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 constitutive1*; 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 stress 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 (*MYELOBLAST*; 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-transcriptional 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 ($K_m = 200 \mu\text{M}$) (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,

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

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

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

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

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

212 **5. SA receptors in plants**

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

227 Another SA receptor, NPR1 (non-expressor of PR proteins 1) emerged as a master regulatory
 228 protein of SA-dependent defence responses by being a transcriptional co-activator of *PR*-
 229 gene expression (Vlot et al. 2009; Wu et al. 2012). Other studies reported that SA also binds
 230 to NPR1 prologues NPR3 and NPR4 (Attaran and He 2012; Fu et al. 2012). At least two
 231 forms of NPR1 exist in cells. An oligomeric NPR1 is the oxidised form localised in the
 232 cytoplasm when the SA concentration is low (i.e., no infection/stress), but SA accumulation
 233 following stress leads to an altered cellular redox status that in turn activates NPR1 by
 234 reducing biologically-inactive NPR1 oligomers to active monomers (Dong 2004). SA binding
 235 to NPR3 and NPR4 triggered reduction of oligomeric into monomeric NPR1 (Fu et al. 2012).
 236 The resulting biologically-active NPR1 monomers are transported into the nucleus, where
 237 they interact with specific transcription factors that activate SA-responsive *PR* genes (Dong
 238 2004; Fu et al. 2012). In fact, more than 90 percent of *PR* genes were NPR1-dependent
 239 (Blanco et al. 2009). In addition to regulating defence genes downstream of SA, the presence
 240 of NPR1 in the nucleus is essential to prevent SA accumulation by inhibiting *ICS1*
 241 (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 μ M SA was supplied exogenously, but inhibition was exaggerated when SA concentration exceeded 100 μ M (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 H₂O₂ form a “self-amplifying feedback loop” in response to various abiotic and biotic

stresses; H₂O₂ induces accumulation of SA, and SA enhances H₂O₂ 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 constitutive1*) 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.

6.3 Photosynthesis and transpiration

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

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

7. 1 Cross-talks with other plant hormones

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

7.2 Cross-talks with Reactive Oxygen Species

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

397 in the conversion of benzoic acid to SA (Dempsey and Klessig 1995). An increase in H₂O₂
 398 concentration by SA is mediated via inhibition of catalase and ascorbate peroxidase enzymes
 399 through SA binding (Durner and Klessig 1995; Durner and Klessig 1996; Horváth et al.
 400 2002).

401 All biotic and abiotic stresses are causally associated with increased ROS concentrations. Salt
 402 stress increases production of various forms of ROS, namely superoxide (O₂⁻), singlet
 403 oxygen (¹O₂), hydrogen peroxide (H₂O₂) and hydroxyl radical ([•]OH) in plants (reviewed in
 404 Parida and Das 2005). The ROS are scavenged by enzymatic and/or non-enzymatic
 405 antioxidants to protect plants from prolonged salt stress (Bose et al. 2014). Indeed, salt stress
 406 tolerance in diverse plant species was positively correlated with increased efficiency of the
 407 antioxidative system (Horváth et al. 2007; Munns and Tester 2008; Ashraf et al. 2010).
 408 Exogenous SA application at physiologically relevant concentrations caused moderate stress
 409 by generating H₂O₂, which induced the anti-oxidative defence system including enzymatic
 410 (superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase) and non-
 411 enzymatic antioxidants (glutathione, ascorbic acid, carotenoids and tocopherols) during
 412 acclimation to salt stress (Durner and Klessig 1995; Durner and Klessig 1996; Gill and Tuteja
 413 2010).

414 Interestingly, SA may generate ROS species in the photosynthetic tissues, thereby enhancing
 415 oxidative damage under salt stress. Indeed, salt-treated wild type plants showed necrotic
 416 lesions in shoot tissues, but these lesions were not observed in salt-treated SA-deficient *NahG*
 417 transgenic plants (Borsani et al. 2001; Hao et al. 2012). High ratios of glutathione to oxidised
 418 glutathione (GSH/GSSG) and ascorbic acid to dehydroascorbate (ASA/DHA) in *NahG* plants
 419 enhanced their antioxidant capacity to mitigate salt-induced oxidative stress (Borsani et al.
 420 2001; Cao et al. 2009; Hao et al. 2012). However, high ratio of GSH/GSSG in rice *NahG*
 421 lines did not result in oxidative stress tolerance (Yang et al. 2004; Kusumi et al. 2006),
 422 questioning the above notion. Moreover, SA-hyperaccumulating mutants, namely *siz1* (*small*
 423 *ubiquitin-like modifier E3 ligase1*) and *aba3-1* (*ABA biosynthesis mutant3-1*) showed
 424 enhanced salt tolerance (Asensi-Fabado and Munné-Bosch 2011; Miura et al. 2011),
 425 implying high SA may be essential in preventing salt-induced oxidative stress. A subsequent
 426 comparison of two SA hyper accumulating *Arabidopsis* mutants namely *nudt7* (contains the
 427 constitutively expressed SA-mediated NPR1-independent and NPR1-dependent defence
 428 genes) and *npr1-5* (formerly known as *sail*, salicylic acid-insensitive1; without the SA-

mediated NPR1-dependent defence response) under salt and oxidative stress revealed that presence of NPR1-mediated SA signalling pathway is essential for salt-induced *in vivo* H₂O₂ production as well as salt and oxidative stress tolerance (Jayakannan et al. 2014).

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

8.1 Sodium transport across the plasma membrane

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

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

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

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

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

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

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

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

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

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

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

10. Conclusions and future work

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

First, similar to other signalling molecules (such as cytosolic free Ca^{2+} , H_2O_2 , or NO) salicylic acid signalling is highly dynamic and should be considered in a strict temporal 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 multifaceted 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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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- β -glucose; SAG= SA O- β -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 (H₂O₂).

Figure 4: Generalised model explaining SA-mediated NPR1-dependent 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-expressor of pathogenous-related gene 1. A question mark denotes pending pharmacological experiments to confirm the role.