8

Signaling in Plant Resistance Responses: Divergence and Cross-Talk of Defense Pathways

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8.1 Introduction

Plants possess inducible defense mechanisms to protect themselves against attack by microbial pathogens and herbivorous insects. The endogenous signaling molecules salicylic acid, ethylene, and jasmonic acid, and the peptide messenger systemin play important roles in the regulation of these induced defense responses. Disease resistance of plants can also be induced by chemical agents, such as 2,6-dichloroisonicotinic acid, benzothiadiazole, and the non-protein amino acid β-aminobutyric acid. In most cases, these chemical agents mimic or ingeniously make use of the same pathways that are activated by the endogenous defense signals. This review is focussed on the current state of research on signal transduction pathways involved in induced resistance against pathogens and insects. Recent advances in induced resistance research revealed that the signaling pathways involved are interconnected, resulting in overlap, synergism, and antagonism between the different signal transduction pathways. Divergence and crosstalk of pathways in defense response signaling provide the plant with flexibility and the opportunity for fine-tuning of resistance responses, thereby enabling it to cope with different forms of stress more efficiently.

8.2 Salicylic Acid Induces Systemic Resistance Responses

Over the past decade it became increasingly clear that the endogenous signal salicylic acid (SA) serves multiple roles in plants. For example, SA is involved in the regulation of cell growth (Vanacker et al., 2001), flowering, and thermogenesis (for reviews, see Malamy and Klessig, 1992; Raskin, 1992; Klessig and Malamy, 1994; Shah and Klessig, 1999). SA also plays a crucial role in plant defense against

pathogens by affecting lesion formation (Weymann et al., 1995) and by activating induced disease resistance (Dempsey et al., 1999; Shah and Klessig, 1999; Nawrath et al., in this volume). The latter is variously referred to as systemic acquired resistance (SAR) or induced systemic resistance (ISR). Although these terms are synonymous (Hammerschmidt et al., 2001), we refer to the SA pathway-dependent, induced disease resistance as SAR. SAR is characterized by a long-lasting resistance against a broad spectrum of pathogens both at the initial infection site and in the distal, uninoculated organs. The most compelling evidence for the important role of SA in the onset of SAR comes from studies with transgenic tobacco and *Arabidopsis* plants expressing the *NahG* gene from *Pseudomonas putida.* This gene encodes a salicylate hydroxylase, which destroys the SA signal by converting it to catechol. Upon pathogen attack, *NahG* transgenic tobacco and *Arabidopsis* plants do not accumulate enhanced levels of SA nor do they establish SAR (Gaffney et al., 1993; Delaney et al., 1994). The SAR state is activated by many microbes that cause tissue necrosis but it can also be induced by exogenous application of SA or its functional analogs 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) (Ryals et al., 1996; Sticher et al., 1997; Dempsey et al., 1999).

The onset of SAR is associated with an early increase in endogenous SA levels and with the immediate expression of a specific set of so-called *SAR* genes, some of which encode pathogenesis-related (PR) proteins (Ryals et al., 1996; Sticher et al., 1997; Dempsey et al., 1999). While it is known that some PR proteins display antimicrobial activity (Van Loon and Van Strien, 1999), their actual role in SAR is still unclear and can depend on the plant-pathogen system. In fact, a strict correlation between increased accumulation of PR proteins before challenge pathogen attack and SAR has not always been observed. To gain a better understanding of the mechanisms that contribute to SAR, it is necessary, therefore, to study further defense-associated cellular events that are induced faster or to a greater extent in attacked, SAR-protected plants. Such events include the activation of defense-related genes other than those encoding PR proteins, and the deposition of callose (Kohler et al., 2002).

In addition to *SAR* gene expression, SAR is also associated with priming (sensitizing) which enhances the plant's capacity for the rapid and effective activation of cellular defense responses, that are induced only upon contact with a (challenging) pathogen (Kuć, 1987; Katz et al., 1998; Conrath et al., 2002). These responses include hypersensitive cell death (Mittler and Lam, 1996), cell wall fortification (Hammerschmidt and Kuć, 1982; Stumm and Gessler, 1986; Schmele and Kauss, 1990), the production of reactive oxygen species (Doke et al., 1996), and the activation of defense-related genes (Ryals et al., 1996; Sticher et al., 1997).

The role of SA in *PR* gene expression as a part of SAR is discussed by Nawrath et al. and will therefore not be discussed here in detail. This section of our review will rather focus on the progress made in elucidating the role of SA in priming for potentiated activation of cellular defense responses.

8.2.1 *Salicylic Acid-Induced Priming in a Cell Culture Model System*

Over the past 13 years, it has been reported that a pretreatment of parsley cell cultures with low doses of the SAR inducers SA, INA, and BTH did not directly induce various assayed, cellular defense responses (Kauss et al., 1992a; 1993; Kauss and Jeblick, 1995; Thulke and Conrath, 1998; Katz et al., 1998; 2002). Yet, a preincubation with the SAR inducers primed the cells for potentiated (augmented) activation of defense responses, that were subsequently induced by otherwise noninducing doses of an elicitor from *Phytophthora sojae* cell walls (Kauss et al., 1992a; 1993; Kauss and Jeblick, 1995; Thulke and Conrath, 1998; Katz et al., 1998; 2002). The potentiated responses include the early oxidative burst (Kauss and Jeblick, 1995), a rapidly induced K^+/pH response (Katz et al., 2002), the incorporation into the cell wall of various phenolics and a lignin-like polymer (Kauss et al., 1993), and the secretion of antimicrobial coumarin phytoalexins resulting from an enhanced activity of coumarin biosynthetic enzymes (Kauss et al., 1992a) and augmented expression of some of the genes encoding these enzymes (Kauss et al., 1992a; 1993; Katz et al., 1998; Thulke and Conrath, 1998). In a similar manner, in soybean suspension cells, physiological concentrations of SA strongly augmented defense gene activation, H_2O_2 accumulation, and the hypersensitive necrosis response (HR) that was induced by treatment with avirulent *Pseudomonas syringae* pv. *glycinea* (Shirasu et al., 1997). However, since the SA-mediated potentiation of defense responses in soybean cells did not depend on prolonged pre-treatment with SA, this mechanism of regulation obviously differs from the time-dependent priming in cultured parsley cells. Together, the observations made with parsley and soybean suspension cells revealed that plant cell cultures can be suitable model systems for studying the SA-, INA-, and BTH-induced priming for potentiated activation of cellular plant defense responses.

8.2.2 *Salicylic Acid Serves a Dual Role in the Activation of Defense Responses*

While elucidating the influence of SA and BTH on the activation of defense-related genes in the parsley cell culture, it became obvious that the inducer's effect on gene activation depends on the gene that is being monitored (Katz et al., 1998; Thulke and Conrath, 1998). One set of genes, such as those encoding anionic peroxidase and mannitol dehydrogenase, was found to be directly induced by relatively low concentrations of the two SAR inducers tested (Katz et al., 1998; Thulke and Conrath, 1998). A second set of parsley defense-related genes, including those encoding phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase, intracellular PR-10 proteins and a hydroxyproline-rich glycoprotein, was only faintly responsive to the treatment with relatively low concentrations of SA or BTH. Yet, already at low inducer concentrations, these genes displayed SA- and BTH-dependent potentiation of their expression following treatment with a low elicitor dose (Katz et al., 1998; Thulke and Conrath, 1998). For instance, more than

0.5 mmolar SA was required to activate *PAL* using only SA, whereas as little as 0.01 mmolar SA greatly potentiated the activation of the *PAL* gene by an otherwise faintly inducing elicitor concentration (Thulke and Conrath, 1998). These results revealed a dual role for SAR inducers in the activation of plant defense responses: a direct one in the immediate induction of certain defense genes at higher inducer concentrations, and an indirect one which requires only low doses of the inducers to prime for potentiated activation of another class of defense genes. As the potentiation by SA and BTH of both elicited *PAL* gene expression and coumarin secretion strongly depended on an extended preincubation period, the SAR inducers are assumed to mediate a time-dependent response that shifts the cells on the alert (Katz et al., 1998; Thulke and Conrath, 1998). Whether this shift includes the proposed synthesis of cellular factors with crucial roles in the coordination and expression of cellular defense responses remained uncertain.

Similar observations to those made in parsley have been reported for cowpea seedlings (Latunde-Dada and Lucas, 2001). The BTH-mediated SAR response of cowpea is associated with rapid and transient increases in the activity of PAL and chalcone isomerase followed by accelerated accumulation of kievitone and phaseollidin phytoalexins in infected hypocotyls. These responses were not observed in induced, uninoculated tissues, suggesting that the protection of cowpea seedlings by BTH is mediated via potentiation of early defense mechanisms (Latunde-Dada and Lucas, 2001). In cucumber hypocotyls with INA-induced SAR (Fauth et al., 1996), and in wounded soybean tissue (Graham and Graham, 1994), potentiation was also detected for the development of elicitation competency. Whether the enhanced induction of elicitation competency is based on a similar priming mechanism to the one described above for parsley cells is unclear.

8.2.3 *Activators of SAR Induce Priming in* Arabidopsis

In *Arabidopsis*, BTH directly activates *PR-1* and primes the plants for potentiated *PAL* gene expression induced by phytopathogenic *Pseudomonas syringae* pv. *tomato* (*Pst*) (Kohler et al., 2002). BTH-induced priming also augments both *PAL* gene activation and callose deposition induced by either mechanically wounding the leaves with forceps or infiltrating them with water (Kohler et al., 2002). These observations with *Arabidopsis* not only confirm the above described dual role for SAR inducers in the activation of cellular plant defense responses, they also suggest that priming might be common to several signaling pathways, mediating crosstalk between pathogen defense and wound or osmotic stress responses (see below).

Intriguingly, when SAR was biologically induced by previous infection of *Arabidopsis* with an avirulent strain of *Pst,* there was potentiated activation of both the *PAL* and the *PR-1* gene upon challenge infection with virulent *Pst* (Cameron et al., 1999; Van Wees et al., 1999; Kohler et al., 2002). Priming is thus likely to play an important role not only in chemically induced but also in pathogen-activated SAR of plants. The same conclusion was drawn from studies with SA-primed transgenic tobacco plants displaying potentiated expression of chimeric

Asparagus officinalis PR-1::GUS and *PAL-3::GUS* defense genes after wounding or pathogen attack (Mur et al., 1996). The *Arabidopsis edr1* mutant constitutively displays enhanced resistance to *Pst* (strain DC3000) and to the fungal pathogen *Erisyphe cichoracearum* (Frye and Innes,1998). Interestingly, *edr1* differs from other enhanced disease resistance mutants because it shows no constitutive expression of *PR-1* and *PR-2*, although transcripts of both of these genes accumulate after pathogen attack. This finding, and the fact that *edr1* shows stronger expression of defense responses, such as the HR and callose deposition, after infection strongly suggest an involvement of EDR1 in priming. *EDR1* codes for a putative mitogen-activated protein kinase kinase kinase (MAPKKK) and mediates disease resistance via SA-inducible defense responses (Frye et al., 2001). Future mutational approaches in *Arabidopsis* are expected to yield more genes that play a role in priming.

The *Arabidopsis npr1* mutant (also known as *nim1* or *sai1*) accumulates wild-type levels of SA when treated with avirulent pathogens but is unable to mount biologically or chemically induced SAR (Cao et al., 1994; Delaney et al., 1995; Shah et al., 1997). Interestingly, the potentiation by BTH-priming of both *Pst*-induced *PAL* gene activation and wound- or water infiltration-induced *PAL* gene expression and callose deposition are absent in *npr1* (Kohler et al., 2002). The *Arabidopsis cpr1* and *cpr5* mutants, on the other hand, which express constitutive SAR in the absence of a pretreatment with SAR inducers (Bowling et al., 1994; 1997), are permanently primed for potentiated *PAL* gene activation by *Pst* infection and for augmented *PAL* gene expression and callose deposition upon wounding or water infiltration (Kohler et al., 2002). Constitutive priming in *cpr1* and *cpr5* could be due to the expression of a multiplicity of defense-related genes in these plants, or the activation of other stress response mechanisms besides SAR (Boch et al., 1998; Clarke et al., 2001), although these possibilities remain remote. More likely, however, the enhanced levels of SA in *cpr1* and *cpr5* (Bowling et al., 1994; 1997) cause a permanently primed (alarm) state. Because of constitutive priming, *cpr1* and *cpr5* might be able to rapidly and effectively induce their various cellular defense mechanisms, thus leading to enhanced resistance to pathogens, wounding, or water infiltration (Kohler et al., 2002). In this context it is noteworthy that the constitutively enhanced pathogen resistance of another *Arabidopsis* mutant, *cpr5-2*, has been ascribed to the potentiated induction of the *PR-1* gene upon infection with virulent *Pseudomonas syringae* strains (Boch et al., 1998). There is evidence that a null *eds1* mutation suppresses the disease resistance of both *cpr1* and *cpr6* but only partially that of *cpr5*, indicating a different requirement of *CPR* genes for EDS1 (Clarke et al., 2001). EDS1 also likely plays a role in priming in connexion with PAD4 (Jirage et al., 2001). Although both proteins act upstream of pathogen-induced SA accumulation, their expression can be potentiated by SA-pre-treatment of the plants. It has been proposed that EDS1 is involved in the amplification of defense responses, possibly by associating with PAD4 (Feys et al., 2001).

The strong correlation between the presence of SAR and priming supports the conclusion that priming is an important mechanism for SAR in plants. This

assumption is further substantiated by the close correlation between the ability of various chemicals to induce SAR against tobacco mosaic virus (TMV) in tobacco (Conrath et al., 1995) and their capability to prime for potentiated *PAL* expression induced by either elicitor treatment in parsley cells (Katz et al., 1998; Thulke and Conrath, 1998) or *Pst* infection, wounding, or water infiltration in *Arabidopsis* plants (Kohler et al., 2002). In addition, in *NahG*-transgenic tobacco plants that are unable to establish SA-mediated priming, both the onset of the HR and the activation of an active oxygen-responsive chimeric *Asparagus officinalis PR-1::GUS* reporter gene were significantly delayed when infected with avirulent *Pseudomonads*. The attenuation of priming and the loss of potentiated production of active oxygen species were accompanied by a lack of resistance to the bacteria (Mur et al., 2000). Furthermore, overexpressing the disease resistance gene *PTI5* in tomato potentiates pathogen-induced defense gene expression and enhances the resistance to *Pst* (He et al., 2001). Finally, a complete or partial inactivation of the MLO protein was shown to prime young barley seedlings for potentiated induction of defense responses associated with enhanced resistance against powdery mildew (Büschges et al., 1997).

8.3 Jasmonic Acid and Ethylene: Important Signals in Plant Defense Responses

Apart from SA, the defense signaling molecules jasmonic acid (JA) and ethylene (ET) have also been implicated in the regulation of resistance responses. In many cases, infection by microbial pathogens and attack by herbivorous insects was shown to be associated with enhanced production of these phytohormones and a concomitant activation of distinct sets of defense-related genes (De Laat and Van Loon, 1981; Gundlach et al., 1992; Peña-Cortés et al., 1993; Mauch et al., 1994; Reymond et al., 2000; Schenk et al., 2000). Compelling evidence for a role of JA and ET in disease resistance came from genetic analyses of mutants and transgenic plants that are affected in the biosynthesis or perception of these compounds. In many plant–pathogen interactions, JA and ET appeared to be involved in local and/or systemic induction of defense responses.

8.3.1 Genetic Evidence for a Role of Jasmonic Acid and Ethylene in Pathogen Resistance

Genetic evidence of a role for JA in plant defense came particularly from analyses of *Arabidopsis* mutants affected in the biosynthesis or perception of JA. The JA-response mutant *coi1* displays enhanced susceptibility to the necrotrophic fungi *Alternaria brassicicola* and *Botrytis cinerea* (Thomma et al., 1998), and the bacterial soft-rot pathogen *Erwinia carotovora* (Norman-Setterblad et al., 2000). Another JA-insensitive *Arabidopsis* mutant, *jar1*, allows enhanced growth of *Pst* in the leaves (Pieterse et al., 1998). These findings demonstrate that JA-dependent defense responses contribute to the basal resistance of *Arabidopsis* against

different microbial pathogens. Furthermore, both *jar1* and the *fad3 fad7 fad8* triple mutant of *Arabidopsis*, which is deficient in the biosynthesis of the JA precursor linolenic acid, exhibit susceptibility to normally nonpathogenic soilborne *Pythium* spp. (Staswick et al., 1998; Vijayan et al., 1998), indicating that JA also plays a role in nonhost resistance. A role for JA in defense against herbivorous insects is indicated by the observation that the *Arabidopsis fad3 fad7 fad8* mutant exhibited extremely high mortality after attack by larvae of the common saprophagous fungal gnat, *Bradysia impatiens* (McConn et al., 1997). Furthermore, a JA-deficient tomato mutant, *def-1*, was found to be compromised in the wound-inducible expression of defense genes and resistance to *Manduca sexta* larvae (Howe et al., 1996).

The role of ET in plant resistance seems more ambiguous. In some cases, ET is involved in disease resistance, whereas in other cases it is associated with symptom development. For instance, several ET-insensitive mutants of *Arabidopsis* have been reported to exhibit enhanced susceptibility to *B. cinerea* (Thomma et al., 1999), *Pst* (Pieterse et al., 1998), and *E. carotovora* (Norman-Setterblad et al., 2000), indicating that ET-dependent defense responses contribute to basal resistance against these pathogens. A similar phenomenon was observed in tomato and soybean mutants with reduced sensitivity to ET, which developed more severe symptoms when infected by the fungal pathogens *B. cinerea* (Díaz et al., 2002), *Septoria glycinea*, or *Rhizoctonia solani* (Hoffman et al., 1999). In addition, ET-insensitive tobacco plants transformed with the mutant ET receptor gene *etr1* from *Arabidopsis* displayed susceptibility to the normally nonpathogenic oomycete *Pythium sylvaticum* (Knoester et al., 1998). Thus, ET obviously also plays a role in nonhost resistance. In other cases, reduced ET sensitivity was associated with disease tolerance. For example, ET-insensitive tomato genotypes allowed growth of virulent *Pst* and *Xanthomonas campestris* pv. *vesicatoria* to levels similar to those in wild-type tomato plants, but developed less severe disease symptoms (Lund et al., 1998; Ciardi et al., 2000). A similar phenomenon was found in the ET-insensitive *ein2* mutant of *Arabidopsis*, which displayed increased tolerance to virulent *Pst* and *X. campestris* pv. *campestris* (Bent et al., 1992). In addition, soybean mutants with reduced sensitivity to ET developed disease symptoms similar or less-severe than those in the wild type when infected with the bacterial pathogen *P. syringae* pv. *glycinea* or the oomycete *Phytophthora sojae* (Hoffman et al., 1999). In these interactions, ET is clearly involved in symptom development, rather than in disease resistance.

The dual role of ET in plant defense might reflect its involvement in various physiological processes in the plant. ET plays an important role in senescence (Abeles et al., 1992) and lesion development of hypersensitively reacting plant tissues (Knoester et al., 2001). Since necrotrophic pathogens feed on dead cells, both functions of ET might be favorable for the development of disease caused by such types of pathogens. Biotrophic pathogens, in contrast, need living cells to complete their life cycle. Thus, the same functions of ethylene might help to restrict these types of pathogens. Support for this hypothesis comes from experiments with hypersensitively reacting *Arabidopsis* plants. On the one hand, the hypersensitively

responding tissue was more susceptible to infection by the necrotrophic fungi *B. cinerea* and *Sclerotinia sclerotiorum*, but, on the other hand, inhibited the growth of biotrophic pathogens (Govrin and Levine, 2000).

8.3.2 *Jasmonic Acid- and Ethylene-Mediated Induced Defenses against Pathogens*

Besides their role in basal resistance, JA and ET also function as key regulators in induced defense responses that act systemically to enhance resistance against subsequent pathogen attack. For instance, infection of *Arabidopsis* with the fungal pathogen *A. brassicicola* results in local and systemic activation of the *PDF1.2* gene, encoding a plant defensin with anti-fungal properties. Mutant analysis revealed that *PDF1.2* gene expression is regulated through a JA- and ET-dependent signaling pathway that functions independently of SA (Penninckx et al., 1996; 1998). Another example comes from studies on the interaction between the bacterial pathogen *E. carotovora* and its host plants tobacco and *Arabidopsis*. Infection of leaves of these plants with *E. carotovora*, or treatment of the leaves with elicitors of this pathogen, activated an SA-independent systemic resistance and a set of defense-related genes that differs from that induced upon exogenous application of SA (Vidal et al., 1997; Norman-Setterblad et al., 2000). Interestingly, most of the *E. carotovora*-induced genes appeared to be regulated by JA and ET.

Another type of JA/ET-dependent induced pathogen resistance is triggered by selected strains of nonpathogenic rhizosphere bacteria. Strains that were isolated from naturally disease-suppressive soils, mainly fluorescent *Pseudomonas* spp., were found to promote plant growth by suppressing soilborne pathogens. This biological control activity is effective under field conditions (Zehnder et al., 2001) and in commercial greenhouses (Leeman et al., 1995), and can be the result of competition for nutrients, siderophore-mediated competition for iron, antibiosis, or secretion of lytic enzymes (Bakker et al., 1991). Some of the biological control strains reduce disease through a plant-mediated mechanism that is phenotypically similar to pathogen-induced SAR, as the induced resistance is systemically activated and is effective against various types of pathogens. This type of induced disease resistance is referred to here as rhizobacteria-mediated induced systemic resistance (ISR) (Van Loon et al., 1998; Pieterse et al., 2002). In *Arabidopsis*, rhizobacteria-mediated ISR activated by *Pseudomonas fluorescens* WCS417r and *Pseudomonas putida* WCS358r has been shown to function independently of SA and *PR* gene activation (Pieterse et al., 1996; Van Wees et al., 1997). Instead, rhizobacteria-mediated ISR signaling requires JA and ET, because *Arabidopsis* mutants impaired in their ability to respond to either of these two phytohormones are unable to express ISR (Pieterse et al., 1998; Ton et al., 2001; 2002a). The state of rhizobacteria-mediated ISR is not only independent of *PR* gene expression, but is also not associated with the activation of other known defense-related genes (Van Wees et al., 1999). Upon challenge with a pathogen, however, ISR-expressing

plants show enhanced expression of certain JA- and ET-responsive genes such as *AtVSP, PDF1.2,* and *HEL* (Van Wees et al., 1999; Hase and Pieterse, unpublished observations), suggesting that ISR-expressing tissue is primed to activate specific JA- and ET-inducible genes faster and/or to a higher level upon pathogen attack. As mentioned above, the priming phenomenon has already been observed in other processes in plants responding to stress signals and is regarded to enhance the plant's ability to defend itself against different types of biotic or abiotic stress (Conrath et al., 2002).

8.3.3 *Priming of Defense Responses During Rhizobacteria-Mediated ISR*

Although expression of rhizobacteria-mediated ISR in *Arabidopsis* requires an intact response to both JA and ET (Pieterse et al., 1998), the analysis of local and systemic levels of these plant hormones revealed that ISR is not associated with changes in the production of these signals (Pieterse et al., 2000). This finding suggests that ISR is based on an enhanced sensitivity to these plant hormones rather than on an increase in their production. If this is true, ISR-expressing plants are primed to react faster or more strongly to JA and ET produced after pathogen attack.

The hypothesis that ISR may be based on an enhanced sensitivity to JA is supported by the finding that the expression of the JA-inducible gene *AtVSP* was potentiated in ISR-expressing leaves after challenge with *Pst* (Van Wees et al., 1999). In the same study, the expression of several other JA-responsive genes was tested as well, but these failed to show an enhanced expression level in ISR-expressing leaves, suggesting that ISR in *Arabidopsis* is associated with potentiation of a specific set of JA-responsive genes. Potentiation of defense responses by JA has been reported in other systems as well. For instance, pre-treatment with methyl jasmonate potentiates the elicitation of various phenylpropanoid defense responses in parsley suspension cell cultures (Kauss et al., 1992b) and primes them for enhanced induction of the early oxidative burst (Kauss et al., 1994). Moreover, JA potentiates the expression of the *PR-1* gene in rice and the level of resistance against *Magnaporthe grisea* induced by low doses of INA (Schweizer et al., 1997).

The role of ethylene in priming is more complex. After treatment with a saturating dose of 1 millimolar of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC), ISR-expressing plants emit significantly more ethylene than ACC-treated control plants (Pieterse et al., 2000). Evidently, the capacity to convert ACC to ethylene is increased in ISR-expressing plants. Because in infected tissues, ACC levels rapidly increase as a result of pathogen-induced ACC synthase activity, the enhanced ACC-converting capacity of ISR-expressing plants likely primes the plant for a faster or greater production of ethylene upon pathogen attack. In *Pst*-infected *Arabidopsis* plants induced for ISR, the production of ET was indeed enhanced during the first 24 hours after infection compared to uninduced plants (Hase and Pieterse, unpublished observations). Interestingly, exogenous application of ACC has been shown to induce resistance against *Pst* in *Arabidopsis*

(Pieterse et al., 1998). Therefore, a faster or greater production of ET in the initial phase of infection might contribute to the enhanced resistance against this pathogen.

8.4 Systemins: Peptide Signals in The Systemic Wound Response

In the early 1970s, Green and Ryan (1972) observed an accumulation of proteinase inhibitors (PIs) in tomato and potato plants after herbivore-induced or mechanical wounding in both the injured leaves and undamaged parts of the plants. In this landmark study, Green and Ryan (1972) suggested this systemic reaction to be an inducible defense response directed against herbivorous insects. It is now clear that the systemic wound response is not limited to proteinase inhibitors but rather includes a large number of proteins which may contribute, directly or indirectly, to enhanced insect resistance in many plant species (Constabel, 1999; Reymond et al., 2000; Ryan, 2000; Walling, 2000). The wound response in the *Solanaceae* attracted considerable attention over the past 30 years and has developed into a model system of long-distance signaling in plants. Much effort has been devoted to the identification of a hypothetical wound signal that is generated at the site of injury, transmitted throughout the aerial parts of the plant, and capable of inducing the expression of defense genes in undamaged tissues. Physical stimuli such as hydraulic waves that result from the release of xylem tension upon wounding or action and variation potentials have been implicated in the wound signal transduction process, as well as chemical signaling molecules including JA, ET, abscisic acid, oligogalacturonides (OGAs), and systemins. The activity of these signals and their contribution to long-distance signal transduction has been covered in several reviews (Schaller and Ryan, 1995; Bowles, 1998; Ryan, 2000; de Bruxelles and Roberts, 2001; León et al., 2001) and is also discussed by Korth and Thompson (this volume). This section will instead focus on systemins, their discovery, activity, and signaling properties.

8.4.1 Systemins in Different Plant Species

The systemic wound response of tomato plants is characterized by the accumulation of a large number of defense proteins (systemic wound response proteins, SWRPs) (Ryan, 2000). A search for the hypothetical signaling molecule(s) that allows tomato plants to respond systemically to a local stimulus (i.e., wounding), led to the identification of the first plant peptide with a signaling function in 1991 (Pearce et al., 1991; Ryan, 1992). A 18-amino-acid peptide was isolated from the leaves of tomato plants on the basis of its ability to induce the expression of SWRPs using a sensitive bioassay. The peptide was named "systemin" to emphasize its central role as an inducing compound and the systemic nature of the response (Pearce et al., 1991). Based on the systemin amino acid sequence, the cDNA and gene of prosystemin were cloned, and found to encode a systemin precursor of

200 amino acids (McGurl and Ryan, 1992; McGurl et al., 1992). The systemin sequence is found close to the C-terminus of the precursor. There is a single gene for prosystemin in the haploid tomato genome from which two different polypeptides are derived by differential splicing of the pre-mRNA. The polymorphism is located in the nonsystemin portion of the polypeptides and does not seem to affect their wound signaling properties (Li and Howe, 2001). Highly similar prosystemins have been identified in closely related plant species (potato, bell pepper, and black nightshade) exhibiting 73–88 % identity with the tomato sequence, but not outside the family of *Solanaceae* (Constabel et al., 1998). Homology-based approaches failed to identify prosystemin in the more distantly related tobacco. A search for tobacco signaling molecules functionally related to tomato systemin identified two 18-amino-acid peptide inducers of PI synthesis in tobacco leaves (Pearce et al., 2001). The two peptides are derived from a single precursor protein of 165 amino acids. The precursor of the tobacco systemins is not homologous to the previously identified prosystemins from other *Solanaceae* but contains sequence motifs present also in hydroxyproline-rich cell wall glycoproteins (Pearce et al., 2001). Likewise, tobacco systemins themselves bear no structural similarity to tomato systemin. Therefore, systemins are now considered to represent a structurally diverse group of polypeptides that are produced in injured plants and function as signaling molecules in the activation of defense genes (Pearce et al., 2001). Systemic responses to herbivore attack have been documented in more than 100 plant species (Karban and Baldwin, 1997). It will be interesting to see which proteins exert systemin function in these plants and whether or not further distinct proteins have evolved to perform systemin's signaling function. In the following discussion of systemin activity and signaling we will focus on the properties of the tomato peptide, presently the only one that has been thoroughly investigated.

8.4.2 The Activity of Tomato Systemin

A wealth of physiological data point toward a role for systemin as a signal molecule in the wound signal transduction pathway in tomato plants. In addition to *SWRP* gene expression, the synthetic tomato peptide triggers physiological reactions that are characteristic to the wound response. Changes in plasma membrane permeability are among the earliest cellular responses to treatment with systemin and oligogalacturonide elicitors of the wound response. The influx of calcium and protons and the efflux of potassium and chloride ions lead to an increase in the cytoplasmic free calcium concentration, intracellular acidification, depolarization of the plasma membrane, and alkalinization of the apoplast (Felix and Boller, 1995; Thain et al., 1995; Moyen and Johannes, 1996; Moyen et al., 1998; Schaller, 1998). These early events are essentially indistinguishable from those triggered in plant cells after pathogen recognition or elicitation (Conrath et al., 1991; Ebel and Mithöfer, 1998; Scheel, 1998; Katz et al., 2002). In both wound and pathogen defense responses, these ion fluxes were shown to be necessary and sufficient for the subsequent activation of defense genes (Fukuda, 1996; Jabs et al., 1997; Schaller and Oecking, 1999; Blume et al., 2000; Schaller and Frasson, 2001).

Both wounding and systemin stimulate the accumulation of calmodulin as well as of polygalacturonase, phospholipase, and protein kinase activities which may all contribute to the transduction of the wound signal in tomato (Conconi et al., 1996; Stankovic and Davies, 1997; Stratmann and Ryan, 1997; Bergey and Ryan, 1999; Bergey et al., 1999; Narváez-Vásquez et al., 1999; Chico et al., 2002) and other plant species (e.g., Seo et al., 1995; Vian et al., 1996; Lee et al., 1997; Rojo et al., 1998; Seo et al., 1999; Dhondt et al., 2000; Jonak et al., 2000; Wang et al., 2000; Ishiguro et al., 2001). Both wounding and systemin stimulate the synthesis and transient accumulation of JA (Peña-Cortés et al., 1993; Doares et al., 1995a), another inducer of defense gene expression (Farmer and Ryan, 1990; Farmer et al., 1991). This finding places the octadecanoid pathway for JA biosynthesis downstream of both wounding and systemin in the signaling pathway that leads to the expression of wound-responsive genes (Farmer and Ryan, 1992). Consistently, a rapid and transient induction of JA biosynthetic enzymes is observed after wounding or systemin treatment and is followed by a delayed and more sustained induction of SWRPs with a direct role in deterring insect herbivores (Ryan, 2000; Strassner et al., 2002). The production of ET is triggered by wounding and systemin treatment (Felix and Boller, 1995; O'Donnell et al., 1996), and both ET and JA were shown to be required for *SWRP* gene activation (O'Donnell et al., 1996). Finally, a local and systemic production of H_2O_2 was observed in tomato plants upon wounding and systemin treatment and was shown to depend on a functional octadecanoid pathway. Hence, a role for H_2O_2 as a second messenger downstream of JA was proposed (Orozco-Cardenas and Ryan, 1999; Orozco-Cárdenas, 2000).

8.4.3 The Role of Tomato Systemin in Wound Signal Transduction

The activities elucidated for tomato systemin are essentially consistent with a model of wound signaling originally proposed by Farmer and Ryan (1992). According to this model, systemin is released from prosystemin as a consequence of wounding, translocated throughout the aerial parts of the plant, and then interacts with a cell-surface receptor in the target tissue. This interaction results in the activation of a lipase, which releases linolenic acid from membrane lipids to serve as a substrate of the octadecanoid pathway for the biosynthesis of JA which, in turn, activates defense genes (Farmer and Ryan, 1992). The model was later refined to account for the requirement of ET for *SWRP* gene activation (O'Donnell et al., 1996), the defense signaling activity of oxylipins other than JA (Stintzi et al., 2001), the action of H_2O_2 as a second messenger downstream of JA (Orozco-Cárdenas, 2000), and the involvement of ion fluxes across the plasma membrane and reversible protein phosphorylation in wound signaling (Schaller, 1999; Ryan, 2000; Schaller, 2001). Important support for this model includes the characterization of a cell-surface binding site for systemin exhibiting characteristics of a functional systemin receptor (Meindl et al., 1998; Scheer and Ryan, 1999; Stratmann et al., 2000; Scheer and Ryan, 2002), as well as data derived from the analysis of transgenic and mutant tomato plants. Transgenic tomato plants

in which the expression of prosystemin was suppressed by the antisense RNA technology were impaired in both the wound-induced accumulation of PIs and resistance to insect larvae demonstrating an absolute requirement of prosystemin for the activation of the wound response in tomato plants (McGurl et al., 1992; Orozco-Cardenas et al., 1993). In a converse manner, constitutive accumulation of SWRPs was observed in tomato plants overexpressing prosystemin under control of the constitutive CaMV 35S promoter (McGurl et al., 1994). Extragenic suppressors of the *35S::prosystemin*-mediated SWRP accumulation were identified and characterized, demonstrating that wounding and systemin induce defense gene expression through a common signaling pathway (Howe et al., 1996; Howe and Ryan, 1999). Surprisingly, when ectopically expressed, prosystemin appears to be sufficient to trigger defense gene activation and, thus, wounding is no longer required. In prosystemin-overexpressing plants, untimely processing of prosystemin may occur, or the ectopic expression of prosystemin even alleviates the need for processing, as full-length prosystemin was shown to be as active as systemin in the induction of *SWRP* gene expression when supplied to tomato plants via the transpiration stream (Dombrowski et al., 1999; Vetsch et al., 2000). Grafting experiments were performed using *35S::prosystemin*-expressing plants as the root stock and wild-type tomato as the scion. SWRPs were found to accumulate in the scion, demonstrating that the overexpression of prosystemin is sufficient to generate a graft-transmissible signal for defense gene activation. Similarly, addition of systemin or prosystemin to wound sites on leaves of prosystemin antisense plants caused *SWRP* gene activation in the distal unwounded leaves (Dombrowski et al., 1999). These observations are consistent with systemin itself being the mobile signal. (Pro)systemin-induced synthesis of another, as yet unidentified signaling molecule, however, cannot be excluded.

A microarray comprising 235 cDNAs was used to analyze the relative changes in gene expression in wounded and distal, unwounded leaves of tomato plants. While transcripts for SWRPs with direct defense function (i.e., the "late" defense genes, e.g., those for PIs; Ryan, 2000) accumulated to high levels in both tissues, the coordinate induction of genes for octadecanoid pathway enzymes dedicated to JA biosynthesis ("early" defense genes; Ryan, 2000) was observed locally but not systemically (Strassner et al., 2002). In this study, JA and its precursor 12-oxophytodienoic acid accumulated in the damaged, but not in distal, leaves of wounded plants (Strassner et al., 2002) which is consistent with previous reports of limited systemic JA accumulation (Bowles, 1998; Rojo et al., 1999; Ziegler et al., 2001). Hence, synthesis and accumulation of JA in systemic leaves do not seem to be required for defense gene activation. However, this does not necessarily imply that systemic *SWRP* gene activation, as suggested by Bowles (1998), is JA-independent: A recent study showed that systemic wound signaling requires the capacity to synthesize JA in the wounded leaf, whereas the ability to perceive JA is required in the systemic leaves. Elegant grafting experiments were performed using tomato mutants that either fail to synthesize (*spr-2*; Howe and Ryan, 1999) or perceive (*jai-1*; Li et al., 2001) the JA signal. When grafted plants were wounded below the graft junction, activation of the wound response in the scion depended

on the ability to perceive JA. Wound- or (pro)systemin-induced activation of the JA biosynthetic pathway, on the other hand, was required in the lower part of the plant for the generation of a graft-transmissible signal, but not for defense gene activation in the scion (Li et al., 2002). The data suggest that the activity of (pro)systemin is required in the wounded leaf to promote the production of a systemic signal, possibly JA or another octadecanoid-derived molecule. Therefore, the model of wound signaling originally proposed by Farmer and Ryan (1992) may describe local rather than systemic wound signal transduction events. Thirty years after the initial report on the phenomenon (Green and Ryan, 1972), the identity of the systemic signal molecule in the wound response of plants is still unclear.

Another level of complexity is added by the cell-type-specific expression of genes involved in the wound response, which is certainly highly relevant for the processes leading to both local and systemic activation of defense genes. "Early genes", i.e., those rapidly induced after wounding, including those encoding prosystemin and some of the JA biosynthetic enzymes, are expressed in vascular bundles (Jacinto et al., 1997; Kubigsteltig et al., 1999; Hause et al., 2000), whereas "late genes", i.e., those for SWRPs with a direct role in plant defense, are expressed in palisade and adjacent spongy mesophyll cells (Shumway et al., 1976; Walker-Simmons and Ryan, 1977; Ryan, 2000). The temporally and spatially separated expression of the two classes of genes led to the suggestion that wound-signaling events may initially be activated in the vascular bundles to produce second messengers (octadecanoids, OGAs, H_2O_2) that will then induce defense gene expression in mesophyll cells (Orozco-Cárdenas, 2000; Ryan, 2000). Some of the second messengers may exert their effects over long distances and contribute to systemic signal transduction.

Further work is needed to precisely understand systemin action and function in tomato plants. Obviously, these studies will have to be extended to other plant species, particularly to *Arabidopsis*. The plethora of signaling mutants available in *Arabidopsis* will be useful to advance our understanding of the complexity of wound signal transduction as well as the interaction of the systemin signal transduction pathway with other defense signaling pathways (see below).

8.5 β-Aminobutyric Acid Activates Resistance Responses

β-Aminobutyric acid (BABA) is a nonprotein amino acid, which is only rarely found in nature. BABA has been described as part of a small, 9-kilodalton proteinaceous inhibitor of trypsin and microbial serine proteinases isolated from *Yersinia pseudotuberculosis* (Burtseva and Kofanova, 1996). In addition, BABA was found in root exudates of tomato plants grown in solarized soil (Gamliel and Katan, 1992). Despite its rare occurrence, BABA is an interesting compound. This is because of its close structural similarity to a highly bioactive substance, the neurotransmitter GABA, whose natural occurrence is well documented in plants (Shelp et al., 1999). Also, BABA is a potent inducer of acquired disease resistance (Jakab et al., 2001). Applied as either a soil drench or foliar spray, BABA has a broad

spectrum of activity against viruses, bacteria, oomycetes, fungi, and nematodes (Jakab et al., 2001). This wide range of activity supports a role for BABA as an inducer of acquired disease resistance, especially since the substance was shown not to be directly toxic to microorganisms (reviewed by Jakab et al., 2001). As BABA is highly water-soluble it is readily taken up by plant roots and then distributed throughout the plant (Cohen and Gisi, 1994; Jakab et al., 2001).

8.5.1 β-Aminobutyric Acid-Induced Priming

Depending on the method of application, mild phytotoxic effects of BABA have been observed. BABA has been sprayed on leaves, injected into stems of plants, supplied via petiole dip, or applied as a soil drench to the root system. When applied as a foliar spray to tobacco plants, BABA, and to a lesser extent α-aminobutyric acid, but not GABA, were phytotoxic at a concentration of 100 μg ml^{-1} (ca. 1 mmolar) (Cohen, 1994). Small necrotic lesions started to form on treated leaves two days after spraying. A rapid induction of necrotic lesions in tobacco was also observed by Siegrist et al. (2000) after foliar treatment with 10 mmolar BABA. Localized necrosis was accompanied by the formation of reactive oxygen species, lipid peroxidation, callose deposition around the lesions, and an increase in the SA content of the leaves (Siegrist et al., 2000). No such effects were observed in plants treated with GABA, even at concentrations as high as 2000 μg ml^{-1}(ca. 20 mmolar) (Cohen, 1994; Siegrist et al., 2000).

In *Arabidopsis*, spraying BABA onto leaves also leads to the formation of small necrotic lesions and to an accumulation of *PR* gene transcripts, with a pattern that is similar to the one observed when SA is used to induce resistance. However, when supplied via the root system, BABA concentrations sufficient to induce resistance, do not induce defense gene expression in *Arabidopsis* (Zimmerli et al., 2000). This observation suggests that the induction of resistance by BABA in *Arabidopsis* is not primarily based on a previous accumulation of defense gene transcripts. Rather, an additional mechanism of resistance induction seems to be present in BABA-treated *Arabidopsis* plants. This conclusion is supported by the observation that BABA induces resistance against the oomycete *Peronospora parasicita* in wild-type *Arabidopsis* plants as well as in plants that are impaired in defense gene expression (Zimmerli et al., 2000), such as the *npr1*, *jar1*, or *etr1* mutants (Bleeker et al., 1988; Staswick et al., 1992; Cao et al., 1994), and *NahG* transgenic *Arabidopsis* plants (Delaney et al., 1994). In this case, resistance is independent of the presence of SA and *PR* or other defense gene activation. Common to the BABA-mediated defense mechanism observed in the different mutant and wild-type plants is a more rapid and stronger deposition of callose-containing papillae at the site of infection by *P. parasitica* (Zimmerli et al., 2000). BABA primes *Arabidopsis* to effectively react to *P. parasitica* infection with papillae deposition, thus making further defense responses obsolete since ingress by *P. parasitica* has already been stopped at this point. Interestingly, a similar observation was made with *NahG* tobacco challenged with downy mildew: there was no difference in the protection by BABA between *NahG* and wild-type plants (Cohen et al., 2000). It is

probable that also in this case priming for potentiated induction of SA-independent defense mechanisms is responsible for the observed protection.

When BABA-pretreated *Arabidopsis* plants are challenged with a virulent strain of *Pst*, priming becomes apparent as a strong potentiation of *PR-1* gene expression (Zimmerli et al., 2000). In this case, the induction kinetics are very similar to those observed in response to avirulent *Pst* (Zimmerli et al., 2000). In the interaction between *Arabidopsis* and *Pst*, priming by BABA is dependent on an intact SA signaling pathway, but independent on a functioning JA/ET pathway as evident from experiments with the same defense response mutants as described above (Zimmerli et al., 2000). Interestingly, in the *Arabidopsis-B. cinerea* interaction, it is *PR-1* that again shows strongly potentiated expression (Zimmerli et al., 2001) and not *PDF1.2* (Thomma et al., 1998) that is commonly thought to play a role in defense against *B. cinerea*.

In contrast to other inducers of SAR, such as SA or BTH (Kohler et al., 2002), BABA itself does not induce *PR* gene expression (Zimmerli et al., 2000). Using BABA, it is possible therefore to clearly separate priming and defense gene activation. This will greatly facilitate the future analysis of priming phenomena in induced resistance.

8.6 Cross-Talk Between Signaling Pathways

Over the past years, evidence has accumulated indicating that the SA-, JA-, ET-, and systemin-dependent defense pathways can affect each other, either positively or negatively. Although the observed pathway interactions vary between species and the type of attacker used, it is becoming increasingly clear that cross-talk between signaling pathways is important for the plant to fine-tune its defense responses. For example, JA and ET have been shown to act synergistically in the activation of genes encoding defense-related plant proteins, such as PIs and defensins (O'Donnell et al., 1996; Penninckx et al., 1998). Moreover, JA and ET have been shown to support the action of SA resulting in enhanced *PR* gene expression (Lawton et al., 1994; Xu et al., 1994; Schweizer et al., 1997). On the other hand, SA, INA, and BTH suppress JA-dependent defense gene expression (Doherty et al., 1988; Peña-Cortés et al., 1993; Bowling et al., 1997; Niki et al., 1998; Fidantsef et al., 1999; Van Wees et al., 1999), possibly through inhibition of JA biosynthesis and action (Peña-Cortés et al., 1993; Doares et al., 1995b; Harms et al., 1998). Consistent with this, Preston et al. (1999) demonstrated that TMV-infected tobacco plants displaying SAR are unable to express normal JA-mediated wound responses, probably due to inhibition of JA signaling by increased SA levels resulting from the TMV infection. Also, in the *Arabidopsis ssi2* mutant, the SA-dependent signaling pathway is constitutively activated, while JA-dependent signaling is suppressed (Kachroo et al., 2001). Conversely, in pathogen-inoculated *NahG* plants, which are unable to accumulate significant SA levels, expression of the JA/ET-responsive defensin gene *PDF1.2* was at least twofold higher than in wild-type plants (Penninckx et al., 1996). Inhibitory effects of salicylates on ET biosynthesis

have also been reported (reviewed by Shah and Klessig, 1999). Thus, activating the SA pathway confers resistance to a broad spectrum of microbial pathogens but, at the same time, may have detrimental effects on the JA/ET-dependent signal transduction mechanism that confers resistance against insects and certain groups of pathogens.

An additional level of antagonistic regulation of wound- and pathogen-induced defense responses is provided by the proton electrochemical gradient across the plasma membrane. In tomato and tobacco plants, activation of the plasma membrane H^+-ATPase by the fungal toxin fusicoccin (FC) induces the accumulation of both basic and acidic PR proteins (Fukuda, 1996; Roberts and Bowles, 1999; Schaller and Oecking, 1999; Frick and Schaller, 2002). Also, expression of a bacterial proton pump induced a lesion mimic phenotype, activated multiple defense responses, and increased the resistance to microbial pathogens in transgenic tobacco and potato plants (Mittler et al., 1995; Abad et al., 1997; Rizhsky and Mittler, 2001). In addition to activating pathogen defense responses, the hyperpolarization of the plasma membrane by FC-treatment resulted in a suppression of wound-, systemin-, OGA-, and JA-induced *SWRP* gene expression (Doherty and Bowles, 1990; Schaller, 1999; Frick and Schaller, 2002). Both the activation of pathogen response genes and the repression of wound-induced genes by FC were shown to be at least partly independent of SA, as they (i) were incompatible with the timing of FC-induced SA accumulation in tomato leaves, and (ii) occurred under conditions of inhibited SA biosynthesis (Schaller et al., 2000). Furthermore, FC induced *PR* gene expression in *NahG* tobacco and tomato plants, i.e. plants unable to accumulate significant amounts of SA (Schaller et al., 2000; Frick and Schaller, 2002).

While activation of the H^+-ATPase induced *PR* gene expression and *SWRP* gene supression, inhibitors of the plasma membrane H^+-ATPase activity and ionophores that dissipate the proton electrochemical gradient induced *SWRP* genes in tomato (Schaller and Oecking, 1999; Schaller and Frasson, 2001). Octadecanoid-dependent signaling was also triggered by the ion-channel-forming peptide alamethicin (Engelberth et al., 2001), and, more generally, a role for the pore-forming properties of elicitors in the induction of defense responses has been discussed (Klüsener and Weiler, 1999). Apparently, wound and pathogen defense signaling pathways are differentially affected by changes in the proton electrochemical gradient. Therefore, the plasma membrane H^+-ATPase may act as a switch activating either wound or pathogen defense responses.

Several studies have provided evidence for trade-offs between SA-dependent pathogen resistance and JA-dependent insect resistance, indicating that the activation of a particular defense mechanism can reduce the resistance to certain groups of pathogens or herbivorous insects. For instance, Moran (1998) demonstrated that SAR in cucumber against *Colletotrichum orbiculare* was associated with reduced resistance against feeding by spotted cucumber beetles (*Diabrotica undecimpunctata howardi*) and enhanced reproduction of melon aphids (*Aphis gossypii*). A similar phenomenon was observed by Preston et al. (1999) who demonstrated that TMV-infected tobacco plants induced for SAR display higher sensitivity to

tobacco hornworm (*Manduca sexta*) grazing when compared with noninduced control plants. Furthermore, it has been shown that transgenic tobacco plants with reduced SA levels, caused by silencing of the *PAL* gene, exhibit reduced SAR against TMV but enhanced herbivore-induced resistance to *Heliothis virescens* larvae (Felton et al., 1999). In a converse manner, *PAL*-overexpressing tobacco displays a strong reduction of herbivore-induced insect resistance, while TMV-induced SAR was enhanced in these plants.

The SAR inducer BTH has in some cases also been shown to reduce insect resistance. Exogenous application of BTH to tomato plants enhanced the level of resistance against *Pst*, but improved the suitability of tomato for feeding by leaf chewing larvae of the corn earworm (*Helicoverpa zea*) (Stout et al., 1999). A similar phenomenon was observed by Thaler et al. (1999) who reported compromised resistance to the beet armyworm (*Spodoptera exigua*) upon application of BTH to field-grown tomato plants. In most cases, the reduced insect resistance of SAR-expressing plants could be attributed to the inhibition of JA production by either BTH or increased SA levels.

8.7 Concomitant Activation of Induced Disease Resistance Mechanisms

Though negative interactions between the SA- and JA/ET-dependent signal transduction pathways have clearly been shown, other studies argue against such a negative relationship. A genetic screen for the isolation of *Arabidopsis* signal transduction mutants that constitutively express the JA/ET-responsive *THI2.1* gene yielded two mutants, which showed concomitant induction of both the SA- and the JA-dependent signaling pathways (Hilpert et al., 2001). The finding that some gene transcripts which increase after *A. brassicicola* infection of *Arabidopsis* leaves also accumulate upon treatment with SA, JA, and ET, also points to an overlap of the different signaling pathways, at least in *Arabidopsis* (Schenk et al., 2000). In this context, it is worthwhile to mention that a pre-treatment with systemin was shown to prime tomato cell suspension cultures for augmented induction of the H_2O_2 burst induced by the addition of OGAs or water (Stennis et al., 1998). In a similar manner, preincubating cultured parsley cells with JA potentiated the subsequent activation of phenylpropanoid defense responses by a *P. sojae* cell wall elicitor (Kauss et al., 1992b). Also, priming *Arabidopsis* plants with BTH (Kohler et al., 2002) or BABA (Jakab et al., 2001) enhanced the subsequent induction of defense responses against biotic and abiotic stresses. Thus, priming likely represents a molecular mechanism at which the systemin, JA/ET, BABA, and SA signaling pathways merge.

Failure to demonstrate a negative relationship between signaling mechanisms was also reported on the level of pathogen or insect resistance. For instance, inoculating lower leaves of tobacco plants with TMV does not affect the growth of tobacco aphid (*Myzus nicotianae*) populations (Ajlan and Potter, 1992). In a similar manner, there is no negative effect of BTH application on the population

growth of whiteflies (*Bemisia argentifolii*) and leaf miners (*Liriomyza* spp.) (Inbar et al., 1998). Interestingly, Stout et al. (1999) have demonstrated that inoculation of tomato leaves with *Pst* induced resistance to both *Pst* and the corn earworm (*Helicoverpa zea*) in distal parts of the *Pst*-inoculated plants. Conversely, feeding by *H. zea* induced resistance against both *Pst* and *H. zea*. A nice demonstration of simultaneous pathogen and insect resistance in the field was provided by Zehnder et al. (2001). The authors observed that rhizobacteria-mediated ISR of cucumber against insect-transmitted bacterial wilt disease, caused by *Erwinia tracheiphila*, was associated with reduced feeding of the cucumber beetle vector. It appeared that induction of ISR was associated with reduced concentrations of cucurbitacin, a secondary metabolite and powerful feeding stimulant for cucumber beetles. Induction of rhizobacteria-mediated ISR against *E. tracheiphila* was also effective in the absence of beetle vectors, suggesting that ISR protects cucumber against bacterial wilt not only by reducing beetle feeding and pathogen transmission, but also through induction of defense responses that act against the bacterial pathogen. These observations indicate that negative interactions between induced pathogen- and insect resistance are by no means general.

The question of whether SA- and JA/ET-dependent resistance against microbial pathogens can be expressed simultaneously was recently addressed by Van Wees et al. (2000). In *Arabidopsis*, SA-dependent, necrosis-triggered SAR and JA/ET-dependent, rhizobacteria-mediated ISR are each effective against various pathogens, although their spectrum of effectiveness partly diverges (Ton et al., 2002b). Both SAR and ISR are effective against *Pst*. Simultaneous activation of both types of induced resistance resulted in an additive effect on the level of induced protection against this pathogen. In *Arabidopsis* genotypes that are blocked in either SAR or ISR, this additive effect was absent. Moreover, induction of ISR did not affect expression of the SAR marker gene *PR-1* in plants expressing SAR. Together, these observations demonstrate that the signaling pathways involved in both types of induced resistance can be compatible and that there is not necessarily significant cross-talk between them. Therefore, combining SAR and ISR can provide an attractive tool for improving disease control in plants.

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