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REVIEW

Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens

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Abstract **Jasmonic acid (JA) is a natural hormone regulator involved in development, responses against wounding and pathogen attack. Upon perception of pathogens, JA is synthesized and mediates a signaling cascade initiating various defense responses. Traditionally, necrotrophic fungi have been shown to be the primary activators of JA-dependent defenses through the JA-receptor, COI1. Conversely, plants infected with biotrophic fungi have classically been associated with suppressing JA-mediated responses. However, recent evidence has shown that certain biotrophic fungal species also trigger activation of JA-mediated responses and mutants deficient in JA signaling show an increase in susceptibility to certain biotrophic fungal pathogens. These findings suggest a new role for JA in defense against fungal biotrophs. This review will focus on recent research advancing our knowledge of JA-dependant responses involved in defense against both biotrophic and necrotrophic fungi.**

Keywords jasmonic acid (JA), methyl jasmonate (MeJA), biotrophic fungi, necrotrophic fungi, *COI1*

Introduction

Plants are under constant bombardment from a wide array of environmental pressures. Unlike mammals, plants lack an adaptive immune response and are unable to produce memory cells to specific stimuli. To respond to these environmental pressures, plants rely on a highly diverse set of receptors to activate an intricate innate immune system. These receptors can perceive a variety of signals: from external signals produced by pathogens such as elicitors to internally produced systemic regulatory signals such as phytohormones. These receptors can play a role in a number of different functions such as perception, signal amplification, or both. In plant defense responses, there are three major pathways that are mediated by the phytohormones jasmonic acid (JA), salicylic acid (SA) and ethylene (ET). Synergistic or antagonistic interactions between these three pathways allow the plant to finely tune responses to a specific pathogen (Penninckx et al., 1998; Thomma et al., 1998; Takahashi et al., 2004). The SA pathway is unregulated in response to the biotrophic pathogens *Golovinomycetes cichoracerum* and *Pseudomonas syringae* (Murray et al., 2007; Fabro et al., 2008). In contrast, the JA- and ET-mediated pathways are specifically unregulated in response to necrotrophic fungi such as *Botrytis cinerea* (Berrocal-Lobo et al., 2002; Pena-Cortes et al., 2004; Glazebrook, 2005). Though each of these pathways plays an important role in resistance against several classes of plant pathogens, this review will focus on the role of JA-mediated signaling in fungal defense.

Jasmonic acid is a natural hormone regulator that induces proteinase inhibitor proteins in response to wounding, pathogen attack and development (Gfeller et al., 2010). Jasmonates are derived from a family of oxygenated fatty acids collectively known as oxylipins, which can be made internally from enzymatic reactions within the plant cell (Wasternack and Kombrick, 2010) or derived externally from a few fungal species (Miersch et al., 1991). JA is synthesized by the conversion of α-linolenic acid to 12-Oxo-phytodienoic acid (OPDA) by a series of enzymatic reactions in the chloroplast (Kazan and Manners, 2008). OPDA is then transported to the peroxisomes where it undergoes a series of β-oxidation reactions to generate JA (Vick and Zimmerman et al., 1984; Wasternack, 2007; Kazan and Manners, 2008).

Pathogen attack and wounding utilize their own unique receptors in order to trigger JA-mediated responses. Once JA

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is generated, it diffuses from the peroxisome into the cytosol where it can undergo subsequent reactions to form various JA derivatives. As illustrated in Figure 1, JA can be converted into volatile methyl jasmonate by the enzyme jasmonic methyl transferase (JMT). The addition of a methyl group to JA increases its lipoxygenase activity and methyl jasmonate (MeJA) has been shown to elicit JA-mediated responses by exogenous application (Avdiushko et al., 1995; Berrocal-Lobo et al., 2010). Exogenous application of MeJA has been shown to enhance resistance against several necrotrophic fungal species. For example, wheat (*Triticum aestivum*) pre-treated with MeJA showed delayed symptom development against *Fusarium pseudograminearum* (Desmond et al., 2005) and enhanced resistance to infection by *Stagonospora nodorum* (Jayaraj et al., 2004). JA and ET application has also been shown to enhance maize resistance to the necrotrophic pathogens *Rhizopus microspores* and *Colletotrichum graminicola* (Schmelz et al., 2011).

Additionally, JA can be conjugated to isoleucine by the JA amino acid synthetase, JAR1 (Staswick and Tiryaki, 2004; Fig. 1). JA and its derivatives then migrate to the nucleus where they are bound by the JA receptor, CORONATINE INSENSITIVE1 (COI1). COI1 contains 16 leucine-rich repeats (LRR) and an F-box motif, an important factor in

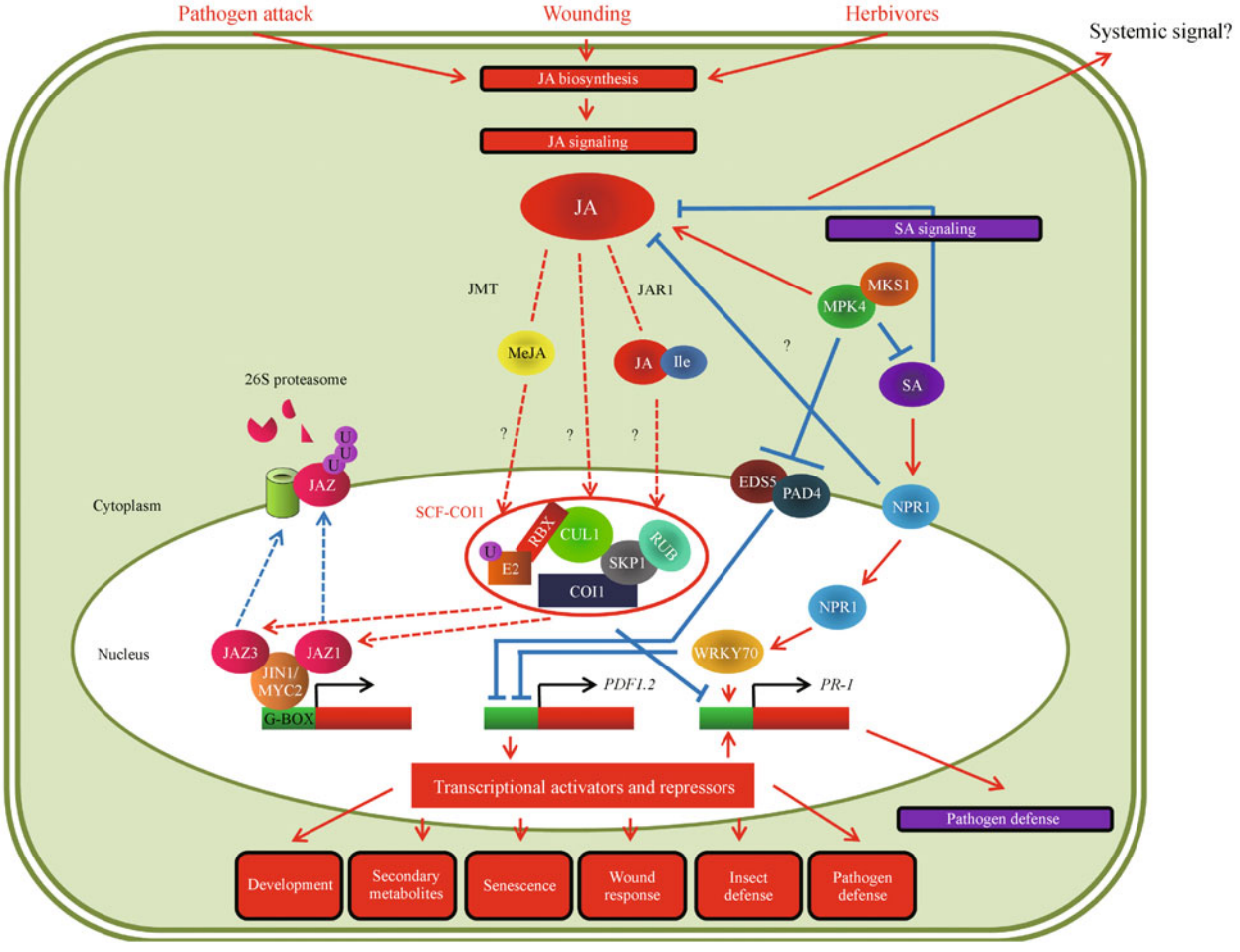

Figure 1 Schematic Diagram of the JA signaling pathway. JA biosynthesis is initiated by necrotrophic fungal infection, wounding, and herbivory. Once synthesized, derivatives of JA can be modified by JMT making methyl jasmonate and JAR1 with the addition of isoleucine. JA and its derivatives migrate across the nuclear envelope where they are bound by the JA-receptor, COI1. SCF-COI1 complex is activated by JA and specifically targets JAZ proteins (JAZ3 and JAZ1) for protein ubiquitination and subsequent degradation by the *26S* proteasome. JAZ proteins are negative regulators of JA-responsive genes. However, once degraded, *MYC2* (also known as *JIN1*) transcription factor can transcribe JA-responsive genes, which can activate or repress several different functions. The SA-mediated pathway acts antagonistically to repress JA signaling. SA can directly inhibit JA, and activate *NPR1*, which also inhibits JA. Conversely, JA can inhibit SA-mediated responses by suppressing *PR-1* expression through the SCF-COI1 complex. JA signaling can be activated and repressed through other proteins in various pathways. In the MAP kinase cascade, *MPK4*, bound to *MKS1*, can activate JA signaling, but the complex of *EDS5* and *PAD4* can suppress JA-responsive gene, *PDF1.2*. Red arrows indicate activation. Blue bars indicate suppression. Red dotted arrows indicate binding. Blue dotted arrows indicate degradation. Modified from Kazan and Manners, 2008.

protein ubiquitination (Xie et al., 1998). Once activated COI1 associates with other proteins forming a large complex (SCF-COI1) that acts as an E3 ubiquitin ligase specifically ubiquitinating JA-repressor proteins (Fig. 1). These repressor proteins, known as JASMONATE ZIM DOMAIN (JAZ) proteins, negatively regulate JA-responsive genes by interacting with MYC2, a JA-regulatory transcription factor (Chini et al., 2007; Fig. 1). Once activated by JA, the SCF-COI1 complex targets JAZ proteins for ubiquitination and subsequent protein degradation by the 26S proteasome (Chini et al., 2007; Thines et al., 2007). Following degradation of the JAZ proteins, MYC2 activates the transcription of several JA-responsive genes that respond to a variety of stimuli though not all JA-responsive genes are regulated by MYC2.

Jasmonic acid acts in defense against necrotrophs via a complex group of signaling molecules

Necrotrophic fungi are organisms that kill host tissue and feed on the remains (Glazebrook et. al., 2003). In response to infection by a biotrophic pathogen, a plant will typically undergo cell death in order to deter further tissue infection and rid itself of the pathogen. Upon necrotroph infection, however, cell death appears to aid the pathogen in its attack so plants have evolved an alternate mechanism of defense that is mediated by JA. JA and its associated signaling pathway respond strongly to infection by necrotrophic fungi and a defense response is initiated that is more effective than resistance gene mediated responses or SA-mediated defenses (Spoel et al., 2007). JA dependent signaling causes increased jasmonic acid synthesis and initiates expression of defense effector genes such as *plant defensin 1.2* (*PDF1.2*), *thionin 2.1* (*thi2.1*) and *vegetative storage protein* (*VSP*) (Glazebrook, 2005).

Despite the fact that the effects of JA on plants have been well characterized, the precise mechanisms by which JA acts are not fully understood (Xu et al., 2002). In *Arabidopsis*, all known activities of JA appear to require the function of the *COI1* gene (Glazebrook 2005). COI1 encodes an F-box protein that binds to JA and its associated proteins along with AtCUL1, AtRBX1, and SKP1-like proteins (ASK1 and ASK2) to form an active SCF/COI1 ubiquitin ligase complex that is involved in protein degradation (Xu et al., 2002). Since COI1 is an essential component in regulating JA-specific responses many studies have characterized its role in fungal defense by testing the response of *coi1* mutants against a variety of necrotrophic fungi such as *Alternaria brassicicola* (Table 1). In *Arabidopsis* JA biosynthesis is upregulated after infection with *A. brassicicola* and synthesis of JA-induced defense genes is amplified in a COI1-dependent manner (van Wees et al., 2003). Additionally, *coi1 Arabidopsis* mutants display an increased susceptibility to the necrotrophic fungi *A. brassicicola*, *Botrytis cinerea*, and *Plectosphaerella cucumerina* (Thomma et al., 1998). The *Arabidopsis* triple mutant *fad3 fad7 fad8*, a JA biosynthetic mutant that is deficient in JA and its precursor metabolites, also shows an increase in susceptibility to *A. brassicicola* (McConn and Browse, 1996; Stintzi et al., 2001) and the JA-signaling mutant *jar1* (JA-insensitive) has been shown to have increased susceptibility to *B. cinerea* (Kachroo and Kachroo, 2009). Reductions in JA-mediated defense responses in *Arabidopsis* have also been experimentally linked to an increase in susceptibility to the necrotrophs *Fusarium oxysporum* f. sp. *Lycopersici* (Thaler et al., 2004), *B. cinerea* (Thomma et al., 1998), *Pythium mastophorum* (Vijayan et al., 1998), *Pythium irregulare* (Staswick and Tiryaki, 2004), and *A. brassicicola* (Penninckx et al., 1996; Thomma et al., 1998). In tomato, the jasmonate-deficient mutant, *deficient in jasmonate1* (*def1*), shows a greater susceptibility to *F. oxysporum* (Thaler et al., 2004), *Verticillium dahlia* (Thaler et al., 2004) and *B. cinerea* (AbuQamar et al., 2008). Furthermore, the tomato homolog of *Arabidopsis coi1*, known as *jasmonate insensitive1* (*jai1*), and the tomato *acyl-coA oxidase* (*acx1*) mutant, which is deficient in JA biosynthesis, both show an increase in susceptibility to *B. cinerea* (AbuQamar et al., 2008). The wheat *phytochrome and flowering time1* (*pft1*) mutation, which is required for JA-mediated defense signaling and encodes the MEDIATOR25 subunit of Mediator, is more susceptible to *F. oxysporum* (Kidd et al., 2009).

Some mutations in JA biosynthesis and signaling result in resistance to necrotrophs

The studies discussed above show that mutations in JA biosynthesis and signaling reduce plant resistance to necrotrophic fungi. This is expected since JA-mediated responses are known to play an important role in defense against these pathogens. However, several recent studies have shown that some mutations linked to JA signaling or synthesis can lead to an *increase in resistance* to several necrotrophic fungi (Thatcher et al., 2009; Makandar et al., 2010; Table 1). Mutations in *JASMONATE INSENSITIVE1/MYC2* (*JIN1*/*MYC2*) are known to exhibit resistance to both *B. cinerea* and *P. cucumerina* (Kachroo and Kachroo, 2009). The tomato mutant *def1* has also been shown to have increased resistance to *Alternaria alternate* (Egusa et al. 2009). Additionally, mutations in *OPDA REDUCTASE3* (*opr3*), *coi1* and *JASMONATE RESISTANT1* (*jar1*) have been shown to exhibit a hyper-resistant response when infected with *Fusarium graminearum* (Makandar et al., 2010). Taken together these results suggest that these genes may contribute in some way to pathogen susceptibility.

Mutations in *OPR3* have also been shown to be more resistant to *A. brasscicola* (Stintzi et al., 2001). This mutant contains a T-DNA insertion in the second intron of *OPR3* and

Table 1 The phenotypes of JA-related mutants when challenged with necrotrophic fungi

Host	Pathogen	Mutant	Phenotype	Source
A. thaliana	*A. brassicicola*	*coi1*	Susceptible	Thomma et al., 1998
A. thaliana	*B. cinerea*	*coi1*	Susceptible	Thomma et al., 1998
A. thaliana	*P. cucumerina*	*coi1*	Susceptible	Thomma et al., 1998
A. thaliana	*F. oxysporum*	*coi1*	Susceptible	Thatcher et al., 2009
A. thaliana	*B. cinerea*	*jar1*	Susceptible	Kachroo and Kachroo, 2009
A. thaliana	*A. brasscicola*	*pad3*	Susceptible	Ferrari et al., 2007
A. thaliana	*B. cinerea*	*pad3*	Susceptible	Ferrari et al., 2007
A. thaliana	*A. brasscicola*	*fad3 fad7 fad8*	Susceptible	Stintzi et al., 2001
A. thaliana	*A. brasscicola*	*opr3*	Resistant	Stintzi et al., 2001
A. thaliana	*B. cinerea*	*opr3*	Resistant	Chehab et., 2011
A. thaliana	*B. cinerea*	*jin1*	Resistant	Kachroo and Kachroo, 2009
A. thaliana	*P. cucumerina*	*jin1*	Resistant	Kachroo and Kachroo, 2009
A. thaliana	*F. graminearum*	*opr3*	Resistant	Makandar et al., 2010
A. thaliana	*F. graminearum*	*coi1*	Resistant	Makandar et al., 2010
A. thaliana	*F. graminearum*	*jar1*	Resistant	Makandar et al., 2010
L. esculentum (Tomato)	*F. oxysporum*	*def1*	Susceptible	Thaler et al., 2004
L. esculentum (Tomato)	*V. dahlia*	*def1*	Susceptible	Thaler et al., 2004
L. esculentum (Tomato)	*B. cinerea*	*def1*	Susceptible	Thaler et al., 2004
L. esculentum (Tomato)	*Fusarium* species	*jai1*	Susceptible	Thaler et al., 2004
L. esculentum (Tomato)	*B. cinerea*	*jai1*	Susceptible	AbuQamar et al., 2008
L. esculentum (Tomato)	*B. cinerea*	*spr2*	Susceptible	Li et al., 2004; AbuQamar et al., 2008
L. esculentum (Tomato)	*B. cinerea*	*acx1*	Susceptible	Li et al., 2006
L. esculentum (Tomato)	*A. alternate*	*def1*	Resistant	Egusa et al., 2009
T. aestivum (Wheat)	*F. oxysporum*	*pft1*	Susceptible	Kidd et al., 2009

accumulates OPDA but is blocked in JA biosynthesis and induction of JA-responsive genes. It has previously been described as null mutant and its resistance to *A. alternate* was attributed to high levels of OPDA though the role of OPDA in defense was unknown (Stintzi and Browse, 2000; Stintzi et al., 2001). In a recent paper, Chehab et al. (2011) conducted a detailed characterization of the *opr3* mutant in an attempt to better understand OPDA's role in defense. Interestingly, the authors determined that *opr3* plants *did accumulate* detectable levels of JA when infected with the virulent pathogen *B. cinerea* but do not produce JA in response to wounding or insect attack (Chehab et al. 2011). The authors go on to show that in *opr3* plants infected with *B. cinerea* full-length transcripts of *OPR3* were present though at a significantly reduced level when compared to wild type (Chehab et al., 2011). They argue that the observed resistance to *B. cinerea* is due to the accumulation of JA in response to the fungus and that *opr3* is not a true loss-of-function mutant. The authors speculate that under certain conditions, such as fungal infection, *opr3* mutants are able to remove the T-DNA insertion via splicing to produce full-length functional transcripts. The exact mechanism that allows the mutant to regain function, however, remains to be determined (Chehab et al., 2011).

JA stimulates phytoalexin production in response to necrotroph infection

Phytoalexins are a large class of antimicrobial compounds that are produced in response to pathogens. Several recent studies suggest that accumulation of phytoalexins is important for defense against necrotrophic pathogens. In *Arabidopsis*, camalexin is the major phytoalexin involved in the inhibition of pathogen growth and encodes the *PHYTOALEXIN DEFICIENT3* (*PAD3*) gene (Zhou et al., 1999). A study conducted by Ferrari et al. (2007) showed that functional *PAD3* is required for resistance to *B. cinerea* in *Arabidopsis*. Mutants in *PAD3* were also shown to be more susceptible to *A. brassicicola* (Thomma et al. 1999; van Wees et al., 2003; Table 1). Camalexin production in *Arabidopsis* has most commonly been observed in plants infected with bacterial pathogens but JA is known to activate camalexin production in other plant species (Thomma et al. 1999, Zhou et al. 1999). These data point to a possible interaction between JA and *PAD3* though additional experiments are needed. The *opr3* mutant was also shown to accumulate camalexin to near wild type levels in response to infection with *B. cinerea* (Chehab et al. 2011). The authors speculate that JA production may be required in some cases for the synthesis

of camalexin (Chehab et al. 2011). In maize, both JA and ET were shown to induce the accumulation of kauralexins, a class of *ent*-kaurane–related diterpenoid phytoalexins that are induced by pathogens (Schmelz et al., 2011). Kauralexin B3 accumulation was shown to enhance maize resistance to the necrotrophic pathogens *Rhizopus microspores* and *Colletotrichum graminicola* (Schmelz et al., 2011).

JA also acts in resistance to biotrophic fungal pathogens

The JA signaling pathway has traditionally been linked to defense responses against necrotrophic fungi leading to necrosis and death of the plant. In contrast, biotrophic fungi require living plant tissue to flourish; therefore, obligate biotrophic fungi must establish a connection with their host to obtain nutrients and produce reproductive structures. Once an infection is established, fungal associated elicitors can trigger defense responses in the plant (Ramonell et al., 2005) that include the upregulation of several defense transcripts like *PDF1.2* (Penninckx et al., 1996) and Pathogenesis Related-1 (*PR-1*; Yalpani et al., 1991). Work by Fabro et al., (2008) showed that in *Arabidopsis* infected with the fungal biotroph, *G. cichoracearum* (powdery mildew), endogenous levels of both SA and JA were increased (Fabro et al., 2008). The SA-insensitive mutant, *noninducible pr1* (*npr1-1*) and *jar1*, were more susceptible to *G. cichoracearum* compared to wild type plants six to eight days post inoculation (Fabro et al., 2008; Table 2). An increase in susceptibility to *G. cichoracearum* was also observed in *coi1-16* mutants (Kloek et al., 2001; Ellis et al., 2002). In contrast a constitutive JA signaling mutant, *constitutive expression of vegetative storage protein1* (*cev1*), showed increased resistance to *Erysiphe orontii* and *G. cichoracearum* (Ellis and Turner, 2001; Ellis et al., 2002). Unlike other constitutive JA-signaling mutants (Hilpert et al., 2001) *cev1* activates JA-dependent signaling and represses SA-mediated defenses (Ellis et al., 2002). The *CEV1* gene encodes cellulose synthase *CESA3* and has reduced cellulose levels in addition to being compromised in the jasmonate pathway. The increased resistance to powdery mildew observed in *cev1* mutants strongly suggests that the resistance is due to the activation of JA-mediated defenses only and is not dependent on SA-mediated responses. Exogenous application of MeJA to barley induced systemic protection against the virulent barley powdery mildew strain, *Blumeria graminis* f. sp. *hordei* (Walters et al., 2002). Interestingly JA-mediated defenses appear to contribute to resistance against the powdery mildews but not against other classes of biotrophic fungal pathogens. For example, in tomato the JA-deficient mutant *def1* showed no difference in the production of fungal conidiophores compared to the wild type when inoculated with the fungal biotrophs *Cladosporium fulvum* and *Oidium neolycopersici* (Thaler et al., 2004; Table 2). Taken together, these studies indicate that JA signaling plays a role in resistance against the powdery mildews but little is known about the molecular mechanisms underlying this resistance.

Crosstalk between the JA, ET and SA signaling pathways in fungal defense

The magnitude of interplay between the major defense pathways is becoming clearer as research studies continue to expand our understanding of these signaling pathways. JA-mediated signaling appears to work in concert with ET-mediated responses and the expression of *PDF1.2* depends on both hormones (Farmer et al., 2003; Guo and Ecker, 2004). In the *cev1* mutant, JA is produced at very high levels leading to the constitutive activation of JA-responsive genes including *PDF1.2* (Ellis et al., 2002). *PDF1.2* expression was repressed in both *cev1/coi1-1* and *cev1/ethylene response factor1 (etr1)* double mutants indicating that *cev1* plants require both JA and ET signaling to induce *PDF1.2* expression (Ellis et al., 2002). Further when infected with *G. cichoracearum*, *cev1* mutants exhibit enhanced resistance compared to wild type plants (Table 2). The enhanced resistance shown in *cev1* mutants against *G. cichoracearum* is likely due to the constitutive expression of JA-regulated defenses (Ellis et al., 2002).

Activation of SA-mediated responses most often results in the inhibition of JA/ET signaling and vice versa (Gupta et al., 2000; Kunkel and Brooks, 2002). However, there are some genes that may be induced by both SA and JA (Kunkel and Brooks, 2002; Glazebrook et al., 2003). The induction of both SA and JA-mediated signaling has been shown to synergistically enhance resistance to *G. cichoracearum* as well as bacterial and insect pathogens (Ellis et al., 2002). During haustorium formation in *G. cichoracearum*, 70 genes were found to have an altered expression pattern during the infection process and several of the genes identified are

Table 2 The phenotypes of JA-related mutants when challenged with biotrophic fungi

Host	Pathogen	Mutant	Phenotype	Source
A. thaliana	*G. cichoracearum*	*coi1*	Susceptible	Kloek et al., 2001; Ellis et al., 2002
A. thaliana	*G. cichoracearum*	*cev1*	Resistant	Xiao et al., 1997; Ellis et al., 2002
A. thaliana	*G. cichoracearum*	*jar1*	Susceptible	Fabro et al., 2008
A. thaliana	*E. orontii*	*cev1*	Resistant	Ellis and Turner, 2001
L. esculentum (Tomato)	*C. fulvum*	*def1*	Neutral	Thaler et al., 2004
L. esculentum (Tomato)	*O. neolycopersici*	*def1*	Neutral	Thaler et al., 2004

known to be sensitive to both SA and JA (Fabro et al., 2008). Infection of *Arabidopsis* by *G. cichoracearum* results in the early induction of specific SA-mediated responses (Frye er al., 2001) and in JA-mediated induction of *PDF1.1*, *PDF1.2*, and *PDF1.3* 18 h post inoculation (Zimmerli et al., 2004). In *jar1-1* and *npr1-1* mutants infected with *G. cichoracearum*, 17 genes showed altered expression patterns (Fabro et al., 2008) including *PDF1.1*. Interestingly, four of the genes identified play a role in energy metabolism and it has been shown that photo-responsive proteins can influence the JA-mediated pathway (Fabro et al., 2008; Frenkel et al., 2009). It is possible that these genes might play a role in reprogramming metabolism and energy allocation necessary for induction of JA-responsive processes during fungal infection (Fabro et al., 2008).

Several studies have focused on the role of a mitogen-activated protein kinase, MPK4 that induces JA/ET signaling responses and represses SA-mediated defense (Petersen et al., 2000; Brodersen et al., 2006; Kazan and Manners, 2008; Gao et al., 2008; Wang et al., 2009). MPK4 plays an important role in the MAP kinase cascade that is induced by pathogen infection and fungal elicitors such as chitin (Wan et al., 2008). In *mpk4/NahG* plants, the JA responsive *PDF1.2* and *THI2.1* genes were not induced by treatment with MeJA despite the fact that both are expressed at normal levels in *NahG* plants alone suggesting that MPK4 is required for the induction of JA-responsive genes (Petersen et al., 2000). Interestingly, *mpk4* mutants show an enhanced resistance to the hemibiotrophic bacterial pathogen, *Pseudomonas syringae* pv. *tomato* DC3000 (Qiu et al., 2008; Cui et al., 2010) though no studies have been conducted to analyze the resistance phenotype of *mpk4* mutants to fungal biotrophs such as *G. cichoracearum*.

Another important signaling molecule is WRKY70, a transcription factor that has been shown to regulate JA- and SA-mediated signaling after pathogen infection (Li et al., 2004,2006). Overexpression lines of *WRKY70* had an increased resistance to *G. cichoracearum*, but enhanced susceptibility to *A. brassicicola*. In contrast, loss-of-function *wrky70* plants were more susceptible to *G. cichoracearum* (Li et al., 2006). Taken together these data illustrate that WRKY70 is necessary to mediate the balance between SA- and JA-induced defense responses against different fungal pathogens (Li et al., 2006).

Conclusions and future directions

JA and its derivatives play diverse roles in several important plant processes such as development, stress responses and pathogen defense. Classically, JA has been shown to be a fundamental component in initiating defense signaling against necrotrophic fungi but mounting recent evidence suggests that JA also plays a role in resistance against specific types of biotrophic fungi. Though the evidence for JA's role in defense against biotrophic fungi is compelling more studies are needed to verify and expand on existing data. To date, the JA-deficient mutants, *jar1* and *coi1*, have been shown to increase susceptibility to the biotroph *G. cichoracearum*. (Ellis et al., 2002; Fabro et al., 2008). Additionally, the JA-constitutive mutant, *cev1*, shows an increased resistance to *G. cichoracearum* (Ellis et al., 2002). More studies are needed that focus on the resistance of other JA-deficient mutants or mutants that are impaired in JA signaling or biosynthesis to *G. cichoracearum* and other biotrophic fungi. For example, inoculating JA mutants that are impaired at various points in JA signaling would allow a more precise determination of components of the pathway that are responsible for biotroph resistance. Analysis of the *fad3 fad7 fad8* triple mutant and *opr3* could be used to pinpoint the role of JA and OPDA biosynthesis with roles in the defense response though studies with *opr3* would have to be carefully monitored because of the potential for JA biosynthesis under certain conditions (Chehab et al., 2011). Studies using *jin1/myc2* transcription factor double mutants would also provide some insight (Lorenzo et al., 2004). Oxidative stress tolerance and wound response are positively regulated by JIN1, but pathogen defense is negatively regulated by JIN1 (Dombrecht et al., 2007; Kazan and Manners, 2008). By inoculating *jin1* mutants with a biotrophic pathogen, one could evaluate the function of JA-mediated gene expression in response to biotroph infection. By using combinations of these mutants in infection studies we may be able to develop a more accurate picture of the role of JA in defense against biotrophic fungi.

This data, however, raises an additional question: is resistance against biotrophs mediated directly by the JA pathway or is JA-mediated defense influenced by crosstalk with other known defense pathways? Previous work has shown that the fungal elicitor chitin activates a MAP kinase cascade (Wan et al., 2008) that includes MPK4 and MPK4 is known to activate JA-mediated signaling (Petersen et al., 2000; Brodersen et al., 2006). However, no studies have tested the defense phenotype of *mpk4* mutants against biotrophic fungi. A previous study showed that a deficiency in the JA-signaling pathway lead to an increase in susceptibility to *G. cichoracearum* (Fabro et al., 2008). Could activated MPK4 be turning on JA-mediated signaling? Studies are needed to observe the expression of JA-responsive genes in inoculated *mpk4* mutants. Crosstalk between defense pathways is known to allow the plant to finely tune its responses to a variety of pathogens, but further study is needed to fully understand these mechanisms in defense.

In addition to observing the role of different JA-signaling mutants against known fungal biotrophs, studies also need to be performed to observe the defense response of common JA-deficient mutants (such as *jar1* and *coi1*) against a larger variety of biotrophic fungi. To date, only mutants in JA signaling have been evaluated for their response to the fungal pathogens *G. cichoracearum, E. orontii*, *C. fulvum*, and *O. neolycopersici* (Ellis and Turner, 2001; Ellis et al., 2002;

Thaler et al., 2004; Fabro et al., 2008). Furthermore, the study of the JA response in other plants (wheat, rice, barley, tomato, etc.) inoculated with various fungal biotrophs would provide a more complete picture of JA's role in defense. For example, exogenous application of MeJA to wheat has been shown to confer resistance against insect pests and necrotrophic fungal pathogens (Desmond et al., 2005; El-Wakeil et al., 2010). Could exogenous application of MeJA to wheat also increase its resistance to the biotrophic rust fungus, *Puccinia graminis* var. *tritici*? More studies are clearly needed to explore these possibilities.

Together these data highlight the complexities of JA-mediated signaling and defense responses. Recent experimental evidence suggests that JA-mediated signaling plays a role in defense against both biotrophic and necrotrophic fungi. A more complete understanding of these defense responses and the interactions between JA-, SA- and ET-mediated defenses should provide new targets for directed improvement in plant defense against fungal pathogens. With increased food output becoming a top priority to feed a growing global population, the implications of these studies resonate far beyond the laboratory.

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References

AbuQamar S, Chai M F, Luo H, Song F, Mengiste T (2008). Tomato protein kinase 1b mediates signaling of plant responses to necrotrophic fungi and insect herbivory. Plant Cell, 20(7): 1964–1983

Avdiushko S, Croft K P, Brown G C, Jackson D M, Hamilton-Kemp T R, Hildebrand D (1995). Effect of volatile methyl jasmonate on the oxylipin pathway in tobacco, cucumber, and arabidopsis. Plant Physiol, 109(4): 1227–1230

Berrocal-Lobo M, Molina A, Solano R (2002). Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J, 29(1): 23–32

Berrocal-Lobo M, Stone S, Yang X, Antico J, Callis J, Ramonell K M, Somerville S (2010). ATL9, a RING zinc finger protein with E3 ubiquitin ligase activity implicated in chitin- and NADPH oxidase-mediated defense responses. PLoS ONE, 5(12): e14426

Brodersen P, Petersen M, Bjørn Nielsen H, Zhu S, Newman M A, Shokat K M, Rietz S, Parker J, Mundy J (2006). *Arabidopsis* MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J, 47(4): 532–546

Chehab E W, Kim S, Savchenko T, Kliebenstein D, Dehesh K, Braam J (2011). Intronic T-DNA insertion renders *Arabidopsis* opr3 a conditional jasmonic acid-producing mutant. Plant Physiol, 156(2): 770–778

Chini A, Fonseca S, Fernández G, Adie B, Chico J M, Lorenzo O, García-Casado G, López-Vidriero I, Lozano F M, Ponce M R, Micol J L, Solano R (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature, 448(7154): 666–671

Cui H, Wang Y, Xue L, Chu J, Yan C, Fu J, Chen M, Innes R W, Zhou J M (2010). Pseudomonas syringae effector protein AvrB perturbs *Arabidopsis* hormone signaling by activating MAP kinase 4. Cell Host Microbe, 7(2): 164–175

Desmond O J, Edgar C I, Manners J M, Maclean D J, Schenk P M, Kazan K (2005). Methyl jasmonate induced gene expression in wheat delays symptom development by the crown rot pathogen *Fusarium pseudograminearum*. Physiol Mol Plant Pathol, 67(3–5): 171–179

Dombrecht B, Xue G P, Sprague S J, Kirkegaard J A, Ross J J, Reid J B, Fitt G P, Sewelam N, Schenk P M, Manners J M, Kazan K (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. Plant Cell, 19(7): 2225–2245

Egusa M, Ozawa R, Takabayashi J, Otani H, Kodama M (2009). The jasmonate signaling pathway in tomato regulates susceptibility to a toxin-dependent necrotrophic pathogen. Planta, 229(4): 965–976

El-Wakeil N E, Volkmar C, Sallam A A (2010). Jasmonic acid induces resistance to economically important insect pests in winter wheat. Pest Manag Sci, 66(5): 549–554

Ellis C, Karafyllidis I, Turner J G (2002). Constitutive activation of jasmonate signaling in an Arabidopsis mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. Mol Plant Microbe Interact, 15(10): 1025–1030

Ellis C, Turner J G (2001). The *Arabidopsis* mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell, 13(5): 1025–1033

Fabro G, Di Rienzo J A, Voigt C A, Savchenko T, Dehesh K, Somerville S, Alvarez M E (2008). Genome-wide expression profiling *Arabidopsis* at the stage of *Golovinomyces cichoracearum* haustorium formation. Plant Physiol, 146(3): 1421–1439

Farmer E E, Alméras E, Krishnamurthy V (2003). Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. Curr Opin Plant Biol, 6(4): 372–378

Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel F M, Dewdney J (2007). Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. Plant Physiol, 144(1): 367–379

Frenkel M, Kulheim C, Jankanpaaa H J, Skogstrom O, Dall'Osto L, Agren J, Bassi R, Moritz T, Moen J, Jansson S (2009). Improper excess light energy dissipation in Arabidopsis results in a metabolic reprogramming. BMC Plant Biol, 9:12

Frye C A, Tang D Z, Innes R W (2001). Negative regulation of defense responses in plants by a conserved MAPKK kinase. Proc Natl Acad Sci USA, 98(1): 373–378

Gao M, Liu J, Bi D, Zhang Z, Cheng F, Chen S, Zhang Y (2008). MEKK1, MKK1/MKK2 and MPK4 function together in a mitogen-activated protein kinase cascade to regulate innate immunity in plants. Cell Res, 18(12): 1190–1198

Gfeller A, Liechti R, Farmer E E (2010). *Arabidopsis jasmonate* signaling pathway. Sci Signal, 3(109): cm4

Glazebrook J (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol, 43

(1): 205–227

Glazebrook J, Chen W, Estes B, Chang H S, Nawrath C, Métraux J P, Zhu T, Katagiri F (2003). Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J, 34(2): 217–228

Guo H, Ecker J R (2004). The ethylene signaling pathway: new insights. Curr Opin Plant Biol, 7(1): 40–49

Gupta V, Willits M G, Glazebrook J (2000). *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jasmonic acid signaling by SA. Mol Plant Microbe Interact, 13(5): 503–511

Hilpert B, Bohlmann H, op den Camp R O, Przybyla D, Miersch O, Buchala A, Apel K (2001). Isolation and characterization of signal transduction mutants of *Arabidopsis thaliana* that constitutively activate the octadecanoid pathway and form necrotic microlesions. Plant J, 26(4): 435–446

Jayaraj J, Muthukrishnan S, Liang G H, Velazhahan R (2004). Jasmonic acid and salicylic acid induce accumulation of β-1,3-glucanase and thaumatin-like proteins in wheat and enhance resistance against *Stagonospora nodorum.* Biol Plant, 48(3): 425–430

Kachroo A, Kachroo P (2009). Fatty Acid-derived signals in plant defense. Annu Rev Phytopathol, 47(1): 153–176

Kazan K, Manners J M (2008). Jasmonate signaling: toward an integrated view. Plant Physiol, 146(4): 1459–1468

Kidd B N, Edgar C I, Kumar K K, Aitken E A, Schenk P M, Manners J M, Kazan K (2009). The mediator complex subunit PFT1 is a key regulator of jasmonate-dependent defense in Arabidopsis. Plant Cell, 21(8): 2237–2252

Kloek A P, Verbsky M L, Sharma S B, Schoelz J E, Vogel J, Klessig D F, Kunkel B N (2001). Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana* coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. Plant J, 26(5): 509–522

Kunkel B N, Brooks D M (2002). Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol, 5(4): 325–331

Li J, Brader G, Kariola T, Palva E T (2006). WRKY70 modulates the selection of signaling pathways in plant defense. Plant J, 46(3): 477–491

Li J, Brader G, Palva E T (2004). The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell, 16(2): 319–331

Lorenzo O, Chico J M, Sánchez-Serrano J J, Solano R (2004). JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. Plant Cell, 16(7): 1938–1950

Makandar R, Nalam V, Chaturvedi R, Jeannotte R, Sparks A A, Shah J (2010). Involvement of salicylate and jasmonate signaling pathways in *Arabidopsis* interaction with *Fusarium graminearum*. Mol Plant Microbe Interact, 23(7): 861–870

McConn M, Browse J (1996). The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. Plant Cell, 8(3): 403–416

Miersch O, Schneider G, Sembdner G (1991). Hydroxylated jasmonic acid and related compounds from *Botryodiplodia theobromae*. Phytochemistry, 30(12): 4049–4051

Murray S L, Ingle R A, Petersen L N, Denby K J (2007). Basal resistance against Pseudomonas syringae in *Arabidopsis* involves WRKY53 and a protein with homology to a nematode resistance protein. Mol Plant Microbe Interact, 20(11): 1431–1438

Pena-Cortes H, Barrios P, Dorta F, Polanco V, Sanchez C, Sanchez E, Ramirez I (2004). Involvement of jasmonic acid and derivatives in plant responses to pathogens and insects and in fruit ripening. J Plant Growth Regul, 23: 246–260

Penninckx I A, Eggermont K, Terras F R, Thomma B P, De Samblanx G W, Buchala A, Métraux J P, Manners J M, Broekaert W F (1996). Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. Plant Cell, 8(12): 2309–2323

Penninckx I A, Thomma B P, Buchala A, Métraux J P, Broekaert W F (1998). Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell, 10(12): 2103–2113

Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johansen B, Nielsen H B, Lacy M, Austin M J, Parker J E, Sharma S B, Klessig D F, Martienssen R, Mattsson O, Jensen A B, Mundy J (2000). *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. Cell, 103(7): 1111–1120

Qiu J L, Zhou L, Yun B W, Nielsen H B, Fiil B K, Petersen K, Mackinlay J, Loake G J, Mundy J, Morris P C (2008). *Arabidopsis* mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. Plant Physiol, 148(1): 212–222

Ramonell K, Berrocal-Lobo M, Koh S, Wan J, Edwards H, Stacey G, Somerville S (2005). Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum*. Plant Physiol, 138(2): 1027–1036

Schmelz E A, Kaplan F, Huffaker A, Dafoe N J, Vaughan M M, Ni X, Rocca J R, Alborn H T, Teal P E (2011). Identity, regulation, and activity of inducible diterpenoid phytoalexins in maize. Proc Natl Acad Sci USA, 108(13): 5455–5460

Spoel S H, Johnson J S, Dong X (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc Natl Acad Sci USA, 104(47): 18842–18847

Staswick P E, Tiryaki I (2004). The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell, 16(8): 2117–2127

Stintzi A, Browse J (2000). The Arabidopsis male-sterile mutant, opr3, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc Natl Acad Sci USA, 97(19): 10625–10630

Stintzi A, Weber H, Reymond P, Browse J, Farmer E E (2001). Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci USA, 98(22): 12837–12842

Takahashi H, Kanayama Y, Zheng M S, Kusano T, Hase S, Ikegami M, Shah J (2004). Antagonistic interactions between the SA and JA signaling pathways in *Arabidopsis* modulate expression of defense genes and gene-for-gene resistance to cucumber mosaic virus. Plant Cell Physiol, 45(6): 803–809

Thaler J S, Owen B, Higgins V J (2004). The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiol, 135(1): 530–538

Thatcher L F, Manners J M, Kazan K (2009). *Fusarium oxysporum* hijacks COI1-mediated jasmonate signaling to promote disease development in *Arabidopsis*. Plant J, 58(6): 927–939

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He S Y, Howe G A, Browse J (2007). JAZ repressor proteins are

targets of the SCF(COI1) complex during jasmonate signalling. Nature, 448(7154): 661–665

Thomma B P, Eggermont K, Penninckx I A, Mauch-Mani B, Vogelsang R, Cammue B P, Broekaert W F (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci USA, 95(25): 15107–15111

Thomma B P, Nelissen I, Eggermont K, Broekaert W F (1999). Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. Plant J, 19(2): 163–171

van Wees S C, Chang H S, Zhu T, Glazebrook J (2003). Characterization of the early response of *Arabidopsis* to *Alternaria brassicicola* infection using expression profiling. Plant Physiol, 132(2): 606–617

Vick B A, Zimmerman D C (1984). Biosynthesis of jasmonic acid by several plant species. Plant Physiol, 75(2): 458–461

Vijayan P, Shockey J, Lévesque C A, Cook R J, Browse J (1998). A role for jasmonate in pathogen defense of *Arabidopsis*. Proc Natl Acad Sci USA, 95(12): 7209–7214

Walters D, Cowley T, Mitchell A (2002). Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. J Exp Bot, 53(369): 747–756

Wan J, Zhang X C, Neece D, Ramonell K M, Clough S, Kim S Y, Stacey M G, Stacey G (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. Plant Cell, 20(2): 471–481

Wang Z, Mao H, Dong C, Ji R, Cai L, Fu H, Liu S (2009). Overexpression of *Brassica napus* MPK4 enhances resistance to *Sclerotinia sclerotiorum* in oilseed rape. Mol Plant Microbe Interact, 22(3): 235–244

Wasternack C (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot (Lond), 100(4): 681–697

Wasternack C, Kombrink E (2010). Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chem Biol, 5(1): 63–77

Xiao, S., Ellwood, S., Findlay, K., Oliver, R.P., and Turner, J.G. (1997). Characterization of three loci controlling resistance of *Arabidopsis thaliana* accession Ms-0 to two powdery mildew diseases. Plant J, 12: 757–768

Xie D X, Feys B F, James S, Nieto-Rostro M, Turner J G (1998). COI1: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science, 280(5366): 1091–1094

Xu L, Liu F, Lechner E, Genschik P, Crosby W L, Ma H, Peng W, Huang D, Xie D (2002). The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. Plant Cell, 14(8): 1919–1935

Yalpani N, Silverman P, Wilson T M, Kleier D A, Raskin I (1991). Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell, 3(8): 809–818

Zhou N, Tootle T L, Glazebrook J (1999). *Arabidopsis* PAD3, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. Plant Cell, 11(12): 2419–2428

Zimmerli L, Stein M, Lipka V, Schulze-Lefert P, Somerville S (2004). Host and non-host pathogens elicit different jasmonate/ethylene responses in *Arabidopsis*. Plant J, 40(5): 633–646

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