Xylem ion loading and its implication for plant abiotic stress tolerance

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ABSTRACT

Plant adaptive potential is critically dependent on efficient communication and coordination of resource allocation and signalling between above and below-ground plant parts. Control of xylem ion loading plays an important role in this process. This review focuses on the molecular identity, tissue-specific expression patterns, and transcriptional and post-translational regulation of transporters mediating xylem loading of Na^+, K^+, and Cl^- in plants grown under abiotic stress conditions such as drought, salinity, and soil flooding. The data is discussed in the context of breeding crops for stress resilience, which remains one of the highest priorities for dealing with the global food security challenge. This resilience was present in wild ancestors, but has been then lost during domestication of crop species and exacerbated by the selection for higher yielding cultivars over the last 100 years. Thus, the progress in the field requires a major rethink of current paradigms in crop breeding and the

targeting of previously unexplored genes and traits. We argue that control of xylem ion loading is one of these traits and represents an unexplored opportunity for genetic improvement of plant germplasms.

I. INTRODUCTION

Abiotic stresses such as drought, salinity, temperature extremes and flooding severely limit crop production worldwide, with estimated losses of over US$120 billion p.a. (FAO, 2005). With the current trends in climate change, these costs will increase. At the same time, global food security requires food production to increase by ~70% by 2050 to match population growth (Tester & Langridge, 2010). This goal is not achievable by simply maintaining current agronomical and breeding practices. There is a growing recognition of the limitations of the first Green Revolution, primarily because the technologies developed did not consider the constraints to production in more marginal environments such as those subjected to drought, salinity or flooding (Pingali, 2012). Tolerance to these stresses was present in wild ancestors, but has been lost during domestication of crop species and exacerbated by the selection for higher yielding cultivars over the last 100 years (Huang et al., 2016). Nonetheless, future agricultural production will inevitably need to utilise marginal land due to increasing urbanisation, global climate trends and the need to match food production to population growth. In their latest concept paper, the Global Plant Council, a peak body comprising a coalition of national and international societies, and affiliates representing plant, crop and agricultural and environmental sciences across the globe, has identified breeding for stress resilience as one of the highest priorities for dealing with the global food security challenge (Davies & Ribaut, 2017). This requires a major rethink of the current paradigms in crop breeding and the targeting of previously unexplored genes and traits. One such trait is the control of xylem ion loading.

II. ESSENTIALITY OF XYLEM ION LOADING FOR ABIOTIC STRESS TOLERANCE

Plant adaptive potential is critically dependent on efficient communication and coordination of resource allocation and signalling between above and below-ground plant parts (Shabala, Bose et al., 2016). This resource allocation becomes especially critical under stress conditions. To maintain cell turgor, so enabling leaf expansion growth under the conditions of reduced soil water potential that are present in saline conditions, plants need to undergo osmotic adjustment. From an energy cost-benefit point of view, this is best achieved by increased delivery of the major inorganic osmolytes (K^+, Na^+, Cl^-) to the shoot. However, because some of these nutrients are cytotoxic, their uptake must be restricted to essential and precisely regulated quantities. In this context, the optimal scenario for a plant would be to quickly send the amount of Na^+ and Cl^- to the shoot that is required to rapidly achieve full osmotic adjustment and maintain the normal growth rate (i.e. no yield penalties) (Shabala, 2013). Once this is achieved, plants need to reduce the rate of xylem Na^+ and Cl^- loading to the absolute minimum required for driving cell turgor in newly growing tissues. Such control can be achieved at the xylem-parenchyma boundary. Not surprisingly, the ability of plants to control xylem ion loading has been repeatedly named as central to salt tolerance (Mäser,

Gierth, & Schroeder, 2002; Berthomieu et al., 2003; Munns & Tester, 2008; Shabala, 2013; Bose, Shabala, et al., 2014; Zhu, Zhou, Shabala, & Shabala, 2017).

Control of xylem ion loading is also essential for plant adaptation to drought, which also reduces the soil water potential. Transcription levels of SKOR (an outward-rectifying K^+ efflux channel in the stele) in roots of the xerophytic *Z. xanthoxylum* plants are several fold higher in plants exposed to osmotic stress (Hu et al., 2016). The reported increase in K^+ delivery to the shoot could contribute to improved drought tolerance via two concurrent mechanisms: directly by contributing to shoot cell sap osmolarity, and indirectly, through regulation of the plant hydraulic conductance. Supporting evidence for the latter mechanism comes from work by Oddo et al. (2011), who showed that increases in xylem sap K^+ in *Laurus nobilis* resulted in a marked increase in plant hydraulic conductance, most likely the result of the interaction of potassium ions with the pectin matrix of intervessel pits.

Equally essential is the control of xylem ion loading in plant adaptive responses to flooding. In aerated roots, the respiration rate in the stele is six-fold higher than in the cortex (Aguilar et al., 2003). The stele is the first to sense oxygen deprivation caused by soil waterlogging or flooding. Hypoxia in the stele inhibits H^+-ATPase activity in the xylem parenchyma, thus diminishes the H^+ gradients, inhibiting secondary energy-dependent ion transport and channel conductances (Colmer & Greenway, 2011). This has major implications for the delivery of essential nutrients to the shoot. Oxygen deficiency in the stele also reduces the concentrations of K^+, Na^+ and Cl^- in the xylem parenchyma cells at the subapical region (Kotula et al., 2015), impeding ion translocation.

Root stellar tissues harbour a large number of membrane transporter proteins that mediate ion loading into the xylem. These are regulated (gated in case of channels) by various signalling molecules and metabolites whose level, in turn, is strongly affected by stress. For example, soil mineral deficiencies result in a substantial accumulation of γ-aminobutyric acid (GABA) in tomato (Sung et al., 2015). GABA is a non-protein amino acid, which modulates anion fluxes across the plasma membrane (Ramesh et al., 2015). A dramatic (several orders of magnitude) increase in the GABA content is one of the most prominent biochemical alterations under flood conditions (Kinnersley & Turano, 2000). In addition, evidence for a role of GABA in sequestering salt load into epidermal bladder cells in a halophyte species (*C. quinoa)* has been recently provided (Kiani-Pouya et al., 2017). Xylem loading of cytokinins and ABA were shown to be involved in the regulation of ion fluxes through xylem parenchyma cells (Lips, 1997), and the levels of both these hormones are altered dramatically under stress conditions. Thus, understanding the molecular identity and control modes of key ion transporters at the xylem-parenchyma interface may offer plant breeders new and previously unexplored opportunities for creating stress-tolerant germplasms.

III. THE MOLECULAR IDENTITY OF THE KEY TRANSPORT SYSTEMS MEDIATING XYLEM ION LOADING

A. Sodium

Despite its critical importance for salinity tolerance, it is still a matter of debate whether the process of Na^+ loading is thermodynamically active or passive (Tester & Davenport, 2003). Most likely, both active and passive transport systems are involved, but their

respective roles may differ depending on the length of time since salinity onset (Shabala, 2013). Hence, a number of candidate genes encoding different transport systems are likely to be involved.

SOS1. Given that the reported membrane potential values for parenchyma cells are in the range of -110 to -140 mV (Wegner et al., 2011) and the xylem Na^+ content typically exceeds 10 mM under saline conditions (Shabala et al., 2010), a thermodynamically active xylem loading mechanism is likely to be required. One of the most likely candidates for active loading is the *SOS1* (salt over sensitive 1)-encoded Na^+/H^+ antiporter present at the xylem/parenchyma interface. SOS1 belongs to the cation proton antiporter (CPA) subfamily of proteins and consists of 10 to 12 transmembrane domains with a 700 amino acid-long tail, which faces the cytoplasm (reviewed by Zhu, 2003).

Bacterial Na^+/H^+ antiporters; NhaA (from *Escherichia coli*) and NhaP1 (from *Methanococcus jannaschii*) are dimers in the membrane containing 12 and 13 transmembrane helices respectively (reviewed by Núñez-Ramírez et al., 2012). In plants, the SOS1 protein is a homodimer, which contains a membrane domain similar to that found in other antiporters of the family and an elongated, large and structured cytosolic domain. Both the transmembrane and cytosolic moieties contribute to the dimerization of the antiporter (reviewed by Núñez-Ramírez et al., 2012).

SOS1 mRNA is unstable under normal, unstressed conditions, but its stability is substantially increased under salt stress and other ionic and dehydration stresses (Chung et al., 2008). The driving force for its antiport activity is a H^+ gradient, created by H^+-translocating ATPases. SOS1 also operates at the plasma membrane of epidermal root cells where it expels Na^+ out of the root, back to the rhizosphere. It has also been suggested to operate as a Na^+ sensor (Sánchez-Barrena, Martínez-Ripoll, & Albert, 2013; Shabala, Wu, & Bose, 2015).

In response to salt stress, the activity of SOS1 is controlled via phosphorylation by SOS2 (a member of calcium-induced protein kinase; CIPK24) (Chinnusamy, Schumaker, & Zhu, 2004), with calcium binding proteins, SOS3 (CBL4) or SCaBP8/CBL10, being required for the activity of this kinase (Quan et al. 2007).

HKT. The high-affinity HKT transporters are present in the xylem parenchyma cells and, despite their name (high affinity K^+ transporters), they show high permeability for Na^+ (Rubio, Gassmann, & Schroeder, 1995; Rubio et al., 1999; Schachtman & Schroeder, 1994). Biophysical transport and phylogenetic analysis have classified the HKT family into two subgroups, Class 1 and Class 2 (Platten et al., 2006). Class 2 HKTs generally show co-transport of Na^+-K^+, while Class 1 shows preferential transport of Na^+ over other cations (reviewed by Horie, Hauser, & Schroeder, 2009). HKT1 is related to the bacterial K^+ transporters such as bacterial Trk and fungal Ktr (Corratgé-Faillie et al., 2010), and an evolutionary correlation is proposed between Trk/Ktr/HKT-type K^+ transporters, K^+ channels and Kdp-type K^+ pumps (present in prokaryotic cells) (reviewed by Hamamoto et al., 2015). AtHKT1;1 consists of a single polypeptide with four repeated domains (reviewed by Hamamoto et al., 2015; Shabala, White et al., 2016), and its major role is assigned to Na^+ retrieval from the xylem (Byrt et al., 2007; Davenport et al., 2007; Horie et al., 2009; Mäser et al., 2002; Ren et al., 2005; Sunarpi et al., 2005). Far less is known about the role of Class 2

HKT transporters. From a biophysical point of view, these transporters are highly suited for active xylem Na^+ loading under conditions that favour passive outward K^+ movement across the parenchyma cell plasma membrane (e.g. the depolarised membrane potential that occurs under saline conditions; Wegner et al., 2011). Supporting this notion, strong OsHKT2;1 expression was reported in the stellar root tissues of rice (Jabnoune et al., 2009).

NSCC. Xylem Na^+ loading could also be mediated by a passive mechanism that involves non-selective cation channels (NSCC). NSCC show diverse characteristics in terms of regulation and functional expression (Demidchik & Maathuis, 2007). NSCC do not strongly differentiate between cations, particularly between K^+ and Na^+; K^+/Na^+ permeability ratios range between 0.3 and 3 (see Pottosin & Dobrovinskaya, 2014 for review). The two major known families of NSCC are the cyclic nucleotide gated channels (CNGC; 20 genes in Arabidopsis) and the ionotropic glutamate receptors (GLR; 20 genes in Arabidopsis), some of which show high expression in root tissues (Demidchik, Davenport, & Tester, 2002; Gobert et al., 2006; Lam et al., 1998). The potential importance of CNGC and GLR in generating cytosolic Ca^{2+} signals in response to stress conditions has been proposed (Swarbreck, Colaço, & Davies, 2013) and their engagement in Na^+ transport at the xylem/symplast boundary is also highly plausible.

CNGC. CNGC are considered likely candidates for the voltage-independent NSCC (Demidchik, Davenport, & Tester, 2002; Kaplan, Sherman, & Fromm, 2007). Heterologously expressed CNGCs act as cation transporters (Gobert et al., 2006; Li et al., 2005) and electrophysiological studies have shown that both AtCNGC1 and AtCNGC4 can transport K^+ and Na^+ (Balague et al., 2003; Leng et al., 2002). Furthermore, CNGC10 is suggested to mediate K^+ efflux and Na^+ influx at the root-soil interface (Guo et al., 2008) and based on altered shoot cation (K^+, Ca^{2+} and Mg^{2+}) content in *atcngc10* mutants, it has also been suggested that this GNGC mediates xylem ion loading (Guo et al., 2010). However, direct evidence is needed to validate this.

GLR. GLRs are an important class of NSCC that transport Na^+, K^+ and Ca^{2+} across the plasma membrane (Demidchik, 2014). At least three GLRs (GLR3.2, GLR3.4 or GLR3.3) are expressed in *Arabidopsis* root tissues (Vincill et al., 2013). Although a direct role for GLR-mediated xylem Na^+ loading remains to be shown, such a scenario is plausible (Vincill et al., 2013).

Aquaporins. Aquaporins are abundant in the parenchyma cells of woody angiosperms (Almeida-Rodriguez & Hacke, 2012; Sakr et al., 2003; Secchi, Pagliarani, & Zwieniecki, 2017). Aquaporins are small proteins (21-34 kD), which consist of six membrane-spanning alpha-helices integrated by five loops. Initially, aquaporins were regarded as merely water channels (Maurel et al., 1995), but recently, Byrt et al. (2017) revealed a new activity for one of most highly expressed aquaporins, AtPIP2;1 (plasma membrane intrinsic protein). AtPIP2:1 was found to induce a Na^+ conductance that is inhibited by low external pH and Ca^{2+} when expressed in heterologous oocyte systems. Given that AtPIP2;1 ion conductance

exhibits characteristics typical of NSCCs (reviewed by Wegner, 2017), it was suggested as one of these (Byrt et al., 2017).

CCC. Cation chloride cotransporters (CCC) mediate symport of Cl^-, Na^+ and K^+ (Colmenero-Flores et al., 2007). Although there are uncertainties regarding their plasma membrane location (Chen et al., 2016; Geilfus, 2018; Henderson, Wege, & Gilliham, 2018), their preferential expression at the xylem/symplast boundary of *Arabidopsis* has been shown (Colmenero-Flores et al., 2007). This suggests a role in long-distance ion transport. Functional support for this role came from pharmacological studies by Zhu et al. (2017), who demonstrated a significant reduction in the magnitude of Na^+ efflux from barley root stelar tissue in the presence of bumetanide, a known inhibitor of mammalian CCC. Because Cl^- transport into the xylem is thermodynamically passive (see next section), it may provide a driving force for Na^+ (secondary) active loading into the xylem.

B. Chloride

Xylem Cl^- loading is considered a thermodynamically passive process, the result of a negative plasma membrane potential in the xylem parenchyma cells and outwardly-directed chloride concentration gradients (Li et al., 2017, Teakle & Tyerman, 2010). Two key anion conductances have been identified in xylem parenchyma cells by patch-clamp studies. One is a quickly activating anion conductance (X-QUAC), the dominant conductance for xylem Cl^- loading, and the other, a more rarely observed, slowly activating anion conductance (X-SLAC) (Gilliham & Tester, 2005; Köhler & Raschke, 2000). More recently, genes encoding the candidate anion channels previously identified by the patch-clamp studies have been discovered. These include the NPF2.4 protein that belongs to the Nitrate Transporter 1/Peptide Transporter (NRT1/PTR) family and two anion channels, SLAH1 and SLAH3, from the SLAC/SLAH (slow-type) family (Cubero-Font et al., 2016, Li et al., 2016).

The NPF2.4 protein in Arabidopsis was the first plasma membrane protein shown to directly catalyse Cl^- transport into the root xylem (Li et al., 2016, 2017). NPF2.4 overexpression resulted in a 23% increase in shoot Cl^- compared with null segregant lines in seedlings exposed to 75 mM NaCl. When expressed in *Xenopus laevis* oocytes with negative membrane potentials equivalent to those in the stele (-100 mV or lower), AtNPF2.4 generated a pH-independent Cl^- efflux, with a four-times greater permeability for Cl^- than NO_3^-. However, the properties of this transporter (lack of rectification, Na^+ and K^+-dependence of the currents and the greater permeability to Cl^- over NO_3^-) indicate that it does not correspond to any of the anion channels detected in patch-clamp studies on xylem parenchyma cells (Li et al., 2016). Two subsequent studies identified SLAH1 and SLAH3 as key players in xylem Cl^- loading. Both co-localised to the xylem pole pericycle cells adjacent to the xylem vessels (Cubero-Font et al., 2016), with the properties for SLAH3 conforming to the X-SLAC based currents found in xylem parenchyma cells. SLAH1 though, was found to be electrically silent. Nonetheless, co-expression studies showed that SLAH1 interacts with SLAH3 forming SLAH1/SLAH3 heteromers. When expressed in *Xenopus* oocytes, these heteromers elicit macroscopic Cl^- currents that override the kinase and nitrate-dependent activation of SLAH3. Further oocyte experiments revealed that the interaction with SLAH1

alters the electrical properties of SLAH3, enhancing the Cl^- conductance and the Cl^-/NO_3^- selectivity of the SLAH3/SLAH1 heteromers compared with the kinase-activated SLAH3 homomers, which are characterised by a 10-fold greater permeability to NO_3^- compared with Cl^- (Cubero-Font et al., 2016). This indicates that SLAH1 and SLAH3 act in tandem to regulate Cl^- and NO_3^- loading in the xylem. Thus, SLAH1 abundance is a key player that determines the degree of Cl^- exclusion from the shoot by activating and influencing the anion selectivity of SLAH3.

However, we are still lacking the candidate gene(s) encoding the X-QUAC type channel, the dominant conductance for Cl^- loading into the xylem (Gilliham & Tester, 2005, Köhler & Raschke, 2000). Hence, in addition to NPF2.4 and SLAH3/SLAH1, other transport pathways are in place in roots for xylem loading of Cl^-. Transcriptional comparisons have been used in the search for additional candidate transport proteins (reviewed in Li B. et al., 2017). Two members of the NRT1/PTR family, AtNPF7.2 and AtNPF7.3 have been identified and given their electrophysiological feature could, respectively, catalyse xylem Cl^- retrieval and loading. Another candidate is the Aluminium-Activated Malate Transporter AtALM12, which is permeable to Cl^- and expressed in the stele of roots (for details see Li et al., 2017). A further potential contender is the Cation Chloride (Cl) Cotransporters, discussed above.

C. Potassium

K^+ contributes about 50% to the xylem sap ion content (Siebrecht et al., 2003), with concentrations ranging from 3 to 5 mM (Watson, Pitchard, & Malone, 2001; Jiang et al., 2013). Control of K^+ xylem loading is vital for supplying adequate K^+ to the photosynthesising shoot tissue. However, far less is known about xylem loading of K^+ and its regulation compared to knowledge of root uptake and transport processes in guard cells - surface cells are much easier to access than cells located deep in the root.

Because membrane potential values for xylem parenchyma cells reportedly range from -80 mV (De Boer & Volkov, 2003) to -130 mV (Shabala et al., 2010), channel-mediated xylem loading of K^+ is feasible. It was shown in the 1990s that two xylem parenchyma-located channel types contribute to loading, K^+ selective Outward Rectifying Channels (KORC) and Non-selective Outward Rectifying Cation (NORC) channels (Wegner & Raschke, 1994; Wegner & De Boer, 1997). While this early work was on barley, the KORC channel was soon after identified in Arabidopsis as the Shaker-like channel Stellar K^+ Outward-Rectifier, SKOR (Gaymard et al., 1998).

Although SKOR is frequently cited as the main transport system involved in K^+ xylem loading in Arabidopsis, *skor* knockout lines only have a 40% reduction in xylem sap K^+ and a 50% reduction in shoot K^+ (Gaymard et al., 1998). Thus a large proportion of K^+ xylem loading is unaccounted for. In these respects, NORCs were proposed early on as being involved in xylem K^+ loading but their molecular identity is still unknown;.

Also localised to the plasma membrane of xylem parenchyma cells is the Arabidopsis AtHKT1 (Mäser et al., 2002; Berthomieu et al., 2003; Sunarpi et al., 2005). Despite its name (High Affinity Potassium Transport) and being initially proposed as a K^+ transporter (Schachtman, & Schroeder, 1994), it is now apparent that its primary role is in Na^+ transport

(Ren et al., 2005; Sunarpi et al., 2005; Horie et al., 2009). Thus, there is no evidence of a significant direct role in xylem K^+ loading in the literature for any of the HKTs in any plant species, although an indirect effect cannot be ruled out (see below). Also, by virtue of its triple function, the Cation Chloride Co-transporter (CCC) may mediate xylem K^+ loading, using Cl^- efflux as a driving force. It was shown that in rice, OsCCC1 RNAi lines accumulated less K^+ than wildtype plants, indicating a role of CCC in K^+ homeostasis (Kong et al., 2011) So despite a paucity of information in the literature, CCCs could contribute towards that "missing" 50% of K^+ xylem loading in Arabidopsis.

Recently, Han et al. (2016) proposed that the KT/HAK/KUP transporter KUP7 mediates K^+ release into the xylem. Xylem sap K^+ content was reduced in *kup7* loss-of-function mutants. Localisation analysis indicated targeting of KUP7 to the plasma membrane, with high expression in the stellar tissues, and KUP7 mediation of K^+ transport was shown in yeast complementation assays. However, the evidence is not unequivocal because *kup7* lines also had lower root K^+ uptake rates, which could be the cause of the reduced xylem content. Furthermore, as a member of the high affinity KT/HAK/KUP transporter family, KUP7 may rather be involved in K^+ uptake into cells than release. For example, it may function in uptake into xylem parenchyma cells for later release to the xylem? However, some HAK transporters have been shown to mediate K^+ efflux in addition to K^+ uptake under some conditions (Bañuelos et al., 2002; Garciadeblas, Benito, & Rodriguez-Navarro, 2002; Osakabe et al., 2013).

Finally, and very recently, Li et al. (2017) reported that NRT1.5/NPF7.3 from the Nitrate Transporter 1/Peptide Transporter (NRT1/PTR) family, functions as a H^+/K^+ antiporter mediating K^+ release from root parenchyma cells into the xylem. Traditionally, this NRT1/PTR family has been cited as nitrate/peptide transporters, but this work shows that NPF7.3/NRT1.5 is important for NO_3^--dependent K^+ translocation in Arabidopsis (Li H. et al., 2017), possibly in association with SKOR (Drechsler et al., 2015).

Most of our current knowledge regarding xylem loading of K^+ is based on the model dicot Arabidopsis and may not apply to all species. Indeed, the literature suggests different systems of xylem loading of K^+ in monocot species. Rice (*Oryza sativa*) for example, possesses two putative outward Shaker channel subunits (Pilot et al., 2003; Véry et al., 2014), OsK5.1 (or OsSKOR), and OsK5.2 (or OsGORK) (Pilot et al., 2003; Kim et al., 2015). While OsK5.1 is mainly expressed in root vasculature (Kim et al., 2015), like AtSKOR. OsK5.2 is expressed in both roots and shoots (Kim et al., 2015) Similar to AtSKOR, OsK5.2 mediates K^+ translocation into the xylem sap in the root, and like the guard cell-localised K^+ outward rectifying channel AtGORK (Ache et al., 2000; Ivashikina et al., 2001), it is involved in K^+ release from guard cells and stomatal movements (Nguyen et al., 2017). Thus, one channel in rice, AsK5.2, combines the functions of two Arabidopsis homologs, AtSKOR and AtGORK.

It is not yet known whether the expression patterns of OsK5.1 and OsK5.2 are shared by homologous genes in other monocots. In maize, the expression pattern of only one gene from the OsK5.2/OsGORK K^+-selective, outward-rectifying subgroup has been partially characterised (Büchsenschütz et al., 2005). Like OsK5.2, the maize channel, *ZORK*, is expressed in both guard cells and roots, and therefore may have a dual function.

In Arabidopsis, AtAKT1 mediates low-affinity K^+ uptake in roots (Lagarde et al., 1996), while AtHAK5 enables high-affinity uptake (Rubio et al., 2010) and there is no evidence of their expression in stellar tissue. In contrast, strong expression of the rice OsAKT1 (Li et al., 2014), OsHAK1 (Bañuelos et al., 2002) and OsHAK5 (Yang et al., 2014) is detected in the vascular bundles as well as in root epidermal cells. This suggests that they could have a direct or indirect role in K^+ translocation, in addition to their roles in root K^+ uptake. Although these transporters could mediate K^+ accumulation in xylem parenchyma cells to enable efficient K^+ release into the xylem, similar to the Arabidopsis AtKUP7, it has been proposed that they themselves mediate K^+ transfer to the xylem (Nieves-Cordones et al., 2016). Support for this is that some HAK transporters can allow K^+ efflux, as noted above (Bañuelos et al., 2002; Garciadeblas et al., 2002; Osakabe et al., 2013).

IV. STRESS-INDUCED REGULATION OF XYLEM ION LOADING AND ITS IMPLICATIONS

A. Sodium

Transcriptional changes. In response to salt stress, both *SOS1* and *HKT* transcript levels are regulated (Tounsi et al., 2017), a process far better optimised in salt-tolerant halophyte species. For example, the halophyte *Salicornia dolichostachya* substantially increases its expression of *SdSOS1* in the root under salinity compared with the taxonomically related glycophyte *Spinacia oleracea* (Katschnig et al., 2015). This process was accompanied by the down-regulation of xylem Na^+ unloading by HKT1;1, leading to increased salt accumulation in the shoot. It should be kept in mind that, when accumulated in shoots, Na^+ can serve as a 'cheap' osmoticum for energetically efficient osmotic adjustment, and this rapid elevation of xylem Na^+ loading may only be transient (Bose, Shabala, et al., 2014; Shabala, 2013).

After completion of osmotic adjustment, the maintenance of non-toxic shoot Na^+ levels and a favourable Na^+/K^+ ratio is crucial for plant survival under prolonged salinity. The expression levels of *PtSOS1* and *PtHKT1;5* in *Puccinellia tenuiflora* (halophytic grass) depend on the capacity of the vacuole to sequester Na^+, mediated by PtNHX1 (Zhang et al., 2017). *PtSOS1* expression is enhanced under mild salinity; a condition under which PtNHX would efficiently sequester Na^+ to the vacuole. Under more severe conditions, when the vacuolar Na^+ capability is exceeded, strong expression of *PtHKT1;5* (mediating xylem Na^+ unloading) is observed (Zhang et al., 2017).

Compared with Class 1 *HKT*s, regulation of *HKT2* expression in the root stele and its role in abiotic stress tolerance are less well understood. When *OsHKT2;1* is heterologously expressed with *OsHKT1* in oocytes, it shows the characteristics of a typical Na^+ transporter (Horie et al., 2009; Horie et al., 2001). A compensatory role of OsHKT2;1 in K^+-starved rice plants was also suggested (Horie et al., 2007). The same upregulation of Class 2 *HKT* genes by K^+ starvation was found in species such as wheat, barley and rice (Garciadeblás et al., 2003; Horie et al., 2001; Wang et al., 1998). Taken together, it can be hypothesised that *HKT2* expression in the root stele mediates loading of Na^+ into the xylem as a substitution for K^+ under saline conditions, when the availability of the latter is limited.

Post-translational regulation and signalling

SOS1 is proposed as the most likely candidate for xylem Na^+ loading, and cytosolic Ca^{2+} plays a crucial role in its activation. An increase in cytosolic Ca^{2+} is referred to as a 'master switch' in a wide range of cell responses, which includes stress responses (Knight, Brandt, & Knight, 1998). The activity of SOS1 is controlled by SOS2 (CIPK24), which is itself activated by the SOS3 protein (CBL4). In brief, CBL4 dimerises upon calcium binding, and interacts with the NAF (asparagine, alanine, phenylalanine) domain of CIPK24. This induces the release of the C-terminal autoinhibition domain of CIPK24 (Quintero et al., 2011). Activated CIPK24 phosphorylates the SOS1 C-terminus, which removes the SOS1 autoinhibitory domain (Quintero et al., 2011), and SOS1 is activated. Although CIPK24 may also affect the activity of HKT1 (Laurie et al., 2002; Rus et al., 2004), its role in Ca^{2+} signalling and control of HKTs is unknown. Most studies proposed that Ca^{2+} signalling occurs as a result of osmotic rather than ionic stress, and that salt/osmotic induced Ca^{2+} elevation is only short-lived (Ca^{2+} is restored within 1-2 minutes) (reviewed by Maathuis, 2014). This phenomenon can support a role for SOS1 in osmotic adjustment by means of Na^+ accumulation.

In addition to Ca^{2+}, changes in cytosolic pH are also reported under stressing environments, including flooding (Felle, 2005) and salinity (D'Onofrio & Lindberg, 2009). This may also induce SOS1 activation (reviewed by Núñez-Ramírez et al., 2012). Indeed, activation of bacterial Na^+/H^+ antiporters can be triggered by changes in pH and in the nonvascular plant *Physcomitrella patens*, SOS1 activity is coupled with cytosolic pH changes (Fraile-Escanciano et al., 2010). Consequently, in higher plants, the mechanism of SOS1 regulation could be a concerted activation by pH and by SOS2-SOS3 (reviewed by Núñez-Ramírez et al., 2012).

While abscisic acid (ABA) plays a key role in signalling under abiotic stress conditions (e.g. drought and salinity), its accumulation also leads to changes in H^+ flux into the xylem. Stimulation by ABA of H^+ extrusion into the xylem, followed by a pH decrease was observed in onion roots (Clarkson & Hanson, 1986). In addition, increases in net H^+ uptake into xylem parenchyma cells (H^+ efflux from the xylem) by direct application of ABA were observed in pea and quinoa (Sun et al., 2017). Although the mechanism of ABA-induced H^+ flux into the xylem is still unknown, this process could be essential for fuelling the activity of SOS1 at the xylem/symplast boundary. In addition to activation of SOS1, ABA application to the barley root stele induced a (direct or indirect) down-regulation of Na^+ retrieval from the xylem (Zhu et al., 2017), likely due to the repression of HKT1 activity. The transcriptional factor abscisic acid insensitive (ABI4), which is known to down-regulate AtHKT1;1 in Arabidopsis (Shkolnik-Inbar, Adler, & Bar-Zvi, 2013), could be involved in this process. Taken together, ABA accumulation and pH changes in the xylem could lead to increased xylem Na^+ concentrations.

Stress conditions frequently result in the increased production of reactive oxygen species (ROS) (e.g. H_2O_2, $O_2^{\cdot-}$, $^{\cdot}OH$ and $^1O_2$) (Miller, Shulaev & Mittler, 2008; Mittler, 2002), which are likely to influence xylem Na^+ loading, and in turn, the xylem Na^+ content.

Apoplastic H_2O_2 production, mediated by NADPH oxidases and causally related ROS-induced Ca^{2+} uptake systems in the root stele, operate upstream of xylem Na^+ loading (Zhu et al., 2017). H_2O_2 has been shown to activate Na^+-permeable NSCCs (Demidchik, Shabala, & Davies, 2007; Zepeda-Jazo et al., 2011), which would result in high xylem Na^+ concentrations. Also possible is an increase in SOS1 activity due to a ROS-induced increase in cytosolic Ca^{2+} (Quintero et al., 2011). ROS also contributes to up-regulation of H^+-ATPases (reviewed by Assaha et al., 2017), which would increase the driving force for SOS1. However, the detailed mechanisms of ROS-induced xylem Na^+ loading are unclear.

NSCC-mediated Na^+ currents have been observed in root stelar tissue (Zhao et al., 2007). Given their voltage-dependence, any change in membrane potential, including those induced by abiotic stress, is likely to affect NSCC-mediated xylem Na^+ loading. One of such factors is reduced ATP availability under stress conditions (e.g. hypoxia; Gibbs & Greenway, 2003). Under oxygen deficiency, xylem parenchyma cells are significantly depolarised. Salinity also causes depolarisation of the root stele (Wegner et al., 2011). This stress-induced depolarisation of the plasma membrane at the xylem/symplast boundary would induce passive xylem loading of Na^+ through NORC or other voltage-dependent NSCC.

Cyclic nucleotides are second messengers in plant adaptive response to the environment and their concentration in the cell increases within seconds after salinity and osmotic stress onset (reviewed by Julkowska & Testerink, 2015; Shabala et al., 2015). Cyclic guanosine monophosphate (cGMP) is one such cyclic nucleotide and its concentration rapidly increases under salinity (reviewed by Donaldson et al., 2004). Cyclic nucleotide-gated channels (CNGCs) are ligand-gated cation channels, which can be activated by direct binding with cyclic nucleotides (Chin, Moeder, & Yoshioka, 2009). Representing a sub-class of NSCC, they potentially modulate xylem Na^+ loading when expressed in the stellar tissue. In addition to CNGCs, cGMP activated-aquaporins could also mediate Na^+ transport into the xylem. Mammalian Aquaporin-1 (AQP1) is activated by intracellular cGMP, and the phosphorylation of tyrosine 253 located at the carboxyl terminal domain, was found to govern the responsiveness of the AQP1 ionic conductance to cGMP (Campbell, Birdsell, & Yool, 2012). Water permeability through AtPIP2;1 is also regulated by phosphorylation (Prado et al., 2013), but the regulation of its ionic conductance (including Na^+) is not yet clear. Due to similar cyclic nucleotide signalling cascades present in animal and plant cells (Isner, Nühse, & Maathuis, 2012; Maurel et al., 1995), the mechanism known to control ion and water transport in mammalian AQP1 (including phosphorylation) should also be explored in AtPIP2;1 (reviewed by Kourghi et al., 2018). In plants, the level of cGMP is increased by ABA perception (Isner et al., 2012), and ABA modulates aquaporin conductance in response to water stress (Parent et al., 2009). Thus, it is plausible that plant aquaporins (e.g. PIP2;1) mediate xylem Na^+ loading in response to osmotic stress caused by drought or salinity.

[Insert Figure 1 here]

B. Chloride

Transcriptional changes. Chloride is the predominant anion in saline soils. Elevated Cl^- concentrations are metabolically toxic to plants and associated with crop yield reduction in salinised plants (Li et al., 2017; White & Broadley, 2001). Therefore, the dominant view on Cl^- regulation in non-halophytes is that the rate of Cl^- transport and exclusion from shoots correlates with salt tolerance. The key gatekeepers in controlling this process are the transporters within the root stelar cells, which are responsible for xylem loading of Cl^- (Li et al., 2017). Consistent with this, the expression of known Cl^- transport systems (e.g., NPF2.4, SLAH1) has been found to decrease after exposure to salt stress. In Arabidopsis for instance, expression profiling by qRT-PCR showed that a five days treatment with 75 mM NaCl reduced the root abundance of NPF2.4 mRNA by almost 90% compared to plants grown at 2 mM Cl^- (Li et al., 2016).

Salt stress was also found to decrease SLAH1 expression in roots in a dose dependent manner, underpinning the view that SLAH1 fine-tunes the degree of Cl^- exclusion from the transpirational stream via the SLAH3 pathway. In contrast, SLAH3 transcript abundance was affected in the short (24 h) but not in the long-term (Cubero-Font et al., 2016, Qiu et al., 2016). This transcriptional regulation of SLAH1 might therefore represent a molecular valve to ensure NO_3^- loading while limiting Cl^- entry into the xylem during salinity stress.

Very little is known about transcriptional regulation of Cl^- transporters in plants following drought stress. As for saline plants, drought stress significantly reduces SLAH1 and SHLA3 transcript abundance in Arabidopsis (Cubero-Font et al., 2016). Given that ABA is involved in both salt and drought stress responses, this similar regulation suggests that in both stresses, ABA acts in the stele and transcriptionally regulates Cl^- transport into the xylem (Cubero-Font et al., 2016, Gilliham & Tester, 2005).

Post-translational regulation and signalling. Anion channels and transporters play a prominent role in a wide spectrum of physiological functions in plants and are under tight post-translational regulation (Barbier-Brygoo et al., 2011, Roberts, 2006). Classic examples are the ABA-mediated regulation of SLAC1 and SLAH3 in guard cells (Hedrich & Geiger, 2017) and the CIPK23-dependent phosphorylation of the NO_3^- transporter NRT1.1, which fine-tunes NO_3^- uptake at the root plasma membrane (Jacquot et al., 2017; Liu & Tsay, 2003). Although our knowledge of the post-translational modifications and the signalling pathways involved in the regulation of these key anion channels in response to environmental stimuli is fairly advanced, the translation of that knowledge into transport regulation in xylem parenchyma cells may prove difficult. Indeed, given their differential role in a wide range of physiological processes throughout the plant, similar anion channel homologs appear to have pronounced differences in their mode of regulation by cytosolic messengers in different cells (e.g., Colcombet et al., 2005; Frachisse et al., 2008). For example, while ABA activates Cl^- efflux through OST1-activated SLAC1 in guard cells (Hedrich & Geiger, 2017; Lind et al., 2015), ABA inhibits Cl^- efflux to the xylem apoplast in xylem parenchyma cells via an ABA-stimulated rise in cytosolic Ca^{2+} (Gilliham & Tester, 2005). This raises the question as to whether other regulatory proteins could act as intermediaries and alter channel regulation by cytosolic messengers, thus explaining this differential regulation in different cells. Possible candidates are aquaporins, whose intermediary role in ABA- and pathogen-triggered stomatal

closure has recently been uncovered in Arabidopsis. Epidermal assays showed that AtPIP2;1 is required for ABA-dependent ROS signalling in guard cells and ABA-triggered stomatal closure (Rodrigues et al., 2017). This, coupled with the fact that AtPIP2;1 was found to interact with OST1–SLAC1 and CPK6/23–SLAC1 complexes and indirectly regulated SLAC1 activity in Arabidopsis guard cells (Wang et al., 2016), invites speculation that aquaporins could play a similar intermediary role in the ABA mediated regulation of Cl^- transport in xylem parenchyma cells

Other candidate cellular signals that deserve further attention are GABA and ATP, which are known to post-translationally affect anion channel activity (e.g. ALMTs), in different parts of the plant (De Angelis et al., 2016; Ramesh et al., 2015). For example, GABA is thought to alter stomatal movement by blocking ALMTs (Mekonnen et al., 2016). Combined with the evidence that both GABA and ATP are key players in plant responses to various stresses including drought and salinity (Bown & Shelp, 2016; De Angelis et al., 2016; Kiani-Pouya et al., 2017, Li et al., 2017, Ramesh et al., 2015), it further supports the importance of investigating their effects on xylem loading.

[Insert Figure 2 here]

C. Potassium

Transcriptional changes. Given that the molecular identity of NORC channels is as yet unknown, most of the knowledge in the field comes from studies on Arabidopsis SKOR. Decreased SKOR transcript accumulation has been reported in response to K^+ deprivation, auxin and cytokinin treatments (Pilot et al., 2003) and importantly with regards to drought, ABA treatment (Gaymard et al., 1998; Pilot et al., 2003). This suggests that under a limited water supply, K^+ translocation to shoots is restricted to retain K^+ in the roots for osmotic adjustment (Sharp, Hsiao, & Silk, 1990; Pritchard, Jones, & Tomos, 1991) and maintenance of root turgor (Ahmad & Maathuis, 2014).

In contrast to drought, salinity increases SKOR transcript levels (Maathuis, 2006), but not systematically (Pilot et al., 2003). The resulting increase in SKOR activity could be crucial in maintaining and possibly restoring shoot K^+ levels under saline conditions, this function being vital in a plant's survival response to salt stress. Importantly, because SKOR is strongly K^+ selective, up-regulation would not increase Na^+ translocation to the shoot as it would if NORC expression/activity were increased - this channel shows equal selectivity for K^+ and Na^+. ABA which decreases SKOR transcript levels is probably not involved in the up-regulation of SKOR during salinity stress. ROS levels, which are known to increase under saline conditions (Schachtman & Shin, 2007; Jacoby, Taylor, & Millar, 2011), are more likely responsible for this upregulation. H_2O_2 is known to activate SKOR at the post-translational level (Garcia-Mata et al., 2010). Whether it also acts at the transcriptional level remains to be tested.

The expression of SKOR, together with that of NRT1.5/NPF7.3, is up-regulated by NO_3^- (Wang et al., 2004) (Lin et al., 2008). This suggests that K^+ and NO_3^- transport into the xylem vessels may be co-ordinated via transcriptional regulation of several types of K^+ transporters (Li H. et al., 2017). Indeed, SKOR seems to mediate root-to-shoot translocation of K^+ under high NO_3^- and low K^+ availability, while NRT1.5/NPF7.3 is more important for K^+ translocation under low NO_3^- availability, irrespective of the K^+ supply (Drechsler et al., 2015). Salinity may affect this balance. Under this stress condition, the chloride component of NaCl frequently reduces root NO_3^- uptake, leading to reduced availability in the plant. The strong induction of SKOR by salt stress may help mitigate this, especially as NRT1.5/NPF7.3 expression is down-regulated when potassium is replaced by sodium (Lin et al., 2008) as frequently occurs under saline conditions.

Very little is known about transcriptional regulation of CCCs. In rice, the expression of the *OsCCC1* gene is significantly enhanced by external KCl treatment (Kong et al., 2011), while in citrus and grapevine, *CCC* genes seem not to be transcriptionally regulated (Sorensson et al., 2012). The only report of transcriptional regulation in KUP7 is that it is not induced by K^+ and that any regulation is at the post-translational/post-transcriptional level (Han et al., 2016).

Post-translational regulation and signalling pathways. Being an outward-rectifying channel, SKOR is activated upon membrane depolarisation. It is also sensitive to the external concentration of K^+, with its open probability decreasing when external K^+ concentration increases. SKOR is therefore in a closed state when the driving force for K^+ is inwardly directed and a far higher membrane potential is then required for its opening. This property minimises the risk of a K^+ influx into the parenchyma cell back from the xylem vessels (Johansson et al., 2006). The mechanism of external K^+ sensing involves interactions between the gating domain present in the sixth transmembrane segment (S6) of the channel polypeptide and the base of the pore helix (Johansson et al., 2006). In addition to this intrinsic regulation by external K^+, SKOR displays post-translational activation by H_2O_2. This process involves a cysteine residue present in the third transmembrane segment (S3) of the channel polypeptide. This H_2O_2 sensitive site is only accessible from the extracellular side of the membrane and when the channel is in an open conformation (Garcia-Mata et al., 2010). In agreement with this sensitivity to H_2O_2, the contribution of SKOR to root-to-shoot K^+ translocation is increased after H_2O_2 treatment (Garcia-Mata et al., 2010).

In addition to transcriptional regulation, ABA also acts post-translationally on SKOR (Gaymard et al., 1998; Roberts, 1998; Roberts & Snowman, 2000). In maize stellar cells, ABA triggers a rapid decrease in outward channel activity, which cannot result from a transcriptional regulation since it occurs within minutes (Roberts, 1998). This inhibition is rather post-translational and may be pH mediated; ABA would induce a decrease in cytosolic pH (Wilkinson & Davies, 2002), leading to inhibition of SKOR.

The SKOR channel indeed exhibits a strong pH-dependence. An internal pH decrease from 7.4 to 7.2 accounts for about 80% of the voltage independent decrease of the macroscopic SKOR current, and an external acidification from pH 7.4 to pH 6.4 has similar effects (Lacombe et al., 2000). Because the macroscopic gating parameters and the single

channel conductance remain unchanged, it was proposed that internal and external pH adjustments regulate K^+ secretion to the xylem by altering the number of channels available for activation (Lacombe et al., 2000). Keeping in mind that stress-induced changes in the xylem pH are well reported (Bahrun et al., 2002; Wang, Liu, & Jemsen, 2012), these findings suggest a causal link between xylem K^+ loading and plant adaptive responses to environmental stresses such as drought and salinity.

A number of regulatory partners have been identified that control the targeting to the plasma membrane, or the activity of plant Shaker channels (to which SKOR belongs). These include β-subunits (Zhang, Ma, & Berkowitz, 1999), 14-3-3 proteins (Sottocornola et al., 2006), different types of protein kinases (Xu et al., 2006; Zhao et al., 2013), phosphatases (Chérel et al., 2002; Lee et al., 2007), and SNAREs (Sutter et al., 2006; Honsbein et al., 2009). However, compared to other, more conveniently-detectable Shaker channels, a few of such regulatory partners have been investigated for SKOR. Yet, it has been reported that the availability of phosphatidylinositol phosphates (PIPs) in the cell membrane is essential to the function of SKOR (Liu, Li, & Luan, 2005), indicating that PIPs are essential regulators for the voltage-dependent and independent activation of plant Shaker-type channels. This study also showed that PIPs are required for SKOR activation by voltage, suggesting that PIPs maintain the channel conformation in a 'ready state'.

The regulation of K^+ transport proteins by calcineurin B-like (CBL)-interacting protein kinases (CIPK) and their regulatory CBL partners has been characterized for a number of Shaker channels in Arabidopsis. Arabidopsis has 26 serine/threonine CIPKs and 10 CBLs, which form a signalling network that is involved in plant responses to environmental cues, including salt stress and K^+deficiency (Luan, 2009; Wang & Wu, 2013). Binding of Ca^{2+} to CBLs is required for CIPK-CBL interaction (Halfter, Ishitani, & Zhu, 2000; Shi et al., 2002). The CIPK23 protein, which is activated by CBL1 or CBL9, and subsequently phosphorylates the AKT1 channel is well described (Xu et al., 2006; Lee et al., 2007). Similarly, the SOS3-SOS2-SOS1 pathway is well known (Halfter et al., 2000; Shi et al., 2002; Gong et al., 2004). Thus far, a similar regulatory pathway has not been found or even investigated for SKOR. Nonetheless, the ankyrin domain which serves as a CIPK interaction site in Shaker channels (Lee et al., 2007) is present in SKOR and therefore this type of regulation probably exists.

NORC channels show strong voltage dependence, are open at zero potential and are controlled by external and cytosolic Ca^{2+}, not by external salt concentration (Pottosin & Dobrovinskaya, 2014; Shabala, Cuin, & Pottosin, 2007). Due to their sensitivity to intracellular Ca^{2+}, it has been proposed that NORC can mediate massive salt loading to the xylem under any stress condition that invokes an increase in cytosolic Ca^{2+}. One such stress is salinity. Unfortunately, NORC is equally selective for K^+ and Na^+ (Wegner & Raschke, 1994), and the far higher Na^+ soil concentration under salinized conditions could result in large amounts of toxic Na^+ reaching the shoots. However, the potentially low K^+ content in the xylem could activate SKOR-mediated K^+ efflux and SKOR is known to be up-regulated by salinity (Maathuis, 2006). In addition, SKOR is activated by H_2O_2, as discussed above and ROS levels increase under salinity (Schachtman & Shin, 2007; Jacoby et al., 2011). Thus, this putative dual regulation of SKOR and NORC activity has implications for salinity

tolerance mechanisms, increasing the K^+/Na^+ selectivity of xylem loading, thereby improving the K^+ status, and as a consequence, the salinity tolerance of the plant.

An indirect effect of HKT1 on SKOR/NORC activity could also be present. Sodium absorption into xylem parenchyma cells via HKT transporters would depolarise xylem parenchyma cells. This could activate the depolarisation-activated SKOR and NORC, thus mediating K^+ efflux (Schroeder, Raschke, & Neher, 1987; Wegner & Raschke, 1994; Wegner & De Boer, 1997). Such a hypothesis corresponds with the frequently observed reduction in K^+ accumulation in the xylem and leaves of *athkt1.1* mutant plants alongside increases in the root K^+ content (Ren et al., 2005; Sunarpi et al., 2005), even though HKT1 is not a K^+ transporter.

[Insert Figure 3 here]

V. IMPLICATIONS FOR PLANT BREEDING

As evident from the analysis above, control of xylem ion loading is essential for plant adaptive responses to abiotic stresses, including drought, salinity and flooding. Surprisingly, our search on the Web of Science has failed to reveal any papers reporting QTLs associated to any of above xylem parenchyma cell-based transporters mediating xylem Na^+, Cl^- and K^+ loading. Several reasons may explain this absence. First, plant xylem is not easily accessible, and the physiological markers that can be used to characterise the activity of these transporters is rather complicated and unsuited for the high throughput assays required for screening doubled haploid (DH) lines. Second, the molecular identity of NSCC remains an enigma, which complicates any gene editing, and makes the direct proof of concept highly challenging. Finally, the kinetics of xylem ion loading is a highly dynamic process, hence the candidate genes need to be targeted at specific time points.

More optimistically, because the xylem loading trait has never been targeted in any breeding programmes, it is an unexplored opportunity for genetic improvement of plant germplasm. In most staple crops, the root stele can be isolated relatively simply by mechanical means. This enables electrophysiological studies and, specifically, allows measurement of the kinetics of ion transport in xylem parenchyma cells by non-invasive vibrating MIFE ion-selective microelectrodes (Shabala et al., 2010; Sun et al., 2017; Zhu et al., 2017). When applied to DH lines, this approach is likely to reveal the appropriate QTLs conferred by one or several transporters that mediate the process of xylem loading. More excitingly, key xylem-specific regulators of these transporters may be uncovered and/or validated. Our previous studies have revealed a large extent of variability in the xylem ion content between contrasting barley (Shabala et al., 2010; Zhu et al., 2017) and wheat (Zhu et al., 2016) varieties. This data can be used to create appropriate DH lines between contrasting parental varieties.

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Figure legends

Fig 1. Membrane transporters mediating xylem Na^+ loading under abiotic stress condition and signalling pathways involved in their control. HKT, High Affinity Potassium Transporter; SOS, Salt Overly Sensitive; NORC, non-selective outward-rectifying channel; PIP, plasma membrane intrinsic protein; NSCC, non-selective cation channel; CCC, chloride-cation cotransporter.

Fig 2. Membrane transporters mediating xylem Cl^- loading under abiotic stress condition and signalling pathways involved in their control. NPF, Nitrate /Peptide Family transporter; ALM, Aluminium-Activated Malate Transporter; SLAH, Slow Anion efflux channel; CCC, chloride-cation cotransporter. CCC, chloride-cation cotransporter

Fig 3. Membrane transporters mediating xylem K^+ loading under abiotic stress condition and signalling pathways involved in their control. SKOR, Shaker-like outward rectifying K^+ channel; NORC, non-selective outward-rectifying channel; HKT, High Affinity Potassium Transporter; CCC, chloride-cation cotransporter.

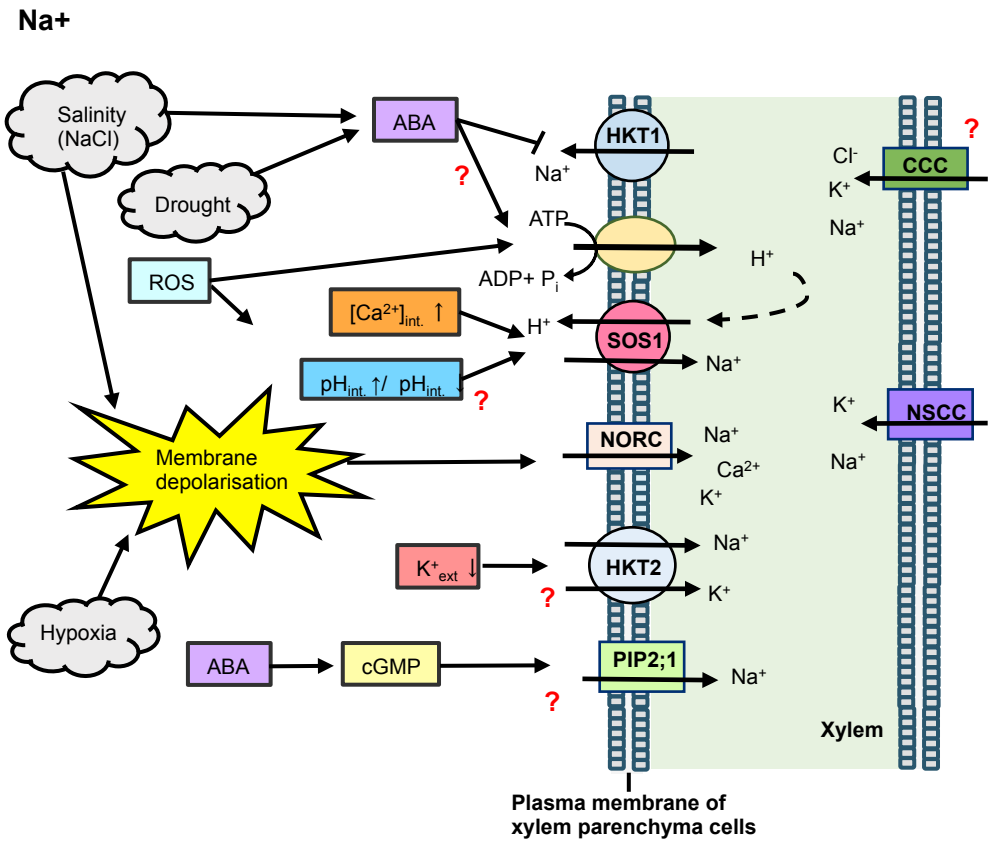

Figure 1

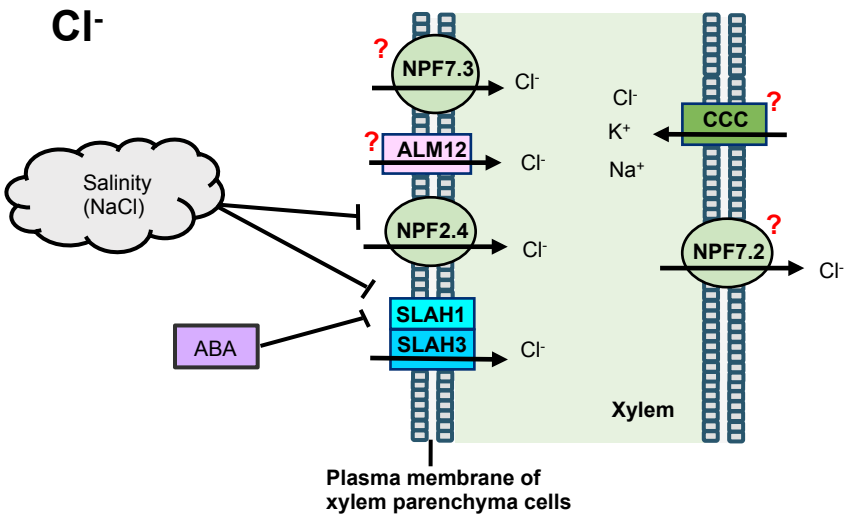

Figure 2

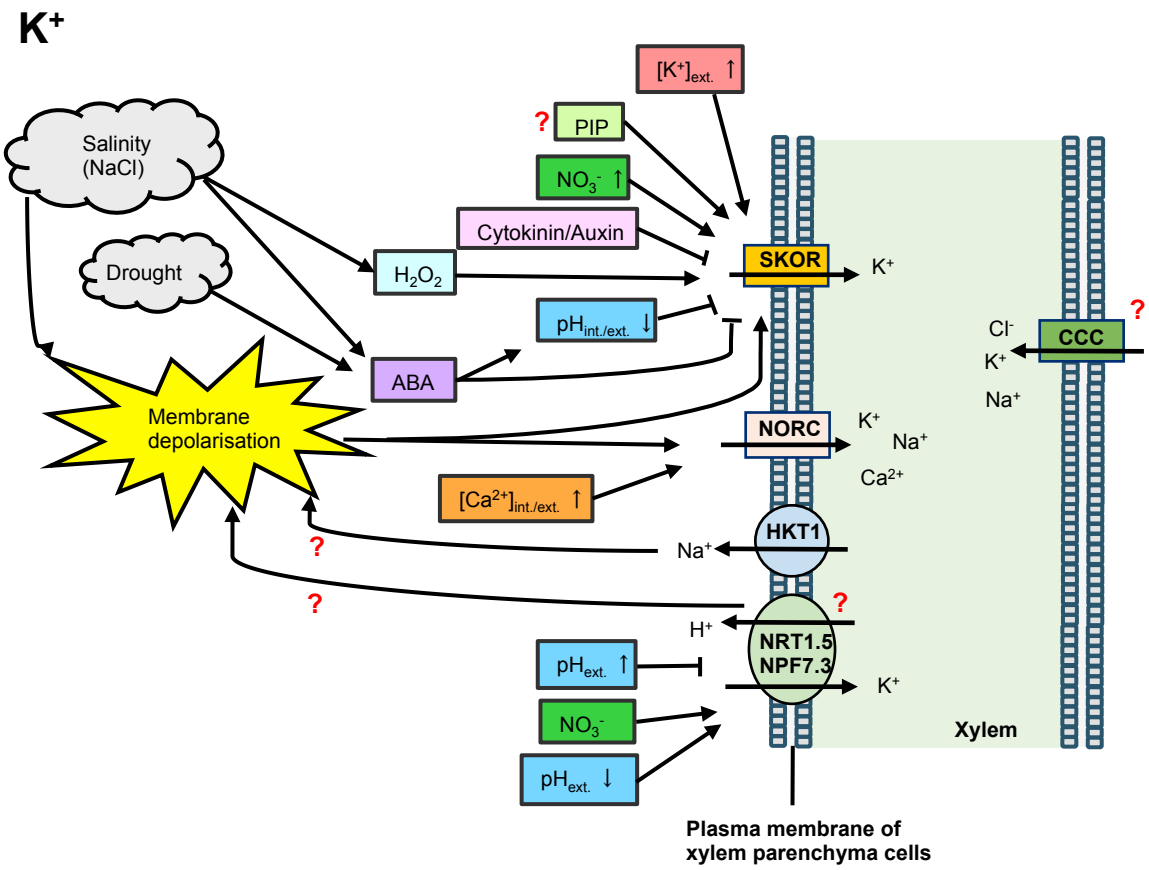
K+
Salinity (NaCl)
Drought
Membrane depolarisation
[K+]ext. ↑
?
PIP
NO3- ↑
Cytokinin/Auxin
H2O2
SKOR
K+
pHint./ext. ↓
ABA
Cl-
K+
Na+
CCC
?
NORC
K+
Na+
Ca2+
[Ca2+]int./ext. ↑
HKT1
Na+
?
?
H+
NRT1.5
NPF7.3
?
pHext. ↑
K+
NO3-
pHext. ↓
Xylem
Plasma membrane of xylem parenchyma cells

Figure 3