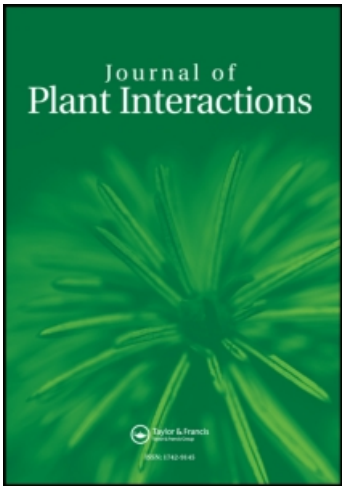

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REVIEW ARTICLE

Transcriptional regulation of plant inducible defenses against herbivores: a mini-review

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Inducible plant defenses against herbivores are controlled by a transient burst of jasmonic acid (JA) and its conversion to the active hormone (3R,7S)-jasmonoyl-L-isoleucine (JA-Ile). JA-Ile shows high affinity for binding to the COI1 protein complex with JAZ repressor protein(s), a multi component JA-Ile receptor, promoting hormone-dependent ubiquitination and degradation of JAZ transcriptional repressors. Degradation of JAZ proteins in *Arabidopsis* leads to the release of a bHLH transcription factor, MYC2, which functions as a master regulator of JA-dependent defense responses. Because the activity of the MYC2 coincides with the presence of active jasmonate in cells, it is unlikely that MYC2, alone, regulates prolonged transcriptional responses of genes encoding enzymes required for the accumulation of defense metabolites. In this review, we focus on MYC2 and a specific group of MYC2-regulated 'secondary' transcription factors as critical components of the JA signal transduction pathway that controls inducible chemical defense responses in plants.

Keywords: bHLH; defense; herbivores; jasmonic acid (JA); MYC2; transcription factor

Introduction

In a world of complex ecological and trophic interactions, the sedentary lifestyle of plants exposes them to a complex array of biotic and abiotic selection pressures. Survival under these stressful conditions requires prompt and adequate responses that can circumvent the pressures without compromising the overall fitness of plants (Heil and Baldwin 2002). Herbivore attack is one of the most severe biotic stresses that plants have endured in their long evolutionary history. Plants evolved various defense strategies against herbivores including constitutive defensive structures such as thorns, trichomes, and tough cuticles, reviewed in Hanley et al. (2007). In contrast to mechanical defenses, direct chemical defenses can be either constitutive or inducible expressed (Kessler and Baldwin 2002), as exemplified by the production and accumulation patterns of anti-digestive – or toxic secondary metabolites. In addition, attacked plants release a blend of volatile organic compounds to attract natural predators or parasitoids of the attacking herbivores as a form of indirect defense (Baldwin 2010). Production and deployment of chemical defense is often costly for plants (Mckey 1974; Heil and Baldwin 2002) utilizing resources that could otherwise be used for growth or reproduction. Hence, the activation of plant defenses must be fine-tuned and titrated with the need for protection.

Phytohormone-mediated responses to stress

Herbivores of different feeding guilds attack plants and activate a complex web of defense signaling pathways (Voelckel and Baldwin 2004; Bodenhausen and Reymond 2007). Jasmonic acid (JA), ethylene (ET), salicylic acid (SA), and abscisic acid (ABA), with their synergistic and/or antagonistic cross-talks, mediate the majority of plant defenses against biotic and abiotic stress (Bari and Jones 2009). JA has been repeatedly shown to be the most important mediator of plant-herbivore interactions (Katsir et al. 2008; Koo and Howe 2009; Rasmann and Agrawal 2009). For example, *Nicotiana attenuata* plants deficient in production of JA (*ir-lox3*) or JA perception (*ir-coi1*) have been shown to be highly vulnerable to the natural herbivore community when transplanted in their natural habitat in Great Basin desert, USA (Halitschke and Baldwin 2003; Paschold et al. 2007). While the central role of JA in plant defense against herbivores is well established, the next important task in understanding plant-herbivore interactions is to uncover how the attack signals are recognized by plants, and how these signals are translated into downstream defense responses.

Early plant responses to herbivore attack

Herbivore attack is usually associated with wounding of plant tissues and direct contact of cells with oral secretions of the herbivores (Howe and Jander 2008). Plant-specific green leaf volatiles (GLVs) are immediately released from the wounded tissues (Turlings et al. 1998). These 'attack-associated' cues are perceived by plants as markers of either imminent or apparent attack (Hilker and Meiners 2010). The exact mechanisms of how the herbivore-associated

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molecular patterns (HAMPSs) are perceived is not yet clear; however, some of the active components in oral – and oviposition – secretions have already been identified and characterized (Bonaventure and Baldwin 2010).

Perception of herbivory is followed by a rapid Ca^{2+}-influx, membrane depolarization, generation of reactive oxygen and nitrogen species, and activation of mitogen associated protein kinases (MAPKs). In *N. attenuata*, activation of MAPK cascade results in rapid phosphorylation of two critical wound – and SA induced protein kinases, WIPK, and SIPK, respectively (Wu and Baldwin 2009). SIPK is proposed to regulate the activity of chloroplastic GLA1 phospholipase (Kallenbach et al. 2010) which releases polyunsaturated fatty acids (PUFAs) from the plastidial membranes. The PUFAs (e.g. 18:3 linolenic acid) are then channeled into the synthesis of JA (for review on JA biosynthesis see Wasternack 2007). In many plant species, JA/JA-Ile burst start immediately after herbivore attack, with the accumulation attaining maximum levels in a few hours. For example, in *N. attenuata* maximum JA accumulations are attained in leaves one hour after simulated herbivore attack (Stork et al. 2009). Similar trends were reported in plants belonging to *Solanaceae*, *Brassicaceae*, *Poaceae,* and *Salicaceae* families (Pauwels et al. 2009; Wasternack and Kombrink 2010). The increase in both JA and JA-Ile is, however, transient as JA and JA-Ile levels in *N. attenuata* decrease to the pre-induced level few hours after elicitation (Halitschke et al. 2001; Stork et al. 2009). This presents an interesting scenario in which plants respond to initial attack and quickly reset their perception and signal transduction systems, preparing themselves for the perception of the next elicitation event. This may apply particularly to cases of discontinuous feeding by herbivores, allowing plants to monitor activity, and possibly, the size or numbers of attacking herbivores.

Rapid metabolism of JA

Owing to a transient nature of the JA burst, JA is rapidly metabolized to various forms, some of which are considered to be activated, transport, storage, or inactive forms of the hormone (Wasternack 2007). JA methylation at carbon 1 by JA carboxyl methyl transferase (JMT) forms a volatile methyl jasmonate (MeJA); JA hydroxylation at carbon 11 or 12 (by yet unknown enzyme) forms 11- or 12-hydroxy JA, which can be further sulfonated by hydroxyjasmonate sulfotransferases into a probable storage forms (Swiatek et al. 2004; Miersch et al. 2008). JA can also be enzymatically conjugated into sugars (forming JA-glucose), ACC (forming JA-ACC) or amino acids. Members of the JAR family of enzymes are responsible for biosynthesis of the JA-amino acid-conjugates, including (3R,7S)-jasmonoyl-L-isoleucine (JA-Ile), which is the major biologically active molecule perceived by the COI1-JAZ receptor complex (Wasternack 2007; Fonseca et al. 2009; Avanci et al. 2010; Wasternack and Kombrink 2010).

JA perception machinery

The translation of the initial JA and JA-Ile increase into downstream defense responses requires specific perception and maintenance mechanisms. The process starts when JA-Ile binds to the coronatine insensitive 1 (COI1) protein that associates with the JAZ repressor protein(s) to form an active receptor for JA-Ile. COI1 is an F-box protein that associates with the SKP-Cullin-F-box (SCF) complex, also known as E3 ubiquitin ligase (Figure 1). In the presence of JA-Ile, E3 ligase specifically recognizes JAZ repressors via COI1-JAZ interaction and mediates ubiquitination and consequent degradation of the JAZ repressors by the 26S proteasome. Degradation of the JAZ repressors releases their target MYC2 transcription factor (TF), and possibly other TFs, which in turn activates defense-related transcriptional reprogramming. Therefore, MYC2 protein plays a key role in translating the JA burst to downstream defense responses. It is believed that in the pre-induced state, JAZ proteins partially or completely repress MYC2 transcriptional activity (Figure 1). Interestingly, JA (possibly via MYC2) activates transcription of multiple JAZ repressors (Thines et al. 2007; Chico et al. 2008), and therefore triggers the attenuation of its own signaling at very early levels of signal transduction. Recently, substantial information on the perception of JA signal, the function of JAZ repressors and the structure of JA-Ile receptor complex has been obtained and thoroughly reviewed (Chini et al. 2007; Wasternack 2007; Chico et al. 2008; Chini et al. 2009; Memelink 2009; Avanci et al. 2010; Pauwels et al. 2010).

MYC2 plays a central role in transcriptional reprogramming of plant cells

MYC2 (also known as JAI1, JIN1, RD22BP1, RAP-1, or ZBF1) belongs to the basic Helix-Loop-Helix (bHLH) superfamily of TFs. TFs of this superfamily are known to regulate cellular processes ranging from cell proliferation and differentiation, neurogenesis, and heart development in animals to controlling light signaling, production of flavonoids, and other secondary metabolites in plants. bHLH TFs are characterized by two conserved motifs (1) the basic region, a stretch of about 15 basic and largely hydrophobic amino acids, that is used for DNA binding and (2) the HLH domain composed of two conserved alpha helices separated by a less conserved loop. The HLH domain is required for hetero- and / or homodimer protein formation (Heim et al. 2003; Toledo-Ortiz et al. 2003; Carretero-Paulet et al. 2010).

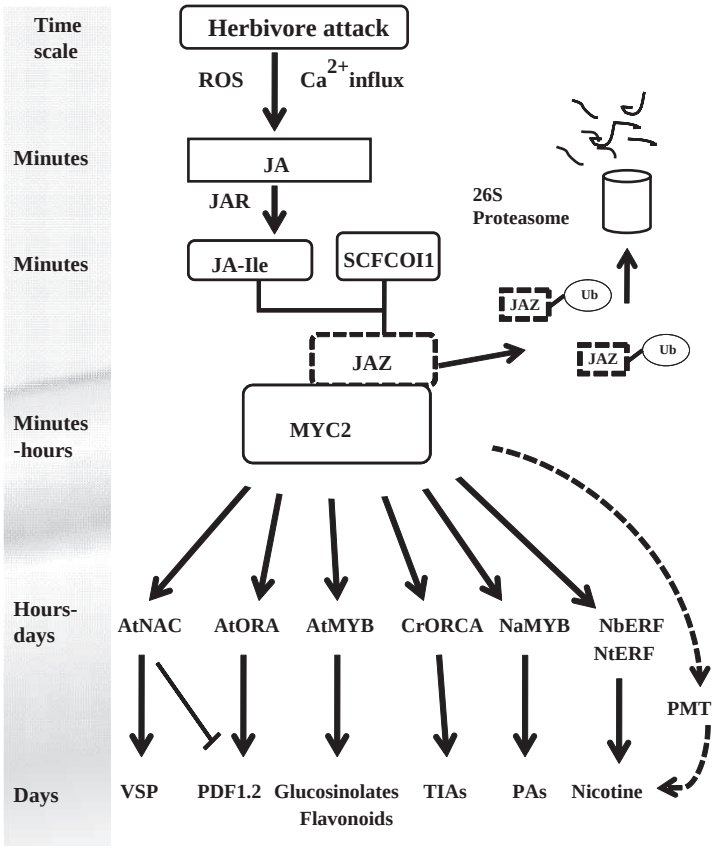

Figure 1. Proposed model of herbivore-induced JA-signaling cascade. Herbivore attack induces JA and JA-Ile bursts. JA-Ile accumulation mediates the SCF^{COI1}-JAZ interaction resulting in degradation of the JAZ repressors and the release of the MYC2 TF. MYC2 is proposed to regulate the transcription of defense-related genes directly (dotted lines) or indirectly through secondary TFs (solid lines) that mediate transcription of defense-related genes and accumulation of defense metabolites. VSP, vegetative storage protein; PDF1.2, plant defensin gene 1.2; TIAs, terpenoid indole alkaloids; PAs, phenolamides; PMT, putrescine N-methyltransferase.

Most TFs in bHLH superfamily have a DNA binding affinity. The MYC2 protein is shown to bind the G-box consensus hexanucleotide sequence, 5'-CACGTG-3', or its variants. The G-box consensus sequence or other G-Box-like sequences are found in many defense-related genes and transcription factors like the promoters of potato proteinase inhibitor 2 (PIN2) (Kim et al. 1992), Arabidopsis VSP1 (Guerineau et al. 2003), tobacco putrescine-N-methyltransferase (PMT1a) (Xu and Timko 2004), and the octadecanoid-derivative responsive AP2 domain genes (Endt et al. 2007). As expected from their general defense-related function, MYC2-like TFs are found in highly conserved forms in many plant species (Heim et al. 2003; Toledo-Ortiz et al. 2003; Li et al. 2006; Dombrecht et al. 2007; Carretero-Paulet et al. 2010; Todd et al. 2010). Moreover, wounding, MeJA or herbivory have been reported to induce transient expression of MYC2 genes in *Arabidopsis thaliana* (Lorenzo et al. 2004), *Catharanthus roseus* (Chatel et al. 2003), M*edicago truncatula* (Naoumkina et al. 2008), *Nicotiana benthamiana* (Todd et al. 2010) and *Nicotiana tabacum* (Shoji et al. 2008). Interestingly, three MYC2-like bHLH TFs have been reported in *N. benthamiana* that either positively (NbbHLH1 and NbbHLH2) or negatively (NbbHLH3) regulate nicotine biosynthesis (Todd et al. 2010).

How can plants shift from transient to prolonged defense responses?

Inferring from the mechanisms involved in jasmonate biosynthesis, degradation and perception, the wound- or herbivore-mediated activation of MYC2 protein should, more or less, coincide with the JA and JA-Ile bursts, starting few minutes after attack, reaching the peak level in about an hour and returning to the pre-induced levels in few hours. However, the accumulation of many, if not all, transcripts required for biosynthesis of defense-related secondary metabolites controlled by MYC2 lasts for many hours to days. Considering the fact that plant defense responses to herbivory last for long time periods, and in comparison to the transient nature of the MYC2 expression, it is tempting to speculate that MYC2 regulates certain defense-related secondary transcription factors which, in turn, regulate the defense metabolite

accumulation (Figure 1; Dombrecht et al. 2007). The following examples serve to elaborate this point further.

Identification of MYC2-dependent suite of secondary transcription factors

In *Arabidopsis thaliana*, AtMYC2 positively regulates flavonoid biosynthesis and alleviates oxidative stress. It also enhances herbivore resistance against the generalist herbivore, *Helicoverpa armigera*. Transcripts of many defense genes (for example, PDF1.2, CHI/PR3, HEL/PR4, TAT, and VSP) as well as several TFs (for example MYB34, MYB75, MYB51, WRKY33, ERF1, ERF4) were reported to have been differentially regulated (up- or down-regulated) in *Atmyc2*-silenced plants, suggesting that MYC2 may use other TFs as secondary messengers to regulate target genes. Consistent with this argument and as mentioned above, the MYC2 binding site (the G-Box or one of its variants) is found on promoters of these transcription factors and genes (Dombrecht et al. 2007).

Gigolashvili et al. (2007) reported the involvement of members of the subgroup 12 R2R3-MYB type TFs in the synthesis of indolic and aliphatic glucosinolates. Amongst them, MYB29, MYB34, MYB51 are wound- or MeJA-inducible TFs that are controlled by AtMYC2. These MYB TFs may be examples of secondary transcription factors in the JA signaling cascade.

A regulatory association between MYB transcription factors and defense metabolites was also shown in non-Arabidopsis plant models. For example, Galis et al. (2006) identified NtMYBJS1 as a MeJA-inducible MYB transcription factor by using large-scale microarray analysis of MeJA-elicited tobacco BY2 cells. NtMYBJS1 regulates the phenylpropanoid-polyamine conjugates (phenolamides, PAs) biosynthesis in tobacco cells. Its over-expression results in selective activation of genes involved in the phenylpropanoid biosynthetic pathway (PAL A, PAL B, 4CL, and C3H) without affecting the expression of genes involved in the synthesis of nicotine or other metabolites. Silencing (antisense) of the NtMYBJS1 resulted in reduced transcript accumulation of the PAL A, PAL B, 4CL1, and 4CL2. A functional homologue of NtMYBJS1 (named NaMYB8) was cloned in *N. attenuata* plants (Kaur et al. 2010). The authors showed that the transcripts of NaMYB8 are transiently induced by mechanical wounding. Interestingly, NaMYB8 transcript accumulation was further amplified by application of oral secretion from the specialist herbivore, *Manduca sexta,* to the wounds. Efficient silencing of NaMYB8 by RNA interference in *N. attenuata* made plants unable to accumulate the PAs, caffeoylputrescine and dicaffeoylspermidine, and rendered the transgenic plants more susceptible for herbivore attack (Kaur et al. 2010). Because the expression of NaMYB8 depends on NaMYC2 in *N. attenuata* (unpublished data Ivan Galis, Melkamu G. Woldemariam, and Ian T. Baldwin), NaMYB8 can be positioned between MYC2 and defense genes as a typical secondary TF in the JA signaling cascade.

In *N. benthamiana*, silencing (by VIGS) of two AtMYC2 homologues, NbbHLH1, and NbbHLH2, resulted in reduction of both the constitutive and MeJA-induced nicotine accumulation in leaves. VIGS silencing of ethylene response factor 1 (NbERF1) and a homeobox domain like TF (NbHB1) also reduced MeJA-induced nicotine levels. Interestingly, PMT promoters in tobacco contain both the G-Box (MYC2 binding site) and the GCC box (AP2/ERF binding site) (Todd et al. 2010). It remains therefore unclear whether these bHLHs exert their regulatory effects at the level of transcription factors or genes, or both. Considering the MYC2 function in Arabidopsis, we propose that bHLH1 and bHLH2, either separately or in concert, regulate the activity of ERF TFs which, in turn, regulate the promoters through their GCC motif.

The involvement of the MeJA-inducible group IX tobacco ERFs (closely related to ORCA2 and ORCA3 of *C. roseus*) in the regulation of nicotine biosynthetic genes was recently demonstrated in low nicotine-containing *nic1|nic2* mutant plants of *N. tabacum* (Shoji et al. 2010). This report emphasizes the importance of ERF family TFs in regulation of alkaloid biosynthesis and accumulation in tobacco. Two other members of the AP2/ERF domain transcription family, NtORC1 and NtJAP, were also shown to positively regulate the activity of the PMT promoters in tobacco (Goossens et al. 2003). Memelink (2009) proposed an MeJA inducible homologue of AtMYC2 (named NtMYC2 in tobacco) to regulate the activities of NtORC1 and NtJAP1. This brief summary is consistent with the view that alkaloid biosynthesis and accumulation in tobacco is under complex regulation which involves MYC2-like genes as one of the major regulatory nodes. Terpenoid indole alkaloids (TIAs) occur as defense metabolites against pathogens and UV exposure in *C. roseus* plants. The octadecanoid-responsive *C. roseus* AP2/ERF TFs, ORCA2, and ORCA3, are JA-inducible TFs that bind to the GCC-like box on jasmonate and elicitor responsive elements (JERE) in the promoter of the strictosidine synthase (STR) gene. STR is a key biosynthetic gene that is directly controlled by ORCA TFs (van der Fits and Memelink 2000, 2001). It is possible to propose that the JA-inducible ORCA TFs could be regulated by the master MYC2 TFs in regulating TIA biosynthetic genes. In *A. thaliana*, ORA59 (octadecanoid-responsive Arabidopsis AP2/ERF 59), a member of the Arabidopsis AP2/ERF family, is rapidly induced by ET and MeJA, contributing to the activation of many JA- and ET-inducible genes. Although the MeJA-inducibility of

ORA59 is COI1- and EIN2.1- dependent, the direct involvement of AtMYC2 remains unknown (Pre et al. 2008).

In Arabidopsis, ANAC019 and ANAC055 are MeJA inducible TFs belonging to the NAC family of proteins. The COI1- and MYC2- dependent induction of these genes by MeJA starts as early as 15 minutes and is maintained for six hours after induction. A double knockout of ANAC019 and ANAC055 reduced expression of AtMYC2-regulated VSP2 and LOX2, whereas their ectopic over-expression increased their respective expression. Moreover, over-expression of ANAC019 partially rescued *Atmyc2* mutation. It is suggested that ANAC019 and ANAC055 act downstream of the AtMYC2 regulator (Bu et al. 2008). If the latter is true, these NAC TFs would play the roles of secondary TFs that directly control the expression of VSP2 and LOX2 upon initial activation by AtMYC2.

Conclusions

The high level of inter-species sequence – and functional-conservation of MYC2, together with the universal presence of its DNA binding sites in a large number of genes and transcription factors mediating defense processes, indicates that MYC2 is a master regulator of plant defense responses that directly regulates defense genes or indirectly regulates transcription factors that in turn control these genes.

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References

Avanci NC, Luche DD, Goldman GH, Goldman MHS. 2010. Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. Genetics and Molecular Research. 9(1):484–505.

Baldwin IT. 2010. Plant volatiles. Current Biology. 20(9):R392–R397.

Bari R, Jones J. 2009. Role of plant hormones in plant defence responses. Plant Molecular Biology. 69(4):473–488.

Bodenhausen N, Reymond P. 2007. Signaling pathways controlling induced resistance to insect herbivores in Arabidopsis. Mol Plant Microbe Interact. 20(11):1406–1420.

Bonaventure G, Baldwin IT. 2010. New insights into the early biochemical activation of jasmonic acid biosynthesis in leaves. Plant Signal Behav. 5(3):287–289.

Bu QY, Jiang HL, Li CB, Zhai QZ, Zhang JY, Wu XQ, Sun JQ, Xie Q, Li CY. 2008. Role of the Arabidopsis thaliana NAC transcription factors ANAC019 and ANAC055 in regulating jasmonic acid-signaled defense responses. Cell Research. 18(7):756–767.

Carretero-Paulet L, Galstyan A, Roig-Villanova I, Martinez-Garcia JF, Bilbao-Castro JR, Robertson DL. 2010. Genome-wide classification and evolutionary analysis of the bHLH family of transcription factors in Arabidopsis, poplar, rice, moss, and algae. Plant Physiology. 153(3):1398–1412.

Chatel G, Montiel G, Pre M, Memelink J, Thiersault M, Saint-Pierre B, Doireau P, Gantet P. 2003. CrMYC1, a *Catharanthus roseus* elicitor–and jasmonate-responsive bHLH transcription factor that binds the G-box element of the strictosidine synthase gene promoter. Journal of Experimental Botany. 54(392):2587–2588.

Chico JM, Chini A, Fonseca S, Solano R. 2008. JAZ repressors set the rhythm in jasmonate signaling. Current Opinion in Plant Biology. 11(5):486–494.

Chini A, Boter M, Solano R. 2009. Plant oxylipins: COI1/JAZs/MYC2 as the core jasmonic acid-signalling module. Febs Journal. 276(17):4682–4692.

Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, et al. 2007. The JAZ family of repressors is the missing link in jasmonate signalling. Nature. 448(7154):666–671.

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM, et al.. 2007. MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. Plant Cell. 19(7):2225–2245.

Endt DV, Silva MSE, Kijne JW, Pasquali G, Memelink J. 2007. Identification of a bipartite jasmonate-responsive promoter element in the *Catharanthus roseus* ORCA3 transcription factor gene that interacts specifically with AT-hook DNA-binding proteins. Plant Physiology. 144(3):1680–1689.

Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C, Solano R. 2009. 7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nature Chemical Biology. 5(5):344–350.

Galis I, Simek P, Narisawa T, Sasaki M, Horiguchi T, Fukuda H, Matsuoka K. 2006. A novel R2R3 MYB transcription factor NtMYBJS1 is a methyl jasmonate-dependent regulator of phenylpropanoid-conjugate biosynthesis in tobacco. Plant J. 46(4):573–592.

Gigolashvili T, Yatusevich R, Berger B, Muller C, Flugge UI. 2007. The R2R3-MYB transcription factor HAG1/MYB28 is a regulator of methionine-derived glucosinolate biosynthesis in *Arabidopsis thaliana*. Plant Journal. 51(2):247–261.

Goossens A, Hakkinen ST, Laakso I, Seppanen-Laakso T, Biondi S, De Sutter V, Lammertyn F, Nuutila AM, Soderlund H, Zabeau M, et al.. 2003. A functional genomics approach toward the understanding of secondary metabolism in plant cells. Proceedings of the National Academy of Sciences of the United States of America. 100(14):8595–8600.

Guerineau F, Benjdia M, Zhou DX. 2003. A jasmonate-responsive element within the *A.thaliana* vsp1 promoter. Journal of Experimental Botany. 54(385):1153–1162.

Halitschke R, Baldwin IT. 2003. Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. Plant Journal. 36(6):794–807.

Halitschke R, Schittko U, Pohnert G, Boland W, Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae)

and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. Plant Physiology. 125(2):711–717.

Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM. 2007. Plant structural traits and their role in anti-herbivore defence. Perspectives in Plant Ecology Evolution and Systematics. 8(4):157–178.

Heil M, Baldwin IT. 2002. Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends in Plant Science. 7(2):61–67.

Heim MA, Jakoby M, Werber M, Martin C, Weisshaar B, Bailey PC. 2003. The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. Mol Biol Evol. 20(5):735–747.

Hilker M, Meiners T. 2010. How do plants "notice" attack by herbivorous arthropods? Biol Rev Camb Philos Soc. 85(2):267–280.

Howe GA, Jander G. 2008. Plant immunity to insect herbivores. Annual Review of Plant Biology. 59:41–66.

Kallenbach M, Alagna F, Baldwin IT, Bonaventure G. 2010. *Nicotiana attenuata* SIPK, WIPK, NPR1, and fatty acid-amino acid conjugates participate in the induction of jasmonic acid biosynthesis by affecting early enzymatic steps in the pathway. Plant Physiology. 152(1):96–106.

Katsir L, Chung HS, Koo AJK, Howe GA. 2008. Jasmonate signaling: a conserved mechanism of hormone sensing. Current Opinion in Plant Biology. 11(4):428–435.

Kaur H, Heinzel N, Schottner M, Baldwin IT, Galis I. 2010. R2R3-NaMYB8 regulates the accumulation of phenylpropanoid-polyamine conjugates, which are essential for local and systemic defense against insect herbivores in *Nicotiana attenuata*. Plant Physiology. 152(3):1731–1747.

Kessler A, Baldwin IT. 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology. 53:299–328.

Kim SR, Choi JL, Costa MA, An GH. 1992. Identification of G-box sequence as an essential element for methyl jasmonate response of potato proteinase inhibitor-II promoter. Plant Physiology. 99(2):627–631.

Koo AJK, Howe GA. 2009. The wound hormone jasmonate. Phytochemistry. 70(13–14):1571–1580.

Li XX, Duan XP, Jiang HX, Sun YJ, Tang YP, Yuan Z, Guo JK, Liang WQ, Chen L, Yin JY, et al. 2006. Genome-wide analysis of basic/helix-loop-helix transcription factor family in rice and Arabidopsis. Plant Physiology. 141(4):1167–1184.

Lorenzo O, Chico JM, Sanchez-Serrano JJ, 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.

Mckey D. 1974. Adaptive patterns in alkaloid physiology. American Naturalist. 108(961):305–320.

Memelink J. 2009. Regulation of gene expression by jasmonate hormones. Phytochemistry. 70(13–14):1560–1570.

Miersch O, Neumerkel J, Dippe M, Stenzel I, Wasternack C. 2008. Hydroxylated jasmonates are commonly occurring metabolites of jasmonic acid and contribute to a partial switch-off in jasmonate signaling. New Phytologist. 177(1):114–127.

Naoumkina MA, He XZ, Dixon RA. 2008. Elicitor-induced transcription factors for metabolic reprogramming of secondary metabolism in Medicago truncatula. Bmc Plant Biology. 8:132.

Paschold A, Halitschke R, Baldwin IT. 2007. Co(i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. Plant Journal. 51(1):79–91.

Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, Perez AC, Chico JM, Vanden Bossche R, Sewell J, Gil E, et al. 2010. NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature. 464(7289):788–791.

Pauwels L, Inze D, Goossens A. 2009. Jasmonate-inducible gene: what does it mean? Trends in Plant Science. 14(2):87–91.

Pre M, Atallah M, Champion A, De Vos M, Pieterse CMJ, Memelink J. 2008. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiology. 147(3):1347–1357.

Rasmann S, Agrawal AA. 2009. Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. Current Opinion in Plant Biology. 12(4):473–478.

Shoji T, Kajikawa M, Hashimoto T. 2010. Clustered transcription factor genes regulate nicotine biosynthesis in tobacco. Plant Cell. 22(10):3390–3409.

Shoji T, Ogawa T, Hashimoto T. 2008. Jasmonate-induced nicotine formation in tobacco is mediated by tobacco COI1 and JAZ genes. Plant and Cell Physiology. 49(7):1003–1012.

Stork W, Diezel C, Halitschke R, Galis I, Baldwin IT. 2009. An ecological analysis of the herbivory-elicited JA burst and its metabolism: plant memory processes and predictions of the moving target model. Plos One. 4(3): e4679.

Swiatek A, Van Dongen W, Esmans EL, Van Onckelen H. 2004. Metabolic fate of jasmonates in tobacco Bright Yellow-2 cells. Plant Physiology. 135(1):161–172.

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu GH, Nomura K, He SY, Howe GA, Browse J. 2007. JAZ repressor proteins are targets of the SCFCO11 complex during jasmonate signalling. Nature. 448(7154):661–666.

Todd AT, Liu EW, Polvi SL, Pammett RT, Page JE. 2010. A functional genomics screen identifies diverse transcription factors that regulate alkaloid biosynthesis in *Nicotiana benthamiana*. Plant Journal. 62(4):589–600.

Toledo-Ortiz G, Huq E, Quail PH. 2003. The Arabidopsis basic/helix-loop-helix transcription factor family. Plant Cell. 15(8):1749–1770.

Turlings TCJ, Lengwiler UB, Bernasconi ML, Wechsler D. 1998. Timing of induced volatile emissions in maize seedlings. Planta. 207(1):146–152.

van der Fits L, Memelink J. 2000. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. Science. 289(5477): 295–297.

van der Fits L, Memelink J. 2001. The jasmonate-inducible AP2/ERF-domain transcription factor ORCA3 activates gene expression via interaction with a

jasmonate-responsive promoter element. Plant Journal. 25(1):43–53.

Voelckel C, Baldwin IT. 2004. Herbivore-induced plant vaccination. Part II. Array-studies reveal the transience of herbivore-specific transcriptional imprints and a distinct imprint from stress combinations. Plant Journal. 38(4):650–663.

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

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

Wu JQ, Baldwin IT. 2009. Herbivory-induced signalling in plants: perception and action. Plant Cell and Environment. 32(9):1161–1174.

Xu BF, Timko MP. 2004. Methyl jasmonate induced expression of the tobacco putrescine N-methyltransferase genes requires both G-box and GCC-motif elements. Plant Molecular Biology. 55(5):743–761.