Salicylic acid in plant salinity stress signalling and tolerance

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Abstract

Soil salinity is one of the major environmental stresses affecting crop production worldwide, costing over $27Bln per year in lost opportunities to agricultural sector and making improved salinity tolerance of crops a critical step for sustainable food production. Salicylic acid (SA) is a signalling molecule known to participate in defence responses against variety of environmental stresses including salinity. However, the specific knowledge on how SA signalling propagates and promotes salt tolerance in plants remains largely unknown. This review focuses on the role of SA in regulation of ion transport processes during salt stress. In doing this, we briefly summarise a current knowledge on SA biosynthesis and metabolism, and then discuss molecular and physiological mechanisms mediating SA intracellular and long distance transport. We then discuss mechanisms of SA sensing and interaction with other plant hormones and signalling molecules such as ROS, and how this signalling affects activity of sodium and potassium transporters during salt stress. We argue that NPR1-mediated SA signalling is pivotal for (i) controlling Na^+ entry into roots and the subsequent long-distance transport into shoots, (ii) enhancing H^+-ATPase activity in roots, (iii) preventing stress-induced K^+ leakage from roots via depolarisation-activated potassium outward-rectifying channel (KOR) and ROS-activated non-selective cation channels (NSCC), and (iv) increasing K^+ concentration in shoots during salt stress. Future work should focus on how SA can regulate Na^+ exclusion and sequestration mechanisms in plants.

Key words: sodium; potassium; reactive oxygen species; intracellular ionic homeostasis; stomatal regulation; H^+-ATPase; membrane transporters; voltage gating

Abbreviations used:

ABA - abscisic acid; *aba3-1 - ABA biosynthesis mutant3-1; acd - accelerated cell death; agd2 - aberrant growth and death2; AHG2* -encoding poly (A)-specific ribonuclease; BA2H-benzoic-acid-2-hydroxylase; *cpr - constitutive expresser of PR; dnd - defence no death; eds - enhanced disease susceptibility* 5; GLR - glutamate receptor channels; GORK - Guard cells Outward-Rectifying depolarisation-activated K^+ channel; HKT - high-affinity K^+ transporter; ICS- isochorismate synthase; IPL- isochorismate pyruvate lyase; *isd1 - lesions simulating disease1;* MeSAG- methyl salicylic acid O-β-glucose; MeSA-methyl salicylate; *NahG - naphthalene hydroxylase G;* NPR1-non-expresser of PR proteins 1; NSCC - non-selective cation channels; *nudt7 - nudix hydrolase7;* PAL- ammonia-lyase; SABP2-SA-binding protein

2; SAG- salicylic acid O-β-glucoside; SAGT-SA glycosyltransferase; SAR-systemic acquired resistance; SA-salicylic acid; SGE-salicyloyl glucose ester; *sid2* -SA-induction-deficient; *siz1 - small ubiquitin-like modifier E3 ligase1; snc1 - suppressor of npr1-1 consitutive1;* SOS1 - Salt overly sensitive1; SUMO -small ubiquitin-related modifier

1. Introduction

Agricultural crop production around the world is severely limited by a variety of abiotic (e.g. drought, salinity, acidity, flooding, temperature extremes) and biotic (pathogens) stresses. Salinity is one of the major abiotic stresses, affecting 7% of the world's total land area (Munns 2005) and imposing over $27Bln penalties to agricultural sector every year (Qadir et al 2014). Salinisation of agricultural land is a continuing natural process, which is further exacerbated by secondary salinisation, resulting from poor irrigation and/or drainage practices. It is estimated that every day between 2000 and 4000 ha (Shabala 2013; Qadir et al 2014) of irrigated land in arid and semiarid areas across the globe are degraded by salinity and become unsuitable for crop production. This questions our ability to increase agricultural food production by 70% by 2050 to match the projected population growth to 9.3 billion (Tester and Langridge 2010; Shabala 2013). At the same time, remediation of salt-affected arable lands is very expensive and time consuming process that is hard to implement on a large scale. Thus, introducing salinity-tolerant cultivars through molecular and plant breeding is the most attractive and viable option to meet the ever-growing food demand (Ondrasek et al. 2011), that can be fulfilled if specific signalling events and/or mechanisms mediating salt tolerance are identified in plants.

Plants respond to any stress by initiating a broad range of signal transduction pathways. Among the signalling molecules, salicylic acid (SA; *o*-hydroxybenzoic acid; Fig. 1) has received a particular attention because of its capacity to regulate various aspects of plant responses to biotic and abiotic stresses through extensive signalling cross-talk with other growth substances (Horváth et al. 2007; Asensi-Fabado and Munné-Bosch 2011).

Apart from involvement in biotic stres responses (Vlot et al. 2009), SA has been shown to play a key role in defence responses against different abiotic stresses, including salinity and osmotic stress (Borsani et al. 2001). Impressive volume of studies have demonstrated that exogenous addition of SA can ameliorate toxicity symptoms induced by salinity stress in

many plant species (reviewed in Horváth et al. 2007; Ashraf et al. 2010; Hayat et al. 2010). Moreover, various Arabidopsis mutants with altered SA synthesis and accumulation have been shown to have altered salt tolerance (Borsani et al. 2001; Cao et al. 2009; Asensi-Fabado and Munné-Bosch 2011; Miura et al. 2011; Hao et al. 2012). However, the exact signalling cascade and downstream mechanisms by which SA protects plants during salinity stress remain obscure. To shed light on this issue, the existing literature pertinent to SA biosynthesis, metabolism, transport, receptors and physiological roles in plants is reviewed in the following sections.

2. SA biosynthesis

SA is synthesised via two distinct pathways (Fig. 2): phenylalanine ammonia-lyase pathway and isochorismate pathway. The phenylalanine pathway occurs in the cytoplasm. In this pathway, SA is synthesised from phenylalanine (Fig. 1) after a series of reactions. In the first step, trans-cinnamic acid (Fig. 1) is produced from phenylalanine by the action of phenylalanine ammonia-lyase (PAL). Trans-cinnamic acid is subsequently converted to benzoic acid (Fig. 1) (Horváth et al. 2007; Mustafa et al. 2009). The enzyme benzoic-acid-2-hydroxylase (BA2H) catalyses the final step that is the conversion of benzoic acid to SA. In rice seedlings, salinity increases endogenous SA levels by increasing BA2H activity, suggesting phenylalanine pathway is mediating endogenous SA elevation during salinity stress (Sawada et al. 2006).

The isochorismate pathway takes place in chloroplasts. In this pathway, SA is produced from chorismate (Fig. 1) via isochorismate (Fig. 1) as an intermediate product in a two-step process involving isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) (Verberne et al. 2000; Strawn et al. 2007). The Arabidopsis genome encodes two ICS enzymes, namely, *ICS1* (also known as *SA-INDUCTION DEFICIENT2*) and *ICS2* (At1g18870). The mutants lacking functional *ICS1* are severely compromised in SA accumulation. However, detection of residual SA in an *ics1/ics2* double mutant confirms that ICS pathway is not the only source of SA production in Arabidopsis (Garcion et al. 2008) and suggests that the phenylalanine ammonia-lyase pathway may be responsible for the residual SA in these mutants (Dempsey et al. 2011).

The bulk of pathogen-induced SA is synthesised by *ICS1* in Arabidopsis, *Nicotiana benthamiana* and tomato (Wildermuth et al. 2001; Vlot et al. 2009), inducing local and

systemic acquired resistance (SAR) in these plants (Wildermuth et al. 2001). Moreover, two Arabidopsis mutants, *SA-induction-deficient (sid)2-1*(Nawrath and Metraux 1999) and *enhanced disease susceptibility (eds)16-1/sid2-2* (Dewdney et al. 2001) are defective in SA biosynthesis and SAR, exhibiting enhanced susceptibility to disease. Subsequent analyses showed that *ICS1* enzymes are affected in these mutants (Wildermuth et al. 2001). Interestingly, *sid2-1* is also sensitive to UV light, ozone and salinity (Ogawa et al. 2005; Dempsey et al. 2011), implying SA biosynthesis through isochorismate pathway is crucial for abiotic stress tolerance in plants.

2.1. Transcriptional and post-transcriptional regulation of SA biosynthesis

The SA biosynthesis is controlled at both transcriptional and post-transcriptional levels. At transcriptional level, MYBs (*MY* ELO *B*LAST; MYB 96 and MYB30), WRKYs (WRKY28 and WRKY 46) and WIPK(Wound-Induced Mitogen-Activated Protein Kinase)-activated transcription factors has been shown to positively regulate *ICS* gene encoding isochorismate synthase pathway thereby increasing SA production in plants (Vidhyasekaran 2015). At post-trancriptional level, the RNA-binding proteins (RBP) has been shown to participate in multiple post-transcriptional processes. In particular, an RBP from *A. thaliana,* AtRBP-defense related 1 (AtRBP-DR1) has been shown control *ICS1*-mediated SA biosynthesis, because (1) loss-of-function *AtRBP-DR1* mutant plants accumulated less SA and overexpression lines showed higher SA than wildtype; and (2) mRNA levels of *SID2* were higher in *AtRBP-DR1* overexpressor lines (Qi et al . 2010).

3. Metabolism of SA

Most SA synthesised in plants is either glycosylated and/or methylated in the cells (Fig. 2). The SA O-β-glucoside (SAG; Fig. 1) is the dominant glucosylated conjugate of SA, formed by glucose conjugation at the hydroxyl group of SA, whereas glucose conjugation at the SA carboxyl group produces salicyloyl glucose ester (SGE; Fig. 1) (Dean and Delaney 2008). These glucose conjugation reactions are catalysed by SA glycosyltransferase (SAGT) that is induced by exogenous application of SA or pathogen attack (Lee and Raskin 1998; Song 2006). SAGT has moderately high affinity for SA (K_m = 200 μM) (Dempsey et al. 2011). Arabidopsis encodes two SAGT enzymes: one favourably converts SA into SAG, whereas the other enzyme may catalyse the formation of SGE from SA (Dean and Delaney 2008). The SAGT appears to be located in the cytosol of tobacco plants (Dean et al. 2005). Further,

148 SAG in Arabidopsis is thought to be produced in the cytosol and then actively transported
149 into the vacuole for storage. Several studies suggest that SAG is inactive and must be
150 converted to SA to induce defence responses (Dempsey et al. 2011). Indeed, SAG injection
151 into tobacco leaves induced expression of SA marker gene PR-1, however such expression is
152 preceded by the conversion of SAG into SA by the action of extracellular glycosidases
153 (Hennig et al. 1993). Moreover, a non-hydrolysable chemical analogue of SAG was unable to
154 induce PR-1 expression (Dempsey et al. 2011).

155 SA is metabolised into methyl salicylate (MeSA; Fig. 1) by the activity of salicylic acid
156 carboxyl methyltransferase (SAMT1, K_m = 16 μM) at relatively low SA concentrations *in*
157 *vivo* (Dempsey et al. 2011). Like SAG, MeSA is biologically inactive, and acts as a mobile
158 endogenous signal carrier that triggers induction of SAR upon converting back into SA
159 (Shulaev et al. 1997; Park et al. 2007; Vlot et al. 2008; Vlot et al. 2009; Manosalva et al.
160 2010). The MeSA can be further glucosylated into methyl salicylic acid O-β-glucose
161 (MeSAG) (Song et al. 2008) (Fig. 2). Unlike SAG, the vacuole is not the predominant
162 organelle for the storage of MeSAG, because MeSAG predominantly accumulates inside the
163 cytosol (Fig. 2) (Dean et al. 2003; Dean et al. 2005).

164 The biological role of MeSAG remains unknown. One possible function is to serve as a non-
165 volatile storage form of MeSA (Dean et al. 2003; Dean et al. 2005) that can be released as
166 MeSA during defence responses. Alternatively, formation of MeSAG may protect plant cells
167 from toxicity caused by high concentration of intracellular MeSA formed during a defence
168 response (Fig. 2).

169 A bacterial (*Pseudomonas putida*) salicylate hydroxylase enzyme, *NahG* (*naphthalene*
170 *hydroxylase G*) has been shown to degrade SA into catechol in plants (Gaffney et al. 1993).
171 Indeed, transgenic *NahG* Arabidopsis, tobacco and rice plants (expressing *Pseudomonas*
172 *putida* salicylate hydroxylase) have been used widely to demonstrate the crucial role of SA in
173 plant responses to biotic and abiotic stresses (Yang et al. 2004; Kazemi et al. 2010). During
174 salt stress, the germination of Arabidopsis *NahG* transgenic seeds was shown to be delayed
175 (Rajjou et al. 2006), slightly accelerated (Lee et al. 2010) or completely unaffected by the
176 salinity stress (Borsani et al. 2001). Moreover, *NahG* plants showed enhanced tolerance to
177 salt and oxidative stresses (Borsani et al. 2001; Lee et al. 2010). The decreased NaCl-induced
178 oxidative damage (Borsani et al. 2001; Cao et al. 2009) and antioxidant properties of catechol
179 (Lee et al. 2010) have been suggested as the reasons for enhanced salt tolerance. However, a

recent study reported that shoot growth of Arabidopsis *NahG* is sensitive to salt stress (Miura et al. 2011). Moreover, expression of *NahG* in Arabidopsis mutants with high endogenous SA decreased SA concentration, but the resulting phenotypes showed either a salt-sensitive (Miura et al. 2011) or a salt-tolerant response (Hao et al. 2012). Hence, a role of *NahG* in plants is unclear.

4. SA transport in plants

4.1. Long-distance transport

SA induces systemic acquired resistance (SAR) in plants and must be transported to other part of plant. This transport occurs in the phloem (Yalpani et al. 1991; Molders et al. 1996) and can be detected within minutes after SA application/induction (Ohashi et al. 2004). Among the various forms of SA, only the methylated form (MeSA) has been shown to travel in plant tissue locally as well as systemically after pathogen infections (Seskar et al. 1998). Thus, MeSA was considered to be the long-distance signalling molecule that moves from infected to uninfected leaves via phloem. Interestingly, MeSA also functions as airborne signal, with MeSA released from *Pseudomonas syringae*-infected Arabidopsis expressing *OsSAMT* (gene from rice), and tobacco mosaic virus-infected tobacco inducing defence genes in neighbouring plants (Shulaev et al. 1997). In addition, MeSA is the only form of SA that could pass through the tough cuticular layer by diffusion independently of cuticular pH (Niederl et al. 1998). Since MeSA is biologically inactive, MeSA does not activate any systemic defence response while being transported.

4.2. Intracellular transport

After biosynthesis, SA can be freely transported in and out of the cells, tissues and organs (Kawano et al. 2004). A radio-tracer study in tobacco cell suspension culture found *de novo* stimulation of free SA secretion across the plasma membrane (Chen 1999; Chen et al. 2001). This secretion was mediated by ROS- and Ca^{2+}-dependent (at 200 µM SA) and ROS- and Ca^{2+}-independent (at 20 µM SA) transporters (Chen 1999; Chen et al. 2001). However, the molecular identity of above transporters remains unknown. A volatile form of SA (MeSA) was shown to move between cells by diffusion (Shulaev et al. 1997).

In soybean, SAG transport into the vacuole (Fig. 2) was mediated by a tonoplast ABC transporter-like protein (Dean and Mills 2004), whereas tonoplast H^+-antiporter was involved

in tobacco suspension culture cells (Dean et al. 2005). Transporters mediating movements of SA or SA conjugates between other cell organelles remain unknown (Fig. 2).

5. SA receptors in plants

To induce defence signalling, SA should bind to some specific receptors (Ross et al. 1999; Forouhar et al. 2005). The search for SA receptors has resulted in identification of few SA-binding proteins. Two enzymes controlling the balance between SA and MeSA were suggested to act as SA receptors (Fig. 2): (i) SA methyl transferase 1 (SAMT1) that generates MeSA from SA (Ross et al. 1999), and (ii) SA-binding protein 2 (SABP2) that is essential for both local and systemic acquired resistance (SAR) following tobacco mosaic virus infection (Kumar and Klessig 2003). This can be explained by the fact that SABP2 displays SA-inhibiting methyl salicylate esterase activity to convert biologically inactive MeSA into active SA (Forouhar et al. 2005). Subsequent studies reported that the activity of SAPB2 and SAMT1 was essential for SAR signal perception in distal tissues (Park et al. 2009). Interestingly, a transcriptomic-profiling study comparing wild halophytic tomato and a salt-sensitive tomato cultivar revealed that SABP2 was induced by salinity only in wild tomato, suggesting involvement of SABP2 in the salt tolerance mechanisms (Sun et al. 2010). However, exact SABP2-mediated signalling during salt stress remains unknown.

Another SA receptor, NPR1 (non-expresser of PR proteins 1) emerged as a master regulatory protein of SA-dependent defence responses by being a transcriptional co-activator of *PR*-gene expression (Vlot et al. 2009; Wu et al. 2012). Other studies reported that SA also binds to NPR1 prologues NPR3 and NPR4 (Attaran and He 2012; Fu et al. 2012). At least two forms of NPR1 exist in cells. An oligomeric NPR1 is the oxidised form localised in the cytoplasm when the SA concentration is low (i.e., no infection/stress), but SA accumulation following stress leads to an altered cellular redox status that in turn activates NPR1 by reducing biologically-inactive NPR1 oligomers to active monomers (Dong 2004). SA binding to NPR3 and NPR4 triggered reduction of oligomeric into monomeric NPR1 (Fu et al. 2012). The resulting biologically-active NPR1 monomers are transported into the nucleus, where they interact with specific transcription factors that activate SA-responsive *PR* genes (Dong 2004; Fu et al. 2012). In fact, more than 90 percent of *PR* genes were NPR1-dependent (Blanco et al. 2009). In addition to regulating defence genes downstream of SA, the presence of NPR1 in the nucleus is essential to prevent SA accumulation by inhibiting *ICS1* (Wildermuth et al. 2001; Zhang et al. 2010). This is a crucial step in the SA signalling

termination following successful induction of a defence response (Fig. 2). If SA accumulation is not controlled that would lead to a hypersensitive response to stresses. Indeed, an Arabidopsis *npr1* mutant accumulated excess SA (Zhang et al. 2010) and was defective in all major SA-dependent defence responses (Cao et al. 1994; Delaney et al. 1995).

The role of NPR1 during salt stress is controversial because (1) Arabidopsis *npr1* mutant showed enhanced growth during salt stress (Hao et al. 2012), and (2) NPR1-hyperaccumulating Arabidopsis double mutant (*npr3npr4*) failed to undergo programmed cell death (Attaran and He 2012; Fu et al. 2012), suggesting NPR1-mediated prevention of programmed cell death may be beneficial during salt stress. The above observations suggest that salt tolerance in plants can be controlled by both NPR1-independent and NPR1-dependent mechanisms (Jayakannan et al. 2014).

6. Physiological processes controlled by SA during salt stress

6.1 Seed germination

Germination of Arabidopsis *sid2* mutant defective in *ICS1*-mediated SA biosynthesis was hypersensitive to salt stress (Lee et al. 2010). Reversal of salt-induced germination inhibition was noted when the expression level of *ICS1* was increased (Alonso-Ramirez et al. 2009). The above results suggest SA synthesis and accumulation are vital for seed germination, especially during salt stress. On the other hand, SA alone inhibited seed germination in Arabidopsis (Nishimura et al. 2005; Lee et al. 2010), maize (Guan and Scandalios 1995) and barley (Xie et al. 2007) in a dose-dependent manner. The above discrepancies seem to be attributed to SA concentrations used in the above studies. For example, an inhibitory effect of salt stress on germination of *sid2* mutant was decreased when less than 50 μM SA was supplied exogenously, but inhibition was exaggerated when SA concentration exceeded 100 μM (Lee et al. 2010). Interestingly, a proteomic study involving SA-deficient *NahG* transgenic plants showed that germination of SA-deficient *NahG* plants was severely delayed under high salinity, but exogenous application of SA reversed this delayed germination of *NahG* (Rajjou et al. 2006). However, other studies reported that germination of *NahG* was not affected during salt stress (Borsani et al. 2001; Lee et al. 2010).

Regulation of ROS balance by SA has been suggested as a mechanism by which SA modulates germination during salt stress (Lee et al. 2010). This may be true because SA and H_2O_2 form a “self-amplifying feedback loop” in response to various abiotic and biotic

stresses; H_2O_2 induces accumulation of SA, and SA enhances H_2O_2 concentration (Shirasu et al. 1997; Rao and Davis 1999).

6.2 Plant growth

Effect of exogenous SA on growth is dependent on concentration and plant species. Usually, SA at relatively low concentrations (less than 100 μM) enhanced, and at relatively high concentrations (more than 1 mM) decreased, growth in diverse plant species (Rivas-San Vicente and Plasencia 2011). An alteration in the status of other hormones (Shakirova 2003) and/or photosynthesis, transpiration and stomatal conductance (Stevens et al. 2006) was suggested as a reason for the above effects.

Characterisation of Arabidopsis mutants with altered SA accumulation has provided direct evidence for the involvement of SA in plant growth. The SA-deficient plants [*sid2, enhanced disease susceptibility* 5 (*eds5/sid1)* and *NahG]* had higher biomass than wild type, whereas SA-hyperaccumulating mutants such as *cpr1/5/6* (*constitutive expresser of PR1/5/6*), *acd1/5/6/11* (*accelerated cell death1/5/6/11*), *dnd1/2* (*defence no death1/2*), *isd1* (*lesions simulating disease1*), *nudt7* (*nudix hydrolase7), agd2* (*aberrant growth and death*), *snc1* (*suppressor of npr1-1 consitutive1*) and *siz1* [*SUMO (small ubiquitin-related modifier) E3 ligase1*] showed dwarfism (reviewed in Miura et al. 2011; Rivas-San Vicente and Plasencia 2011). Negative regulation of cell division and cell enlargement by SA has been suggested as a reason for the above growth differences (Xia et al. 2009; Hao et al. 2012).

The growth of mutants with altered SA concentrations did not show any clear pattern during salt stress. Some studies have found that SA-deficient Arabidopsis *NahG* exhibited higher growth compared with the wild type and SA-hyperaccumulating (*snc1*) mutant during salinity stress (Borsani et al. 2001; Cao et al. 2009; Hao et al. 2012). However, in other studies SA-hyperaccumulating mutants, namely *siz1* (*small ubiquitin-like modifier E3 ligase1*) showed enhanced growth (Miura et al. 2011) and *aba3-1* (*ABA biosynthesis mutant3-1*) showed no change in growth (Asensi-Fabado and Munné-Bosch 2011), whereas severe growth reduction was observed in SA-deficient plants (*NahG*, *sid2* and *eds5*) during salt stress (Asensi-Fabado and Munné-Bosch 2011; Miura et al. 2011). Moreover, growth of *NahG siz1* double mutant was retarded (Miura et al. 2011), whereas *NahG snc1* had enhanced growth (Hao et al. 2012) during salt stress. Thus, more research is needed to decipher the exact role of SA in plant growth during salt stress.

304 **6.3 Photosynthesis and transpiration**

305 An effect of exogenous SA on photosynthesis is concentration-dependent (Ashraf et al.
306 2010). At low concentrations (less than 10 µM), SA alleviated a salt-induced decrease in
307 photosynthesis by increasing photosynthetic rate (Stevens et al. 2006; Nazar et al. 2011),
308 carbon fixation, transpiration, stomatal conductance (Stevens et al. 2006; Poór et al. 2011a)
309 and antioxidant activity (Szepesi et al. 2008) in many plant species. The opposite effects were
310 noted at high (1-5 mM) SA concentrations (Nazar et al. 2011). Indeed, millimolar
311 concentrations of SA decreased net photosynthetic rate (Nemeth et al. 2002), hampered
312 synthesis of Rubisco (Pancheva and Popova 1997), decreased chlorophyll concentration
313 (Moharekar et al. 2003), and resulted in an increase in chloroplast volume, swelling of
314 thylakoid grana, and coagulation of stroma (Uzunova and Popova 2000). However,
315 characterisation of Arabidopsis plants with altered endogenous SA concentration did not
316 reveal any clear pattern. In one study, SA-deficient *NahG* showed higher chlorophyll
317 concentration and variable-to-maximum fluorescence ratio (F_v/F_m; indicator of damage to the
318 PSII) in comparison with SA-hyperaccumulating *snc1* (Hao et al. 2012). In another study,
319 there was no significant difference between SA-deficient *(sid2* and *eds5*) and
320 hyperaccumulating (*aba3*) Arabidopsis mutants in chlorophyll concentration and *Fv/Fm* ratio
321 (Asensi-Fabado and Munné-Bosch 2011) during salt stress. Thus, more studies are needed to
322 decipher the exact role of SA in influencing photosynthetic parameters during salt stress.

323 Stomata play a major role in processes involved in maintenance of photosynthetic capacity.
324 In particular, stomatal closure and opening affect the transpiration and photosynthetic
325 capacity, and thus plant adaptation to different stresses. Abscisic acid (ABA) is a
326 phytohormone known to play an important role in stomatal closure and resistance to
327 drought/water deficit. ABA affects stomatal closure through production of ROS species by
328 NADPH oxidase (Acharya and Assmann 2009). SA antagonised the ABA-induced stomatal
329 closure (Rai et al. 1986). However, 0.4 mM SA induced stomatal closure in Arabidopsis
330 within 2 h, decreasing stomatal gas exchange by 4-fold (Mateo et al. 2004; Rivas-San Vicente
331 and Plasencia 2011). Specifically, the *Arabidopsis wrky54wrky70* mutant, known to
332 accumulate high levels of endogenous SA, exhibited tolerance to PEG-induced osmotic
333 stress, which was correlated with improved water retention and enhanced stomatal closure (Li
334 et al. 2013). Moreover, bacteria-induced stomatal closure was not observed in SA-deficient
335 *NahG* transgenic plants and SA-biosynthesis mutant *eds16-2*, indicating the essential role of

SA in stomatal closure (Melotto et al. 2006; Melotto et al. 2008). The SA-induced stomatal closure is also mediated by ROS that are generated in a reaction catalysed by peroxidase instead of NADPH oxidase (Miura et al. 2013; Miura and Tada 2014). Interestingly, Arabidopsis mutant with high endogenous SA concentration (*siz1*) showed decreased stomatal aperture (Miura et al. 2013) and increased salt tolerance (Miura et al. 2011), implying that SA-mediated stomatal closure may be beneficial during salt stress.

6.4 Nutrient acquisition

Exogenous application of SA is well known to ameliorate the effect of salinity. Some studies suggested maintenance of optimum K^+/Na^+ ratio under saline conditions as a reason for enhanced salt tolerance in plants (reviewed in Horváth et al. 2007; Ashraf et al. 2010; Hayat et al. 2010). Usually, exogenous SA minimises Na^+ uptake while increasing tissue concentrations of K^+, Ca^{2+}, Mg^{2+} (Gunes et al. 2005; Yildirim et al. 2008), Fe^{2+}, Mn^{2+}, Cu^{2+} (El-Tayeb 2005; Gunes et al. 2005; Yildirim et al. 2008), P (El-Tayeb 2005; Gunes et al. 2005; Yildirim et al. 2008), N (Gunes et al. 2007; Yildirim et al. 2008; Nazar et al. 2011) and S (Nazar et al. 2011) in many plant species. However, there are some contrary results as well. The exogenous application of SA decreased concentrations of K^+ and P in shoot and root tissues of maize (Gunes et al. 2007) and in barley shoots (El-Tayeb 2005) under salinity stress. Surprisingly, Na^+ and Cl^- concentrations in salinised spinach roots and shoots were not affected by SA (Eraslan et al. 2008). Further, application of SA to tomato plants inhibited K^+ uptake and increased Na^+ uptake (Szepesi et al. 2009). Hence, a role of SA in maintenance of ionic homeostasis under salinity stress is poorly understood.

Most of the results mentioned above are based on prolonged salt exposure (days to months). Hence, the reported effects are likely to be indirect and strongly dependent on doses of SA used, plant species studied, intensity and duration of salt stress (reviewed in Horváth et al. 2007). Moreover, the critical role of SA in modulation of specific ion transporters in roots during salt stress has been overlooked. Hence, relevant information regarding membrane transporters controlling K^+ homeostasis, Na^+ uptake and Na^+ redistribution during salt stress is reviewed in the following sections.

7. SA signalling networks

365 **7. 1 Cross-talks with other plant hormones**

366 SA exerts its role in a variety of plant developmental processes via cross-talk with
367 gibberellins, abscisic acid, jasmonic acid and ethylene (Yasuda et al. 2008; Alonso-Ramirez
368 et al. 2009; Khan et al. 2014). Interestingly, exogenous application of gibberellins (50 μM)
369 under NaCl (150 mM) stress slightly improved germination of SA-deficient *sid2* mutant
370 (Alonso-Ramirez et al. 2009), implying gibberellins can offset SA deficiency. In general, SA
371 is antagonistic to ABA during development of systemic acquired resistance (SAR);
372 exogenous application of ABA hampered the induction of SAR, whereas activation of SAR
373 by SA suppressed ABA signalling (Yasuda et al. 2008). In addition, *AHG2* (encoding poly
374 (A)-specific ribonuclease) controlled ABA sensitivity and promoted expression of SA-
375 inducible genes (Nishimura et al. 2005). On the other hand, SA and ABA play a similar role
376 in stomatal closure, albeit through a different pool of ROS (see above), suggesting the
377 interaction between SA and ABA may be either positive or negative depending on conditions.
378 Recently, the Arabidopsis *siz1* mutant defective in SUMO (small ubiquitin-related modifier)
379 E3 ligase showed ABA sensitivity, high SA accumulation and expression of SA-regulated
380 genes (Lee et al. 2006; Miura et al. 2009). Moreover, high endogenous concentrations of SA
381 in two ABA-sensitive mutants (*aba3* and *siz1*) improved salt tolerance (Asensi-Fabado and
382 Munné-Bosch 2011; Miura et al. 2011), implying that suppression of ABA signalling by SA
383 is critical for salt tolerance.

384 **7.2 Cross-talks with Reactive Oxygen Species**

385 Redox homeostasis in plants is maintained by the appropriate balance between ROS
386 generation and scavenging (Apel and Hirt 2004). In general, low concentrations of SA
387 facilitate tolerance to abiotic stresses, whereas high concentrations induce oxidative stress
388 due to exacerbated generation of ROS species, leading to cell death (Shirasu et al. 1997; Lee
389 et al. 2010; Poór et al. 2011b; Miura and Tada 2014). Similar to SA, H_2O_2 (a ROS species) at
390 low concentrations acts as a signalling molecule, inducing tolerance to several biotic and
391 abiotic stresses, but at high concentrations triggers apoptosis-like and autophagic cell death
392 (Love et al. 2008; Quan et al. 2008). A "self-amplifying feedback loop" concept (Fig. 3) has
393 been proposed to explain the interaction between SA and H_2O_2 during various abiotic and
394 biotic stresses; H_2O_2 induces accumulation of SA, and SA increases H_2O_2 concentration
395 (Shirasu et al. 1997; Harfouche et al. 2008). A H_2O_2-mediated increase in endogenous SA
396 concentration can be explained by the catalytic activity of H_2O_2 on BA2H enzyme involved

in the conversion of benzoic acid to SA (Dempsey and Klessig 1995). An increase in H_2O_2 concentration by SA is mediated via inhibition of catalase and ascorbate peroxidase enzymes through SA binding (Durner and Klessig 1995; Durner and Klessig 1996; Horváth et al. 2002).

All biotic and abiotic stresses are causally associated with increased ROS concentrations. Salt stress increases production of various forms of ROS, namely superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) in plants (reviewed in Parida and Das 2005). The ROS are scavenged by enzymatic and/or non-enzymatic antioxidants to protect plants from prolonged salt stress (Bose et al. 2014). Indeed, salt stress tolerance in diverse plant species was positively correlated with increased efficiency of the antioxidative system (Horváth et al. 2007; Munns and Tester 2008; Ashraf et al. 2010). Exogenous SA application at physiologically relevant concentrations caused moderate stress by generating H_2O_2, which induced the anti-oxidative defence system including enzymatic (superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase) and non-enzymatic antioxidants (glutathione, ascorbic acid, carotenoids and tocopherols) during acclimation to salt stress (Durner and Klessig 1995; Durner and Klessig 1996; Gill and Tuteja 2010).

Interestingly, SA may generate ROS species in the photosynthetic tissues, thereby enhancing oxidative damage under salt stress. Indeed, salt-treated wild type plants showed necrotic lesions in shoot tissues, but these lesions were not observed in salt-treated SA-deficient *NahG* transgenic plants (Borsani et al. 2001; Hao et al. 2012). High ratios of glutathione to oxidised glutathione (GSH/GSSG) and ascorbic acid to dehydroascorbate (ASA/DHA) in *NahG* plants enhanced their antioxidant capacity to mitigate salt-induced oxidative stress (Borsani et al. 2001; Cao et al. 2009; Hao et al. 2012). However, high ratio of GSH/GSSG in rice *NahG* lines did not result in oxidative stress tolerance (Yang et al. 2004; Kusumi et al. 2006), questioning the above notion. Moreover, SA-hyperaccumulating mutants, namely *siz1* (*small ubiquitin-like modifier E3 ligase1*) and *aba3-1* (*ABA biosynthesis mutant3-1*) showed enhanced salt tolerance (Asensi-Fabado and Munné-Bosch 2011; Miura et al. 2011), implying high SA may be essential in preventing salt-induced oxidative stress. A subsequent comparison of two SA hyper accumulating Arabidopsis mutants namely *nudt7* (contains the constitutively expressed SA-mediated NPR1-independent and NPR1-dependent defence genes) and *npr1-5* (formerly known as *sai1*, salicylic acid-insensitive1; without the SA-

429 mediated NPR1-dependent defence response) under salt and oxidative stress revealed that
430 presence of NPR1-mediated SA signalling pathway is essential for salt-induced *in vivo* H_2O_2
431 production as well as salt and oxidative stress tolerance (Jayakannan et al. 2014).

432 8. SA-mediated control of Na^+ uptake and sequestration

433 8.1 Sodium transport across the plasma membrane

434 Several transporters contribute to Na^+ uptake during salt stress. High-affinity potassium
435 transporters (HKT) have been reported in many plant species (Rubio et al. 1995; Gassmann et
436 al. 1996; Garciadeblas et al. 2003; Horie et al. 2006; Munns et al. 2012) and involved in both
437 high-affinity Na^+ uptake (Haro et al 2010) and Na^+ redistribution within the plant (Munns et
438 al 2012). Interestingly, SA pre-treatment in the Arabidopsis wild type (Jayakannan et al.
439 2013) and high endogenous-SA mutant *nudt7* decreased the shoot Na^+ concentration during
440 prolonged salt stress (Jayakannan et al. 2014). Considering that another mutant with high SA
441 content (*npr1-5*) accumulated higher Na^+ in shoot than the wild type and showed
442 hypersensitivity to salt stress, it is clear that the NPR1-dependent SA signalling is critical for
443 salt tolerance by restricting Na^+ into the shoots (Fig. 4) (Jayakannan et al. 2014). However, it
444 remains unclear whether prevention of Na^+ loading into the shoots or enhanced Na^+ removal
445 from xylem responsible for lower Na^+ in shoots.

446 Exogenous SA pre-treatment for 1 h did not cause any significant difference in Na^+ influx
447 during the acute salt stress in Arabidopsis roots (Jayakannan et al. 2013). Among the
448 constitutively high endogenous SA Arabidopsis mutants, *nudt7* recorded lowest Na^+ influx
449 and the NPR1-signalling blockage mutant *npr1-5* recorded the highest Na^+ influx
450 (Jayakannan et al. 2014). The above observations suggest that the exogenous SA require
451 longer than 1 h to act on Na^+ transporters, and the SA action occurs at post-transcriptional
452 level because absence of NPR1 (a transcriptional co-activator of SA genes) has resulted in
453 highest Na^+ influx and salt hypersensitivity (Jayakannan et al. 2014). As aforementioned
454 studies have measured net Na^+ fluxes, it is hard to pinpoint whether SA inhibited Na^+ entry
455 pathways and/or enhanced the activity of Na^+/H^+ exchangers (Fig. 4). Additional experiments
456 are needed to address this issue.

457 Weakly voltage-dependent non-selective cation channels (NSCC) are considered to be the
458 main pathway for Na^+ entry into roots exposed to high NaCl concentrations (Tyerman et al.
459 1997; Amtmann and Sanders 1999; Tyerman and Skerrett 1999; Davenport and Tester 2000;

460 Horie et al. 2001; Tyerman 2002; Tester and Davenport 2003; Horie and Schroeder 2004;
461 Horie et al. 2006). There are two sub groups within the NSCC channels that can mediate Na^+
462 uptake in plants: cyclic-nucleotide-gated channels and glutamate receptor-like channels
463 (GLRs). The latter may be suggested as possible downstream targets of SA. Indeed, salt
464 stress increased the glutamate synthase activity in tomato leaves (Berteli et al. 1995), and
465 exogenous SA modulated glutamate dehydrogenase activity in maize roots (Jain and
466 Srivastava 1981). Thus, it is plausible to suggest that SA can modulate GLRs involved in Na^+
467 entry and redistribution in plants.

468 A low cytosolic Na^+ concentration is maintained by the Na^+/H^+ antiporter (SOS1 -SALT
469 OVERLY SENSITIVE1) that extrudes excess Na^+ from the cytosol (Hasegawa et al. 2000;
470 Sanders 2000; Shi et al. 2000; Zhu 2002, 2003) (Fig. 4). SOS1 promoter activity has been
471 identified in virtually all tissues, but the greatest activity is found in root epidermal cells,
472 particularly at root tips and in the cells bordering the vascular tissue. SOS1 plays three major
473 roles: (i) mediates Na^+ efflux from cytosol to the rhizosphere, (ii) increases the time available
474 for Na^+ storage in the vacuole by slowing down Na^+ accumulation in the cytoplasm, and (iii)
475 controls long-distance Na^+ transport between roots and shoots through Na^+ retrieval (Zhu
476 2003). The inherent stability of SOS1 mRNA was poor, with the half-life of only 10 minutes
477 (Chung et al. 2008). In a SOS1-overexpresser line this stability was increased by H_2O_2 in a
478 rapid (within 30 min) concentration-dependent manner, but not by SA. This is surprising
479 because SA controls H_2O_2 balance via a "self-amplifying feedback loop" in plants (see
480 above; Fig. 3). Hence, more research is needed to decipher role of SA in SOS1 expression
481 and functioning in plants, if any (Fig. 4).

482 A stress-inducible plasma membrane localised PMP3 (PLASMA MEMBRANE PROTEIN 3)
483 has been shown to participate in Na^+ efflux dependent on a Na^+/H^+ exchanger (Fig. 4) or Na^+-
484 ATPase during salt stress (Inada et al. 2005; Mitsuya et al. 2005). The PMP3 homologues
485 have been identified in a few halophyte monocotyledons, rice and Arabidopsis (Inada et al.
486 2005; Mitsuya et al. 2005; Chang-Qing et al. 2008). *In situ* hybridisation study in a halophyte
487 sheep grass (*Aneurolepidium chinense*) has revealed that *AcPMP3* is localised in root cap and
488 root epidermis (Inada et al. 2005). Interestingly, *AcPMP3* expression was up-regulated within
489 15 min of H_2O_2 and 30 min of SA treatments (Inada et al. 2005), implying SA may control
490 AcPMP3 operation during salt stress.

8.2 Sodium transport across the tonoplast

Vacuolar Na^+ sequestration is important for the maintenance of low cytosolic Na^+ concentrations and is considered as a key attribute of salinity tolerance mechanism employed by salt tolerant species, including halophytes (Shabala 2013). This sequestration is mediated by tonoplast Na^+/H^+ exchangers (NHX) (Apse et al. 1999; Gaxiola et al. 1999) that belong to the CPA family of cation/proton antiporters (Apse and Blumwald 2007; Rodríguez-Rosales *et al*. 2008). At least six NHX isoforms have been found in Arabidopsis; with their expression pattern, both tissue- and stress-specific (Rodríguez-Rosales *et al*. 2009). NHX exchangers are constititively expressed in halophytes and and inducible in salt-tolerant glycophyte species (Shabala and Mackay 2011). Overexpression of NHX1 increased salinity tolerance of Arabidopsis (Apse et al. 1999), *Brassica napus* (Zhang et al. 2001), tomato (Zhang and Blumwald 2001) and maize (Zörb et al. 2005). These results confirm that increased capacity for vacuolar Na^+ sequestration is important for salinity tolerance. Interestingly, AtNHX1 and AtNHX2 can mediate K^+ transport along with Na^+/H^+ exchange (Zhang and Blumwald 2001; Apse et al. 2003; Bassil et al. 2011; Barragán et al. 2012). While there is no direct proof of SA regulating NHXs, the NHX1 expression was upregulated by ABA and/or SA treatments in diverse plant species (Wu et al. 2004; Guan et al. 2011). Further, SA interacts with ABA during abiotic stresses (see below). Hence, it may be possible that SA may regulate Na^+ and K^+ vacuolar sequestration through NHXs.

9. Membrane transporters controlling K^+ homeostasis during salt stress

Salinity stress operates through ionic, hyperosmotic and oxidative components that severely hamper cell metabolism. All these components affect ion transport processes, particularly K^+ uptake and retention. Under salt conditions, entry of Na^+ ions causes K^+ leakage, thereby depleting the cytosolic K^+ pool available for metabolic functions, which eventually leads to cell death (Shabala and Cuin 2008; Shabala 2009). Thus, maintenance of K^+ homeostasis has emerged as a fundamental component of salt tolerance mechanism (Maathuis and Amtmann 1999; Shabala and Cuin 2008; Demidchik et al. 2010). Indeed, several studies reported a strong positive correlation between the capacity of roots to retain K^+ and salt tolerance in barley (Chen et al. 2005; Chen et al. 2007a; Chen et al. 2007b), wheat (Cuin et al. 2008), lucerne (Smethurst et al. 2008) and Arabidopsis (Shabala et al. 2005; Shabala et al. 2006; Jayakannan et al. 2011; Bose et al. 2013; Jayakannan et al. 2013). Moreover, divalent cations (Shabala et al. 2003; Shabala et al. 2006), polyamines (Pandolfi et al. 2010) and compatible

523 solutes (Cuin and Shabala 2005; Cuin and Shabala 2007) were able to prevent NaCl-induced
524 K^+ loss and improve salt tolerance. In several plant species, SA ameliorated detrimental
525 effects of salinity (Horváth et al. 2007; Ashraf et al. 2010; Hayat et al. 2010) and increased
526 K^+ concentration in roots (He and Zhu 2008), but it remained unclear whether enhanced K^+
527 uptake or prevention of K^+ loss played a major role in this ameliorative effect. Recent work
528 in our laboratory have proved that prevention of salt-induced K^+ loss through K^+-outward
529 rectifying channel (Fig. 4) plays a major role in SA mediated salt tolerance in plants
530 (Jayakannan et al. 2013).

531 In many species, NaCl-induced K^+ efflux from mesophyll is mediated by depolarisation-
532 activated outward-rectifying K^+ channels (GORK in Arabodopsis)(Shabala and Cuin 2008;
533 Anshutz et al 2014). Interestingly, pre-treating Arabidopsis roots with physiologically
534 relevant concentration of SA (<0.5 mM) has decreased K^+ leak through GORK channel (Fig.
535 4) suggesting prevention of K loss through GORK is the main mode of action for SA during
536 salt stress (Jayakannan et al. 2013). Further, decreased K^+ leak through GORK channel is
537 NPR1 mediated because *npr1-5* mutant unable to decrease K^+ loss through depolarisation-
538 activated KOR channel (Jayakannan et al. 2014).

539 Being a voltage-gated channel, GORK operation is strongly affected by the plasma
540 membrane H^+-ATPase that plays a crucial role in regulating membrane potential (Palmgren
541 and Nissen 2010). The activation of proton pumps by salt stress (Kerkeb et al. 2001) is
542 positively correlated with salinity tolerance, and this effect is stronger in salt-tolerant than
543 salt-sensitive species (Chen et al. 2007b; Sahu and Shaw 2009; Bose et al. 2013; Jayakannan
544 et al. 2013). Such an increase in H^+ pumping could act in two parallel pathways. First,
545 enhanced activity of H^+-ATPase would down-regulate depolarisation-activated outward-
546 rectifying K^+ channels, thus preventing K^+ leakage via KOR channels (Chen et al. 2007b).
547 Indeed, the SA pre-treatment under salinity conditions enhanced the H^+-ATPase activity in a
548 dose- and time-dependent manner (Fig. 4), helping plants to maintain membrane potential at
549 more negative values thereby decreasing NaCl-induced K^+ leakage via depolarization-
550 activated KOR channels in Arabidopsis (Jayakannan et al. 2013). Interestingly, the above SA
551 effects were absent in *npr1-5* mutant but present in *nudt7* mutant, implying SA up-regulates
552 H^+-ATPAse activity through NPR1 (Jayakannan et al. 2014). Secondly, H^+ pumping would
553 provide a driving force for the plasma membrane Na^+/H^+ exchanger (SOS1) to remove Na^+
554 from the cytoplasm to the apoplast (Shi et al. 2000; Apse and Blumwald 2007), thus

555 decreasing Na^+/K^+ ratio in the cytoplasm. The SA pre-treatment increased the activity of the
556 plasma membrane H^+-ATPase in grape and peas during temperature stress (Liu et al. 2008;
557 Liu et al. 2009); hence, each of the two pathways mentioned above may potentially be
558 affected by SA. Overall, it appears that beneficial effects of SA during salt stress may be
559 related to up-regulation of the plasma membrane H^+-ATPase activity and the consequent
560 effects on intracellular ionic homeostasis of Na^+ and K^+.

561 Another major pathway of K^+ leak from the cytosol under saline condition is via ROS-
562 activated K^+ permeable channels (Shabala and Pottosin 2014; Anschutz et al 2014). Various
563 ROS species are produced during salt stress in various cellular compartments including
564 apoplast, chloroplasts (in leaves) and mitochondria (reviewed in Miller et al. 2009). Some of
565 these ROS species ($^{\cdot}OH$ and H_2O_2) can activate either GORK or NSCC channels to induce
566 K^+ loss and trigger programmed cell death during salt stress (e.g Shabala et al. 2007;
567 Demidchik et al. 2010; Poór et al. 2011b). Hence, prevention of K^+ loss through ROS-
568 activated NSCC during salt stress is critical for salt tolerance in plants. Given the reported
569 cross-talks between SA and ROS signalling pathways (see below), SA can control K^+ loss
570 though ROS-activated NSCC (Fig. 4). Indeed, the results from two Arabidopsis mutants with
571 high endogenous SA concentration and altered SA signalling (*nudt7* and *npr1-5*)
572 demonstrated that SA decreased the oxidative damage and hypersensitivity to oxidative stress
573 only if NPR1 was present (Jayakannan et al. 2014). The above conclusion is proposed based
574 on the fact that *npr1-5* mutant showed higher K^+ efflux and higher sensitivity during ROS
575 stress than *nudt7* mutant (Jayakannan et al. 2014).
576

577 ## 10. Conclusions and future work

578 Exogenous application of SA is widely used as a possible remedy to ameliorate toxicity
579 symptoms induced by salinity stress in many plant species (Horváth et al. 2007; Ashraf et al.
580 2010). Also popular is an idea of overexpressing SA biosynthesis through isochorismate
581 synthase (ICS) pathway and NPR1 in glycophytes. Yet, neither of these methods has fully
582 negated detrimental effects of salinity on plant performance. Several reasons may contribute
583 to this.

584 First, similar to other signalling molecules (such as cytosolic free Ca^{2+}, H_2O_2, or NO)
585 salicylic acid signalling is highly dynamic and should be considered in a strict temporal
586 context. This condition is often not met. In this context, a constitutive overexpression of SA

biosynthesis may interfere with other signal transduction pathways negating all the beneficial effects gained. Can we talk about stress-specific SA "signatures", in a manner similar to those reported for cytosolic free Ca^{2+} (Dodd et al 2010) or H_2O_2 (Bose et al 2014a)? This aspect warrants proper investigation in a future.

The NPR1 mediated SA signalling not only improve salt tolerance but also offer tolerance to many biotic and abiotic stresses and, thus, may be considered as an important part of the cross-tolerance mechanism. However, as shown above NPR1-dependent SA signalling may control numerous physiological traits by (i) minimising Na^+ entry into roots and the subsequent long-distance transport into shoots, (ii) enhancing H^+-ATPase activity in roots, (iii) preventing stress-induced K^+ leakage from roots via depolarisation-activated KOR and ROS-activated non-selective cation channels (NSCC), and (iv) increasing K^+ concentration in shoots under salt and oxidative stresses. Each of these traits, however, should be considered in a context of the tissue specificity. Salinity stress tolerance is a physiologically multi-faceted trait, and the latter are not always mutually compatible. Say, reduced Na^+ entry into roots and lesser Na^+ accumulation in the shoot (Jayakannan et al 2013) will jeopardise the plant's ability to adjust to hyperosmotic conditions imposed by salinity. Thus SA-mediated Na^+ reduction from uptake should be complemented by plant's ability to achieve osmotic adjustment by increase *de novo* synthesis of compatible solutes.

The energy cost of some of above enhanced traits should be also not neglected. It was reported before that both halophytes (Bose et al 2014b) and salt-tolerant glycophytes cultivars (Chen et al 2007b) have intrinsically higher rate of H^+ pumping and thus are able to maintain more negative membrane potential, preventing NaCl-induced K^+ loss via GORK channels. However, this comes with the yield penalties. Thus, a constitutive enhancement of SA production and associated increase in root H^+-pumping (Jayakannan et al 2013) may result in reduced plant yield under control conditions. Thus, enhanced SA biosynthesis through either isochorismate synthase (ICS) and NPR1 pathways should be achieved only by using stress-inducible promoters, to avoid associated yield penalties resulting from (otherwise futile) H^+ pumping to maintain highly negative membrane potential.

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References

Acharya BR, Assmann SM (2009) Hormone interactions in stomatal function. Plant Mol Biol 69: 451-462

Alonso-Ramirez A, Rodriguez D, Reyes D, Jimenez JA, Nicolas G, Lopez-Climent M, Gomez-Cadenas A, Nicolas C (2009) Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in Arabidopsis seeds. Plant Physiol 150: 1335-1344

Amtmann A, Sanders D (1999) Mechanisms of Na^+ uptake by plant cells. Adv Bot Res 29: 75-112

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55: 373-399

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. Science 285: 1256-1258

Apse MP, Blumwald E (2007) Na^+ transport in plants. FEBS Lett 581: 2247-2254

Apse MP, Sottosanto JB, Blumwald E (2003) Vacuolar cation/H^+ exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of AtNHX1, the *Arabidopsis* vacuolar Na^+/H^+ antiporter. Plant J 36: 229-239

Asensi-Fabado M, Munné-Bosch S (2011) The *aba3-1* mutant of *Arabidopsis thaliana* withstands moderate doses of salt stress by modulating leaf growth and salicylic acid levels. J Plant Growth Regul 30: 456-466

Ashraf M, Akram NA, Arteca RN, Foolad MR (2010) The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit Rev Plant Sci 29: 162-190

Attaran E, He SY (2012) The long-sought-after salicylic acid receptors. Molecular Plant 5: 971-973

649 Barragán V, Leidi EO, Andrés Z, Rubio L, De Luca A, Fernández JA, Cubero B, Pardo JM
650 (2012) Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to
651 regulate cell turgor and stomatal function in *Arabidopsis*. The Plant Cell 24: 1127-1142

652 Bassil E, Tajima H, Liang Y-C, Ohto M-a, Ushijima K, Nakano R, Esumi T, Coku A,
653 Belmonte M, Blumwald E (2011) The Arabidopsis Na^+/H^+ antiporters NHX1 and NHX2
654 control vacuolar pH and K+ homeostasis to regulate growth, flower development, and
655 reproduction. The Plant Cell 23: 3482-3497

656 Berteli F, Corrales E, Guerrero C, Ariza MJ, Pliego F, Valpuesta V (1995) Salt stress
657 increases ferredoxin-dependent glutamate synthase activity and protein level in the leaves of
658 tomato. Physiol Plant 93: 259-264

659 Blanco F, Salinas P, Cecchini N, Jordana X, Hummelen P, Alvarez M, Holuigue L (2009)
660 Early genomic responses to salicylic acid in *Arabidopsis*. Plant Mol Biol 70: 79-102

661 Borsani O, Valpuesta V, Botella MA (2001) Evidence for a role of salicylic acid in the
662 oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant
663 Physiol 126: 1024-1030

664 Bose J, Rodrigo-Moreno A, Shabala S (2014a) ROS homeostasis in halophytes in the context
665 of salinity stress tolerance. J Exp Bot 65: 1241-1257

666 Rapid regulation of the plasma membrane H+-ATPase activity contributes to salinity
667 tolerance in two halophyte species, Atriplex lentiformis and Chenopodium quinoa

668 Bose J, Rodrigo-Moreno A, Lai D, Xie Y, Shen W, Shabala S (2014b) Rapid regulation of
669 the plasma membrane H^+-ATPase activity is essential to salinity tolerance in two halophyte
670 species, *Atriplex lentiformis* and *Chenopodium quinoa*. Ann Bot (in press;
671 10.1093/aob/mcu219)

672 Bose J, Xie Y, Shen W, Shabala S (2013) Haem oxygenase modifies salinity tolerance in
673 *Arabidopsis* by controlling K^+ retention via regulation of the plasma membrane H^+-ATPase
674 and by altering SOS1 transcript levels in roots. J Exp Bot 64: 471-481

675 Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an Arabidopsis mutant
676 that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6: 1583-1592

677 Cao Y, Zhang ZW, Xue LW, Du JB, Shang J, Xu F, Yuan S, Lin HH (2009) Lack of salicylic
678 acid in Arabidopsis protects plants against moderate salt stress. Zeitschrift fur
679 Naturforschung C, Journal of Biosciences 64: 231-238

680 Chang-Qing Z, Shunsaku N, Shenkui L, Tetsuo T (2008) Characterization of two plasma
681 membrane protein 3 genes (*PutPMP3*) from the alkali grass, *Puccinellia tenuiflora,* and
682 functional comparison of the rice homologues, *OsLti6a/b* from rice. Biochemistry and
683 Molecurar Biology Reports 41: 448-454

684 Chen H-J (1999) Ca^{2+}-dependent excretion of salicylic acid in tobacco cell suspension
685 culture. Botanical Bulletin of Academia Sinica 40: 267-273

686 Chen H-J, Hou W-C, Kuc J, Lin Y-H (2001) Ca^{2+} dependent and Ca^{2+} independent excretion
687 modes of salicylic acid in tobacco cell suspension culture. J Exp Bot 52: 1219-1226

688 Chen Z, Cuin TA, Zhou M, Twomey A, Naidu BP, Shabala S (2007a) Compatible solute
689 accumulation and stress-mitigating effects in barley genotypes contrasting in their salt
690 tolerance. J Exp Bot 58: 4245-4255

691 Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S (2005) Screening plants for
692 salt tolerance by measuring K^+ flux: a case study for barley. Plant Cell Environ 28: 1230-
693 1246

694 Chen Z, Pottosin II, Cuin TA, Fuglsang AT, Tester M, Jha D, Zepeda-Jazo I, Zhou M,
695 Palmgren MG, Newman IA, Shabala S (2007b) Root plasma membrane transporters
696 controlling K^+/Na^+ homeostasis in salt-stressed barley. Plant Physiol 145: 1714-1725

697 Chung JS, Zhu JK, Bressan RA, Hasegawa PM, Shi H (2008) Reactive oxygen species
698 mediate Na^+-induced *SOS1* mRNA stability in Arabidopsis. Plant J 53: 554-565

699 Cuin T, Shabala S (2007) Amino acids regulate salinity-induced potassium efflux in barley
700 root epidermis. Planta 225: 753-761

701 Cuin TA, Betts SA, Chalmandrier R, Shabala S (2008) A root's ability to retain K^+ correlates
702 with salt tolerance in wheat. J Exp Bot 59: 2697-2706

703 Cuin TA, Shabala S (2005) Exogenously supplied compatible solutes rapidly ameliorate
704 NaCl-induced potassium efflux from barley roots. Plant Cell Physiol 46: 1924-1933

705 Davenport RJ, Tester M (2000) A weakly voltage-dependent, nonselective cation channel
706 mediates toxic sodium influx in wheat. Plant Physiol 122: 823-834

707 Dean JV, Delaney SP (2008) Metabolism of salicylic acid in wild-type, *ugt74f1* and *ugt74f2*
708 glucosyltransferase mutants of *Arabidopsis thaliana*. Physiol Plant 132: 417-425

709 Dean JV, Mills JD (2004) Uptake of salicylic acid 2-O-β-D-glucose into soybean tonoplast
710 vesicles by an ATP-binding cassette transporter-type mechanism. Physiol Plant 120: 603-612

711 Dean JV, Mohammed LA, Fitzpatrick T (2005) The formation, vacuolar localization, and
712 tonoplast transport of salicylic acid glucose conjugates in tobacco cell suspension cultures.
713 Planta 221: 287-296

714 Dean JV, Shah RP, Mohammed LA (2003) Formation and vacuolar localization of salicylic
715 acid glucose conjugates in soybean cell suspension cultures. Physiol Plant 118: 328-336

716 Delaney TP, Friedrich L, Ryals JA (1995) Arabidopsis signal transduction mutant defective
717 in chemically and biologically induced disease resistance. Proc Natl Acad Sci 92: 6602-6606

718 Demidchik V, Cuin TA, Svistunenko D, Smith SJ, Miller AJ, Shabala S, Sokolik A, Yurin V
719 (2010) Arabidopsis root K^+-efflux conductance activated by hydroxyl radicals: single-
720 channel properties, genetic basis and involvement in stress-induced cell death. J Cell Sci
721 123: 1468-1479

722 Dempsey DA, Klessig DF (1995) Signals in plant disease resistance. Bulletin de l'Institut
723 Pasteur 93: 167-186

724 Dempsey DMA, Vlot AC, Wildermuth CM, Klessig FD (2011) Salicylic acid biosynthesis
725 and metabolism. The Arabidopsis Book 9: e0156 doi:0110.1199/tab.0156

726 Dewdney J, Reuber TL, Wildermuth MC, Devoto A, Cui J, Stutius LM, Drummond EP,
727 Ausubel FM (2001) Three unique mutants of Arabidopsis identify *eds* loci required for
728 limiting growth of a biotrophic fungal pathogen. Plant J 24: 205-218

729 Dodd AN, Kudla J, Sanders D (2010) The language of calcium signaling. Annu Rev Plant
730 Biol 61: 593-620

731 Dong X (2004) NPR1, all things considered. Curr Opin Plant Biol 7: 547-552

732 Durner J, Klessig DF (1995) Inhibition of ascorbate peroxidase by salicylic acid and 2, 6-
733 dichloroisonicotinic acid, two inducers of plant defense responses. Proc Natl Acad Sci 92:
734 11312-11316

735 Durner Jr, Klessig DF (1996) Salicylic acid is a modulator of tobacco and mammalian
736 catalases. J Biol Chem 271: 28492-28501

737 El-Tayeb MA (2005) Response of barley grains to the interactive effect of salinity and
738 salicylic acid. Plant Growth Regul 45: 215-224

739 Eraslan F, Inal A, Pilbeam DJ, Gunes A (2008) Interactive effects of salicylic acid and silicon
740 on oxidative damage and antioxidant activity in spinach (*Spinacia oleracea* L. cv. *Matador*)
741 grown under boron toxicity and salinity. Plant Growth Regul 55: 207-219

742 Forouhar F, Yang Y, Kumar D, Chen Y, Fridman E, Park SW, Chiang Y, Acton TB,
743 Montelione GT, Pichersky E (2005) Structural and biochemical studies identify tobacco
744 SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. Proc Natl
745 Acad Sci 102: 1773-1778

746 Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, Oka N, Mohan R, Spoel SH, Tada Y, Zheng N
747 (2012) NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature
748 486: 228-232

749 Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H,
750 Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired
751 resistance. Science 261: 754-756

752 Garciadeblas B, Senn ME, Banuelos MA, Rodriguez-Navarro A (2003) Sodium transport and
753 HKT transporters: the rice model. Plant J 34: 788-801

754 Garcion C, Lohmann A, Lamodiere E, Catinot J, Buchala A, Doermann P, Metraux J-P
755 (2008) Characterization and biological function of the ISOCHORISMATE SYNTHASE2
756 gene of Arabidopsis. Plant Physiol 147: 1279-1287

757 Gassmann W, Rubio F, Schroeder JI (1996) Alkali cation selectivity of the wheat root high-
758 affinity potassium transporter HKT1. Plant J 10: 869-882

759 Gaxiola RA, Rao R, Sherman A, Grisafi P, Alper SL, Fink GR (1999) The *Arabidopsis*
760 *thaliana* proton transporters, *AtNhx1* and *Avp1*, can function in cation detoxification in yeast.
761 Proc Natl Acad Sci 96: 1480-1485

762 Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress
763 tolerance in crop plants. Plant Physiol Biochem 48: 909-930

764 Guan B, Hu Y, Zeng Y, Wang Y, Zhang F (2011) Molecular characterization and functional
765 analysis of a vacuolar Na^+/H^+ antiporter gene (*HcNHX1*) from *Halostachys caspica*. Mol
766 Biol Rep 38: 1889-1899

767 Guan L, Scandalios JG (1995) Developmentally related responses of maize catalase genes to
768 salicylic acid. Proc Natl Acad Sci 92: 5930-5934

769 Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N (2007) Salicylic acid induced
770 changes on some physiological parameters symptomatic for oxidative stress and mineral
771 nutrition in maize (*Zea mays* L.) grown under salinity. J Plant Physiol 164: 728-736

772 Gunes A, Inal A, M.Alpaslan, Cicek N, Guneri E, Eraslan F, Guzelordu T (2005) Effects of
773 exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral
774 nutrition in maize (*Zea mays* L.). Arch Agron Soil Sci 51: 687-695

775 Hao L, Zhao Y, Jin D, Zhang L, Bi X, Chen H, Xu Q, Ma C, Li G (2012) Salicylic acid-
776 altering Arabidopsis mutants response to salt stress. Plant Soil 354: 81-95

777 Harfouche AL, Rugini E, Mencarelli F, Botondi R, Muleo R (2008) Salicylic acid induces
778 H_2O_2 production and endochitinase gene expression but not ethylene biosynthesis in
779 *Castanea sativa in vitro* model system. J Plant Physiol 165: 734-744

780 Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular
781 responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51: 463-499

782 Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of exogenous salicylic acid under
783 changing environment: A review. Environ Exp Bot 68: 14-25

784 He Y, Zhu Z (2008) Exogenous salicylic acid alleviates NaCl toxicity and increases
785 antioxidative enzyme activity in *Lycopersicon esculentum*. Biologia Plantarum 52: 792-795

786 Hennig J, Malamy J, Grynkiewicz G, Indulski J, Klessig DF (1993) Interconversion of the
787 salicylic acid signal and its glucoside in tobacco. Plant J 4: 593-600

788 Horie T, Horie R, Chan WY, Leung HY, Schroeder JI (2006) Calcium regulation of sodium
789 hypersensitivities of *sos3* and *athkt1* mutants. Plant Cell Physiol 47: 622-633

790 Horie T, Schroeder JI (2004) Sodium transporters in plants. Diverse genes and physiological
791 functions. Plant Physiol 136: 2457-2462

792 Horie T, Yoshida K, Nakayama H, Yamada K, Oiki S, Shinmyo A (2001) Two types of HKT
793 transporters with different properties of Na^+ and K^+ transport in *Oryza sativa*. Plant J 27:
794 129-138

795 Horváth E, Janda T, Szalai G, Páldi E (2002) *In vitro* salicylic acid inhibition of catalase
796 activity in maize: differences between the isozymes and a possible role in the induction of
797 chilling tolerance. Plant Sci 163: 1129-1135

798 Horváth E, Szalai G, Janda T (2007) Induction of abiotic stress tolerance by salicylic acid
799 signaling. Journal of Plant Growth Regulation 26: 290-300

800 Inada M, Ueda A, Shi W, Takabe T (2005) A stress-inducible plasma membrane protein 3
801 (AcPMP3) in a monocotyledonous halophyte, *Aneurolepidium chinense*, regulates cellular
802 Na^+ and K^+ accumulation under salt stress. Planta 220: 395-402

803 Jain A, Srivastava HS (1981) Effect of salicylic acid on nitrite reductase and glutamate
804 dehydrogenase activities in maize roots. Physiol Plant 53: 285-288

805 Jayakannan M, Babourina O, Rengel Z (2011) Improved measurements of Na^+ fluxes in
806 plants using calixarene-based microelectrodes. J Plant Physiol 168: 1045-1051

807 Jayakannan M, Bose J, Babourina O, Rengel Z, Shabala S (2013) Salicylic acid improves
808 salinity tolerance in *Arabidopsis* by restoring membrane potential and preventing salt-
809 induced K^+ loss via a GORK channel. J Exp Bot 64: 2255-2268

810 Jayakannan M, Bose J, Babourina O, Shabala S, Massart A, Poschenrieder C, Rengel Z
811 (2014) NPR1-dependent salicylic acid signalling pathway is pivotal for enhanced salt and
812 oxidative stress tolerance in Arabidopsis. J Exp Bot (accepeted)

813 Kawano T, Furuichi T, Muto S (2004) Controlled salicylic acid levels and corresponding
814 signaling mechanisms in plants. Plant biotechnology 21: 319-335

815 Kazemi N, Khavari-Nejad RA, Fahimi H, Saadatmand S, Nejad-Sattari T (2010) Effects of
816 exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme
817 activities in leaves of *Brassica napus* L. under nickel stress. Scientia Horticulturae 126: 402-
818 407

819 Kerkeb L, Donaire JP, Rodríguez-Rosales MP (2001) Plasma membrane H^+-ATPase activity
820 is involved in adaptation of tomato calli to NaCl. Physiol Plant 111: 483-490

821 Khan MIR, Asgher M, Khan NA (2014) Alleviation of salt-induced photosynthesis and
822 growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna*
823 *radiata* L.). Plant Physiology and Biochemistry 80: 67-74

824 Kumar D, Klessig DF (2003) High-affinity salicylic acid-binding protein 2 is required for
825 plant innate immunity and has salicylic acid-stimulated lipase activity. Science Signalling
826 100: 16101

827 Kusumi K, Yaeno T, Kojo K, Hirayama M, Hirokawa D, Yara A, Iba K (2006) The role of
828 salicylic acid in the glutathione-mediated protection against photooxidative stress in rice.
829 Physiol Plant 128: 651-661

830 Lee H-i, Raskin I (1998) Glucosylation of salicylic acid in *Nicotiana tabacum* Cv. Xanthi-nc.
831 Phytopathology 88: 692-697

832 Lee J, Nam J, Park HC, Na G, Miura K, Jin JB, Yoo CY, Baek D, Kim DH, Jeong JC (2006)
833 Salicylic acid-mediated innate immunity in Arabidopsis is regulated by *SIZ1 SUMO E3*
834 ligase. Plant J 49: 79-90

835 Lee S, Kim SG, Park CM (2010) Salicylic acid promotes seed germination under high
836 salinity by modulating antioxidant activity in Arabidopsis. New Phytol 188: 626-637

837 Li J, Besseau S, Törönen P, Sipari N, Kollist H, Holm L, Palva ET (2013) Defense-related
838 transcription factors WRKY70 and WRKY54 modulate osmotic stress tolerance by
839 regulating stomatal aperture in *Arabidopsis*. New Phytol 200: 457-472

840 Liu Y, Liu H, Pan Q, Yang H, Zhan J, Huang W (2009) The plasma membrane H^+-ATPase is
841 related to the development of salicylic acid-induced thermotolerance in pea leaves. Planta
842 229: 1087-1098

843 Liu Y, Zhang J, Liu H, Huang W (2008) Salicylic acid or heat acclimation pre-treatment
844 enhances the plasma membrane-associated ATPase activities in young grape plants under
845 heat shock. Scientia Horticulturae 119: 21-27

846 Love AJ, Milner JJ, Sadanandom A (2008) Timing is everything: regulatory overlap in plant
847 cell death. Trends Plant Sci 13: 589-595

848 Maathuis FJM, Amtmann A (1999) K^+ nutrition and Na^+ toxicity: The basis of cellular
849 K^+/Na^+ ratios. Ann Bot 84: 123-133

850 Manosalva PM, Park S-W, Forouhar F, Tong L, Fry WE, Klessig DF (2010) Methyl esterase
851 1 (StMES1) is required for systemic acquired resistance in potato. Mol Plant-Microbe
852 Interact 23: 1151-1163

853 Mateo A, Mühlenbock P, Rustérucci C, Chang CC-C, Miszalski Z, Karpinska B, Parker JE,
854 Mullineaux PM, Karpinski S (2004) LESION SIMULATING DISEASE 1 is required for
855 acclimation to conditions that promote excess excitation energy. Plant Physiol 136: 2818-
856 2830

857 Melotto M, Underwood W, He SY (2008) Role of stomata in plant innate immunity and
858 foliar bacterial diseases. Annu Rev Phytopathol 46: 101

859 Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006) Plant stomata function in
860 innate immunity against bacterial invasion. Cell 126: 969-980

861 Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2009) Reactive oxygen species homeostasis
862 and signalling during drought and salinity stresses. Plant Cell Environ 33: 453-467

863 Mitsuya S, Taniguchi M, Miyake H, Takabe T (2005) Disruption of *RCI2A* leads to over-
864 accumulation of Na^+ and increased salt sensitivity in *Arabidopsis thaliana* plants. Planta 222:
865 1001-1009

866 Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM (2009) Sumoylation of ABI5 by the
867 *Arabidopsis* SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling. Proc Natl
868 Acad Sci 106: 5418-5423

869 Miura K, Okamoto H, Okuma E, Shiba H, Kamada H, Hasegawa PM, Murata Y (2013) SIZ1
870 deficiency causes reduced stomatal aperture and enhanced drought tolerance via controlling
871 salicylic acid-induced ROS accumulation in Arabidopsis. Plant J 73: 91-104

872 Miura K, Sato A, Ohta M, Furukawa J (2011) Increased tolerance to salt stress in the
873 phosphate-accumulating Arabidopsis mutants *siz1* and *pho2*. Planta 234: 1191-1199

874 Miura K, Tada Y (2014) Regulation of water, salinity, and cold stress responses by salicylic
875 acid. Frontiers in plant science 5: doi: 10.3389/fpls.2014.00004

876 Moharekar S, Lokhande S, Hara T, Tanaka R, Tanaka A, Chavan P (2003) Effect of salicylic
877 acid on chlorophyll and carotenoid contents of wheat and moong seedlings. Photosynthetica
878 41: 315-317

879 Molders W, Buchala A, Metraux J-P (1996) Transport of salicylic acid in tobacco necrosis
880 virus-infected cucumber plants. Plant Physiol 112: 787-792

881 Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167: 645-663

882 Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman
883 SD, Tester M, Darren Plett, Gilliham M (2012) Wheat grain yield on saline soils is improved
884 by an ancestral Na^+ transporter gene. Nat Biotechnol 30: 360-364

885 Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651-
886 681

887 Mustafa NR, Kim HK, Choi YH, Erkelens C, Lefeber AWM, Spijksma G, Heijden Rvd,
888 Verpoorte R (2009) Biosynthesis of salicylic acid in fungus elicited *Catharanthus roseus*
889 cells. Phytochemistry 70: 532-539

890 Nawrath C, Metraux J-P (1999) Salicylic acid inductional deficient mutants of Arabidopsis
891 express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation.
892 Plant Cell 11: 1393-1404

893 Nazar R, Iqbal N, Syeed S, Khan NA (2011) Salicylic acid alleviates decreases in
894 photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and
895 antioxidant metabolism differentially in two mungbean cultivars. J Plant Physiol 168: 807-
896 815

897 Nemeth M, Janda T, Horvath E, Paldi E, Szalai G (2002) Exogenous salicylic acid increases
898 polyamine content but may decrease drought tolerance in maize. Plant Sci 162: 569-574

899 Niederl S, Kirsch T, Riederer M, Schreiber L (1998) Co-permeability of ^{3}H-labeled water and
900 ^{14}C-labeled organic acids across isolated plant cuticles investigating cuticular paths of
901 diffusion and predicting cuticular transpiration. Plant Physiol 116: 117-123

902 Nishimura N, Kitahata N, Seki M, Narusaka Y, Narusaka M, Kuromori T, Asami T,
903 Shinozaki K, Hirayama T (2005) Analysis of ABA hypersensitive germination2 revealed the
904 pivotal functions of PARN in stress response in Arabidopsis. Plant J 44: 972-984

905 Ogawa D, Nakajima N, Sano T, Tamaoki M, Aono M, Kubo A, Kanna M, Ioki M, Kamada
906 H, Saji H (2005) Salicylic acid accumulation under O_3 exposure is regulated by ethylene in
907 tobacco plants. Plant Cell Physiol 46: 1062-1072

908 Ohashi Y, Murakami T, Mitsuhara I, Seo S (2004) Rapid down and upward translocation of
909 salicylic acid in tobacco plants. Plant Biotechnology 21: 95-101

910 Ondrasek G, Rengel Z, Veres S (2011) Soil salinisation and salt stress in crop production. *In*
911 A Shanker, B Venkateswarlu, eds, Abiotic stress in plants - mechanisms and adaptations.
912 InTech (ISBN: 978-953-307-394-1, DOI: 10.5772/22248), pp 171-190

913 Palmgren M, Nissen P (2010) P-type ATPases. Annual Review of Biophysics 40: 243-266

914 Pancheva TV, Popova LV (1997) Effect of salicylic acid on the synthesis of ribulose-1,5-
915 bisphosphate carboxylase/oxygenase in barley leaves. J Plant Physiol 220: 381-386

916 Pandolfi C, Pottosin I, Cuin T, Mancuso S, Shabala S (2010) Specificity of polyamine effects
917 on NaCl-induced ion flux kinetics and salt stress amelioration in plants. Plant Cell Physiol
918 51: 422-434

919 Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol
920 Environ Saf 60: 324-349

921 Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF (2007) Methyl salicylate is a critical
922 mobile signal for plant systemic acquired resistance. Science 318: 113-116

923 Park SW, Liu PP, Forouhar F, Vlot AC, Tong L, Tietjen K, Klessig DF (2009) Use of a
924 synthetic salicylic acid analog to investigate the roles of methyl salicylate and its esterases in
925 plant disease resistance. J Biol Chem 284: 7307-7317

926 Poór P, Gémes K, Horváth F, Szepesi Á, Simon ML, Tari I (2011a) Salicylic acid treatment
927 via the rooting medium interferes with stomatal response, CO_2 fixation rate and carbohydrate
928 metabolism in tomato, and decreases harmful effects of subsequent salt stress. Plant Biol 13:
929 105-114

930 Poór P, Szopkó D, Tari I (2011b) Ionic homeostasis disturbance is involved in tomato cell
931 death induced by NaCl and salicylic acid. In Vitro Cell Dev Biol Plant 48: 377-382

932

933 Qadir M, Quillérou E, Nangia V, Murtaza G, Singh M, Thomas RJ, Drechsel P, Noble AD
934 (2014) Economics of salt-induced land degradation and restoration. Natural Res Forum
935 (DOI: 10.1111/1477-8947.12054)

936 Qi Y, Tsuda K, Joe A, Sato M, Nguyen LV, Glazebrook J, Alfano JR, Cohen JD, Katagiri F
937 (2010) A putative RNA-binding protein positively regulates salicylic acid-mediated
938 immunity in Arabidopsis. Molecular plant-microbe interactions 23: 1573-1583

939 Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule
940 of the reactive oxygen species network. J Integr Plant Biol 50: 2-18

941 Rai V, Sharma S, SHARMA S (1986) Reversal of ABA-induced stomatal closure by
942 phenolic compounds. J Exp Bot 37: 129-134

943 Rajjou L, Belghazi M, Huguet R, Robin C, Moreau A, Job C, Job D (2006) Proteomic
944 investigation of the effect of salicylic acid on Arabidopsis seed germination and
945 establishment of early defense mechanisms. Plant Physiol 141: 910-923

946 Rao MV, Davis KR (1999) Ozone-induced cell death occurs via two distinct mechanisms in
947 *Arabidopsis*: the role of salicylic acid. Plant J 17: 603-614

948 Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant
949 growth and development. J Exp Bot 62: 3321-3338

950 Ross JR, Nam KH, D'Auria JC, Pichersky E (1999) S-adenosyl-L-methionine: salicylic acid
951 carboxyl methyltransferase, an enzyme involved in floral scent production and plant defense,
952 represents a new class of plant methyltransferases. Arch Biochem Biophys 367: 9-16

953 Rubio F, Gassmann W, Schroeder JI (1995) Sodium driven potassium uptake by the plant
954 potassium transporter *hkt1* and mutations conferring salt tolerance. Science 270: 1660-1663

955 Sahu BB, Shaw BP (2009) Salt-inducible isoform of plasma membrane H^+-ATPase gene in
956 rice remains constitutively expressed in natural halophyte, *Suaeda maritima*. J Plant Physiol
957 166: 1077-1089

958 Sanders D (2000) Plant biology: The salty tale of *Arabidopsis*. Curr Biol 10: R486-R488

959 Sawada H, Shim I-S, Usui K (2006) Induction of benzoic acid 2-hydroxylase and salicylic
960 acid biosynthesis-modulation by salt stress in rice seedlings. Plant Sci 171: 263-270

961 Seskar M, Shulaev V, Raskin I (1998) Endogenous methyl salicylate in pathogen-inoculated
962 tobacco plants. Plant Physiol 116: 387-392

963 Shabala L, Cuin TA, Newman IA, Shabala S (2005) Salinity-induced ion flux patterns from
964 the excised roots of Arabidopsis *sos* mutants. Planta 222: 1041-1050

965 Shabala S (2009) Salinity and programmed cell death: unravelling mechanisms for ion
966 specific signalling. J Exp Bot 60: 709-712

967 Shabala S (2013) Learning from halophytes: physiological basis and strategies to improve
968 abiotic stress tolerance in crops. Ann Bot 112: 1209-1221

969 Shabala S, Cuin TA (2008) Potassium transport and plant salt tolerance. Physiol Plant 133:
970 651-669

971 Shabala S, Cuin TA, Prismall L, Nemchinov LG (2007) Expression of animal CED-9 anti-
972 apoptotic gene in tobacco modifies plasma membrane ion fluxes in response to salinity and
973 oxidative stress. Planta 227: 189-197

974 Shabala S, Demidchik V, Shabala L, Cuin TA, Smith SJ, Miller AJ, Davies JM, Newman IA
975 (2006) Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from Arabidopsis root and leaf
976 cells by controlling plasma membrane K^+-permeable channels. Plant Physiol 141: 1653-1665

977 Shabala S, Shabala L, Van Volkenburgh E (2003) Effect of calcium on root development and
978 root ion fluxes in salinised barley seedlings. Funct Plant Biol 30: 507-514

979 Shakirova FM (2003) Changes in the hormonal status of wheat seedlings induced by salicylic
980 acid and salinity. Plant Sci 164: 317-322

981 Shi HZ, Ishitani M, Kim CS, Zhu JK (2000) The *Arabidopsis thaliana* salt tolerance gene
982 SOS1 encodes a putative Na^+/H^+ antiporter. Proceedings of the National Academy of
983 Sciences 97: 6896-6901

984 Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C (1997) Salicylic acid potentiates
985 an agonist-dependent gain control that amplifies pathogen signals in the activation of defense
986 mechanisms. Plant Cell 9: 261-270

987 Shulaev V, Silverman P, Raskin I (1997) Airborne signalling by methyl salicylate in plant
988 pathogen resistance. Nature 385: 718-721

989 Smethurst CF, Rix K, Garnett T, Auricht G, Bayart A, Lane P, Wilson SJ, Shabala S (2008)
990 Multiple traits associated with salt tolerance in lucerne: revealing the underlying cellular
991 mechanisms. Funct Plant Biol 35: 640-650

992 Song JT (2006) Induction of a salicylic acid glucosyltransferase, AtSGT1, is an early disease
993 response in *Arabidopsis thaliana*. Mol Cells 22: 233-238

994 Song JT, Koo YJ, Seo HS, Kim MC, Choi YD, Kim JH (2008) Overexpression of AtSGT1,
995 an Arabidopsis salicylic acid glucosyltransferase, leads to increased susceptibility to
996 *Pseudomonas syringae*. Phytochemistry 69: 1128-1134

997 Stevens J, Senaratna T, Sivasithamparam K (2006) Salicylic acid induces salinity tolerance in
998 tomato (*Lycopersicon esculentum* cv. *Roma*): Associated changes in gas exchange, water
999 relations and membrane stabilisation. Plant Growth Regul 49: 77-83

1000 Strawn MA, Marr SK, Inoue K, Inada N, Zubieta C, Wildermuth MC (2007) Arabidopsis
1001 isochorismate synthase functional in pathogen-induced salicylate biosynthesis exhibits
1002 properties consistent with a role in diverse stress responses. J Biol Chem 282: 5919-5933

1003 Sun W, Xu X, Zhu H, Liu A, Liu L, Li J, Hua X (2010) Comparative transcriptomic profiling
1004 of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. Plant Cell Physiol
1005 51: 997-1006

1006 Szepesi A, Csiszar J, Gemes K, Horvath E, Horvath F, Simon ML, Tari I (2009) Salicylic
1007 acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and
1008 abscisic acid accumulation, and increases Na^+ content in leaves without toxicity symptoms in
1009 *Solanum lycopersicum* L. J Plant Physiol 166: 914-925

1010 Szepesi A, Poór P, Gémes K, Horváth E, Tari I (2008) Influence of exogenous salicylic acid
1011 on antioxidant enzyme activities in the roots of salt stressed tomato plants. Acta Biologica
1012 Szegediensis 52: 199-200

1013 Tester M, Langridge P (2010) Breeding technologies to increase crop production in a
1014 changing world. Science 327: 818-22

1015 Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 91:
1016 503-527

1017 Tyerman SD (2002) Nonselective cation channels. Multiple functions and commonalities.
1018 Plant Physiol 128: 327-328

1019 Tyerman SD, Skerrett IM (1999) Root ion channels and salinity. Scientia Horticulturae 78:
1020 175-235

1021 Tyerman SD, Skerrett M, Garrill A, Findlay GP, Leigh RA (1997) Pathways for the
1022 permeation of Na^+ and Cl^- into protoplasts derived from the cortex of wheat roots. J Exp Bot
1023 48: 459-480

1024 Uzunova A, Popova L (2000) Effect of salicylic acid on leaf anatomy and chloroplast
1025 ultrastructure of barley plants. Photosynthetica 38: 243-250

1026 Verberne MC, Verpoorte R, Bol JF, Mercado-Blanco J, Linthorst HJM (2000)
1027 Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen
1028 resistance. Nat Biotechnol 18: 779-783

1029 Vidhyasekaran P (2015) Salicylic Acid Signaling in Plant Innate Immunity. *In* Plant
1030 Hormone Signaling Systems in Plant Innate Immunity, Vol 2. Springer Netherlands, pp 27-
1031 122

1032 Vlot AC, Dempsey DMA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to
1033 combat disease. Annu Rev Phytopathol 47: 177-206

1034 Vlot AC, Liu P-P, Cameron RK, Park S-W, Yang Y, Kumar D, Zhou F, Padukkavidana T,
1035 Gustafsson C, Pichersky E, Klessig DF (2008) Identification of likely orthologs of tobacco
1036 salicylic acid-binding protein 2 and their role in systemic acquired resistance in *Arabidopsis*
1037 *thaliana*. Plant J 56: 445-456

1038 Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required
1039 to synthesize salicylic acid for plant defence. Nature 414: 562-565

1040 Wu C-A, Yang G-D, Meng Q-W, Zheng C-C (2004) The cotton *GhNHX1* gene encoding a
1041 novel putative tonoplast Na^+/H^+ antiporter plays an important role in salt stress. Plant Cell
1042 Physiol 45: 600-607

1043 Wu Y, Zhang D, Chu JY, Boyle P, Wang Y, Brindle ID, De Luca V, Després C (2012) The
1044 *Arabidopsis* NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell
1045 Reports 1: 639-647

1046 Xia J, Zhao H, Liu W, Li L, He Y (2009) Role of cytokinin and salicylic acid in plant growth
1047 at low temperatures. Plant Growth Regul 57: 211-221

1048 Xie Z, Zhang Z-L, Hanzlik S, Cook E, Shen QJ (2007) Salicylic acid inhibits gibberellin-
1049 induced alpha-amylase expression and seed germination via a pathway involving an abscisic-
1050 acid-inducible WRKY gene. Plant Mol Biol 64: 293-303

1051 Yalpani N, Silverman P, Wilson TM, Kleier DA, Raskin I (1991) Salicylic acid is a systemic
1052 signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell 3:
1053 809-818

1054 Yang YN, Qi M, Mei CS (2004) Endogenous salicylic acid protects rice plants from
1055 oxidative damage caused by aging as well as biotic and abiotic stress. Plant J 40: 909-919

1056 Yasuda M, Ishikawa A, Jikumaru Y, Seki M, Umezawa T, Asami T, Maruyama-Nakashita A,
1057 Kudo T, Shinozaki K, Yoshida S (2008) Antagonistic interaction between systemic acquired
1058 resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. Plant Cell
1059 20: 1678-1692

1060 Yildirim E, Turan M, Guvenc I (2008) Effect of foliar salicylic acid applications on growth,
1061 chlorophyll, and mineral content of cucumber grown under salt stress. J Plant Nutr 31: 593-
1062 612

1063 Zhang HX, Blumwald E (2001) Transgenic salt-tolerant tomato plants accumulate salt in
1064 foliage but not in fruit. Nat Biotechnol 19: 765-768

1065 Zhang HX, Hodson JN, Williams JP, Blumwald E (2001) Engineering salt-tolerant *Brassica*
1066 plants: Characterization of yield and seed oil quality in transgenic plants with increased
1067 vacuolar sodium accumulation. Proc Natl Acad Sci 98: 12832-12836

1068 Zhang X, Chen S, Mou Z (2010) Nuclear localization of NPR1 is required for regulation of
1069 salicylate tolerance, *isochorismate synthase 1* expression and salicylate accumulation in
1070 Arabidopsis. J Plant Physiol 167: 144-148

1071 Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:
1072 247-273

1073 Zhu JK (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6: 441-
1074 445

1075 Zörb C, Noll A, Karl S, Leib K, Yan F, Schubert S (2005) Molecular characterization of
1076 Na^+/H^+ antiporters (*ZmNHX*) of maize (*Zea mays* L.) and their expression under salt stress. J
1077 Plant Physiol 162: 55-66
1078
1079
1080

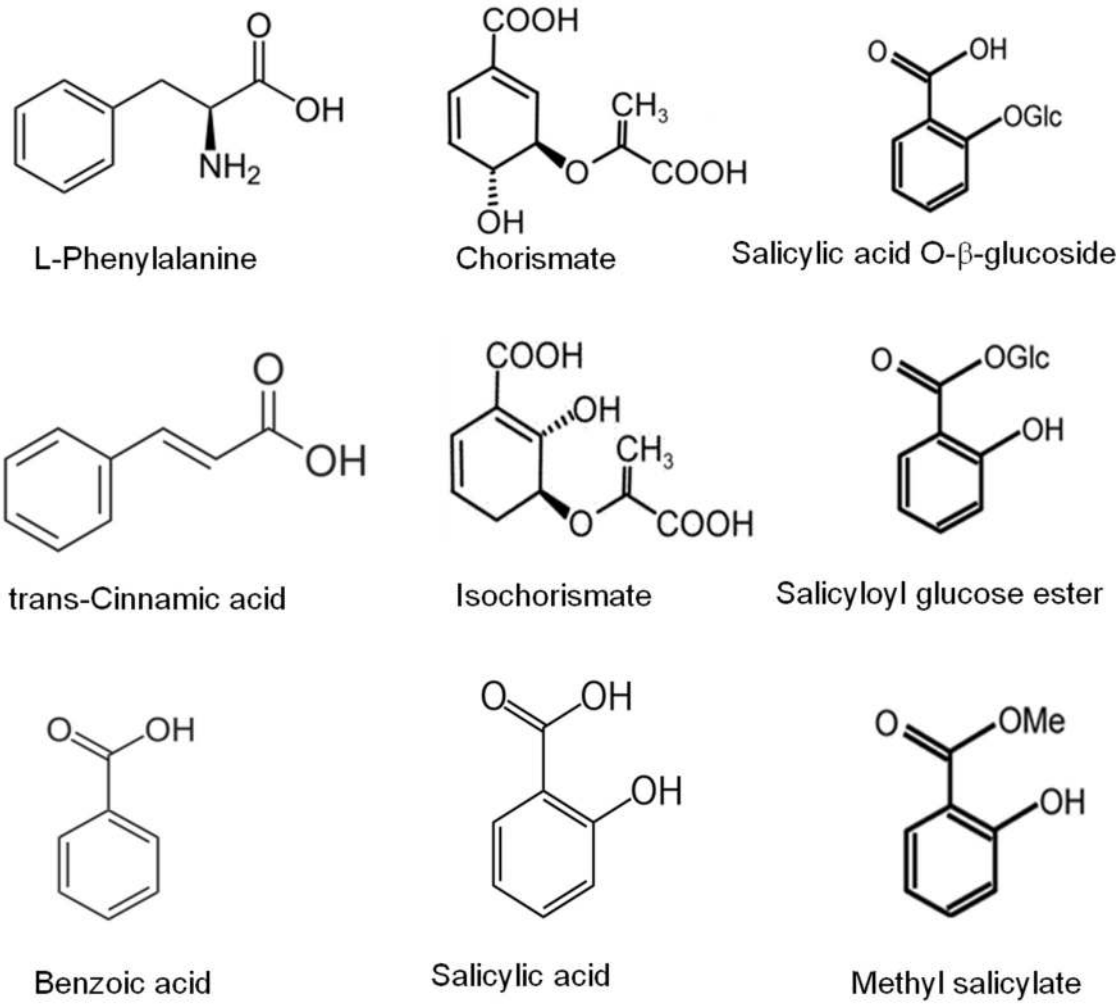

Figure 1: Chemical structure of phenolic compounds that participates in salicylic acid (*o*-hydroxybenzoic acid) biosynthesis and metabolism.

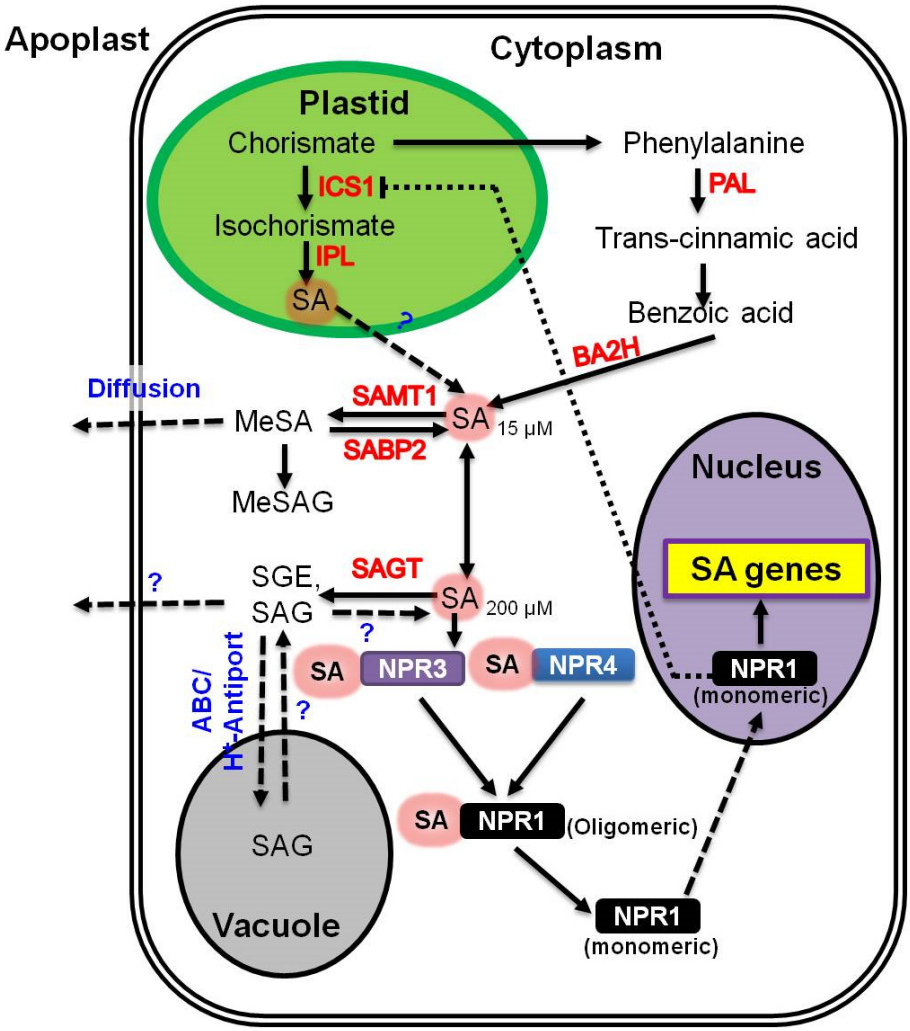

Figure 1: SA biosynthesis, metabolism, homeostasis, transport and signalling during biotic and abiotic stresses. Enzymes involved in SA production and metabolism are shown in red fonts. BA2H= benzoic-acid-2-hydroxylase; ICS1= isochorismate synthase 1; IPL= isochorismate pyruvate-lyase; PAL= phenylalanine ammonia-lyase; SABP2= SA-binding protein 2; SAGT= SA glycosyltransferase; SAMT1= SA carboxyl methyltransferase. Concentrations shown are K_m values of SAMT1 (15 µM) and SAGT (20 µM). Dashed lines with arrows indicate transport across the membranes. Blue text denotes mode of transport. Question marks denote unidentified mechanisms. ABC denotes ATP-binding cassette transport protein. SA surrounded by pink colour indicates free SA. SA conjugated forms are MeSA= methyl salicylate; MeSAG= methyl SA O-ß-glucose; SAG= SA O-ß-glucoside; SGE= salicyloyl glucose ester. SA-binding receptors are depicted in boxes. NPR1/2/3= non-expresser of PR (pathogenesis-related) proteins 1/2/3. 'Oligomeric' means the oxidised form of NPR1, whereas 'monomeric' denotes reduced form of NPR1. Dotted line connecting NPR1 in the nucleus and ICS1 depicts the inhibition of ICS1 following activation of defence response.

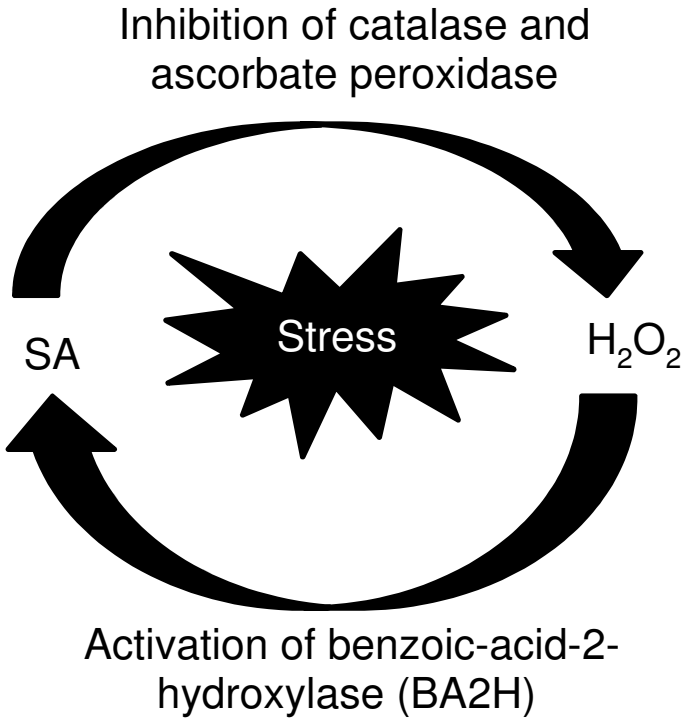

Figure 3. Thematic diagram of "self-amplifying feedback loop" between salicylic acid (SA) and hydrogen peroxide (H_2O_2).

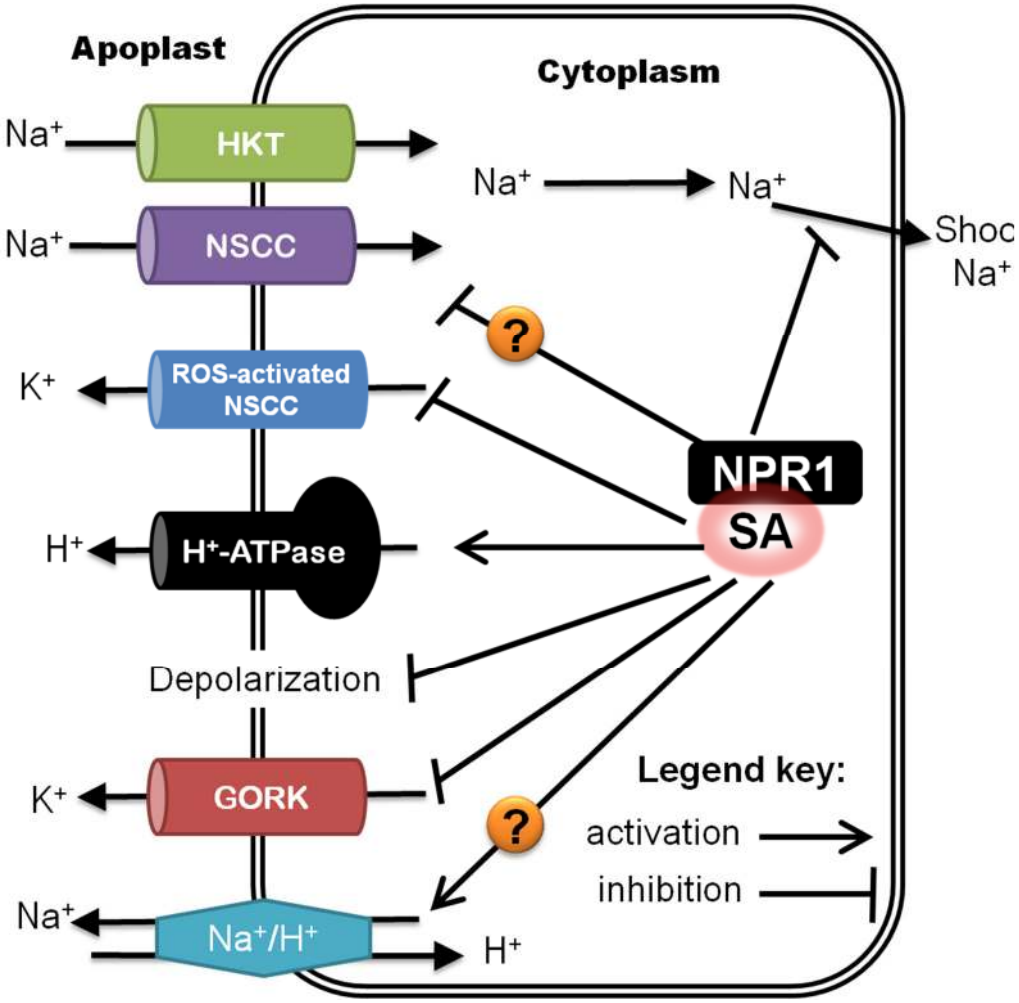

Figure 4: Generalised model explaining SA-mediated NPR1-dependant salt tolerance mechanisms in plants. HKT-High affinity K^+ transporter; NSCC, non-selective cation channels; ROS, reactive oxygen species; GORK, guard cell outward-rectifying K^+ channel; NPR1, non-expresser of pathogenous-related gene 1. A question mark denotes pending pharmacological experiments to confirm the role.