

Role of Salicylic Acid in Plant Abiotic Stress

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Salicylic acid (SA) plays many roles in plant physiology. Besides pathogenesis-related resistance, SA is involved in the response to abiotic stress. However, the effects of SA on plant resistance to abiotic stress were found contradictory, and the actual role of SA in abiotic stress remains unresolved. Generally, deficiency of SA or a very high level of SA increase the plant susceptibility to abiotic stress. The optimal levels for the highest stress tolerance range from 0.1 mM to 0.5 mM for most plants. But the role of SA at a certain level in moderate and severe abiotic stress may be different. This can be attributed to redox regulations in plant cells. In this paper, we discuss the relationship between reactive oxygen species (ROS) and SA, and propose a subsequent intracellular signal transduction network of SA and ROS under abiotic stress. Anti-stress substances besides antioxidant enzymes induced by SA are also summarized.

Key words: Abiotic Stress, Antioxidant Enzymes, Salicylic Acid, Reactive Oxygen Species

Introduction

Salicylic acid (SA) is a phenolic compound synthesized throughout the plant kingdom via the phenylpropanoid pathway (Metraux, 2002). Research efforts over the past decade have focused on this molecule to elucidate its many roles in plant physiology. Detailed evidence implicates SA in pathogenesis-related (PR) gene expression, systemic acquired resistance, and the hypersensitive response (Shah, 2003). SA also seems to be involved in responses to abiotic stresses, such as ozone (Rao and Davis 1999; Koch *et al.*, 2000), salt and osmotic (Borsani *et al.*, 2001; Molina *et al.*, 2002), UV-B (Surplus *et al.*, 1998; Nawrath *et al.*, 2002), drought (Nemeth *et al.*, 2002; Munne-Bosch and Penuelas, 2003), paraquat (Yang *et al.*, 2004), heat (Clarke *et al.*, 2004; He *et al.*, 2005; Shi *et al.*, 2006), cold (Janda *et al.*, 1999; Kang and Saltveit, 2002; Kang *et al.*, 2003; Tasgin *et al.*, 2003; Scott *et al.*, 2004) and metal stress (Metwally *et al.*, 2003; Yang *et al.*, 2003; Pal *et al.*, 2005). Stress-influenced developmental transitions, including flowering (Hatayama and Takeno, 2003; Martinez *et al.*, 2004), tuberization (Lopez-Delgado and Scott, 1997; Stacey *et al.*, 2006), and senescence (Morris *et al.*, 2000), also involve SA. However, the effects

of salicylic acid on plant resistances to abiotic stress usually contradict with each other. The same pre-treatment with exogenous SA results in opposite responses in different plant species (Yang *et al.*, 2004). Even the same SA concentration promotes resistance to one kind of stress meanwhile decreases the resistance to another stress (Nemeth *et al.*, 2002). The fact that SA can exert different effects under various stress situations or with different species is not in contradiction but rather illustrates the fact that different stresses can either be dependent or independent on an SA pathway and that the molecule does not have the same effect on various species.

SA Concentration-Dependant ROS Level Determines Susceptibility to Stress

SA deficiency

Arabidopsis NahG (expressing a bacterial salicylate hydroxylase), *sid2* (SA induction deficient) and *eds5* (enhances disease susceptibility), which have a very low level of SA (below 1 µg/g fresh weight), accumulate a high level of reactive oxygen species (ROS) and therefore more severe damages in abiotic stress (Lederer and Böger, 2003; Nawrath and Metraux, 1999; Rao and Davis,

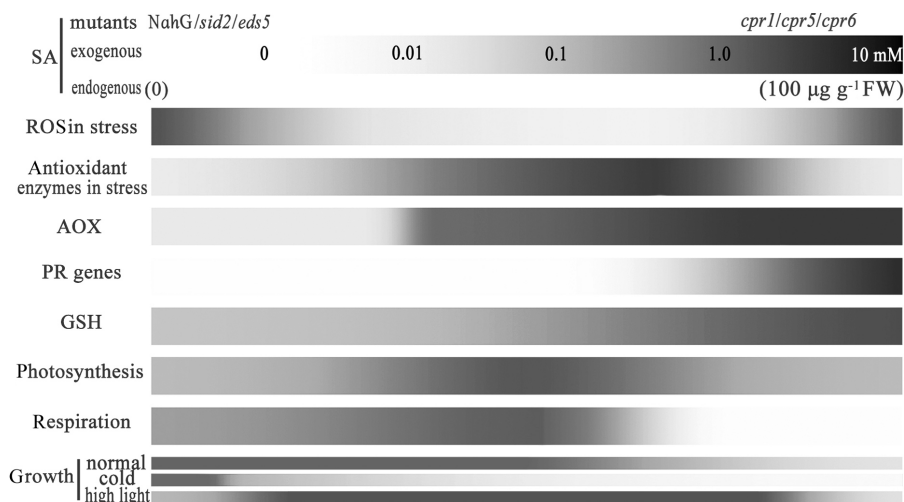


Fig. 1. Relationship between concentration of salicylic acid and plant growth, metabolism and resistance to stresses. 0–10 mM in the SA bar means the commonly used concentration of exogenous salicylic acid. The possibly corresponding endogenous salicylic acid content is marked in parentheses. The SA-less plants NahG, *sid2* and *eds5*, and the SA-over-accumulating mutants *cpr* are shown above the corresponding SA content. Reactive oxygen species (ROS) and antioxidant enzyme activities under stress change as the SA level increases. Contents of alternative oxidase (AOX), pathogenesis-related (PR) genes and reduced glutathione (GSH), and photosynthesis rate and respiration rate are also tightly related with the SA level. Under normal conditions (23 °C, low light), a high level of SA inhibits plant growth. However, SA-deficient *Arabidopsis* plants grow best at 5 °C (cold condition), and SA-over-accumulating mutants have a similar growth rate to the wild type in high light condition (450 μmol m⁻² s⁻¹). Depth of black in each bar indicates each substance's content or activity.

1999; Nawrath *et al.*, 2002; Clarke *et al.*, 2004). Although NahG transgenic plants and *sid2/eds5* mutants are functionally different, the NahG plants can synthesize SA in the chloroplasts but SA is reduced in the cytosol, while *sid2* plants are not able to synthesize SA in the chloroplasts (Friedrich *et al.*, 1995), they respond to abiotic stress similarly. SA is required for the inducement of stress resistance proteins, such as antioxidant enzymes and heat shock protein (HSP). Therefore SA-deficient plants usually can not weave an effective stress defense system (Fig. 1) and be more sensitive to abiotic stress (Rao and Davis, 1999; Nawrath *et al.*, 2002; Clarke *et al.*, 2004). For example, SA promotes thermotolerance during heat shock and induces the heat shock protein *Hsp17.6*, while NahG has a much decreased thermotolerance and a low level of *Hsp17.6* (Clarke *et al.*, 2004).

0.01–0.05 mM exogenous SA

For wheat and tomato, pre-treatment with 0.01–0.05 mM exogenous SA (diluted in water and sprayed) is enough for a significant increase of tolerance to cold stress (Ding *et al.*, 2002; Tasgin *et*

al., 2003). During this pre-treatment, so little SA could not largely induced the ROS accumulation, but it significantly promotes stress tolerance. Some stress-protective proteins, such as catalase (Ding *et al.*, 2002), alternative oxidase (AOX) (Norman *et al.*, 2004) and HSP (Clarke *et al.*, 2004) could be induced by SA at this level. The minimum inducing level of exogenous SA to AOX and HSP is about 0.01 mM. These two proteins decrease stress injuries by eliminating ROS and preserving the membrane integrity (Sun *et al.*, 2001; Torok *et al.*, 2001; Norman *et al.*, 2004). This is contradictory to PR genes, which require much higher levels of exogenous SA (typically in the range of 0.5–5 mM) (Clarke *et al.*, 2004).

0.1–0.5 mM exogenous SA

The optimal levels for the highest stress tolerance usually range from 0.1 mM to 0.5 mM for most low-level-SA plants (*i.e.* maize and *Arabidopsis*; SA was fed through hydroponics) (Janda *et al.*, 1999; Ding *et al.*, 2002; Kang and Saltveit, 2002; Kang *et al.*, 2003; Nemeth *et al.*, 2002; Tasgin *et al.*, 2003; He *et al.*, 2005; Shi *et al.*, 2006). SA pre-treatment at this concentration increases the ROS lev-

els by inhibiting antioxidant enzymes, including ascorbate peroxidase (APX) and catalase (CAT) and maybe others. Then ROS acts as a secondary stress signal to enhance activities of cellular protective enzymes during subsequent abiotic stress, such as CAT, APX, superoxide dismutase (SOD), guaiacol peroxidase (GPX), glutathione reductase (GR), and AOX, HSP as mentioned above (Janda *et al.*, 1999; Kang and Saltveit, 2002; Kang *et al.*, 2003; Tasgin *et al.*, 2003; He *et al.*, 2005; Shi *et al.*, 2006). Inhibition of ROS during pre-treatment largely decreases the later protective effect of SA, and exogenous H_2O_2 treatment without SA similarly increases the stress tolerance also by activating cellular protective enzymes (Kang *et al.*, 2003; Wahid *et al.*, 2007). Therefore, SA in this level protects plants mainly through a ROS-dependent but SA-independent pathway. This contradicts with the facts in biotic stress, where inducement of PR genes requires both SA and ROS (Surplus *et al.*, 1998). Benzo(1,2,3)thiadiazole-7-carbothioic acid S-methylester (BTH) also induces antioxidant enzymes similar to SA. But it does not have disadvantage effects when the concentration is high, and it is a safe compound against peroxidizing herbicides (Knörzer *et al.*, 1999).

Very high level of SA

When exogenous SA is more than 1 mM or some SA-over-accumulating mutants *cpr1*, *cpr5*, *cpr6* (constitutive inducer of PR proteins) are used, plants usually trend to oxidative burst and cell death (Fig. 1) (Rao and Davis, 1999; Tasgin *et al.*, 2003; Mateo *et al.*, 2006). Actually, not all SA-over-accumulating mutants trend to cell death, such as *dnd1* (defence no death) (Yu *et al.*, 1998) and *mpk4* (MAP kinase 4 mutant) (Petersen *et al.*, 2000), but very high levels of SA (times of normal level) almost result in oxidative burst and cell death. What are the mechanisms?

Originally, H_2O_2 was proposed to function downstream of SA on the basis of evidence that high levels of SA can bind and inhibit H_2O_2 -removing enzymes, such as CAT, APX (Durner and Klessig, 1996; Chamnongpol *et al.*, 1998) and dehydrins (Sun *et al.*, 2006). Furthermore, APX is post-transcriptionally suppressed by SA (Mittler *et al.*, 1998; Yuan and Lin, 2004), and CAT is downregulated at the level of steady-state mRNA (Dorey *et al.*, 1998). Later, it was found that increased H_2O_2 levels also act upstream of SA to induce endog-

enous SA (Leon *et al.*, 1995). SA and H_2O_2 compose a positive feedback at this concentration. Thus, the plant simultaneously produces more ROS and at the same time diminishes its own capacity to scavenge H_2O_2 , resulting in the over-accumulation of ROS and the activation of cell death under stresses (Mittler, 2002). Ethylene, NO and jasmonic acid (JA) were found to participate in this SA-ROS self-amplifying loop. Stress-induced redox regulation is usually accompanied by accumulations of ethylene, NO and JA (Dat *et al.*, 2003; Van Breusegem and Dat, 2006). NO is an inhibitor of the respiratory cytochrome c oxidase and may increase the electron flow from ubiquinone towards oxygen, thereby stimulating the superoxide and H_2O_2 formation. In addition, NO can chemically react with superoxide, generating the cytotoxic compounds peroxynitrite and hydroxyl radical, therefore inducing oxidative burst and cell death (Van Camp and Van Montagu,

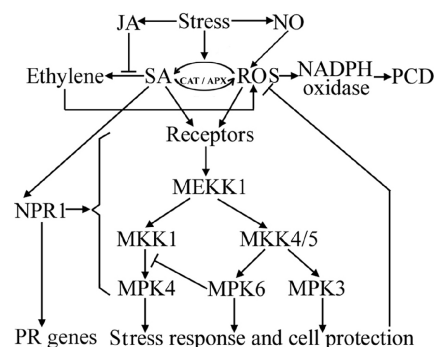


Fig. 2. Hypothetical model showing the role of ROS and SA in the induction of anti-stress gene expression and cell death during abiotic stress. SA can bind and inhibit ROS-removing enzymes, such as CAT and APX, while ROS induces SA directly. SA and H_2O_2 compose a positive feedback. Stress-induced redox regulation is usually accompanied by accumulations of ethylene, NO and JA. Ethylene and NO were found to participate in this SA-ROS self-amplifying loop and the subsequent NADPH oxidase-dependent programmed cell death (PCD). SA promotes ethylene, while JA inhibits SA-induced ethylene biosynthesis and ethylene-mediated PCD. SA signalling is mediated by at least two mechanisms, one requires the *NPR1* gene and the second is independent of *NPR1* but requires MAPK. SA induces abiotic-stress-protective genes almost through the second mechanism. Transmission of SA signal takes place via at least three MAPK signalling cascades. MPK6 action inhibits MPK4 activation, while MPK3 stimulates SA-signalling. MPK4 is under control of MKK1, and MPK6/3 is under control of MKK4/5. MEKK1 is an upstream activator of MKK1/4/5. Moreover, MAPK and some transcription factors also could be promoted by *NPR1*.

1998). Ethylene also promotes the superoxide-dependent cell death. SA promotes ethylene, while JA inhibits the ethylene biosynthesis (Salzman *et al.*, 2005; Seo *et al.*, 2007). Involvement of the plasma membrane NADPH oxidase in ozone-triggered ROS accumulation also has been, for example, shown in the *Arabidopsis thaliana* mutant *rcd1* (radical-induced cell death 1) (Overmyer *et al.*, 2000). In this work, application of the NADPH oxidase inhibitor diphenylene iodonium inhibited ROS accumulation and reduced leaf damage of the *rcd1* mutant (Overmyer *et al.*, 2000). The complexity of ethylene, NO, JA and NADPH oxidase in SA/ROS-induced oxidative burst and cell death is illustrated in Fig. 2.

The Roles of SA in Moderate and Severe Abiotic Stress May Be Contradictory

The above discussion holds for most severe abiotic stresses. However, SA-deficient mutants or SA-over-accumulating mutants may have growth advantages under moderate stress. NahG *Arabidopsis* grew at similar rates as wild type plants at 23 °C, and the growth of both genotypes was slowed by transfer to 5 °C. However, at 5 °C, NahG plants displayed relative growth rates about one-third greater than wild type plants (Fig. 1). In contrast, the *cpr1 Arabidopsis* mutant at 5 °C accumulated very high levels of SA, and its growth was much more inhibited than in wild type plants (Scott *et al.*, 2004). The phenomenon can be attributed to the growth inhibitory properties of SA. The long-term growth at 5 °C causes no detectable injury in wild type *Arabidopsis*. Therefore, the role of SA suggested by this study is likely to be distinct from that in the severe cold stress. (SA-deficient plants should adapt poorly to abiotic stress, as discussed above.)

Wild type plants germinated under moderate light conditions in media supplemented with 100 mM NaCl or 270 mM mannitol showed intensive necrosis in the shoot. In contrast, NahG *Arabidopsis* plants germinated under the same conditions remained green and developed true leaves. The authors suggest that SA potentiates the generation of ROS in photosynthetic tissues during salt and osmotic stress (Borsani *et al.*, 2001; Yuan *et al.*, 2005). However, NahG actually does not produce effective antioxidant enzymes, such as GPX (Borsani *et al.*, 2001). It was supposed that protective mechanisms other than antioxidant en-

zymes may be promoted in NahG and hampered in wild type plants, such as reduced glutathione. On the other hand, NahG plants accumulate catechol as product of SA enzymatic degradation, and catechol could act as antioxidant molecule under light condition (Yang *et al.*, 2004). This might be another reason for the better adaption of NahG. Nevertheless, lack of SA in NahG *Arabidopsis* the seedlings cannot protect seedlings at very high levels of NaCl and mannitol (Borsani *et al.*, 2001).

Wild type *Arabidopsis* with the normal SA level and some *cpr* mutants with a little higher SA level adapt better (higher biomass accumulation) to high light condition ($450 \mu\text{mol m}^{-2} \text{s}^{-1}$) than SA-deficient mutants (Fig. 1). A high level of SA is important for an optimal photosynthetic performance and growth under high light conditions (Mateo *et al.*, 2006). However, this high light treatment is a kind of moderate stress condition and is distinct from light stress or excess light (beyond $750 \mu\text{mol m}^{-2} \text{s}^{-1}$). Both NahG and *cpr* mutants present more severe injuries than wild type plants in excess light (Mateo *et al.*, 2006). The advantage of a high level of SA only exists under moderate light stress.

Does SA Protect Plants through Substances Other than Antioxidant Enzymes?

Endogenous SA correlates with basal thermotolerance, SA-deficient and SA-accumulating *Arabidopsis* mutants having the lowest and highest thermotolerance, respectively (Clarke *et al.*, 2004). According to the criteria mentioned above, SA-accumulating mutants should have a higher level of ROS and a higher susceptibility to heat stress. However, the electrolyte leakage is lower in *cpr5* mutants than in the wild type during heat shock treatment. Expression of some *HSP* genes may contribute to the increased thermotolerance in *cpr5* mutants (Clarke *et al.*, 2004).

Pre-treatment of SA at a 0.5 mM concentration protected the seedlings from cadmium (Cd) toxicity during the following growth period (Metwally *et al.*, 2003). However, SA treatments strongly or completely suppressed the Cd-induced upregulation of the antioxidant enzyme activities. It can be concluded that SA alleviates Cd toxicity not at the level of antioxidant defense but by affecting other mechanisms of Cd detoxification. One mechanism may include binding of Cd resulting in a lowered level of plasmatic free Cd. Alternatively, SA stim-

ulates the expression of certain ABC transporters (Eichhorn *et al.*, 2006). Such transporters have been implicated in the vacuolar sequestering of the products of Cd action therefore releasing the Cd stress (Rea *et al.*, 1998).

SA levels are paralleled by glutathione, which constitutes one of the major components of the antioxidant defence system, and it is also the major determinant of the cellular redox status in plants (Foyer and Noctor, 2005). SA could promote stress tolerances by increasing the glutathione content (Mateo *et al.*, 2006). 0.5 mM SA added to the hydroponic solution of maize increased its tolerance to low temperature stress (Janda *et al.*, 1999; Nemeth *et al.*, 2002), and the significantly increased polyamines level may partly contribute to the protective role of SA (Nemeth *et al.*, 2002). Furthermore, SA increased the freezing tolerance in wheat leaves by increasing the ice nucleation activities for apoplastic proteins, which contain not only antioxidant enzymes but also antifreeze proteins (Tasgin *et al.*, 2003).

Different Species and Organs Respond Differently to SA under Abiotic Stress

All above discussion is suitable for the most plant species, which contain low basal levels of SA (0.1 $\mu\text{g/g}$ fresh weight). Rice has a very high basal SA level (5–30 $\mu\text{g/g}$ fresh weight) (Yang *et al.*, 2004). Depletion of high levels of endogenous SA in transgenic rice (NahG) does not measurably affect the PR gene expression, but reduces the plant's capacity to detoxify ROS. SA-deficient transgenic rice contains elevated levels of superoxide and H_2O_2 , and exhibits spontaneous lesion formation in an age- and light-dependent manner (Yang *et al.*, 2004). We suppose that the SA level in NahG transgenic rice during stress is above the threshold for inducing defense genes, but below the threshold for inducing antioxidant enzymes. Research on SA in rice should be distinct from other plant species.

Root and aerial portions of a plant are differently regulated by SA. For example, when 50 μM $\text{Cd}(\text{NO}_3)_2$ was applied to maize roots, Cd was translocated into the leaves, inducing an increase of the SA level, and subsequently oxidative damage. However, Cd did not affect free or conjugated SA, or the antioxidant enzyme activities in the roots (Pal *et al.*, 2005). For maize and rice and cucumber, SA treatments that induced the chilling

tolerance and activation of GR and GPX in the aerial portion of the seedlings did not induce the chilling tolerance in the radicles, even though the SA treatments were applied to the radicles (Kang and Saltveit, 2002). The shoots of NahG *Arabidopsis* seedlings weighed around seven times of the wild type after 15 d of 100 mM NaCl treatment. However, no significant difference was found in terms of fresh weights of the roots (Borsani *et al.*, 2001). In summary, the effectual SA level for roots may be different from the one for shoots, and the antioxidant enzymes in roots may be insensitive to SA.

SA Signal Transduction under Abiotic Stress

Accumulation of SA is usually accompanied with ROS. Correspondingly, the SA signal and oxidative signal largely overlap. In the transmission of SA/ROS signal, MAPK are involved. *Arabidopsis thaliana* encodes 10 MAPKKKK, 80 MAPKKK, 10 MAPKK and 23 MAPK, which form complex signalling networks with synergistic and antagonistic links (Fujita *et al.*, 2006). The MAP kinases MPK3, MPK4 and MPK6 are activated by various abiotic stresses and might be central elements of SA-ROS signal transduction (Baier *et al.*, 2005; Kangasjarvi *et al.*, 2005; Fujita *et al.*, 2006). MPK6 and MPK3, which are the *Arabidopsis* homologues of SIPK and WIPK, respectively, are activated by the MAP kinase kinases MKK4 and MKK5 (Baier *et al.*, 2005). In MPK6-silenced plants MPK4 is activated indicating that MPK6 suppresses MPK4 activation (Menke *et al.*, 2004). As MPK4 is under control of MKK1, Menke *et al.* (2004) suggested that in the response to stress at least two MAPK cascades act in parallel. All the three MPKs (MPK3, MPK4 and MPK6) are activated by MEKK1 (a kind of MAPKKK), which response to both abiotic stress and SA/ROS (Fujita *et al.*, 2006; Suarez-Rodriguez *et al.*, 2007).

Gene targets of the SA signal through MAPKs are also well documented. In response to SA, transcript levels of peroxidase, lipid transfer protein (Chini *et al.*, 2004), glutathione *S*-transferase (Gruhler *et al.*, 2005), dehydrin-like proteins, HSP, AOX (Salzman *et al.*, 2005; Rajjou *et al.*, 2006) and SOD (Rajjou *et al.*, 2006) were much elevated. Except for pathogen-related genes, other SA-inducible genes are involved in stress protection and ROS elimination. *NPR1* (nonexpressor of PR genes) induced by SA is required for the expres-

sion of PR genes or disease resistance, but not for genes involved in stress protection as mentioned above (Blanco *et al.*, 2005). In other words, SA signalling is mediated by at least two mechanisms, one requiring the *NPR1* gene and a second that is independent of *NPR1* but requires MAPK (Shah, 2003). SA induces abiotic-stress-protective genes almost through the second mechanism. However, genes involved in signal transduction, such as protein kinases (MAPK) and transcription factors, also could be promoted by *NPR1* (Blanco *et al.*, 2005). The complexity of the SA signal pathways is illustrated in Fig. 2.

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