THE ROLE OF ALTERNATIVE SPLICING IN THE REGULATION OF JASMONATE SIGNALING

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Cell and Molecular Biology Program

2011

ABSTRACT

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The plant hormone jasmonate (JA) is studied primarily for its role in plant defense. In response to attack by pathogens and herbivores, the JA-amino acid conjugate JA-isoleucine (JA-Ile) accumulates and promotes the association of the E3 ubiquitin ligase SCF^{COI1} with its substrates, the Jasmonate-ZIM domain (JAZ) repressor proteins. JAZ proteins bind the F-box protein COI1 in a hormone-dependent manner and are targeted for ubiquitination and degradation by the 26S proteasome pathway. JAZ degradation releases transcription factors from repression, thereby promoting transcription of JA-responsive genes. Among the earliest response genes to be transcribed are the *JAZ* genes themselves. Many *JAZ* genes are subject to alternative splicing events that reduce the ability of JAZ proteins to interact with COI1 in a hormone-dependent manner. These truncated JAZ proteins are hypothesized to attenuate JA responses. In this study, *JAZ* transcripts were quantified in response to JA elicitation and in various tissues. The results show that the relative abundance of *JAZ* splice variants is not significantly affected by JA elicitation or tissue type, thus suggesting that alternative splicing of *JAZ* pre-mRNA is controlled by the efficiency with which *cis*-acting splice sites are selected by the spliceosome. Additionally, transgenic plants ectopically expressing cDNAs encoding truncated splice variants of JAZ2 and JAZ4 have reduced sensitivity to JA, indicating that these proteins act as dominant repressors of JA signaling. This research highlights the importance of alternative splicing as an adaptive response of plants to environmental stress.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my advisor, Dr. Gregg Howe. Throughout my years of working in his lab, I have come to truly admire and respect his extraordinary abilities as a scientist, a professor, and a mentor. His continuous support and encouragement have been critical to my successes and my development as a scientist.

I would also like to thank members of my thesis committee: Dr. David Arnosti, Dr. Eva Farre, Dr. Beronda Montgomery-Kaguri, and Dr. Curtis Wilkerson, for their critical insight and their understanding.

My unforgettable experience in the Howe lab was largely attributed to the interaction and collaboration among members of the lab. First, I would like to thank a former graduate student, Dr. Hoo Sun Chung, for providing much of the preliminary work on *JAZ10*. Second, I would like to thank Dr. Marco Herde for his invaluable assistance in deciphering the results of our RNA-seq data. I am indebted to Dr. Javier Moreno for his assistance in designing and troubleshooting cloning projects. I would like to thank Dr. Yuki Yoshida for his preliminary analysis of the *jaz10-1* root length phenotype. I would like to thank Dr. Abe Koo, Dr. Jin Ho Kang, and Christine Shyu for their advice, cooperation, and constructive criticism. Finally, I would like to thank my beloved comrades, Dr. Ian Major and Marcelo Campos, for their unlimited support, wholehearted sympathy, and friendship.

I am extremely grateful to my family and friends for their compassion and assistance throughout my graduate career. Their constant care and appreciation is the basis for all of my achievement.

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LIST OF ABBREVIATIONS

35S-JAZ10G	*35S-JAZ10*-genomic
AFP	ABI-FIVE BINDING PROTEIN
bHLH	basic helix loop helix
BiFC	Bimolecular fluorescent complementation assays
COI1	Coronatine Insensitive-1
Col-0	*Arabidopsis thaliana* ecotype Columbia-0
COR	Coronatine
EAR	ETHYLENE RESPONSIVE FACTOR-associated amphiphilic repression
EMS	ethyl methanesulfonate
GA	Gibberellic acid
GFP	Green Fluorescent Protein
GUS	β-Glucuronidase
JA	Jasmonic Acid
JAR1	JASMONATE RESISTANT 1
JAZ	Jasmonate-ZIM domain
JMT	JASMONIC ACID CARBOXYLMETHYLTRANSFERASE
LRR	Leucine-rich repeat
MeJA	Methyl Jasmonate
NINJA	Novel Interactor of JAZ
NPR1	NONEXPRESSOR of PR GENES1
OPCL1	OPC-8:0 Co-A LIGASE 1
OPDA	12-oxo-phytodienoic acid
OPR3	12-OXOPHYTODIENOIC ACID REDUCTASE
PAMP	Pathogen associated molecular patterns
PTC	Premature Termination Codon
PTI	Pathogen triggered immunity
qPCR	Quantitative reverse-transcriptase polymerase chain reaction
RPKM	Reads per kilobase pair per million mapped reads of model
SA	Salicylic Acid
SCF	Skp/Cullin/F-box protein complex
TAP	Tandem Affinity Purification tag
TIR1	Transport Inhibitor Response 1
TPL	TOPLESS
YFP	Yellow Fluorescent Protein

INTRODUCTION

As sessile organisms, plants have evolved complex yet efficient means of responding to a variety of abiotic and biotic stresses to optimize their fitness. Plants are routinely exposed to stress, which often requires significant remodeling of transcriptional and metabolic networks. Upon pathogen infection or herbivory, plants activate a diverse array of direct and indirect defense responses, which are often adapted to specific pathogens or pests (Howe and Jander, 2008). Direct defense responses include production of anti-nutritive enzymes and toxic secondary metabolites (Wittstock and Burow, 2010; Gonzales-Vigil et al., 2011). Some of the best studied defensive enzymes, protease inhibitors, are effective in the insect midgut to directly prevent nutrient accessibility and digestion (Ryan, 1990). Indirect defenses include the release of volatile compounds that attract nearby predators of the attacking herbivore (Herde et al., 2008). Although defensive mechanisms vary amongst plant species, a number of these defense responses are inducible and regulated by the plant hormone jasmonate (JA).

In unperturbed tissue, JA regulates a variety of physiological processes such as cell division, carbon partitioning, photomorphogenesis, as well as the development of reproductive tissues, trichomes and vascular cambium (McConn and Browse, 1996; Creelman and Mullet, 1997; Li et al., 2004; Feys et al., 1994; Yoshida et al., 2009; Sehr et al., 2010). Plants also utilize the JA signaling pathway to respond to abiotic stresses such as ozone, ultraviolet irradiation, and high salinity (Conconi et al., 1996; Rao et al., 2000; Glazebrook, 2005; Browse and Howe, 2008) JA is primarily studied for its involvement in defense responses (Conconi et al., 1996; Rao et al., 2000; Glazebrook, 2005; Browse and Howe, 2008; Howe and Jander, 2008; Acosta and Farmer, 2010).

A key aspect of the JA signaling pathway is the regulation of transcriptional repressors by the Skp1-Cul1-Fbox (SCF)-ubiquitin-proteasome pathway in response to environmental stimuli (Figure 1) (Howe, 2010). During optimal growth conditions, levels of bioactive JA-isoleucine conjugate (JA-Ile) are low, and Jasmonate-ZIM domain (JAZ) proteins suppress defense responses through repressive interaction with transcription factors such as MYC2, MYC3 and MYC4 (Lorenzo et al., 2004; Chini et al., 2007; Fernandez-Calvo et al., 2011). Under these conditions, JAZ proteins also interact with Novel Interactor of JAZ (NINJA), which recruits the corepresseor TOPLESS, creating a multi-protein, repressor complex (Pauwels et al., 2010; Pauwels et al., 2011). Upon pathogen or insect attack, JA-Ile is massively produced and enables the interaction of SCF^{COI1} and the JAZ proteins (Koo et al., 2009; Sheard et al., 2010). The SCF^{COI1} complex acts as an E3 ubiquitin ligase that targets JAZ transcriptional repressors for degradation by the 26S proteasome (Chini et al., 2007; Thines et al., 2007; Katsir et al., 2008a; Chung and Howe, 2009). The degradation of JAZ proteins allows the transcription factors to promote expression of JA responsive genes. Further studies of JA signal transduction will reveal its role in balancing growth and defense, and uncover basic principles of hormone action in plants.

JA Biosynthesis and Metabolism

Mechanical wounding and herbivory stimulate the biosynthesis and accumulation of JA (Reymond et al., 2000; Koo et al., 2009). JA biosynthesis begins with the release of α-linolenic acid from the plastid membrane through the action of phospholipases and fatty acid desaturases

Figure 1. The JA Signaling Pathway. (A) During optimal growth conditions, levels of bioactive JA-Ile are low and JAZ proteins (domain 1- D1, ZIM- Z, and Jas- J) suppress defense responses. The Jas domain (J) of JAZ proteins interact with transcription factors such as MYC2 (activation domain-AD, binding domain- BD). JAZ proteins also interact with NINJA through the ZIM domain (Z), which recruits the corepresseor TOPLESS, creating a multi-protein repressor complex. (B) Upon wounding or pathogen attack, JA-Ile is massively produced and enables the interaction of COI1 and the JAZ proteins. The SCF^{COI1} complex acts as an E3 ubiquitin ligase that targets JAZ transcriptional repressors for degradation by the 26S proteasome. The degradation of JAZ proteins allows MYC2 and RNA polymerase (RNAP) to promote expression of JA responsive genes, including *JAZ* genes themselves. Many *JAZ* genes produce alternative splice variants in which the Jas motif is removed or truncated (J with dotted lines). (C) JAZ splice variants have reduced affinity for COI1 and thus are more stable in the presence of high levels of JA-Ile. As a consequence, JAZ splice variants continue to repress MYC2 in the presence of high JA-Ile levels as part of a negative feedback mechanism to attenuate JA responses. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

FIGURE 1 (cont'd)

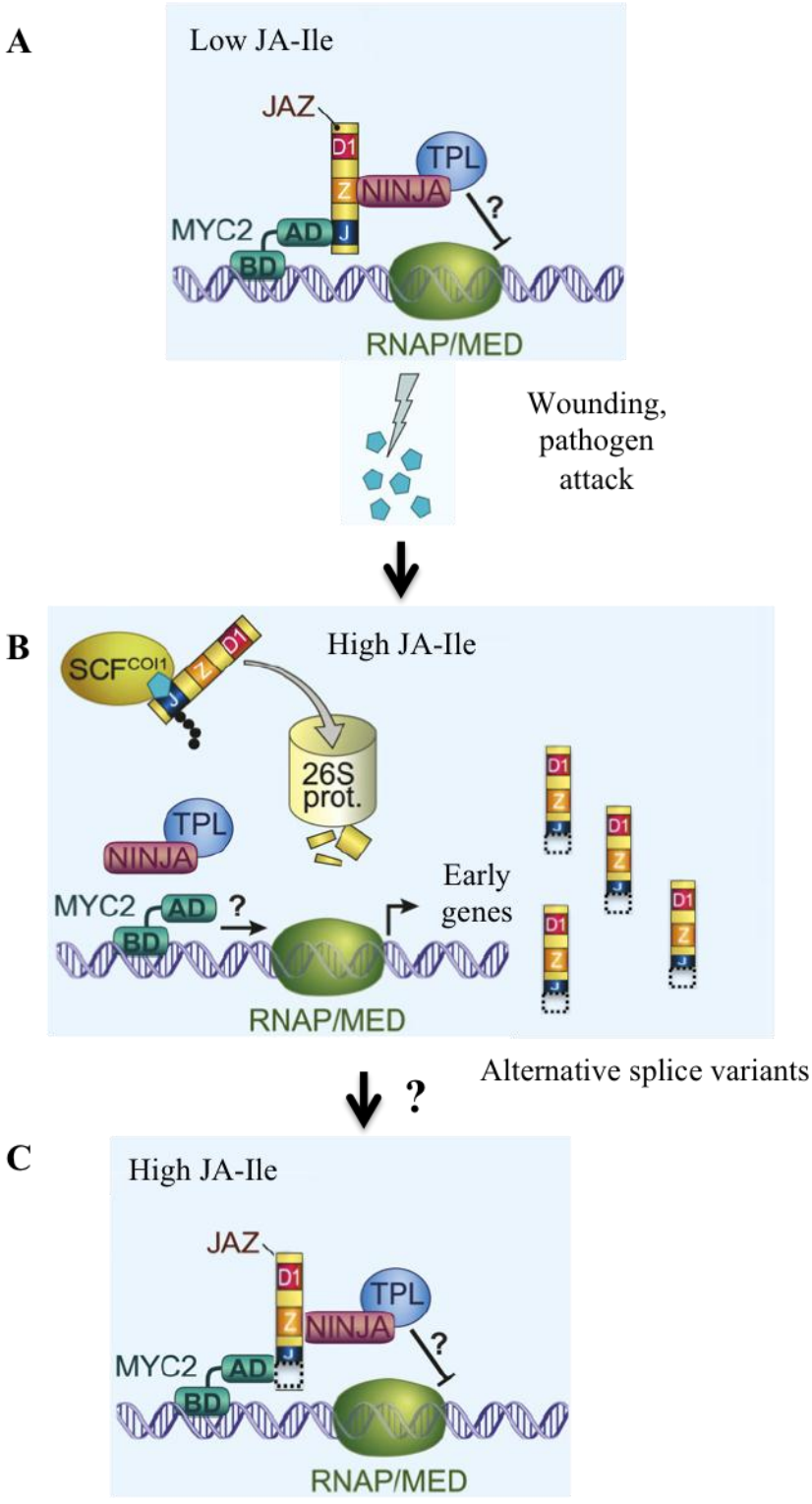

Figure 1. The JA Signaling Pathway. Modified from Howe (2010).

Figure 2. The JA Biosynthetic Pathway. Wounding and other stress-related conditions activate the biosynthetic pathway for the production of JA-Ile. The process begins with the release of linolenic acid from the chloroplast membrane, which is further metabolized by the chloroplast-residing enzymes LOX, AOS and AOC to form OPDA. OPDA is transported from the chloroplast to the peroxisome through the ATP-binding cassette (ABC) COMATOSE transporter, but small amounts may also be transported passively. Within the peroxisome, OPDA is reduced by OPR3 and conjugated to CoA by OPCL1. Three cycles of β-oxidation yield jasmonic acid (JA). Conjugation of JA to isoleucine by JAR1 yields the bioactive hormone, (3R, 7S)-JA-Ile.

FIGURE 2 (cont'd)

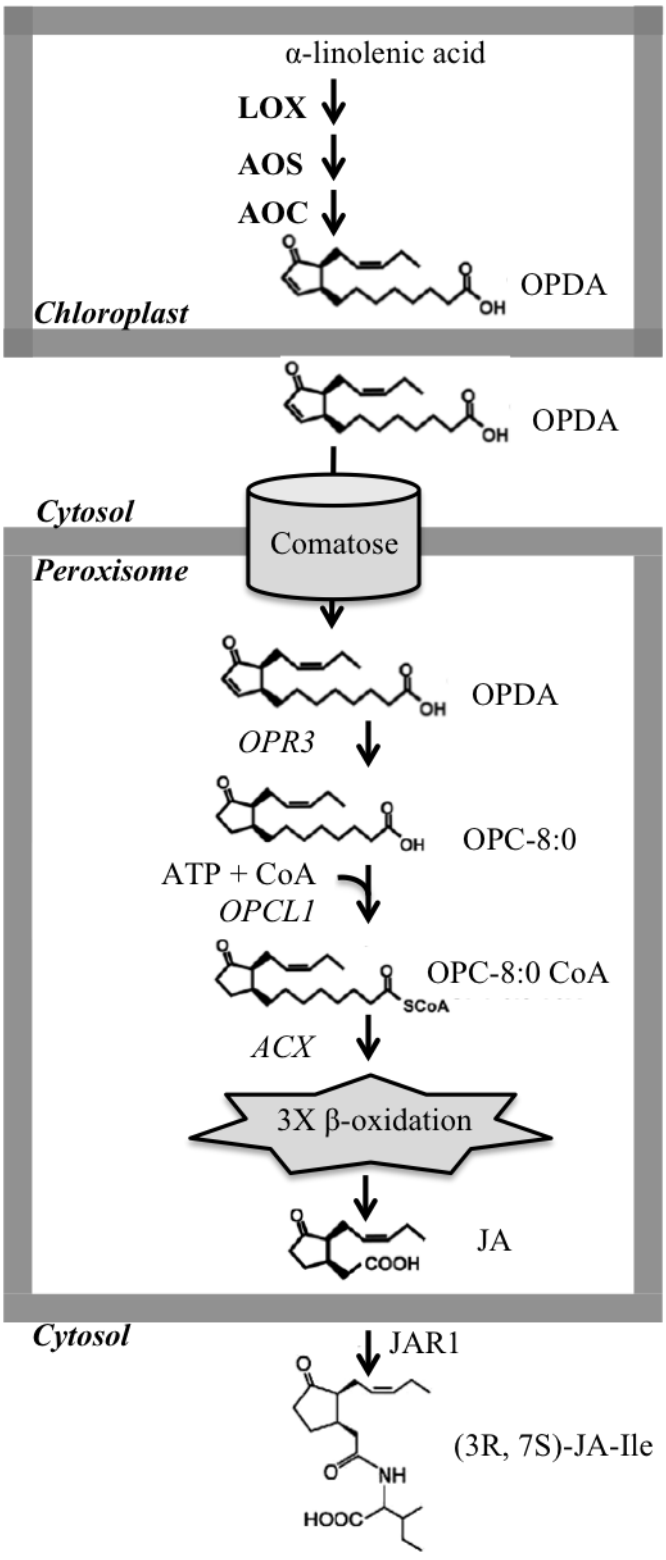

Figure 2. The JA Biosynthetic Pathway. Modified from Koo and Howe (2007).

(Figure 2) (McConn and Browse, 1996; Ishiguro et al., 2001). Linolenic acid is further metabolized by the chloroplast-residing enzymes lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) to form oxo-phytodienoic acid (OPDA). OPDA is transported from the chloroplast to the peroxisome through an ATP-binding cassette transporter COMATOSE, but small amounts may also be transported passively (Theodoulou et al., 2005). Once OPDA is in the peroxisome, it is reduced by 12-OXOPHYTODIENOIC ACID REDUCTASE (OPR3), and conjugated to CoA by OPC-8:0 Co-A LIGASE 1 (OPCL1) for three cycles of β-oxidation yielding JA (Stintzi and Browse, 2000; Koo et al., 2006; Schaller and Stintzi, 2009). In plant tissues, JA occurs as the (3R,7S) stereoisomers. This isomer can be converted to the more thermodynamically stable (3R,7R) isomer during extraction from plant tissues (Creelman and Mullet 1997; Fonseca et al., 2009).

JA can be further metabolized and conjugated by various enzymes. Methylation of JA occurs through the action of JASMONIC ACID CARBOXYLMETHYLTRANSFERASE (JMT). Transgenic plants ectopically expressing JMT have increased expression of JA response genes and enhanced resistance to *B. cinerea* infection (Seo et al., 2001). Ectopic expression of JMT results in increased production of MeJA in *Arabidopsis* and tobacco (Seo et al., 2001; Stitz et al., 2011). JMT is expressed in rosette leaves, cauline leaves, and flowers, and is induced in local and systemic tissues upon wounding or exogenous MeJA treatment (Seo et al., 2001). Additionally, ectopic expression of *Arabidopsis* JMT in *Nicotiana attenuata* suggested a metabolic source-sink relationship in regulating the production and conjugation of JA (Stitz et al., 2011). This study also provided evidence that JA metabolism is regulated in a tissue-specific manner.

Another important enzyme in the JA biosynthesis pathway is JASMONATE RESISTANT 1 (JAR1), which conjugates (3R,7S)-JA to isoleucine (Staswick and Tiryaki, 2004) to yield (3R, 7S)-JA-Ile, which is the bioactive hormone (Fonseca et al., 2009). The *jar1* mutant shows JA insensitivity in roots and increased susceptibility to the soil fungus *Pythium irregulare* (Staswick et al, 1998). The *jar1* mutant also has reduced levels of JA-Ile (Staswick and Tiryaki, 2004). Silencing of *JAR4*, the homolog of *JAR1* in *Nicotiana attenuata*, generated plants with increased susceptibility to herbivory by *Manduca sexta* larvae and this effect could be restored to wild-type levels by supplementation of JA-Ile (Kang et al., 2006). Clearly, impaired production of JA-Ile affects JA-mediated defense responses, supporting its role as the bioactive hormone.

Recent research has implicated the cytochrome P450 CYP94B3 and closely related members of the family in the inactivation of JA-Ile by 12-hydroxylation. A T-DNA insertion mutant (*cyp94b3*) hyper-accumulates JA-Ile, has reduced levels of 12-hydroxy-JA-Ile, and increased expression of JA responsive genes. Ectopic expression of CYP94B3 causes male sterility, root insensitivity to JA and increased susceptibility to insect feeding (Koo et al., 2011). Thus, CYP94B3 likely plays a role in the attenuation of JA responses by reducing levels of available bioactive JA-Ile.

The Role of the Ubiquitin-Proteasome Pathway in JA Signaling

Plant hormones utilize the ubiquitin-proteasome pathway as an essential part of hormone perception and signaling. Until a receptor was identified, the mechanism by which JA affected downstream changes in gene expression remained elusive. The bacterial phytotoxin coronatine (COR), which is produced by the plant pathogen *Pseudomonas syringae*, is a structural mimic of

(3R,7S)-JA-Ile (Brooks et al., 2004; Katsir et al., 2008b; Fonseca et al., 2009). Ethyl methanesulfonate (EMS), a chemical mutagen, was used to introduce mutations in *Arabidopsis* seedlings, which were then screened for resistance to COR (Feys et al. 1994). Using this forward genetics approach, mutants were identified that had a suite of JA-insensitive phenotypes, including male sterility due to defects in anther dehiscence, root insensitivity to JA, and decreased anthocyanin accumulation. Using a map-based cloning approach, the mutation was found to be in a gene encoding the F-box protein CORONATINE INSENSITIVE1 (COI1) (Xie et al., 1998). COI1 associates with the SKP1-CUL1 protein complex and functions as the specificity determinant of the E3 ubiquitin ligase SCF^{COI1} (Xie et al., 1998; Turner et al., 2002; Xu et al., 2002).

The identification of COI1 as an F-box protein implied the existence of a negative regulator that is ubiquitinated by SCF^{COI1} in response to JA. For nearly a decade after the discovery of COI1, much research was dedicated to finding the COI1-interacting substrates by various experimental methods. The mechanism by which COI1 regulates JA responses remained elusive until the discovery of Jasmonate-ZIM domain (JAZ) proteins (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). Transcriptional profiling experiments revealed that expression of *JAZ* genes is rapidly induced upon JA treatment, suggesting that these genes might be important for the JA response pathway (Thines et al., 2007). In addition, a dominant negative mutation (*jai1-3*) that causes insensitivity to JA was shown to map to a *JAZ* gene (Chini et al., 2007). Subsequent studies showed that JAZ proteins interact with COI1 in the presence of (3R,7S)-JA-Ile and COR, and that this interaction results in JAZ degradation via the 26S proteasome pathway (Thines et al., 2007; Chini et al., 2007; Chung and Howe, 2009; Pauwels et al., 2011).

Jasmonate-ZIM domain (JAZ) Proteins

Arabidopsis has 12 JAZ proteins that belong to the larger family of TIFYXG-containing proteins (Vanholme et al., 2007). JAZ proteins share three conserved regions: Domain 1, the ZIM domain and the Jas domain (Shikata et al., 2004; Thines et al., 2007; Chung et al., , 2009). The N-terminal Domain 1 is loosely conserved and contains an acidic region with unknown functional significance (Thines et al., 2007). The ZIM domain is a 28-amino-acid sequence containing the highly conserved TIFYXG motif (Vanholme et al., 2007). The ZIM domain is necessary for protein-protein interactions, including the formation of homo- and heteromeric JAZ-JAZ complexes (Chini et al., 2009; Chung and Howe, 2009). JAZ proteins have a C-terminal Jas motif with the highly conserved ELPIARRASLX_2FX_2KRX_2RX_5PY sequence (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chung et al., 2010). In the case of nine of the 12 JAZ proteins in *Arabidopsis,* the genomic DNA encoding the 27-amino-acid Jas domain is encoded by two exons that are separated by an intron, referred to as the Jas intron (Figure 3) (Chung et al., 2010). The N-terminal 20 amino acids of the Jas domain are necessary and sufficient for hormone-dependent binding to COI1 and have been designated as the JAZ degron (Sheard et al., 2010). Analyses of JAZ truncation mutants have also implicated the Jas domain in binding of the transcription factor MYC2 (Chini et al., 2007; Thines et al., 2007; Katsir et al., 2008b; Chini et al., 2009; Fonseca et al., 2009). Furthermore, analysis of JAZ1 truncation mutants fused to GFP suggests that the conserved PY sequence at the C-terminal end of the Jas motif plays a role in nuclear localization (Grunewald et al., 2009).

Although there are 12 JAZ proteins in *Arabidopsis,* the role of each protein in the regulation of JA responses is unclear. Biochemical approaches have revealed that differences in amino acid

sequence may alter the ability of JAZ proteins to interact with other proteins. For example, JAZ proteins vary in their ability to homo- or heterodimerize, or participate in protein-protein interactions with MYC2 or COI1 (Chini et al., 2009; Chung and Howe, 2009; Pauwels and Goossens, 2011). With the exception of the *jaz10-1* mutant, all available *jaz* T-DNA insertion mutants have no phenotype (Thines et al., 2007). *jaz10-1* shows a phenotype of increased cell growth in the stem base and altered JA responses in interfascicular-derived cambium tissues (Sehr et al., 2010). Recently, *jaz10-1* was also shown to have a JA-hypersensitive phenotype in roots, and increased susceptibility to *Pseudomonas syringae* infection, similar to a *JAZ10* RNAi line (Yan et al., 2007; Demianski et al., 2011). The general paucity of phenotypes in *jaz* single mutants suggests there is functional redundancy amongst the gene family.

JA Perception

The recent elucidation of the x-ray crystal structure of the COI1-JAZ complex revealed the molecular mechanism of JA-Ile and COR binding to the receptor. COI1 shares structural similarity to the auxin receptor TIR1 (TRANSPORT INHIBITOR RESPONSE 1) (Katsir et al., 2008a; Tan et al., 2007). Both COI1 and TIR1 have an N-terminal F-box motif and 18 leucine-rich repeats (LRR) that are arranged in a solenoid structure and bind inositol polyphosphates (Tan et al., 2007; Sheard et al., 2010). *In vitro* ligand binding assays performed with recombinant ASK1, COI1, and JAZ proteins were used to define the biochemical features of the receptor (Sheard et al., 2010). The highly conserved N-terminal region of the JAZ degron consists of ELPIARR residues that are critical for high affinity binding of (3R,7S)-JA-Ile or COR (Sheard et al., 2010). Using mass spectrometry and nuclear magnetic resonance data, inositol

pentakisphosphate was found to co-purify with the COI1-ASK1 complex (Sheard et al., 2010). It was concluded that the high-affinity receptor for (3R,7S)-JA-Ile and COR is composed of a complex of COI1-JAZ and inositol pentakisphosphate (Sheard et al., 2010). Evidence that COI1 is required for E3 ubiquitin ligase activity was shown by the detection of ubiquitinated JAZ6 (Saracco et al., 2009).

Mechanism of Transcriptional Repression by JAZ Proteins

In a screen for JAZ-interacting proteins, a tandem affinity purification (TAP) tagged derivative of JAZ1 was found to interact with Novel Interactor of JAZ (NINJA) (Pauwels et al., 2010). Analysis of the NINJA sequence revealed three conserved domains, named A, B, and C. The C domain was previously described in members of the ABI-FIVE BINDING PROTEIN (AFP) family as being necessary and sufficient for binding to the transcription factor ABI5 (Garcia et al., 2008). Yeast two-hybrid assays showed that domain C of NINJA interacts with the TIFY motif in the ZIM domain in all JAZ proteins except JAZ7 and JAZ8 (Pauwels et al., 2010). Because the TIFY motif is also involved in JAZ-JAZ dimerization and the C domain of NINJA binds ABI5, it is unclear whether these domains compete for binding with other proteins or create large, multi-protein repressor complexes (Geerinck et al., 2010).

The A domain in NINJA contains an ETHYLENE RESPONSIVE FACTOR-associated amphiphilic repression (EAR) motif that has been previously shown to recruit the Groucho/Tup1 type co-repressor TOPLESS (TPL) (Geerinck et al., 2010; Kagale et al., 2010; Pauwels et al., 2010). Previous research showed that EAR motifs are present in many Aux/IAA proteins, and that the EAR motif recruits TPL to repress the expression of auxin-responsive genes (Szemenyei

et al., 2008). NINJA interacts with TPL in yeast and in plant nuclei, as determined by *in vivo* bimolecular fluorescent complementation (BiFC) assays (Pauwels et al., 2010).

The TPL co-repressor is involved in multiple hormone pathways (Kagale et al., 2010; Pauwels et al, 2010). TPL was first studied for its role in auxin signaling in the determination of apical polarity (Long et al., 2006). A *tpl1-1* mutant was identified in which the shoot pole is transformed into a root pole during the transition stage of early embryogenesis, generating a double-rooted plant that is "topless" (Long et al., 2006). TPL is a 1131-amino-acid protein containing C-terminal WD40 repeats, a lissenchephaly type 1-like homolog domain (LiSH) and a C-terminal to LiSH domain (CTLH), that latter of which interacts with EAR motifs (Long et al., 2006, Pauwels et al., 2010). TPL also has a proline-rich region that is similar to the known repressors in the Groucho/TUP1 family (Long et al., 2006). TPL may actively repress transcription by recruiting the histone deacetylase HDA19 (Long et al., 2006).

JAZ-interacting Transcription Factors

Genetic screens for JA response mutants resulted in the identification of a JA-*insensitive 1* (*jin1*) mutant (Lorenzo et al., 2004). Using a map-based cloning approach, the *JIN1* locus was found to encode the bHLH transcription factor MYC2, which positively regulates JA responses in a COI1-dependent manner (Lorenzo et al., 2004; Chini et al., 2007). The JA-insensitivity of *jin1* mutants is relatively weak in comparison to the fully insensitive and male sterile *coi1* mutant, suggesting that additional transcription factors are involved in promoting JA responses (Lorenzo et al., 2004).

MYC2 belongs to a family of bHLH transcription factors that also includes MYC3 and MYC4 (Boter et al., 2004; Fernandez-Calvo et al., 2011; Niu et al., 2011). The *myc2myc3myc4* triple mutant is more insensitive to JA than the single *myc2* (*jin1*) mutant, but still not as insensitive as the *COI1* null mutant *coi1-1* (Fernandez-Calvo et al., 2011). The *myc2myc3myc4* triple mutant is more susceptible to *S. littoralis* feeding than single or double *myc* mutants, and is as susceptible as *coi1-1* (Fernandez-Calvo et al., 2011). Additionally, ectopic expression of *MYC3* and *MYC4* resulted in increased accumulation of anthocyanins, which are known to be regulated by the JA pathway (Niu et al., 2011).

MYC2, MYC3 and MYC4 share approximately 56% sequence similarity, with the highest degree of similarity in the N-terminus (Niu et al., 2011). JAZ proteins interact with both MYC3 and MYC4 in yeast two-hybrid and *in vitro* pull down assays (Niu et al., 2011; Fernandez-Calvo et al., 2011). Structure-function studies further showed that a 67-amino-acid region in the N-terminus of MYC2 ($MYC2^{D93\text{-}160}$) is conserved in MYC3 and MYC4, and is sufficient to bind JAZ proteins (Fernandez-Calvo et al., 2011). Genome-wide analysis of MYC2 binding sites indicated preferential binding to the G-box motif CACGTG, which is overrepresented in promoters of JA-responsive genes (Dombrecht et al., 2007; Chini et al., 2007). Interestingly, MYC3 and MYC4 have identical preference for binding G-box motifs (Fernandez-Calvo et al., 2011)

Analysis of promoter-GUS fusions of MYC3 showed that *MYC3* is expressed in most vegetative tissues, whereas *MYC4* is expressed primarily in vasculature tissues (Fernandez-Calvo et al., 2011). *MYC2* promoter-GUS fusions show high basal expression in roots (Chen at al., 2011). It was proposed that MYC3 and MYC4 work in concert with MYC2 to regulate JA responses and are not fully functionally redundant. MYC2, MYC3 and MYC4 may also form

homo- and heterodimers *in vivo* (Fernandez-Calvo et al., 2011). Other transcription factors may also play a synergistic role in regulating JA responses.

Alternative Splicing in the Arabidopsis Genome

Many intron-containing plant genes undergo alternative splicing (Filichkin *et al*., 2010; Reddy, 2007). More than 80% of genes in the *Arabidopsis* genome harbor one or more introns (Reddy, 2007). It is estimated that 42% of *Arabidopsis* intron-containing genes are alternatively spliced, and further suggested that splicing might be regulated by abiotic stress (Filichkin et al., 2010). Among 18 *Arabidopsis* accessions, the most differentially expressed genes are those involved in defense responses and stress responses (Gan et al., 2011). To date, there are relatively few examples of functionally relevant alternative splicing events in plants. But among these few examples, many are involved in stress responses (Kazan et al., 2003). An example of this is the *Arabidopsis* R gene, *RPS4*, which undergoes intron retention and splicing of a cryptic intron within the 3rd exon (Zhang and Gassmann, 2003; Gassmann et al., 2008). Ectopic expression of *RPS4* cDNA confers susceptibility to *Pseudomonas syringae* pv *tomato* strain DC3000, suggesting that intron retention and splicing of the cryptic intron is critical to conferring defense responses (Zhang and Gassmann, 2003). Increasing evidence suggests that alternative splicing may be an important mechanism for regulating the expression of stress-responsive genes (Ali and Reddy, 2008; Gassmann, 2008).

Alternative Splicing of JAZ Genes

A key feature of most *JAZ* genes is their strong expression in response to JA treatment or stress-related cues that activate JA synthesis. JA-induced expression of *JAZ* genes may serve as a mechanism of negative feedback regulation (Thines et al., 2007; Chung et al., 2009). Upon methyl-jasmonate (MeJA) treatment or wounding, *JAZ* genes are highly induced and alternatively spliced transcripts of several *JAZ* genes are produced (Yan et al., 2009; Chung and Howe, 2009). Nine of the 12 *Arabidopsis JAZ* genes are predicted to produce alternative splice variants in which the Jas domain is truncated due to Jas intron retention (Chung et al., 2010). *JAZ10* (At5g13220) naturally produces three alternative splice variants in which the Jas domain is truncated or missing (Figure 3) (Chung and Howe, 2009). *JAZ10.1* encodes the full-length protein isoform. *JAZ10.2* is produced by retention of the Jas intron. A premature termination codon (PTC) within the Jas intron results in the loss of 12 amino acids from the C-terminus. *JAZ10.3* also retains the Jas intron. Unlike *JAZ10.2*, however, *JAZ10.3* has another alternative splicing event at the first GT within the retained Jas intron, resulting in a 3' UTR that is shorter than that of *JAZ10.2*. *JAZ10.2* and *JAZ10.3* encode identical proteins. A fourth *JAZ10* splice variant, *JAZ10.4*, is produced by use of an alternate splice donor site in the third exon. This splicing event causes a frame-shift mutation that results in the deletion of the entire Jas domain (Chung and Howe, 2009). In addition to *JAZ10*, several other *JAZ* genes undergo alternative splicing events involving retention of the Jas intron. Similar to the case of *JAZ10.2/JAZ10.3*, these transcripts are predicted to produce proteins that lack the C-terminal end (X5PY) of the Jas motif (Chung et al. 2010).

Figure 3. Gene Models of *JAZ10* Alternative Splice Variants. *JAZ10.1* (A), consists of five exons (black boxes) and four introns (lines) and encodes the full-length isoform containing a complete Jas domain, which is encoded by the last two exons. *JAZ10.2* retains the last intron, referred to as the Jas intron. A premature termination codon located near the 5' end of the Jas intron results in production of a truncated protein lacking seven amino acids from the C-terminal end of the Jas motif. *JAZ10.3* retains a portion of the Jas intron but uses an alternative splice acceptor site within the Jas intron to generate a transcript that encodes a protein identical to JAZ10.2. *JAZ10.4* is produced from the use of an alternative splice donor site located in the third exon. This splicing event causes a frameshift and truncation of the entire Jas domain. Open boxes represent 5' and 3' untranslated regions. Schematic representation of proteins produced for each *JAZ10* alternative splice variant (B). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

* indicate unique splicing events

FIGURE 3 (cont'd)

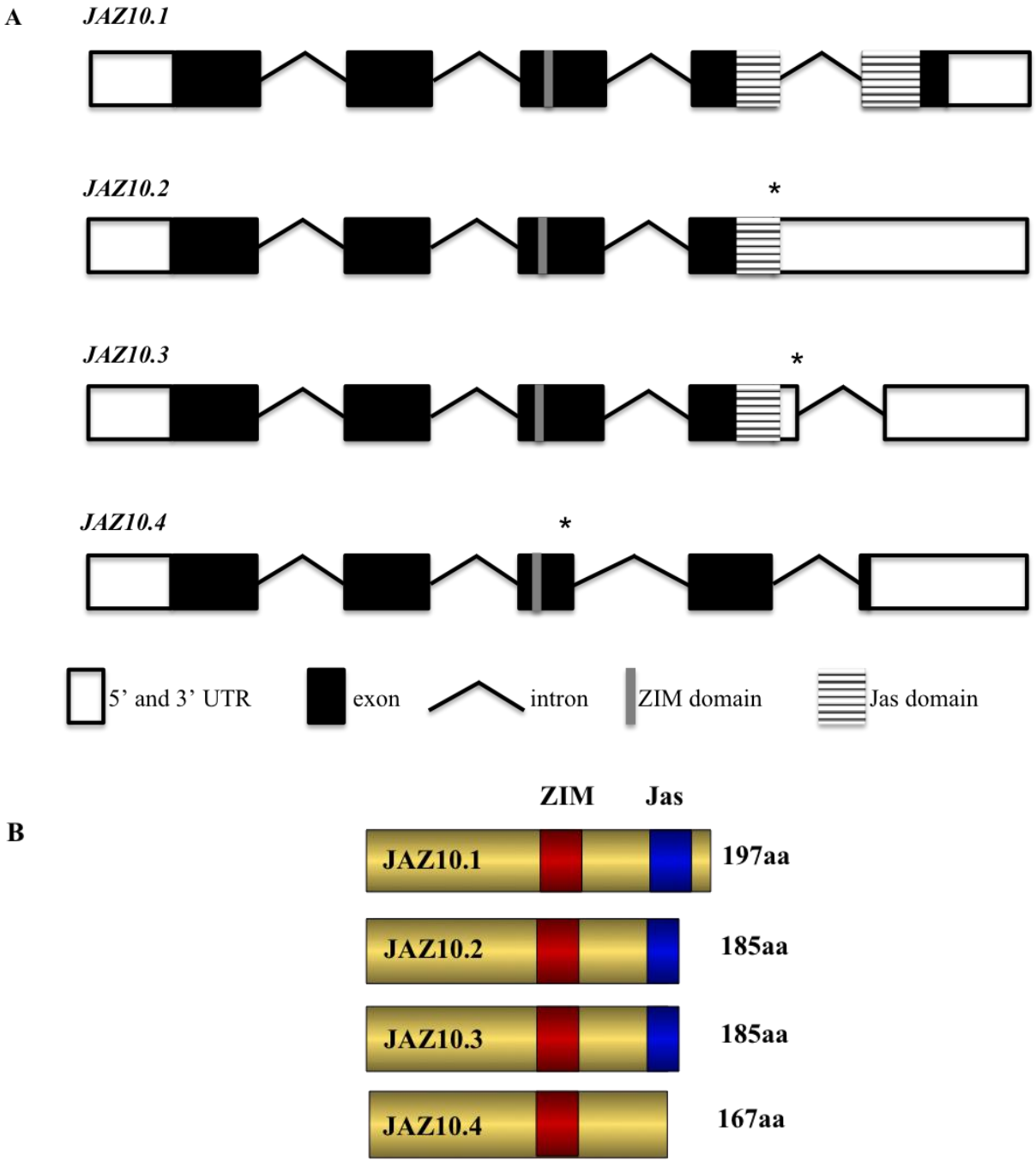

Figure 3. Gene Models of *JAZ10* Alternative Splice Variants.

JAZ proteins in which the Jas domain is truncated or missing are more stable than their full-length counterparts, have decreased affinity for COI1 and thus are stable in the presence of JA (Chung and Howe, 2009; Chung et al., 2010; Sheard et al., 2011). Transgenic plants overexpressing these truncated isoforms exhibit decreased sensitivity to JA (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chung and Howe, 2009; Chung et al., 2010). Interestingly, the Jas intron is also conserved in *JAZ* genes found in all land plant species, including the evolutionarily distant *Physcomitrella patens* (Chung et al., 2010). Thus, alternative splicing events affecting the Jas domain may be an important control point in regulating JA responses.

The Role of JA in Fertility

Previous research has revealed an essential role for JA in the development of male reproductive tissues in *Arabidopsis* (Park et al., 2002; Stintzi and Browse, 2000). The JA perception mutant *coi1*, as well as various JA biosynthetic mutants, are male-sterile due to defects in anther dehiscence and anther filament elongation (Park et al., 2002; Stintzi and Browse, 2000; McConn and Browse, 1996; Feys et al., 1994). Transcriptional profiling experiments of JA treated *Arabidopsis* stamens revealed strong induction of flower-specific transcription factors MYB21 and MYB24 in comparison to untreated samples (Mandaokar et al., 2006). The *myb21myb24* double knockout is male sterile in a similar manner to JA biosynthetic and JA perception mutants, suggesting a regulatory role of these transcription factors in male fertility (Mandaokar et al., 2006). The ability of MYB21 and MYB24 to interact with several JAZ proteins suggests a role for JAZs in regulating male fertility (Song et al., 2011). In support of this idea, ectopic expression of MYB21 in the *coi1* mutant partially restored fertility (Song et

al., 2011). Overexpression of various JAZ derivatives that lack the Jas domain (e.g., JAZ10.4) also results in male sterility (Thines et al., 2007; Chung et al., 2009; Song et al., 2011). The mechanism by which MYB transcription factors and JAZ proteins regulate stamen and anther development remains to be determined.

The Role of JAZ Proteins in the Integration of Other Hormone Signaling Pathways

JA-Auxin

There are many similarities between auxin and JA signaling that enable cross-regulation between these two hormones. Among the ~700 F-box proteins encoded by the *Arabidopsis* genome, the most closely related to COI1 is TIR1 and TIR1-like proteins (Katsir et al., 2008a; Tan et al., 2007). At the protein level, COI1 and TIR1 are approximately 33% similar and share overall structural features, including 18 tandem LRRs arranged in a solenoid array (Tan et al., 2007; Sheard et al., 2010). Both COI1 and TIR1 bind inositol polyphosphates, which may be involved in regulating receptor function (Tan et al., 2007; Sheard et al., 2010). Both TIR1 and COI1 are components of SCF-type E3 ubiquitin ligases and share other subunits of the complex (Hoffmann et al., 2011). Some mutants affected in the formation of SCF complexes, such as the *cul1* mutant, show both auxin- and JA-related phenotypes (Hoffmann et al., 2011). Upon hormone perception, SCF^{TIR1} and SCF^{COI1} function as E3 ubiquitin ligases, targeting Aux/IAA and JAZ repressor proteins, respectively, for degradation by the 26S proteasome (Pauwels et al., 2010). Auxin and JA pathways utilize EAR motif-containing proteins to recruit TPL (Pauwels et al., 2010).

JA-GA:

The gibberellic acid (GA) signaling pathway is involved in numerous aspects of plant growth and development and has recently been integrated in the JA signaling network. GA signaling is similar to that of auxin and JA. The hormone receptor SCF^{GID1} binds DELLA transcriptional repressors and targets them for degradation by the ubiquitin-26S proteasome pathway (Ueguchi-Tanaka et al., 2007). Recently, it was shown that DELLA proteins compete with MYC2 for interaction with JAZ proteins (Hou et al., 2010). The DELLA quintuple mutant exhibits reduced expression of JA responsive genes, which can be even further reduced when treated with a GA biosynthesis inhibitor (Hou et al., 2010). Chromatin immunoprecipitation and *in vitro* transactivation assays provide evidence that MYC2 can bind DNA more efficiently when levels of DELLA proteins are high and that this process is dependent on COI1 (Hou et al., 2010). This suggests a model in which DELLAs bind JAZ proteins under low GA or high JA levels to allow MYC2 to bind DNA and promote transcription (Hou et al., 2010).

JA-Ethylene:

Ethylene is a plant hormone involved in a number of plant developmental processes and is also known to be important for defense against necrotrophic fungi (Dong, 1998; Browse, 2009). Ethylene and JA induce defense genes more strongly in combination with each other, suggesting a synergistic effect between these two pathways (Lorenzo et al., 2003). The transcription factors EIN3 and EIL1 are necessary and sufficient for induction of ethylene responses (Alonso et al., 1999). EIN3 and EIL1 interact directly with JAZ1, JAZ3 and JAZ9 in yeast two-hybrid and *in vivo* BiFC assays (Zhu et al., 2011). The *ein3 eil1* double mutant is partially insensitive to JA in root length assays, suggesting that EIN3 and EIL1 are positive regulators of JA responses. This

study also showed that the ability of EIN3 to activate ethylene responsive genes depends in part on JAZ1 (Zhu et al., 2011). These results provide a molecular mechanism for the role of JAZ proteins in the integration of JA and ethylene hormone signaling pathways.

JA-Phytochome:

Arabidopsis possesses five types of phytochromes, phyA-phyE, that mediate different aspects of light signaling (Sharrock and Quail, 1989). PhyA is responsible for the detection of far-red light in the range of 600-750 nm and controls the expression of genes leading to inhibition of hypocotyl elongation, opening of the apical hook, expansion of cotyledons, accumulation of anthocyanin, and blockage of greening (Chory et al., 1996; Yang et al., 2005). There is much experimental evidence supporting the crosstalk between the JA and light signaling pathways. A mutant screen for suppressors of the *constitutive photomorphogenesis 1* mutation identified a mutant allele of *JAR1* (Hsieh et al., 2000). The *myc2* mutant was also discovered in a screen for proteins binding specific elements of light responsive promoters (Yadav et al., 2005). The ecological importance of this was demonstrated in an experiment in which plants grown in high density or under far-red light had reduced JA responses and were more susceptible to herbivores (Moreno et al., 2009). Interestingly, the *phyA* mutant is insensitive to JA in root length assays, and *JAZ1* transcript is more abundant in plants grown under far-red light (Robson et al., 2010). Ectopic expression of JAZ1-GUS in the phyA mutant has revealed that PhyA is required for the degradation of JAZ1 in response to wounding and JA treatment (Robson et al., 2010).

In summary, the transcriptional regulation of alternative splicing of *JAZ* genes in response to JA is not fully understood. It is necessary to determine whether splicing occurs constitutively or whether splicing is regulated by stress-induced changes in the spliceosome machinery. Truncated JAZ proteins that are stable in the presence of JA may serve to attenuate defense responses and JA responsive gene expression. This research aims to clarify the regulation of alternative splicing of *JAZ* genes, as well as characterize their functional significance. Information gained from these studies will help to elucidate the role of alternative splicing in JA signaling and related responses to pathogen attack and herbivory.

RESULTS

Regulation of JAZ Alternative Splicing

Upon MeJA treatment or wounding, *JAZ* gene expression is highly induced and may serve as a mechanism of negative feedback regulation. In addition to high expression of *JAZ* genes, alternatively spliced transcripts of several *JAZ* genes are detected (Thines et al., 2007; Chung and Howe, 2009). Nine of the 12 *Arabidopsis JAZ* genes are predicted to produce alternative splice variants in which the Jas domain is truncated due to Jas intron retention (Chung et al., 2010). Several *JAZ* genes, including *JAZ2*, *JAZ6* and *JAZ10* are subject to Jas intron retention, and produce similarly truncated proteins (Figure 11).

To determine whether the relative levels of each *JAZ* splice variant remains constant during induction of *JAZ* expression or if there is a change in splice-site preference, the level of full-length (*JAZ10.1*) and alternative splice variants (*JAZ10.2*, *JAZ10.3*, *JAZ10.4*) were quantified using quantitative real time polymerase chain reaction (qPCR) and splice variant-specific primer sets. In mock treated seedlings (Figure 4A, "0"), *JAZ* genes were expressed at a low basal level, where the full-length transcript (*JAZ10.1*) predominates and the alternatively spliced transcripts are present at very low to undetectable levels. The level of all four *JAZ10* splice variant mRNAs increased in response to MeJA treatment, with *JAZ10.1* being the predominant form. The relative abundance of each transcript, however, was generally maintained throughout the time course (Figure 4A). For example, levels of *JAZ10.3* and *JAZ10.4* remain at approximately 3-10% of the

Figure 4. Levels of *JAZ10* Alternatively Spliced Transcripts in MeJA-Treated Seedlings. Col-0 seedlings were grown in liquid MS media for nine days and then treated with 100 μM MeJA or a mock control (0.007% ethanol) for two hours. At the indicated time after treatment, seedlings in each biological replicate (a pool of 15-20 seedlings per sample) were harvested for isolation of RNA and qPCR analysis of *JAZ10* (A), *JAZ2* (B), and *JAZ6* (C) transcript levels. Relative expression refers to the level of a specific alternatively *JAZ* transcript after normalization to the reference genes *PP2A* and *YLS8*. Data points show the mean and standard error of three biological replicates. Due to the lack of a unique region in *JAZ10.1*, qPCR primers for *JAZ10.1* detect both *JAZ10.1* and *JAZ10.4* splice variants.

FIGURE 4 (cont'd)

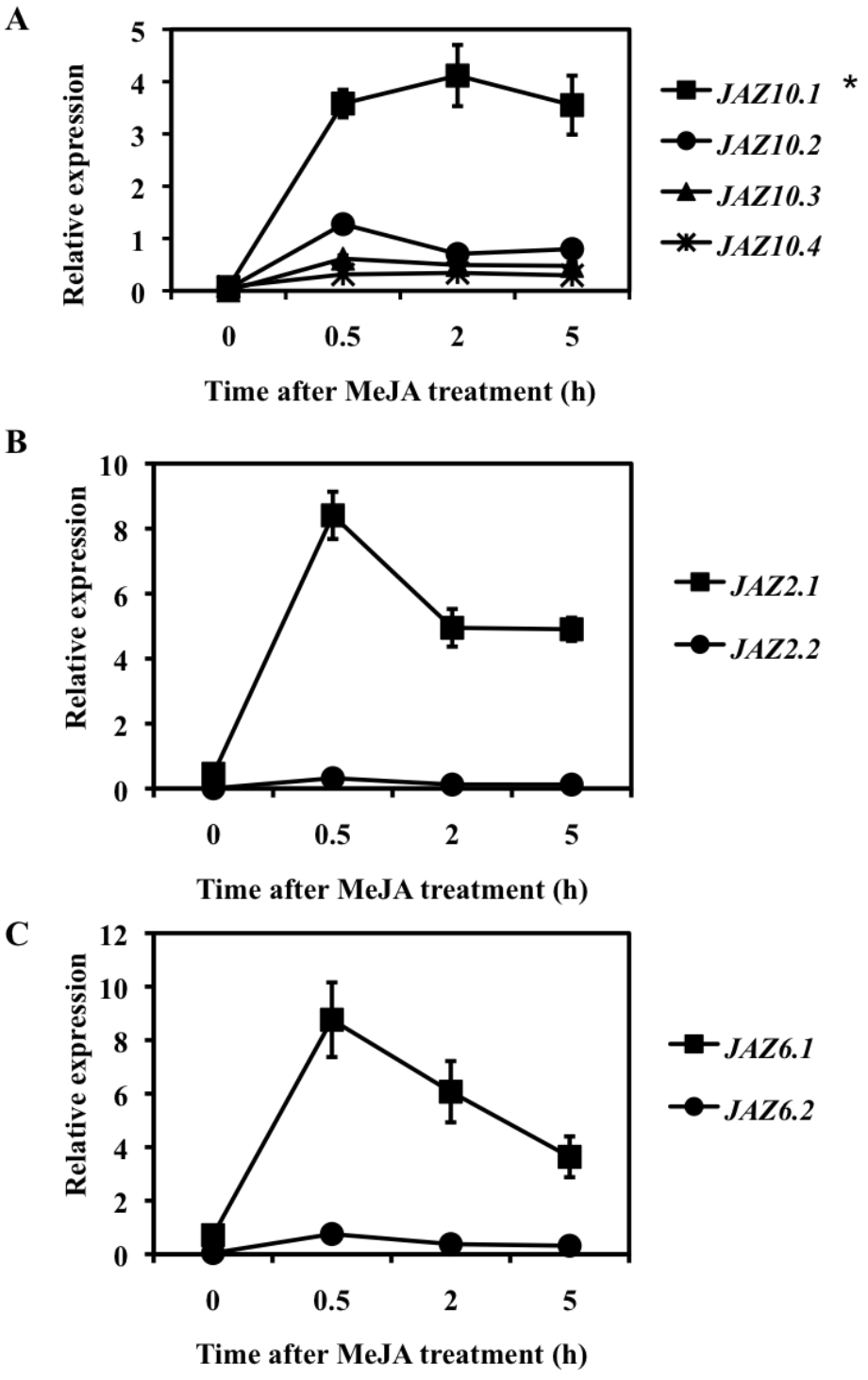

Figure 4. Levels of *JAZ10* Alternatively Spliced Transcripts in MeJA-Treated Seedlings.

levels of *JAZ10.1*. One notable exception to this was *JAZ10.2*, which accumulated to approximately 35% of the level of *JAZ10.1*.

The same RNA samples were used to quantify alternatively spliced forms of *JAZ2* and *JAZ6*, which are subject to *JAZ10.3*-like splicing events involving retention of the Jas intron. Similar to the results obtained with *JAZ10*, the full-length *JAZ2.1* and *JAZ6.1* transcripts predominated over the alternatively spliced *JAZ2.2* and *JAZ6.2* transcripts in response to MeJA treatment (Figure 4B and C). Relative levels of *JAZ2.2* were approximately 2-5% that of *JAZ2.1*. The basal expression of *JAZ6.1* in unwounded leaves was relatively high in comparison to that observed in the MeJA-treated samples (Figure 5C). Variation in the basal level expression among the *JAZ* genes has been previously reported, including a high basal expression of *JAZ6* (Chung et al., 2008). After MeJA treatment, relative levels of *JAZ6.2* were approximately 5-9% that of *JAZ6.1*.

To determine whether the results obtained for MeJA-treated seedlings extends to other stages of plant development, the levels of alternatively spliced *JAZ* transcripts were also measured in adult plants subject to mechanical wounding (Figure 5). In unwounded leaf tissue (Figure 5A, "0"), *JAZ* genes are expressed at a low basal level, where the full-length transcript (*JAZ10.1*) predominates and the alternatively spliced transcripts are present at very low to undetectable levels. The level of all four *JAZ10* mRNAs increased in response to wounding, with *JAZ10.1* being the predominant form. Similar to the results obtained with MeJA treatment (Figure 4), the relative abundance of each transcript was maintained throughout the time course (Figure 5A). Levels of *JAZ10.3* and *JAZ10.4* remained at approximately 3-11% of the levels of *JAZ10.1*, similar to MeJA-treated seedlings. The peak level of *JAZ10.2* was nearly 40% of *JAZ10.1*.

Figure 5. Levels of *JAZ* Alternatively Spliced Transcripts in Wounded Leaves. Col-0 plants were grown for 25 days on soil. Each biological replicate is a pool of three wounded leaves from two plants. Wounded leaves were harvested for isolation of RNA and qPCR analysis of *JAZ10* (A), *JAZ2* (B), and *JAZ6* (C) transcript levels. Data points show the mean and standard error of four biological replicates. Due to the lack of a unique region in *JAZ10.1*, qPCR primers for *JAZ10.1* detect both *JAZ10.1* and *JAZ10.4* splice variants.

FIGURE 5 (cont'd)

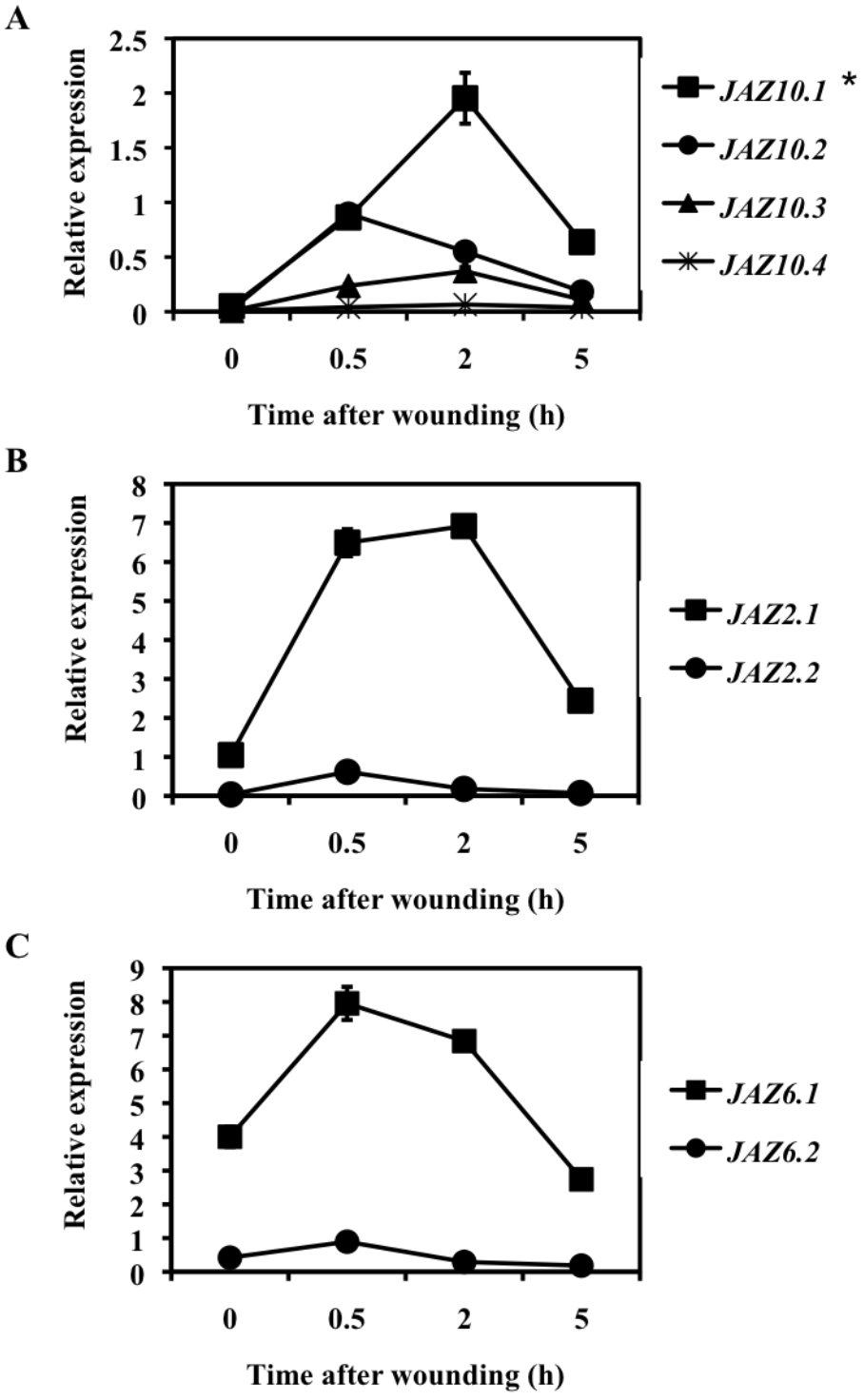

Figure 5. Levels of *JAZ* Alternatively Spliced Transcripts in Wounded Leaves.

Alternative Splicing of JAZ Genes in Flowers and Roots

In animals, alternative splicing is often regulated by tissue-specific factors that alter the spliceosome machinery (Grabowski and Black, 2001). To test the hypothesis that alternative splicing of *JAZ10* is regulated in a tissue-specific manner, qPCR was utilized to measure the levels of *JAZ10* alternative splice variants in floral tissue. These experiments were performed with RNA isolated from Col-0 (wild-type; WT) flowers, as well as flowers from a transgenic line (*35S-JAZ10G*) that overexpresses a *JAZ10* genomic clone (Chung et al., 2010). As shown in Figure 6**,** the level of all *JAZ10* alternative splice variants in *35S-JAZ10G* flowers was much higher (approximately 30-fold) than that in WT flowers. The relative abundance of each splice variant in WT and *35S-JAZ10G* flowers, however, was similar. *JAZ10.1* was the predominant transcript in both genotypes. The level of *JAZ10.2* was approximately 50% of the level of *JAZ10.1* Levels of *JAZ10.3* and *JAZ10.4* ranged from 5-15% of the level of *JAZ10.1*, similar to the results obtained with RNA isolated from vegetative tissues (Figures 4 and 5).

Using the same RNA, levels of full-length and alternatively spliced *JAZ2* and *JAZ6* were also measured in WT and *35S-JAZ10G* flowers (Figure 7). Similar to the results obtained for *JAZ10*, the full-length *JAZ2.1* and *JAZ6.1* transcripts were the predominately expressed forms, with *JAZ2.2* and *JAZ6.2* accounting for less than 10% of the total transcript level. The level of each *JAZ2* and *JAZ6* transcript in WT and *35S-JAZ10G* flowers was similar, indicating that ectopic expression of *JAZ10* does not alter the expression of the endogenous *JAZ2* and *JAZ6* genes.

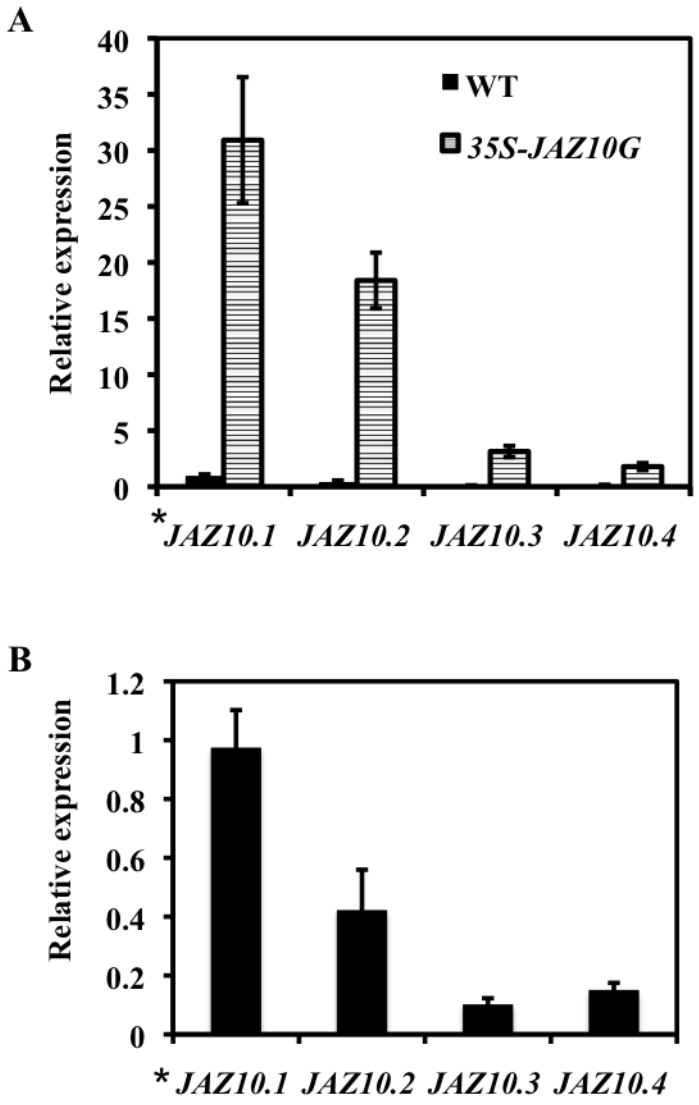

Figure 6. Levels of *JAZ10* Alternatively Spliced Transcripts in WT and *35S-JAZ10G* Flowers. (A) Wild-type (WT) and *35S-JAZ10G* plants were grown for seven weeks on soil. Data show the mean and standard error of three biological replicates. Each replicate of RNA were derived from flowers obtained from four plants. (B) Re-plotting of WT data shown in panel A, using a different scale.

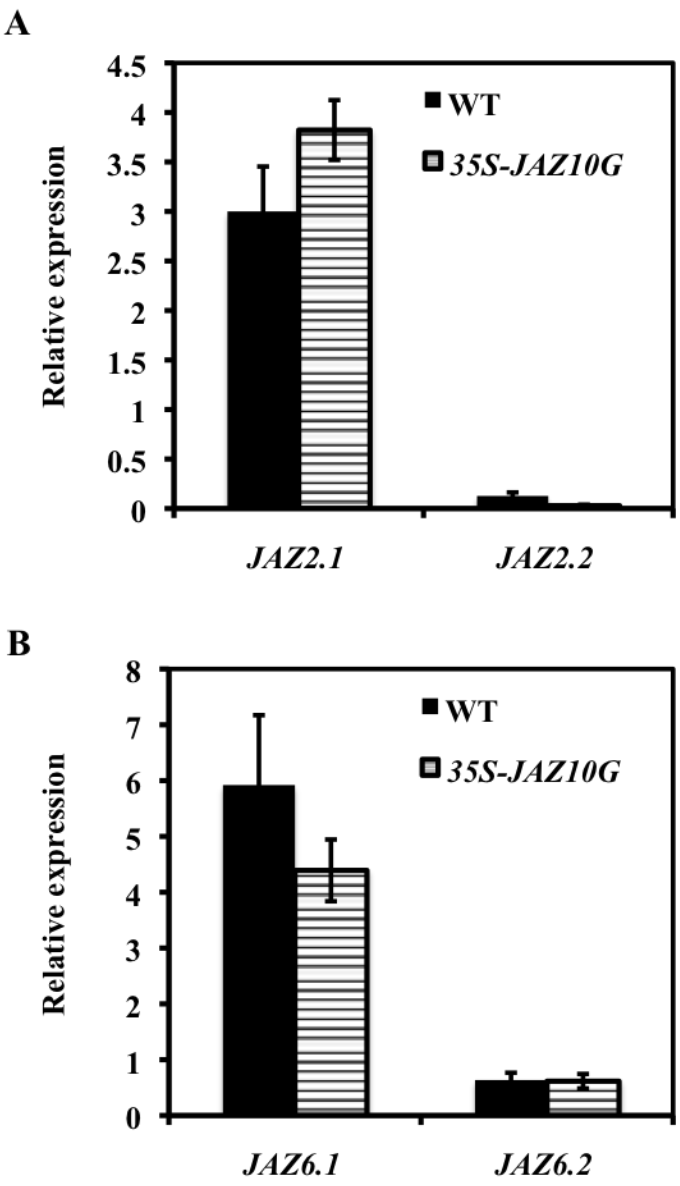

Figure 7. Levels of *JAZ2 and JAZ6* Alternatively Spliced Transcripts in WT and *35S-JAZ10G* Flowers. The same RNA used for the experiment described in Figure 6 was analyzed by qPCR for the expression of alternatively spliced forms of *JAZ2* (A) and *JAZ6* (B). Data points show the mean and standard error of three biological replicates.

In addition to flowers, *JAZ* transcripts were also quantified in root tissues of WT and *35S-JAZ10G* seedlings. As seen in Figure 8, the *35S-JAZ10G* transgene was highly expressed in roots. Similar to results obtained with flowers, increased expression of the *35S-JAZ10G* transgene in roots did not significantly alter the ratio of *JAZ10* splice variants. However, the level of all *JAZ10* splice variants was several-fold higher in roots compared to flowers. The difference in *35S-JAZ10G* expression may explain the previous observation that *35S-JAZ10G* roots are strongly insensitive to JA, whereas *35S-JAZ10G* flowers do not exhibit JA-related reproductive phenotypes typically observed in strong JA-insensitive mutants (Chung et al., 2010).

JAZ2 and *JAZ6* transcripts were also quantified in root tissues (Figure 9). Similar to the results obtained for *JAZ10*, the full-length *JAZ2.1* and *JAZ6.1* transcripts were the predominantly expressed forms, with *JAZ2.2* and *JAZ6.2* accounting for less than 5% of the total transcript level. Interestingly, the levels of all *JAZ2* and *JAZ6* transcripts were greatly reduced in roots of *35S-JAZ10G* seedlings compared to WT seedlings. Consistent with the proposed role of *JAZ10* in negative regulation of JA responses (Yan et al., 2007; Chung and Howe, 2009; Sehr et al., 2010; Demianski et al., 2011), these data suggest increased expression of one or more *JAZ10* splice variants in roots represses the expression of *JAZ2* and *JAZ6*.

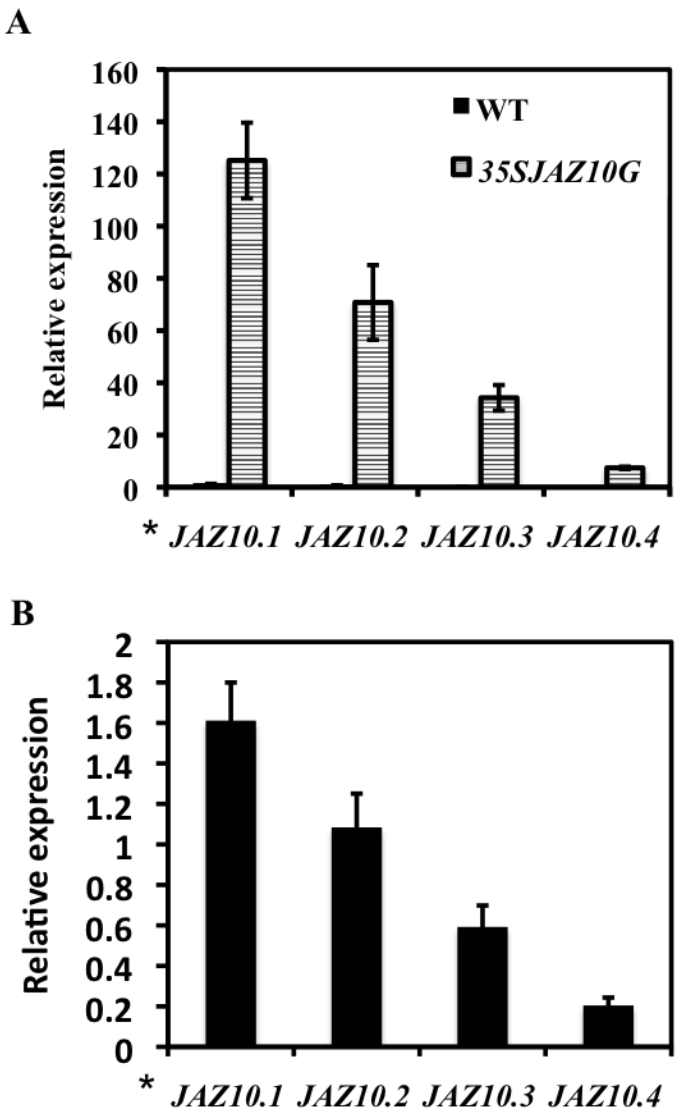

Figure 8. Quantification of *JAZ10* Alternatively Spliced Transcripts in WT and *35S-JAZ10G* Roots. (A) Wild-type (WT) and *35S-JAZ10G* seedlings were grown for nine days on MS plates (supplemented with 0.8% sucrose) in continuous light prior to RNA isolation from root tissue. Data points show the mean and standard error of three biological replicates. (B) Re-plotting of WT data shown in panel A, using a different scale.

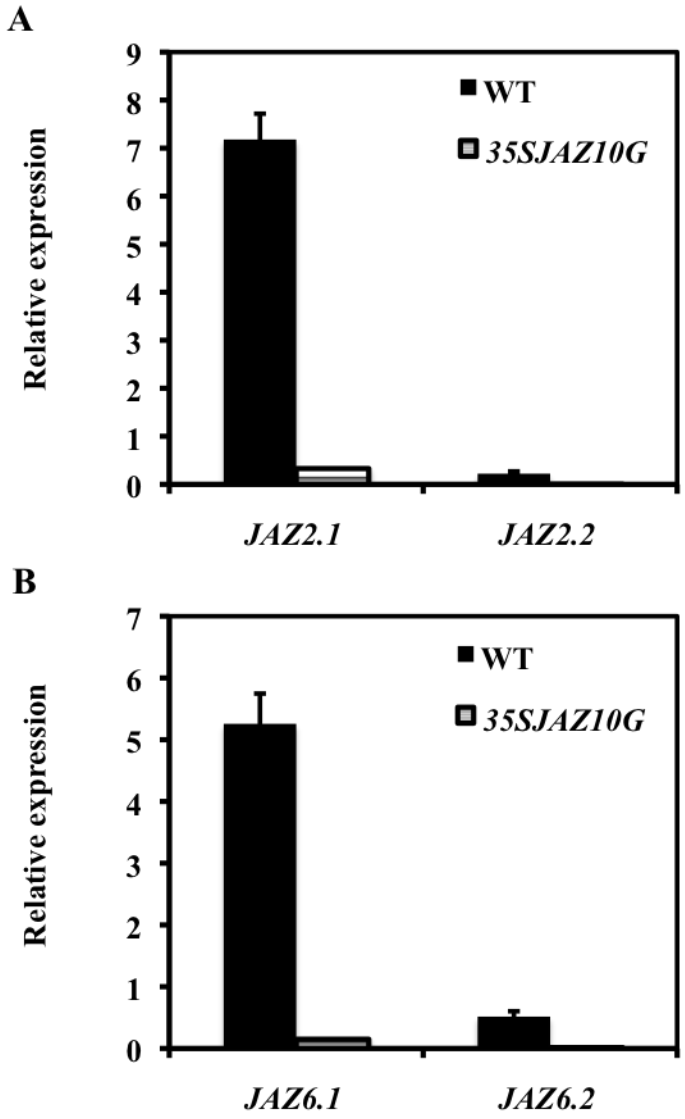

Figure 9. Quantification of *JAZ2* and *JAZ6* Alternatively Spliced Transcripts in WT and *35S-JAZ10*G Roots. The same RNA used for the experiment described in Figure 8 was analyzed by qPCR for the expression of alternatively spliced forms of *JAZ2* (A) and *JAZ6* (B). Data points show the mean and standard error of three biological replicates.

Detection of JAZ Alternative Splice Variants Using RNA Sequencing Data

Illumina sequencing was performed on cDNA samples from MeJA- and coronatine-treated seedlings, as well as from wounded leaves. Tophat was used to map reads to *Arabidopsis* TAIR 9 full-length cDNAs. Under all treatment conditions, *JAZ* genes were highly induced, as shown by the increase in the total number of reads for each gene, compared to sequencing of samples from control plants (Table 1A). In this experiment, coronatine treatment was generally the most effective in inducing *JAZ* gene expression. Some *JAZ* genes, including *JAZ6* and *JAZ12*, showed a relatively high basal level of expression in mock-treated samples, which is consistent with previous RNA blot analyses (Chung et al., 2008) (Table 1B).

To determine the relative expression levels of each *JAZ* splice variant, reads were aligned and normalized to a unique region for each splice variant. Using Illumina data, the number of reads per kilobase pair per million mapped reads of model (RPKM) was calculated. Only one biological replicate was available for each treatment condition, thus excluding the ability to perform statistical analysis for each treatment condition.

For *JAZ* genes in which Jas intron retention was predicted but were not annotated in TAIR 9 reference cDNAs, we created a new gene model in which the Jas intron was retained in an otherwise fully spliced transcript. In some cases, the number of unique reads for the full-length transcript could not be calculated because of the lack of a unique region distinct from alternative splice variants. For example, no unique reads align to *JAZ10.1* because it lacks a region that is distinct from *JAZ10.2, JAZ10.3* or *JAZ10.4* (Figure 10). In order to obtain the number of reads for *JAZ10.1,* the total reads were normalized to the entire cDNA, and unique reads for

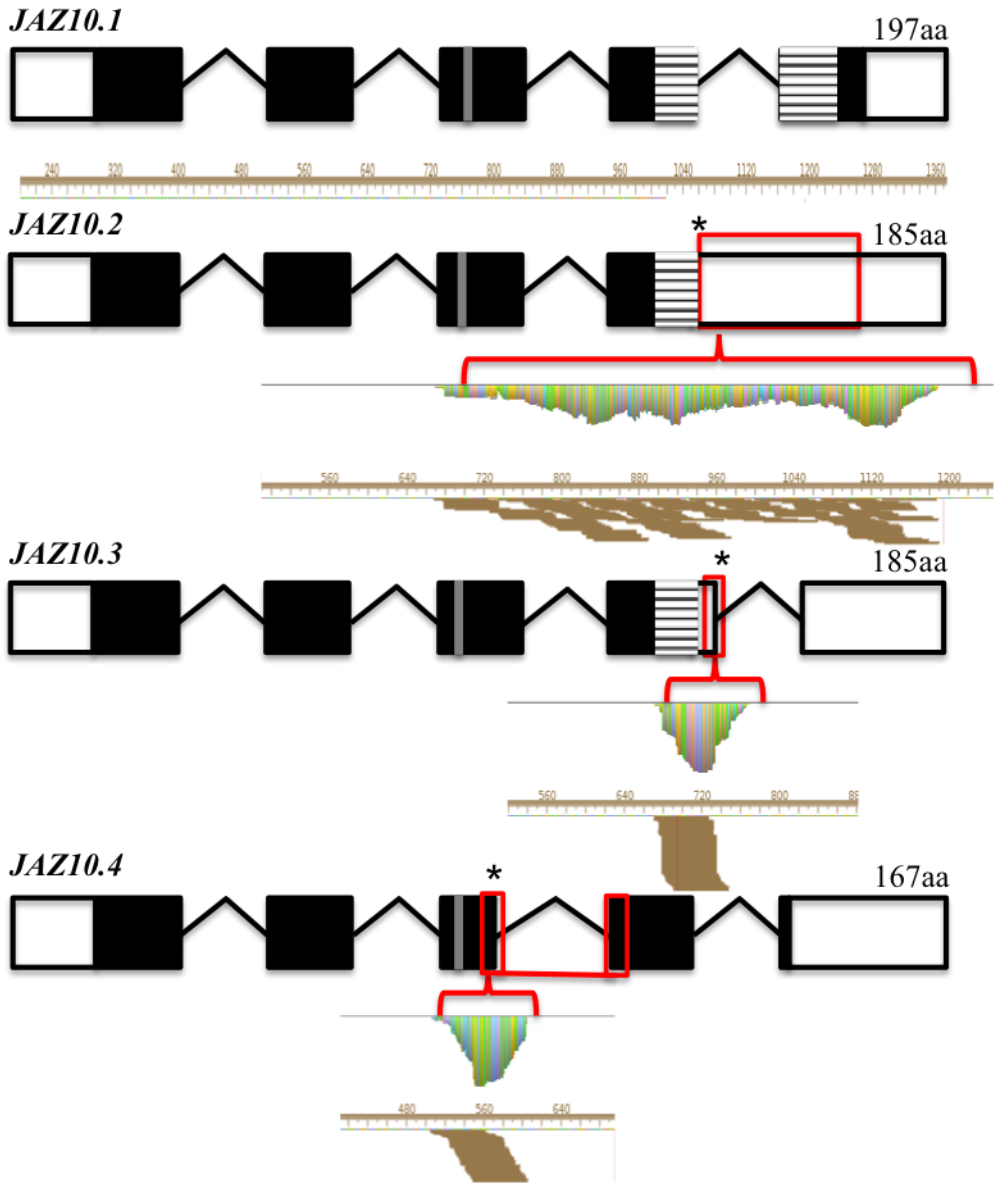

Figure 10. Evidence of *JAZ10* Alternatively Spliced Transcripts in RNA-seq Data. Gene models for alternative splicing events are supported by reads that align to unique regions of each *JAZ10* splice variant. No reads are available for *JAZ10.1* due to lack of sequence region that is specific for *JAZ10.1* Colored vertical bars indicate coverage over a unique region, whereas brown horizontal bars are reads that align to the unique region. Data are from Dr. Marco Herde. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Table 1. Evidence of *JAZ* Gene Induction and Jas Intron Retention in RNA-seq data. (A) *JAZ* genes are highly induced in response to JA elicitation compared to mock or unwounded samples. (B) Reads per kilobase pair per million mapped reads of model were calculated for gene models with alternative splicing events. Identification of alternative splicing events is supported by reads that align to unique regions for each splice variant. *JAZ9.1* and *JAZ10.1* were calculated from normalization of total reads to the size of the full-length cDNA and subtracting the unique reads from unique regions of other splice variants, and is outlined in dots. * indicates strong evidence for Jas intron retention.

A

AT Locus	Name	Mock	MeJA	Mock	COR	Unwounded	Wounding
AT1G19180.1	JAZ1	63	585	38	2136	49	2270
AT1G74950.1	JAZ2	0	19	0	71	5	111
AT3G17860.1	JAZ3	802	6355	840	24899	850	18591
AT1G48500.1	JAZ4	6	11	3	0	1	8
AT1G17380.1	JAZ5	1	9	1	0	0	0
AT1G72450.1	JAZ6	72	2322	38	6668	65	8068
AT2G34600.1	JAZ7	992	8248	1295	15626	1952	14434
AT1G30135.1	JAZ8	13	190	27	4016	7	2689
AT1G70700.1	JAZ9	173	4049	383	4498	419	7608
AT5G13220.1	JAZ10	12	299	7	577	3	486
AT3G43440.1	JAZ11	278	319	405	293	281	231
AT5G20900.1	JAZ12	1011	1887	1406	3070	1632	6161

B

	AT Locus	Name	Mock	MeJA	Mock	COR	Unwounded	Wounded
	AT1G74950.1	*JAZ2.1*	12.716	52.624	8.635	292.918	6.025	175.231
*	AT1G74950.2	*JAZ2.2*	0.694	7.478	0.654	37.227	0	24.749
	AT3G17860.2	*JAZ3.2*	0	0	0	0	0	0.356
	AT3G17860.3	*JAZ3.3*	0	0.704	0	0	0	1.392
	AT3G17860.4	*JAZ3.4*	0	0.000	0	0.341	0.168	1.680
	AT1G48500.1	*JAZ4.1*	1.774	1.939	0.836	0	0.274	0
	AT1G48500.2	*JAZ4.2*	0	0	0	0	0	0
	AT1G48500.3	*JAZ4.3*	0.115	0.968	0.108	0	0	0
	AT1G17380.1	*JAZ5.1*	1.774	36.006	0.279	113.495	0.822	127.590
*	AT1G17380.2	*JAZ5.2*	0	1.523	0	9.177	0	9.247
	AT1G72450.1	*JAZ6.1*	20.405	134.052	21.727	280.122	36.699	255.455
*	AT1G72450.2	*JAZ6.2*	1.099	10.581	0.920	19.014	1.753	18.262
	AT1G70700.1	*JAZ9.1*	25.023	535.639	52.977	491.865	59.417	865.103
	AT1G70700.2	*JAZ9.2*	0.887	6.647	0.334	2.337	0.164	1.479
*	AT1G70700.3	*JAZ9.3*	0.168	5.825	0.475	7.906	1.712	12.918
	AT5G13220.1	*JAZ10.1*	3.354	199.376	2.185	219.558	1.219	255.135
*	AT5G13220.2	*JAZ10.2*	0.305	8.636	0.173	23.265	0.113	17.358
*	AT5G13220.3	*JAZ10.3*	0.392	19.819	0.369	29.121	0	30.840
*	AT5G13220.4	*JAZ10.4*	0	17.172	0.557	24.479	0	17.249
	AT5G20900.1	*JAZ12.1*	16.265	27.143	22.005	45.343	29.304	100.210
	AT5G20900.2	*JAZ12.2*	0.311	0.291	0.293	0.975	0.096	3.072

Table 1. Evidence of *JAZ* Gene Induction and Jas Intron Retention in RNA-seq Data.

JAZ10.2, *JAZ10.3* and *JAZ10.4* were subtracted from this value. This approach was also used in the case of *JAZ9.1*, where a unique region distinct from *JAZ9.2* and *JAZ9.3* was not found.

Based on the number of unique reads found in the data set, *JAZ* gene models in which there was Jas intron retention were separated into those with "strong" or "weak" evidence (Table 1B). *JAZ* genes with strong evidence of producing alternative splice variants by Jas intron retention include: *JAZ2*, *JAZ5*, *JAZ6*, *JAZ9*, and *JAZ10*. In the case of *JAZ10*, *JAZ2* and *JAZ6*, Jas intron retention is also supported by qPCR results (Figures 4 and 5). Similar to qPCR results obtained for *JAZ10*, *JAZ2* and *JAZ6*, the full-length transcript is by far the most abundant form produced. *JAZ5.2* and *JAZ9.3* do not have a PTC close to the intron retention site, but are much farther into the Jas intron sequence than other *JAZ*s (Chung et al., 2010).

Jas intron retention is weakly supported for *JAZ3*, *JAZ4* and *JAZ12*. In these cases, it is difficult to determine whether Jas intron retention is occurring or not due to low expression of the gene and lack of replicates. For example, *JAZ4.2* was not supported in the data set, likely due to low expression levels.

Ectopic Expression of JAZ2.2 and JAZ4.2 Results in Decreased Sensitivity to JA

Seven of the 12 *JAZ* genes in *Arabidopsis* produce alternative splice variants in which the Jas domain is truncated as a result of Jas intron retention (Chung et al., 2010). Because of the presence of a PTC at the 5'-end of the Jas intron, these transcripts are predicted to encode proteins that lack the conserved X_5PY sequence that defines the C-terminal end of the Jas domain (Figure 11).

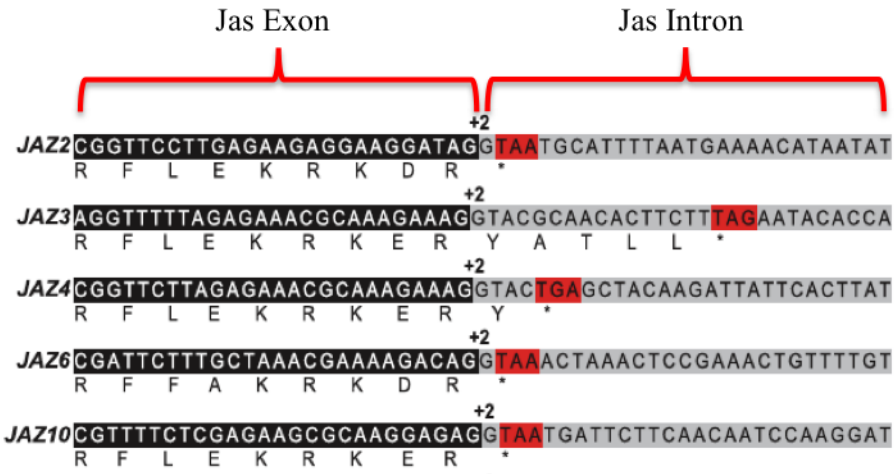

Figure 11. Intron/Exon Structure for Select *JAZ* Genes Containing the Jas Intron. Exon sequence is highlighted in black, Jas intron sequence is highlighted in grey, with predicted amino acid sequence. In-frame premature stop codons are highlighted in red. Modified from Chung et al., 2010. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Previous studies showed that one such alternative splice variant, *JAZ10.3*, functions as a dominant repressor of JA signaling when ectopically expressed in *Arabidopsis* (Yan et al., 2007; Chung and Howe, 2009). To determine whether other truncated ΔPY splice isoforms exert similar effects *in vivo*, transgenic plants expressing *35S-JAZ2.2* (Figure 12) were constructed and tested for altered sensitivity to JA-induced root growth inhibition. The results showed that *35S-JAZ2.2* plants have a JA-insensitive phenotype that is quantitatively similar to that of *35S-JAZ10.3* plants (Figure 12).

Ectopic expression of *JAZ* alternative splice variants containing a PTC at similar positions in the Jas intron as *JAZ10.3* may aid in understanding the functional physiological role of splicing in regulating JA responses. Besides *JAZ10* and *JAZ2*, other *JAZ* genes may produce splice variants with variable C-termini, depending on the location of the PTC in the retained Jas intron. The splice variant *JAZ4.2* retains a Jas intron that harbors a PTC one codon 3' of where it occurs for *JAZ10.3* and *JAZ2.2* (Figure 11) (Chung et al., 2010). Transgenic plants expressing the full-length *JAZ4.1* and splice variant *JAZ4.*2 from the 35S promoter were generated. *35S-JAZ4.1* seedlings were as sensitive to JA as WT plants in the root length assay (data not shown). Ten independent *35S-JAZ4.2* lines (T2 generation) that were segregating for the transgene were screened for JA insensitivity in the root growth assay; all lines segregated for individuals whose root length was significantly greater than that of JA-treated WT roots (Figure 13). These findings provide genetic evidence that *JAZ2.2*, like *JAZ10.3* and *JAZ4.2*, acts as a dominant repressor of JA signaling.

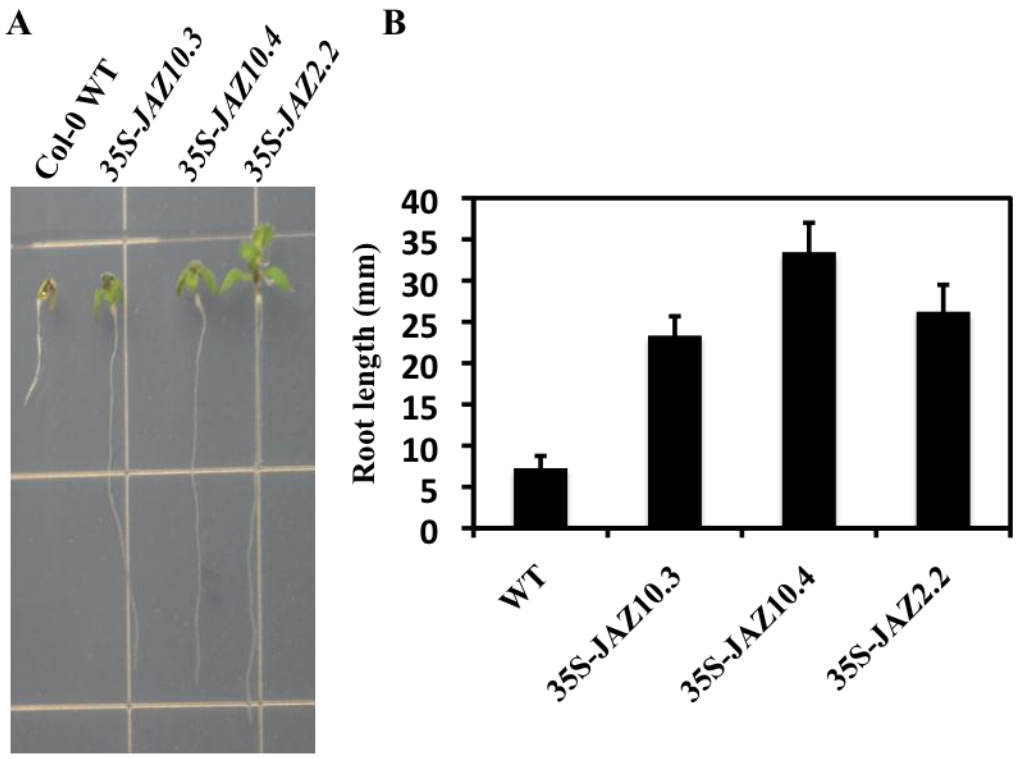

Figure 12. The MeJA-induced Root Growth Phenotype of *35S-JAZ2.2* Seedlings is Similar to that of *35S-JAZ10.3* Seedlings. Root length measurements were made with seedlings of the indicated genotype. (A) All seedlings were grown for nine days on MS medium containing 0.8% sucrose and 50 μm MeJA. (B) Data points represent the mean ± SD of the following number of seedlings per genotype: wild type (WT), n = 17; *35S-JAZ10.3*, n = 8; *35S-JAZ10.4*, n = 7; *35S-JAZ2.2*, n = 28. The mean root length of *35S-JAZ2.2* seedlings was most similar to that of *35S-JAZ10.3* seedlings, and was significantly different from WT ($P < 0.0001$), *35S-JAZ10.4* ($P < 0.0001$), and *35S-JAZ10.3* ($P < 0.025$) seedlings. Modified from Chung et al., 2010. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

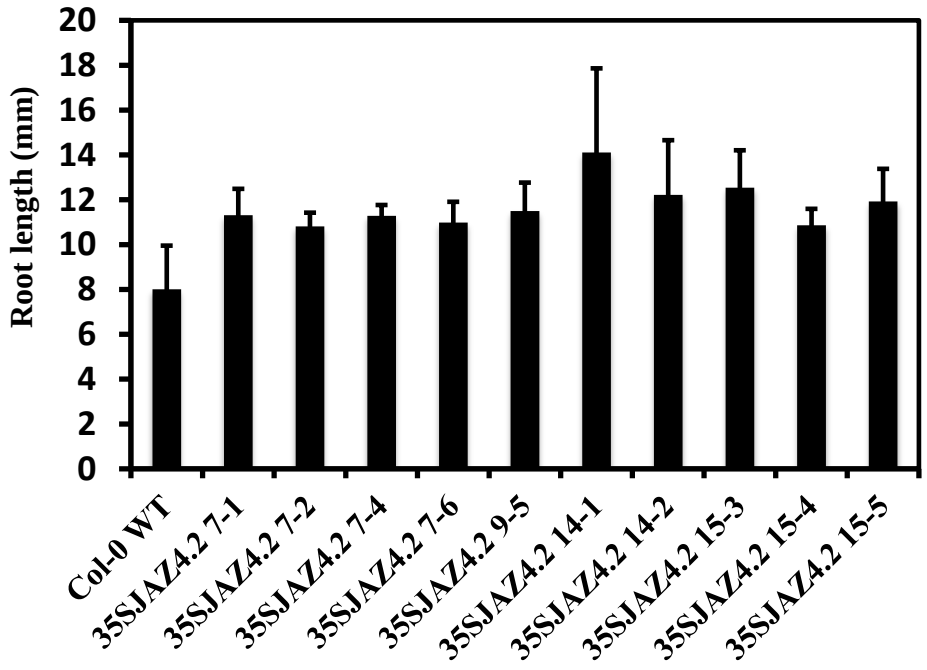

Figure 13. MeJA-Induced Root Growth Phenotype of *35S-JAZ4.2*. All seedlings were grown for nine days on MS media. Seedlings from independently transformed lines (T2 generation) grown in the presence of 20 μm MeJA. Root length measurements were made for each indicated genotype: wild type (WT), n = 34; (7-1), n=21; (7-2), n=11; (7-4), n=9; (7-6), n=16; (9-5), n=32; (14-1), n=22; (14-2), n=12; (15-3), n=22; (15-4), n=17; (15-5), n=24. The mean root length of all transgenic lines was statistically greater ($p<0.0005$) than WT.

DISCUSSION

The data presented here describe the analysis of alternative splicing of *JAZ* genes in specific tissues and in response to stress-related cues (JA treatment and wounding). Prior to these studies, there were two major hypotheses to explain how alternative splicing of *JAZ* genes is regulated. First, alternative splicing may occur constitutively through splice site competition. A second hypothesis is that alternative splicing is regulated by *trans*-acting factors, such as components within the spliceosome that alter splice-site selection in response to developmental or environmental cues. If the latter hypothesis is true and there is a change in splice site preference in JA stimulated cells, then the ratio of various alternative splice variants should change in response to JA treatment. The results of this research supports the first hypothesis, namely that the absolute level of all *JAZ* alternative splice variants increase in response to JA treatment and wounding. The abundance of all *JAZ* alternatively spliced transcripts increased upon MeJA treatment and wounding. For all *JAZ* genes analyzed, the full-length transcript was the most abundant form at all time points during the time course. On average, alternatively spliced *JAZ* transcripts represent approximately 3-10% of the level of the full-length transcript. In some cases (e.g., JAZ10.2), alternatively spliced transcripts accumulated to higher levels. Maintenance of full-length and splice variant ratios in mock and treated samples suggest that spliceosome machinery does not alter splice site preference upon JA treatment. Similar results were obtained for plants subject to mechanical wounding.

Wound- and JA-induced transcription of *JAZ* genes results in increased levels of *JAZ* pre-mRNA, which is alternatively spliced in a constitutive manner that does not cause major changes in the relative abundance of each transcript. It is likely that differences in splice acceptor sites are

responsible for determining the relative amount of each splice variant for a particular gene. Differences in the sequence of splice donor and acceptor sites may provide more or less competition for the spliceosome machinery, leading to a constant ratio of each alternative splice variant. The results do not exclude a role for altered mRNA stability in determining the accumulated level of each transcript. In summary, the data suggest that the relative abundance of splice variants for a particular *JAZ* gene is determined by the efficiency with which a particular splice site is selected by the spliceosome apparatus, and does not result from changes in stress-induced splice-site preference. In general, full-length splice variants are the predominantly expressed forms, which is consistent with previous studies on alternative splicing in plants (Ali and Reddy, 2008).

The qPCR results showed that *35S-JAZ10G* is expressed at much higher levels in roots than flowers. This observation may explain why this transgenic line shows JA insensitivity in roots but not in flowers, as determined by the absence of reproductive defects. Based on normalized expression to references genes, *JAZ10.1* and other *JAZ10* splice variants accumulated to much higher levels in roots as compared to flowers. Reduced expression of *35S-JAZ10G* in flowers compared to roots may be related to the developmental stage of the plants or the efficiency of transgene expression in these tissues. Consequently, expression levels of *35S-JAZ10G*, principally the production of *JAZ10.4*, in flowers may not be sufficient to cause male sterility. Expression of *JAZ10.1* in WT roots and flowers are similar, suggesting endogenous *JAZ10.1* levels are not regulated in a tissue-specific manner.

Stress responsive genes may be expressed in a tissue specific manner (Zhang and Gassmann, 2003; Ali and Reddy 2008). It was therefore of interest to compare the level of *JAZ* alternative splice variants in different tissues, including seedlings, leaves, roots and flowers. In

general, there was much higher expression of *JAZ10* and *JAZ2* in roots than in flowers, whereas *JAZ6* was expressed at similar levels in these tissues. Similar to the results obtained for analysis of basal *JAZ* expression in untreated seedlings and unwounded leaves, *JAZ* transcripts encoding full-length proteins were the predominant form. Variation in *JAZ* expression patterns was observed in different tissues. This could reflect variation in JA levels, the amount of stress perceived by different tissues, or disparity in transcriptional activity in different tissue types. For example, the expression of *JAZ6.1* was similar in roots, flowers, and unwounded leaves, but was much lower in liquid grown seedlings. As previously reported, the level of expression of each *JAZ* gene varies (Chung et al., 2008). Nonetheless, this data indicate that *JAZ* genes are alternatively spliced in the same manner that is independent of tissue type or level of transcriptional induction within a given tissue.

Many alternatively spliced *JAZ* transcripts, including *JAZ10.3*, *JAZ2.2*, *JAZ6.2* and *JAZ4.2*, are produced through retention of the Jas intron. The *JAZ* transcripts encode proteins lacking the C-terminal $X_5\Delta PY$ amino acids. In all tissues examined, *JAZ10*, *JAZ2* and *JAZ6* full-length transcripts are the predominant form and alternative splice variants are produced at low to undetectable levels. It is interesting to note that in all treatment conditions and tissues examined, *JAZ10.2* was the most abundant of these transcripts. Since *JAZ10.2* and *JAZ10.3* essentially produce the same protein, this distinction in transcript levels and abundance provides insight as to which splice variant is involved in the attenuation of JA responses. The ΔPY form of JAZ10 is also likely to be abundant because the protein products of *JAZ10.2* and *JAZ10.3* are identical. Retention of the entire Jas intron in *JAZ10.2* results in a long 3' UTR. It is unknown what role, if any, the 3' UTR may have in *JAZ* mRNA stability and translation. Microarray studies suggest that some *JAZ* transcripts are highly unstable, but it remains to be determined how transcript

instability impacts JAZ protein levels (Gutierrez et al., 2002). In summary, this data suggest ΔPY JAZ variants are relatively abundant in JA stimulated tissues, have increased stability in response to JA, and that these isoforms play an important role in attenuation of JA responses.

High throughput sequencing was used to determine whether *JAZ* genes produce alternative splice variants in response to various stress-related treatments. *JAZ* gene expression increases in response to MeJA treatment, COR treatment and wounding (Table 1A). Reads aligned to unique splice junctions for each splice variant of *JAZ10*, but the full-length transcript (*JAZ10.1*) was confirmed to be the predominant form in all conditions. Similarly, the RNA-seq data support the existence of *JAZ2* and *JAZ6* transcripts in which the Jas intron is retained (Table 1B). Interestingly, predictions of Jas intron retention in *JAZ5* and *JAZ9* splice variants are strongly supported by the Illumina data, which has not been previously reported. Confirmation of *JAZ5.2* and *JAZ9.3* splice variants by qPCR, together with ectopic expression of these variants in transgenic plants, may reveal their functional role in the regulation of JA signaling.

Some *JAZ* transcript models were weakly supported by the RNA-seq data, which may occur for several reasons. Jas intron retention was identified by mapping reads to all JAZ isoforms in TAIR9 and additional predicted retention events in Chung et. al., 2010. The inclusion of additional predicted splice variants as mapping templates to identify unique reads often prevented reads mapping to the full-length transcript due to sequence redundancy. Evidence of Jas intron retention for *JAZ3* and *JAZ12* were supported weakly by the RNA-seq data. Some TAIR9-supported gene models, such as *JAZ4.2*, also were not supported in this data set. In the case of *JAZ3*, this is likely attributed to the low expression of the gene. It is also important to note these data require replication. Comparison across treatments is difficult due to different developmental stages, tissue types and treatment conditions. Weakly supported gene models

maybe be further confirmed by sequencing additional replicates and may require validation by other methods such as qPCR.

The availability of a *jaz10-1* null mutant provides a useful tool for further analysis of the function of *JAZ10* alternative splice variants (Sehr et al., 2010; Demianski et al., 2011). It is currently unknown which *JAZ10* splice variants are involved in attenuation of JA responses. To address this question, transgenic plants expressing each *JAZ10* splice variant from the native *JAZ10* promoter may be used to complement the JA hypersensitive phenotype of *jaz10-1* plants. It would also be interesting to measure the level of JAZ proteins and their splice variants, and to determine the extent to which protein levels correlate with transcript levels.

Ectopic expression of alternative splice variants provides insight into the function of alternatively spliced JAZ proteins. The transgenic lines *35S-JAZ10.3*, *35S-JAZ10.4* and *35S-JAZ10G* were previously shown to exhibit JA insensitivity in roots and, in the case of *35S-JAZ10.4*, male sterility (Chung and Howe, 2009; Chung et al., 2010). In addition to *JAZ10*, *JAZ2.2* also showed JA insensitivity in root length assays (Chung et al., 2010). *35S-JAZ2.2* and *35S-JAZ10.3* confer similar levels of JA insensitivity in root length assays. Other overexpression constructs, such as *35S-JAZ3.4*, did not confer reduced sensitivity to JA in root length assays (Chung et al., 2010). Consistent with this finding, JAZ3.4 interacts with COI1 in a similar manner as full-length JAZ3.1 in yeast two hybrid assays and *in vitro* pull down assays (Chung et al., 2010). The JA responsiveness of *35S-JAZ4.1* seedlings was identical to that of WT, whereas *35S-JAZ4.2* lines showed a significant reduction in JA sensitivity. Validation of this conclusion will require further analysis of homozygous lines. Preliminary data suggest that *35S-JAZ4.2* seedlings are not as insensitive as seedlings that overexpress *JAZ10.3*, *JAZ10.4* or *JAZ2.2*.

Variation in JA insensitivity among transgenic lines that ectopically express different *JAZ* splice variants appears to correlate with differences in position of the PTC within the retained Jas intron (Figure 11). It is possible that the position of the PTC in the Jas intron affects the stability of the α-helix that is part of the JAZ degron, thereby altering interactions with COI1 (Sheard et al., 2010). It is possible that removal of the X_5PY motif through alternative splicing disrupts the integrity of the degron, thereby impeding efficient hormone-dependent COI1-JAZ binding. Analysis of other truncated JAZ proteins, such as *JAZ6.2*, may provide a means to test this hypothesis. Additional experiments in which the length of the C-terminus is systematically altered may also be used to test this hypothesis.

The phenomenon of alternative splicing as a mechanism to regulate stress responses may be widespread in plants (Ali and Reddy, 2008; Gassmann, 2008; Filichkin et al., 2010). In addition to *JAZ* genes, there may be numerous, functionally relevant alternative splicing events that are involved in biotic stress response. Understanding the role of alternative splicing in the regulation of JA signaling will provide valuable insight into how plants cope with biotic and abiotic stress.

AT LOCUS	NAME	PRIMER SEQUENCE
AT5G13220	*JAZ10.1* FP	5'-GAAGCGCAAGGAGAGATTAG-3'
	JAZ10.3 FP	5'-AAGGAGAGGTAATGATTCTTCAACAAT-3'
	JAZ10.1/10.3 RP	5'-AGCCAAATCCAAAAACGAACA-3'
	JAZ10.4 FP	5'-GCTAATGAAGCAGCATCTAAGAAAGA-3'
	JAZ10.4 RP	5'-GCGATGGGAAGATCGAAAGA-3'
	JAZ10.2 FP	5'-CCCCCAAATAATTAAAGAAAGGTTTTT-3'
	JAZ10.2 RP	5'-AAGCATGTGCGTTGTTGAACA-3'
AT1G74950	*JAZ2.1/2.2* FP	5'-CAAAAACCGCAGCACAAGAG-3'
	JAZ2.1 RP	5'-CCTTTGATGTGATCCTATCCTTCCT-3'
	JAZ2.2 RP	5'-CAAGATATTATGTTTTCATTAAAATGCATTAC-3'
AT1G72450	*JAZ6.1/6.2* FP	5'-CCGGGAACAATGAAGATCAAG-3'
	JAZ6.1 RP	5'-CCACAGCCCTGTCTTTTCGT-3'
	JAZ6.2 RP	5'-AGTTTCGGAGTTTAGTTTACCTGTCTTT-3'
AT1G13320	*PP2A* FP	5'-AAGCAGCGTAATCGGTAGG-3'
	PP2A RP	5'-GCACAGCAATCGGGTATAAAG-3'
AT5G08290	*YLS8* FP	5'-CTCTCAAGGACAAGCAGGAGTTCATT-3'
	YLS8 RP	5'-CGGTATTTGGTGGAGTAATCTTTTGG-3'

Table 2. qPCR Primers for the Quantification of *JAZ* Alternative Splice Variants. Primer pairs were designed across unique splice junctions for each *JAZ* alternative splice variant. In the case of *JAZ10.1* the primer pair detects two transcripts, *JAZ10.1* and *JAZ10.4*. Primer pairs were confirmed to have over 90% PCR efficiency.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis (ecotype Columbia-0; Col-0) was used as the wild type (WT) for all experiments. Seeds were surface sterilized with 40% (v/v) bleach for ten minutes, washed with water ten times, and stratified at 4^{o} C for three days. Seedlings were inoculated in baffled, 125-ml flasks containing 50 ml MS medium supplemented with 0.8% sucrose. Flasks were incubated with mild shaking (200 rpm) under long-day conditions (16 h light/8 h dark) at 21^{o} C and 100 μE m^{-2} s^{-1} of light. For plants grown on soil, seeds were surface sterilized and stratified as described above prior to sowing on autoclaved soil. *35S-JAZ10G*, *35SJAZ2.2*, *35S-JAZ4.2* transgenic lines were previously described in Chung et al., 2010.

Plant treatments

Liquid-grown seedlings were treated with either 100 μm MeJA (Sigma-Aldrich) or a mock control (0.007% ethanol) as described in the legend of Figure 4. For experiments involving wounding, leaves of similar size on 25-day-old soil-grown plants were mechanically wounded twice along the leaf midvein with a hemostat. Wounded and unwounded control leaves harvested, and pooled from two plants per biological replicate. Primary and secondary inflorescences were harvested from soil grown WT and *35S-JAZ10G* plants. Flowers at various developmental stages (stages 1-12 as defined by Smyth et al., 1990) prior to bud opening were pooled from four, seven-week old plants. Root tissue was collected from vertically growing

seedlings on MS agar (% 0.8 agar) plates for nine days in continuous light. Autoclaved nylon mesh (Sefar America Inc.) was placed on top of the agar prior to sowing seeds. Prior to harvesting, a razor blade was used to excise the roots below the hypocotyl. Approximately 100 mg of tissue was collected and frozen in liquid nitrogen in fast-prep tubes (MP biomedical) containing metal balls.

RNA extraction and cDNA synthesis

Prior to RNA extraction, tissue was homogenized in frozen blocks using the Qiagen TissueLyser® II at a frequency of 25 s^{-1} for 1.5 min. RNA was extracted with the RNeasy® Plant Mini RNA kit (Qiagen) according to manufacturer's protocol. To ensure removal of genomic DNA, RNA was treated with RNase Free DNase (Qiagen) for 15 min prior to elution with diethlypyrocarbonate treated, RNase-free water. RNA was quantified with a Nanodrop spectrophotometer. Assessment of RNA quality was done by determining the A260/A280 ratio, and only those samples with a ratio of 1.8 or higher were used. RNA samples for submission to Illumina sequencing were also analyzed for integrity on a microfluidics chip (Agilent Bioanalyzer). Only those RNA samples with and RNA integrity number higher than seven were used for Illumina sequencing (Bustin et al., 2009).

Approximately 100 ng of RNA was used as a template for cDNA synthesis using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). cDNA was diluted 1:10 for qPCR assays. In the case RNA isolated form roots, 500 ng of RNA was used at a 1:5 dilution. Linearity of the reverse transcriptase (RT) reaction was tested using serial dilutions of RNA for cDNA synthesis and showed no inhibition of the RT reaction with R^2 values above 90%. Primer

efficiency was calculated by testing serial dilutions of cDNA concentrations and performing qPCR and showed primers were efficient in amplifying transcripts with R^2 values above 90%.

Quantitative real-time polymerase chain reaction (qPCR)

Primer pairs were optimized for Power SYBR Green (Applied Biosystems®) using the PrimerExpress® software provided by Applied Biosystems®. All primer sets were checked for specificity against the *Arabidopsis* genome using BLAST. For each primer set, dissociation curves were generated to confirm the presence of a single product. Primers were designed to unique splice-site junctions to ensure specificity. In the case of *JAZ10.1*, it was not possible to identify a unique region that is not shared by the other splice variants. To resolve this problem, a primer set was designed to detect both *JAZ10.1* and *JAZ10.4*.

qPCR was performed using SYBR Green chemistry and the 7500 Fast Real-Time PCR (Applied Biosystems) system with default settings. Ct values were determined using default auto Ct settings. In the case of *JAZ10.1*, values shown are a sum of *JAZ10.1* and *JAZ10.4*. *JAZ* transcript levels were normalized to the reference genes *PP2A* (AT1G13320.1) and *YLS8* (AT5G08290), using the geometric mean of the two reference genes and the ΔCt method (Czechowski et al., 2005; Livak and Schmittgen, 2001). The expression level of these reference genes did not change in response to JA treatment or in different tissues. Unless otherwise indicated, error bars indicate standard error of at least three technical and biological replicates.

Illumina sequencing

Six RNA samples were analyzed by Illumina sequencing. One set of samples was obtained from nine-day-old seedlings treated with 100 μm MeJA or a mock control (0.007% ethanol) for two hours. The second set of RNA samples was obtained from -day-old seedlings treated with 5 μm coronatine (vendor) or a mock control (0.007% ethanol) for one hour. The third set of samples was obtained from control (unwounded) or wounded leaves, which were harvested one hour after mechanical wounding (see above). RNA was extracted as described above. All samples were sequenced (55 nt paired-end reads) on the Illumina Genome Analyzer II. The program Tophat was used to map unique, forward reads to the TAIR9 cDNAs of *Arabidopsis*.

Transgenic plants

Col-0 plants were grown on soil for 4 to 6 weeks until flowering. Plants were transformed with *Agrobacterium tumefaciens* strain C58C1 as previously described (Clough and Bent, 1998). Transformed T1 seeds were screened for resistance to kanamycin (50 μg/ml) and vancomycin (100 μg/ml) on MS agar plates. T2 seedlings were screened for altered sensitivity to JA in the root growth assays

Root length assays

Seeds of each genotype were surface sterilized with 40% (v/v) bleach for 10 min, washed ten times with autoclaved water, and then stratified for three days at 4^{o} C. Seeds were sown on MS media supplemented with 0.8% sucrose with or without 50 μM MeJA. Seedlings were grown vertically for nine days in continuous light. Photographs of roots were taken with a Nikon D80 DSLR camera, and root lengths were measured with ImageJ software.

REFERENCES

REFERENCES

Acosta IF, Farmer EE (2010) Jasmonates. The Arabidopsis Book. Rockville, MD: American Society of Plant Biologists. http://www.bioone.org/doi/pdf/10.1199/tab.0129

Ali GS, Reddy ASN (2008) Regulation of alternative splicing of pre-mRNAs by stresses. Curr Topics Microbiol Immunol **326:** 257-275

Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, Ausubel FM, Ecker JR (2003) Five components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in *Arabidopsis*. Proc Natl Acad Sci USA **100:** 2992–2997

Boter M, Ruiz-Rivero O, Abdeen A, Prat S (2004) Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. Genes and Dev **18:** 1577-1591

Brooks DM, Hernandez-Guzman G, Kloek AP, Alarcon-Chaldez F, Sreedharan A, Rangaswamy V, Penaloza-Vazquez A, Bender CL, Kunkel BN (2004) Identification and characterization of a well-defined series of coronatine biosynthetic mutants of *Pseudomonas syringae* pv. *tomato* DC3000. MPMI **17:** 162-174

Browse J, Howe GA (2008) New weapons and a rapid response against insect attack. Plant Physiol. **146**: 832–838

Browse J (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. Annu Rev Plant Biol **60**: 183-205

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chem **55:** 611-622

Chen Q, Sun J, Zhai Q, Zhou W, Qi L, Wang B, Chen R, Jiang H, Qi J, Li X, Palme K, Li C (2011) The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in *Arabidopsis*. Plant Cell doi/10.1105/tpc.111.089870

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

Chini A, Fonseca S, Chico J, Fernandez-Calvo P, Solano R (2009) The ZIM domain mediates homo- and heteromeric interactions between *Arabidopsis* JAZ proteins. Plant J **59:** 77-87

Chory J, Chatterjee M, Cook RK, Elich T, Fankhauser C, Li J, Nagpal P, Neff M, Pepper A, Poole D, Reed J, Vitart, V (1996) From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc Natl Acad Sci USA **93:** 12066–12071

Chung HS, Koo AJ, Gao X, Jayanty S, Thines B, Jones AD, Howe GA (2008) Regulation and function of *Arabidopsis* JASMONATE ZIM-domain genes in response to wounding and herbivory. Plant Physiol **146:** 952–64

Chung HS, Howe GA (2009) A critical role for the TIFY motif in repression of jasmonate signaling by a stabilized splice variant of the JASMONATE ZIM-domain protein JAZ10 in *Arabidopsis*. Plant Cell **21:** 131-145

Chung HS, Cooke TF, DePew CL, Patel LC, Ogawa N, Kobayashi Y, Howe GA (2010) Alternative splicing expands the repertoire of dominant JAZ repressors of jasmonates signaling. Plant J **63:** 613-622

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J **16:** 735-743

Conconi A, Smerdon MJ, Howe GA, Ryan CA (1996) The octadecanoid signalling pathway in plants mediates a response to UV radiation. Nature **383:** 826–29

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Phys and Plant Mol Biol **48:** 355–81

Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR (2005) Genome-wide identification of testing of superior reference genes for transcript normalization in *Arabidopsis*. Plant Physiol **139:** 5-17

Demianski AJ, Chung KM, Kunkel BN (2011) Analysis of *Arabidopsis JAZ* gene expression during *Pseudomonas syringae* pathogenesis. Mol Plant Pathol **13:** 46-57

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

Dong X (1998) SA, JA, ethylene, and disease resistance in plants. Curr Opin Plant Biol **1:** 316-323

Fernandez-Calvo P, Chini A, Fernandez-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, Pauwels L, Witters E, Puga MI, Paz-Ares J, Goossens A, Reymond P, De Jaeger G, Solano R (2011) The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant

Cell doi/10.1105/tpc.110.080788

Feys B, Benedetti CE, Penfold CN, Turner JG (1994) *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell **6:** 751–759

Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, Wong WK, Mockler TC (2010) Genome-wide mapping of alternative splicing in *Arabidopsis thaliana*. Genome Res **20:** 45-58

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 jasmonates. Nat Chem Bio **5:** 344-350

Gan X, Stegle O, Behr J, Steffen JG, Drewe P, Hildebrand KL, Lyngsoe R, Schultheiss SJ, Osborne EJ, Sreedharan VT, Kahles A, Bohnert R, Jean G, Derwent P, Kersey P, Belfield EJ, Harberd NP, Kemen E, Toomajian C, Kover PX, Clark RM, Ratsch G, Mott R (2011) Multiple reference genomes and transcriptomes for *Arabidopsis thaliana*. Nature **477:** 419-423

Garcia ME, Lynch T, Peeters J, Snowden C, Finkelstein R (2008) A small plant specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. Plant Mol Biol **67:** 643-658

Gassmann W (2008) Alternative splicing in plant defense. Curr Topics Microbiol and Immunol **326:** 219-232

Geerinck J, Paulwels L, De Jaeger G, Goosens A (2010) Dissection of the one-MegaDalton JAZ1 protein complex. Plant Signal Behav **5:** 1-3

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol **43:** 205–227

Gonzales-Vigil E, Bianchetti CM, Philips GN, Howe GA (2011) Adaptive evolution of threonine deaminase in plant defense against insect herbivores. Proc Natl Acad Sci USA **108:** 5897-5902

Grabowski PJ, Black DL (2001) Alternative RNA splicing in the nervous system. Prog Neurobiol **65:** 289-308

Grunewald W, Vanholme B, Pauwels L, Plovie E, Inze D, Gheysen G, Goossens A (2009) Expression of the *Arabidopsis* jasmonate signaling repressor JAZ1/TIFY10A is stimulated by auxin. EMBO Rep **10:** 923-928

Gutierrez RA, Ewing RM, Cherry M, Green PJ (2002) Identification of unstable transcripts in *Arabidopsis* by cDNA microarray analysis: rapid decay is associated with a group of

touch- and specific clock-controlled genes. Proc Natl Acad Sci USA **99:** 11513-11518

Hause B, Stenzel I, Miersch O, Maucher H, Kramell R, Ziegler J, Wasternack C (2000) Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. Plant J **24**: 113-126

Herde M, Gartner K, Kollner TG, Fode B, Boland W, Gershenzon J, Gatz C, Tholl D (2008) Identification and regulation of TPS04/GES, an *Arabidopsis* geranyllinalool synthase catalyzing the first step in the formation of the insect-induced volatile C_{16}-homoterpene TMTT. Plant Cell **20:** 1152-1168

Hoffmann M, Hentrich M, Pollmann S (2011) Auxin-oxylipin crosstalk: relationship of antagonists. J Intergr Plant Biol **53:** 429-445

Hou X, Lee L, Xia K, Yan Y, Yu H (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell **19:** 884-894

Howe GA (2010) Ubiquitin ligase-coupled receptors extend their reach to jasmonate. Plant Physiol **154:** 471-474

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol **59:** 41–66

Hsieh, HL, Okamoto H, Wang M, Ang LH, Matsui M, Goodman H, Deng XW (2000) FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of *Arabidopsis* development. Genes Dev **14:** 1958–1970

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The *DEFECTIVE IN ANTHER DEHISCENCE1* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. Plant Cell **13:** 2191-2209

Kagale S, Links MG, Rozwadowski K (2010) Genome-wide analysis of ethylene-responsive element binding factor-associated amphiphilic repression motif-containing transcriptional regulators in *Arabidopsis*. Plant Physiol **152:** 1109-1134

Kang JH, Wang L, Giri A, Baldwin IT (2006) Silencing threonine deaminase and JAR4 in *Nicotiana attenuata* impairs jasmonic-acid-isoleucine-mediated defenses against *Manduca sexta*. Plant Cell **18:** 3303-3320

Katsir L, Chung HS, Koo AJK, Howe GA (2008a) Jasmonate signaling: a conserved mechanism of hormone sensing. Curr Opin Plant Biol **11:** 428–435

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA (2008b) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc

Natl Acad Sci USA **105:** 7100–7105

Kazan K (2003) Alternative splicing and proteome diversity in plants: the tip of the iceberg has just emerged. Trends in Plant Sci **8:** 468-471

Koo AJ, Chung HS, Kobayashi Y, Howe GA (2006) Identification of a peroxisomal acyl-activating enzyme involved in the biosynthesis of jasmonic acid in *Arabidopsis*. J Biol Chem **281:** 33511-33520

Koo AJ, Howe GA (2007) Role of peroxisomal β-oxidation in the production of plant signaling compounds. Plant Signal Behav **2:** 20-22

Koo AJK, Gao X, Jones AD, Howe GA (2009) A rapid wound signal activates systemic synthesis of bioactive jasmonates in *Arabidopsis*. Plant J **59:** 974-986

Koo AJK, Cooke TF, Howe GA (2011) Cytochrome P450 CYP94B3 mediates catabolism and inactivation of the plant hormone jasmonoyl-L-isoleucine. Proc Natl Acad Sci USA **108:** 9298-9303

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonates-signaled defense responses, and glandular trichome development. Plant Cell **16:** 126-143

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ method. Methods **25:** 402-408

Long JA, Ohno C, Smith ZR, Meyerowitz EM (2006) TOPLESS regulates apical embryonic fate in *Arabidopsis*. Science **312:** 1520-1523

Lorenzo O, Chico JM, Sánchez-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:** 1938–1950

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell **15:** 165-178

Mandaokar A, Thines B, Shin B, Lange BM, Choi G, Koo YJ, Yoo YJ, Choi YD, Choi G, Browse J (2006) Transcriptional regulators of stamen development in *Arabidopsis* identified by transcriptional profiling. Plant J **46:** 984-1008

McConn M, Browse J (1996) The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. Plant Cell **8:** 403–16

Moreno JE, Tao Y, Chory J, Ballare CL (2009) Ecological modulation of plant defense via

phytochrome control of jasmonate sensitivity. Proc Natl Acad Sci USA **106:** 4935–4940

Niu Y, Figueroa P, Browse J (2011) Characterization of JAZ-interacting bHLH transcription factors that regulate jasmonates responses in *Arabidopsis*. J Exp Bot **62:** 2143–2154

Park J, Halitschke R, Kin H, Baldwin I, Feldmann K, Feyereisen R (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J **31:** 1-12

Pauwels L, Goossens A (2011) The JAZ proteins: a crucial interface in the jasmonate signaling cascade. The Plant Cell Review doi/10.1105/tpc.111.089300

Pauwels L, Barbero GF, Geerinck J, Tilleman S, Grunewald W, Perez AC, Chico JM, Bossche RV, Sewell J, Gil E, Garcia-Casado G, Witters E, Inze D, Long JA, De Jaeger G, Solano R (2010) NINJA connects the co-repressor TOPLESS to jasmonate signaling. Nature **46:** 788-793

Rao MV, Lee H, Creelman RA, Mullet JE, Davis KR (2000) Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. Plant Cell **12:** 1633-1646

Reddy ASN (2007) Alternative splicing of pre-messenger RNAs in plants in the genomic era. Annu Rev Plant Biol **58:** 267–294

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell **12:** 707-720

Robson F, Okamoto H, Patrick E, Harris SR, Wasternack C, Brearley C, Turner JG (2010) Jasmonate and Phytochrome A signaling in *Arabidopsis* wound and shade responses are integrated through JAZ1 stability. Plant Cell **22:** 1143-1160

Ryan CA (1990) Protease inhibitors in plants: genes for improving defenses against insects and pathogens. Annu Rev Phytopathol **28:** 425–49

Saracco SA, Hansson M, Scalf M, Walker JM, Smith LM, Vierstra RD (2009) Tandem affinity purification and mass spectrometric analysis of ubiquitylated proteins in *Arabidopsis*. Plant J **59:** 344-358

Schilmiller AL, Koo AJK, Howe GA (2007) Functional diversification of Acyl-Coenzyme A Oxidases in jasmonic acid biosynthesis and action **143:** 812-824

Schaller A, Stintzi A (2009) Enzymes in jasmonate biosynthesis-structure, function, regulation. Phytochemistry **70:** 1532–1538

Sehr EM, Agusti J, Lehner R, Farmer EE, Schwarz M, Greb T (2010) Analysis of secondary growth in the *Arabidopsis* shoot reveals a positive role of jasmonates signalling in

cambium formation. Plant J **63:** 811-822

Seo HK, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Seob J, Do Choi Y (2001) Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonates-regulated plant responses. Proc Natl Acad Sci USA **98:** 4788-4793

Sharrock, RA, Quail PH (1989) Novel phytochromes sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev **3:** 1745-1757

Sheard LB, Tan X, Mao H, Withers J, Nissan GB, Hinds TR, Hsu FF, Sharon M, Browse J, He SY, Rizo-Rey J, Howe GA, Zheng N (2010) Mechanism of jasmonates recognition by an inositol phosphate-potentiated COI1-JAZ co-receptor. Nature **468**: 400-405

Shikata M, Matsuda Y, Ando K, Nishii A, Takemura M, Yokota A, Kohchi T (2004) Characterization of *Arabidopsis ZIM*, a member of a novel plant-specific GATA factor gene family. J Exp Bot **55:** 631–39

Song S, Qi T, Huang H, Ren Q, Wu D, Chang C, Peng W, Liu Y, Peng J, Xie D (2011) The jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect jasmonates-regulated stamen development in *Arabidopsis*. Plant Cell **23:** 1000-1013.

Staswick PE, Yuen GY, Lehman CC (1998) Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. Plant J **15:** 747-754

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

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

Stitz M, Gase K, Baldwin IT, Gaquerel E (2011) Ectopic expression of AtJMT in *Nicotiana attenuata*: creating a metabolic sink has tissue-specific consequences for the jasmonate metabolic network and silences downstream gene expression. Plant Physiol **157:** 341-354

Szemenyei H, Hannon M, Long JA (2008) TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. Science **319:** 1384-1386

Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature **446:** 640-645

Theodoulou FL, Job K, Slocombe SP, Footitt S, Holdsworth M, Baker A, Larson TR,

Graham IA (2005) Jasmonic acid levels are reduced in COMATOSE ATP-binding cassette transporter mutants. Implications for transport of jasmonate precursors into peroxisomes. Plant Physiol **137:** 835-840

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. Nature **448:** 661–665

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell **14:** S153-S164

Ueguchi-Tanaka M, Nakajima M, Katoh E, Ohmiya H, Asano K, Saji S, Hongyu X, Ashikari M, Kitano H, Yamaguchi I, Matsuoka M (2007) Molecular interactions of a soluble gibberellin receptor GID1, with a rice DELLA protein, SLR1, and gibberellin. Plant Cell **19:** 2140-2155

Vanholme B, Grunewald W, Bateman A, Kohchi T, Gheysen G (2007) The tify family previously known as ZIM. Trends Plant Sci **12:** 239-244

Wittstock U and Burow M (2010) Glucosinolate breakdown in *Arabidopsis*: mechanism, regulation and biological significance. The *Arabidopsis* Book 8: e0134. DOI: 10.1199/tab.0134

Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) *COI1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science **280:** 1091–1094

Xu L, Liu F, Lechner E, Genschik P, Crosby WL, 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:** 1919–1935

Yadav V, Mallappa C, Gangappa SN, Bhatia S, Chattopadhyay S (2005) A basic helix-loop-helix transcription factor in *Arabidopsis*, MYC2, acts as a repressor of blue light mediated photomorphogenic growth. Plant Cell **17:** 1953-1966

Yan Y, Stolz S, Chételat A, Reymond P, Pagni M, Dubugnon L, Farmer EE (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell **19:** 2470–2483

Yang J, Lin R, Sullivan J Hoecker U, Liu B, Xu L, Deng XW, Wang H (2005) Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in *Arabidopsis*. Plant Cell **17:** 804-821

Yoshida Y, Sano R, Wada T, Takabayashi J, Okada K (2009) Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in *Arabidopsis*. Devel **136:** 1039-1048

Zhang XC, Gassmann W (2003) *RPS4*-mediated disease resistance requires the combined presence of *RPS4* transcripts with full-length and truncated open reading frames. Plant

Cell **15:** 2333-2342

Zhu Z, An F, Feng Y, Li P, Xue L, A M, Jiang Z, Kim JM, To TK, Li W, Zhang X, Yu Q, Dong Z, Chen WQ, Seki M, Zhou JM, Guo H (2011) Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonates and ethylene signaling synergy in *Arabidopsis*. Proc Natl Acad Sci USA **108:** 12539-12544