele confers less  
332 efficient Na<sup>+</sup> exclusion and differs from the ‘Aromatic’ allele at the same four amino acid  
333 residues identified by Ren et al. (2005). In the study by Platten et al. (2013), only HKT1;5  
334 allelic variation was examined in the rice accessions in relation to salinity tolerance. Analysis  
335 of the ‘3000 rice genome’ rice data set by Pulipati et al. (in press) shows that, based on non-  
336 synonymous substitutions, ten major *OsHKT1;5* haplotypes can be identified, of which seven  
337 haplotypes correspond with *OsHKT1;5* sequences reported by Platten et al. (2013). The  
338 seven major allelic groups reported by Platten et al. (2013) have also been reported  
339 previously (Negrão et al., 2013; Yang et al., 2018). Within the remaining three haplotypes  
340 identified by Pulipati et al. (in press), one haplotype consists of tropical *japonica* accessions  
341 while another haplotype has predominantly *indica*- accessions (Wang et al., 2018b).  
342 However, no functional assessments of haplotypic *OsHKT1;5* variants identified by Platten  
343 et al. (2013) and Pulipati et al. (in press) have been carried out so far in heterologous systems  
344 to confirm possible changes in Na<sup>+</sup> transport characteristics.

345 Another approach to analyzing HKT1;5 structure-function relationships is to  
346 compare transporter function between closely related glycophytic and halophytic species.  
347 *Oryza coarctata* is a halophytic wild rice that occurs as a mangrove associate in fluctuating  
348 salinity at the seawater-estuarine interface in Indian and Bangladeshi coastal regions



349 (Rajakani et al., 2019). Transport activity of OcHKT1;5 from *O. coarctata* and OsHKT1;5  
350 (a salt sensitive version found in *japonica* rice) has been compared in *Xenopus* oocytes  
351 (Somasundaram et al., 2020). Similar to OsHKT1;5, OcHKT1;5 is a plasma membrane-  
352 localized Na<sup>+</sup>-selective transporter. Surviving in Na<sup>+</sup>-rich soils, it would be expected that  
353 halophytic species would have more efficient Na<sup>+</sup> transport systems. However, OcHKT1;5  
354 displays 16-fold lower affinity for Na<sup>+</sup> and 3.5-fold higher maximal conductance compared  
355 to OsHKT1;5 (salt sensitive version), attributable to the presence of a positively charged  
356 residue at the ion pore (K239) entrance, repelling entry of the positively charged Na<sup>+</sup>.  
357 Reciprocal mutagenesis of OcHKT1;5(K239E) and OsHKT1;5 (E270K) had opposite effects  
358 (Fig. 3). In the OcHKT1;5(K239E) mutant, Na<sup>+</sup> transporter affinity increased 9-fold and  
359 maximal conductance decreased by 48%. Reciprocally, for the OsHKT1;5(E270K) mutant,  
360 Na<sup>+</sup> affinity decreased 3-fold and maximal conductance increased by 52%. Differences in  
361 Na<sup>+</sup> transport affinity and maximal conductance between the two transporters (OcHKT1;5  
362 versus OsHKT1;5) could explain differences in xylem Na<sup>+</sup> sap concentrations under salinity  
363 in *O. coarctata* compared to *O. sativa*. Targeted overexpression of OcHKT1;5 in cultivated  
364 rice would be required to confirm if a definitive link exists between xylem sap desalination  
365 and OcHKT1;5 function in *O. coarctata*. Wild sea barley (*Hordeum marinum*) has higher  
366 salt tolerance compared to cultivated *H. vulgare*, attributable to lower root Na<sup>+</sup> uptake as  
367 well as lower translocation to shoots (Huang et al., 2019). Plasma membrane localized  
368 HmHKT1;5 shows lower root specific expression under salinity compared to HvHKT1;5.  
369 While HmHKT1;5, shows Na<sup>+</sup>- specific transport activity in oocytes, it has lower affinity for  
370 Na<sup>+</sup> compared to cultivated barley HvHKT1;5. Thus, it seems that both halophytic species  
371 *O. coarctata* and *H. marinum* whose Na<sup>+</sup> tolerance mechanisms are based on Na<sup>+</sup> exclusion  
372 express lower affinity HKT1;5 transport systems in roots compared to their glycophytic  
373 counterparts (Huang et al., 2019; Somasundaram et al., 2020), that may allow higher Na<sup>+</sup>

374 retrieval from xylem sap in high  $\text{Na}^+$  concentration conditions and hence play a crucial role  
375 in driving low sodium accumulation in shoots.

### 376 **6. What determines which allele/haplotype will prevail?**

377 It is undisputed that within the Type I HKT transporters, HKT1;5 transporters are critical for  
378 maintaining plant fitness under saline conditions (An et al., 2017; Kobayashi et al., 2017;  
379 Busoms et al., 2018; Houston et al., 2020). However, the apparent contradictions with regard  
380 to their strong association with both ‘ion exclusion’ and ‘ion accumulation’ needs to be  
381 delineated. Hence, it may be hypothesized that ‘hyper-functional’ HKT1;5 alleles/haplotypes  
382 (higher root specific expression under salinity and/or more efficient  $\text{Na}^+$  transport) occur in  
383 plant species and/or genotypes that grow in soil conditions where salinity variations are  
384 sudden and pronounced during the growing period (due to a combination of  
385 edaphic/climatic/geographical conditions), conferring ion exclusion. On the other hand, in  
386 habitats that have near continuous fluctuations in salinity, there is a need to utilize excess  
387  $\text{Na}^+$  as an osmoticum for turgor driven shoot growth (partly to compensate for  $\text{K}^+$  loss due to  
388 salinity). In such habitats, ‘weak’ HKT1;5 alleles/haplotypes (lower root specific or altered  
389 tissue expression and/or less efficient  $\text{Na}^+$  transport) are beneficial as they become functional  
390 in sequestering  $\text{Na}^+$  from xylem only at a point when any further transport of  $\text{Na}^+$  to the shoot  
391 would result in ion toxicity. (Fig. 2). Weak ‘HKT1;5’ alleles/haplotypes, that confer leaf ion  
392 accumulation, may give a competitive advantage to plants grown under conditions of high  
393 salinity, as they also appear to co-occur in accessions equipped with efficient tissue tolerance  
394 mechanisms (e.g.  $\text{Na}^+$  vacuolar sequestration as well as cytosolic  $\text{K}^+$  retention; Shabala et al.,  
395 2020). Thus, a ‘one size fits all’ approach is too simplistic and counterproductive to account  
396 for salinity tolerance. A subtle shift in crop breeding for salinity tolerance is required  
397 targeting *HKT1;5*-mediated  $\text{Na}^+$  exclusion under moderate salinities but relying on  
398 halophytic traits under conditions of severe soil salinity. Thus, naturally occurring variation

399 in crop salinity tolerance (balancing Na<sup>+</sup> exclusion against accumulation) can be utilized for  
400 a range of salinity scenarios.

401 **7. What balancing mechanisms occur in plants to support the two extreme *HKT1;5***  
402 **allele/haplotype forms?**

403 Halophytic species and/or genotypes (e.g., *Chenopodium quinoa*, *Salicornia*, *Suaeda*) that  
404 depend on Na<sup>+</sup> for osmotic adjustment rapidly increase xylem sap Na<sup>+</sup> content for osmotic  
405 adjustment and turgor maintenance in the shoot to minimize energy costs (Shabala, 2013;  
406 Bose et al., 2014; Tyerman et al., 2019). Once this process is achieved, they decrease the rate  
407 of Na<sup>+</sup> loading to the minimum required to control cell turgor in new tissues. Further, it is  
408 also seen that halophytic species or those growing in soils that have consistently ‘higher’  
409 salinity, also have highly controlled xylem Na<sup>+</sup> loading mechanisms (Zarei et al., 2020). This  
410 ‘fine-tuning’ of the xylem loading process requires several concurrently operating  
411 mechanisms that will assist in delivery of the required amounts of Na<sup>+</sup> for shoot osmotic  
412 adjustment. Under a majority of physiologically relevant scenarios, xylem Na<sup>+</sup> loading is a  
413 thermodynamically active process (Shabala, 2013). Kinetics of xylem ion loading is a highly  
414 dynamic process and varies in a real time manner. Thus, it may be up- or down-regulated at  
415 a given timepoint and determined dynamically by the plant physiological requirements. The  
416 most likely candidate transporters involved in xylem loading are SOS1 (plasma membrane  
417 Na<sup>+</sup>/H<sup>+</sup> exchanger) and CCC (chloride/cation exchangers; Ishikawa et al., 2018).

418 SOS1 belongs to the cation proton antiporter (CPA) subfamily of proteins (Isayenkov  
419 et al., 2020; Zhao et al., 2020). SOS1 has 10 to 12 transmembrane domains with a 700 amino  
420 acid-long C-terminal tail that contains an auto-inhibitory domain and phosphorylation sites  
421 that are required for protein-protein interaction (Quintero et al., 2011) and dimerization  
422 (Ullah et al., 2016). *SOS1* transcripts are abundant in the xylem parenchyma tissue under  
423 salinity (Shi et al., 2000). SOS1 is autoinhibited under normal conditions, and this inhibition

424 is released by the phosphorylation of Ser1044 by a serine/threonine protein kinase SOS2 that  
425 localizes to the plasma membrane after activation by SOS3, a calcium binding protein  
426 (Quintero et al., 2011). Based on mathematical modelling, it is suggested that, in *Arabidopsis*,  
427 SOS1 functions to restrict cytosolic Na<sup>+</sup> accumulation in mature roots and apex due to tissue  
428 specific expression outer mature root (at least one tissue) and epidermis respectively (Foster  
429 and Miklavcic, 2019). In the mature stele (xylem parenchyma: xylem interface), SOS1  
430 functions to actively load Na<sup>+</sup> into the xylem transpiration stream, enhancing the transport of  
431 Na<sup>+</sup> and water to the shoot. Increasing evidence also suggests that AtHKT1;1/HKT1;5 and  
432 SOS1 interplay is crucial to conferring salinity tolerance in plants. Consistent with the above  
433 notion, *hkt1-1*, *sos1-1* double mutants of *Arabidopsis* suppress the salt sensitivity phenotype  
434 of the *Arabidopsis* salt over-accumulating *sos1-1* mutant (Rus et al., 2004). Salinized roots  
435 of rice *sos1* mutants show reduced *OsHKT1;5* expression, presumably to protect roots from  
436 accumulating lethal levels of Na<sup>+</sup> (due to loss of SOS1 function and recirculation of Na<sup>+</sup> from  
437 xylem to root by HKT1;5; El Mahi et al., 2019). Data from recent electrophysiological  
438 studies also show that *OsHKT1;5* (*SKC1*) NIL lines (showing higher *OsHKT1;5* expression)  
439 do not show Na<sup>+</sup> reabsorption by root xylem parenchyma compared to the wild type parent  
440 (Al Nayef et al., 2020). Instead, changes in the expression level of *OsHKT1;5* correlates with  
441 alterations in expression of membrane transporters (including *SOS1*). Tolerant wild barley  
442 show up-regulation of *SOS1* and down-regulation of *HKT1;5*, maximizing Na<sup>+</sup> loading into  
443 xylem in the initial time period of application of salinity (Zhu et al., 2017). On the other  
444 hand, sensitive barley genotypes maximize Na<sup>+</sup> retrieval from the xylem by increasing  
445 *HKT1;5* expression substantially and having relatively lower *SOS1* transcripts expressed in  
446 the stele relative to the tolerant wild or domesticated barley cultivars. Similarly, in halophytic  
447 *Salicornia dolichostachya*, constitutive *SOS1* expression under salinity is balanced by the  
448 near lack of expression of *HKT1*, contributing to shoot Na<sup>+</sup> accumulation (Katschnig et al.,

449 2015). In halophytic species, xylem Na<sup>+</sup> loading is important for osmotic balance so a highly  
450 functional *SOS1* allele to increase shoot sodium content may function in tandem with a weak  
451 *HKT1;5* allele (low ion exclusion), to allow Na<sup>+</sup> transfer to the shoot (Fig. 2). High tissue  
452 tolerance may also be a crucial component associated with high sodium content in halophytic  
453 root and shoot tissues under salinity. In glycophytes, an opposite mechanism must prevail  
454 i.e.: strong *HKT1;5* to confer Na<sup>+</sup> exclusion matched by lowered *SOS1* function to reduce  
455 xylem Na<sup>+</sup> loading.

456         These findings suggest that a complex feedback regulation of transporter activity is  
457 involved in maintenance of plant ionic homeostasis and signaling under stress conditions.  
458 This feedback regulation might operate at transcriptional, post-transcriptional and post-  
459 translational levels. *SOS1* function is known to be altered by post-translational changes  
460 (phosphorylation altering activity; Quintero et al., 2011). In addition, post-translational  
461 modifications have the potential to alter transporter function by altering activity,  
462 dimerization, protein stability/turnover, membrane aggregation etc. Thus, evidence from  
463 forward and reverse genetics needs to be supported by other functional assays and proteomic  
464 analysis to determine controls operative on transporter function. Another transporter, CCC,  
465 is also preferentially expressed at the xylem/parenchyma interface in *Arabidopsis*  
466 (Colmenero-Flores et al., 2007). This transporter has a 1:1:1 stoichiometry between Cl<sup>-</sup>, Na<sup>+</sup>  
467 and K<sup>+</sup> and may use the downhill energy of passive Cl<sup>-</sup> transport to load both Na<sup>+</sup> and K<sup>+</sup>  
468 into the xylem against their electrochemical gradient. Indeed, given highly negative  
469 membrane potential of the xylem parenchyma cells (-120 to -140 mV; Shabala et al., 2010;  
470 Wegner et al., 2011) and the fact that cytosolic Cl<sup>-</sup> concentration is at least as high as that in  
471 the xylem sap, passive chloride movement through CCC protein will be able to energize  
472 transport of both cations (Na<sup>+</sup> and K<sup>+</sup>) *via* a symport mechanism. While the physical location  
473 of CCC transporters in some species is still a matter of debate (Golgi network; Henderson et

474 al., 2018), pharmacological studies in barley (Zhu et al., 2017) as well as localization studies  
475 in rice (Kong et al., 2011; Chen et al., 2016) also support the concept of CCC operation at  
476 the plasma membrane of xylem Together with HKT1 and SOS1, CCC transporters may,  
477 therefore, enable a fine tuning of xylem Na<sup>+</sup> loading and delivery to the shoot.

478         Given that xylem Na<sup>+</sup> loading precedes xylem Na<sup>+</sup> retrieval, it is interesting that it is  
479 “only” AtHKT1;1/HKT1;5 that seems to be associated with soil salinity. To precisely  
480 identify determinants of xylem Na<sup>+</sup> loading, it may be necessary to screen plant accessions  
481 systematically, comprehensively and exhaustively for xylem Na<sup>+</sup> content and gene  
482 expression at numerous, defined timepoints during salinity treatment. This will enable one  
483 to examine the gamut of changes occurring in plants in the interval between ‘Na<sup>+</sup> delivery to  
484 the shoot for osmotic adjustment’ to a stage wherein xylem Na<sup>+</sup> loading ceases to prevent  
485 uncontrolled Na<sup>+</sup> accumulation. It is also crucial to couple this functional analysis with  
486 examining tissue specific HKT1 and SOS1 expression (e.g. *in situ* PCR, *in situ* hybridization,  
487 immunocytochemistry, single cell RNAseq) and not the whole root. Finally, given the  
488 multiplicity of HKT transporters, HKT1;5 alone does not confer xylem Na<sup>+</sup> exclusion in  
489 cereals. In rice, the OsHKT1;4 is a high affinity Na<sup>+</sup> transporter that appears to function in  
490 xylem sap desalinization in a wider concentration range (submillimolar to salt stress  
491 conditions; Khan et al., 2020). Furthermore, a role for OsHKT1;1 in Na<sup>+</sup> recirculation via  
492 the phloem has been proposed (Wang et al., 2015; Campbell et al., 2017). However,  
493 AtHKT1;1/HKT1;5 and SOS1 interplay appears to be crucial to controlling Na<sup>+</sup> homeostasis,  
494 with HKT1;5 allelic forms, being selected for by soil salinity and the level of plant tolerance  
495 to salt.

## 496 **8. Conclusions**

497 Increasing evidence suggests that HKT1;5 haplotypes seen in plant species are strongly  
498 related to the soil salinity and, most likely, also to limiting soil K<sup>+</sup> content. Given the large

499 heterogeneity in salinity in both agricultural and native soils, a ‘one size fits all’ approach  
500 may be not suitable for plants to adapt to adverse saline conditions. Within the spectrum of  
501 extreme HKT1;5 alleles/haplotypes (hyper-functional *vs* weak) seen in a given species that  
502 confer either ion exclusion or ion accumulation, there occur a spectrum of intermediate  
503 haplotypes that confer an adaptive benefit in a specified set of edaphic, climatic and/or  
504 geographical conditions Given that salinity effects are not uniform, the role of intermediate  
505 haplotypes can be assessed by taking advantage of the availability of genome sequences for  
506 crop species. Generating resequencing data for accessions coupled with GWAS for a range  
507 of salinity scenarios, will help to identify allele(s)/haplotype(s) that confer a beneficial  
508 advantage to plants growing in complex saline soils in the future. This knowledge can be  
509 then used to engineer germplasm that will be most suitable for specific climatic/soil  
510 conditions, to maximise its productivity in farmers’ fields.

#### 511 **Contributions**

512 G.V., S.S., G.N.H., Suji S, S.P., G.S., M.H., K.K., A.-A.V., L.S., M.Z., Z-H. C: Analyzed  
513 literature and critically assessed data. G.V. and S.S. assembled the first draft. A.-A.V., L.S.,  
514 M.Z., Z-H.C. critically assessed paper and contributed to discussion. All authors read and  
515 approved the final manuscript. All authors declare they have no professional, personal or  
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#### 517 **Declaration of competing interest**

518 The authors declare that they have no competing interest.

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856

## 857 **Legends**

858 **Fig. 1. A:** High-Affinity K<sup>+</sup> Transporters (HKT) have a common structural organization  
859 consisting of four consecutively arranged MPM domains. Selectivity filters (SF) within each  
860 pore domain confer cation specificity In Type I HKTs, a serine residue is present in the first  
861 SF while Type II HKTs show a glycine residue (Type I: S-G-G-G vs Type II: G-G-G-G). **B:**  
862 Alignment of HKT1;5 sequences, rice (*OsjHKT1;5* and *OsiHKT1;5*; Ren et al., 2005), wheat  
863 (*Triticum durum*: *TmHKT1;5A*; *Triticum aestivum*; *TaHKT1;5D*; Xu et al., 2018) and barley  
864 (*HvHKT1;5*; Houston et al., 2020), and *Arabidopsis AtHKT1;1* (Uozumi et al., 2000). Sodium-  
865 specific selectivity filter residues 'S-G-G-G' are marked with a star and occur in pore helices

866 marked P<sub>A</sub>, P<sub>B</sub>, P<sub>C</sub> and P<sub>D</sub> respectively and underlined in black. Residues surrounding the  
 867 selectivity filter are conserved and marked by red boxes.

868 **Fig. 2.** Allelic variants of AtHKT1;1 or HKT1;5 conferring contrasting phenotypes (shoot Na<sup>+</sup>  
 869 exclusion or accumulation), are strongly selected by soil salinity in *Arabidopsis*,  
 870 wild/cultivated accessions of barley and rice. Hyper-functional *HKT1;5* alleles (conferring Na<sup>+</sup>  
 871 exclusion) may result from a combination of strong salinity induced root-specific  
 872 *AtHKT1;1/HKT1;5* expression [Control (C) vs salinity (S); shown in red] and/or efficient Na<sup>+</sup>  
 873 sequestration in xylem parenchyma (XP) cells from xylem (higher Na<sup>+</sup> affinity and/or  
 874 conductance of HKT1;5) and are selected for in soils with sudden and pronounced variations  
 875 in salinity. Weak *HKT1;5* alleles predominate in habitats with near continuous fluctuations in  
 876 salinity, selected by a need to utilize Na<sup>+</sup> as an osmoticum for turgor driven shoot growth.  
 877 Weak alleles derive from low or absent root specific *AtHKT1;1/HKT1;5* expression/altered  
 878 tissue specific expression under salinity and/or less efficient Na<sup>+</sup> transport mechanisms  
 879 [lowered Na<sup>+</sup> affinity/conductance attributable to alterations in amino acid residues near the  
 880 ion pore entrance (green) or transmembrane helix (pink)]. Opposite balancing mechanisms  
 881 involving xylem ion loading controlled by transporters such as SOS1 or CCC must occur in  
 882 these extreme phenotypes. Species/accessions with weak HKT1;5 alleles often show high  
 883 tissue Na<sup>+</sup> tolerance.

884 Fig. 3: Amino acid residues at the ion pore entrance (green) in OsHKT1;5-Ni and OcHKT1;5.  
 885 A and B, on the left, a triad of negatively charged residues in OsHKT1;5-Ni (D366, E270 and  
 886 D81) mutually repel each other to allow for entry of Na<sup>+</sup> ions. Attraction between E270 and  
 887 K244 in OsHKT1;5-Ni also helps increase ion pore entrance size. In OcHKT1;5 on the other  
 888 hand (B, on the left), the presence of positively charged K239 (in place of E270 in OsHKT1;5-  
 889 Ni) attracts both D334 and E81, narrowing the ion pore site. However, the pore size is slightly  
 890 increased upon approach of Na<sup>+</sup> ions (indicated by dotted residues and dotted black arrows;

891 based on modelling and simulation analysis in Somasundaram et al., 2020). Approach of Na<sup>+</sup>  
892 ions causes K239 to move towards positively charged K213 in OcHKT1;5, causing repulsion.  
893 E83 and R214 in OcHKT1;5 move towards one another, reinforcing K239-K213 repulsion.  
894 The salt bridge between D334 and K239 is hence, more reinforced, opening the ion pore  
895 entrance, in the presence of Na<sup>+</sup>. Residue swapping by mutagenesis (shown on the right-hand  
896 side in A and B) has opposite effects: i.e. reduces pore size in OsHKT1;5E270K and increases  
897 pore size in OcHKT1;5K239E. Repulsion of residues is indicated blue dotted arrows while  
898 attraction between residues is shown by red arrows. The triad of negatively charged residues  
899 at the ion pore entrance as well as 'GKG' in OsHKT1;5-Ni occur in MPM domains 1-3.-  
900

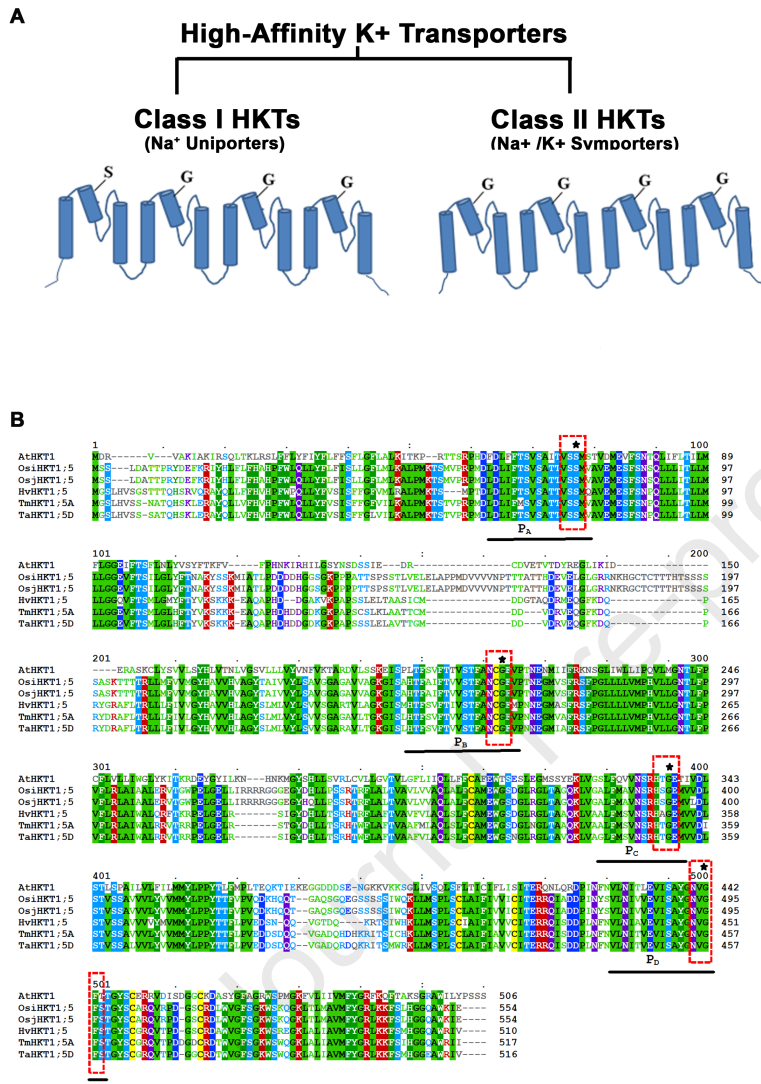


Figure 1



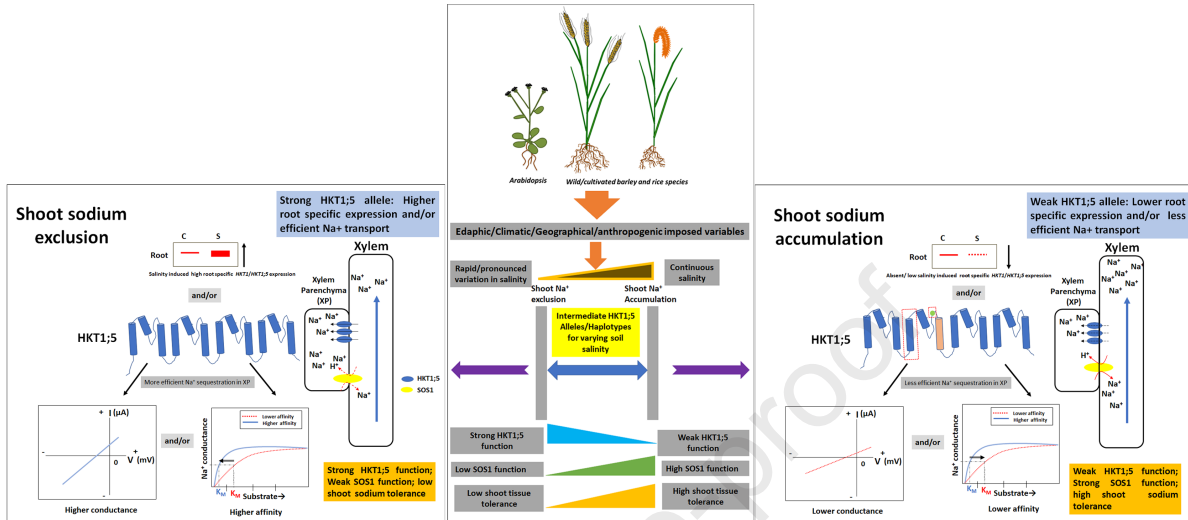


Figure 2

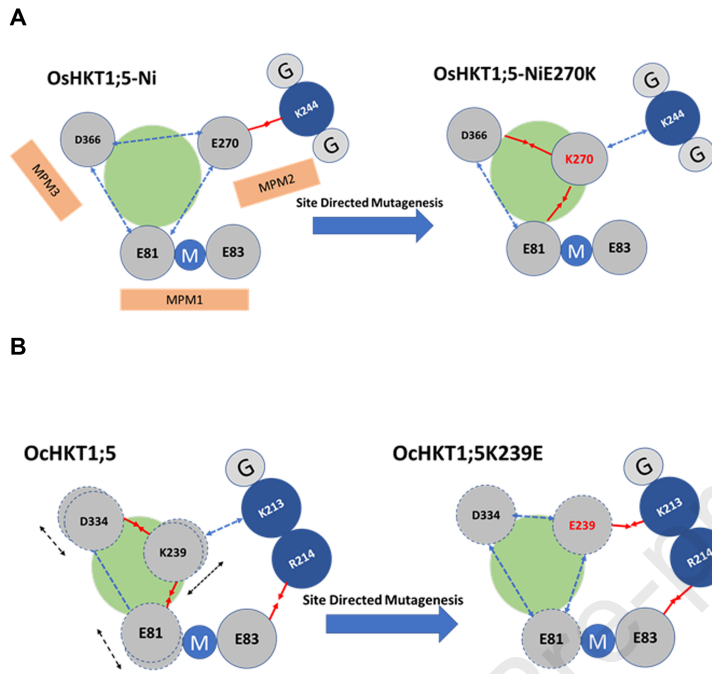


Figure 3

**Highlights**

- High-affinity K<sup>+</sup> (HKT) family of transporters play a critical role in K<sup>+</sup> and Na<sup>+</sup> homeostasis in plants
- *HKT1/HKT1;5* operate in both shoot Na<sup>+</sup> exclusion and shoot Na<sup>+</sup> accumulation
- Contrasting phenotypes are attributable to either hyper-functional or weak *HKT1/HKT1;5* alleles/haplotypes

**Contributions**

G.V., S.S., G.N.H., Suji S, S.P., G.S., M.H., K.K., A.-A.V., L.S., M.Z., Z-H. C: Analyzed literature and critically assessed data. G.V. and S.S. assembled the first draft. A.-A.V., L.S., M.Z., Z-H.C. critically assessed paper and contributed to discussion. All authors read and approved the final manuscript. All authors declare they have no professional, personal or financial conflicts of interest.

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**Declaration of competing interest**

The authors declare that they have no competing interest.

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