Review Article

Cross-talk between plant defence signalling pathways: boost or burden?

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Abstract

Plants are exposed to very different attackers, including microbial pathogens and herbivorous insects. To protect themselves, plants have evolved defensive strategies to counteract potential invaders. Recent advances in plant defence signalling research have revealed that plants are capable of differentially activating inducible, broad-spectrum defence mechanisms, depending on the type of invader encountered. The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are major players in the network of defence signalling pathways. Cross-talk between SA-, JA- and ET-dependent signalling pathways is thought to be involved in fine-tuning the defence reaction, eventually leading to the activation of an optimal mix of defence responses to resist the intruder. Genetic engineering of the biosynthetic pathways of these signalling compounds and the development of protective chemicals mimicking their mode of action provide useful tools for the development of new strategies for crop protection. However, there is evidence for antagonism between SA-dependent resistance to microbial pathogens and JA-dependent resistance to herbivorous insects: once a plant is conditioned to express resistance against microbial pathogens it may become more susceptible to attack by herbivores, and vice versa. Yet, the evidence for trade-offs between pathogen and insect resistance is contradictory. This review is focused on recent experimental evidence on the relationship between SA-, JA- and ET-dependent induced resistance to microbial pathogens and herbivorous insects. In addition, we will address the question whether manipulation of defence signalling pathways, either through genetic engineering or through application of defence signal-mimicking plant protectants, will boost the plant's immunity to potential invaders or will be a burden in crop protection strategies.

Introduction

Plants require a broad range of defence mechanisms to effectively combat invasion by microbial pathogens or attack by herbivorous insects. These mechanisms include pre-existing physical and chemical barriers, as well as inducible defence responses that become activated upon pathogen infection or insect herbivory. A concerted action of these defensive activities helps the plant to minimize damage caused by the attacker. In addition to localized defences, plants possess various inducible defence mechanisms that establish an enhanced defensive capacity in plant parts distant from the site of primary attack, thereby protecting the plant systemically against subsequent invasion. Recent studies on defence signalling pathways revealed that induced defences against microbial pathogens and herbivorous insects are regulated by a network of interconnecting signalling pathways in which the plant signal molecules SA, JA and ET play a dominant role (for reviews see Dong, 1998; Reymond and Farmer, 1998; Bostock, 1999; Glazebrook, 1999; Maleck and Dietrich, 1999; Pieterse and Van Loon, 1999; Feys and Parker, 2000). However, so far little is known about how plants integrate signals generated by different inducers of resistance into specific defence responses. An well-accepted hypothesis is that this is accomplished by modulation of different signalling pathways. Generally SA-dependent defences are activated more strongly in response to necrosis-inducing microbial pathogens and JA- and ET-dependent defences are activated to a higher extent in response to insect herbivory (Reymond and Farmer, 1998; Bostock, 1999; Maleck and Dietrich, 1999; Pieterse and Van Loon, 1999).

There is ample evidence that SA-, JA-, and ET-dependent defence pathways can affect each other's signalling, either positively or negatively. This 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 activa-

tion of a particular defence pathway over another, thereby providing an optimal defence against the invader encountered. It is mostly assumed that SA-dependent defences and JA/ET-dependent defences are mutually exclusive due to negative cross-talk. 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 defence-signalling pathways, or upon treatment with chemical plant protectants that mimic the action of specific defence signalling molecules.

SA, JA and ET: important signals in primary defence

The defence signal molecules SA, JA and ET have repeatedly been implicated in the regulation of primary resistance responses. In many cases, infection by microbial pathogens and attack by herbivorous insects is associated with enhanced production of these hormones and a concomitant activation of distinct sets of defence-related genes (Maleck *et al.*, 2000; Reymond *et al.*, 2000; Schenk *et al.*, 2000). Moreover, exogenous application of these compounds often results in an enhanced level of resistance. Compelling evidence for the role of SA, JA and ET came from recent genetic analyses of plant mutants and transgenics that are affected in the biosynthesis or perception of these compounds.

SA

A central role for SA became apparent with the use of NahG transformants. NahG plants constitutively express the bacterial *NahG* gene, encoding salicylate hydroxylase, which converts SA into inactive catechol. Tobacco and *Arabidopsis thaliana* NahG plants show enhanced disease susceptibility to a broad range of oomycete, fungal, bacterial and viral pathogens (Delaney *et al.*, 1994; Kachroo *et al.*, 2000). Recently, genetic screens in *Arabidopsis* to unravel plant defence pathways have identified recessive mutants affected in SA signalling that also show enhanced susceptibility to pathogen infection. For instance, the *sid1*, *sid2*, and *pad4* mutants are defective in SA accumulation in response to pathogen infection. As a result, these mutants display enhanced susceptibility to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* or the oomycete pathogen *Peronospora parasitica* (Nawrath and Métraux, 1999; Zhou *et al.*, 1998), confirming the importance of SA in basal resistance against different types of pathogens.

JA

Genetic evidence for the role of JA in plant defence came predominantly 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 mutant of *Arabidopsis*, *jar1*, allows enhanced levels of growth of *P. syringae* pv. *tomato* in the leaves (Pieterse *et al.*, 1998). This clearly demonstrates that JA-dependent defences contribute to basal resistance against different microbial pathogens. Furthermore, both the *jar1* mutant 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 non-pathogenic soil-borne *Pythium* spp. (Staswick *et al.*, 1998; Vijayan *et al.*, 1998), indicating that JA plays a role in non-host resistance against this type of pathogens. In another study, mutant *fad3 fad7 fad8* showed extremely high mortality from attack by larvae of the common saprophagous fungal gnat, *Bradysia impatiens* (McConn *et al.*, 1997), demonstrating an important role of JA in primary defence against herbivorous insects.

ET

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 disease susceptibility to *B. cinerea* (Thomma *et al.*, 1999), *P. syringae* pv. *tomato* (Pieterse *et al.*, 1998) and *E. carotovora* (Norman-Setterblad *et al.*, 2000), indicating that ET-dependent defences contribute to basal resistance against these pathogens. A similar phenomenon was observed in soybean mutants with reduced sensitivity to ET, which developed more-severe symptoms in response to infection by the fungal pathogens *Septoria glycines* and *Rhizoctonia solani* (Hoffman *et al.*, 1999). In addition, Knoester *et al.* (1998) reported that ET-insensitive tobacco transformed with the mutant ET receptor gene *etr1* from *Arabidopsis*, displayed susceptibility to the normally non-pathogenic oomycete *Pythium sylvaticum*. Thus, ET plays a role in non-host resistance as well. In other cases, reduced ET sensitivity was associated with disease tolerance. For instance, ET-insensitive tomato genotypes allowed wild-type levels of growth of virulent *P. syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria*, but developed less-severe symptoms of disease (Lund *et al.*, 1998; Ciardi *et al.*, 2000). A similar phenomenon was observed in the *Arabidopsis* ET-insensitive *ein2* mutant, which displayed increased tolerance to virulent strains of both *P. syringae* pv. *tomato* and *X. campestris* pv. *campestris* (Bent *et al.*, 1992). In addition, soybean mutants with reduced sensitivity to ET developed similar or less-severe disease symptoms in response to the bacterial pathogen *P. syringae* pv. *glycinea* and the oomycete *Phytophthora sojae* (Hoffman *et al.*, 1999). In these interactions, ET is clearly involved in symptom development, rather than disease resistance.

SA, JA and ET: important signals in induced resistance against pathogens

SA-dependent induced resistance

Plants possess several pathogen-inducible defence mechanisms that are active against microbial pathogens. A classic example of such a systemically induced resistance is activated after primary infection with a necrotizing pathogen, rendering distant, uninfected plant parts more resistant towards a broad spectrum of virulent pathogens, including viruses, bacteria and fungi (Kuc, 1982). This form of induced resistance is often referred to as systemic acquired resistance (SAR; Ross, 1961), and has been demonstrated in many plant-pathogen interactions (Ryals *et al.*, 1996; Sticher *et al.*, 1997). SAR is typically characterized by a restriction of pathogen growth and a suppression of disease symptom development compared to non-induced plants infected by the same pathogen (Hammerschmidt, 1999). The onset of SAR is associated with increased levels of SA both at the infection site and systemically (Mauch-Mani and Métraux, 1998). Moreover, SAR is associated with the coordinate activation of a specific set of genes encoding pathogenesis-related (PR) proteins, some of which possess antimicrobial activity (Van Loon, 1997). 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). Transgenic NahG plants that cannot accumulate SA, and the recessive mutants *sid1*, *sid2*, and *pad4* which are comprised in pathogen-induced SA accumulation, are incapable of developing SAR and do not show *PR* gene activation upon pathogen infection (Gaffney *et al.*, 1993; Lawton *et al.*, 1995; Zhou *et al.*, 1998; Nawrath and Métraux, 1999). All together this indicates that SA is a necessary intermediate in the SAR signalling pathway.

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. This implicates NPR1 as a key regulatory factor that functions downstream of SA in the SAR signalling pathway (Cao *et al.*, 1994, Delaney *et al.*, 1995). Recently, Zhang *et al.* (1999) demonstrated that, 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. This suggests a direct link between NPR1 activity and regulation of *PR* gene expression.

JA- and ET-dependent induced resistance

Upon pathogen infection other, SA-independent systemic resistance responses can be activated as well. For instance, infection of *Arabidopsis* with the fungal pathogen *A. brassicicola* results in the systemic activation of the *PDF1.2* gene, encoding a plant defensin with anti-fungal properties. *PDF1.*2 gene expression is regulated through a JA- and ET-dependent signalling pathway that functions independently of SA (Penninckx *et al.*, 1998). Another example comes from studies on the interaction between the bacterial pathogen *E. carotovora* and the hosts tobacco and *Arabidopsis*. Infection of the leaves by *E. carotovora*, or treatment of the leaves with elicitors of this pathogen, activated a SA-independent systemic resistance and a set of defence-related genes that is different from that induced upon exogenous application of SA (Vidal *et al.*, 1997; Norman-Setterblad *et al.*, 2000). Most of the *E. carotovora*-induced genes appeared to be regulated by JA and/or ET. Interestingly, *E. carotovora*-induced gene expression was antagonised by exogenous application of SA, whereas SA-induced *PR* gene expression was antagonised by *E. carotovora*-derived elicitors. This nicely demonstrates that plants differentially activate either SA-dependent or JA/ET-dependent defences, depending on the type of pathogen encountered.

Another SA-independent type of induced pathogen resistance is triggered by selected strains of non-pathogenic rhizosphere bacteria. Saprophytic rhizosphere bacteria are present in large numbers on plant root surfaces, where root exudates and lysates provide nutrients (Lynch and Whipps, 1991). Strains that were isolated from naturally disease-suppressive soils, mainly fluorescent *Pseudomonas* spp., were found to promote plant growth by suppressing soil-borne 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 these 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 microbial pathogens. This type of induced disease resistance is often referred to as rhizobacteria-mediated induced systemic resistance (ISR; reviewed by Van Loon *et al.*, 1998; Pieterse *et al.*, 2001). In *Arabidopsis*, rhizobacteria-mediated ISR has been shown to function independently of SA and *PR* gene activation (Pieterse *et al.*, 1996). Instead, ISR signalling requires JA and ET, because *Arabidopsis* mutants impaired in their ability to respond to either of these phytohormones are unable to express ISR (Pieterse *et al.* 1998; Ton *et al.*, 2001a). The state of rhizobacteria-mediated ISR is not only independent of the transcriptional activation of genes encoding PRs, but is not associated with increases in the expression of other known defence-related genes either (Pieterse *et al.*, 1996; 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, also referred to as “potentiation” or “sensitization”, is an emerging new topic in the field of plant defence signalling research (Cameron *et al.*, 1999; Conrath *et al.*, 2001; Zimmerli *et al.*, 2000).

Although pathogen-induced SAR and rhizobacteria-mediated ISR follow distinct signalling pathways, they are both blocked in mutant *npr1* plants. Elucidation of the sequence of ISR-signalling events revealed that NPR1 functions downstream of the JA and ET response in the ISR pathway (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 defence 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. In *Arabidopsis*, basal resistance against the oomycete pathogen *P. parasitica* and turnip crinkle virus (TCV) seems to be controlled predominantly by a SA-dependent pathway. Only SA-nonaccumulating NahG plants exhibit enhanced disease susceptibility to these pathogens (Delaney *et al.*, 1994; Kachroo *et al.*, 2000), whereas mutants affected in JA or ET signalling do not (Thomma *et al.*, 1998; Kachroo *et al.*, 2000). In contrast, basal resistance against the fungal pathogens *A. brassicicola* and *B. cinerea* is reduced only in JA- and ET-insensitive mutants, and not in NahG plants (Thomma *et al.*, 1998; 1999). Interestingly, basal resistance against the bacterial pathogens *P. syringae* pv. *tomato* and *X. campestris* pv. *armoraciae* was found to be affected in both NahG plants and in JA- and ET-response mutants (Pieterse *et al.*, 1998; Ton *et al.*, 2001b), suggesting that basal resistance against these pathogens is controlled by a combined action of SA, JA and ET. 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). Thus, SAR seems to constitute an enhancement of SA-dependent defences, whereas ISR seems to be based on an enhancement of JA- and ET-dependent defences.

SA, JA and ET: important signals in induced insect resistance

Induced defence against herbivorous insects is triggered upon insect feeding and involves two levels. The first level is direct defence, such as the production of secondary chemicals that act as toxins or feeding deterrents. The second level is an indirect defence, and consists of the production of a blend of volatiles to attract carnivorous enemies of the herbivores (Farmer and Ryan 1992; Karban and Baldwin 1997; Dicke 1999).

Direct defence against insects

A classic example of induced direct defence is the observation that following herbivore attack, tomato leaves systemically accumulate proteinase inhibitor proteins. These proteins inhibit digestive serine proteinases of herbivorous insects and reduce further insect feeding (Farmer and Ryan, 1992). As a result of wounding, an 18-amino acid peptide, systemin, is generated through cleavage from a larger protein, pro-systemin. This peptide is translocated via the phloem and its perception in distant leaves leads to phospholipid hydrolysis and the release of the JA precursor linolenic acid. Linolenic acid is rapidly metabolized via the octadecanoid pathway into JA, which in turn activates genes encoding proteinase inhibitors (Farmer and Ryan 1992; Wasternack and Parthier, 1997). Interestingly, ET has been shown to be co-required in this process (O’Donnell *et al.*, 1996).

Another example of direct defence is the induction of nicotine synthesis in the roots of *Nicotiana* species in response to wounding or herbivore attack of the leaves (Baldwin *et al.*, 1997). The toxic alkaloid nicotine is translocated to above-ground plant parts leading to dramatic systemic increases in vegetative and reproductive tissues, thereby protecting the plant against herbivores. JA is implicated in nicotine synthesis, because the increase in JA levels upon wounding or herbivore attack is strongly correlated with the whole-plant nicotine response.

Indirect defence against insects

Leaves continuously release small quantities of volatile chemicals, but when herbivorous insects damage a plant high amounts of volatiles are released. The blend of volatiles is characteristic for the plant and the herbivorous insect species. This induced production of a specific blend of volatiles is a form of indirect defence which attracts and allows parasitic and predatory insects to distinguish between infested and non-infested plants, and thus help in locating hosts or prey (Takabayashi and Dicke, 1996). JA is the major phytohormone involved in the induced production of plant volatiles that attract carnivorous enemies of the herbivores (Dicke *et al.*, 1999). SA has been implicated in induced defence against herbivory as well. Herbivores such as spider mites induce the emission of methyl salicylate (MeSA) in many plant species (Takabayashi and Dicke, 1996), which can lead to the activation of SA-inducible defence-related genes (Arimura *et al.*, 2000). Moreover, certain combinations of JA and SA treatments induce a blend of volatiles that is similar to the blend induced by spider mite feeding (Dicke *et al.*, 1999; Ozawa *et al.*, 2000) and attracts carnivorous enemies that can exterminate the herbivore population (Dicke *et al.* 1990).

Genetic engineering of defence pathways for improved resistance

SA-dependent pathway

Genetic engineering for pathogen resistance has mainly been focussed on the construction of plants that constitutively express individual defensive genes, such as *PR* genes, to reduce pathogen growth and symptom expression, consistent with a role of PRs in the expression of SAR (for review see Van Loon, 1997). Although in specific cases this approach has been proven successful, increased resistance as a result of overexpression of *PR* genes is by no means general. Novel insights in plant defence signalling have been instrumental in developing of new approaches to engineer plants with improved resistance by manipulating master switches of inducible plant defence pathways. Several approaches involve activating the SAR pathway to confer constitutive, broad-spectrum resistance against microbial pathogens. Verberne *et al.* (2000) transformed tobacco with two bacterial genes coding for isochorismate synthase and isochorismate-pyruvate lyase to convert chorismate into SA by a two-step process. When the two enzymes were targeted to the chloroplasts, the transgenic plants showed a 500- to 1,000-fold increased accumulation of free and conjugated SA compared to control plants. Transgenic SA-overproducing plants constitutively expressed *PR* genes and showed enhanced resistance to infection by tobacco mosaic virus (TMV) and the fungal pathogen *Oidium lycopersici* resembling SAR in non-transgenic plants. A similar approach was undertaken by Mauch *et al.* (2001), who engineered a novel hybrid enzyme with SA synthase activity by fusing an isochorismate synthase gene and an isochorismate-pyruvate gene from the bacterium *Pseudomonas aeruginosa*. The fusion gene was overexpressed in *Arabidopsis* and with the protein targeted to the chloroplasts, transgenic plants showed increased levels of free and conjugated SA and enhanced disease resistance toward the oomycete pathogen *P. parasitica*. Cao *et al.* (1998) investigated the possibility of generating broad-spectrum disease resistance through overexpression of the SAR regulatory protein NPR1. Indeed, NPR1-overexpressing *Arabidopsis* plants showed enhanced resistance towards the bacterial pathogen *P. syringae* pv. *maculicola* and the oomycete *P. parasitica*. Thus, engineered activation of key steps of the SAR pathway provides an attractive tool for controlling plant diseases.

ET-dependent pathway

The ET-signalling pathway has also been used to improve resistance against microbial pathogens. ET is perceived by a family of ET receptors, which, when mutated, give rise to dominant ET insensitivity, indicating that the ET response is negatively regulated (Stepanova and Ecker, 2000). Several genes encoding ET receptors have been isolated from *Arabidopsis* and tomato. The ET-insensitive *Never ripe* (*Nr*) mutant of tomato contains a mutation in the ET receptor gene *NR*, which is homologous the *Arabidopsis* ET receptor gene *ERS1*. Interestingly, the *Nr* mutant showed increased tolerance to the fungal pathogen *Fusarium oxysporum*, and the bacterial pathogens *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria* (Lund *et al.*, 1998). Overexpression of the wild-type *NR* gene in tomato, resulting in a stronger negative regulation of the ET response and reduced ET sensitivity, conferred increased tolerance to *X. campestris* pv. *vesicatoria* as well (Ciardi *et al.*, 2000). However, the effect of ET insensitivity varies greatly in different plant-microbe interactions. For instance, transformation of tobacco plants with the mutant *Arabidopsis etr1* gene, conferring dominant ET insensitivity, resulted in the loss of non-host resistance against the normally non-pathogenic soil-borne oomycete *Pythium sylvaticum* (Knoester *et al.*, 1998).

Insect resistance

Of the approaches taken to engineer resistance against insects, the use of toxins is already well developed. Most toxin genes have been derived from *Bacillus thuringiensis*, which is an ubiquitous spore-forming soil bacterium that produces crystals containing insecticidal proteins, called *Bt* toxins. Generally, transgenic plants expressing genes encoding native or engineered *Bt* toxins cause high mortality of target pests in the field (Schuler *et al.*, 1998; Peferoen, 1997). Other approaches make use of genes encoding inhibitors of digestive enzymes and lectins. Although JA signalling clearly plays an important role in defence against herbivorous insects, genetic engineering of its signalling pathway to obtain enhanced insect resistance has, to our knowledge, not been described yet.

Cross-talk between plant defence pathways

The previous sections have illustrated that SA, JA and ET play important roles in the regulation of defence responses and that genetic engineering of the corresponding signalling pathways can effectively enhance resistance. However, evidence is accumulating that components from the SA-, JA-, and ET-dependent defence pathways can affect each other's signalling. For instance, SA and its functional analogues INA and BTH suppress JA-dependent defence gene expression (Peña-Cortés *et al.*, 1993; Bowling *et al.*, 1997; Doherty *et al.*, 1988; Fidantsef *et al.*, 1999; Van Wees *et al.*, 1999), possibly through the inhibition of JA biosynthesis and action (Peña-Cortés *et al.*, 1993; Doares *et al.*, 1995; Harms *et al.*, 1998). In agreement herewith, Preston *et al.* (1999) demonstrated that TMV-infected tobacco plants expressing SAR are unable to develop normal JA-mediated wound responses, probably because of inhibition of JA signalling by increases in SA levels resulting from TMV infection. Thus, overexpression of the SA pathway, conferring resistance to a broad spectrum of microbial pathogens, could have detrimental effects on the JA/ET pathway that confers resistance against insects and certain groups of pathogens. For JA and ET, positive interactions have been reported: JA and ET act in concert in activating genes encoding defensive proteins, such as proteinase

inhibitors and plant defensins (O'Donnell *et al.*, 1996; Penninckx *et al.*, 1998). In some cases, JA and ET have been shown to stimulate SA action leading to enhanced *PR* gene expression (Lawton *et al.*, 1994; Xu *et al.*, 1994; Schweizer *et al.*, 1997).

Effects of cross-talk between pathways on the level of resistance against pathogens and insects in plants that are manipulated through genetic engineering of defence pathways or through application of defence signal-mimicking plant protectants, are to be expected. Recent studies start to provide some clarity in this complex matter (reviewed by Felton and Korth, 2000).

Negative effects of pathway cross-talk

Several studies have shown that activation of a particular pathway negatively affects resistance to certain groups of pathogens or insects. For instance, Moran (1998) demonstrated that in cucumber pathogen-induced SAR against the fungus *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-inoculated tobacco plants expressing SAR are more suitable for grazing by tobacco hornworm (*Manduca sexta*) than non-induced control plants. Furthermore, Felton *et al.* (1999) demonstrated that transgenic tobacco plants with reduced SA levels, caused by silencing of the phenylalanine ammonia-lyase (PAL) gene, exhibited reduced SAR against TMV but enhanced herbivore-induced resistance to *Heliothis virescens* larvae. Conversely, PAL-overexpressing tobacco plants showed a strong reduction of herbivore-induced insect resistance, while TMV-induced SAR was enhanced in these plants.

Application of the SAR inducer BTH has been shown to negatively effect insect resistance as well. For instance, BTH induced resistance against the bacterial pathogen *P. syringae* pv. *tomato* but improved suitability of tomato leaves 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 showed that application of BTH to field-grown tomato plants compromised resistance to the beet armyworm (*Spodoptera exigua*). In most cases, reduced insect resistance observed in SAR-expressing plants is attributed to the inhibition of JA production by BTH or increased SA levels.

Concomitant expression of induced defence pathways

Although negative interactions between pathogen and insect resistance have been clearly demonstrated, other studies failed to demonstrate such a negative relationship. For instance, Ajlan and Potter (1992) found that inoculation of the lower leaves of tobacco with TMV had no effect on population growth of tobacco aphids (*Myzus nicotianae*). Similarly, Inbar *et al.* (1998) found no negative effect of BTH application on population growth of whiteflies (*Bemisia argentifolii*) and leaf miners (*Liriomyza* spp.). Interestingly, Stout *et al.* (1999) showed that inoculation of tomato leaves with the bacterial pathogen *P. syringae* pv. *tomato* induced resistance against both *P. syringae* pv. *tomato* and the corn earworm *H. zea* in distal plant parts. Conversely, feeding by *H. zea* likewise induced resistance against both *P. syringae* pv. *tomato* and *H. zea.* A nice demonstration of simultaneous pathogen and insect resistance in the field was provided by Zehnder *et al.* (2001). In field experiments with cucumber they observed that induction of rhizobacteria-mediated ISR against the 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 plant metabolite and powerful feeding stimulant for cucumber beetles. Induction of 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 transmission of the pathogen, but also through the induction of defence responses that are directly active against the pathogen. These observations indicate that negative interactions between induced pathogen and insect resistance are by no means general.

The question whether SA- and JA-dependent induced resistance against microbial pathogens can be expressed simultaneously was addressed by Van Wees *et al.* (2000). In *Arabidopsis*, SA-dependent SAR, triggered by necrotizing pathogens, and JA/ET-dependent ISR, triggered by non-pathogenic rhizobacteria, are each effective against various pathogens, although their spectrum of effectiveness partly diverges (Ton *et al.*, 2001b). Both SAR and ISR are effective against *P. syringae* pv. *tomato*. 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. Therefore, combining SAR and ISR provides an attractive tool for improvement of disease control.

Concluding remarks

Genetic and molecular analyses of mutant and transgenic plants altered in their resistance to pathogens or insects revealed that SA-, JA-, and ET-dependent signalling pathways play a dominant role in plant defence. The three signals are involved in the activation of distinct sets of defence-related genes resulting in a differential activation of resistance against specific groups of microbial pathogens and insects. There is ample evidence that SA-, JA-, and ET-dependent pathways can affect each other's signalling, either positively or negatively. This 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 one particular pathway over another, thereby providing an optimal defence against the invader encountered.

Knowledge on defence signalling pathways has been proven to be instrumental for 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, cross-talk between SA- and JA-dependent defence pathways may be a burden when enhanced pathogen resistance is associated with reduced insect resistance. Fortunately, negative cross-talk between SA- and JA-dependent defences appears to be confined to specific inducer-plant-attacker combinations. Only in those cases in which the inducer strongly activates the SAR pathway, there seems to be an antagonistic effect on resistance against attackers that are normally resisted through JA-dependent defences. In other cases there seems to be little or no antagonism and can SA- and JA-dependent defences be expressed concomitantly to boost the plant's immunity to potential invaders. Thus, the general notion that SA-dependent pathogen resistance and JA-dependent insect resistance are mutually exclusive needs to be adjusted.

Future research on the molecular mechanisms of cross-talk between plant defence pathways will provide more insight into how plants are able to integrate signals into appropriate defences. Ultimately, this

will not only provide fundamental insights into how plants cope with different enemies, but will also help to assess the consequences of genetic engineering of specific plant defence pathways.

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