Salicylic Acid and Defense Responses in Plants

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Abstract Salicylic acid (SA) is a simple phenolic compound distributed in a wide range of plant taxa. Depending on the plant species, developmental stage, and growth conditions, it can be synthesized from cinnamic acid produced by phenylalanine ammonia-lyase in the cytosol or from isochorismic acid generated by isochorismate synthase in chloroplasts. However, a fully defined SA biosynthetic pathway is still unavailable in plants. Besides its role in regulating various aspects of plant growth and development, SA is a plant immune signal essential for both local defense response and systemic acquired resistance. Significant progress has been made recently in understanding SA-mediated defense signaling networks including identification of SA receptors and elucidation of the crucial role of NPR1 (nonexpressor of pathogenesis-related genes 1) in SA signal execution. Understanding of SA-mediated plant defense has facilitated the development of disease-resistant crops through genetic manipulation of the SA signaling pathway. Although the use of *NPR1* and its orthologs in developing broad-spectrum transgenic disease resistance has been successfully extended to a variety of crop species, commercial application of these transgenic crops has been hampered by ethical concerns. In this regard, cisgenesis may hold the potential for application of bioengineered disease-resistant crops in agriculture.

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Introduction

Salicylic acid (SA, 2-hydroxy benzoic acid) is a small phenolic compound synthesized by a wide range of prokaryotic and eukaryotic organisms. It has a broad distribution in the plant kingdom as free phenolic acid and/or conjugated forms generated by glucosylation, methylation, amino acid conjugation, sulfonation, or hydroxylation (Pridham 1965; Pierpoint 1994; Vlot et al. 2009; Dempsey et al. 2011). Among these natural SA derivatives, salicin (β-glucoside salicylic alcohol) is the best known one. It accumulates to high levels in several willow species including *Salix alba*, *S. purpurea*, *S. daphnoides*, and *S. fragilis* whereby the name of salicylic acid was derived from (Raskin 1992; Foster and Tyler 1999). However, the highest levels of total SA were found in inflorescence of thermogenic plants and in spice herbs (Raskin et al. 1990). Under optimal conditions, rice, crabgrass, green foxtail, barley, and soybean have SA levels in excess of 1 μg g^{-1} fresh weight (FW) (Raskin et al. 1990). In the model plant *Arabidopsis thaliana*, basal levels of total SA range from 0.25 μg to 1 μg g^{-1} FW (Nawrath and Métraux 1999; Wildermuth et al. 2001; Brodersen et al. 2005). However, basal SA levels differ widely among species (up to 100-fold differences), even among members of the same family (Yalpani et al. 1991; Malamy et al. 1992; Navarre and Mayo 2004). As ubiquitous distributed secondary metabolites, salicylates (the general name of SA and its derivatives) have been known to possess medicinal properties since the fifth century BC when Hippocrates prescribed salicylate-rich willow leaf and bark for pain relief during childbirth (Weissman 1991). It eventually led to the development of aspirin, one of the world's most widely used drugs, in the 1890s (Raskin 1992). Recently, SA has been established as a distinct class of plant hormone because of its important regulatory roles in seed germination (Rajou et al. 2006), seedling establishment (Alonso-Ramírez et al. 2009), cell growth (Rate et al. 1999; Vanacker et al. 2001), trichome development (Traw and Bergelson 2003), flowering (Cleland 1974; Cleland and Ajami 1974; Martínez et al. 2004), thermogenesis (Raskin et al. 1987), nodulation (Stacey et al. 2006), respiration (Norman et al. 2004), stomatal responses (Manthe et al. 1992; Lee 1998), senescence (Morris et al. 2000; Rao and Davis 2001; Rao et al. 2002), and responses to biotic and abiotic stresses (Janda et al. 2007; Vlot et al. 2009).

The best-established role for SA is as a signal molecule functioning in plant immune responses (Enyedi et al. 1992; Alvarez 2000; Nishimura and Dangl 2010). Due to sessile nature and lacking specialized immune cells, plants have developed the capability to sense pathogen and mount immune response through individual cells. Recognition of pathogen-associated molecular patterns (PAMPs) leads to PAMP-triggered immunity (PTI) that prevents pathogen colonization. While PTI is sufficient to prevent further colonization by many microbes, some pathogens have evolved effectors to dampen PAMP-triggered signals. In turn, host plants have

evolved resistance (R) proteins to detect the presence of pathogen effectors and induce effector-triggered immunity (ETI) including hypersensitive response (HR) (Jones and Dangl 2006). Activation of defense signaling pathways (PTI or ETI) results in the generation of a mobile signal(s) that moves from local infected tissue to distal tissues to induce systemic acquired resistance (SAR), which is a long-lasting immunity against a broad spectrum of pathogens (Fu and Dong 2013). SA-mediated immune responses are important parts of PTI and ETI and also essential for the activation of SAR (Durrant and Dong 2004). Efforts to elucidate the crucial role of SA in immune responses have uncovered that pathogen infection leads to SA accumulation not only in the local infected tissue but also in systemic tissues that develop SAR (Malamy et al. 1990; Métraux et al. 1990) and that SA accumulation usually parallels or precedes the increase in expression of *pathogenesis-related* (*PR*) genes and development of SAR. Consistently, exogenous application of SA and its functional analogs induces *PR* gene expression and resistance against viral, bacterial, oomycete, and fungal pathogens in both dicotyledonous and monocotyledonous plants (Malamy and Klessig 1992; Wasternack et al. 1994; Gorlach et al. 1996; Ryals et al. 1996; Morris et al. 1998; Shah and Klessig 1999; Pasquer et al. 2005; Makandar et al. 2006). Conversely, blocking SA accumulation through expression of a bacterial *naphthalene* (*nah*)-catabolic gene *nahG*, which encodes a salicylate hydroxylase that converts SA to catechol, in transgenic tobacco and *Arabidopsis* plants compromises both HR and SAR (Gaffney et al. 1993; Delaney et al. 1994). Similarly, mutations of genes involved in SA biosynthesis and inhibition of SA biosynthesis have been shown to enhance susceptibility to pathogens, yet the resistance can be restored through exogenous SA application (Mauch-Mani and Slusarenko 1996; Nawrath and Métraux 1999; Wildermuth et al. 2001; Nawrath et al. 2002). Therefore, SA is an important endogenous marker and determinant of plant disease resistance.

In the past two decades, intensive studies have revealed a complex network of SA biosynthesis and signaling in plant immunity. Increasing knowledge of SA-mediated immunity in model systems has led to translational research on developing disease-resistant crop cultivars through transgenic approaches. Genetic screens, transcriptomics, proteomics, and protein interaction studies predominantly in *Arabidopsis* have provided a large number of candidate genes for biotechnological manipulation in crops. At the same time, outcomes of genetic engineering have enhanced our understanding of the SA-mediated immune responses in different plant species. Here, we describe the recent progresses in our understanding of SA biosynthesis, signal perception and execution, and their biotechnological applications in improvement of crop disease resistance.

Salicylic Acid Biosynthesis

Studies of SA biosynthesis in plants have discovered two distinct and differentially compartmentalized pathways: the phenylalanine ammonia-lyase (PAL) pathway starting in the cytosol and the isochorismate synthase (ICS) pathway operative in

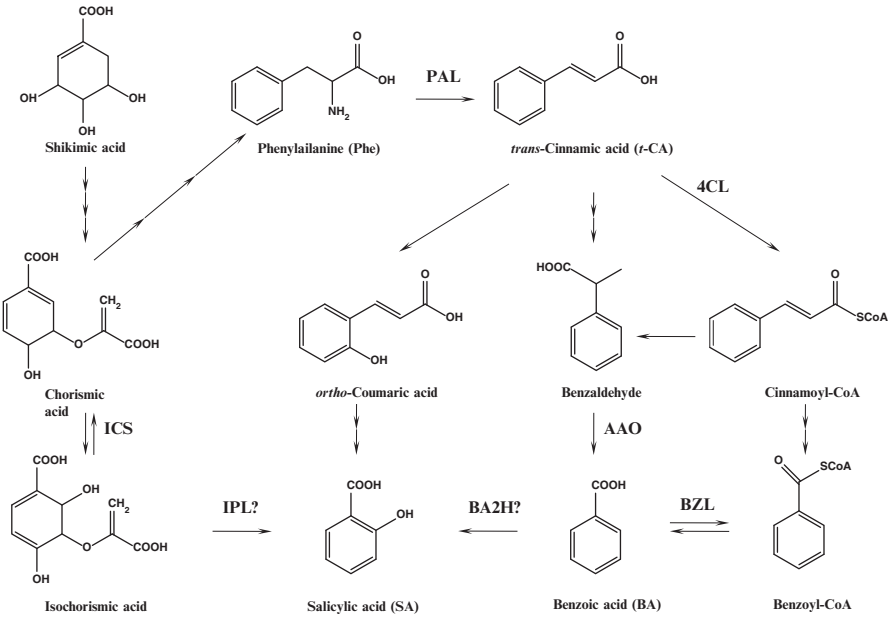

Fig. 1 Salicylic acid biosynthetic pathways in *Arabidopsis thaliana. AAO Arabidopsis* aldehyde oxidase, *BZL* benzoyl-CoA ligase, *BA2H* benzoic acid-2-hydroxylase, *4CL* 4-coumaroyl:CoA ligase, *ICS* isochorismate synthase, *IPL* isochorismate pyruvate lyase, *PAL* phenylalanine ammonia-lyase. Enzymes that have not been identified so far are marked with a question marker

chloroplasts (Fig. 1). Both pathways require the primary metabolite chorismate. However, to date neither biosynthetic route has been fully resolved.

The PAL Pathway

PAL (EC 4.3.1.5) is the first enzyme in the phenylpropanoid pathway, which catalyzes phenylalanine (Phe) to *trans*-cinnamic acid (*t*-CA) and NH_3 via a non-oxidative deamination reaction (Raes et al. 2003; Rohde et al. 2004). Early radiolabeling studies with Phe, *t*-CA, or benzoic acid (BA) suggested that SA is synthesized from Phe via *t*-CA, which is then converted to SA through two possible routes depending on the plant species and growing conditions (Klämbt 1962; El-Basyouni et al. 1964; Chadha and Brown 1974).

1. Hydroxylation of *t*-CA to *ortho*-coumaric acid followed by its decarboxylation to SA (Fig. 1). Feeding of ^{14}C-labled Phe and *t*-CA to young *Primula acaulis* and *Gaultheria procumbens* leaf segments leads to accumulation of *ortho*-coumaric acid and SA, indicating the function of *ortho*-coumaric acid pathway in SA biosynthesis (Griesebach and Vollmer 1963; El-Basyouni et al. 1964). Similarly, upon *Agrobacterium tumefaciens* infection, young tomato seedlings synthesize

SA through hydroxylation of *t*-CA to *ortho*-coumaric acid (Chadha and Brown 1974). Although the conversion of *t*-CA to *ortho*-coumaric acid is believed to be catalyzed by *trans*-cinnamate-4-hydroxylase in multiple species (Russel and Conn 1967; Alibert and Ranjeva 1971, 1972; Gabriace et al. 1991), the activity of 2-hydroxylation of *t*-CA to form *ortho*-coumaric acid was only detected in the suspension of chloroplasts instead of the cytosol of the sweet clover (*Melilotus alba* Desr.) (Gestetner and Conn 1974). Nevertheless, the enzyme(s) that catalyzes the conversion of *ortho*-coumaric acid to SA has not yet been identified.

2. Decarboxylation of the side chains of *t*-CA to generate BA followed by hydroxylation at C_2 position (Fig. 1). A growing body of evidence indicates that plants can potentially develop three biosynthetic subroutes to BA, including an β-oxidative route from cinnamoyl Co-A, a non-oxidative route from cinnamoyl Co-A, and a non-oxidative route from *t*-CA to BA (Wildermuth 2006). Radiolabeling studies using Phe or putative pathway intermediates performed in tobacco mosaic virus (TMV)-infected tobacco, smoke-treated coyote tobacco, or cucumber detected incorporation of radiolabeled carbon into BA and SA but not benzaldehyde, suggesting that SA is synthesized through the cinnamoyl-Co-A β-oxidative subroute (Ribnicky et al. 1998; Jarvis et al. 2000). Similar studies have not been performed in *Arabidopsis* to probe downstream components of SA biosynthesis via PAL pathway. However, a study of BA production in developing seeds identified an *Arabidopsis* aldehyde oxidase4 (AAO4) that catalyzes the conversion of benzaldehyde to BA, which is then incorporated into benzoyl glucosinolates (Ibdah et al. 2009). Additionally, the formation of [^{14}C]BA from [^{14}C]Phe through [^{14}C]*t*-CA was observed in *Tsuga canadensis*, young *Gaultheria procumbens* tissue, and uninfected tomato seedlings (Zenk and Muller 1964; Ellis and Amrhein 1971; Chadha and Brown 1974). Furthermore, ^{14}C-tracer studies with tobacco cell suspensions or TMV-inoculated leaves indicated that the label moves from *t*-CA to SA via BA (Yalpani et al. 1993). Similarly, rice shoots can convert both [^{14}C]*t*-CA and [^{14}C]BA to SA (Silverman et al. 1995).

The direct conversion of [^{14}C]BA to [^{14}C]SA discovered in etiolated *Helianthus annuus* hypocotyls, *Solanum tuberosum* tubers, *Pisum sativum* internodes, and infected cucumber plants was proposed to be catalyzed by an inducible BA 2-hydroxylase (BA2H) (Klämbt 1962; Meuwly et al. 1995). BA2H activity was further detected in ozone-exposed tobacco leaves, heat-treated pea plants, and salt-stressed rice seedlings (León et al. 1995; Ogawa et al. 2005; Sawada et al. 2006; Pan et al. 2006). Biochemical characterization indicated that tobacco BA2H is a soluble P450 oxygenase that specifically hydroxylates the *ortho* position of BA (León et al. 1995). Although there has been no subsequent report describing a BA2H-encoding gene in plants, similar activity has been observed in *Arabidopsis*, which converts neonicotinoid metabolite 6-chloropyridinyl-3-carboxylic acid to the SA mimic 6-chloro-2-hydroxypyridinyl-3-carboxylic acid *in planta* (Ford et al. 2010). Studies conducted in poplar and tobacco indicated that it might also be possible that the glucose-conjugated ester of BA acts as an intermediate for the synthesis of the SA glucose ester and SA (Chong et al. 2001; Ruuhola and Julkunen-Tiitto 2003).

The preference of SA biosynthetic route in the PAL pathway depends on plant species and growth conditions. Isotope-feeding experiments revealed that SA is mainly synthesized from BA in some plant species such as tobacco, rice, potato, cucumber, sunflower, and pea (Klämbt 1962; Yalpani et al. 1993; León et al. 1995; Silverman et al. 1995; Sticher et al. 1997), while other plant species can form SA through the route of *ortho*-coumaric acid (Yalpani et al. 1993; León et al. 1995; Silverman et al. 1995). However, feeding of ^{14}C-labeled Phe, *ortho*-coumaric acid, and BA to young *Primula acaulis* and *G. procumbens* leaf segments all leads to SA, suggesting that both routes are probably utilized in SA biosynthesis (El-Basyouni et al. 1964). Similarly, SA is formed mostly via BA in young tomato seedlings, but after infection with *A. tumefaciens*, SA biosynthesis is shifted to the route of hydroxylation of cinnamate to *ortho*-coumaric acid (Chadha and Brown 1974).

Elucidation of the above PAL pathway largely relied on isotope feeding of the perspective SA biosynthetic precursors to suspension cells or plant segments. Since isotope feeding is not an accurate reflection of *in planta* metabolism, the results might be misleading. Further supports to the PAL pathway in SA biosynthesis came from the evidence that pathogen-resistant tobacco and *Arabidopsis* show increased *PAL* expression and SA levels (Pellegrini et al. 1994; Mauch-Mani and Slusarenko 1996; Dempsey et al. 1999). Additionally, loss of PAL activity, due to sense suppression or treatment with the PAL inhibitor 2-aminoindan-2-phosphonic acid (AIP), reduces pathogen-induced SA accumulation in tobacco, cucumber, and *Arabidopsis*, and the defense phenotypes of PAL-inhibited plants can be complemented by exogenous SA application (Meuwly et al. 1995; Mauch-Mani and Slusarenko 1996; Pallas et al. 1996). Moreover, increases in BA2H activity parallel or precede SA accumulation induced by TMV infection, UV exposure, or treatment with BA or hydrogen peroxide in tobacco (Léon et al. 1993; Yalpani et al. 1993; León et al. 1995). Similarly, salinity induces BA2H activity and SA biosynthesis in rice seedlings, and the induced SA accumulation can be inhibited by uniconazole, a BA2H inhibitor, suggesting that inhibition of BA2H can prevent salinity-induced SA accumulation (Sawada et al. 2006). Importantly, genetic analysis of the *pal* quadruple mutant (*pal1 pal2 pal3 pal4*) revealed a ~75 % reduction in the basal level of total SA as compared with wild-type plants and a ~50 % reduction in total SA levels following avirulent bacterial pathogen infection (Huang et al. 2010). Therefore, it is generally believed that SA can be synthesized through the PAL pathway (Raskin 1992; Lee et al. 1995; Coquoz et al. 1998; Dempsey et al. 2011).

The ICS Pathway

Although early studies suggested that plants might synthesize SA through the PAL pathway, there have been accumulating data questioning its role in the overall SA biosynthesis. In some of the radiolabeling studies described above, the incorporation rate of labeled precursor into SA is lower than expected, particular under infection/induction conditions (Chadha and Brown 1974; Yalpani et al. 1993;

Coquoz et al. 1998). Inhibiting PAL activity by AIP can only reduce chemical- or pathogen-induced SA accumulation by several folds in potato or *Arabidopsis*, respectively (Mauch-Mani and Slusarenko 1996; Coquoz et al. 1998). These pieces of evidence indicated that there might be another pathway in plants leading to SA biosynthesis (Fig. 1).

Bacteria in several genera have been shown to synthesize SA in the production of iron-chelating siderophores (Garcion and Métraux 2006). In the bacterial pathway, chorismate is converted to SA through an isochorismate (IC) intermediate (Verberne et al. 1999). In some bacterial species, like *Pseudomonas aeruginosa* and *P. fluorescens*, chorismate is first converted to IC by isochorismate synthase (ICS, EC 5.4.4.2) and followed by conversion to SA and pyruvate by another unifunctional enzyme, isochorismate pyruvate lyase (IPL, EC 4.2.99.21) (Serino et al. 1995; Mercado-Blanco et al. 2001). In contrast, SA synthesis in *Yersinia enterocolitica* and *Mycobacterium tuberculosis* is achieved through a sole, bifunctional enzyme named SA synthase (SAS) that directly converts chorismate to SA via an isochorismate intermediate (Pelludat et al. 2003; Kerbarh et al. 2005; Harrison et al. 2006). Structurally, ICS and SAS are similar and contain conserved active sites (Harrison et al. 2006; Kerbarh et al. 2005; Kolappan et al. 2007; Parsons et al. 2008). Functionally, both enzymes begin with nucleophilic attack at C_2 of chorismate, with water as the nucleophile, concomitant with displacement of the C_4 hydroxyl group in an S_N2 reaction (He et al. 2004); however, reactions on SAS is followed by elimination of pyruvate and release of SA.

In plants, chorismate is synthesized in the plastid (Poulsen and Verpoorte 1991; Schmid and Amrhein 1995). Considering the fact that many plastid-localized pathways are derived from prokaryotic endosymbionts, it is possible that plants may also utilize a similar ICS pathway for SA biosynthesis (Verberne et al. 1999; Wildermuth et al. 2001). To assess whether plants contain an endogenous pathway to synthesize SA through IC, Wildermuth et al. (2001) identified two putative *ICS* genes in the *Arabidopsis* genome. ICS1 (At1g74710) and ICS2 (At1g18870) share 78 % identity at the amino acid level and ICS1 is 57 % identical to a *Catharanthus roseus* ICS, whose activity has been confirmed biochemically (van Tegelen et al. 1999; Garcion et al. 2008). However, only *ICS1* transcript is accumulated in leaves infected with fungal (*Golovinomyces orontii*) and bacterial (*P. syringae* pv. *maculicola*) pathogens (Wildermuth et al. 2001). *ICS1* expression correlates with SA accumulation and expression of the SA-inducible *PR1* gene. Subsequent analyses indicated that *ICS1* transcripts also accumulate in response to a variety of biotic or abiotic stresses, including UV light, ozone, PAMPs, (hemi)biotrophic pathogens, and exogenous SA treatment (Ogawa et al. 2005; Killian et al. 2007; Nobuta et al. 2007; Postel et al. 2010; Dempsey et al. 2011; Harrower and Wildermuth 2011). Two *Arabidopsis* mutants, *sid2-1* (*salicylic acid induction-deficient2-1*) and *eds16-1* (*enhanced disease susceptibility16-1*) (Nawrath and Métraux 1999; Dewdney et al. 2000), which can accumulate only 5–10 % of the wild-type level of SA following infection of virulent or avirulent pathogens, were found to contain lesions in the *ICS1* gene (Wildermuth et al. 2001). Exogenous SA application can complement their enhanced disease susceptibility phenotype (Wildermuth et al. 2001).

Biochemical and molecular analyses provided further evidence supporting the role of ICS1 in SA biosynthesis. As expected, ICS1 contains a putative plastid transit sequence and a cleavage site (Wildermuth et al. 2001). The high affinity of ICS1 for chorismate allows ICS1 to compete successfully with other pathogen-induced enzymes that use chorismate as their substrate, such as anthranilate synthase (Strawn et al. 2007; Ziebart and Toney 2010). Unlike the bifunctional SAS, the recombinant ICS1 only converts chorismate to IC, since no SA was detected in the products of this reaction (Strawn et al. 2007). Additional analyses revealed that proper function of ICS1 requires Mg^{2+}. However, ICS1 displays maximal activity over a broad range of pH and temperature, which is suitable for the light-mediated changes in the stromal environment.

Similarly to *ICS1*, *ICS2* encodes a functional ICS enzyme that can be imported into the chloroplast stroma (Strawn et al. 2007; Garcion et al. 2008). The fact that null *ics1* mutant still accumulates some SA suggests a likely role for ICS2 in SA biosynthesis. Comparison of SA accumulation in *ics1* and the double mutant *ics1 ics2* demonstrated that ICS2 indeed participates in the biosynthesis of SA. Upon UV exposure, *ics1* and *ics1 ics2* accumulate roughly 10 and 4 % of total SA compared to wild type, respectively. Therefore, the majority of SA (about 95 %) is synthesized from the ICS pathway in UV-treated *Arabidopsis* plants with the remaining through an alternative pathway (Garcion et al. 2008).

ICS homologs have also been identified in a wide variety of plant species (van Tegelen et al. 1999; Ogawa et al. 2005; Uppalapati et al. 2007; Yuan et al. 2007; Catinot et al. 2008). Given their role in phylloquinone synthesis, it is very likely that *ICS* homologs are present in all plant species. However, identification of an *ICS* gene in a given plant species is not sufficient to confirm its role in SA biosynthesis. Nevertheless, isotope-feeding experiment, with the intension to reflect *in planta* metabolism, revealed that most SA is synthesized via the ICS pathway in *Pythium aphanidermatum*-elicitated *C. roseus* cells. In addition, virus-induced gene silencing of *ICS* expression in *N. benthamiana* or tomato suppresses UV- and/or pathogen-induced SA accumulation (Uppalapati et al. 2007; Catinot et al. 2008).

Although it is becoming clear that SA is synthesized via the ICS pathway in various plant species, how isochorismate, the product of ICS, is converted to SA is still unclear. This conversion should be accomplished by an enzymatic reaction since nonenzymatic synthesis of SA from IC is negligible when the reactants are incubated under conditions consistent with chloroplast stroma (Strawn et al. 2007). In addition, it is expected that the enzyme(s) involved in SA synthesis from IC is plastid localized, as transgenic *Arabidopsis* expressing *nahG* fused to a chloroplast localization sequence fails to accumulate SA upon pathogen infection or UV treatment (Fragnière et al. 2011). However, no plant genes encoding IPL activity have been reported (Chen et al. 2009). Thus, whether plants contain IPLs that are structurally unrelated to or highly divergent from the bacterial counterparts or use a metabolic pathway distinct from that in bacteria and, consequently, catalyzed by enzymes unrelated to IPL merits further investigation.

Signal Perception and Execution of Salicylic Acid-Induced Responses

Over the past more than two decades, many genetic screens have been conducted to identify genes that are involved in SA biosynthesis/metabolism, perception, and signal transduction in *Arabidopsis*. These screens have yielded numerous mutants with genetic lesions either upstream or downstream of SA biosynthesis. Furthermore, recent studies have revealed the involvement of epigenetic factors in SA-mediated plant defense signaling. All these have sketched an integrated model for regulation of SA accumulation and a finely tuned SA-mediated defense signaling network. Here, we focus on SA perception and downstream signal execution. For regulation of SA accumulation, readers are referred to the recent review in The *Arabidopsis* Book (Dempsey et al. 2011).

SA Receptors

Although SA plays a pivotal role in galvanizing immune responses, until very recently it was unclear how plant cells perceived SA. There have been serious efforts to identify SA receptors using biochemical purification of SA-binding proteins (SABPs). To date, four types of SABPs have been identified including a catalase, a methyl salicylate esterase, a cytoplasmic ascorbate peroxidase, and a chloroplastic carbonic anhydrase (Du and Klessig 1997; Slaymaker et al. 2002; Kumar and Klessig 2003; Park et al. 2007; Vlot et al. 2008, 2009). Although these SABPs are involved in mediating some aspects of SA metabolism or action, genetic analyses suggested that none of them fulfill the criteria for a bonafide SA receptor, because these molecules do not have functional roles in plant immune signaling. Using different ligand-receptor binding methods, two research groups recently reported that NPR1 (nonexpressor of pathogenesis-related genes1) and NPR1-related proteins, NPR3 and NPR4, are the long-sought-after SA receptors in *Arabidopsis* (Fu et al. 2012; Wu et al. 2012). NPR1, NPR3, and NPR4 are all characterized by a conserved N-terminal BTB/POZ (broad complex, tramtrack, and bric-à-brac/poxvirus, zinc finger) domain and an ankyrin repeat in the middle of the proteins (Cao et al. 1997; Kinkema et al. 2000; Liu et al. 2005).

Using a special equilibrium dialysis ligand binding method, Wu et al. (2012) demonstrated that NPR1 binds to SA when NPR1 and SA are in equilibrium. SA binds strongly to a C-terminal transactivation (TA) domain of NPR1 through Cys^{521} and Cys^{529} via the transition metal copper (Rochon et al. 2006; Wu et al. 2012). Mutations of cysteines to serines or metal chelation abolish the binding of SA by NPR1. In the absence of SA, the NPR1 TA domain is inhibited by the BTB domain and thus fails to activate the expression of SA response genes. However, increased SA concentration upon pathogen infection facilitates binding of SA to Cys^{521}

and Cys^{529} through coordinated copper. Thus, the direct binding of NPR1 to SA and the functional importance of this interaction in plant immunity indicate NPR1 may be an SA receptor in *Arabidopsis*.

The presence of a BTB domain in NPR1 suggests that, like other BTB domain-containing proteins, NPR1 may interact with Cullin 3 (CUL3) E3 ligase and mediate substrate degradation. Even though the substrate for NPR1 has yet to be identified, NPR1 protein itself can be degraded by the proteasome both before and after SAR induction (Spoel et al. 2009). NPR1 paralogs NPR3 and NPR4 are adaptor proteins for the CUL3 E3 ligase that specifically targets NPR1 for degradation in an SA concentration-dependent manner (Fu et al. 2012). NPR1 and NPR4 interact with one another in the absence of SA; SA disrupts this interaction and promotes interaction between NPR1 and NPR3 instead. Using conventional ligand-receptor binding assays, Fu and colleagues (2012) found that the NPR1 protein does not have considerable SA-binding activity under different conditions but two NPR1-related proteins, NPR3 and NPR4, bind to SA with different affinity. Since NPR4 has high affinity for SA (nanomolar range) while NPR3 has low affinity for SA (micromolar range), low SA levels should reduce NPR1 degradation, whereas high SA levels should enhance it. According to the proposed model, in the absence of pathogen infection, NPR4 constantly removes most of the NPR1 protein through CUL3-NPR4-mediated degradation, and basal SA disrupts some of the NPR1–NPR4 interactions, allowing some NPR1 to escape degradation, which is required for keeping basal immunity (PTI). Following pathogen infection, recognition of pathogen effectors by plant resistance proteins induces a high level of SA in local infected tissue, which promotes interaction between NPR1 and NPR3, triggering CUL3-NPR3-mediated NPR1 degradation. As NPR1 is likely a negative regulator of programmed cell death (PCD) during ETI, degradation of NPR1 allows PCD to occur at the site of infection. In systemic tissues, on the other hand, an intermediate level of SA is insufficient to bring about NPR1–NPR3 interaction but high enough to disrupt NPR1–NPR4 interaction and, consequently, enables NPR1 to accumulation, leading to SAR activation. Thus, as SA receptors, NPR3 and NPR4 appear to regulate the homeostasis of NPR1, thus modulating the function of NPR1 in basal immunity, ETI, and SAR.

The seemingly conflicting results on the identification of SA receptors can be attributed to the different experimental approaches used to test the direct binding of SA to NPR1. Crystal structure analysis of NPR1, NPR3, and NPR4 will be the next crucial step to further unravel the binding sites and the exact SA-sensing mechanisms of these receptors. NPR3 and NPR4 may not be the merely SA-binding proteins that facilitate SA-mediated degradation of NPR1 and additional proteins are yet to be discovered (Kaltdorf and Naseem 2013). Alternatively, SA could be perceived by both NPR1 and NPR3/NPR4, resembling the multireceptor sensing of other phytohormones like abscisic acid (Spartz and Gray 2008). Given the fact of the existence of SA-dependent but NPR1-independent defense signaling pathway, in which NPR3/NPR4 may not participate, additional SA perception mechanisms may be present. Furthermore, it has now been well established that SA is also a prominent regulator of plant growth, development, and response to abiotic stresses

(Vicente and Plasencia 2011), suggesting the possible existence of additional SA receptors in plants. Regardless, identification of NPR1, NPR3, and NPR4 as SA receptors represents a great step forward in elucidation of SA immune signaling and is expected to have a long-lasting impact on future research in plant immunity.

NPR1-Dependent SA Signaling

As a central transcription coactivator, NPR1 is responsible for controlling approximately 95 % of SA-dependent genes, thus represents a key node in signaling downstream from SA (Dong 2004; Durrant and Dong 2004; Pieterse and van Loon 2004). The *NPR1* gene promoter contains W-box sequences, which are binding sites of WRKY transcription factors. Mutations in the W-box region of the *NPR1* gene affect its expression, suggesting that WRKY transcription factor(s) is crucial in mediating SA-induced *NPR1* expression (Yu et al. 2001). SA treatment or pathogen inoculation enhances *NPR1* expression. SA also promotes the translocation of NPR1 from cytoplasm to the nucleus. SA-induced changes in cellular redox state lead to reduction of disulfide bonds formed among conserved cysteine residues such as Cys^{82} and Cys^{216} likely though the function of TRX-H5 (thioredoxin-H5) and/or TRX-H3 (Mou et al. 2003; Tada et al. 2008). SA binding to the NPR1 protein appears to also play a role in this oligomer-to-monomer transition (Wu et al. 2012). Nevertheless, mutation of either Cys82 or Cys216 elevates the level of monomeric, nuclear localized NPR1, and consequently upregulates *PR1* gene expression (Mou et al. 2003). Since the NPR1 protein does not have DNA-binding capability, relaying NPR1-mediated signaling requires other transcription factors. Indeed, genome-wide expression profiling analysis indicated that several members of the WRKY transcription factor family act downstream of NPR1 (Wang et al. 2006), and protein–protein interaction assays revealed that NPR1 interacts with at least seven TGA (TGACG motif-binding factor) transcription factors (Zhang et al. 1999; Després et al. 2000; Zhou et al. 2000; Subramaniam et al. 2001; Song et al. 2011) and three structurally related NIMIN (noninducible immunity1 (NIM1)-interacting) proteins (Weigel et al. 2001, 2005).

The TGA transcription factors can directly interact with *PR1* gene promoter through binding to the activator sequence-1 (as-1) element in the promoter (Lebel et al. 1998). *In planta* analyses showed that the interaction between NPR1 and TGA1 and/or TGA4 needs the presence of SA (Després et al. 2000) and that the ability of TGA2 and TGA3 to activate transcription of downstream genes requires both SA and NPR1 (Johnson et al. 2003). In another study, however, interaction between NPR1 and TGA2 was detected in the absence of SA, but the interaction is weaker than in the presence of SA (Fan and Dong 2002). More recent studies suggested that the repressor activity of TGA2 is transformed into an activator activity by its incorporation into a transactivation complex with NPR1 (Rochon et al. 2006; Boyle et al. 2009). All these results indicate that SA and NPR1 likely enhance the DNA-binding activity of certain TGA factors and thus affect the transcription of *PR*

genes (Durrant and Dong 2004). Indeed, mutant characterization confirmed that TGA2, TGA5, and TGA6 function redundantly in SA signaling and SAR and that TGA3 and TGA7 are required for SA-mediated basal immunity (Zhang et al. 2003; Kesarwani et al. 2007; Song et al. 2011).

The NIMIN proteins appear to regulate SA/NPR1 signaling in a negative manner. While *NIMIN3* is expressed constitutively at a low level, both *NIMIN1* and *NIMIN2* are responsive to SA treatment (Weigel et al. 2001; Hermann et al. 2013). Overexpression of *NIMIN1* compromises ETI and SAR, whereas reducing its expression enhances SA-induced *PR1* gene expression (Weigel et al. 2005). NIMIN3 appears to also suppress SA-induced *PR1* gene expression, though to a lesser extent than NIMIN1 (Hermann et al. 2013). It was proposed that the NIMIN proteins act in a strictly consecutive and SA-regulated manner on NPR1 to repress the *PR1* gene at the onset of SAR (Hermann et al. 2013).

In a genetic screen for suppressors of *npr1*, a mutant named *sni1* (*suppressor of npr1-1*, *inducible1*) was identified (Li et al. 1999). The *sni1* mutation restores SA inducibility of *PR* genes and resistance to *npr1-1* and renders plants with a wild-type copy of the *NPR1* gene more sensitive to SAR signals. SNI1 is a nuclear protein with limited similarity to the mouse retinoblastoma protein, a negative transcription regulator, suggesting that SNI1 is likely a negative regulator of SAR (Mosher et al. 2006). Further genetic screens for suppressors of the *sni1* mutation identified a group of proteins including RAD51D (RAS associated with diabetes51d), BRCA2A (breast cancer2a), and SSN2 (suppressor of SNI1,2) that are required for SA-mediated defense gene transcription (Durrant et al. 2007; Wang et al. 2010; Song et al. 2011). Since RAD51D, BRCA2A, and SSN2 are all involved in homologous recombination or DNA repair, these results demonstrated that proteins from homologous recombination or DNA repair pathways play important roles in SA- and NPR1-mediated defense signaling (Moore et al. 2011).

Recent progresses have defined the function of a number of plant Mediator (MED) subunits in SA-mediated plant immune responses. As a conserved multiprotein cofactor of RNA polymerase II (RNAPII), the Mediator complex is recognized as an important player to fine-tune gene-specific and pathway-specific transcriptional reprogramming by acting as an adaptor/coregulator between sequence-specific transcription factor and RNAPII. Mutations in genes encoding the Mediator subunits MED14, MED15, and MED16 all affect SA-induced *PR* gene expression, compromise basal resistance against biotrophic bacterial pathogens, and block biological induction of SAR (Canet et al. 2012; Wathugala et al. 2012; Zhang et al. 2012b, 2013a). However, only *med15* causes SA hyperaccumulation and reduced SA tolerance like *npr1* (Canet et al. 2012). MED16 and NPR1 function largely independently of each other in basal immunity, whereas MED14 and NPR1 have significant overlapping functions in regulating basal immunity. Unlike the *med16* mutation, which differentially affects expression of several SAR positive and negative regulators, *med14* inhibits induction of a large group of defense genes including both SAR positive and negative regulators (Zhang et al. 2012b, 2013a). Both MED14 and MED15 appear to function downstream of NPR1 and do not affect NPR1 nuclear localization and/or stability (Canet et al. 2012; Zhang et al. 2013a),

whereas MED16 positively contributes to NPR1 protein accumulation (Zhang et al. 2012b). Interestingly, although the *med8* mutant displays enhanced susceptibility to bacterial pathogens, it has no significant defects in biological induction of SAR (Kidd et al. 2009; Zhang et al. 2012b). Furthermore, mutations in *MED25* attenuate the induction of SA-responsive genes but have no significant effects on resistance to biotrophic bacterial pathogens and biological induction of SAR (Kidd et al. 2009; Zhang et al. 2012b). Thus, these Mediator subunits employ distinct mechanisms to regulate SA-mediated defense gene expression and pathogen resistance.

NPR1-Independent SA Signaling

In *Arabidopsis*, ETI is suppressed by expression of the *nahG* gene, but not by the *npr1* mutation, suggesting the presence of NPR1-independent SA signaling in plant immunity (Raridan and Delaney 2002; Kachroo et al. 2001; Takahashi et al. 2002). The existence of NPR1-independent SA signaling is further supported by the results from characterization of a group of *Arabidopsis* mutants that either display SA inducibility of *PR* genes or constitutively accumulate SA and *PR* gene transcripts in the absence of a functional *NPR1* gene. The *sni1* mutation confers SA inducibility of *PR* genes to the *npr1-1* mutant, suggesting an NPR1-independent mechanism (Li et al. 1999). More components in the NPR1-independent SA signaling pathway were identified through screening for suppressors of the *npr1-5* mutant. The *ssi* (*suppressor of SA insensitivity*) *npr1* double mutants *ssi1 npr1*, *ssi2 npr1*, and *ssi4 npr1* constitutively accumulate SA and exhibit heightened resistance to a variety of pathogens (Shah et al. 1999, 2001; Shirano et al. 2002). The *ssi1* and *ssi2* single mutants accumulate higher levels of *PR1* gene transcripts than the *ssi1 npr1* and *ssi2 npr1* double mutants, respectively, indicating an NPR1-independent pathway functioning additively with the NPR1-dependent pathway (Shah et al. 1999, 2001). Another *npr1* suppressor, *snc1* (*suppressor of npr1-1 constitutive1*), displays constitutive SA-dependent, NPR1-independent resistance owning to a mutation in a Toll-interleukin-1 receptor-nucleotide binding site-leucine-rich repeat type *R* gene. The gain-of-function *snc1* mutation leads to constitutive activation of the R protein and downstream immune responses without the presence of pathogens. The *snc1* mutant also accumulates high levels of SA, constitutively expresses *PR* genes, and displays enhanced resistance to pathogens (Li et al. 2001). Further genetic screens for suppressors of *snc1* identified a series of *mos* (*modifier of snc1*) mutations affecting signal transduction downstream of *snc1* (Zhang and Li 2005). New members of the *snc* mutants such as *snc2-1D* (*suppressor of npr1-1*, *constitutive 2-1D*) and *snc4-1D* have been identified and characterized (Bi et al. 2010; Zhang et al. 2010b). Moreover, a set of genes that may be involved in SA-regulated, NPR1-independent signaling pathway encode WHIRLY (WHY) and MYB transcription factors. The single-stranded DNA-binding activity of WHY1 is stimulated by SA treatment in both wild-type and *npr1* mutant plants (Desveaux et al. 2002, 2004), indicating its important role in NPR1-independent *PR1* expression and resistance against

pathogens. The *Arabidopsis MYB30* (*myeloblastosis30*) gene positively regulates the HR in an SA-dependent, NPR1-independent manner (Raffaele et al. 2006). Additionally, the *cpr5* (*constitutive expressor of PR genes5*), *cpr6*, and *hrl1* (*hypersensitive response-like lesions1*) mutants exhibit NPR1-independent and SA-dependent immune phenotypes (Clarke et al. 2000; Devadas et al. 2002). Interestingly, the *cpr5*, *cpr6*, and *hrl1* mutations also activate jasmonic acid (JA)- and ethylene (ET)-mediated immune responses, indicating that the SA-dependent, NPR1-independent signaling may function synergistically with the JA/ET-mediated defense pathways (Clarke et al. 2000; Devadas et al. 2002).

In a genetic screen for suppressors of the *npr1* mutant based on its intolerance to SA, an *elp2* (*Elongator subunit2*) mutant allele was isolated (DeFraia et al. 2010). ELP2 is one of the six subunits of the Elongator complex, which interacts with elongating RNAPII to facilitate transcription (Winkler et al. 2002; Close et al. 2006). Despite the structural diversity of the Elongator subunits, loss of any Elongator subunit generally compromises its integrity and renders the complex inactive (Versées et al. 2010; Glatt et al. 2012). The Elongator catalytic subunit ELP3/ELO3 (ELONGATA3) harbors a C-terminal histone acetyltransferase (HAT) domain and an N-terminal cysteine-rich motif that resembles an iron-sulfur radical *S*-adenosylmethionine (SAM) domain (Chinenov 2002; Winkler et al. 2002; Nelissen et al. 2005). Both the HAT and SAM domains are required for Elongator's function in plant immunity (DeFraia et al. 2013). Mutations in *ELP2* and *ELP3* restore SA tolerance to *npr1*, suppress *npr1*-mediated hyperaccumulation of SA, and delay the induction of SA accumulation and defense gene expression (DeFraia et al. 2010, 2013). Although Elongator regulates the NPR1 transcriptional cascade, Elongator and NPR1 appear to function largely independently of each other in ETI, and mutations in *ELP2* and *ELP3* do not affect SAR (DeFraia et al. 2010, 2013). Further mutant characterization revealed that ELP2 is an epigenetic regulator required for *P. syringae*-induced rapid transcriptome reprogramming likely through maintaining histone acetylation levels in defense genes, modulating genomic DNA methylation landscape, and influencing pathogen-induced dynamic DNA methylation changes (Wang et al. 2013). Such chromatin modification has recently been described as an additional layer of regulation on plant immunity. Several reports have shown that the state of histone acetylation or DNA methylation is associated with SA-mediated defense responses (Mosher et al. 2006; Butterbrodt et al. 2006; Koornneef et al. 2008; van den Burg and Takken 2009; Choi et al. 2012; Luna et al. 2012). Compared with other epigenetic regulators, Elongator is unique in that it regulates both histone acetylation and DNA methylation status of defense-related genes (Winkler et al. 2002; Nugent et al. 2010; Xu et al. 2012). The NPR1 transcriptional cascade exemplifies a signal cascade where Elongator modulates the chromatin structure of both the key transcription regulator and its target genes, forming a transcriptional feed-forward loop and determining the kinetics of the transcription. However, the mechanism of the cooperative interaction between the specific transcription regulator NPR1 and the chromatin modulator Elongator in regulating gene transcription during immune responses is still unclear.

Biotechnological Manipulation of Salicylic Acid Signaling and Biosynthesis in Agriculture

Disease is a major threat to the yield and quality of crop plants worldwide. One major goal in plant science is the production of crops with increased and durable resistance to a spectrum of pathogens. Compared with other approaches employed to develop disease-resistant crops, genetic engineering is faster and allows transference of individual traits into crops in a calculated manner. Strategies for developing transgenic disease resistance have been evolved from overexpression of a single or combination of a small number of genes, which suffer from either incomplete efficacy or durability, to modification of existing innate signaling pathways, which can activate a battery of defense responses (Collinge et al. 2010). The accumulating knowledge of SA-mediated defense signaling pathways provides new opportunities for manipulating plant disease resistance. Several genes have received attention with respect to possible exploitation for developing transgenic disease-resistant crops. Among them *NPR1* is the most promising gene for generating broad-spectrum disease-resistant crop plants.

The *NPR1* gene was originally discovered in several independent genetic screens performed in *Arabidopsis*. The *npr1* (also known as *nim1* and *sai1* (*salicylic acid-insensitive1*)) mutants are unable to either mount a SAR response or accumulate *PR* transcripts and are hypersusceptible to biotrophic pathogens (Cao et al. 1994; Delaney et al. 1994; Shah et al. 1997). The original study in *Arabidopsis* using *NPR1* showed that overexpression of this gene increases resistance to two diverse biotrophic pathogens, the bacterium *P. syringae* pv. *maculicola* and the oomycete *Hyaloperonospora arabidopsidis* (Cao et al. 1998; Table 1). Since then transgenic studies using *NPR1* or its orthologs from other species have been extended to a large group of crop plants for resistance against pathogens with either biotrophic or necrotrophic lifestyle (Tables 1 and 2). In addition, overexpression of *NPR1* seems to enhance resistance to insect and root-knot nematode in tobacco plants (Meur et al. 2008; Priya et al. 2011). Interestingly, the majority of the transgenic plants display little or no constitutive expression of *PR* genes; rather, the transgenic plants exhibit a "primed" phenotype where induction of *PR* genes is faster, at higher intensity, and for a longer duration, resulting in a heightened capacity to undergo SAR when challenged with pathogens or treated with SA analogs. However, transgenic rice expressing either *NPR1* or the rice ortholog *OsNH1* (*Oryza sativa NPR1 HOMOLOGUES1*) is different, which exhibits constitutive expression of *PR* genes (Fitzgerald et al. 2004; Quilis et al. 2008).

Another avenue for boosting SA-mediated plant immunity is to manipulate SA biosynthesis. Tobacco plants overexpressing heterologous *PAL* transgenes display enhanced resistance to the fungal pathogen *Cercospora nicotianae* and the oomycete *Phytophthora parasittica* pv. *nicotianae* (Felton et al. 1999; Way et al. 2002). However, based on comparison of *PAL*-overexpressing plants and *PAL-overexpressing* plants harboring a *nahG* gene, which compromises SA accumulation, it has been suggested that the accumulation of phenylpropanoid intermediates

Table 1 Use of the *Arabidopsis NPR1* gene in transgenic disease resistance

Recipient plant	Pathogen resistance	Other resistance	Note	Reference
Arabidopsis thaliana	*Pseudomonas syringae* pv. *maculicola* and pv. *tomato*, *Erysiphe cichoracearum*, *Hyaloperonospora arabidopsidis*, and *Fusarium graminins*	N/A	Pathogens resistance is proportional to the NPR1 protein level; without notable yield penalty; observed fitness disadvantage in some conditions	Friedrich et al. (2001), Cao et al. (1998), Makandar et al. (2006), Heidel and Dong (2006)
Grapefruit/sweet orange	*Xanthomonas citri* subsp. *citri*	N/A	Grapefruit has fewer lesions and lower bacterial populations; no significant difference for sweet oranges	Zhang et al. (2010a)
Cotton	*Verticillium dahliae* isolate TS2, *F. oxysporum* f.sp. *Vasinfectum*, *Rhizoctonia solani*, and *Alternaria alternata*	Reniform nematode	Overexpression plants phenotypically normal; not resistance to all *V. dahliae* isolates	Parkhi et al. (2010a, b), Kumar et al. (2013)
Carrot	*Botrytis cinerea*, *Alternaria radicina*, *Sclerotinia sclerotiorum*, *E. heraclei*, *X. hortorum*, and *Thielaviopsis basicola*	N/A	Overexpression plants phenotypically normal	Wally et al. (2009)
Tomato	*F. oxysporum*, *Stemphylium solani*, *Ralstonia solanacearum*, and *X. campestris*	N/A	No adverse effects on growth or yield; enhanced susceptibility to *B. cinerea*	Lin et al. (2004), El Oirdi et al. (2011)
Rice	*X. oryzae*, *Erwinia chrysanthemi*, *Magnaporthe oryzae*, and *F. verticillioides*	N/A	Deleterious effect on rice growth; BTH- and environment-induced lesion-mimic/cell death phenotype; increased sensitivity to salt and virus	Fitzgerald et al. (2004), Quilis et al. (2008)
Tobacco	N/A	Nematode and insect	Up to 50 % improved resistance to both; proportional to *NPR1* expression levels; enhanced oxidative stress tolerance	Meur et al. (2008), Srinivasan et al. (2009), Priya et al. (2011)
Wheat	*F. graminearum*	N/A	Rapid defense response; 25 % infection level comparing to wild type; no yield penalty in lab	Makandar et al. (2006)
Canola	*P. syringae* pv. *tomato*	N/A	Effectively enhances basal resistance against *P. syringae* pv. *tomato*	Potlakayala et al. (2007)

Table 2 Use of *NPR1* orthologs in transgenic disease resistance

Source gene	Recipient plant	Pathogen resistance	Notes	Reference
Mustard *NPR1*	Mung bean	*Rhizoctonia solani*	No dry rot symptoms on transgenic shoots	Vijayan and Kirti (2012)
Soybean *NPR1*	*Arabidopsis*	*Pseudomonas syringae* pv. *tomato*	Soybean *NPR1-1* and *NPR1-2* complement the *Arabidopsis npr1-1* mutation; comparable levels of protection from both soybean orthologs as from *AtNPR1*	Sandhu et al. (2009)
Malus hupehensis NPR1	Tobacco	*Botrytis cinerea*	Increased resistance to fungus *B. cinerea*	Zhang et al. (2012a)
Malus hupehensis NPR1	Fuji apple	*Podosphaera leucotricha*	Induces *PR* gene expression and promotes SAR	Chen et al. (2012)
Malus pumila NPR1	Galaxy and M26 apple varieties	*Erwinia amylovora*, *Venturia inaequalis*, and *Gymnosporangium juniperi-virginianae*	Both varieties show significantly increased disease resistance	Malnoy et al. (2007)
Rice *NPR1*	*Arabidopsis*	*P. syringae* pv. *tomato*	Partially complements the *Arabidopsis npr1* mutation	Yuan et al. (2007)
Rice *NPR1*	Rice	*Magnaporthe oryzae* and *X. oryzae* pv. *oryzae*	Overexpressors are more resistant; RNAi lines are more susceptible; spontaneous lesions observed	Chern et al. (2005); Yuan et al. (2007); Feng et al. (2011)
Cacao *NPR1*	*Arabidopsis*	*P. syringae* pv. *tomato*	Partially complements the *Arabidopsis npr1* mutation	Shi et al. (2010)
Canola *NPR1*	*Arabidopsis*	*P. syringae* pv. *tomato*	Restores *PR1* gene expression; enhanced basal defense and SAR against *P. syringae* pv. *tomato*	Potlakayala et al. (2007)
Canola *NPR1*	Canola	*P. syringae* pv. *tomato*	Effectively enhances basal resistance against *P. syringae* pv. *tomato*	Potlakayala et al. (2007)
Grape *NPR1*	*Arabidopsis*	*P. syringae* pv. *maculicola*	Complements the *Arabidopsis npr1* mutation; increases tolerance to salinity but has not effect on the drought tolerance	Le Henanff et al. (2009, 2011), Bergeault et al. (2010), Zhang et al. (2013b)
Pepper *NPR1*	Tobacco	*Ralstonia solanacearum*	Resistance to *R. solanacearum* is coupled with enhanced transcript levels of defense-related maker genes	Dang et al. (2012)

such as chlorogenic acid is primarily responsible for the enhanced resistance to *C. nicotianae* in *PAL*-overexpressing plants, whereas SA accumulation has limited contributions (Shadle et al. 2003). Nevertheless, targeting the bacterial SA biosynthesis enzymes ICS and IPL to chloroplasts in transgenic tobacco plants increases SA and SA glucoside accumulation, leading to constitutive expression of defense genes and resistance to viral and fungal infection (Verberne et al. 2000). Importantly, overaccumulation of SA in transgenic tobacco plants does not affect plant growth, which is crucial for engineering disease-resistant crops. However, targeting a functional fusion enzyme of the bacterial ICS and IPL to chloroplasts in *Arabidopsis* strongly inhibits plant growth and significantly reduces seed production (Mauch et al. 2001).

As an increasing number of important SA signaling components are discovered, the list of candidate genes for genetic manipulation grows. Interestingly, many of the SA signaling components also plays important roles in nonhost resistance, which is the most common form of resistance exhibited by plants against a wide variety of microbial pathogens (An and Mou 2011). Therefore, manipulating these genes in crop species hold the potential to boost both host and nonhost resistance. However, limited investigations have been conducted on utilizing nonhost resistance to develop disease-resistant crops. Furthermore, manipulating SA-mediated immune responses through suppression of negative regulators or activation of positive regulators represents an attractive strategy for engineering disease resistance (Gurr and Rushton 2005b; Salomon and Sessa 2012). Thus far, the function of many defense regulators in manipulating disease resistance has been tested in *Arabidopsis*, but the efforts of translating these technologies to crops still lag behind.

It should be noted that because of the involvement of SA in diverse physiological processes other than plant immunity, increasing SA biosynthesis or signaling might lead to fitness penalties. Although little evidence for fitness penalties has been found for overexpression of *NPR1* in the laboratory, one study using controlled environments suggested that there seem to be fitness penalties for overexpression of *NPR1* under high nutrient conditions (Heidel and Dong 2006). To minimize the cost of defense activation on plant growth, pathogen- or chemical-inducible and tissue-specific promoters may be useful as they limit the cost of resistance by controlling temporal and spatial expression of the defense genes (Gurr and Rushton 2005a).

Although our understanding of the role of SA in plant defense against pathogens has increased considerably over the last two decades, much still remains to be elucidated. Among them, SA biosynthesis in plants is still not fully understood and the central signaling components, such as NPR1, still require more in-depth studies. Additionally, SA-mediated defense signaling pathways and other defense pathways are not isolated but rather interconnected to form a well-regulated network. Elucidating genetic components, especially those connecting multiple defense pathways, will continue to be a major task of the research community. On the other hand, understanding of SA-mediated plant defense has facilitated development of more effective ways for controlling important crop diseases. While gene efficacy in transgenic plants has often been good, field trials of transgenic disease-resistant crops have been hampered by ethical concerns. In this regard, the recently

developed cisgenic approach (Schouten et al. 2006), which utilizes target crop-derived genes and regulatory elements (promoters) together with improved transformation methods that do not rely on or subsequently eliminate selective marker genes, has the potential to develop resistant cultivars more acceptable to consumers.

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