Salicylic Acid: A Double-Edged Sword for Programed Cell Death in Plants

Ana Radojičić1, Xin Li1,2 and Yuelin Zhang1*

1 Department of Botany, The University of British Columbia, Vancouver, BC, Canada, 2 The Michael Smith Laboratories, The University of British Columbia, Vancouver, BC, Canada

In plants, salicylic acid (SA) plays important roles in regulating immunity and programed cell death. Early studies revealed that increased SA accumulation is associated with the onset of hypersensitive reaction during resistance gene-mediated defense responses. SA was also found to accumulate to high levels in lesion-mimic mutants and in some cases the accumulation of SA is required for the spontaneous cell death phenotype. Meanwhile, high levels of SA have been shown to negatively regulate plant cell death during effector-triggered immunity, suggesting that SA has dual functions in cell death control. The molecular mechanisms of how SA regulates cell death in plants are discussed.

Keywords: salicylic acid, hypersensitive reaction, programed cell death, effector-triggered immunity, plant immunity

Salicylic acid (SA) is a plant hormone that plays key roles in defense signaling (Vlot et al., 2009). Pathogen infection induces SA biosynthesis and accumulation. Two groups of Arabidopsis mutants, salicylic acid induction deficient2 (sid2) and enhanced disease susceptibility5 (eds5), are deficient in pathogen-induced SA accumulation and exhibit increased susceptibility to biotrophic pathogens (Nawrath and Metraux, 1999; Dewdney et al., 2000). sid2 mutants carry mutations in the isochorismate synthase ICS1, suggesting that SA is synthesized from chorismate following pathogen infection via ICS1 (Wildermuth et al., 2001). EDS5 encodes a multi-antimicrobial extrusion protein (MATE) transporter (Nawrath et al., 2002). The exact role of EDS5 in SA metabolism is unclear. It is likely to be involved in exporting SA or a precursor of SA out of plastids (Serrano et al., 2013).

SA is perceived by two groups of receptors, NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) and NPR3/NPR4, all of which display high affinity with SA (Fu et al., 2012; Wu et al., 2012; Manohar et al., 2015; Ding et al., 2018). However, they have opposite roles in transcriptional regulation of defense gene expression (Ding et al., 2018). NPR1 functions as a transcriptional activator that promotes SA-induced defense gene expression and pathogen resistance (Fan and Dong, 2002). Loss of NPR1 results in reduced SA-induced PR gene expression and increased susceptibility to pathogens (Cao et al., 1994; Delaney et al., 1995). On the other hand, NPR3 and NPR4 serve as redundant transcriptional co-repressors that prevent activation of defense gene expression when the SA level is low (Ding et al., 2018). When SA levels are high, SA inhibits the transcriptional repression activity of NPR3/NPR4 to activate the expression of SA-responsive genes. The NPR4-4D mutant protein that is unable to bind SA constitutively represses defense gene expression and blocks SA-induced immunity, rendering the mutant plants with enhanced disease susceptibility (Ding et al., 2018). Regulation of defense genes by NPR1 and NPR3/NPR4 is directly facilitated by a group of redundant bZIP transcription factors, including TGA2, TGA5, and TGA6, which interact with both NPR1 and NPR3/NPR4 (Zhang et al., 1999, 2003, 2006; Despres et al., 2000; Zhou et al., 2000).
Increased SA accumulation is associated with hypersensitive response (HR), a form of programmed cell death often induced by effector-triggered immunity (ETI), as well as spontaneous cell death in lesion-mimic mutants. Early studies showed that activation of N gene-mediated defense responses by tobacco mosaic virus led to about 20-fold increase in endogenous SA levels in the infected tobacco leaves (Malamy et al., 1990). Activation of ETI by Pseudomonas effectors AvrRpm1 and AvrRpt2 in Arabidopsis also results in dramatic increases in local SA levels in a SID2 and EDS5-dependent manner (Nawrath and Metraux, 1999). Meanwhile, in mutants with spontaneous cell death, SA accumulates at much higher levels than in wild type (Bruggeman et al., 2015). However, in autoimmune mutants with no spontaneous lesion formation, such as suppressor of npr1-1, constitutive1 (snc1) and defense, no death1 (dnd1), SA levels are still dramatically increased (Yu et al., 1998; Li et al., 2001), suggesting that cell death is not required for the activation of SA biosynthesis and high levels of SA alone are not sufficient to activate cell death.

Salicylic acid has been shown to be required for spontaneous cell death in several lesion-mimic mutants (Table 1). Treatment with low levels of SA activates runaway cell death in lesion simulating disease 1 (lsd1) (Dietrich et al., 1994). Blocking SA accumulation by expressing the SA hydroxylase encoded by the bacterial NahG gene suppresses lesion formation in lsd6, lsd7, accelerated cell death 6 (acd6), and acd11 mutants (Weymann et al., 1995; Rate et al., 1999; Brodersen et al., 2005). In the syntaxin of plants 121 (syp121) syp122 double mutant, spontaneous cell death is also attenuated when SA biosynthesis or SA perception is blocked (Zhang et al., 2007). However, not all lesion-mimic mutants require SA accumulation for activation of spontaneous cell death. For example, expression of NahG does not affect lesion formation in lsd2 and lsd4 mutants (Dietrich et al., 1994; Hunt et al., 1997).

### Table 1 | SA levels and cell death phenotypes of Arabidopsis thaliana mutants.

| Mutant | SA levels | Cell death phenotype | Reference |
|--------|-----------|----------------------|-----------|
| lsd1   | High      | Spontaneous cell death | Dietrich et al., 1994 |
| lsd2   | ND*       | Spontaneous cell death | Dietrich et al., 1994 |
| lsd2 nahG | Low      | Spontaneous cell death | Dietrich et al., 1994; Hunt et al., 1997 |
| lsd4   | ND*       | Spontaneous cell death | Dietrich et al., 1994 |
| lsd4 nahG | Low      | Spontaneous cell death | Dietrich et al., 1994; Hunt et al., 1997 |
| lsd6   | High      | Spontaneous cell death | Weymann et al., 1995 |
| lsd6 nahG | Low      | No spontaneous cell death | Weymann et al., 1995 |
| lsd7   | High      | Spontaneous cell death | Weymann et al., 1995 |
| lsd7 nahG | Low      | No spontaneous cell death | Weymann et al., 1995 |
| acd6   | High      | Spontaneous cell death | Rate et al., 1999 |
| acd6 nahG | Low      | No spontaneous cell death | Rate et al., 1999 |
| acd11  | High      | Spontaneous cell death | Brodersen et al., 2005 |
| acd11 nahG | Low      | No spontaneous cell death | Brodersen et al., 2005 |
| syp121 syp122 | High      | Spontaneous cell death | Zhang et al., 2007 |
| syp121 syp122 nahG | Low      | Reduced spontaneous cell death | Zhang et al., 2007 |
| syp121 syp122 sid2 | Low      | Reduced spontaneous cell death | Zhang et al., 2007 |
| snc1   | High      | No spontaneous cell death | Li et al., 2001 |
| dnd1   | High      | No spontaneous cell death; reduced AvrRpt2-induced cell death | Yu et al., 1998 |
| dnd2   | High      | No spontaneous cell death; reduced AvrRpt2- and AvrRpm1-induced cell death | Jukowski et al., 2004 |
| agd2   | High      | Spontaneous cell death; reduced AvrRpt2- and AvrRpm1-induced cell death | Rate and Greenberg, 2001 |
| agd2 nahG | Low      | Spontaneous cell death; restored AvrRpm1-induced cell death | Rate and Greenberg, 2001 |
| agd2 npr1 | ND*      | Reduced spontaneous cell death; restored AvrRpt2- and AvrRpm1-induced cell death | Rate and Greenberg, 2001 |
| hrl1   | High      | Spontaneous cell death; reduced AvrRpm1-induced cell death | Devadas and Raina, 2002 |
| hrl1 nahG | Low      | Delayed spontaneous cell death; restored AvrRpm1-induced cell death | Devadas and Raina, 2002 |
| hrl1 npr1 | High      | Delayed spontaneous cell death; restored AvrRpm1-induced cell death | Devadas and Raina, 2002 |
| npr3 npr4 | WT-like  | No spontaneous cell death; reduced AvrRpt2-induced cell death | Zhang et al., 2006; Fu et al., 2012 |

*ND, not determined; WT, wild type.*
Interestingly, pre-treatment of Arabidopsis Col-0 plants with SA blocks HR activated by *Pseudomonas syringae pv maculicola* (*P.s.m.*) ES4326 carrying *avrRpm1* (Devadas and Raina, 2002). In transgenic plants overexpressing NPR1, activation of cell death by the bacteria is also attenuated (Rate and Greenberg, 2001). In addition, increased ion leakage was observed in eds5-3 compared to wild type following treatment with *Pseudomonas syringae pv tomato* (*P.s.t.*) DC3000 with *avrRpt2* (Figure 1A), indicating that *AvrRpt2*-induced cell death is enhanced in eds5-3. These findings suggest that activation of SA signaling plays an important role in negative regulation of cell death during ETI.

Consistent with the role of pathogen-induced SA in negative regulation of cell death in ETI, enhanced cell death was observed in the npr1-1 mutant compared to wild type following treatment with *P.s.m.* ES4326 carrying *avrRpm1* (Rate and Greenberg, 2001), suggesting that perception of SA by NPR1 is critical for the attenuation of *AvrRpm1*-induced cell death. When npr1-1, npr4-4D, and the npr1-1 npr4-4D double mutant plants were challenged with *P.s.t.* DC3000 carrying *avrRpt2*, cell death in the npr1-1 and npr4-4D single mutants was similar to that in wild type, whereas npr1-1 npr4-4D exhibited enhanced cell death (Figure 1B), suggesting that npr1-1 and npr4-4D have additive effect on *AvrRpt2*-induced cell death. These data also suggest that SA signaling mediated by both NPR1 and NPR3/NPR4 plays critical roles in dampening cell death during ETI.

Consistent with the effects of pathogen-induced SA accumulation on inhibition of HR, avirulent pathogen-induced cell death in several autoimmune mutants with high SA levels was found to be greatly reduced. For example, cell death induced by *P.s.m.* ES4326 strains carrying *avrRpt2* or *avrRpm1* is dramatically reduced in *aberrant growth and death 2* (agd2) plants (Rate and Greenberg, 2001). The reduced cell death can be restored back to wild type level by introducing *NahG* into *agd2*, suggesting that the high SA level in *agd2* is responsible for the suppression of cell death activated during ETI. In the *hypersensitive response like lesions 1* (*hrl1*) mutant, cell death induced by *AvrRpt2* and *AvrRpm1* is also greatly reduced (Devadas and Raina, 2002). Similarly, introducing *NahG* or npr1-1 into *hrl1* leads to restoration of RPM1-mediated cell death. In another class of autoimmune mutants, including *dnd1* and *dnd2*, gene-for-gene resistance is normal, but there is almost no HR following infection by avirulent bacterial pathogens (Yu et al., 1998; Jurkowski et al., 2004). Both *dnd1* and *dnd2* accumulate high levels of SA in the absence of pathogen infection, which is likely responsible for the lack of ETI-induced HR in these mutants.

Arabidopsis NPR3 and NPR4 function redundantly in negative regulation of defense gene expression. *npr3* *npr4* double mutants accumulate similar levels of SA as wild type plants, but constitutively express *PR* genes and exhibit enhanced resistance to virulent pathogens (Zhang et al., 2002). Interestingly, HR activated by *AvrRpt2* is almost completely blocked in *npr3* *npr4* double mutant plants (Fu et al., 2012). *AvrRpt2*-induced HR is restored in the *npr3* *npr4* *npr1* triple mutant [9], suggesting that constitutive activation of SA response in *npr3* *npr4* mutants is responsible for the suppression of cell death activated by *AvrRpt2*. This is consistent with reduced ETI-induced cell death in autoimmune mutants with high SA levels.

In conclusion, SA plays dual roles in the regulation of programmed cell death in plants. The exact mechanism of how SA regulates cell death is currently still unclear. Analysis of early SA-responsive genes by RNA-sequencing revealed that a large number of positive regulators of defense signaling are strongly
up-regulated 1 h after SA treatment (Ding et al., 2018). Induction of these defense regulators may play critical roles in potentiating defense signaling leading to activation of cell death. Meanwhile, many known negative regulators of plant immunity are also rapidly induced after SA treatment. Induction of such negative immune regulators could lead to negative feedback regulation of defense responses and cell death, which is critical in controlling the magnitude of cell death and preventing the spread of cell death beyond the infection site. The key regulatory components downstream of the SA receptors that are involved in SA-mediated inhibition of ETI-induced cell death remain to be determined in the future.

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AUTHOR CONTRIBUTIONS

YZ designed the experiments. AR performed the experiments. All authors wrote the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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