Cross-talk Between Salicylate- and Jasmonate-Dependent Induced Defenses in Arabidopsis

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Plants possess inducible defense mechanisms to effectively combat invasion by microbial pathogens or attack by herbivorous insects. Research on defense signaling pathways revealed that induced defenses against pathogens and herbivores are regulated by a network of interconnecting signaling pathways in which the plant signal molecules salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play a dominant role (Glazebrook, 2001; Pieterse and Van Loon, 1999). In many cases, attack by pathogens or herbivores is associated with enhanced production of these hormones and a concomitant activation of distinct sets of defense-related genes (Maleck et al., 2000; Reymond et al., 2000; Schenk et al., 2000). Moreover, exogenous application of SA, JA or ET often results in an enhanced level of resistance (Van Wees et al., 1999).

Little is known about how plants integrate signals generated by different inducers of resistance into specific defense responses. An well-accepted hypothesis is that this is accomplished by modulation of different signaling pathways. There is ample evidence that SA-, JA-, and ET-dependent defense pathways can affect each other's signaling, either positively or negatively (for review see Pieterse et al., 2001a). This so-called cross-talk between pathways provides a great regulatory potential for activating multiple resistance mechanisms in varying combinations and may help the plant to prioritize the activation of a particular defense pathway over another, thereby providing an optimal defense against the invader encountered. It is often assumed that SA-dependent defenses and JA/ET-dependent defenses are mutually exclusive due to negative cross-talk (Felton and Korth, 2000). This may have an enormous impact on crop plants that gained improved resistance to certain diseases or pests, either through genetic engineering of key factors of defense-signaling pathways, or upon

treatment with chemical plant protectants that mimic the action of specific defense signaling molecules.

SA, JA and ET: Important Signals in Induced Resistance

A classic example of systemically induced resistance is activated after primary infection with a necrotising pathogen, rendering distant, uninfected plant parts more resistant towards a broad spectrum of virulent pathogens. This form of induced resistance is often referred to as systemic acquired resistance (SAR). The onset of SAR is associated with increased levels of SA (Métraux, 2001), and the coordinate activation of a specific set of genes encoding pathogenesis-related (PR) proteins (Van Loon, 1997). Transgenic NahG plants that cannot accumulate SA are incapable of developing SAR and do not show *PR* gene activation upon pathogen infection indicating that SA is a necessary intermediate in the SAR signaling pathway (Gaffney et al., 1993). Another key component in the SAR pathway is the regulatory protein NPR1. Mutants affected in the *NPR1* gene accumulate normal levels of SA in response to pathogen infection but fail to mount SAR (Cao et al., 1994). Upon induction of SAR, NPR1 activates *PR-1* gene expression by physically interacting with a subclass of basic leucine zipper protein transcription factors that bind to promoter sequences required for SA-inducible *PR* gene expression (Zhang et al., 1999), suggesting a direct link between NPR1 activity and regulation of *PR* gene expression.

Another type of induced resistance is triggered by selected strains of non-pathogenic, biological control bacteria that colonize plant roots. Similar to pathogen-induced SAR, the induced resistance is systemically activated and is effective against various pathogens. This type of induced disease resistance is often referred to as rhizobacteria-mediated induced systemic resistance (ISR; for reviews see Van Loon et al., 1998; Pieterse et al., 2001b). In Arabidopsis, ISR has been shown to function independently of SA and *PR* gene activation (Pieterse et al., 1996; Van Wees et al., 1997). Instead, ISR signaling requires an intact response to both JA and ET (Pieterse et al. 1998; Ton et al., 2001a). The state of ISR is not associated with increases in the expression of known defense-related genes (Van Wees et al., 1999). However, upon challenge with a pathogen, ISR-expressing plants show an enhanced expression of certain JA-responsive genes, suggesting that ISR-expressing tissue is primed to activate specific JA-inducible genes faster or to a higher level upon attack (Van Wees et al., 1999). This phenomenon of priming has also been described for other types of induced resistance (Conrath et al., 2001; Zimmerli et al., 2000).

Although SAR and ISR follow distinct signaling pathways, they are both blocked in mutant *npr1* plants. Elucidation of the sequence of ISR-signaling events revealed that NPR1 functions downstream of the JA and the ET response (Pieterse et al., 1998). Evidently, NPR1 is not only required for the

SA-dependent expression of *PR* genes that are activated during SAR, but also for the JA- and ET-dependent activation of so far unidentified defense responses in rhizobacteria-mediated ISR.

Differential Effectiveness of SAR and ISR

SA, JA and ET are involved to different extents in basal resistance against specific pathogens. Mutant analyses in Arabidopsis showed that basal resistance against *Peronospora parasitica* and turnip crinkle virus (TCV) is controlled predominantly by a SA-dependent pathway (Delaney et al., 1994; Kachroo et al., 2000; Thomma et al., 1998). By contrast, basal resistance against *Alternaria brassicicola* is dependent on JA (Thomma *et al.*, 1998), whereas basal resistance against *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Xanthomonas campestris* pv. *armoraciae* was found to be controlled by a combined action of SA, JA and ET (Pieterse et al., 1998; Ton et al., 2001b). Comparison of the effectiveness of SA-dependent SAR and JA/ET-dependent ISR against these different Arabidopsis pathogens, revealed that SAR is predominantly effective against pathogens that in non-induced plants are resisted through SA-dependent basal resistance mechanisms, whereas ISR is predominantly effective against pathogens that in non-induced plants are resisted through JA/ET-dependent basal resistance responses (Ton et al., 2001b).

No Cross-talk Between SAR and ISR

Negative interactions between SA- and JA/ET-dependent defense pathways have been repeatedly demonstrated, feeding the notion that SA- and JA/ET-dependent defenses are mutually exclusive (for reviews see Felton and Korth, 2000; Pieterse et al., 2001a). To investigate the possibility of negative cross-talk between the SAR and the ISR signaling pathway, we activated both pathways simultaneously and determined the level of protection against *Pst*, which is sensitive to both SAR and ISR. Simultaneous activation of SAR and ISR 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 not evident. Moreover, induction of ISR did not affect the expression of the SAR marker gene *PR-1* in plants expressing SAR. Together, these observations demonstrate that the SAR and the ISR pathway are compatible and that there is no significant cross-talk between these pathways (Van Wees et al., 2000). Therefore, combining SAR and ISR provides an attractive tool for improvement of disease control.

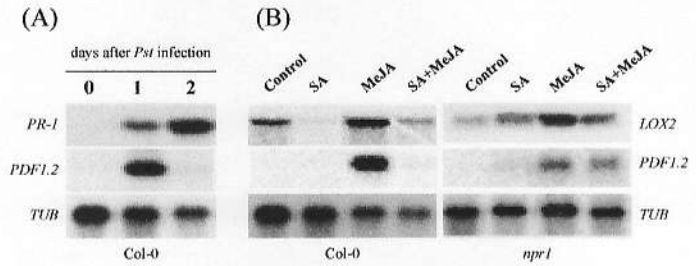

Fig. 1: (A) The JA-responsive *PDF1.2* gene is down-regulated in later stages of *Pst* infection when the SA-inducible *PR-1* gene is highly expressed. (B) Exogenous application of SA suppresses both steady-state and MeJA-induced mRNA levels of the JA-responsive genes *LOX2* and *PDF1.2* in wild-type Col-0 plants but not in mutant *npr1 plants.* The blots were also hybridized with a probe for β-tubulin (*TUB*) to check for equal loading.

Negative Cross-talk Between SA- and JA-Dependent Defenses

Although Van Wees et al. (2000) demonstrated that SA- and JA-dependent defenses are not necessarily mutually exclusive, other studies have shown that activation of the SAR pathway can negatively affect certain JA-dependent resistance responses. This negative cross-talk is thought to be caused by SA-mediated suppression of JA-responsive gene expression, possibly through the inhibition of JA biosynthesis and action (see Pieterse et al. 2001a and references herein). Previously, Van Wees et al. (1999) showed that the JA-responsive genes *PDF1.2*, *VSP*, and *LOX2* are transiently expressed upon infection with *Pst*. After a strong induction in early stages of infection, the genes were significantly down-regulated in the later stages when SA-inducible *PR*-genes were maximally expressed (see Fig. 1A for results of *PDF1.2* and *PR-1*). From this it was postulated that cross-talk between SA- and JA-dependent pathways is involved in the orchestration of the defense response against the invading pathogen.

To investigate the molecular mechanism of SA-mediated suppression of JA signaling, we monitored biosynthesis of JA and the expression of JA-responsive genes in Arabidopsis genotypes Col-0, NahG and *npr1*. Upon infection with *Pst*, NahG plants accumulated 32-fold more JA than wild-type Col-0 plants (1990 versus 61 ng/g FW), suggesting that JA biosynthesis was significantly suppressed by SA in wild-type plants. Consistent with this, infected NahG plants accumulated significantly higher levels of transcripts of the JA-responsive genes *PDF1.2*, *VSP*, and *LOX2*.

Furthermore, exogenous application of SA inhibited steady-state and/or MeJA-induced *PDF1.2*, *VSP*, and *LOX2* mRNA levels in wild-type Col-0 plants. However, in mutant *npr1* plants this inhibition was not apparent (see Fig. 1B for results of *PDF1.2* and *LOX2*), indicating that SA-mediated inhibition of JA-inducible gene expression functions via NPR1.

LOX2 encodes a key enzyme in the octadecanoid pathway leading to biosynthesis of JA. Thus, SA-mediated inhibition of *LOX2* gene expression might be sufficient to inhibit JA production. To investigate this we monitored JA production in *Pst*-infected transgenic S-12 plants, which have severely reduced levels of the LOX2 isozyme due to co-suppression of the *LOX2* gene (Bell et al., 1995). The reduced LOX2 levels in S-12 plants have no effect on the steady-state JA level, but the wound-induced production of JA is blocked (Bell *et al.*, 1995). Two days after *Pst* infection, infected Col-0 leaves showed an 8-fold increase in JA levels compared to the water control (115 versus 15 ng/g FW). In contrast, water-treated and *Pst*-infected S-12 plants showed similar, low basal levels of JA (8 versus 19 ng/g FW), indicating that suppression of the *LOX2* gene is sufficient to block pathogen-induced production of JA (Pieterse et al., 2000).

In view of the above mentioned results we hypothesize that SA-mediated inhibition of JA signaling is based on an NPR1-dependent suppression of JA-responsive gene expression. Consequently, suppression of the JA-responsive *LOX2* gene, and possibly also other JA-responsive genes involved in JA biosynthesis, leads to an inhibition of JA biosynthesis. The mode of action of the NPR-dependent down-regulation of JA-responsive gene expression by SA is currently investigated.

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