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Chapter 11

INDUCED RESISTANCE – ORCHESTRATING DEFENCE MECHANISMS THROUGH CROSSTALK AND PRIMING

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Abstract: In nature, plants interact with a wide range of microbial pathogens and herbivorous insects. During the evolutionary arms race between plants and their attackers, primary and secondary immune responses evolved to recognise common or highly specialised features of the attacker encountered, resulting in sophisticated mechanisms of induced defence. Induced resistance mechanisms are characterised by a broad-spectrum effectiveness and often act systemically in plant parts distant from the site of primary attack, thereby protecting the plant against subsequent invaders. Plant hormones are key players in the regulation of the defence signalling pathways involved. Because induced defence responses entail ecological fitness costs, plants must possess elaborate regulatory mechanisms that efficiently coordinate the activation of attacker-specific defences so that fitness costs are minimised while optimal resistance is attained. A major focus in plant defence signalling research is to uncover key mechanisms by which plants tailor their responses to different attackers, and to investigate how plants cope with simultaneous interactions with multiple aggressors. Pathway crosstalk and priming for enhanced defence emerged as important regulatory mechanisms that enhance the efficiency of the plant's inducible defence arsenal. Here, we review the current knowledge on the signalling cascades involved in different types of induced pathogen and insect resistance, and the regulatory mechanisms by which plants are able to orchestrate their inducible defences in a cost-effective manner.

Keywords: crosstalk; priming; induced resistance; defence signalling; phytohormones

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11.1 Introduction

11.1.1 Constitutive defence

Plants are abundant on earth and form the basis of most food webs. Each of the ~300 000 plant species interacts with a range of organisms, some of which are harmful (e.g. pathogens or herbivorous insects) and others beneficial (e.g. growth-promoting rhizobacteria, mycorrhizal fungi and predatory enemies of herbivores). Intriguingly, plants are resistant to the majority of their attackers. This resistance is based on an array of defensive mechanisms. Some of these mechanisms are pre-existing and prevent or attenuate the invasion of potential attackers. Thorns, needles and trichomes are examples of constitutively present defensive structures that are designed to harm or deter herbivores. On a smaller scale, the cell wall poses a pre-existing physical barrier to many microorganisms. In addition, plants constitutively produce secondary metabolites that inhibit growth of microbes or render the tissue less attractive for herbivores (Osbourn, 1996; Tierens *et al.*, 2001).

11.1.2 PAMP-triggered immunity

Despite the diversity of pre-existing defensive barriers, many microbes and insects succeed in breaking through this first layer of defence. However, as a second line of defence, a wide spectrum of inducible plant defences becomes activated that help the plant to prevent the attacker from causing further damage, either by blocking its colonisation or by directly targeting the attacker's physiology (see Chapter 8). For this second line of defence, plants have evolved sophisticated strategies to 'perceive' their attacker and translate this 'perception' into an effective defence response (Chisholm *et al.*, 2006; Jones and Dangl, 2006). This primary immune response recognises common features of microbial pathogens, such as flagellin, chitin, glycoproteins and lipopolysaccharides (Bittel and Robatzek, 2007). These microbial determinants are referred to as pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) (Chisholm *et al.*, 2006; Jones and Dangl, 2006; Bittel and Robatzek, 2007). PAMPs activate pattern-recognition receptors, which in turn initiate diverse downstream signalling events that ultimately result in the activation of a basal resistance that is called PAMP-triggered immunity (PTI; Chisholm *et al.*, 2006; Jones and Dangl, 2006) (see Chapter 2).

11.1.3 Effector-triggered immunity

During the co-evolutionary arms race between plants and their microbial attackers, pathogens acquired the ability to suppress PTI via the delivery of specific effector proteins. In turn, plants acquired immune receptors (R proteins) that are able to recognise these attacker-specific effector proteins

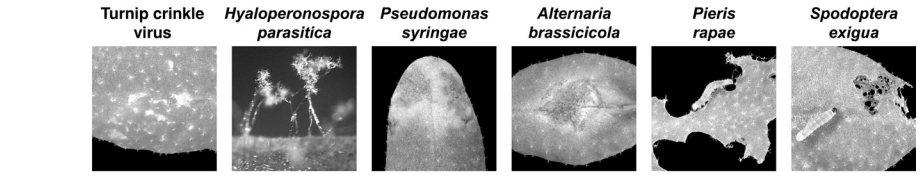
(in this context known as avirulence proteins), resulting in a second line of defence called 'effector-triggered immunity' (ETI; Chisholm *et al.*, 2006; Jones and Dangl, 2006). ETI results in *R* gene-mediated resistance which is often associated with a highly effective hypersensitive response (HR) that arrests further pathogen ingress. The co-evolutionary arms race between plants and herbivorous insects has been intensely debated (Schoonhoven *et al.*, 2005). However, knowledge of the underlying molecular mechanisms is relatively limited in comparison to well-studied mechanisms involved in the arms race between pathogens and their host plants.

11.1.4 Systemically induced resistance

In addition to PTI and ETI that act locally and are activated upon attacker-specific recognition, plants can activate yet another line of defence that is referred to as 'induced resistance'. Over the past three decades, distinct forms of induced resistance have been identified, mainly defined by differences in the signal transduction pathways. Induced resistance can be activated by microbial pathogens and insect herbivores, but also by beneficial microorganisms, such as mycorrhizal fungi and plant growth-promoting rhizobacteria (Kessler and Baldwin, 2002; Dicke and Hilker, 2003; Pozo *et al.*, 2004). While PTI and ETI are thought to be directed specifically against the microbial invader encountered, induced resistance is typically characterised by a broad spectrum of effectiveness. Moreover, induced resistance often also acts systemically in plant parts distant from the site of primary attack, thereby protecting the plant against subsequent invaders. Systemically induced resistance can be activated upon a local PTI or ETI response, but there are also other means of activating systemic-induced resistance. Several biologically induced systemic defence responses have been characterised in detail: systemic acquired resistance (SAR), which is triggered by pathogens causing limited infection, such as hypersensitive necrosis (Durrant and Dong, 2004); rhizobacteria-induced systemic resistance (ISR), which is activated upon colonisation of roots by selected strains of non-pathogenic rhizobacteria (Van Loon *et al.*, 1998), and wound-induced resistance (WIR), which is typically elicited upon tissue damage, such as caused by insect feeding (Kessler and Baldwin, 2002; Howe, 2004). In addition, broad-spectrum resistance can be induced by chemicals such as the non-protein amino acid β-aminobutyric acid (BABA) (Zimmerli *et al.*, 2000). An overview of the spectrum of effectiveness of four types of induced resistance in *Arabidopsis* is presented in Fig. 11.1.

11.1.5 Hormonal regulation of induced resistance

The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) emerged as key players in the regulation of signalling pathways involved in these induced defence responses (Durrant and Dong, 2004; Van Poecke and Dicke, 2004; Von Dahl and Baldwin, 2007). Other plant hormones,



	Turnip crinkle virus	*Hyaloperonospora parasitica*	*Pseudomonas syringae*	*Alternaria brassicicola*	*Pieris rapae*	*Spodoptera exigua*
SAR	+	+	+	–	–	+
ISR	–	+/–	+	+	–	+
WIR	+	nd	+	–	+	nd
BABA-IR	nd	+	+	+	nd	nd

Figure 11.1 Spectrum of effectiveness of systemically induced resistance in *Arabidopsis thaliana*. Induced resistance is indicated by + and ineffective resistance by – (nd; not determined). SAR is effective against (hemi-)biotrophic pathogens such as turnip crinkle virus (TCV), *Hyaloperonospora parasitica* and *Pseudomonas syringae* (Ton *et al.*, 2002b). By contrast, ISR is also effective against necrotrophic pathogens such as *Alternaria brassicicola* (Ton *et al.*, 2002b). Both SAR and ISR are effective against the generalist herbivore *Spodoptera exigua*, whereas the specialist herbivore *Pieris rapae* is unaffected by both induced resistance responses (Van Oosten, 2007). WIR, induced by *P. rapae* caterpillars, confers resistance against subsequent infestation by *P. rapae* and against TCV and *P. syringae*, but not against *A. brassicicola* (De Vos *et al.*, 2006). BABA-IR is effective against all attackers shown here (Zimmerli *et al.*, 2000; Ton and Mauch-Mani, 2004). BABA-IR, β-aminobutyric acid-induced resistance; ISR, induced systemic resistance; SAR, systemic acquired resistance; WIR, wound-induced resistance.

including abscisic acid (ABA) (Mauch-Mani and Mauch, 2005), brassinosteroids (Nakashita *et al.*, 2003) and auxins (Navarro *et al.*, 2006; Wang *et al.*, 2007), have also been reported to have a role in induced plant defence responses, but their significance is less well studied. Upon pathogen or insect attack, plants respond with the production of a specific blend of these alarm signals which varies greatly in quantity, composition and timing, and results in the activation of differential sets of defence-related genes that eventually determine the nature of the defence response against the attacker encountered (Reymond and Farmer, 1998; Rojo *et al.*, 2003; De Vos *et al.*, 2005). Global expression profiling of various *Arabidopsis*-attacker interactions revealed substantial crosstalk between SA-, JA- and ET-dependent defence pathways (Glazebrook *et al.*, 2003; De Vos *et al.*, 2005). Cross-communication between these pathways provides a powerful regulatory potential that allows the plant to fine-tune its defence responses.

11.1.6 Priming for enhanced defence

Induced resistance is often associated with the production of defensive compounds such as pathogenesis-related (PR) proteins with anti-microbial activity (Van Loon *et al.*, 2006), proteinase inhibitors (PIs) that affect insect feeding (Howe, 2004) or volatiles that attract parasitoids and predators of the

herbivores that feed on the plant (Van Poecke and Dicke, 2004). However, in many cases, the enhanced defensive capacity in induced plants cannot be attributed to direct activation of defence-related genes. Instead, broad-spectrum protection of an induced plant is based on a faster and stronger activation of basal defence mechanisms upon exposure to either microbial pathogens or herbivorous insects. It is therefore hypothesised that the broad-spectrum characteristic of induced resistance is largely based on this conditioning of the tissue to react more effectively to a stress condition. By analogy with a phenotypically similar phenomenon in animals and humans, this enhanced capacity to express basal defence mechanisms is called 'priming' (Conrath *et al.*, 2002, 2006).

In this chapter, we aim to review the current status of induced disease resistance signalling research. We focus on the roles of SA, JA and ET in the signalling cascades involved in the different types of induced resistance. We also discuss two regulatory mechanisms that enhance the efficiency of the plant's inducible defence arsenal: cross-communication between defence signalling pathways and priming of induced defence.

11.2 Induced resistance signalling

11.2.1 Systemic acquired resistance

The classical form of induced resistance is called systemic acquired resistance (SAR) and develops in systemic tissue upon primary infection with a necrosis-inducing pathogen (Durrant and Dong, 2004). Avirulent pathogens that activate ETI resulting in an HR are potent inducers of SAR. However, PTI activated by PAMPs that induce the SA signalling pathway can also trigger SAR (Mishina and Zeier, 2006). SAR has been demonstrated in many plant–pathogen interactions (Ryals *et al.*, 1996; Sticher *et al.*, 1997) and is typically characterised by a restriction of pathogen growth and suppression of disease symptom development compared to non-induced plants infected by the same pathogen (Hammerschmidt, 1999). Although SAR is effective against a broad range of pathogens, including viruses, bacteria, fungi and oomycetes (Kuc, 1982), it seems predominantly effective against pathogens with a (hemi-)biotrophic lifestyle (Ton *et al.*, 2002b).

11.2.1.1 The onset of SAR

The onset of SAR is accompanied by a local and systemic increase in endogenous levels of SA (Malamy *et al.*, 1990; Métraux *et al.*, 1990). Moreover, SAR is associated with the coordinate activation of a specific set of genes encoding PR proteins, some of which possess antimicrobial activity (Van Loon *et al.*, 2006). Exogenous application of SA or its functional analogues 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH) induces SAR and

activates the same set of *PR* genes (Ryals *et al.*, 1996). The importance of SA in the regulation of SAR became evident through experiments with transgenic NahG plants that convert SA into catechol through the activity of an introduced bacterial salicylate hydroxylase (*NahG*) gene. Transgenic NahG plants that cannot accumulate SA are incapable of developing SAR and do not show *PR* gene activation upon pathogen infection (Gaffney *et al.*, 1993; Lawton *et al.*, 1995), indicating that SA is a necessary intermediate in the SAR signalling pathway. Interestingly, NahG tobacco and *Arabidopsis* plants are not only incapable of accumulating SA and mounting SAR but also more susceptible to many different pathogens, including bacteria, viruses, fungi and oomycetes (Delaney *et al.*, 1994; Kachroo *et al.*, 2000). Similarly, *Arabidopsis* mutants that are not able to enhance the production of SA upon pathogen infection, such as *eds1* (*enhanced disease susceptibility 1*), *sid1* (*salicylic acid induction-deficient 1*) (*eds5*), *sid2* (*eds16*) and *pad4* (*phytoalexin-deficient 4*), display a higher level of susceptibility to different pathogens, indicating that SA also plays an important role in basal defence (Rogers and Ausubel, 1997; Zhou *et al.*, 1998; Nawrath and Métraux, 1999; Feys *et al.*, 2001; Wildermuth *et al.*, 2001).

Although SA is an essential molecule in the signal transduction of SAR, it seems that this molecule does not function as the systemically transported signal of SAR. Using grafts of wild-type and SA-degrading NahG tobacco rootstocks and scions, Vernooij *et al.* (1994) demonstrated that SA production is not required for the generation of the mobile signal in SAR. On the other hand, Shulaev *et al.* (1995) demonstrated that radioactively labelled SA, synthesised at the site of primary infection, is transported throughout the plant. Seskar *et al.* (1998) proposed that methyl salicylate (MeSA), synthesised from SA in the locally infected leaves and systemically transported throughout the plant, acts in the systemic target tissues by being converted back to SA. This hypothesis was supported by later findings in tobacco, demonstrating that SA methyl transferase and the MeSA esterase SA-BINDING PROTEIN 2 are essential for the expression of SAR in locally infected and systemic leaves, respectively (Kumar and Klessig, 2003; Forouhar *et al.*, 2005; Park *et al.*, 2007).

11.2.1.2 Lipid-derived components involved in SAR

Besides SA and MeSA, lipid-derived components have been implicated as important systemic signals during SAR. Analysis of an *Arabidopsis* T-DNA insertion line identified the *DEFECTIVE IN INDUCED RESISTANCE 1* (*DIR1*) gene encoding a putative apoplastic lipid transfer protein required for pathogen-induced SAR (Maldonado *et al.*, 2002). Assessment of the ability of petiole exudates from wild-type and mutant *dir1* plants to induce SAR gene expression indicated that *dir1* mutant plants are incapable of producing or transmitting the mobile signal that is essential for the systemic expression of SAR. Remarkably, *dir1* still accumulated SA during the establishment and manifestation stages of SAR. The authors suggested that DIR1 interacts with a lipid-derived molecule to allow long-distance signalling. Hence, analogous

to the agonist function of SA in ETI (Shirasu *et al.*, 1997), the role of SA in SAR might be to amplify a DIR1-dependent signal. Moreover, SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY 1 (SFD1), required for systemic activation of SAR, also appeared to be involved in lipid metabolism (Nandi *et al.*, 2004). Furthermore, Truman *et al.* (2007) suggested a central role for jasmonates in SAR signalling. However, other lines of evidence demonstrate that mutants disrupted in JA signalling, e.g. *jar1* (*jasmonate resistant 1*) and *eds8*, are still able to mount wild-type levels of SAR (Pieterse *et al.*, 1998; Ton *et al.*, 2002a). Therefore, the exact role for JAs in SAR needs to be explored further.

11.2.1.3 SAR signal transduction

Transduction of the SA signal to activate *PR* gene expression and SAR requires the function of the regulatory protein NPR1 (NONEXPRESSOR OF *PR*-GENES 1), also known as NIM1 (NON IMMUNITY 1), or SAI1 (SALICYLIC ACID-INSENSITIVE 1) (Cao *et al.*, 1994; Delaney *et al.*, 1995; Shah *et al.*, 1997). Mutations in the *NPR1* gene render the plant largely unresponsive to pathogen-induced SA production, thereby blocking the induction of SA-dependent *PR* genes and SAR (Cao *et al.*, 1994; Delaney *et al.*, 1995; Shah *et al.*, 1997). *NPR1* is expressed throughout the plant at low levels and shows only modest induction upon pathogen infection or SA treatment in wild-type *Arabidopsis* (Cao *et al.*, 1997; Ryals *et al.*, 1997). Overexpression of NPR1 does not result in a massive induction of the *PR-1* gene, indicating that NPR1 requires post-translational activation in order to transduce the SA signal (Cao *et al.*, 1998; Friedrich *et al.*, 2001). Indeed, SA-induced redox changes have been shown to reduce intermolecular disulphide bonds that hold NPR1 together as an inactive oligomer. This reduction converts the inactive oligomeric complex into an active monomeric form which is translocated into the nucleus to activate *PR* gene expression (Mou *et al.*, 2003).

Although NPR1 acts as a modulator of *PR* gene expression, it does not bind to DNA itself (Cao *et al.*, 1997). Yeast two-hybrid analyses indicated that NPR1 functions through members of the TGA subclass of the basic leucine zipper (bZIP) family of transcription factors (TGAs) that bind to the *as-1* promoter element of the *PR-1* gene (Dong, 2004). Electromobility shift assays showed that NPR1 substantially increases binding of TGA2 to SA-responsive promoter elements in the *Arabidopsis PR-1* gene (Després *et al.*, 2000), suggesting that NPR1-mediated DNA binding of TGAs is important for *PR* gene activation. TGA factors act either as positive or as negative regulators of *PR-1* gene expression (Kesarwani *et al.*, 2007), indicating that the *PR* gene expression during SAR must be under tight regulatory control. Recently, a genomics-directed approach demonstrated that upon induction of SAR, a select group of *WRKY* transcription factor genes is induced after nuclear translocation of NPR1 monomers (Wang *et al.*, 2006). Like the TGAs, WRKY transcription factors have also either a positive or a negative effect on the expression of *PR* genes, thereby further contributing to the complexity

of the SA- and NPR1-dependent signalling network involved in SAR (Wang *et al.*, 2006).

In addition to the regulation of *PR* genes, NPR1 was shown to target the transcription of genes that are involved in the protein secretory pathway. Expression of these proteins ensures proper processing of *PR* transcripts and secretion of PR proteins which contributes to SA-based resistance (Wang *et al.*, 2005). Other important regulators involved in NPR1-dependent *PR* gene expression and SAR have been identified (e.g. NIMIN1, SNI1). Their role in defence signalling will be discussed elsewhere in this book.

Recently, a novel signalling component in SAR was identified. Mishina and Zeier (2006) demonstrated in *Arabidopsis* that FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1) plays a critical role in the onset of SAR. Transcription of the *FMO1* gene was induced locally and systemically upon inoculation with an avirulent strain of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000). Analysis of a T-DNA knockout mutant revealed that FMO1 is important in the amplification of the SAR signal in the systemic tissues (Mishina and Zeier, 2006). A future challenge represents further characterisation of the FMO1-dependent pathway, and how this pathway exerts its role in the onset of the systemically expressed resistance during SAR.

Besides being important for systemic SAR signalling (Koch *et al.*, 2006), FMO1 contributes local basal resistance against *Hyaloperonospora parasitica* and *P. syringae* (Bartsch *et al.*, 2006). Bartsch *et al.* (2006) demonstrated that FMO1 mediates an SA-independent branch of EDS1 signalling. In some studies, *eds1* mutant plants were also found to be defective in SAR signalling (Truman *et al.*, 2007; C. Vlot, L. Jorda and J.E. Parker, unpublished results), while in another report (Mishina and Zeier, 2006) EDS1 appeared not to contribute to SAR. Hence, the roles of EDS1 and FMO1 in SAR need further clarification.

11.2.2 Induced systemic resistance

Beneficial microorganisms, such as mycorrhizal fungi and non-pathogenic rhizobacteria, have been demonstrated to induce a systemic resistance response that is phenotypically similar to SAR (Van Loon *et al.*, 1998; Pozo *et al.*, 2004). Large numbers of non-pathogenic rhizobacteria are attracted to plant exudates produced at the surface of plant roots. Selected strains of these non-pathogenic rhizobacteria are capable of stimulating plant growth (Bloemberg and Lugtenberg, 2001). Growth promotion results mainly from the suppression of soilborne pathogens and other deleterious microorganisms. Fluorescent *Pseudomonas* spp. are among the most effective plant growth-promoting rhizobacteria and have been shown to be responsible for the reduction of soilborne diseases in naturally disease-suppressive soils (Weller *et al.*, 2002). This type of natural biological control can result from competition for nutrients, siderophore-mediated competition for iron, production of antibiotic compounds or production of lytic enzymes (Van Loon and Bakker, 2005).

Apart from such direct antagonistic effects on soilborne pathogens, some rhizobacterial strains are capable of reducing disease incidence in aboveground plant parts through a plant-mediated defence mechanism called ISR (Van Loon *et al.*, 1998). Like SAR, rhizobacteria-mediated ISR has been demonstrated in many plant species, e.g. bean, carnation, cucumber, radish, tobacco, tomato and *Arabidopsis*, and is effective against a broad spectrum of plant pathogens, including oomycetes, fungi, bacteria and viruses (Van Loon *et al.*, 1998). While SAR is predominantly operative against biotrophic pathogens that are resisted through SA-dependent defences, ISR also functions against necrotrophic pathogens that are susceptible to JA-dependent responses, such as *Alternaria brassicicola* (Ton *et al.*, 2002b).

11.2.2.1 The onset of ISR

In contrast to SAR, relatively little is known about the onset of ISR. Although ISR-inducing rhizobacteria show little specificity in their colonisation of roots of different plant species (Van Loon *et al.*, 1998), their ability to induce ISR is dependent on the bacterium–host combination. For instance, *Pseudomonas fluorescens* WCS374r is capable of inducing ISR in radish but not in *Arabidopsis* (Leeman *et al.*, 1995; Van Wees *et al.*, 1997). Conversely, *Arabidopsis* is responsive to *Pseudomonas putida* WCS358r, whereas radish is not (Leeman *et al.*, 1995; Van Wees *et al.*, 1997). *P. fluorescens* WCS417r is capable of inducing ISR in both *Arabidopsis* and radish (Van Wees *et al.*, 1997) as well as in other species, e.g. carnation, radish, tomato and bean but not in *Eucalyptus* (Van Loon and Bakker, 2005). Besides interspecies differences in ISR-inducibility, intraspecies variation is also observed. *Arabidopsis* accessions Columbia (Col-0) and Landsberg *erecta* (L*er*-0) are both responsive to ISR induction by WCS417r but accessions Wassilewskija (Ws-0) and RLD1 are not (Van Wees *et al.*, 1997; Ton *et al.*, 1999). These latter accessions are compromised in a common trait governing a step between the recognition of the bacterium and the expression of ISR. These data clearly indicate that ISR is genetically determined.

Several bacterially derived MAMPs have been implicated in the elicitation of rhizobacteria-mediated ISR (Van Loon and Bakker, 2005). Examples are flagella, cell wall components such as lipopolysaccharides and excreted metabolites such as siderophores and antibiotics. Although conclusive evidence is still lacking, the striking homologies with sensitive perception mechanisms for pathogen-derived PAMPS that function in PTI suggest that non-pathogenic rhizobacteria are similarly recognised by general immune surveillance mechanisms.

Research on the molecular mechanism of rhizobacteria-mediated ISR was initially focused on the role of PR proteins, as the accumulation of these proteins was considered to be strictly correlated with induced disease resistance. However, radish plants whose roots were treated with ISR-inducing WCS417r bacteria did not accumulate PR proteins, although these plants clearly showed enhanced resistance against *Fusarium* wilt disease (Hoffland

et al., 1995). Similarly, *Arabidopsis* plants expressing WCS417r-mediated ISR showed enhanced resistance against *Fusarium oxysporum* f.sp. *raphani* and *Pst* DC3000, but this did not coincide with the activation of the SAR marker genes *PR-1*, *PR-2* and *PR-5* (Pieterse *et al.*, 1996; Van Wees *et al.*, 1997). Determination of SA levels in ISR-expressing *Arabidopsis* plants revealed that ISR is not associated with increased accumulation of SA (Pieterse *et al.*, 2000). Moreover, WCS417r-mediated ISR was expressed normally in SA non-accumulating *Arabidopsis* NahG plants (Pieterse *et al.*, 1996; Van Wees *et al.*, 1997). This led to the conclusion that *P. fluorescens* WCS417r-mediated ISR is an SA-independent resistance mechanism and that WCS417r-mediated ISR and pathogen-induced SAR are regulated by distinct signalling pathways. SA-independent ISR has been shown not only in *Arabidopsis* (Pieterse *et al.*, 1996 Iavicoli *et al.*, 2003; Ryu *et al.*, 2003) but also in tobacco (Press *et al.*, 1997; Zhang *et al.*, 2002) and tomato (Yan *et al.*, 2002). The wide range of induction of ISR suggests that the ability of these *Pseudomonas* strains to activate an SA-independent pathway controlling systemic resistance is a common feature of these non-pathogenic rhizobacteria.

11.2.2.2 ISR signal transduction

Since SA is not required for WCS417r-elicited ISR, the *Arabidopsis* JA response mutant *jar1* and the ET response mutant *etr1* (*ethylene response 1*) were tested for their ability to express ISR. Both mutants were unable to mount resistance against *Pst* DC3000 after colonisation of the roots by WCS417r (Pieterse *et al.*, 1998), indicating that ISR requires responsiveness to both JA and ET. Another indication for involvement of the JA signalling pathway came from the analysis of *Arabidopsis* mutant *eds8* which was previously shown to exhibit enhanced susceptibility to *P. syringae* (Glazebrook *et al.*, 1996). This mutant was impaired in both WCS417r-mediated ISR (Ton *et al.*, 2002a) and JA signalling (Ton *et al.*, 2002b; Glazebrook *et al.*, 2003). To elucidate further the role of ET in the ISR signalling pathway, a large set of well-characterised ET signalling mutants was analysed. None of these mutants showed an ISR response against *Pst* DC3000 after colonisation of the roots by WCS417r (Knoester *et al.*, 1999). These results confirmed that an intact ET signalling pathway is required for the establishment of ISR. Particularly interesting was the analysis of the *eir1* (*ethylene insensitive root 1*) mutant, which is ET insensitive in the roots, but not in the shoot. This *eir1* mutant was incapable of showing ISR after root colonisation by WCS417r. In contrast, after leaf infiltration with WCS417r it exhibited ISR, indicating that responsiveness to ET is required at the site of rhizobacterial induction (Knoester *et al.*, 1999).

To investigate possible involvement of the SAR regulatory protein NPR1 in ISR signalling, the *Arabidopsis npr1* mutant was tested in the ISR bioassay. Surprisingly, *npr1* was incapable of displaying WCS417r-mediated ISR (Pieterse *et al.*, 1998; Van Wees *et al.*, 2000). This result showed that WCS417r-mediated ISR, like SA-dependent SAR, is an NPR1-dependent defence response. Further analysis of the ISR signal transduction pathway revealed that NPR1

acts downstream of the JA- and ET-dependent steps (Pieterse *et al.*, 1998). Because SAR is associated with NPR1-dependent *PR* gene expression, and ISR is not, the action of NPR1 in ISR must be different from that in SAR. These different activities are not mutually exclusive because simultaneous activation of ISR and SAR can lead to an enhanced defensive activity compared to that observed with either type of induced resistance alone (Van Wees *et al.*, 2000). These results suggest that the NPR1 protein is important in regulating and connecting different hormone-dependent defence pathways. As ISR is neither dependent on SA nor associated with *PR* gene expression, the function of NPR1 in ISR is still unknown. Recently, Wang *et al.* (2005) demonstrated a role for NPR1 in the stress-induced augmentation of the protein secretory pathway. Since NPR1 influences the transcription rate of genes involved in protein secretion differently from that of *PR* genes (Wang *et al.*, 2005), it is tempting to speculate that NPR1-dependent stimulation of this process may also be involved in the expression of rhizobacteria-mediated ISR.

Microarray analysis revealed that the R2R3-MYB-like transcription factor gene *MYB72* is specifically activated in roots of *Arabidopsis* upon colonisation by WCS417r (Verhagen *et al.*, 2004). T-DNA knockout mutants *myb72-1* and *myb72-2* appeared to be incapable of mounting ISR against different pathogens, indicating that MYB72 is an essential ISR signalling component that is required in the roots during early signalling steps of broad-spectrum ISR (Van der Ent *et al.*, 2008).

Although the majority of ISR-inducing rhizobacterial strains tested to date trigger an SA-independent signalling pathway, some exceptions have been reported (Van Loon and Bakker, 2005). For instance, an SA-overproducing mutant of *Pseudomonas aeruginosa* 7NSK2 was shown to trigger the SA-dependent SAR pathway in bean and tobacco by producing SA at the root surface (De Meyer *et al.*, 1999). In *Arabidopsis*, the rhizobacterial strain *Paenibacillus alvei* K165 induced systemic resistance against *Verticillium dahliae* which was blocked in SA signalling mutants *eds5* and *sid2* (Tjamos *et al.*, 2005), indicating that this rhizobacterial strain activated an SA-dependent defence pathway.

11.2.3 Induced resistance against herbivorous insects

To fend off insect herbivores, plants have adapted two distinct strategies: induced defence directed against the attacker, referred to as direct defence, and induced defence aimed at exploiting the natural enemies of the attacker, referred to as indirect defence. Both types of defence can be triggered upon insect feeding. Direct defence includes induced responses such as the production of secondary chemicals or enzymes that act as toxins or feeding deterrents (Kessler and Baldwin, 2002; Howe, 2004), whereas indirect defence can involve production of a blend of volatiles that attracts predatory or parasitic enemies of the herbivores (Turlings and Ton, 2006).

11.2.3.1 Direct defence

One of the best-studied examples of induced direct defence against herbivores is the rapid and systemic induction of PIs after wounding or insect feeding in tomato (*Lycopersicon esculentum*) (Howe, 2004). Upon consumption of induced tissues by the herbivore, PIs bind to and inhibit digestive proteases in the insect gut, leading to reduced feeding (Farmer and Ryan, 1992). Several PI-inducing signals have been discovered including oligogalacturonides (OGAs) and systemin. In response to wounding, OGAs are produced from cell wall components, and the 18-amino acid peptide systemin is generated by cleavage from its precursor protein prosystemin. This eventually leads to JA synthesis via the octadecanoid pathway and induction of PIs and other defence genes (Farmer and Ryan, 1992). However, the signal transduction events that couple perception of OGAs and systemin at the plasma membrane to the subsequent activation of JA synthesis in the chloroplast remain to be elucidated (Howe, 2004).

The key role of JAs in induced direct defence against insect herbivores has been demonstrated in many plant–herbivore interactions. For instance, *Pieris rapae* caterpillars (small cabbage white butterfly) gained significantly more weight when they fed on the *Arabidopsis* JA signalling mutant *coi1* (*coronatine insensitive 1*) than on wild-type plants (Reymond *et al.*, 2004). Likewise, the population of the aphid *Myzus persicae* (green peach aphid) increased faster on *coi1* than on wild-type *Arabidopsis* (Ellis *et al.*, 2002). Conversely, on the constitutive JA signalling *Arabidopsis* mutant *cev1* (*constitutive expression of VSP1*), population levels of *M. persicae* were reduced (Ellis *et al.*, 2002). The tomato mutant *def1* (*defenseless 1*), deficient in JA biosynthesis, has compromised resistance to tissue-chewing *Manduca sexta* (tobacco hornworm) and *Spodoptera exigua* (beet armyworm) larvae as well as the cell-content feeding *Tetranychus urticae* (two-spotted spider mite) and *Frankliniella occidentalis* (Western flower thrips) (Howe *et al.*, 1996; Li *et al.*, 2002; Thaler *et al.*, 2002). The JA biosynthesis mutant *fad3fad7fad8* (*fatty acid desaturation 3, 7, 8*) of *Arabidopsis* is extremely sensitive to larvae of the fungal gnat, *Bradysia impatiens* (McConn *et al.*, 1997). Moreover, the ET insensitive *Arabidopsis* mutant *ein2* is less resistant to larvae of *Spodoptera littoralis* (Egyptian cotton worm) (Stotz *et al.*, 2000). In addition, *Arabidopsis* mutants and transgenics that are compromised in SA-dependent defence responses exhibit enhanced resistance against feeding by the cabbage looper *Trichoplusia ni* (Cui *et al.*, 2002). Thus, whereas JA plays a main role, ET and SA also influence plant resistance against insects.

11.2.3.2 Indirect defence

Insect feeding induces production of volatile chemicals in the plant which are effective in attracting parasitic and predatory insects (Van Poecke and Dicke, 2004; Turlings and Ton, 2006). JA is the major signalling molecule involved in the induced production of plant volatiles (Van Poecke and Dicke *et al.*, 2004). Treatment of plants with JA leads to the emission of a volatile blend that is similar, but not identical, to the blend of herbivore-infested plants. Moreover,

the volatiles induced by JA treatment are attractive to carnivorous enemies of the herbivores (Van Poecke and Dicke, 2004).

ET and SA can also play a role in indirect defence. ET was shown to enhance JA-mediated volatile emission in lima bean (*Phaseolus lunatus*) (Horiuchi *et al.*, 2001). Herbivores such as spider mites induce the emission of MeSA in many plant species (Ament *et al.*, 2004; De Boer and Dicke, 2004), which can lead to the activation of SA-inducible defence-related genes (Arimura *et al.*, 2000; Kant *et al.*, 2004). In line with these results, feeding by *P. rapae* larvae induced MeSA production in *Arabidopsis* (Van Poecke and Dicke, 2002). In *Arabidopsis* NahG plants, MeSA was not produced upon *P. rapae* feeding, leading to decreased attractiveness of the induced volatile blend to the parasitoid wasp *Cotesia rubecula* (Van Poecke and Dicke, 2002). Similarly, the volatiles induced upon feeding of *P. rapae* in the transgenic *Arabidopsis* S-12 line with reduced JA biosynthesis were less attractive to *C. rubecula* (Van Poecke and Dicke, 2002). These results illustrate that JA, ET and SA all play a role in induced indirect defence against insects.

Airborne volatile organic compounds (VOCs) that are produced upon insect herbivory not only are important in the attraction of natural enemies of the herbivore but can also enhance the level of resistance in neighbouring plants that is effective against future insect attack (Baldwin *et al.*, 2006; Turlings and Ton, 2006). From an evolutionary perspective, it has always been puzzling how this form of plant–plant communication can persist, as it benefits the receiver plant, rather than the emitter plant. It seems more likely therefore that VOCs play an important role as systemic within-plant signalling components in insect-induced resistance. Indeed, recent findings by Heil and Silva Bueno (2007) and Frost *et al.* (2007) support a within-plant signalling role of VOCs. Surrounding organisms, such as neighbouring plants, herbivorous insects and predatory or parasitoid insects, have merely evolved the ability to 'eavesdrop' on this airborne within-plant signalling.

11.2.3.3 JA signal transduction

In the past 20 years, JA and its functionally active derivates (e.g. JA isoleucine (JA-Ile) and methyl JA (MeJA)) emerged as important regulators of induced plant defence. JAs are produced by the octadecanoid pathway after insect herbivory or pathogen attack (Wasternack, 2007). Downstream target genes include defence-related genes such as the defensin *PDF1.2* (*PLANT DEFENSIN 1.2*) and thionin *Thi2.1* (*THIONIN 2.1*), but also genes that are required for the biosynthesis of JA. All plant responses to JA that have been described so far are dependent on an intact COI1 protein (Feys *et al.*, 1994; Xie *et al.*, 1998). COI1 encodes an F-box protein (Xie *et al.*, 1998) which is part of an SCF^{COI1} E3 ubiquitin ligase complex that is involved in proteasome-mediated protein degradation (Xu *et al.*, 2002). The F-box protein confers specificity to the E3 ligase complex by interacting with proteins that are targeted for ubiquitination and subsequent degradation. Therefore, COI1 is thought to mediate the removal

of repressors that keep JA responses inactive (Devoto *et al.*, 2003). Recently, JAZ (jasmonate ZIM-domain) proteins have been identified as likely candidates for COI1-targeted transcriptional repressors of JA-responsive genes (Chini *et al.*, 2007; Thines *et al.*, 2007). JAZ proteins repress JA-responsive gene expression by active suppression of transcriptional activators of JA-responsive genes such as AtMYC2 (Chini *et al.*, 2007). Upon stimulation of the JA response, the physical interaction of JA-Ile with JAZ proteins allows COI1 to target JAZ proteins for degradation by the proteasome (Thines *et al.*, 2007). As a result, repression by the JAZ proteins is lifted, causing enhanced transcription of JA-responsive genes. Notably, JAZ biosynthesis genes are induced by JA themselves, indicating a negative feedback loop that allows for a pulsed response to the JA-inducing stimulus (Chini *et al.*, 2007; Thines *et al.*, 2007).

11.2.4 Chemically induced resistance

In addition to biological stimuli, the application of certain chemicals can induce resistance of distal plants parts. Often, these chemicals induce a similar resistance response as biologically induced SAR, as is the case with applications of INA, BTH and SA (Durrant and Dong, 2004). However, the non-protein amino acid BABA seems to induce a somewhat different induced resistance response. Application of BABA induces resistance in many different plant species (Jakab *et al.*, 2001; Cohen, 2002) and is effective against biotrophic and necrotrophic pathogens (Siegrist *et al.*, 2000; Zimmerli *et al.*, 2000; Ton and Mauch-Mani, 2004), insects (Hodge *et al.*, 2005) and some forms of abiotic stress such as osmotic stress and heat stress (Jakab *et al.*, 2005). This remarkably wide range of effectiveness of BABA-induced resistance (BABA-IR) suggests that multiple resistance responses are involved. Indeed, Zimmerli *et al.* (2000) demonstrated that BABA-IR against the oomycete *H. parasitica* was still functional in *Arabidopsis* genotypes impaired in SA-dependent signalling, whereas BABA-IR against *Pst* DC3000 was blocked in these *Arabidopsis* genotypes. Hence, expression of BABA-IR involves both SA-dependent and SA-independent resistance mechanisms, and the importance of these mechanisms varies according to the nature of the challenging pathogen.

By screening previously characterised *Arabidopsis* mutants for BABA-IR, ABA was identified as an additional regulator of BABA-IR against the necrotrophic fungi *A. brassicicola* and *Plectosphaerella cucumerina*. Mutants impaired in SA, JA and ET signalling as well as camalexin production remained unaffected in BABA-IR against these fungi (Ton and Mauch-Mani, 2004). These findings suggested a novel role for ABA in the regulation of induced resistance against fungal pathogens. The role for ABA in BABA-IR was further confirmed by the identification of the mutant *ibs3* (*impaired in BABA-induced sterility 3*). This mutant is affected in the transcriptional regulation of the ABA biosynthetic gene *ABA1* and concomitantly fails to express

wild-type levels of BABA-IR against the oomycete *H. parasitica* (Ton *et al.*, 2005).

11.3 Crosstalk between defence signalling pathways

Recent genomics research has revealed that the capacity of plants to respond to the enormous diversity of attackers and beneficial organisms is highly flexible (Verhagen *et al.*, 2004; De Vos *et al.*, 2005; Sanchez *et al.*, 2005; Kempema *et al.*, 2007). The signalling networks that are activated by the plant in response to parasitic, herbivorous and beneficial organisms overlap, indicating that the regulation of the adaptive response of the plant is finely balanced between protection against aggressors and acquisition of benefits. The signalling pathways that are controlled by endogenous accumulation of defence signals such as SA, JA and ET regulate different defence responses that are effective against partially distinct classes of attackers. For many years, it was assumed that pathogens with a biotrophic lifestyle are predominantly inhibited by SA-dependent defences, whereas necrotrophic pathogens and herbivorous insects are resisted by JA/ET-dependent defences (Thomma *et al.*, 1998; Kessler and Baldwin, 2002; Glazebrook, 2005). However, over the past years it became clear that there are exceptions to this partition (Thaler *et al.*, 2004), suggesting additional layers of regulation to the plant's defensive response. In fact, plants react with a surprisingly specific response to attack by different pathogens or insects which is reflected by a highly specific amplitude and timing in the production of defence signalling compounds such as SA, JA and ET. It is thought that this so-called signal signature contributes to the specificity of the plant's primary induced defences (De Vos *et al.*, 2005). However, cross-communication between the corresponding pathways has been put forward as an additional mechanism by which plants fine-tune their defence responses (Reymond and Farmer, 1998).

11.3.1 Crosstalk between SA and JA signalling

A well-characterised example of defence-related signalling crosstalk is the interaction between the SA and JA response pathways (Rojo *et al.*, 2003; Bostock, 2005; Beckers and Spoel, 2006). Although most reports indicate a mutually antagonistic interaction between SA- and JA-dependent signalling (Bostock, 2005), synergistic interactions have been described as well (Schenk *et al.*, 2000; Van Wees *et al.*, 2000; Mur *et al.*, 2006). As a result of negative crosstalk between SA and JA, activation of the SA response should render a plant more susceptible to attackers that are resisted via JA-dependent defences and vice versa. Indeed, trade-offs between SA-dependent resistance against biotrophic pathogens and JA-dependent defence against insect herbivory have been reported (Pieterse *et al.*, 2001; Bostock, 2005). However, comparative analysis of a large number of plant–microbe and plant–insect interactions revealed

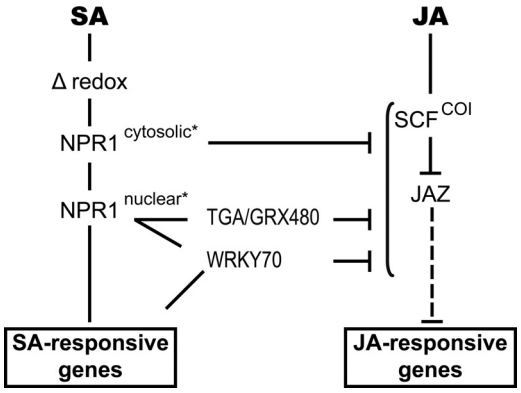


Figure 11.2 Schematic representation of molecular players in the NPR1-dependent crosstalk between salicylic acid (SA) and jasmonic acid (JA) signalling. SA-activated cytosolic NPR1 mediates downregulation of JA-responsive gene expression. The transcription factor WRKY70 and glutaredoxin GRX480 mediate suppression of JA responses in an NPR1-dependent fashion. The exact target of the JA signalling route (indicated by the extended bracket) remains to be determined. SCF^{COI}, SKP1/Cullin/F-box protein-COI1 complex; JAZ, jasmonate ZIM-domain protein. See text for details.

a more complex reality which can partially be explained by differences in experimental conditions, thereby making predictions about the outcome of such tripartite interactions difficult (Stout *et al.*, 2006).

Elucidating the molecular mechanism underlying antagonistic interactions between SA- and JA-dependent defence signalling pathways provides an excellent model to unravel the multifaceted signal interactions that shape the plant immune response. Over the past years, various regulatory components have been identified in the crosstalk between SA- and JA-dependent signalling pathways. These include proteins with stimulatory or repressive functions in either SA-dependent signalling (NPR1, WRKY70 and GRX480) or JA-dependent signalling (MPK4 and SSI2). Although different individual components have been placed in the 'crosstalk signalling network' (Fig. 11.2), their exact position and role in this network often remains to be determined. Below, the molecular players of SA/JA crosstalk identified to date are described in more detail.

11.3.1.1 NPR1

Several key regulatory proteins involved in pathway crosstalk have been identified. In *Arabidopsis*, SA-mediated suppression of JA-inducible gene expression is blocked in the *npr1* mutant, demonstrating that NPR1 plays a critical role in the crosstalk between SA and JA signalling (Spoel *et al.*, 2003). Using *npr1* plants expressing recombinant NPR1 protein with a glucocorticoid receptor hormone binding domain, Spoel *et al.* (2003) showed that nuclear localisation of NPR1 is not required for SA-mediated suppression

of the JA response. This indicates that the SA-induced suppression of the JA response is controlled by a novel cytosolic function of NPR1. Recently, a similar function of NPR1 in crosstalk was reported in rice (*Oryza sativa* L.). Overexpression of cytosolic *OsNPR1* suppressed JA-responsive transcription and enhanced the level of susceptibility to insect herbivory. Moreover, NPR1-dependent suppression of the JA response was no longer present in plants expressing OsNPR1 that was constitutively targeted to the nucleus (Yuan *et al.*, 2007).

11.3.1.2 WKRY transcription factors

WRKY transcription factors have been shown to play an important role in the regulation of SA-dependent defence responses (Maleck *et al.*, 2000; Wang *et al.*, 2006). Several WRKY transcription factors were implicated in the cross-communication between SA and JA signalling. In *Arabidopsis*, WRKY70 was identified as a node of convergence between SA and JA signalling when Li *et al.* (2004) showed that overexpression of WRKY70 caused enhanced expression of SA-inducible *PR* genes but concomitantly suppressed MeJA-induced *PDF1.2* expression. Hence, WRKY70 acts as a positive regulator of the SA response, but also as a repressor of the JA response. The exact position of WRKY70 in the crosstalk signalling network is, however, still unclear. On the one hand, WRKY70 acts as a downstream target of NPR1 (Li *et al.*, 2004; Wang *et al.*, 2006). On the other hand, transgenic plants overexpressing *WRKY70* in the *npr1-1* mutant background are impaired in *PDF1.2* repression, indicating functional NPR1 is required for WRKY70-dependent repression of this JA-responsive gene (Li *et al.*, 2006). Furthermore, recent studies with T-DNA insertion lines of *WRKY70* revealed rather diverse phenotypes, including increased levels of *PDF1.2* and *PR-1* gene expression (AbuQamar *et al.*, 2006; Li *et al.*, 2006; Ülker *et al.*, 2007), increased accumulation of SA (Wang *et al.*, 2006), enhanced resistance to *A. brassicicola* (Li *et al.*, 2006) and enhanced susceptibility to *Erysiphe cichoracearum* (Li *et al.*, 2006), *B. cinerea* (AbuQamar *et al.*, 2006) and *H. parasitica* (Knoth *et al.*, 2007). These data indicate that WRKY70 indeed affects both SA and JA signalling, but in a rather complex manner.

Besides WRKY70, WRKY11 and WRKY17 have also been implicated in crosstalk between SA and JA signalling. In *Arabidopsis*, double knockout mutants in *WRKY11* and *WRKY17* displayed enhanced levels of *PR-1* gene expression but decreased levels of JA-inducible gene expression. The expression of *WRKY70* expression was upregulated in this double mutant, suggesting that WRKY11 and WRKY17 function as negative regulators of WRKY70 (Journot-Catalino *et al.*, 2006). Recently, WRKY62 was added to the list of WRKY factors with a putative role in the crosstalk between SA and JA signalling. Mao *et al.* (2007) reported that the expression of *WRKY62* was synergistically induced by SA and JA in wild-type Col-0 plants but not in mutant *npr1-3*. Furthermore, transposon-tagged *wrky62* plants showed enhanced MeJA-induced transcription of *LOX2* (*LIPOXYGENASE 2*) and *VSP2*

(*VEGETATIVE STORAGE PROTEIN 2*), whereas overexpression of *WRKY62* resulted in suppression of these genes. These findings point to a repressive effect of WRKY62 on the JA response. Whether the observed WRKY62-dependent suppression of JA-inducible genes is actually activated by SA and dependent on NPR1 remains to be investigated.

11.3.1.3 Glutaredoxin GRX480

Another putative regulator in the crosstalk between SA and JA signalling is the glutaredoxin GRX480. Glutaredoxins catalyse thiol disulphide reductions and have been implicated in redox-dependent regulation of protein activities (Lemaire, 2004). Recently, Ndamukong *et al.* (2007) identified this glutaredoxin in a two-hybrid screen for interactors with TGA transcription factors. Expression of *GRX480* was found to be inducible by SA and dependent on NPR1. Interestingly, overexpression of *GRX480* completely abolished MeJA-induced *PDF1.2* expression but hardly affected the induction of JA-responsive LOX2 and VSP. This suggests that GRX480 regulates only a part of SA-induced suppression of the JA response. The suppressive effect of GRX480 on *PDF1.2* induction was abolished in the *tga2tga5tga6* triple mutant, indicating that the interaction between GRX480 and TGA transcription factors is essential for the GRX480-dependent crosstalk (Ndamukong *et al.*, 2007). These results suggest a model in which NPR1 induces GRX480, which in turn interacts with TGA transcription factors to suppress JA-responsive gene induction.

11.3.1.4 MPK4

Apart from NPR1-dependent crosstalk between SA and JA signalling pathways, several NPR1-independent routes have also been described. Petersen *et al.* (2000) identified MAP kinase 4 (MPK4) as a negative regulator of SA signalling and positive regulator of JA signalling in *Arabidopsis*. Inactivation of MPK4 resulted in elevated SA levels and constitutive *PR* gene expression. MPK4 was shown to function upstream of SA accumulation, but independently of NPR1 (Petersen *et al.*, 2000). Inactivation of MPK4 in mutant *mpk4* resulted in enhanced susceptibility to *A. brassicicola*, which is sensitive to JA-dependent defences. Moreover, *mpk4* blocked JA-responsive gene expression independently of SA accumulation, as *mpk4*/NahG transgenics still exhibited increased susceptibility to *A. brassicicola* and suppression of MeJA-induced *PDF1.2* expression (Petersen *et al.*, 2000; Brodersen *et al.*, 2006). Thus, MPK4 is required for JA-responsive gene expression. EDS1 and PAD4 were identified as downstream effectors of MPK4 function, having the opposite effect of MPK4 by behaving as activators of SA signalling and repressors of JA signalling (Brodersen *et al.*, 2006). Another target of MPK4 is its substrate MKS1 (MAP kinase 4 substrate 1). Phosphorylation of MKS1 is thought to repress SA signalling, since *MKS1*-RNAi could partially rescue the *PR-1*-overexpressing phenotype of *mpk4*. However, over- or underexpression of MKS1 did not affect *PDF1.2* gene expression, indicating that other downstream targets of MPK4 are involved in JA signalling. MKS1 interacted with two WRKY

transcription factors, WRKY25 and WRKY33, that could both be phosphorylated by MPK4 (Andreasson *et al.*, 2005). These WRKYs might be downstream targets of MPK4 that contribute to the repression of SA responses, as overexpression of both WRKY25 and WRKY33 resulted in decreased pathogen-induced *PR-1* expression and enhanced susceptibility to *P. syringae* (Zheng *et al.*, 2006, 2007).

11.3.1.5 SSI2

Mutant *ssi2* (*suppressor of SA insensitivity 2*) is defective in stearoyl ACP desaturase, resulting in an altered fatty acid content. Also, this mutant shows *NPR1*-independent constitutive *PR-1* expression and enhanced resistance to *H. parasitica* but is impaired in *PDF1.2* transcription and resistance to *B. cinerea*. Inhibition of *PDF1.2* is not dependent on elevated SA levels, since *ssi2*/NahG plants were still unable to express JA-induced *PDF1.2* (Kachroo *et al.*, 2001). Mutations that restored the lowered 18:1 fatty acid levels rescued the *ssi2* mutant phenotype, suggesting a role for fatty acid signalling in SA/JA crosstalk (Kachroo *et al.*, 2003, 2004).

11.3.1.6 Targets of the SA/JA antagonism

By analogy to the inhibitory effect of aspirin (acetyl salicylic acid) on the production of the octadecanoid prostaglandin in mammalian systems, evidence suggests that the antagonistic effect of SA on the JA response in plants is targeted at the level of octadecanoid biosynthesis (Pan *et al.*, 1998). Several reports have described suppression of JA biosynthesis genes by SA or its acetylated form, suggesting that SA targets the octadecanoid biosynthesis pathway to suppress downstream JA signalling (Peña-Cortés *et al.*, 1993; Doares *et al.*, 1995; Spoel *et al.*, 2003). However, it is not known whether suppression of the JA biosynthesis pathway is essential in transducing the SA antagonism. Recent evidence shows that *Arabidopsis* mutants that are blocked in JA biosynthesis have normal levels of SA-mediated suppression of MeJA-induced *PDF1.2* expression, suggesting that downstream components in the JA signalling pathway are targeted by SA (H.A. Léon Reyes and C.M.J. Pieterse, unpublished results). In view of its importance in the JA signalling pathway, it is tempting to speculate that the SCF^{COI1} complex is the target of SA in SA/JA crosstalk. Alternatively, downstream components of COI1 might be involved in the SA/JA antagonism. The recently identified JAZ repressor proteins can also be an attractive target for the SA-mediated suppression of JA signalling. SA-mediated stabilisation of these repressors would inhibit JA signalling and a broad-spectrum effect on JA target genes would be achieved.

11.3.2 Crosstalk between other defence signals

Besides crosstalk between SA and JA signalling, other phytohormone pathways have been shown to interact and affect each other's downstream

defence responses. In *Arabidopsis*, for example, JA-inducible defence mechanisms against pathogens and insects are antagonistically co-regulated by ET and ABA via MYC2 and ERF1 (ETHYLENE RESPONSE FACTOR 1), respectively (Anderson *et al.*, 2004; Lorenzo *et al.*, 2004). These transcription factors regulate divergent branches of the JA signalling route that are involved in the response to wounding and pathogen attack, respectively. MYC2-dependent gene induction is synergistically induced by JA and ABA, while ERF1-dependent gene induction is controlled by the combined action of JA and ET. These findings show that the antagonistic crosstalk between ABA and ET signalling not only influences developmental process (Pierik *et al.*, 2006) but also contributes to the fine-tuning of defence against pathogens and insects (Anderson *et al.*, 2004; Lorenzo *et al.*, 2004).

11.3.3 Decoys of plant defences

Crosstalk between defence pathways is thought to provide the plant with a powerful regulatory potential that helps it to prioritise and 'decide' which defensive strategy to follow, depending on the type of attacker. Yet, it seems that attackers and beneficial organisms have also evolved to manipulate plants for their own benefit by shutting down induced defences or modulating the signalling network (Pieterse and Dicke, 2007). Some microbial pathogens have acquired the ability to manipulate the plant's signalling infrastructure by producing phytohormones or their functional mimics to 'trick' the plant into activating inappropriate defences (Robert-Seilaniantz *et al.*, 2007). For instance, coronatine is a phytotoxin produced by virulent *P. syringae* strains (Nomura *et al.*, 2005) that functions as an extremely potent mimic of JA-Ile. It is assumed that the coronatine from the pathogen triggers a hypersensitive induction of JA-Ile responses, causing suppression of SA-dependent defences through crosstalk (Brooks *et al.*, 2005; Cui *et al.*, 2005). Recently, coronatine was also demonstrated to prevent PAMP-induced stomatal closure, thereby facilitating bacterial entry into the leaf (Melotto *et al.*, 2006).

Insects can also induce ineffective plant signalling cascades as a decoy mechanism. For instance, *Bemisia tabaci* (silverleaf whitefly) nymphs trigger SA-responsive gene expression and, as a consequence, suppress the induction of effectual JA- and ET-dependent genes (Zarate *et al.*, 2007). Further analysis of mutant and transgenic *Arabidopsis* lines revealed that JA-regulated rather than SA-dependent defences contributed to basal resistance against silverleaf whitefly nymphs. Hence, the nymphs are capable of exploiting negative crosstalk between SA and JA to make plant tissue more accessible for infestation (Zarate *et al.*, 2007). Recently, egg-derived elicitors from *Pieris brassicae* and *P. rapae* have been suggested to suppress JA-dependent responses through mechanisms of SA-induced crosstalk as an insect strategy to benefit hatching larvae (Little *et al.*, 2007).

11.4 Priming for enhanced defence

Different signal signatures, pathway crosstalk and attacker-mediated suppression of host defence signalling are major molecular mechanisms by which the defence response of the plant is shaped. Priming for enhanced defence adds another layer of complexity to the way plants can adapt to their biotic environment. The primed state can be induced biologically by beneficial rhizobacteria, mycorrhizal fungi, pathogens or insect herbivores, but also chemically, for example, by exogenous application of low doses of SA, JA or BABA (Conrath *et al.*, 2002, 2006). In primed plants, defence responses are not activated directly by the priming agent but are accelerated following perception of biotic or abiotic stress signals, resulting in an enhanced level of resistance (Conrath *et al.*, 2002, 2006). By studying the costs and benefits of priming in *Arabidopsis*, it was shown that the fitness costs of priming are lower than those of constitutively activated defences such as expressed in the constitutive SAR-expressing mutant *cpr1* (*constitutive expresser of PR genes*; Van Hulten *et al.*, 2006). The fitness benefits of priming outweighed its costs under pathogen pressure, suggesting that priming functions as an ecological adaptation of the plant to respond faster to a hostile environment.

11.4.1 Priming during SAR

Remarkably, first implications for the involvement of priming in SAR arose from studies using elicitors of chemical-induced resistance. Low amounts of BTH and SA did not directly activate defence responses but rather accelerated the expression of *PAL* and *PR* genes (Mur *et al.*, 1996; Kohler *et al.*, 2002). Mutant analyses demonstrated a role for NPR1 in priming of SA-mediated defences. Besides being blocked in direct activation of defence genes, *npr1* plants were not able to prime the expression of *PAL* for a faster response to *Pst* DC3000 when pretreated with BTH or an avirulent strain of *Pst* DC3000 (Kohler *et al.*, 2002). Hence, NPR1 is involved in priming for enhanced expression of SA-mediated defences. Also, BTH-primed deposition of callose-containing papillae at *H. parasitica* penetration sites was disrupted in *npr1* (Fig. 11.3a). Together with antimicrobial components residing in the callose matrix, these papillae may form a physical and/or chemical barrier to certain pathogens, preventing them from invading the plant tissue.

Besides *npr1*, *edr1* (*enhanced disease resistance 1*) that is mutated in an *MAPKKK* (Frye *et al.*, 2001) has an altered priming phenotype. The enhanced protective level against various pathogens of *edr1* is not based on constitutive activation of defence responses (Frye and Innes, 1998; Van Hulten *et al.*, 2006). Rather, *edr1* plants are constitutively primed for augmented expression of diverse defences such as *PR-1* expression, callose deposition and HR (Frye and Innes, 1998; Van Hulten *et al.*, 2006), suggesting that the EDR1 protein is a repressor of priming.

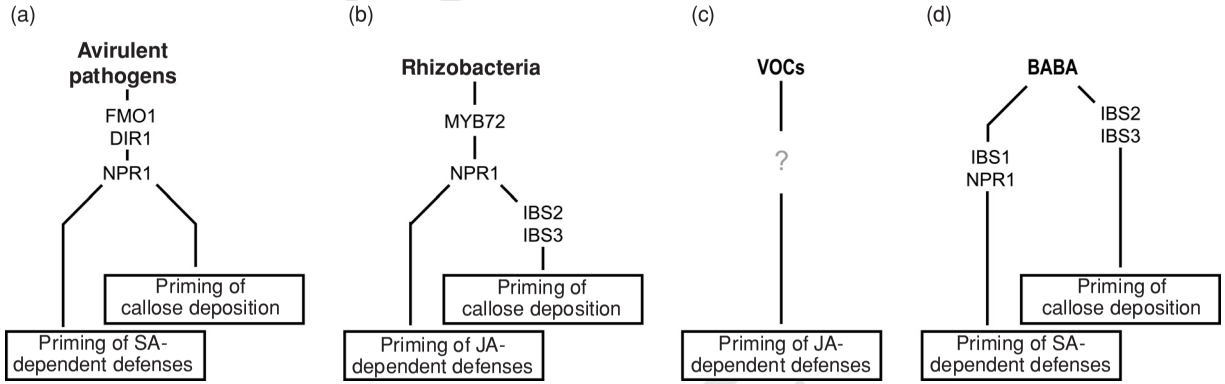


Figure 11.3 Priming pathways for enhanced defence in *Arabidopsis*. (a) Priming during systemic acquired resistance (SAR). The SAR response requires FMO1 and DIR1 for propagation of the systemic signal and requires intact NPR1 for both priming of enhanced SA-dependent defence gene expression and priming of increased callose deposition. (b) Priming during induced systemic resistance (ISR). Rhizobacteria-mediated ISR requires MYB72 and NPR1 and leads to priming of enhanced JA-dependent defence gene expression and priming of accelerated callose deposition via the IBS2 and IBS3 proteins. (c) Priming by VOCs. Volatiles released upon insect herbivory prime plants for both direct and indirect JA-dependent defence responses. (d) Priming by β-aminobutyric acid (BABA). BABA can prime for both enhanced SA-dependent defence gene expression and increased callose deposition upon pathogen attack. NPR1 and IBS1 are required for priming of SA-dependent responses, but not for priming of callose deposition. Rather, IBS2 and IBS3 are needed for priming of increased formation of callose-rich papillae. SA, salicylic acid; JA, jasmonic acid; VOCs, volatile organic compounds.

11.4.2 Priming during ISR

In contrast to SAR, ISR is not associated with direct induction or priming of *PR* gene expression (Van Wees *et al.*, 1999). To detect ISR-induced genes in systemic tissue of *Arabidopsis*, Verhagen *et al.* (2004) analysed the transcriptome of leaves upon colonisation of the roots by ISR-inducing WCS417r rhizobacteria. Despite elevated levels of resistance, no differences in gene expression could be observed between the distal parts of ISR- and control-treated plants prior to pathogen infection. However, a similar analysis of induced systemic tissue after pathogen infection led to the identification of a set of genes that responded faster and stronger to pathogen attack (Verhagen *et al.*, 2004), which was in line with earlier observations of selected defence-related marker genes (Van Wees *et al.*, 1999). In particular, genes regulated by JA or ET exhibited a primed response upon pathogen attack, corresponding with the requirement of intact JA and ET signal transduction pathways for the expression of WCS417r-mediated ISR. Similarly, ISR-inducing *P. putida* LSW17S was demonstrated to prime JA- and ET-dependent defence responses of *Arabidopsis* (Ahn *et al.*, 2007). In many other interactions between plants and plant growth-promoting rhizobacteria, increased resistance arises from a potentiated expression of defence-related genes (Benhamou *et al.*, 1996; De Meyer *et al.*, 1999; Ahn *et al.*, 2002; Kim *et al.*, 2004; Tjamos *et al.*, 2005). Recent findings suggest that WCS417r-mediated ISR in *Arabidopsis* against the oomycete pathogen *H. parasitica* is based on an augmented deposition of callose-rich papillae. This priming response was dependent on NPR1 as well as the IBS2 and IBS3 proteins that play a role in BABA-IR against fungi and oomycetes (S. van der Ent and J. Ton, unpublished results) (Fig. 11.3b).

11.4.3 Priming by airborne signals

Priming by airborne signals such as VOCs produced following insect herbivory is a major topic in molecular ecological research on plant–herbivore and plant–plant interactions (Baldwin *et al.*, 2006; Turlings and Ton, 2006). Analogous to chemicals such as INA and BTH, VOCs can either directly activate defence responses of recipient plants or prime them to respond faster and stronger to stress exposure (Baldwin *et al.*, 2006; Turlings and Ton, 2006). Engelberth and co-workers (2004) demonstrated that green leafy volatiles produced by corn plants after insect feeding prime neighbouring plants for enhanced JA-dependent defence against herbivory, rather than directly activating it (Fig. 11.3c). In a laboratory study with maize, VOCs were similarly demonstrated to prime neighbouring plants for enhanced direct and indirect defence, resulting in reduced performance of caterpillars of the Egyptian cotton leafworm *S. littoralis* (direct defence) and improved attractiveness to parasitoid *Cotesia marginiventris* wasps that feed on the insect herbivores

(indirect defence) (Ton *et al.*, 2007). Also in the field, herbivory-induced VOCs have been demonstrated to prime nearby plants for enhanced direct and indirect defence responses (Kessler *et al.*, 2006), indicating that priming has a role in plant defence under ecological conditions. Another demonstration of VOC-induced priming in nature was provided by Heil and Silva Bueno (2007). They showed that VOCs released by beetle-infested 'emitter' leaves of lima bean plants growing in their natural habitat primed nearby 'receiver' leaves for enhanced secretion of extrafloral nectar, resulting in prolonged visitation by predatory arthropods. Although the active players in VOC-mediated priming differ among plant species, it seems to be a common defence strategy in plants (Baldwin *et al.*, 2006).

11.4.4 Priming during BABA-IR

Application of high concentrations of BABA directly activates defence responses that are regulated either by SA or by ABA (Van Hulten *et al.*, 2006; J. Ton and M. Van Hulten, unpublished results). However, lower amounts of BABA prime for enhanced induction of NPR1-dependent *PR* gene expression as well as NPR1-independent deposition of callose-containing papillae at entry sites of the pathogen (Fig. 11.3d) (Zimmerli *et al.*, 2000; Ton *et al.*, 2005). Augmented callose deposition is also involved in and even essential for BABA-IR against the fungal pathogens *A. brassicicola* and *P. cucumerina* (Ton and Mauch-Mani, 2004).

Screening for mutants that are impaired in BABA-induced sterility (*ibs*) resulted in the identification of three genes (*IBS1*, *AtSAC1b/IBS2* and *ABA1/IBS3*) with a regulatory role in BABA signalling (Ton *et al.*, 2005). While BABA-IR is also impaired in *ibs1*, *ibs2* and *ibs3*, the three mutants show normal levels of basal resistance. IBS1 is involved in the SA-dependent component of BABA-IR, while AtSAC1b/IBS2 and ABA1/IBS3 are required for ABA-regulated callose deposition and subsequent BABA-IR (Fig. 11.3d).

11.4.5 Molecular mechanisms of priming

Priming is associated with different types of induced resistance (Conrath *et al.*, 2002, 2006), and it provides the plant with an enhanced capacity for rapid and effective activation of cellular defence responses once a pathogen contacts. Such a defence system could explain the broad-spectrum action that is often associated with induced resistance. The molecular mechanisms underlying priming are still poorly understood. Hypothetically, the primed state could be based on the accumulation or post-translational modification of one or more signalling proteins that, after being expressed or modified, still remain inactive. Upon subsequent perception of a stress, a second signalling event could 'hyperactivate' the signalling protein, triggering a potentiated signal

transduction. Another hypothesis suggests that priming enables accelerated defence gene expression by inducing the accumulation of essential transcription factors. After stress recognition, signal transduction in primed cells then could directly induce an appropriate set of defence genes, thereby avoiding a preliminary step of transcription factor expression.

11.5 Concluding remarks

Plant diseases and pests are responsible for large crop losses in agriculture. Conventional crop protection is based on resistance breeding and application of chemical agents. Classic resistance breeding depends on the availability of *R* genes that often have limited durability. The use of chemical agents and their persistence in soil are potentially harmful to the environment, especially when chemicals are applied repeatedly in large amounts such as in the control of soilborne fungal pathogens. Moreover, these disease control strategies are directed against a single or a small group of plant pathogens. Induced disease resistance might be an attractive alternative form of plant protection, as it is based on the activation of extant resistance mechanisms in the plant and is effective against a broad spectrum of plant pathogens (Kuc, 1982; Van Loon *et al.*, 1998).

Knowledge of defence signalling pathways has been proven to be instrumental in the development of new strategies for broad-spectrum disease resistance. Examples are genetic engineering of the SAR pathway and the development of defence signal-mimicking chemicals such as BTH. However, crosstalk between SA- and JA-dependent defence pathways may be a burden when enhanced pathogen resistance is associated with reduced resistance against insects.

Priming fits well in the ecological context of induced resistance. While constitutive activation of inducible defences involves major costs that affect plant growth and reproduction (Heil, 2002), priming for enhanced defence is associated with significant fitness benefits when disease occurs (Van Hulten *et al.*, 2006). Consequently, plants in the primed state are effectively protected against stress without major trade-off effects on commercially and ecologically important traits, such as growth and seed set. Thus, from an economic perspective, priming could be the plant's solution to the trade-off dilemma between disease protection and the cost of defence activation.

Future research on the molecular mechanisms of induced resistance, crosstalk between plant defence pathways and priming for enhanced defence will provide more insight into how plants are able to integrate signals into appropriate defences cost-effectively. Ultimately, this will not only provide a deeper understanding of how plants cope with different enemies, but also be instrumental in developing strategies for biologically based, environmentally friendly and durable crop protection.

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