

1 **Chloride on the move**

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15 ALMT9

16

17 **Abstract**

18 Chloride ( $\text{Cl}^-$ ) is an essential plant nutrient, but under saline conditions can  
19 accumulate to toxic levels in leaves; limiting this accumulation improves the salt  
20 tolerance of some crops. The rate-limiting step for this process – the transfer of  $\text{Cl}^-$   
21 from root symplast to xylem apoplast, which can antagonize delivery of the  
22 macronutrient nitrate ( $\text{NO}_3^-$ ) to shoots – is regulated by abscisic acid (ABA) and is  
23 multigenic. Until recently the molecular mechanisms underpinning this salt tolerance  
24 trait were poorly defined. Here, we discuss how recent advances highlight the role of  
25 newly identified transport proteins, some that directly transfer  $\text{Cl}^-$  into the xylem, and  
26 others that act on endo-membranes in ‘gatekeeper’ cell types in the root stele to  
27 control root-to-shoot delivery of  $\text{Cl}^-$ .

28

29 ***Chloride - a problem nutrient?***

30 Chloride ( $\text{Cl}^-$ ) is a plant nutrient with proposed regulatory roles in photosynthesis,  
31 transpiration, fertilization, nutrition and growth; how the accumulation of  $\text{Cl}^-$  and its  
32 movement across membranes influence these key plant physiological processes via  
33 changes in membrane potential, enzyme stability, charge balance, pH,  
34 osmoregulation, volume control and turgor have been discussed elsewhere [1-3]. As  
35 it is a charged solute,  $\text{Cl}^-$  moves into cells and between cellular compartments  
36 predominantly through ion transport proteins (**transporters**) embedded within  
37 cellular membranes. This occurs either passively down its difference in  
38 electrochemical potential through ion channels or actively through carrier proteins  
39 such as **symporters** or **antiporters** that can move  $\text{Cl}^-$  using the difference in  
40 electrochemical potential for another ion (such as protons,  $\text{H}^+$ ). Over the past ten  
41 years, and particularly the last two, a number of plant transport proteins permeable  
42 to  $\text{Cl}^-$  have been identified and characterized, which has advanced our knowledge of  
43 plant  $\text{Cl}^-$  transport from the biochemical, physiological and electrophysiological level  
44 into the molecular domain. Outlined in Figure 1 are major transport steps across  
45 cellular membranes that affect nutritive  $\text{Cl}^-$  uptake, translocation and storage. The  
46 key rate-limiting ‘**gatekeeper**’ step modulating  $\text{Cl}^-$  accumulation in the shoot has  
47 been shown to be the loading of  $\text{Cl}^-$  from the root **stelar symplast** into the xylem  
48 **apoplast**, which is regulated following drought and **salinity** stress via abscisic acid  
49 (**ABA**) [2, 4-10]. In the root, ABA inhibits xylem loading of  $\text{Cl}^-$ , but ABA has no effect  
50 on its uptake [7, 9]. This leads to accumulation of  $\text{Cl}^-$  in the root under saline

51 conditions, contributing to shoot salt exclusion. Whilst other processes such the  
52 regulation of root epidermal uptake or compartmentation of  $\text{Cl}^-$  in the root vacuole  
53 are acknowledged to affect shoot  $\text{Cl}^-$  accumulation they have been reported to vary  
54 less between the materials examined, and are not the key process regulated by ABA  
55 [1, 2].

56

57 In **saline** conditions, limiting accumulation of  $\text{Cl}^-$  in leaves is a sub-trait of salt (NaCl)  
58 tolerance and is a **multigenic** process [2] (Fig 1). However, the molecular factors  
59 that limit long-distance  $\text{Cl}^-$  transport have been relatively neglected compared to  
60 those for the other ionic component of salt, sodium ( $\text{Na}^+$ ) [11]. Here we review the  
61 latest findings related to the regulation of  $\text{Cl}^-$  transport from root to shoot, including  
62 an overview of newly identified  $\text{Cl}^-$  transporting proteins associated with the root  
63 **stele**. This includes proteins that directly catalyze the movement of  $\text{Cl}^-$  from the root  
64 **symplast** to the xylem [12-14], and several endo-membrane transport proteins that  
65 appear to influence long-distance  $\text{Cl}^-$  transport and salt tolerance [15-18]. We  
66 evaluate these studies and propose potential future research directions for studies  
67 with the aim of improving  $\text{Cl}^-$  exclusion of  $\text{Cl}^-$  sensitive plants [19]. Although the  
68 scope of this review is mostly limited to the root **stele**, we discuss the similarities and  
69 differences in  $\text{Cl}^-$  transport processes with stomatal guard cells. We do this to  
70 emphasize two important concepts that highlight the plants' flexibility in its transport  
71 regulation: (i) plant cells appear to repurpose particular proteins in different tissues to  
72 perform novel roles; and, (ii) plants use closely related proteins in different ways in  
73 different cell types.

74

### 75 ***Chloride – a neglected component of salt toxicity?***

76 **Salinity**-induced yield reductions in our conventional staple or high value crops are  
77 common in coastal and arid regions, and are substantial and increasing for irrigated  
78 agriculture [1]. It is estimated that up to 8% of rain-fed (dryland) agriculture and 20%  
79 of irrigated agriculture is currently affected by **salinity**, with both figures expected to  
80 double by 2050 [20]. **Saline** soils are more prevalent in arid and semi-arid countries.  
81 For instance, in Australia over 67% of its cropping region has the potential to develop  
82 **salinity** issues in any one season [20]. There are at least three sub-traits that confer  
83 **salinity** tolerance – **leaf salt exclusion**, **tissue tolerance** and **osmotic adjustment**

84 – each sub-trait is under the influence of multiple genes acting on multiple underlying  
85 components [11, 21, 22].

86

87 To date, Na<sup>+</sup> is the ion that has most commonly been associated with crop yield  
88 reductions due to salt accumulation in the soil solution [11]. In fact, Na<sup>+</sup> and salt are  
89 often used interchangeably in the literature. However, in woody perennial crops such  
90 as grapevine (*Vitis* spp. L.), citrus (*Citrus* spp. L.), and avocado (*Persea americana*  
91 L.), and legumes such as soybean (*Glycine max* L.) and faba bean (*Vicia faba* L.) it  
92 is the accumulation of Cl<sup>-</sup> in leaves, not Na<sup>+</sup>, that is often best correlated with  
93 decreased transpiration, photosynthesis, crop yield and quality – and eventually  
94 plant death [2, 5, 6, 10, 23-26]. This association exists not because Cl<sup>-</sup> is  
95 metabolically more toxic than Na<sup>+</sup> to these species, but because they are able to  
96 secrete a greater proportion of their Na<sup>+</sup> in roots and/or woody stems. This limits Na<sup>+</sup>  
97 transport to the leaves to reduce its potential impact upon cellular metabolism within  
98 photosynthetic organs [2, 21]. For instance, trifoliolate orange (*Poncirus trifoliata* L.)  
99 was able to maintain shoot Na<sup>+</sup> exclusion in treatments below 100 mM by secreting it  
100 into the woody tissue of roots and the basal stem, presumably through xylem  
101 retrieval, whereas leaves accumulated high concentrations of Cl<sup>-</sup> even when under a  
102 25 mM NaCl treatment [27]. It is now emerging that even cereals classically thought  
103 to be Na<sup>+</sup> sensitive are also sensitive to shoot Cl<sup>-</sup> accumulation (see below).

104

105 In horticulture, the scion of salt-sensitive species such as grapevine and citrus are  
106 grafted to **rootstocks** that limit the delivery of Cl<sup>-</sup> to the root xylem (e.g. trifoliolate  
107 orange) to confer a degree of shoot salt exclusion and improve growth and yield in  
108 saline environments [6, 10, 25, 26]. Comparative genomics of these **rootstocks** is  
109 being used to uncover the molecular determinants that control the delivery of Cl<sup>-</sup> to  
110 the shoot [6, 28]. The hope is that this will allow manipulation of specific Cl<sup>-</sup>  
111 **transporters** to improve crop salt tolerance as has occurred for the manipulation of  
112 Na<sup>+</sup> transporters [19, 29-31].

113

114 There are likely to be multiple molecular targets of Cl<sup>-</sup> (and Na<sup>+</sup>) toxicity, most of  
115 which are unknown [11]. For Cl<sup>-</sup>, some are likely to occur via interference in its  
116 nutritive roles. All that has been observed so far are the phenotypes associated with  
117 salt accumulation. During salt stress, the effects of Cl<sup>-</sup> can be additive and/or

118 synergistic to Na<sup>+</sup>. Treatments of NaCl can affect the growth and physiology of a  
119 variety of species more than treatments that contain only high concentrations of one  
120 of salt's constituent ions (Cl<sup>-</sup> or Na<sup>+</sup>). This occurs even in wheat, barley and rice that  
121 are classically thought to be more Na<sup>+</sup> sensitive [32-36]. Whilst it is difficult to  
122 separate Cl<sup>-</sup> toxicity from Na<sup>+</sup> toxicity, and other components of salt stress, some  
123 toxic effects have been shown to be relatively Cl<sup>-</sup> specific when using complex  
124 mixed salt solutions. For instance, in faba bean, a significant decline in leaf  
125 chlorophyll was observed following a treatment containing 100 mM Cl<sup>-</sup> without Na<sup>+</sup>,  
126 but not following a 100 mM Na<sup>+</sup> treatment lacking Cl<sup>-</sup> [34]. It has also been shown  
127 that exposure of roots to Cl<sup>-</sup> can inhibit gas exchange via an indirect long-distance  
128 signal, which induces an alkalinisation of the leaf **apoplastic** pH – resulting in a  
129 redistribution of leaf ABA and stomatal closure [37]. Although the interpretations of  
130 experiments that alter either Na<sup>+</sup> or Cl<sup>-</sup> in isolation remain controversial, as they  
131 have to change the concentration of several counterions at the same time, these  
132 treatments are consistent with Cl<sup>-</sup> toxicity being a significant contributor to salt stress.  
133 Such approaches could also be used to identify key players in Cl<sup>-</sup> transport  
134 pathways and the targets of ion specific toxicity.

135

136 An important accompanying and detrimental effect of increased root Cl<sup>-</sup> uptake, and  
137 its accumulation in shoot vacuoles, is the reduction in the uptake and storage of the  
138 major biological building block nitrogen. This well-documented effect occurs through  
139 antagonism of NO<sub>3</sub><sup>-</sup> transport and accumulation of Cl<sup>-</sup> (e.g. [12, 13, 38]). Both are  
140 monovalent anions with a similar ionic radius, can be transported through the same  
141 or different proteins (see transport selectivity section below), and both perform a role  
142 in charge balance and turgor regulation. We propose that the ratio of NO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> in  
143 the shoot may be a useful indicator of salt tolerance similar to the well described  
144 **K<sup>+</sup>/Na<sup>+</sup> ratio** [2], as depression of the NO<sub>3</sub><sup>-</sup>/Cl<sup>-</sup> ratio correlates with a reduction in  
145 growth [13]. As the severity of sub-lethal Cl<sup>-</sup> exposure increases, and Cl<sup>-</sup>  
146 accumulates to high concentrations, marginal necrosis occurs (also known as leaf  
147 burn) [39], as well as a depression in fertilization and yield [40]. Application of NO<sub>3</sub><sup>-</sup>  
148 to reduce Cl<sup>-</sup> uptake, is sometimes successful in reducing Cl<sup>-</sup> toxicity [39].

149

150 ***Getting Cl<sup>-</sup> from root-to-shoot – what's known, what's new?***

151 To limit accumulation of  $\text{Cl}^-$  in leaves, the net transfer of  $\text{Cl}^-$  into the root xylem  
152 should be minimized. This can occur by limiting  $\text{Cl}^-$  entry into the xylem and/or  
153 maximizing  $\text{Cl}^-$  retrieval from the xylem (Fig 1 and 2). The movement of  $\text{Cl}^-$  across  
154 the plasma membrane of the **stelar** cells into the root xylem **apoplastic** space is  
155 passive, down a difference in **electrochemical potential** for  $\text{Cl}^-$ , and thus it does not  
156 require direct expenditure of energy. Figure 2 describes the biophysics for the  
157 loading/unloading of  $\text{Cl}^-$  into xylem vessels. It was hypothesized that plants down-  
158 regulate the activity and expression of  $\text{Cl}^-$  transporters that load  $\text{Cl}^-$  into the xylem  
159 during salt exposure, or following drought, via an ABA mediated pathway [9]. Such a  
160 phenomenon is known to occur for  $\text{K}^+$  loading via the **SKOR** channel during water  
161 stress [41-43], and this putative property has been used as a framework to identify  
162 proteins that regulate loading  $\text{Cl}^-$  into the xylem. In non-stressed conditions, there is  
163 an electrochemical potential difference for protons between the root **symplast** and  
164 the xylem-associated **apoplast**, so it is possible that a  $\text{Cl}^-/\text{H}^+$  **symporter** could  
165 actively retrieve  $\text{Cl}^-$  from the xylem **apoplastic** space back into the root symplast, if  
166 present on this membrane. Thermodynamically, this action against the  
167 **electrochemical potential** difference for  $\text{Cl}^-$  could resemble the  $\text{Cl}^-/2\text{H}^+$  symport  
168 described for the initial uptake of  $\text{Cl}^-$  into root hair cells [44] (Fig 1). As such, this  
169 would be less energy efficient than reducing the passive movement of  $\text{Cl}^-$  into the  
170 xylem apoplast as it requires direct use of some of the difference in electrochemical  
171 potential for  $\text{H}^+$ , built up by the  $\text{H}^+$ -ATPases on the plasma membrane of these cells.  
172 Retrieval of  $\text{Cl}^-$  from the root xylem through the direct action of a **CCC** has been  
173 proposed [45], although others have shown this is an endo-membrane protein so is  
174 not likely to be directly involved [16] (see **CCC section below**). Furthermore, recent  
175 evidence has been used to suggest that the disruption of cytosolic  $\text{Cl}^-$  through  
176 knockout of vacuolar  $\text{Cl}^-$  transporters has knock-on effects on root-to-shoot transfer  
177 of  $\text{Cl}^-$  [18] (see **ALMT9 section below**).

178

179 In the following section, we highlight proteins implicated in modulating shoot  $\text{Cl}^-$   
180 accumulation either through facilitating transfer of  $\text{Cl}^-$  transport to the root xylem or  
181 the regulation of this process (Fig 3), we also comment on several further candidates  
182 that are yet to be examined for their role in long-distance  $\text{Cl}^-$  transport (Fig 3).

183

184 ***Nitrate transporter1/Peptide transporter (NPF) proteins***

185 A recent microarray screen identified the first protein proposed to directly catalyze  
186  $\text{Cl}^-$  transport into the root xylem, *Arabidopsis thaliana* L. (arabidopsis, At) AtNPF2.4  
187 [14]. Protoplasts were specifically isolated from the stele or epidermis/cortex to probe  
188 for transcripts that: 1) were expressed preferentially in the stele; 2) had their  
189 expression negatively regulated by ABA and NaCl; and 3) were likely to encode an  
190 anion transport protein, as it was deemed from previous literature that these were  
191 properties that transporters controlling root-to-shoot transport of  $\text{Cl}^-$  may possess  
192 [14]. Only two genes fulfilled these criteria, *AtNPF2.4* and *AtNRT1.5/AtNPF7.3*, with  
193 the former being chosen for further characterization. The *AtNPF2.4* promoter drove  
194 expression specifically within root stelar cells, the protein was localized to the plant  
195 plasma membrane, overexpression of *AtNPF2.4* resulted in a 23% increase in shoot  
196  $\text{Cl}^-$ , and when *AtNPF2.4* was expressed in *Xenopus laevis* oocytes it catalyzed  $\text{Cl}^-$   
197 efflux at membrane potentials equivalent to those in the stele (around -120 mV) [14].  
198 The currents ascribed to AtNPF2.4 were channel-like and were not pH-dependent,  
199 but were unlike the major conductance thought to be responsible for xylem loading of  
200  $\text{NO}_3^-$  and  $\text{Cl}^-$  (**X-QUAC**) [9, 46, 47], they were small in magnitude, non-rectifying, did  
201 not carry  $\text{NO}_3^-$  and were dependent upon external  $\text{K}^+$  or  $\text{Na}^+$  [14]. This means that  
202 AtNPF2.4 does not encode an **X-QUAC** type channel, or that specific regulatory  
203 factors not present in oocytes are required for it to function as it does in the plant (e.g.  
204 kinases/phosphatases). Alternatively, arabidopsis may not contain **X-QUAC**, with no  
205 equivalent experiments to that in barley and maize being yet conducted [9, 46, 47].  
206 Regardless, it is clear that loading of  $\text{Cl}^-$  to the arabidopsis xylem is a multigenic trait  
207 (as it appears to be in grapevine [10, 26, 28], maize [9] and barley [46, 47], see  
208 Figure 3) as silencing of *AtNPF2.4* resulted in only a ~20-30% reduction in shoot  $\text{Cl}^-$   
209 concentration [14].

210 AtNPF2.4 is a member of NAXT subfamily (Nitrate excretion transporters) (7  
211 members) named after AtNAXT1/AtNPF2.7, a root  $\text{NO}_3^-$  efflux transporter [48].  
212 AtNPF2.5, the closest homolog of AtNPF2.4 among the NAXTs, was recently shown  
213 to be predominantly localized to the plasma membrane of root cortical cells and is  
214 likely to mediate  $\text{Cl}^-$  efflux [49]. As being salt inducible, *AtNPF2.5* was suggested to  
215 encode a transporter that modulates  $\text{Cl}^-$  efflux from the root, contributing to  $\text{Cl}^-$   
216 exclusion from the shoot [49]. The  $\text{Cl}^-$  transport activity of both AtNPF2.4 and  
217 AtNPF2.5 indicated the potential involvement of other root specific NAXTs in  $\text{Cl}^-$   
218 excretion from the root (Fig 3). In that regard, it would be instructive to test the

219 expression profiles of the NAXTs of unknown function for their root tissue localization  
220 and their regulation by salt.

221

222 The *NPF* gene family in arabidopsis encodes several other candidate proteins for  $\text{Cl}^-$   
223 transport to the stele. The expression of some of these genes is regulated by salt  
224 stress, and some have a demonstrated role in affecting  $\text{NO}_3^-$  distribution among  
225 tissues [48, 50-54]. Amongst these, the stelar-specific *AtNRT1.5/AtNPF7.3* was the  
226 only other transcript to encode an anion transporter identified in the microarray  
227 screen described above [14]. This protein was designated as one of the transporters  
228 that loads  $\text{NO}_3^-$  directly into the root xylem and affects its delivery from root-to-shoot  
229 [50]. Salt stress significantly down-regulates *AtNPF7.3* expression [52], which might  
230 be a cause, or be a consequence of, the antagonism between  $\text{Cl}^-$  and  $\text{NO}_3^-$  shoot  
231 accumulation. Interestingly, knockout of *AtNPF7.3* resulted in greater salt tolerance,  
232 which was attributed to an increase in root  $\text{NO}_3^-$  content [52]. *AtNRT1.8/AtNPF7.2* is  
233 a designated retriever of  $\text{NO}_3^-$  from the root xylem, its expression is significantly  
234 induced by salt stress and overexpression increases salt tolerance [51]. It would be  
235 interesting to examine root and shoot  $\text{NO}_3^-/\text{Cl}^-$  ratios in the *Atnpf7.2* and *Atnpf7.3*  
236 mutants and corresponding overexpression plants. *AtNPF7.3* may also facilitate  $\text{Cl}^-$   
237 transport *in planta* and its loss may reduce shoot  $\text{Cl}^-$ , although this remains to be  
238 tested. Similarly, increased *AtNPF7.2* abundance may increase  $\text{Cl}^-$  retrieval from the  
239 xylem, energized by the difference in electrochemical potential for  $\text{H}^+$ , and reduce  
240 shoot  $\text{Cl}^-$  accumulation (Fig 2 and 3). However, it is known that *AtNPF7.2*  
241 overexpression and *AtNPF7.3* knockout also results in improved tolerance to biotic,  
242 cadmium, cold and osmotic stresses, so increased  $\text{NO}_3^-$  accumulation in roots may  
243 simply provide a general increase in stress tolerance [55].

244

### 245 ***Slow-type anion channel associated homolog 1 (SLAH1)***

246 In guard cells of arabidopsis, the Slow-Type Anion Channel-associated 1 protein  
247 (AtSLAC1) is responsible for the major component of anion efflux ( $\text{Cl}^-$  and malate),  
248 important for stomata closure [56, 57]. The AtSLAC1-homolog 1 (*AtSLAH1*) is only  
249 expressed in root pericycle cells [57]. Two recent reports indicate that *AtSLAH1* has  
250 a major role in shoot accumulation of  $\text{Cl}^-$  [12, 13]. Overexpression of *AtSLAH1* either  
251 specifically in the root stele, or constitutively, led to increased shoot  $\text{Cl}^-$  accumulation  
252 and decreased salt tolerance [13], and knockdown or knockout of *AtSLAH1* reduced



253 shoot accumulation of  $\text{Cl}^-$  by about 30% [12, 13]. AtSLAH1 is electrically silent when  
254 expressed by itself in *X. laevis* oocytes [12, 13], as are AtSLAC1 and AtSLAH3, but  
255 pore mutations that make AtSLAC1 and AtSLAH3 constitutively active in oocytes do  
256 not work for AtSLAH1 [12]. It was hypothesized that AtSLAH1 is a silent subunit and  
257 does not transport  $\text{Cl}^-$  directly [13]; instead AtSLAH1 interacts with AtSLAH3 to  
258 seemingly changes AtSLAH3 transport properties increasing its capacity to transport  
259  $\text{Cl}^-$  [13]. AtSLAH3, is expressed in the pericycle and stomatal guard cell and is much  
260 more selective for  $\text{NO}_3^-$  over  $\text{Cl}^-$  when activated by phosphorylation with  $\text{Ca}^{2+}$   
261 dependent kinase 21 (AtCPK21), as occurs in stomatal guard cells [12, 58, 59]. It  
262 was shown that the interaction between AtSLAH1 and AtSLAH3 was specific  
263 amongst the AtSLAC1 homologs in arabidopsis (e.g. not AtSLAC1 or AtSLAH2/4),  
264 but AtSLAH1s from other species such as medicago, poplar, and the Venus fly trap  
265 could all activate AtSLAH3 currents [12]. This work highlights that the interaction  
266 between  $\text{NO}_3^-$  and  $\text{Cl}^-$  transport is at least in part due to competition in the transport  
267 of both anions through the same set of transporters [60].

268

### 269 **Cation-Chloride Co-transporters (CCCs)**

270 Cation-Chloride Co-transporters (CCCs) were first characterized in animal cells  
271 where they catalyze  $\text{Cl}^-$ -cation co-transport (with  $\text{Na}^+$ ,  $\text{K}^+$ , or both); they regulate  
272 cellular  $\text{Cl}^-$  concentration and so influence neuronal excitability, cell volume control  
273 and osmoregulation in kidneys [61]. In animals, there are routinely multiple CCC  
274 genes found in one organism, in plants only one or few CCC representatives are  
275 commonly found per species [16, 45]. The first plant CCC characterized was from  
276 arabidopsis [45]. When expressed in *X. laevis* oocytes AtCCC co-transported  $\text{Cl}^-$ ,  
277  $\text{Na}^+$  and  $\text{K}^+$ , and knockout of AtCCC increased shoot  $\text{Cl}^-$  accumulation, whilst  
278 decreasing its accumulation in roots. Coupled to its expression in the root  
279 vasculature, this led to the conclusion that CCC may retrieve  $\text{Cl}^-$  from the root xylem  
280 [45]. Subsequently, CCC in rice, grapevine and citrus have been investigated as  
281 candidates for improving plant salinity tolerance, and their misexpression has led to  
282 altered shoot  $\text{Cl}^-$ , but the mechanism by which this occurs remains inconclusive [6,  
283 16, 62, 63]. OsCCC was localized to the plasma membrane [62, 63]; however, it  
284 would seem pertinent to revisit this as AtCCC and the grapevine CCC localized to  
285 the Golgi and trans-Golgi Network (TGN) [16]. If it is an endo-membrane protein  
286 CCC is likely to affect root-to-shoot  $\text{Cl}^-$  distribution indirectly, or via a complex

287 mechanism that is not mediated at the plasma membrane (Fig 3). CCC expressed in  
288 multiple tissues and knockout plants of *AtCCC* and *OsCCC* have similar severe  
289 dwarf phenotypes and low fertility in the absence of salinity, so it is clear these  
290 proteins have important functions that are unrelated to those caused by small  
291 changes in xylem  $\text{Cl}^-$  [16, 45, 63]. In rice, *OsCCC* was shown to be vital for cell  
292 osmoregulation and elongation through a control of cytosolic  $\text{Cl}^-$  concentrations [63],  
293 which would be another explanation for the stunted growth phenotype observed for  
294 *Atccc* plants.

295

### 296 **Cation/ $\text{H}^+$ exchanger (CHX)**

297 Soybean is a moderately salt tolerant crop species with  $\text{Cl}^-$  exclusion implicated to  
298 be a major mechanism contributing to its salt tolerance [64]. A cation/ $\text{H}^+$  exchanger  
299 (of the **CPA2** family), *GmSALT3/CHX1*, was shown to localize to the endoplasmic  
300 reticulum of root vasculature-associated cells, and affects  $\text{Na}^+$  exclusion and salt  
301 tolerance of soybean [15]. *GmSALT3* appears to also affect  $\text{Cl}^-$  transport to the  
302 shoot [17], which again suggests the involvement of vascular endo-membrane  
303 transporters in regulating homeostasis in xylem sap as shown for CCCs. It is unclear  
304 whether *GmSALT3/CHX1* directly affects cytosolic  $\text{Cl}^-$  (and  $\text{Na}^+$ ) concentration to  
305 impact plasma membrane transport as suggested for **ALMTs** (see below) as the  
306 characterized members of this family from arabidopsis are considered to be  $\text{K}^+/\text{H}^+$   
307 exchangers [15]. Though, this is a possibility as two CPA2 proteins from drosophila  
308 (*Drosophila melanogaster*) were recently characterized, one as a  $\text{H}^+/\text{Cl}^-$  symporter  
309 and one as a  $\text{Na}^+/\text{H}^+$  exchanger [65]. Alternatively, the unidentified mechanism of  
310 shoot  $\text{Cl}^-$  regulation by root vascular endosomal transporters may occur through  
311 endosomal pH effects or vesicle trafficking such as those noted for the CPA1 type  
312 endosomal **NHX** proteins – that also predominantly transport  $\text{K}^+$  [66].

313

### 314 **Aluminum Activated Malate Transporters (ALMTs)**

315 Aluminum Activated Malate Transporters (ALMTs) were named after the first protein  
316 cloned from this family, from wheat, which carries aluminum ( $\text{Al}^{3+}$ )-activated malate  
317 efflux – this chelates  $\text{Al}^{3+}$  and confers tolerance [67]. ALMT are now known to be  
318 multigenic (with 14 in arabidopsis and 9 in rice), and most that have been  
319 characterized subsequently are not  $\text{Al}^{3+}$  activated nor have any role in  $\text{Al}^{3+}$  tolerance  
320 [68]. Instead, they play diverse physiological roles such as stomatal aperture control,

321 anion homeostasis, fruit quality and seed development, and are widely expressed in  
322 plant tissues [69-71]. Some ALMT are permeable to  $\text{Cl}^-$ , making them good  
323 candidates for studying for a role in long distance  $\text{Cl}^-$  transport (e.g. **AtALMT9** and  
324 **AtALMT12**, Fig 3) [18, 69-72]. AtALMT9, previously characterized as carrying  $\text{Cl}^-$   
325 into the stomatal guard cell vacuole, and for a role in facilitating stomatal opening,  
326 was recently shown to regulate long distance transport of  $\text{Cl}^-$  and  $\text{Na}^+$  [18, 69]. In  
327 *Atalmt9* plants,  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation in the shoot decreased within a day of a  
328 100 mM NaCl treatment but was restored to wildtype levels by 7 days [68]. It was  
329 speculated that the likely increase in cytosolic  $\text{Cl}^-$  (and  $\text{Na}^+$ ) brought about by a  
330 reduced capacity for  $\text{Cl}^-$  storage in the vacuole of root stelar cells constituted a  
331 signal to increase transcription of transporters important for regulating long distance  
332 transport of  $\text{Na}^+$ , *CHX21* and *High-Affinity  $\text{K}^+$  Transporter1* (*AtHKT1.1*) so reducing  
333 shoot salt load [18]. Pleiotropic compensation for AtALMT9 knockout may be the  
334 reason that longer-term effects on salt accumulation are not seen. Another ALMT,  
335 the plasma membrane localized AtALMT12 is expressed in guard cells conducts  
336 cellular anion efflux ( $\text{Cl}^-$  and malate), and is a major component of the ABA-activated  
337 R-type anion current [70]. Interestingly, AtALMT12 is also found in root stelar cells  
338 like AtALMT9 [73]. Therefore, it is a good candidate for being another channel that  
339 catalyzes direct xylem loading of  $\text{Cl}^-$  downstream of ABA signaling, although its role  
340 in this process is yet to be examined.

341

### 342 **Chloride channel (CLC)**

343 Plant CLCs (Chloride channels) that localize to the tonoplast regulate vacuolar  
344 sequestration of  $\text{Cl}^-$  and  $\text{NO}_3^-$ , which makes them possible players in the regulation  
345 of  $\text{Cl}^-$  homeostasis. Of the seven CLCs in arabidopsis, two directly transport  $\text{Cl}^-$ , with  
346 the others transporting  $\text{NO}_3^-$  [74-79]. Tonoplast AtCLCc secretes  $\text{Cl}^-$  into root  
347 vacuoles and helps improve salt tolerance, and regulates gas exchange through its  
348 role in light induced opening and ABA-induced closure [74]; AtCLCg, localized to  
349 mesophyll tonoplast, is predicted to have a similar role for  $\text{Cl}^-$  compartmentation in  
350 leaf mesophyll as its knockout showed increased sensitivity to salt [75]. *CLC* have  
351 their expression upregulated by salt in rice, maize and citrus [80-82], and  
352 overexpression of tonoplast localized GmCLC1 in soybean increased its salt  
353 tolerance [83, 84], indicating, again that direct transfer of  $\text{Cl}^-$  is not the only factor

354 controlling Cl<sup>-</sup>-related tolerance in plants. For further information on other CLC  
355 members, which also localize to other endo-membrane, we refer readers to [85].

356

### 357 ***ABC transporters and ICl<sub>n</sub>***

358 Multidrug-Resistance Protein 4 (MRP4) is a member of the ATP-Binding Cassette  
359 (ABC) family that was shown to be involved in S-type anion channel activity in guard  
360 cells [86]. *AtMRP4* was is expressed in primary roots and its expression is up-  
361 regulated by salt stress [86]. A functional study of the effect of *AtMRP4* (or its  
362 homologs) on Cl<sup>-</sup> transport in the root is therefore needed. Chloride-Conductance  
363 Regulatory Protein (ICl<sub>n</sub>) in animal cells performs as an anion channel in artificial  
364 membranes [87]. Although microarray experiments indicated that the ICl<sub>n</sub> homolog in  
365 arabidopsis (AT5G62290) did not respond to salt stress [88], in citrus, *CcICl<sub>n</sub>* is  
366 differentially expressed in rootstocks with differing Cl<sup>-</sup> exclusion capacities,  
367 suggesting the involvement of *CcICl<sub>n</sub>* in Cl<sup>-</sup> transport [6].

368

### 369 ***Transport selectivity***

370 A molecular basis for transporter selectivity of Cl<sup>-</sup> and NO<sub>3</sub><sup>-</sup> has been revealed  
371 through the manipulation of the selectivity filter of the transport proteins **AtCLCa** and  
372 **AtSLAH2**. Single (but different) amino acid mutations in either proteins shifts the  
373 anion specificity between the two anions. This occurs through a conformational  
374 change in the transport pore (S228 in **AtSLAH2**; or P160 in **AtCLCa**), or by a  
375 mechanism likely to be related to gating (E203 in **AtCLCa**) [89-92]. Conceivably,  
376 mutagenesis of a range of endogenous transporters to improve selectivity for NO<sub>3</sub><sup>-</sup>  
377 over Cl<sup>-</sup> in the root (particularly within the stele) could reduce the shoot transfer of  
378 Cl<sup>-</sup> and improve salt tolerance, although this could interfere with the nutritional roles  
379 of Cl<sup>-</sup> at low salinity [3].

380

### 381 ***Transport regulation – different cells, different stories***

382 The regulation of ion efflux from cells surrounding the xylem and stomatal guard cells  
383 is opposite (i.e. ABA activates ion efflux in guard cells but inhibits this process in  
384 xylem-associated cells). As such, it is not surprising that different transporters  
385 facilitate these processes (such as **SKOR** in the stele, versus **GORK** in guard cells –  
386 for K<sup>+</sup>), and for those transporters that are the same in both cell types (such as  
387 *AtSLAH3*), a different suite of ABA regulatory proteins are likely to be present. In

388 guard cells, AtSLAH3 is a component of the S-type anion current involved in (ABA-  
389 activated) guard cell closure [58]; recently AtSLAH3 was also shown to impede  
390 stomatal opening through inhibiting the uptake of  $K^+$  via direct interaction with the  $K^+$   
391 channel **AtKAT1**, along with AtSLAC1 [93]. Therefore, it would be interesting to  
392 examine if both AtSLAH3 and AtSLAH1 interact with other proteins in root pericycle  
393 cells, and whether these are kinases such as the AtCPKs that regulate AtSLAC1  
394 homologs in guard cells, or are other transporters.

395

396 One such candidate set of proteins is the aquaporins. In citrus genotypes, *PIP1*  
397 expression appears to be associated with  $Cl^-$  accumulation, probably due to the rate  
398 of water movement into the xylem, hydraulic conductance and transpiration [94].  
399 Recently, a PIP2;1 aquaporin was shown to interact with OST1-SLAC1 and  
400 CPK6/23-SLAC1 complexes in arabidopsis [95]. Although this work focuses on  $CO_2$   
401 regulation of AtSLAC1 transport, it invites speculation that the AtSLAH1/AtSLAH3  
402 complex may also interact with aquaporins in the xylem stelar cells, thus coupling  
403 water flow and hydraulic conductance in the xylem to  $Cl^-$  transport. AtPIP2;1 was  
404 also recently shown to transport  $Na^+$  so the coupling of ion and water transport may  
405 occur through a single aquaporin within the stele, which could be balanced by  
406 movement of  $Cl^-$  [96].

407

408 Other post-translational signals, in addition to ABA, that may regulate  $Cl^-$  loading of  
409 the xylem include **ROS**, which was associated with  $Cl^-$  exclusion in soybean [97],  
410 GABA and ATP, which have been shown to inhibit ALMT activity [68, 98, 99]. ATP  
411 also regulates CLC activity so may affect root storage capacity for  $Cl^-$ , and therefore  
412 affect delivery of  $Cl^-$  to the xylem [74, 100]. All three have effects in guard cells [98,  
413 101, 102], but their effects on xylem loading are yet to be determined.

414

415 Another way ABA may differentially regulate  $Cl^-$  efflux across stelar and guard cell  
416 plasma membranes is by regulating transporters differentially. It was recently found  
417 that *AtSLAH1* expression was down-regulated by salt and ABA, but differences were  
418 found in regulation of *AtSLAH3* expression [12, 13]. In one study, no significant  
419 regulation of *AtSLAH3* by salt or ABA was found, which was interpreted as being a  
420 potential mechanism to sustain  $NO_3^-$  loading to the shoot and would assist in  
421 maintaining a higher shoot  $NO_3^-/Cl^-$  ratio [13]. However, *AtSLAH3* was found to be

422 downregulated by ABA in another study [12], although less significantly than  
423 *AtSLAH1*. We interpret this as being due to differences in growth conditions, and  
424 propose that there is likely to be additional factors regulating the expression and  
425 activity of anion transporters in the root stele.

426

427 ABA-responsive elements were identified in the promoter region of *AtNPF2.4*, which  
428 could be explored in further detail to determine how expression of *AtNPF2.4* is  
429 downregulated by ABA. Expression of *AtHKT1.1*, which regulates shoot  $\text{Na}^+$  by  
430 retrieval of  $\text{Na}^+$  from the xylem into root xylem parenchyma cells, has been reported  
431 to be downregulated by root-specific *ABA-insensitive 4* (*ABI4*) binding to elements  
432 within its promoter. This is particularly interesting as simultaneous  $\text{Na}^+$  and  $\text{Cl}^-$   
433 accumulation in the shoot would need to be coordinated with that of other ions to  
434 ensure charge balance (as was exemplified by the *Atalmt9* phenotypes). It is yet to  
435 be shown how ABA regulates **SKOR** or *SLAH* expression, but it plausible that there  
436 are common elements or transcription factors regulating a number of these  
437 transporters involved in shoot delivery of solutes.

438

### 439 **Concluding remarks**

440  $\text{Cl}^-$  toxicity in plants is a significant issue. An important mechanism to reduce  $\text{Cl}^-$   
441 toxicity is to reduce accumulation of  $\text{Cl}^-$  in the shoot, which requires alteration of  
442 transport processes. It appears that key **gatekeepers** of shoot  $\text{Cl}^-$  accumulation are  
443 root stelar cells, and transporters within these cells facilitate loading of  $\text{Cl}^-$ , some  
444 known and with some yet to be identified (Fig 3). Amongst these, NPF and SLAH  
445 proteins have demonstrated roles in modulating long-distance transport of  $\text{Cl}^-$  and  
446 are currently being targeted to improve  $\text{Cl}^-$  exclusion and salinity tolerance of crop  
447 plants. Endo-membrane  $\text{Cl}^-$  transporters (*AtALMT9*, CCCs and CHXs) are emerging  
448 with their roles in regulating long distance  $\text{Cl}^-$  transport, as well as the established  
449 role of CLCs. However, research revealing the identity of regulatory proteins for  
450 these and other stelar  $\text{Cl}^-$  transporters in response to signals such as ABA and  $\text{Ca}^{2+}$   
451 is still at an early stage. The existence of post-translational mechanisms that affect  
452 transport activity (e.g. heterodimerisation and phosphorylation) suggests that  $\text{Cl}^-$   
453 transport is tightly regulated for nutritional reasons that are still to be determined.

454

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462

463 **References**

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## Glossary

**ABA:** Abscisic Acid – is a plant hormone that plays a central role in responses to abiotic and biotic stress, e.g. salinity, drought, low temperature and pathogen attack. Salt stress triggers the synthesis and distribution of ABA throughout the plant, which induces expression of numerous salt stress-related genes, as well as a range of physiological processes, such as the closure of stomata.

**Antiport(er):** A type of carrier transporter that is able to transport different solutes across a membrane in the opposite direction at the same time. It is a type of co-transporter, with a similar principle of action to a symporter, except moving solutes in the opposite direction, rather than the same direction. Normally, the movement of one ion is down its difference in electrochemical potential, allowing the other ion to be pumped against its difference in electrochemical potential.

**Apoplast:** The volume outside the plasma membrane through which water and small molecules (such as  $\text{Cl}^-$ ) can move. In the mature root, apoplastic flow is greatly slowed by the hydrophobic Casparian Strip in the endodermal cell layer.

**AtCLCa:** Arabidopsis Chloride Channel a, a  $\text{NO}_3^-/\text{H}^+$  exchanger localized to the tonoplast in plants. The Thr38 residue is important for its function in stomatal closure in guard cells ( $\text{Cl}^-$  efflux).

**AtSLAH2:** Arabidopsis SLAC1 Homologue 2, a  $\text{NO}_3^-$  selective anion channel. This channel can become more selective for  $\text{Cl}^-$  by mutation of the Ser-228 residue.

**CCC:** Cation- $\text{Cl}^-$  co-transporters, that appear to be located in the endo-membranes of stelar cells, that affect root-to-shoot transfer of  $\text{Cl}^-$ .

**CPA:** Cation/proton antiporter super family. It divides into family 1 and 2. CPA1 contains NHX transporters ( $\text{Na}^+/\text{H}^+$  exchangers). CPA2 contains CHX transporters (cation/ $\text{H}^+$  exchangers).

**Electrochemical potential difference:** The difference between two phases in the electrochemical potential of a particular solute, determined by the respective activities of the solutes in the two phases, the charge of the solute and, if the solute is charged, the difference in electrical potential (voltage) between the two phases.

**Gatekeeper:** Used to denote a process or cell type that is, or controls, a rate-limiting step (see [8]).

**GORK:** Guard cell  $\text{K}^+$  outwardly rectifying  $\text{K}^+$  channel, a protein mediating  $\text{K}^+$  efflux out from guard cells.

**HKT:** High-Affinity  $\text{K}^+$  Transporter1 (AtHKT1.1), which regulates shoot  $\text{Na}^+$  by retrieval of  $\text{Na}^+$  from the xylem into root xylem parenchyma cells.

772

773 **KAT1:** An inward-rectifying K<sup>+</sup> channel.

774

775 **K<sup>+</sup>/Na<sup>+</sup> ratio:** The ratio of tissue or cytosolic K<sup>+</sup> concentration to Na<sup>+</sup> concentration.

776 For technical reasons, it usually refers to the ratio in whole tissue, although the more

777 biologically relevant ratio is that found in the cytosol. It is often used to show the

778 ability of plants to exclude Na<sup>+</sup> under saline condition while maintaining uptake of K<sup>+</sup>.

779

780 **Leaf salt exclusion:** The exclusion of Na<sup>+</sup> and Cl<sup>-</sup> from the cytoplasm of leaves, the

781 primary site of salt damage, under saline conditions.

782

783 **Multigenic:** Being due to more than one gene.

784

785 **NHX:** Na<sup>+</sup>/H<sup>+</sup> exchanger, belonging to CPA1 family. NHX in arabidopsis has 6

786 members. AtNHX1-4 localize to tonoplast and sequester Na<sup>+</sup> and K<sup>+</sup> into vacuoles.

787 AtNHX5 and 6 localize to Golgi and trans-Golgi network, and show higher affinity to

788 K<sup>+</sup> compared to Na<sup>+</sup>.

789

790 **Osmotic adjustment:** The adjustment of intracellular osmotic pressure, to enable

791 maintenance of leaf expansion, leaf turgor and stomatal conductance as a means to

792 minimize the toxic effects of the osmotic component of salt stress.

793

794 **Rootstocks:** Often refers to the underground part of a plant. In grafting, it refers to

795 an already-established healthy plant root system, onto which a cutting or a bud from

796 another plant can be grafted. The use of rootstocks is commonly for better fruiting

797 and resistance to abiotic/biotic stresses of woody perennials such as grapevines and

798 fruit trees.

799

800 **ROS:** Reactive oxygen species. Reactive chemicals containing oxygen (e.g.

801 peroxides, superoxides, hydroxyl radical and singlet oxygen), induced by

802 environmental stresses, which can cause damage to plant cells.

803

804 **Saline/Salinity:** High concentrations of NaCl in the soil (or hydroponic) solution.

805

806 **SKOR:** Stelar K<sup>+</sup> outward rectifier, a K<sup>+</sup> channel responsible for loading K<sup>+</sup> into the

807 xylem apoplast from the stelar parenchyma cells.

808

809 **Stele:** The inner part of roots and stems of vascular plants, containing the xylem and

810 phloem, providing the transport system between root and shoot. In roots, this is

811 delimited by the endodermal cell layer.

812

813 **Symplast:** The volume inside the plasma membrane of cells, with the symplasm of

814 neighboring cells connected by plasmodesmata. It allows the direct flow of water and

815 small molecules (such as Cl<sup>-</sup>) from the cytoplasm of one cell to another. In the root,

816 symplastic flow refers to cell-to-cell movement through the plasmodesmata

817 connecting cells of the epidermis, cortex and endodermis. Water and solutes moving

818 by this path eventually reach the stele, for long-distance transport to the shoot.

819

820 **Symport(er):** A type of carrier transporter that is able to transport different solutes

821 across a membrane in the same direction at the same time. It is a type of co-



822 transporter, with a similar principle of action to an antiporter, except moving solutes  
823 in the same direction, rather than the opposite direction. Normally, the movement of  
824 one ion is down its difference in electrochemical potential, allowing the other ion to  
825 be moved against its difference in electrochemical potential.

826

827 **Tissue tolerance:** Tolerance of tissue, usually leaf, to accumulated  $\text{Na}^+$  and/or  $\text{Cl}^-$ .  
828 This often refers to the cellular compartmentation of both ions into the vacuole, to  
829 protect the cytoplasm. It also refers to intracellular compartmentation of the both ions  
830 in epidermal cells of leaves to protect mesophyll cells, where most photosynthesis  
831 occurs.

832

833 **Transporters:** Transmembrane proteins that move solutes across a membrane.  
834 There are three types of membrane transporters (and transport classifications):  
835 channels (passive), carriers (secondarily active) and pumps (primary active).  
836 Channels moves solutes across membrane through its pore by selective diffusion.  
837 Carriers moves solutes across membrane by selective binding and significant  
838 conformational change. Pumps use energy, such as from the hydrolysis of ATP, to  
839 actively move ions such as  $\text{H}^+$  or  $\text{Ca}^{2+}$  across membrane.

840

841 **X-IRAC:** Xylem-inwardly rectifying anion conductance – is low in occurrence and  
842 abundance, but up-regulated by ABA and  $\text{Ca}^{2+}$ .

843

844 **X-QUAC:** Xylem-quickly activating anion conductance – rapid activation and a  
845 transport capacity at physiological relevant membrane potentials sufficient to load all  
846  $\text{Cl}^-$  and  $\text{NO}_3^-$  to the xylem; its activity is significantly inhibited by ABA and cytosolic  
847  $\text{Ca}^{2+}$ .

848

849 **X-SLAC:** Xylem-slowly activating anion conductance – is very low in transport  
850 capacity and has not been measured in maize stelar cells.

851 **Figure legends**

852

853 **Fig 1 Mechanisms contributing to Cl<sup>-</sup> exclusion from the leaf cytosol and thus Cl<sup>-</sup>**  
854 **tolerance.** Circles in green: processes that positively regulate Cl<sup>-</sup> tolerance. Circles in red:  
855 processes that require inhibition to reduce cytosolic Cl<sup>-</sup> load. X, xylem; P, phloem. In the root:  
856 (1) Minimizing net uptake across the root epidermis and cortex by increasing Cl<sup>-</sup> efflux and  
857 decreasing its influx. Cl<sup>-</sup> ordinarily enters root cells through secondarily active uptake whilst  
858 passive influx occurs at high salinities [103]. A salt-induced Cl<sup>-</sup> efflux may exist to reduce the  
859 net uptake of Cl<sup>-</sup> during salinity. (2) Maximizing intracellular compartmentation in vacuoles to  
860 reduce cytoplasmic Cl<sup>-</sup>. This occurs in many cell types of plants; the root cortex may be a  
861 major location. Evidence has shown that the sequestration of Cl<sup>-</sup> into root vacuoles can  
862 affect root-to-shoot long distance transport of Cl<sup>-</sup>. (3) Minimizing net xylem loading (focus of  
863 the current review). This appears to be a major rate-limiting step for Cl<sup>-</sup> exclusion from the  
864 shoot, and includes a reduction of passive loading and an increase of active retrieval. In the  
865 shoot: (4) Compartmentalizing Cl<sup>-</sup> within leaf epidermis. This is to protect more important  
866 mesophyll cells, where photosynthesis occurs. (5) Maximizing phloem translocation from the  
867 newly expanded leaves to older leaves. Young leaves tend to be more sensitive to salt  
868 damage. Translocation of Cl<sup>-</sup> from older leaves to younger ones could maintain/improve  
869 growth under salt stress. (6) Salt glands and bladders in halophytes. These are structurally  
870 specialized cells that can store or excrete Cl<sup>-</sup> out onto the leaf surface. The excretion can be  
871 significant, but is unique to halophytes.

872

873 **Fig 2 Thermodynamics of Cl<sup>-</sup> transport between the xylem and surrounding cells.**  
874 Likely differences in Cl<sup>-</sup> activity, pH and membrane potential between the two compartments  
875 are indicated. Inside the cells of the root **symplast**, there is a higher concentration of Cl<sup>-</sup> and  
876 a more negative membrane potential – both these favor the passive movement of Cl<sup>-</sup> out of  
877 the cells into the xylem **apoplast**, down a difference in electrochemical potential. Cl<sup>-</sup> retrieval  
878 can occur via coupling with the transport of H<sup>+</sup>. This uses the difference in electrochemical  
879 potential for H<sup>+</sup> built up mainly by the H<sup>+</sup>-ATPase, therefore this expends energy. Under salt  
880 stress. down-regulation of passive Cl<sup>-</sup> loading and maximizing of retrieval are the processes  
881 that underpin the rate-limiting gatekeeper step in loading of Cl<sup>-</sup> to the xylem (adapted from  
882 Fig 3b in [21]).

883

884 **Fig 3 A model showing known and predicted Cl<sup>-</sup> transporters affecting xylem Cl<sup>-</sup>**  
885 **transport and Cl<sup>-</sup> tolerance.** The **symplastic** pathway for Cl<sup>-</sup> in the root is highlighted in  
886 grey while the **apoplastic** pathway is highlighted in blue (dark blue in epidermis and cortex;  
887 light blue in stele). Candidates down-regulated by salt stress are highlighted in red, whereas  
888 those that are up-regulated are highlighted in green. This regulation can be transcriptional  
889 and/or posttranslational. Discussion and references for each candidate can be found in the  
890 manuscript. Also included are anion transporters that affect Cl<sup>-</sup> exclusion in other cell types.  
891 In the shoot: AtCLCa [76, 92], AtCLCc [74], AtCLCg [75] and AtALMT9 [18]. In the root:  
892 AtNPF2.4 [14], AtNPF2.5 [49], AtSLAHs [12, 13], AtALMT9 [18], AtCLCc, CCCs [16, 45, 62,  
893 63], AtNPF7.2 [51], AtNPF7.3 [50, 52] and GmSALT3 [15] and AtALMT12. Active Cl<sup>-</sup> influx:  
894 Cl<sup>-</sup> influx of root epidermal cells mediated by Cl<sup>-</sup>/2H<sup>+</sup> symporters as described in [44].  
895 Passive Cl<sup>-</sup> influx: passive Cl<sup>-</sup> influx of root epidermal cells when in saline conditions  
896 (membrane potential depolarized by Na<sup>+</sup> entry) as described in [103]. Passive Cl<sup>-</sup> efflux of  
897 root epidermal cells favored by the electrochemical difference as reviewed in [2]. ALMT,  
898 Aluminium Activated Malate Transporter; CLC, Chloride Channel; CCC, Cation-Chloride Co-

899 transporter; SLAH, SLAC1 Homolog; NPF, NRT1/PTR Protein Family; GmSALT3: salt  
900 tolerance-associated protein encoded on chromosome 3 (also referred to as GmCHX1/20).  
901 NAXTs: Nitrate excretion transporters.