frontiers in Plant Science

PERSPECTIVE
published: 07 August 2018
doi: 10.3389/fpls.2018.01133

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

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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

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Specialty section:
This article was submitted to Plant Microbe Interactions, a section of the journal Frontiers in Plant Science

Received: *13 May 2018*
Accepted: *13 July 2018*
Published: *07 August 2018*

Citation:
Radojičić A, Li X and Zhang Y (2018) Salicylic Acid: A Double-Edged Sword for Programed Cell Death in Plants. Front. Plant Sci. 9:1133. doi: 10.3389/fpls.2018.01133

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 programed 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-induced cell death	Jurkowski 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-induced 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 death2* (*agd2*) plants (Rate and Greenberg, 2001). The reduced cell death can be restored back to wild type level by introducing *NahG* or *npr1-1* 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 lesions1* (*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., 2006). 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 np4 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.

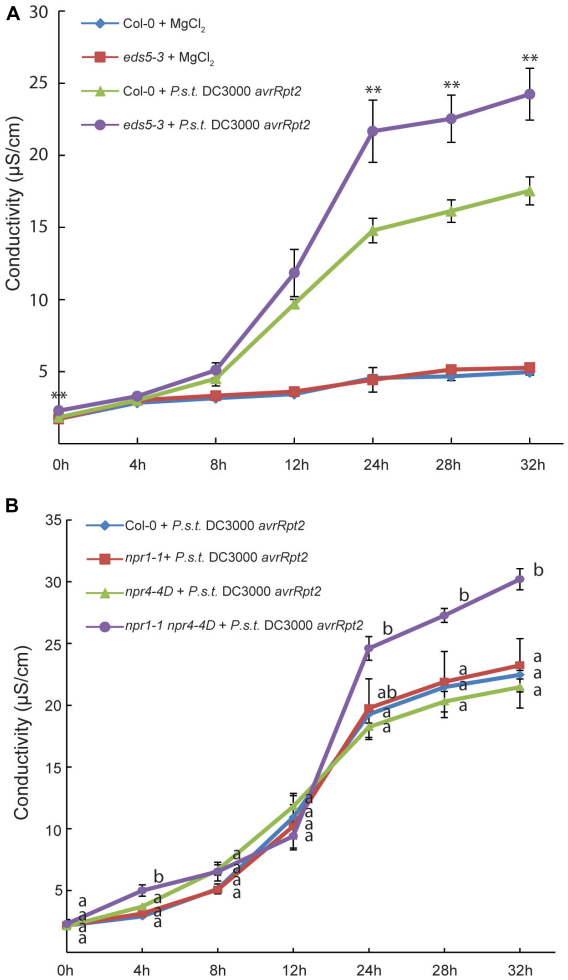

FIGURE 1 | Analysis of ion leakage in *eds5-3*, *npr1-1*, *npr4-4D,* and *npr1-1 npr4-4D* plants after treatment with *P.s.t.* DC3000 *avrRpt2*. Leaves of 4-week-old plants of the indicated genotypes grown under 12 h/12 h light/dark photoperiod at 23°C were infiltrated with mock (10 mM $MgCl_2$) or *P.s.t.* DC3000 *avrRpt2* (OD_{600} = 0.02). For each plant, two leaves were infiltrated and one leaf disk was cut from each leaf immediately after infiltration. The leaf disks were subsequently washed twice in distilled water. Six leaf disks from three plants, representing one biological replicate, were transferred into a 50-ml plastic tube containing 20 ml of distilled water and electrical conductivity was measured at different time points after infiltration using a VWR EC meter (Model 2052). Each data point on the graph represents the mean ± SD of three biological replicates. In **(A)**, Two-tailed *t*-test was performed for each time point between wild type (Col-0) and *eds5-3* plants treated with *P.s.t.* DC3000 *avrRpt2* (**$p < 0.01$). In **(B)**, one way ANOVA with *post hoc* Tukey HSD test was performed for each time point among the different genotypes. Different letters (a,b) indicate statistically significant differences between the samples ($p < 0.01$).

In conclusion, SA plays dual roles in the regulation of programed 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.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

We thank Yuli Ding, Tongjun Sun, and Di Wu (The University of British Columbia) for assistance with the ion leakage analysis and Natural Sciences and Engineering Research Council (NSERC) of Canada and Canada Foundation for Innovation (CFI) for the financial support.

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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.