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Chapter 16

Jasmonate Biosynthesis, Perception and Function in Plant Development and Stress Responses

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1. Introduction

The oxidation products of unsaturated fatty acids are collectively known as oxylipins. These compounds represent a highly diverse group of substances that are involved in a number of developmental processes and various stress responses in plants (Andersson et al., 2006). Plant oxylipins can be formed enzymatically, by initial oxidation by lipoxygenases (LOXs) or α-dioxygenases (α-DOXs); however, non-enzymatic autoxidation of polyunsaturated fatty acids (PUFA) also contribute to oxylipin formation in plant (Göbel and Feussner, 2009). An array of these substances are known to exert protective activities either as signaling molecules in plants during development, wounding, and insect and pathogen attack, or direct anti-microbial substance that are toxic to the invader. Despite the recent progress in deciphering the function of some oxylipins, the role of the vast majority of plant oxylipins remains unclear. Particularly well studied examples of the plant oxylipins are jasmonates (JAs) including jasmonic acid (JA) and its derivatives such as methyl jasmonate (MeJA), *cis*-jasmone, jasmonoyl isoleucine (JA-Ile), jasmonoyl ACC (JA-ACC) and several other metabolites. Another important group of plant oxylipins is green leaf volatiles (GLV). Increasing evidence supports GLVs function in defense responses against herbivore. GLVs are C6 aldehydes, alcohols, and their esters formed through the hydroperoxide lyase (HPL) pathway downstream of LOXs. GLV can further trigger local and systemic volatile organic compounds (VOC) emissions upon insect feeding (Farag and Paré, 2002). A large number of VOC including monoterpenes, sesquiterpenes and carotenoid-type compounds can be biosynthesized in plants from the shikimic, lipidic and terpenic pathways (Fons et al., 2010). Most VOCs are not products of the LOX pathway but similar to LOX derivatives serve as signals for insects to choose a suitable host or to lay eggs (Müller and Hilker, 2001). The third better studied group of plant oxylipins is phytoprostanes, a category of non-

enzymatically formed oxylipins, which play overlapped roles with OPDA in plant stress responses (Eckardt, 2008).

JA biosynthesis and signaling pathways have been extensively investigated in dicotyledonous plants such as *Arabidopsis*, tobacco and tomato. In monocotyledonous species, only a scant number of JA biosynthetic enzymes have been described (Tani et al., 2008; Yan et al., 2012). Jasmonates are formed from the LOX-catalyzed peroxidation of trienoic fatty acids at carbon atom 13 to form 13-hydroperoxide, which is modified to an allene oxide fatty acid and subsequently cyclized to the compound 12-oxo-phytodienoic acid (OPDA). Jasmonic acid (JA) is synthesized from OPDA by the reduction of a double bond and three consecutive rounds of β-oxidation. The pathway can accept C_{18}-PUFA (linolenic acid) as well as C_{16}-PUFA (hexadecatrienoic acid), in the latter case the intermediate is the so-called dinor-OPDA that may also be metabolized to JA. JA can be further enzymatically converted into numerous derivatives or conjugates, some of which have well-described biological activity such as free JA, MeJA, *cis*-jasmone and JA–Ile.

JA signaling pathway, the transition process of JA-Ile as a chemical signal to biological signal, was elucidated in recent years. JA initiates signaling process upon formation of a SCF^{COI1}-JA-Ile-JAZ ternary complex (JAZ: jasmonate ZIM-domain protein; Sheard et al., 2010), in which the JAZ repressors are ubiquitinated and subsequently degraded to release transcription factors, e.g., MYC2, causing downstream transcription activation of defense responses or developmental regulation (Chini et al., 2007; Thines et al., 2007). The only jasmonate receptor identified to date has been the COI1 protein (Katsir et al., 2008; Yan et al., 2009), but interestingly, only JA-Ile was found as a ligand of the SCF^{COI1} E3 ubiquitin ligase complex (Thines et al., 2007).

Since discovered in the 1960s as secondary metabolites from the oils of jasmine flowers (Demole *et al.*, 1962), the biological roles of JA have received increased attention of researchers in the past decades. Jasmonates have gradually become realized as a defense and fertility hormone, and as such modulate numerable processes relating to development and stress responses. In *Arabidopsis* and tomato, JAs are directly involved in stamen and trichome development, vegetative growth, cell cycle regulation, senescence, anthocyanin biosynthesis regulation, and responses to various biotic and abiotic stresses (Creelman and Mullet, 1997; Wasternack, 2007; Howe and Jander, 2008; Browse, 2009; Avanci et al., 2010; Pauwels and Goossens, 2011). In monocots, much less is known about the role of JAs, however, it has been shown they are required for sex determination, reproductive bud initiation and elongation, leaf senescence, pigmentation of tissues and responses to the attack by pathogens and insects (Engelberth et al., 2004; Tani et al., 2008; Acosta et al., 2009; Yan et al, 2012).

In plants, the JA signal acts co-operatively with other plant hormones. A number of studies have already attracted attention to plant hormone cross-talk as it relates to defense responses. In *Arabidopsis,* JA was shown to interact synergistically with ethylene (Xu et al 1994), and, depending on particular stress, both synergistically and antagonistically with salicylic acid (Beckers and Spoel, 2006) and abscisic acid (ABA) (Anderson et al 2004) in

plant-pathogen or -insect interactions. Gibberellins (GA) interact with JA to control flower fertility in *Arabidopsis*. In maize, JA positively regulates ABA and ET biosynthesis in senescing leaves (Yan et al., 2012). In summary, it is clear that JA signaling exert its functions via interaction with multiple plant hormones; however the crossroads of these interactions still remain to be explored.

2. JA biosynthesis pathway and regulation

2.1. The scheme of JA biosynthesis pathway

In 1962, a floral scent compound, the methyl ester of jasmonic acid (MeJA) was isolated for the first time from the aromatic oil of *Jasminum grandiflorum* (Demole et al., 1962). However, the physiological effects of MeJA or its free acid (JA) were unknown until the 1980's when a senescence-promoting effect of JA (Ueda and Kato, 1980) and growth inhibition activity of MeJA to *Vicia faba* (Dathe, 1981) were observed. Now JA and derivatives (JAs) are the best characterized group of oxylipins in plants and are regarded as one of the the major hormones regulating both defense and development.

Biosynthesis of JAs originates from polyunsaturated fatty acids (PUFA) and is synthesized by one of the seven distinct branches of the lipoxygenase (LOX) pathway, the allene oxide synthase (AOS) branch (Feussner and Wasternack, 2002). The remaing six branches form other oxylipins including GLVs as well as epoxy-, hydroxy-, keto- or ether PUFA and epoxyhydroxy-PUFA (Feussner and Wasternack, 2002) (Figure 1). In the oxylipin biosynthesis (Figure 1), only 13-hydroperoxide from α-linolenic acid (18:3, α-LeA) can be utilized by the AOS branch for JA production. Other fatty acid hydroperoxides such as 9- 13- and 2-hydroperoxide, oxygenated by 9-LOX, 13-LOX and α-dioxygenase (α-DOX), respectively, or those whose substrates originate from α-LeA, hexadecatrienoic acid (16:3, HTA) or linoleic acid (18:2, LA) may be channeled to form other oxylipin subgroups. Biological functions of the majority of estimated 400-500 oxylipins is mostly unknown.

The biosynthesis of JA and MeJA was elucidated by Vick and Zimmerman (1983), and Hamberg and Hughes (1988). The original precursors PUFA are released from chloroplast membranes by the action of lipid hydrolyzing enzymes. Upon α-LeA liberation, a molecular oxygen is incorporated by a 13-LOX at carbon atom 13 of the substrate leading to the formation of a fatty acid hydroperoxide, 13-HPOT (13*S*-hydroperoxy-(*9Z*,11*E*,15)-octadecatrienoic acid) (Figure 2). This intermediate compound can proceede to seven distinct enzymatic branches (Figure 1), one of which is dehydration by the allene oxide synthase (AOS) to an unstable allene oxide, 12,13-EOT ((9*Z*,13*S*,15*Z*)-12,13-oxido-9,11,15-octadecatrienoic acid) which can be cyclized to racemic 12-oxo-phytodienoic acid (OPDA). In the presence of an allene oxide cyclase (AOC), preferential product is the enantiomer, 9*S*,13*S*/*cis* (+)-OPDA (Figure 2). All the reactions from α-LeA to OPDA take place within a plastid. *cis* (+)-OPDA is subsequently transported into the peroxisome, where it is further converted into (+)-7-*iso*-JA by 12-oxo-phytodienoic acid reductase (OPR) and three beta oxidation steps involving three peroxisomal enzymatic functions (acyl-CoA oxidase, multi-functional protein, and l-3-ketoacyl-CoA thiolase) (Figure 2). (+)-7-*iso*-JA often epimerizes

into a more stable *trans* configuration, (-)-JA or undergoes modifications to produce diverse JA derivatives including MeJA and (+)-7-*iso*-JA-Ile. The latter one is the bioactive form of JA produced by conjugation of JA to isoleucine by the enzyme encoded by the *JA resistant 1* (*JAR1*) gene (Staswick and Tiryaki, 2004).

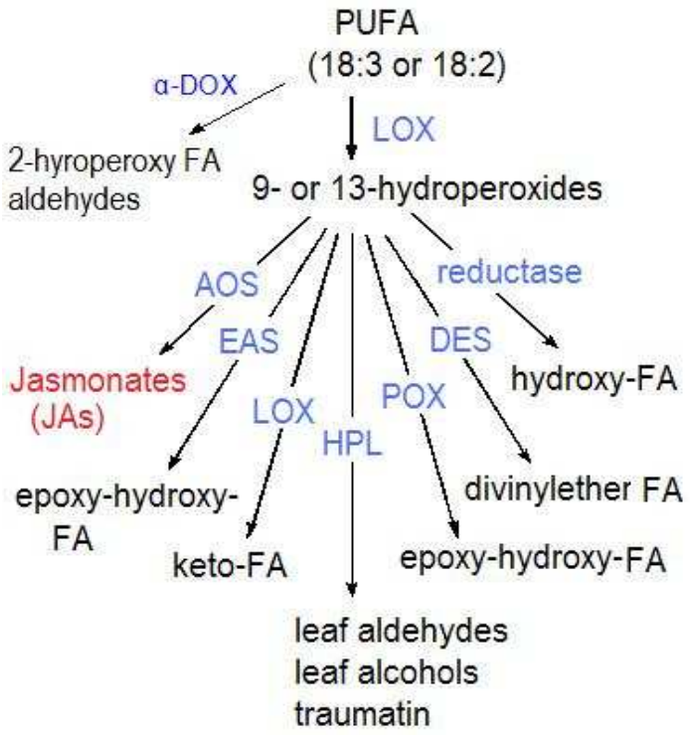

Figure 1. Overview of the oxylipin biosynthesis pathways in plants (Andreou et al., 2009)
In the first reaction, free fatty acids (18:3 or 18:2) are oxidized by the addition of molecular oxygen yielding hydroperoxides (HPOT, hydroperoxy-octadecatrienoic acid; HOPD, hydroperoxy-octadecadienoic acids) through activity of oxygenases, lipoxygenase (LOX) or α-dioxygenase (α-DOX). Hydroperoxide products formed by LOXs are further metabolized by other enzymes: allene oxide synthase (AOS), hydroperoxide lyase (HPL), divinyl ether synthase (DES), peroxygenase (POX) and epoxyalcohol synthases (EAS).

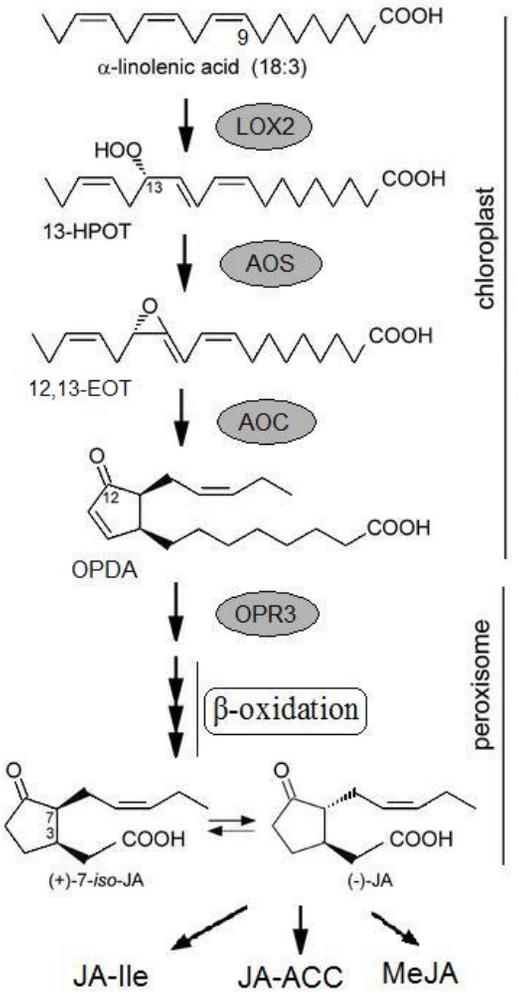

Figure 2. Scheme of JA biosynthesis pathway in *A. thaliana* (Delker et al., 2006)
The enzymes and the intermediates are indicated as LOX2 for lipoxygenase 2, AOS for allene oxide synthase, AOC for allene oxide cyclase, and OPR3 for 12-oxophytodienoate reductase 3; 13-HPOT for 13*S*-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid, 12,13-EOT for 12,13-epoxyoctadecatrienoic acid, OPDA for 12-oxophytodienoic acid, JA for jasmonates, JA-Ile for jasmonoyl-isoleucine, JA-ACC for jasmonoyl-1-amino-1-cyclopropane carboxylic acid, and MeJA for methyl jasmonates.

2.2. The alternative routes of JA biosynthesis

JA biosynthesis beginning with free linolenic acid (18:3) as the substrate is referred to as the Vick and Zimmermann pathway (Schaller et al., 2004) which was considered the major source of JA production in plants. However, there are several variations of this pathway considered as the alternative routes of JA biosynthesis.

Linoleic acid (LA, 18:2) is a ubiquitous component of plant lipids. All seed oils of commercial importance including corn, sunflower and soybean oils usually contain over 50% of linoleate. Previously, LA was considered analogous to α-LeA for metabolism by the Vick and Zimmermann pathway (Schaller et al., 2004) yielding 9,10-dihydro-JA (DH-JA). This product was widely detected *in vivo* in some plant species (Miersch et al., 1999; Blechert

et al., 1995; Gundlach and Zenk, 1998), but not others, suggesting DH-JA biosynthesis from LA through Vick and Zimmermann pathway is not conserved (Gundlach and Zenk, 1998). Investigation by Gundlach and Zenk (1998) revealed that allene oxide cyclase (AOC), unlike most of the other enzymes of the Vick and Zimmerman pathway, discriminates between 18:3 and 18:2-derived pathway intermediates. This implies that AOC is the bottleneck for DH-JA production or alternatively, DH-OPDA (precursor of DH-JA) may result from the spontaneous cyclization of the 18:2-derived allene oxide (Gundlach and Zenk, 1998).

Hexadecatrienoic acid (16:3) was proposed as an analog of linolenic acid for JA biosynthesis through the Vick and Zimmermann pathway (Weber et al., 1997), which is characterized by forming dinor-oxophytodienoic acid (dn-OPDA), a 16-carbon cyclopentanoic acid analog of *cis* (+)-OPDA. First identified in leaf extracts of *Arabidopsis* and potato plants (Weber et al., 1997), dn-OPDA dramatically accumulated upon wounding, suggesting an important role of this molecule in wounding response (Weber et al., 1997) (Figure 3). Although dn-OPDA forms after the first β-oxidation of *cis* (+)-OPDA (Figure 2), the detected dn-OPDA in wounded leaves believed to from 16:3 (Figure 3). Convincing genetic evidence for the role of 16:3 in JA biosynthesis came from the analysis ofthe *Arabidopsis* mutant *fad5* incapable of synthesizing 16:3 and JA (Weber et al., 1997).

OPDA and dn-OPDA are also constituents of arabidopsides (Figure 4), which are considered other alternative substrates for JA production in *Arabidopsis* (Gfeller et al., 2010). Arabidopsides are OPDA- and/or dn-OPDA-containing monogalactosyl-diacylglycerides

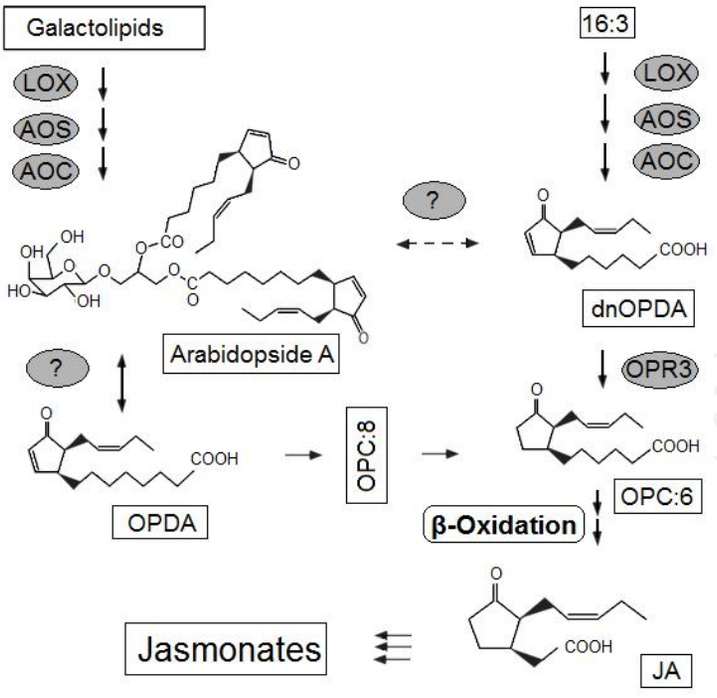

Figure 3. Alternative JA biosynthesis pathway (Schaller et al., 2004; Gfeller et al., 2010)
See the abbreviations of the enzymes in the Fig. 2. dnOPDA indicated the intermediate dinor-oxophytodienoic acid, OPC:8 is 3-oxo-2-(2'-[Z]-pentenyl)-cyclopentane-1-octanoicacid and OPC:6 is 3-oxo-2-(2'-pentenyl) cyclopentanehexanoic acid.

(MGDG) or digalactosyl-diacylglyceride (DGDG). Arabidopsides A, C, E and F have dino-OPDA at *sn2* position of glycerol backbone. Except for arabidopside F, all arabidopsides contain OPDA (Figure 4) (Hisamatsu et al., 2003; Hisamatsu 2005). At present, it is unclear whether the lipid-bound OPDA/dn-OPDA in the membranes is synthesized in situ from MGDG or DGDG or alternatively, OPDA/dn-OPDA is synthesized from free 18:3/16:3 and then incorporated into glycerol (Schaller et al., 2004). The latter possibility is supported by substantial amount of free 18:3 and 18:2 that were detected in tomato leaves after wounding (Conconi et al., 1996). However, Buseman et al. (2006) have shown that within the first 15 min after wounding, levels of OPDA-dnOPDA MGDG, OPDA-OPDA MGDG, and OPDA-OPDA DGDG increased 200 to 1000 folds. Yet in untreated leaves, the levels of these oxylipin-containing complex lipid species remained low, suggesting lipid-bound OPDA/dn-OPDA in wounding response synthesize on the esterified galactolipids rather than via the free fatty acids. Furthermore, OPDA and dn-OPDA sequestered in form of MGDG-O or DGDG-O may provide an abundant resource of OPDA/dn-OPDA, which may rapidly release under appropriate stress conditions for signaling or further metabolism (Schaller et al., 2004).

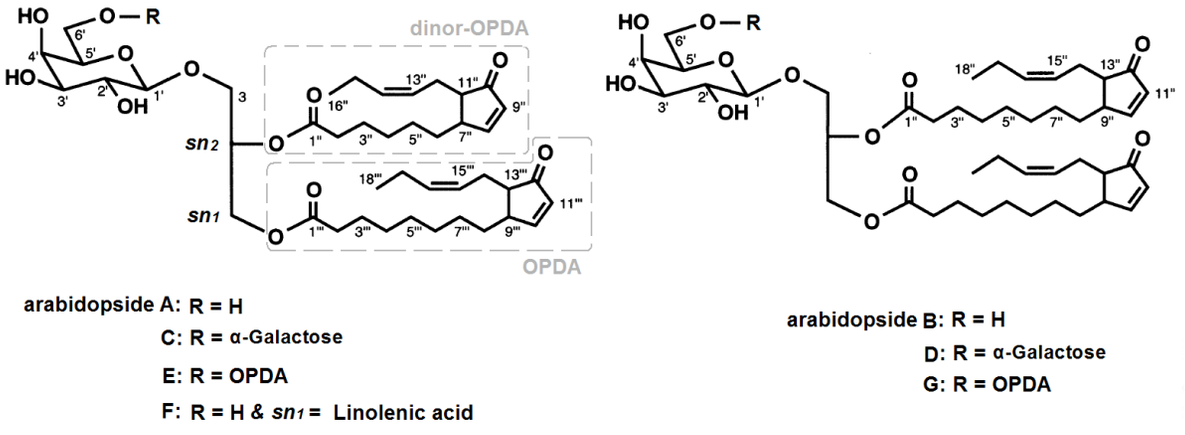

Figure 4. Arabidopsides of *Arabidopsis thaliana* (Hisamatsu et al., 2003, 2005)

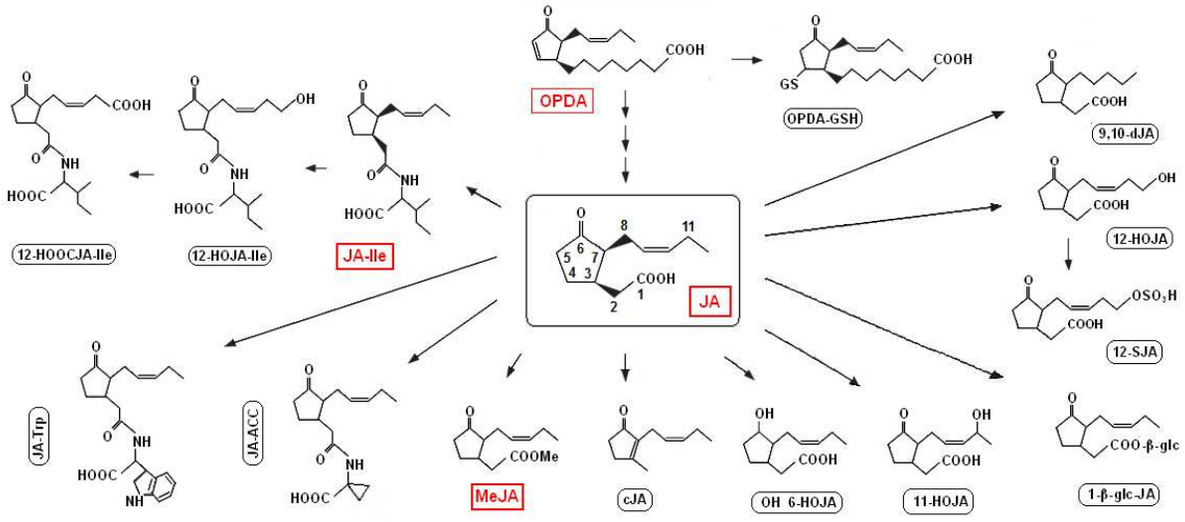

Figure 5. The metabolites produced from JA in plants (Gfeller et al., 2010)

2.3. Derivatives and Metabolites of JA

The JA biosynthetic pathway from linolenic acid yields (+)-7-*iso*-JA (3*R*,7*S*-JA) as the final product (Sembdner and Parthier, 1993). However, this molecule readily isomerizes to the thermodynamically favored stereoisomer (-)-JA (3*R*,7*R*-JA) (Figure 2) resulting in a molar equilibrium of about 9: 1 ((-)-JA : (+)-7-*iso*-JA) under normal conditions (Sembdner and Parthier, 1993). In addition to isomerization, JA undergoes a series of molecular modifications to form a variety of metabolites in plants (Figure 5).

1. C_1 carboxyl group can be methyl-esterified or conjugated with amino acids or with 1-Aminocyclopropane-1-carboxylic acid (ACC).
2. C_1 carboxyl group can be decarboxylated.
3. Glycosylation of C_1 carboxyl group.
4. Reduction of C_6 carbonyl group.
5. Reduction of $C_{9,10}$ double bond.
6. Hydroxylation of carbon at C_{11} or C_{12}.

With the above reactions, (+)-7-*iso*-JA can be converted into more than 30 distinct jasmonates which were found to be widespread in *Angiospermae*, *Gymnospermae*, *Pteridophyta*, *Algae* such as *Euglena*, *Spirulina*, and *Chiarella,* and the red alga *Gelidium* (Sembdner and Parthier, 1993). However, just several jasmonates *e.g.,* free JA, *cis*-jasmone, MeJA and JA-Ile, are considered to be the major bioactive JA forms in plants (Fonseca et al., 2009). Other jasmonate derivatives or conjugates have been viewed as clearance metabolites playing important roles in hormone homeostasis (Sembdner and Parthier, 1993), in which JA biosynthesis (and deconjugation) and JA degradation (or conjugation) are balanced to control the actual active JA level for fine-tuning developemental and defensive events.

2.4. The Enzymes for JA biosynthesis and derivation

The enzymes of JA biosynthesis and metabolism have been extensively investigated in *Arabidopsis* and several articles have reviewed structures, biochemical activities, and functional regulation (Schaller and Stintzi, 2009; Schaller, 2001; Delker et al., 2006). Here we focus on the genes encoding the enzymes for JA biosynthesis and metabolism.

2.5. Phosholipase A (PLA)

According to the classical Vick and Zimmerman pathway (Vick and Zimmerman, 1983), JA biosynthesis initiates by release of 18:3 from chloroplast membrane galactolipids by a lipase. The lipase belongs to one of the following five enzyme (Wasternack, 2007; Delker et al., 2006): (1) phospholipase A_1 (PLA1), which cleaves the acyl group of phospholipid and glycerolipids at the *sn*-1 position; (2) phospholipase A_2 (PLA2), which cleaves the acyl group in *sn*-2 position; (3) patatin-like acyl hydrolases, which has little *sn-1*/*sn-2* specificity and is homologous to animal Ca^{2+}-independent PLA_2; (4) DAD-like lipase with activity of phospholipid and galactolipid acyl hydrolase that may have *sn-1* or *sn-2* specificity; (5) SAG

(senescence-associated gene) 101-like acyl hyrolase. The free hexadecatrienoic acid (16:3) liberated by a phospholipase A2 from the *sn-2* position of MGDG or DGDG can also become substrate for JA biosynthesis to form dn-OPDA (Figure 3). Alternatively, 13-LOX may oxygenate 18:3 and 16:3 esterified in galactolipids (Buseman et al., 2006; Kourtchenko et al., 2007). The resulting hydroperoxy galactolipids may then be substrates for AOS and AOC, yielding arabidopsides, which belong to galactolipid species containing esterified OPDA and dnOPDA in plastidic membranes. These lipid species may serve as storage lipids that may allow the rapid release of OPDA and dnOPDA, the JA biosynthetic intermediates. Conclusively, lipase activity is essential to supply the JA biosynthesis pathway with either free PUFA (18:3 and 16:3) or OPDA/dnOPDA as precursors.

The first reported lipase involved in JA biosynthesis was DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1), a chloroplastic glycerolipid lipase. DAD1 belongs to phospholipase A_1 (PLA1) family. *Arabidopsis dad1* T-DNA insertion mutants are male sterile from decreased JA accumulation required for reproduction (Ishiguro et al., 2001), but remain capable of synthesizing JA, indicating that other lipases contribute to JA production.

A homolog of DAD1, the DONGLE protein was characterized as an essential lipase involved in the early wound response in *Arabidopsis* leaves. A DGL-overexpressing mutant *dgl-D* displayed dwarfism with small round leaves, extremely high basal JA accumulation, increased expression of JA-responsive genes, and increased resistance to the necrotrophic fungus *Alternaria brassicicola* (Hyun et al., 2008).

Arabidopsis PLA1 family comprises seven PLA1 with predicted plastidic transit signaling peptides: DGL (At1g05800; PLA-Iα1), DAD1 (At2g44810; PLA-Iβ1), At2g31690 (PLA-Iα2), At4g16820 (PLA-Iβ2), At1g06800 (PLA-Iγ1), At2g30550 (PLA-Iγ2), and At1g51440 (PLA-Iγ3) (Ryu, 2004). In addition to these seven PLA_1 lipases (DAD1-like lipases), Ellinger et al (2010), identified 14 additional putative lipases with predicted plastid transit peptides suggesting, up to 21 lipases may contribute to JA production in *Arabidopsis*. Mutant lines of 18 different lipases, including DGL and DAD1, have been assessed for wound-induced jasmonate levels. However, none of the single lipase mutants or the quadruple mutant line (*pla-Iβ2* / *Iγ1* / *Iγ2* / *Iγ3*) were completely abolished in JA formation under basal and wound-induced conditions (Ellinger et al., 2010), indicating that multiple lipases with both *sn-1* and *sn-2* (or dual *sn-1/sn-2*) galactolipid substrate specificity participate in JA formation in plant.

2.6. Lipoxygenase (LOX)

Lipoxygenases (LOXs) are non-heme iron-containing dioxygenases widely distributed in yeast, algae, fungus, plant, and animal species (Shin et al, 2008). LOX is the first synthesis enzyme of Vick and Zimmermann pathway. LOX isozymes catalyze the incorporation of molecular oxygen at either position 9 or 13 of polyunsaturated fatty acids (PUFA) such as linoleic (LA,18:2) and α-linolenic (LeA,18:3) acid to produce PUFA hydroperoxides (HPOT and HPOD), which can be further converted to different oxidized fatty acids (oxylipins) through the action of enzymes participating in seven LOX-pathway branches (Figure 1).

Plant LOXs are classified with respect to their positional specificity of LA dioxygenation. LOXs adding O_2 to C-9 or C-13 of the hydrocarbon backbone of LA are designated as 9-LOX or 13-LOX (Feussner and Wasternack, 2002). However, in plants some LOXs have dual positional specificity to C-9 and C-13 of LA and produce both 9- and 13-hydroperoxides of linoleic acid (Hughes *et al*. 2001, Kim *et al*. 2002, Garbe *et al*. 2006). Plants LOXs were found in several subcellular compartments including chloroplast, vacuole and cytosol. According to their localization and sequence similarity, plant LOXs can be classified into type 1- and type 2-LOXs. Type 1-LOXs harbor no chloroplast-transit peptide but the members of this group share a high similarity (>75%) of amino acid sequence to one another. Type 2-LOXs carry a putative chloroplast transit peptide and show only a moderate overall similarity (~ 35%) of amino acid sequence to one another. To date, these LOX forms all belong to the subfamily of 13-LOXs (Feussner and Wasternack, 2002). After LOX activity and fluxing through the lipoxygenase pathway branches, PUFA (mainly LA and LeA) can be converted to hundreds of oxylipin species which physiological roles are largely unclear in plants. However, the jasmonates, a small group of oxylipins, whose members are well known signal molecules mediating defense responses against pathogens and insects. JA alone does not completely describe the effects of lipoxygenase activity, but the other hundreds of oxylipin compounds posses biochemical roles in determing a wide spectrum of responses. Plant LOXs have been correlated with seed germination, vegetative and reproductive growth, fruit maturation, plant senescence, and responses to pathogen attacks and insect wounding (Porta and Rocha-Sosa, 2002).

Every plant species harbors several LOX isozymes, encoded by a LOX gene family. For example, the *Arabidopsis* genome contains six LOX genes (Bannenberg et al., 2009), while rice and maize have 14 (Umate, 2011) and 13 LOX genes (Nemchenko et al. 2006), respectively. All LOX isoforms may contribute to oxylipin production, but only 13-LOXs with chloroplast-transit peptide participate in Vick and Zimmerman pathway for JA production. Additionally, several LOX genes may function for JA biosynthesis, e.g., LOX2, LOX3, LOX4 and LOX6 in *Arabidopsis* contain chloroplast signaling peptides and show 13S-lipoxygenase activity, both required for JA biosynthesis (Bannenberg et al., 2009). However, no LOX gene in *Arabidopsis* shows dual 9-/13-LOX activity (Bannenberg et al., 2009). Compelling evidence establishes LOXs involvment in JA biosynthesis in plants. LOX2 of *Arabidopsis* localizes to chloroplasts (Bell et al., 1995), and transgenic plants lacking LOX2 no longer produced JA as observed in control plants, indicating requirement of LOX2 for wound-induced accumulation of jasmonates in leaves (Bell et al., 1995). *lox3 lox4* double mutant is male sterilie, revealing redundant role of LOX3 and LOX4 in florescence JA biosynthesis (Caldelari et al., 2011). In maize strong evidence establishes *TS1* encoding ZmLOX8, as indispensible for JA biosynthesis in tassel (Acosta et al., 2009). In the *ts1* mutant, the male sex determination process – abortion of pistil primordia in bisexual floral meristem, fails from deficient lipoxygenase activity and subsequent low endogenous JA concentrations (Acosta et al., 2009). In addition to peroxidation of JA percurors, LOXs may indirectly regulate JA biosynthesis in plants. For example, the maize disruption mutant, *lox10*, is devoid of green leaf volatiles (GLV) and reduced JA production (Christensen et al., 2012).

2.7. Allene oxide synthase (AOS)

9-/13-HPOT, the product of LOXs can serve as substrate for several enzymes (Figure 1). Allene oxide synthase (AOS) catalyzes 9-/13-HPOT to the unstable epoxide, either 9,10-EOT (9,10-Epoxyoctadecatrienoic acid) or 12,13-EOT (12,13-Epoxyoctadecatrienoic acid). The unstable epoxide can be either hydrolysed non-enzymatically to α- and γ -ketols or cyclized to 12-oxo-phytodienoic acid (OPDA). Only 12, 13-EOT provides substrate for the following enzyme in JA biosynthesis, allene oxide cyclase (AOC) (Figure 1). AOS and other HPOT-utilizing enzymes such as HPL (hydroperoxide lyase) and DES (divinyl ether synthase) belong to the family of CYP74 enzymes, which are independent from molecular oxygen and NADPH, exhibit low affinity to CO, and use an acyl hydroperoxide as the substrate and the oxygen donor (Stumpe and Feussner, 2006). CYP74 enzymes have been phylogenetically classified into CYP74A, CYP74B, CYP74C, and CYP74D (Stumpe and Feussner, 2006). With some exceptions, plant AOS enzymes belong to CYP74A (Stumpe and Feussner, 2006). According to the specificity of AOS to the substrates, 9-/13-hydroperoxides, AOS enzymes specialize into 9- or 13-AOS, which use either 9- or 13-hydroperoxide, respectively, as substrate. AOS enzymes from barley and rice show no substrate specificity for either (9*S*)-hydroperoxides or (13*S*)-hydroperoxides, and designated 9/13-AOS (Stumpe and Feussner, 2006). Like LOXs, only 13-AOS functions in JA biosynthesis. All 13-AOS carry a plastid-transit peptide except AOS from guayule (Pan et al., 1995) and barley (Maucher at al., 2000), indicating that during JA biosynthesis, AOS localizes to chloroplast. Interestingly, barley AOS, which lacks plastid-transit peptide, was also found localized in plastid (Maucher at al., 2000). Plant species may contain one or multiple AOS genes. For example, *Arabidopsis* jus has a single copy of AOS gene while rice may have four AOS genes (Agrawal et al., 2004). AOS genes from plant species such as flax (Song *et al*., 1993), guayule (Pan *et al*., 1995), *Arabidopsis* (Laudert *et al*., 1996), tomato (Howe *et al*., 2000), barley (Maucher *et al*., 2000), rice (Ha et al., 2002; Agrawal et al., 2004) and corn (Utsunomiya *et al*., 2000) have been cloned or purified so far. The diagram of oxylipin biosynthesis (Figure 1) clearly showed AOS branch competes with other HPOT-using branches for substrate, indicating AOS activity is crucial to control influx of HPOT into JA biosynthesis (Figure 1 & 2). Overexpression of flax AOS in transgenic potato plants led to 6-12 folds increased of basal JA level (Harms et al., 1995). However, overexpression of *Arabidopsis* AOS in either *Arabidopsis* or tobacco did not alter the basal level of JA (Laudert et al., 2000), indicating that the basal expression level of AOS varied in plant species which may be the bottleneck or not for JA production in rest plants. One important property of *AOS* genes in plants is that they are strongly induced by wounding and JA- / MeJA-, and OPDA- treatment in many plant species (Harms et al., 1995; Laudert and weiler, 1998). Other plant hormones such and Ethylene and abscisic acid (ABA) can also induce AOS *Arabidopsis* (Laudert and weiler, 1998). *Arabidopsis* JA-deficient mutant *aos* (Park et al., 2002) or *dde2* (*delayed-dehiscence2*) (von Malek et al., 2002) showed male-sterile phenotype and no JA induction in wounding response, demonstrating AOS enzyme is essential for JA biosynthesis pathway in plants.

2.8. Allene oxide cyclase (AOC)

Allene oxide cyclase (AOC) catalyzes the stereospecific cyclization of the unstable allene oxide, the product of AOS into the cis-(+) enantiomer OPDA, the precursor of JA (Figure 2). The unstable allene oxide is either 9,10-EOT (9,10-Epoxyoctadecatrienoic acid) or 12,13-EOT (12,13-Epoxyoctadecatrienoic acid), corresponding to 9-/13-HPOT, the substrates of AOS. These unstable substrates of AOC, 9,10-EOT and 12,13-EOT can spontaneously and rapidly hydrolyze to a mixture of α- and γ- ketols ($t_{1/2}$ < 30 minutes in water) (Schaller et al., 2004). However, *in vivo* α- and γ- ketols are not detectable (Schaller et al., 2004), suggesting tight coupling of AOS and AOC reactions, which effectively convert HPOT into OPDA. AOC was firstly purified as a 47kDa dimer from maize kernals (Ziegler et al., 1997) and was found to accepted only 12,13-EOT (12,13(*S*)-epoxylinolenic acid) but not 12,13- EOD (12,13(*S*)-epoxylinoleic acid) as a substrate (Ziegler et al., 1999). This is in contrast to AOS, which produces both allene oxides using 13(*S*)-hydroperoxy 18:3 and 18:2. Thus, it appears AOC provides additional specificity to the octadecanoid pathway for JA production in plants (Schaller et al., 2004). To date, one *AOC* gene from tomato (Ziegler et al., 2000), one from barley (Maucher et al., 2004) and four from *Arabidopsis* (Stenzel et al., 2003) have been cloned. Monocot *AOC* genes are less stuied, but at least two exisist in the rice genome (Agrawal et al., 2004). *Arabidopsis* AOCs are enzymatically active and form *cis*-(+)-OPDA, with AOC2 having greatest activity. The N-terminal of cloned *AOC* genes revealed the presence of chloroplast-transit peptide and localization in chloroplast was confirmed immunohistochemically (Ziegler et al., 2000; Stenzel et al., 2003), supporting OPDA production of JA biosynthesis is localized in chloroplast. *Arabidopsis* and rice AOC genes, in particular AOC2 and AOC1, respectively are differentially regulated upon wounding, JA-treatment, and environmental stresses (Agrawal et al., 2004).

2.9. Oxo-phytodienoic acid reductase (OPR)

The second half of the JA biosynthesis pathway, beginning with *cis*-(+)-OPDA, occurs in the peroxisome, requiring OPDA or its CoA ester to transport from the chloroplast into the peroxisome. An OPDA-specific transporter is not yet known, however a peroxisomal ABC transporter protein COMATOSE (CTS, Footitt et al., 2002), also known as PXA1 (Zolman et al., 2001) or PED3 (Hayashi et al., 2002), may mediate transportation of OPDA into peroxisome. While *cts* mutants are JA-deficient, suggesting involvement of CTS with JA-production, substantial residual JA implicates CTS-independent OPDA transport, possiblely by ion trapping of OPDA (Theodoulou et al., 2005).

The first step of peroxisomal JA biosynthesis is the conversion of OPDA, a cyclopentenone to cyclopentanone (3-oxo-2-(2′(*Z*)-pentenyl)-cyclopentane-1-octanoic acid, OPC-8:0) catalyzed by OPDA reductase (OPR). OPR enzymes belong to Old Yellow Enzyme (OYE) (EC 1.6.99.1), initially isolated from brewer's bottom yeast and shown to possess a flavin cofactor. Despite extensive biochemical and spectroscopic characterization, the physiological role of the enzyme remained obscure. OYE has been described as a diaphorase catalyzing the oxidation of NADPH in presence of molecular oxygen, but the physiological oxidant

remains unknown (Schaller, 2001). A number of compounds containing an olefinic bond of α,β-unsaturated ketones and aldehydes may be substrates of OYE (Schaller, 2001). Several OYE homologues have been identified in prokaryotic and eukaryotic organisms (Vaz et al., 1995; Kohli and Massey, 1998; Xu et al., 1999). The first identified OYE in higher plant is OPR1 of *Arabidopsis* (Schaller and Weiler, 1997). Plant OPR isomers are encoded by small gene families identified in across broad plant genre, (Schaller et al., 2004). Better-studied OPR families include five *OPRs* (three of which are characterized) in *Arabidopsis* (Sanders et

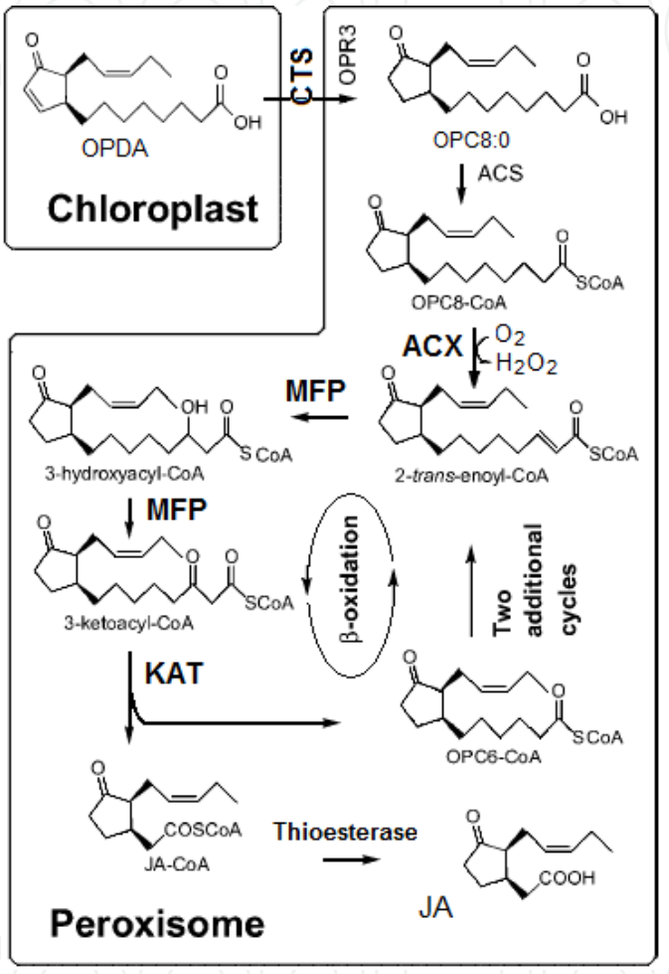

Figure 6. β-Oxidation Scheme of JA Biosynthesis (Li et al., 2005)
Abbreviations: ACS (acyl-CoA synthetase), ACX (acyl-CoA oxidase), MFP (multifunctional protein), KAT (3-ketoacyl-CoA thiolase), and OPC8:0 (3-oxo-2(2′[*Z*]-pentenyl)-cyclopentane-1-octanoic acid)

al., 2000), six in pea (Matsui et al., 2004), three in tomato (Strassner et al., 2002), 13 in rice (Agrawal et al., 2004), and eight in maize (Zhang et al., 2005). All theses OPRs can catalyze the reduction of α,β-unsaturated carbonyls (conjugated enones) in a wide spectrum of substrates in including four stereoisomers of OPDA(Sanders et al., 2000). Earlier studies on the enzymatic activity of OPRs in *Arabidopsis* and tomato revealed that different OPR isomers have distinct substrate preferences to warrant classification into separate groups, group I and II, depending on their substrate specificity to OPDA stereoisomers (Schaller et al., 1998). OPR group I enzymes preferentially catalyze the reduction of (9*R*,13*R*)-12-oxo-

10,15(*Z*)-octadecatrienoic acid (9*R*,13*R*-OPDA), while OPR group II enzymes preferentially catalyze (9*S*,13*S*)-12-oxo-10,15(*Z*)-octadecatrienoic acid (9*S*,13*S*-OPDA), a intermediate biosynthetic precursor in JA biosynthesis (Schaller et al., 1998). OPR3 of *Arabidopsis* and tomato, belonging to group II, have been shown to efficiently reduce the natural isomer 9*S*,13*S*-OPDA to OPC 8:0, the precursor of JA (Schaller et al., 1998). In contrast, OPR group I enzymes such as OPR1/2 of *Arabidopsis* and tomato have very low affinity for 9*S*,13*S*-OPDA (Schaller et al., 1998) and unlikely to be involved in JA biosynthesis but instead function in other yet unknown biochemical processes. In addition, to uncover the molecular determinants of substrate specificity between OPR group I and II, crystal structural comparison and mutational analysis of tomato OPR1/3 in complex with OPDA enantiomers revealed that two active-site residues, i.e., Tyr78 and Tyr246 in OPR1 and Phe74 and His244 in OPR3 of tomato, are critical for substrate specificity (Breithaupt et al. 2009). Thus, the biochemical studies conclude OPR3 rather than OPR1 and OPR2, is responsible for JA production in *Arabidopsis* and tomato.

Numerous genetic studies identifed JA biosynthetic OPR enzymes across several plant species. A knockout *Arabidopsis* mutant, *dde1*/*opr3*, displayed male sterilility and compromised defense responses resulting from JA deficiency, indicating OPR3 is essential for the JA biosynthesis pathway and other OPR isomers such as OPR1/2 can not substitute for OPR3 in JA production (Sanders et al., 2000; Stintzi and Browse, 2000). One orthologue of OPR3, i.e., OsOPR7, was identified as a JA biosynthetic OPR in rice (Tani et al., 2008). *OPR7* and *OPR8* in maize, orthologous to *OPR3* of *Arabidopsis*, are segmentally duplicated genes, sharing 94.5% identity in amino acid sequence to each other and responsible for JA biosynthesis in maize (Yan et al., 2012). *opr7 opr8* double mutant showed a number of genetic phenotypes such as tasselseed and susceptibility to insect and pathogen, reflecting JA essential functions in monocotyledonous plants (Yan et al., 2012). Thus in plants, the physiological role of OPR group II enzymes in plants is primarily for production of the jasmonates, which mediate many development and defense-related processes (Yan et al., 2012). However, the biological significance of plants with multiple OPR group I enzymes is not clearly understood so far.

2.10. β-Oxidation enzymes

β-oxidation in lipid metabolism was believed to be located in the peroxisomes of all higher plants (Masterson and Wood, 2001) but also detected in mitochondria in a non-oilseed plant (Masterson and Wood, 2001). The terminal steps of peroxisomal JA biosynthesis are three β-oxidation reactions, which shorten the carboxyl side chain from the intermediates OPC-8:0 or OPC-6:0 produced from OPDA or dn-OPDA (Vick and Zimmernan, 1983). Prior to entry into the β-oxidation reactions, the carboxylic group of OPC-8:0 or OPC-6:0 must activate as a CoA ester. *Arabidopsis* possesses an acyl-activating superfamily containing 63 different genes, whose proteins are potential acyl-activating enzymes (AAEs) (Shockey et al., 2003). Within this superfamily, a subgroup, called the 4-coumarate:CoA ligase (4CL)-like family, contains 13 members shown to possess peroxisomal acyl-activating activity involved in the biosynthesis of jasmonic acid (Koo et al., 2006). One of these 13 genes, At1g20510 was

confirmed as an OPC-activating enzyme, designated OPCL1 (OPC-8:CoA ligase 1) (Koo et al., 2006). Loss-of-function mutants for *OPCL1* hyper-accumulate OPC-8:0, OPC-6:0, and OPC-4:0, suggesting a metabolic block in OPC-CoA ester formation. The mutants are also compromised in wound-induced JA accumulation. However, about 50% of wild-type levels remain in the mutants indicating that OPCL1 is responsible for only part of the wound-induced JA production, and that additional acyl-CoA synthetases may be involved in OPC-8:0-activation (Koo et al., 2006). Another two 4CL-like proteins At4g05160 and At5g63380, were selected as acyl-CoA synthetases for JA biosynthesis. The recombinant At4g05160 protein showed, *in vitro,* a distinct activity with broad substrate specificity including, medium-chain fatty acids, long-chain fatty acids, as well as, OPDA and OPC-8:0. The closest paralogue of At4g05160, At5g63380, showed high activity with long-chain fatty acids and OPDA (Schneider et al., 2005), suggesting OPDA-CoA could function as substrate for OPR3 to form OPC-CoA, the substrate of β-oxidation.

Peroxisomal β-oxidation (Figure 6) of JA biosynthesis is catalyzed by three proteins (1) acyl-CoA oxidase (ACX), (2) the multifunctional protein (MFP) which exhibits 2-*trans*-enoyl-CoA hydratase, L-3-hydroxyacyl-CoA dehydrogenase, D-3-hydroxyacyl-CoA epimerase and Δ^3, Δ^2-enoyl-CoA isomerase activities, and (3) L-3-ketoacyl-CoA thiolase (KAT) (Schaller et al., 2004). The first *ACX* gene, named *ACX1A* was isolated from tomato. ACX1A was shown to catalyze the first step in the peroxisomal beta-oxidation stage of JA biosynthesis (Li et al., 2005). Recombinant ACX1A exhibited a preference for C12 and C14 straight-chain acyl-CoAs and also was active in the metabolism of cyclopentanoid-CoA precursors of JA (Li et al., 2005). *acx1* tomato mutant produced very little JA in wounded leaves (for 1-hour wound, 5% of wild type) and impaired in wound-induced defense gene activation and insect resistance (Li et al., 2005). *Arabidopsis* genome contains six *ACX* genes, designated *ACX1 to ACX6* (Rylott et al., 2003). *acx1* mutant of Arabidopsis produced 20% of JA production in wild type while *acx1/5* double mutant showed severe JA deficiency symptoms including impaired male fertility and susceptible to leaf-chewing insect (Schilmiller et al., 2007). For MFP roles in JA biosynthesis Arabidopsis *aim1* mutant, in which one of two MFP i.e. MFP2 is disrupted, showed impairment in wound-induced JA accumulation and defensive gene expression (Delker et al., 2007). In tobacco a similar result was obtained, that is, a stress-responsive MFP orthologous to *Arabidopsis* AIM1 are involved in β-oxidation (Ohya et al., 2008). Among five *KAT* genes in *Arabidopsis*, *KAT2* was shown to play a major role in driving wound-activated responses by participating in the biosynthesis of JA in wounded leaves (Castillo et al., 2004). The final step of JA biosynthesis is that jasmonyl-CoA releases free acid by jasmonyl-thioesterase. There is no report of cloning of jasmonyl-thioesterase in plant so far except that in *Arabidopsis* two peroxyisomal acyl-thioesterases, ACH1 and ACH2 have showed thioesterase activity of hydrolyzing both medium and long-chain fatty acyl-CoAs but not jasmonyl-CoA (Tilton et al., 2004).

2.11. Carboxyl methyltransferase (JMT)

The floral scent methyl jasmonate (MeJA) has been identified as a vital cellular regulator that mediates diverse developmental processes and defense responses against biotic and

abiotic stresses (Cheong and Choi, 2003). The enzyme converting JA to methyl jasmonates (MeJA) is JA carboxyl methyltransferase (JMT), which was first cloned and characterized from *Arabidopsis* (Seo et al., 2001). As JMT does not carry any transit signal peptides, it is presumably a cytoplasmic enzyme (Seo et al., 2001). JMT is constitutively expressed in almost all the organs of mature plants, but not in young seedling (Seo et al., 2001), indicating young plant avoids to produce MeJA. However, JMT can be induced by both wounding and MeJA treatment (Seo et al., 2001). Transgenic *Arabidopsis* overexpressing *JMT* exhibited constitutive expression of jasmonate-responsive genes, including *VSP* and *PDF1.2*, and enhanced level of resistance against the virulent fungus *Botrytis cinerea* (Seo et al., 2001), indicating JMT is a key enzyme for airborne-jasmonate-regulated plant responses.

2.12. JA-amino acid synthetase (JAR1)

Since jasmonoyl-L-isoleucine (JA-Ile) is the only one bioactive ligand, known so far, involving in JA signaling, JAR1 (JASMONATE RESISTANT 1), a JA amino acid synthetase that conjugates isoleucine to JA (Staswick and Tiryaki, 2004), was assumed a most important JA derivation enzyme in plants. Recent studies indicate JA-Ile promotes binding of the JAZ proteins to SCF^{COI1} complexes and results in subsequent degradation of JAZ by the ubiquitination/26S-proteasomes (Thines et al., 2007). *JAR1* is one of 19 closely related *Arabidopsis* genes that are similar to the auxin-induced soybean *GH3* gene family (Staswick et al., 2002). Analysis of fold-predictions for this protein family suggested that JAR1 might belong to the acyl adenylate-forming firefly luciferase superfamily. These enzymes activate the carboxyl groups of a variety of substrates including JA, indole-3-acetic acid (IAA) and salicylic acid (SA) for their subsequent biochemical modification (Staswick et al., 2002), thereby regulating hormone activity. The first *jar1* mutant was identified that affected signaling in the jasmonate pathway. *jar1* plants have reduced sensitivity to root growth inhibition in the presence of exogenous JA (Staswick *et al*., 2002). *jar1* was shown to be susceptible to soil oomycete (Staswick et al., 1998) and necrotrophic pathogens (Antico et al., 2012). In wounding *JAR1* transcript was found increased dramatically in wounded tissue and JA–Ile accumulated mostly near the wound site with a minor increase in unwounded tissue (Suza and Staswick, 2008). However, the reduced accumulation of JA–Ile had little or no effect on several jasmonate-dependent wound-induced genes such as *VSP2*, for *LOX2*, *PDF1.2*, *WRKY33*, *TAT3* and *CORI3*. Morphologically, *jar1* mutation is male fertile while JA biosynthesis and signaling mutants are male sterile (Suza and Staswick, 2008).

2.13. JA Biosynthesis Regulation

The levels of jasmonic acid in plants vary with developmental stage, organs, and are variable in response to different environmental stimuli (Creelman and Mullet, 1995). High levels of jasmonates are found in flowers, pericarp tissues of developing fruit, and in the chloroplasts of illuminated plants; Jasmonate levels increase rapidly in response to mechanical perturbations such as tendril coiling and when plants suffer wounding (Creelman and Mullet, 1995). There are several strategies applicable for plants to regulate generation or activities of jasmontes. The first strategy is to regulate the expression of the

enzymes in JA biosynthesis pathway. Many studies show that most enzyme genes for JA biosynthesis such as *LOX*, *AOS*, *AOC*, *OPR3*, *JMT* and *JRA1* are induced by JA treatment (Wasternack, 2007) or wounding (Schaller, 2001), implying that JA biosynthesis is a JA-dependent process in plants. Monitoring of MeJA-responsive genes in *Arabidopsis* by cDNA microarrays also concluded that JA biosynthesis is regulated by a positive feedback loop (Sasaki et al., 2001). Further evidence for this conclusion has come from mutants with constitutively up-regulated JA levels such as *cev1* and *fou2*. These mutants showed typical phenotypes associated with exogenous JA treatment such as roots/shoots growth inhibition, anthocyanin accumulation in the leaves, and over-expression of JA-dependent genes (Ellis and Turner, 2001; Bonaventure et al., 2007). However, the expression levels of JA biosynthetic enzymes do not determine the actual output of JA biosynthesis in limited substrate conditions. The second strategy for JA biosynthesis regulation is controlling the substrate availability. For example, the fully expanded leaves of *Arabidopsis* carry LOX, AOS, and AOC proteins abundantly; however, JA formation is at a substantially low level. JA production rapidly occurs only upon strong external stimuli such as wounding, which largely induces JA biosynthesis enzymes and releases the substrate LA from the membranes (Stenzel et al., 2003). Furthermore, wound induction of JA is transient and appears before the expression induction of *LOX*, *AOS*, and *AOC* genes (Howe et al., 2000). These data clearly show that JA biosynthesis is regulated by enzyme activities and substrate availability. The third strategy of JA biosynthesis regulation is to store or reuse intermediates or conjugates of jasmonate in case of over-produced or quick release release is required for healing in situation of insect or pathogen attacks. Recently, esterified OPDA was found in galactolipids (monogalactosyldiacylglycerol, MGDG and digalactosyldiacylglycerol, DGDG) (Stelmach et al., 2001). A novel oxylipin category, so-called arabidopsides A, B, C, D, E, F, G and F (Figure 4) were found containing OPDA and/or dino-OPDA (Hisamatsu et al., 2003; Hisamatsu, 2005). These compounds could accumulate to 7-8% of total lipids in plants if challenged by pathogens (Anderson et al., 2006). In addition, JA derivatives including jasmonyl-amino acids such as JA-Ile (jasmonoyl-isoleucine), JA-Leu (jasmonoyl-leucine), and JA-Val (jasmonoyl-valine) and jasmonyl-ACC (1-amino- cyclopropane-1-carboxylic acid) conjugates are present and all, except JA-Ile, are considered storage forms of jasmonates in plants (Staswick et al., 2002).

3. JA perception and signaling pathway

3.1. Bioactive JA forms and JA signaling ligand

The initial product (+)-7-*iso*-JA, synthesized in peroxisomes epimerizes simultaneously to a more thermo-stable *trans* configuration, (-)-JA, which is generally known as jasmonic acid (JA) (Wasternack, 2007; Creelman and Mullet, 1997). Both (-)-JA and (+)-7-iso-JA are bioactive but the former is more active (Wasternack, 2007). JA can convert into a number of derivatives and conjugates (Figure 5). The JA precursor OPDA, the free acid and methyl ester of JA, i.e., JA and MeJA, and conjugates JA-Ile and JA-trp (jasmonyl-L-tryptophan) are assumed the most active JA forms in plants (Fonseca ea al., 2009). However, in COI1-JAZ (Coronatine Insensitive 1 and Jasmonate ZIM-domain-containing protein, respectively)

binding experiments JA-Ile rather than OPDA, JA, and MeJA can promote COI1-JAZ binding, indicating only JA-Ile is the direct JA signaling ligand in plants (Thines et al, 2007). The trans configuration, (-)-JA-L-isoleucine was demonstrated to be the active molecule form of JA for COI1-JAZ binding (Thines et al, 2007). However, the recent study showed that (+)-7-iso-JA-L-Ile, which is also structurally more similar to coronatine, is highly active. The previously proposed active form (-)-JA-L-Ile, which contains a small amount of the C7 epimer (+)-7-iso-JA-L-Ile, if purified, is inactive (Fonseca ea al., 2009). In summary, currently (+)-7-iso-JA-L-Ile is the only proven ligand for JA signaling in plants.

3.2. Ubiquitination-based JA receptor: COI1-JAZ complex

Critical to comprehending hormonal control of development and defense events is the understanding of hormone perception. In the past decades, several mechanisms of plant hormone perception have been elucidated (Spartz and Gray, 2008; Chow and MeCourt, 2006). Cytokinins (CK) and ethylene were found to be perceived by two-component-based hormone receptors while brassinosteroids (BR) by leucine-rich repeat (LRR)-based hormone receptors (Chow and MeCourt, 2006) and ABA (abscisic acid) by nuclear RCAR/PYR1/PYL–PP2C complexes (Raghavendra et al, 2010). More recently, auxin, JA, GA (gibberellic acid), and SA (salicylic acid) are found to be perceived by nuclear SCF^{TIR1}, SCF^{COI1}, SCF^{DELLA}, and SCF^{NPR} complexes respectively (Chow and MeCourt, 2006; Lumba et al., 2010; Fu et al. 2012).

Early researchers believed that screening for Arabidopsis mutants insensitive to growth inhibition by bacterial coronatine, which is structurally analogous to JA and MeJA, would result in discovering JA receptor protein(s) in plants. Exhaustive screens identified only the alleles of *coronatine insensitive 1 (coi1)* and *jasmonates resistant 1 (jar1),* suggesting COI1 and JAR1 function in JA perception in plant. However, cloning of COI1 and JAR1 showed that COI1 encodes an F-box protein (Xie et al., 1998) and JAR1 an auxin-induced GH3 protein (Staswick and Tiryaki, 2004), and neither protein shows homology to known plant receptor proteins. The investigators reasoned that COI1, rather than JAR1, is a potential JA-receptor or a component of a receptor complex from two lines of evidence. First, *coi1* mutant displays severe JA signal-phenotypes such as male sterility, defective responses to JA-treatment and wounding, and high susceptiblity to insect and necrotrophic pathogens whereas *jar1* is fertile and only partially defective to JA-treatment and wounding. Secondly, *COI1* locus encodes an F-box protein which is known to associate with SKP1, Cullin, and Rbx proteins to form an E3 ubiquitin ligase, known as the SCF complex. Several SCF complexes in plant have been implicated in a number of important processes, for example, SCF^{TIR1} complex is an auxin receptor, implying SCF^{COI1} may function as analog of SCF^{TIR1} for JA signaling. The components of SCF^{COI1} complex were demonstrated to exist in Arabidopsis and mutations in the components of SCF resulted in reduced JA-dependent responses (Xu et al., 2002). Now the question becomes: what is the substrate(s) of SCF^{COI1} E3 ubiquitin ligase complex? This substrate was anticipated to function as a key negative regulator in JA signaling (Turner et al., 2002; Browse, 2005). Later, three laboratories simultaneously found the substrates of SCF^{COI1} complex, which were called JAZ proteins consisting of 12 members in Arabidopsis (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). Bioactive JA forms JA, OPDA, MeJA,

and JA-Ile were tested for affinity in COI1-JAZ1 binding. Surprisingly, only JA-Ile functioned as ligand for COI1-JAZ interaction (Thines et al., 2007). JA-Ile is a conjugate product of JA with isoluecine by JAR1, a JA-amino acid conjugation enzyme similar to auxin-responsive GH3 family proteins of soybean (Staswick and Tiryaki, 2004). This provided biochemical explination as to why *jar1* mutant showed the phenotype 'JA-RESISTANT' and demonstrating JAR1 as a provider of a JA signal rather than a component of JA perception machinery in plant. More recently, inositol pentakisphosphate (IP5) was found as an existing cofactor of COI1 crystal structure and COI1 protein lacking IP5 lost ligand-binding acivity (Sheard et al., 2010). Based on the information available so far, the true jasmonates receptor is a co-repressor complex, consisting of the SCF^{COI1} E3 ubiquitin ligase complex, JAZ degrons (JAZ1 to JAZ12), and a newly discovered third component, inositol pentakisphosphate (IP5) (Sheard et al., 2010).

3.3. JA Signaling Model: SCF^{COI1}/JAZ Proteins Imitates SCF^{TIR1}/AUX/IAA Proteins

coi1 is a completely insensitive mutant to JA/coronatine. COI1 was map-cloned and revealed as an F-box protein that functions as the substrate-recruiting element of the Skp1–Cul1–F-box protein (SCF) ubiquitin E3 ligase complex. As described above, JAZ family proteins are transcriptional repressors and SCF^{COI1} substrate targets, which associate with COI1 in a hormone-dependent manner. Recent research established JA signaling model (Figure 7) (Chini et al, 2007; Thines et al., 2007; Sheard et al., 2010). In the absence or low level of hormone signal, JAZ repressor complex, including JAZ proteins, adaptor protein NINJA, and co-repressor TPL, actively repress the activity of JA-responsive transcription factors

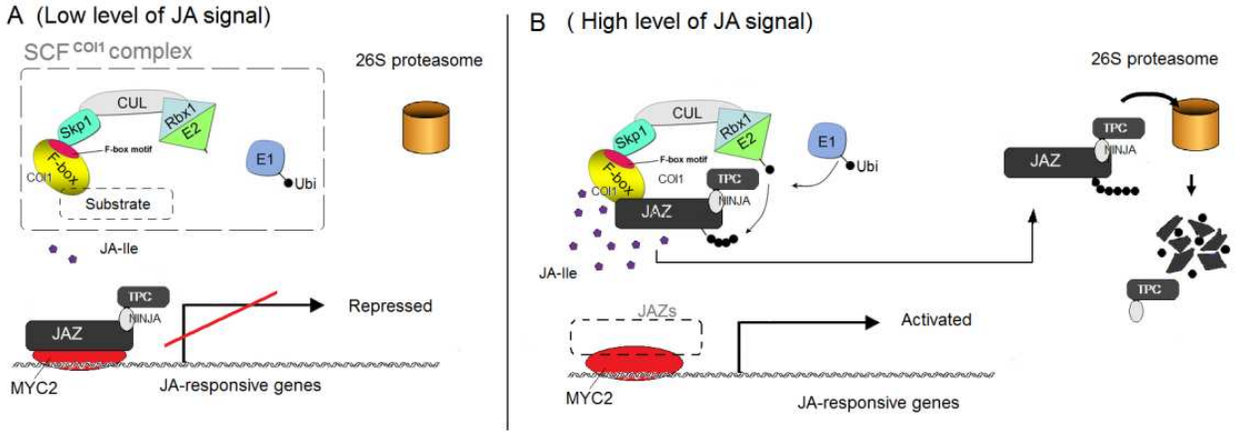

Figure 7. Model of JA signaling in *Arabidopsis* (Browse and Howe, 2008). (A) At low intracellular levels of JA signal (JA-Ile), SCF^{COI1} complex has no essential activity of E3 ubiquitin ligase, resulting in accumulation of JAZ proteins which repress the activity of transcription factors such as MYC2 that positively regulate JA-responsive genes. (B) At high level of JA signal such as upon wounding, rapid accumulation of bioactive JA-Ile promotes SCF^{COI1}-mediated ubiquitination and subsequent degradation of JAZ proteins via the 26S proteasome. JA-induced removal of JAZ proteins causes derepression of transcription factors and the activation of JA-responsive genes.

(e.g., MYC2), which bind to *cis*-acting elements of jasmonate-response genes, preventing transcription activity. In response to stimuli such as wounding JA-Ile stimulates the specific binding of JAZ proteins to COI1, leading to poly-ubiquitination and subsequent degradation of JAZs by the 26S proteasome. JAZ degradation relieves repression of MYC2 and other transcription factors, permitting the expression of jasmonate-responsive genes such as *PDF1.2*. The role of COI1-mediated JAZ degradation in jasmonate signaling is analogous to auxin signaling through the receptor SCF^{TIR1} complex, which degrades the AUX/IAA transcriptional repressors in hormone-dependent manner (Gray et al., 2001; Kepinski and Leyser, 2005). Supported by its sequence homology and functional similarity to TIR1, COI1 is recognized for a critical role in the direct perception of the jasmonate signal (Xie et al., 1998; Katsir et al., 2008; Sheard et al., 2010). Mimicking AUX/IAA proteins which are induced specifically in response to auxin (Gray et al., 2001), JAZ proteins are highly inducible by JA/MeJA treatment or wounding (Thines et al., 2007; Chung et al., 2008).

3.4. Characterization of JA Signaling Repressors: JAZs, NINJA, and TPL

JAZs proteins were designated as JAZs because they were annotated as ZIM-domain containing proteins (ZIM: Zinc-finger protein expressed in Inflorescence Meristem) and their expression depends on jasmonates (Chini et al, 2007; Thines et al., 2007). Thines et al., (2007) in their study found that eight ZIM-domain containing unknown proteins (JAZs) were significantly induced in stamens and seedlings of *opr3* mutant after JA application. JAZ1 protein, one out of the eight JAZs that were tested for activity as a substrate of SCF^{COI1} complex, acts to repress transcription of jasmonate-responsive genes. Jasmonate treatment causes JAZ1 degradation and JA–Ile promotes physical interaction between COI1 and JAZ1 proteins in the absence of other plant proteins (Thines et al., 2007). Additionally, *JAZ1Δ3A*, a mutant with distruption of conserved domain 3A (i.e., Jas domain in Yan et al., 2007) shows typical JA-signaling phenotypes such as male sterility and root growth insensitivity to JA (Thines et al., 2007). In a separate study, Chini et al., (2007) characterized the mutant *jasmonate-insensitive3-1* (*jai3-1*) they identified in a genetic screen for JA-insensitivity. Positional cloning of the *jai3-1* mutation revealed that a base substitution in JAI3 results in a truncated protein that causes a jasmonate-insensitive phenotype and impaired transcriptional responses to jasmonate (Chini et al., 2007). *jai3-1* is a dominant mutant with a missing conserved CT/Jas domain of JAI3, which encodes JAZ3.,This mutant showed some JA-deficiency phenotypes such as root growth insensitivity to JA treatment (Chini et al., 2007). In the third independent study, Yan et al. (2007) profiled the transriptome depletion of *aos* mutant compared to wild type and identified 35 JA-dependent genes responsible for JA signaling depletion in *aos* mutant. Three of these genes encode ZIM-domain containing proteins. Overexpression of a predicted alternatively spliced transcript At5g13220.3, called *Jasmonate-Associated 1* (*JAS1,* identical to *JAZ10*), resulted in reduced sensitivity to MeJA and elevated growth of roots and shoots under MeJA treatment (Yan et al., 2007). In total, 12 *JAZ* genes (Figure 8) have been identified so far in *Arabidopsis* (Chini et al., 2007; Yan et al., 2007; Browse, 2009). All are believed to have redundant function in JA signaling pathway as transcription repressors (Chini et al., 2007). The gene family of ZIM-domain containing

proteins is also known as TIFY family (Vanholme et al., 2007). 12 of 18 TIFY proteins are JAZs. The functions of the remaining TIFYs are unknown, except PPD1 and PPD2, which showed, to additively repress the proliferation of dispersed meristematic cells (DMCs) in leaves (Pauwels and Goossens, 2011).

The first prominent characteristic of JAZ proteins is that they possess two domains, ZIM and Jas domains, and both are important for JAZs function (Thines et al., 2007). The former may have two biochemical roles *in vivo*. 1) JAZ1ΔJas and JAZ3ΔJas can not be degraded by SCF^{COI1} complex plus 26S proteasome but still can suppress JA signaling, indicating JAZ may interact with MYC2 via the first conserved domain ZIM (Chini et al., 2007; Thines et al., 2007). 2) ZIM domains are responsible for homo- and hetero dimerization (Chini et al., 2009; Chung and Howe, 2009). All JAZs except JAZ7 are prone to form homo-/hetero-dimers (Pauwels and Goossens, 2011). The biological meaning of JAZ dimerization *in vivo* remains elusive but extensive dimerization of JAZs or JAZ with other proteins such as MYC2 and NINJA may help to establish insensitivity to SCF^{COI1} E3 ubiquitin ligase in the situation of low JA signal. The second prominent feature of JAZs is "logically-paradoxical"; most *JAZ* genes are highly induced upon JA treatment or by wounding in JA-dependent manner (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007) but, on the other hand, JAZ proteins are degraded during activated signaling (Chini et al., 2007; Thines et al., 2007). Similarly, AUX/IAA proteins have the same paradoxical feature, that is, AUX/IAA genes are induced by auxin but the proteins disappear rapidly (Abel et al., 1994). The third important feature of JAZ genes is that several splice-variant transcripts of single *JAZ* gene exist naturally in plants (Figure 9). For example, *JAZ10* has four alternative splice variants and three of them, *At5g13220.2, At5g13220.3,* and *At5g13220.4* encode stable JAZΔJas isomers, which attenuate JA signal output (Yan et al., 2007 Chung and Howe, 2009).

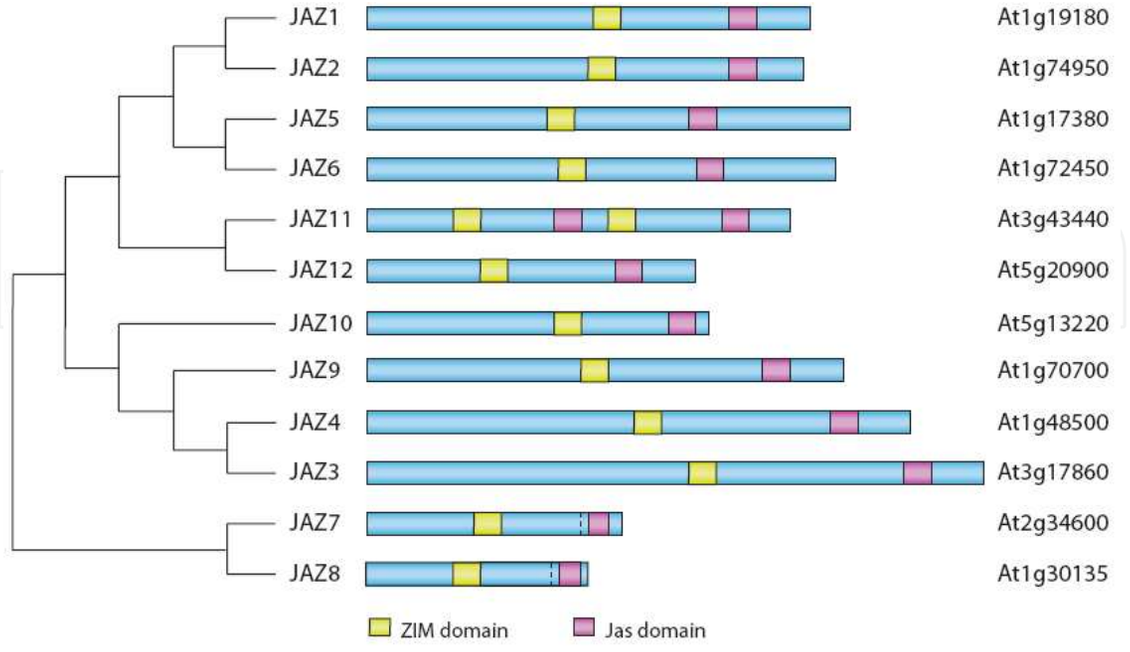

Figure 8. The structures and phylogeny of *Arabidopsis* ZIM-Domain containing proteins JAZs. ZIM domain and Jas domain are shown in yellow and pink bars, respectively (Browse, 2009).

The molecular mechanism by which JAZ proteins repress downstream gene expression is unknown. Pauwels et al. (2010) reported a mechanism that JAZ proteins co-repress JA signal by recruiting the Groucho/Tup1-type co-repressor TOPLESS (TPL) and TPL-related proteins (TPRs) through a newly characterized adaptor protein, designated Novel Interactor of JAZ (NINJA). NINJA acts as a transcriptional repressor whose activity is mediated by a functional TPL-binding motif EAR (ERF-associated amphiphilic repression). Accordingly, both NINJA and TPL proteins function as negative regulators of JA response (Pauwels et al., 2010).

Protein Name	Protein #	Sequence of ZIM domain	Sequence of Jas domain
JAZ1	At1g19180.1	PLTIFYAGQVIVFNDFSAEKAKEVINLA	PIARRASLHRFLEKRKDRVTSKAPY
	At1g19180.2	PLTIFYAGQVIVFNDFSAEKAKEVINLA	PIARRASLHRFLEKRKDRVTSKAPY
JAZ2	At1g74950.1	PLTIFYGGRVMVFDDFSAEKAKEVIDLA	PIARRASLHRFLEKRKDRITSKAPY
JAZ3	At3g17860.1	QLTIFYAGSVCVYDDISPEKAKAIMLLA	PLARKASLARFLEKRKERVTSVSPY
	At3g17860.2	QLTIFYAGSVCVYDDISPEKAKAIMLLA	PLARKASLARFLEKRKERVTSVSPY
	At3g17860.3	QLTIFYAGSVCVYDDISPEKAKAIMLLA	PLARKASLARFLEKRKERVTSVSPY
JAZ4	At1g48500.1	QLTIFYAGSVLVYQDIAPEKAQAIMLLA	PQTRKASLARFLEKRKERVINVSPY
	At1g48500.2	QLTIFYAGSVLVYQDIAPEKAQAIMLLA	PQTRKASLARFLEKRKERY*
	At1g48500.3	QLTIFYAGSVLVYQDIAPEKAQAIMLLA	PQTRKASLARFLEKRKERY*
JAZ5	At1g17380.1	LTIFFGGKVLVYNEFPVDKAKEIMEVA	RIARRASLHRFFAKRKDRAVARAPY
JAZ6	At1g72450.1	QLTIFFGGKVMVFNEFPEDKAKEIMEVA	RIARRASLHRFFAKRKDRAVARAPY
JAZ7	At2g34600.1	ILTIFYNGHMCVSSDLTHLEANAILSLA	KASMKRSLHSFLQKRSLRIQATSPY
JAZ8	At1g30135.1	RITIFYNGKMCFSSDVTHLQARSIISIA	KASMKKSLQSFLQKRKIRIQATSPY
JAZ9	At1g70700.1	QLTIFYGGTISVFNDISPDKAQAIMLCA	PQARKASLARFLEKRKERLMSAMPY
	At1g70700.2	QLTIFYGGTISVFNDISPDKAQAIMLCA	PQARKASLARFLEKRKERLMSAMPY
JAZ10	At5g13220.1	MTIFYNGSVSVFQVSRNKAGEIMKVA	PIARRKSLQRFLEKRKERLVSTSPY
	At5g13220.2	MTIFYNGSVSVFQVSRNKAGEIMKVA	PIARRKSLQRFLEKRKER*
	At5g13220.3	MTIFYNGSVSVFQVSRNKAGEIMKVA	PIARRKSLQRFLEKRKER*
	At5g13220.4	MTIFYNGSVSVFQVSRNKAGEIMKVA	*
JAZ11	At3g43440.1	QLTIIFGGSFSVFDGIPAEKVQEILHIA	PIARRRSLQRFFEKRRHRFVHTKPY
	At3g43440.2	----------GVPAQKVQEILHIA	PIARRRSLQRFFEKRRHRFVHTKPY
JAZ12	At5g20900.1	QLTIFFGGSVTVFDGLPSEKVQEILRIA	PIARRHSLQRFLEKRRDRLVNKNPY
	At5g20900.2	QLTIIFGGSCRVFNGVPAQKVQEILHIA	PIARRRSLQRFFEKRRHRFVHTKPY

Figure 9. The sequences of ZIM and Jas domains in the transcripts of JAZs (Yan et al., 2007)

3.5. JAZ proteins control the MYC-type transcription factors activity

Transcription factors dependent on JA signal are supposed to be the key components for JA signaling pathway. Up to date, MYC2 is the only transcription factor known to interact directly with JAZ proteins. According to the current model of JA signaling (Figure 7), MYC2 is the most important transcription factor to activate transcription of the early JA-responsive genes including downstream transcription factors (such as WRKYs, MYBs, and AP2/ERFs), JA biosynthesis genes, and JAZ proteins (Lorenzo et al., 2004; Chini et al.,

2007; Chung and Howe, 2009). MYC2 was identified as a key regulator of JA signaling, acting as a basic helix-loop-helix (bHLH, also called MYC) transcription factor for JA signal transduction. MYC2 was map-cloned from *jai1* (*jasmonate-insensitive 1*) mutant mutant (Lorenzo et al., 2004), which is allelic to the previously characterized mutant *jin1* (*methyl jasmonate-insensitive 1*) (Berger et al., 1996). MYC2 differentially regulates two branches of JA-mediated responses. That is, it positively regulates a wound-responsive gene set, including *VSP2*, *LOX3*, and *TAT*, but represses the expression of a pathogen-responsive gene set such as *PR4*, *PR1*, and *PDF1.2* (Lorenzo et al., 2004). Interestingly, the ethylene-responsive transcription factor ERF1 also co-regulated these two gene sets, but in opposite direction, i.e., ERF1 activated pathogen-responsive genes but represses wound-responsive genes (Lorenzo et al., 2004).

In Arabidopsis, there are 133 bHLH genes, constituting one of the largest families of transcription factors (Heim et al., 2003). Based on the amino acid sequence similarity of both the entire protein and of the bHLH domain, Arabidopsis bHLH proteins are divided into 12 major groups and 25 subgroups (Heim et al., 2003). MYC2 is a member of the subgroup IIIe, along with MYC3 (At5g46760), MYC4 (At4g17880), and MYC5 (At5g46830) (Heim et al., 2003). In contrast to severe JA-synthesis and JA-perception mutants such as *aos* and *coi1*, *myc2* plants are male-fertile and only partially defense-compromised (Lorenzo et al., 2004). This indicates that other JAZ-interacting transcription factors activate the expression of early JA-responsive genes following JA-mediated ubiquitination-proteasomal removal of JAZ repressors. The close paralogues MYC3 and MYC4, but not MYC5, showed to interact with JAZ1, JAZ3, and JAZ9 proteins in both pull-down and yeast two-hybrid assays. Although *myc3* and *myc4* loss-of-function mutants did not show an evident JA-related phenotype, the triple mutant *myc2 myc3 myc4* is as impaired *as coi1-1* in the activation of several, but not all, JA-mediated responses such as the defense against bacterial pathogens and insect herbivory. Moreover, overexpression of cDNAs encoding MYC3 and MYC4 proteins resulted in anthocyanin accumulation and higher transcript levels of JA-responsive genes compared to wild type. In addition, similar to plants overexpressing MYC2, MYC3 overexpression plants were hypersensitive to JA-mediated root growth inhibition. Based on these results, it is concluded that in addition to previously characterized MYC2, MYC3 and MYC4 are also JAZ-interacting transcription factors that activate JA-responses through SCF^{COI1} complex plus 26S proteasome (Niu et al., 2011; Brown et al., 2003; Cheng et al., 2011; Fernández-Calvo et al., 2011).

3.6. AP2/ERF Transcription Factors Involve in JA Signaling Network

AP2/ERF transcription factors are considered the second important group of transcription factors that belong to a large plant-specific APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) superfamily, containing at least 122 members in *Arabidopsis* (Nakano et al., 2006). Many ERF genes have been shown to be regulated by a variety of stress related stimuli, such as wounding, JA, ethylene, salicylic acid, or infection by different types of pathogens (Pré et al., 2008). Four transcription factors, ERF1, AtERF2, AtERF14, and ORA59, were suggested to function as positive regulators involving in JA signing pathway while AtERF4 was

identified as a negative regulator for this process (Lorenzo et al., 2003; Oñate-Sánchez et al., 2007; Pré et al., 2008; McGrath et al., 2005).

The expression of *ERF1* can be activated rapidly by ethylene or jasmonate in wild-type plant but not in JA or ethylene (ET) signaling mutants *coi1* or *ein2* (*ethylene insensitive2*), suggesting *ERF1* expression depends on JA and/or ethylene signal. Constitutive overexpression of *ERF1* activates the expression of several defense-related genes, including *PLANT DEFENSIN1.2* (*PDF1.2*) and *BASIC CHITINASE* (*ChiB*) (Lorenzo et al., 2003), and was shown to confer resistance to necrotrophic fungi such as *Botrytis cinerea* and *Plectosphaerella cucumerina* (Lorenzo et al., 2003). All these results suggest that ERF1 acts downstream of the intersection between ethylene and jasmonate pathways and suggest that this transcription factor is a key element in the integration of both signals for the regulation of defense response genes (Lorenzo et al., 2003). *ORA59*, a close paralogue of *ERF1* in *Arabidopsis*, has also been shown to integrate JA and ET signals in defense responses against *B. cinerea*. Overexpression line of ORA59 showed a severe dwarf phenotype under normal growth conditions, similar to plant overexpressing *ERF1* (Pré et al., 2008). RNAi-silencing of *ORA59* compromises JA- and ET-induced expression of several defense-related genes such as *PDF1.2*, *HEL,* and *ChiB* (Pré et al., 2008). Two more *ERF1*-like genes, *AtERF2* and *AtERF14* have shown to behave similarly as *ERF1* and *ORA59*. Constitutive overexpression of *AtERF2* or AtERF14 causes high levels of *PDF1.2* and *ChiB* gene expression in transgenic *Arabidopsis* plants (Brown et al., 2003; Oñate-Sánchez et al., 2007). In contrast to *ERF1*, *ORA59*, *AtERF2,* and *AtERF14*, AtERF4 (At3g15210) negatively regulates the expression of *PDF1.2* (McGrath et al., 2005). Loss-of-function mutants of *AtERF4* showed impaired induction of defense genes following exogenous ET treatment and increased susceptibility to *Fusarium oxysporum*. Moreover, the expression of other *ERF* genes such as *ERF1* and *AtERF2* depends on *AtERF14* expression (McGrath et al., 2005). Collectively, several of members of the ERF family negatively and positively control the expression of a number of defense genes mediated by jasmonates.

3.7. JA-signaling controlling anthocyanin accumulation and trichome development via transcription factors WD40/bHLH/R2R3-MYB complex

Current genetic and physiological evidence shows that JA regulates the activity of "WD-repeat/bHLH/MYB complex", which mediates anthocyanin accumulation and trichome initiation in a *COI1*-dependent manner. Overexpression of the MYB transcription factor MYB75 and bHLH factors such as GL3 (GLABRA 3) and EGL3 (ENHANCER OF GLABRA 3) restored anthocyanin accumulation and trichome initiation in the *coi1* mutant, respectively (Qi et al., 2011). Anthocyanin biosynthesis and trichome initiation are both inducible by JA (Maes et al., 2008; Qi et al., 2011). This induction requires both the JA receptor component COI1 and the GL3/EGL3/TT8-type bHLH proteins (Maes et al., 2008; Qi et al., 2011). Interestingly, the major JA signaling players, MYC2/MYC3/MYC4 are also involved in the JA-mediated anthocyanin accumulation (Lorenzo et al., 2004; Niu et al., 2011), but may not be required for trichome induction. bHLH factors GL3, EGL3 and TT8 (TRANSPARENT TESTA 8) function in complexes in which they interact directly with the

WD40 protein TTG1 (TRANSPARENT TEXTA GLABRA 1) and R2R3-MYB proteins such as MYB75 and GL1 (GLABRA 1). Apart from anthocyanin biosynthesis and trichome formation, GL3/EGL3/TT8 complex may be involved in many other processes including root hair formation, flavonoid biosynthesis, stomata patterning, and seed coat mucilage production (Pauwels and Goossens, 2011). JAZ-interacting domain (JID) was found in a number of bHLH transcription factors including MYC2/MYC3/MYC4, indicating that JAZs may have much wider function spectrum than currently known. Indeed, JID domain is present in GL3, EGL3 and TT8, and interaction of these proteins with eight different JAZs has been detected (Qi et al., 2011).

3.8. JA-Signaling regulates Male Fertility via Transcription Factors MYB21 and MYB24

JA was repeatedly shown to be essential for male fertility in *Arabidopsis*. Many JA biosynthesis and signaling mutants such as *dad1*, *fad3/7/8*, *lox3/4*, *aos*, *opr3*, and *coi1* are male sterile because of a combination of defective anther dehiscence, insufficient filament elongation, and severely reduced pollen viability (Browse, 2009). Transcriptome analysis of JA-treated stamens in *opr3* and wild type identified two R2R3 MYB proteins, MYB21 and MYB24, as key regulators of the stamen maturation processes triggered by JA (Mandaokar et al., 2006). Overexpression of *MYB21* in the *coi1-1* or *opr3* mutants could partially restore male fertility (Cheng et al., 2009), whereas the *myb21-1* knockout mutant had strong reduction of fertility that could not be rescued by exogenous JA (Mandaokar et al., 2006).

On the other hand, as the major JA-signaling components, JAZ proteins were found directly involved in stamen development. Overexpression of JAZ1ΔJas (Thines et al., 2007) and JAZ10.4 (Chung and Howe, 2009), both of which lack the full Jas domain and are resistant to degradation by SCFCOI1/26S proteasome, results in male sterility. However, the JAZ3 splice acceptor mutant *jai3-1*, which expresses *JAZ3* without the Jas domain (Chini et al., 2007) and JAZ10.3 (Yan et al., 2007), which lost a portion of Jas domain, are still fertile. This suggests a threshold level of JA signaling determines fertility. This notion was also strongly supported by the findings that COI1 leaky mutant allele coi1-16 is only partially male-sterile (Xiao et al., 2004). Interestingly, JAZ proteins were shown to regulate MYB21/MYB24, the transcription factors responsible for stamen and pollen maturation. A select set of JAZ proteins (JAZ1, JAZ8, and JAZ11) interact directly with MYB21 and MYB24, revealing a mechanism in which JA triggers COI1-dependent JAZ degradation to control MYB21 and MYB24 levels and thereby stamen development (Song et al., 2011). In addition, GA was found to promote JA biosynthesis in flower to control the expression of MYB21, MYB24, and MYB57 in the filament of the flower (Cheng et al., 2009).

3.9. JA Signal Interaction with GA, SA, Ethylene and ABA

As an important signal for plant development and defense, JA does not act independently but cooperatively with other phytohormonal signaling pathways including GA (gibberellin), SA (salicylic acid), Ethylene, and ABA (abscisic acid). For JA-GA interaction, a "relief of

repression" model has been proposed; in which DELLAs compete with MYC2 for binding to JAZ1 in *Arabidopsis*. Without GA, stabilized DELLA proteins bind to JAZ1 and release MYC2 to promote JA signaling. GA triggers degradation of DELLAs, which releases free JAZ1 to bind to MYC2 and, thus, attenuates JA signaling (Hou et al., 2010). Furthermore, GA significantly suppresses JA-activation of JA-responsive genes, whereas, GA alone does not significantly affect the expression of JA-responsive genes (Hou et al., 2010). This study suggested GA negatively regulates JA signaling. However, GA was found to mobilize the expression of DAD1, a key enzyme of JA biosynthesis in flowers. This is consistent with the observation that the JA content in the young flower buds of the GA-deficient quadruple mutant *ga1-3 gai-t6 rga-t2 rgl1-1* is much lower than that in the WT. The conclusion of these observervations suggests that GA promotes JA biosynthesis to control the expression of MYB21, MYB24, and MYB57, which are essential for male anther development (Cheng et al., 2009).

The mutually antagonistic interactions between SA and JA pathways were shown by analysis of SA- and JA-marker gene expression in SA and JA signaling mutants of *Arabidopsis*. JA-signaling mutant *coi1* displayed enhanced basal and inducible expression of SA marker gene *PR1*, while SA signaling mutant *npr1* (*non-repressor of pr genes 1*) showed concomitant increases in basal or induced levels of JA marker gene *PDF1.2* (Mur et al., 2006). Interestingly, exogenous SA promotes JA-dependent induction of defense gene *PDF1.2* when applied at low concentrations. However, at higher SA concentrations, JA-induced induction of *PDF1.2* is suppressed, suggesting the interaction between these pathways may be dose dependent (Mur et al., 2006). The antagonistic interaction between SA and JA is mediated by NPR1, the centrral regulator of SA signaling (Spoel et al., 2003). WRKY70 is a versatile transcription factor with roles in multiple signaling pathways and physiological processes. It regulates the antagonistic interactions between SA and JA pathways. Overexpression of WRKY70 leads to the constitutive expression of the SA-responsive *PR* genes and increased resistance to SA-sensitive pathogens but reduces resistance to JA-sensitive pathogens. In contrast, suppression of WRKY70 leads to increased expression of JA-responsive genes and increased resistance to a pathogen sensitive to JA-dependent defenses (Li et al., 2004). Another important negative regulator of SA signaling is MPK4. The *Arabidopsis mpk4* mutant exhibits increased SA levels, constitutive expression of *PR1*, and increased resistance to *P. syringae* in the absence of pathogen attack. In contrast, the JA-dependent induction of the *PDF1.2* gene was abolished in the *mpk4* mutant (Petersen et al., 2000).

A number of studies provide evidence for positive interactions between the JA and ET signaling pathways. For example, both JA and ET signaling are required for the expression of the defense-related gene *PDF1.2* in response to infection by *Alternaria brassicicola* (Penninckx et al., 1998). Evidence that JA and ET coordinatively regulate many other defense-related genes was obtained in an *A. thaliana* microarray experiment, which showed nearly half of the genes that were induced by ET were also induced by JA treatment (Schenk et al., 2000). Some evidence suggest also antagonistic interactions between the JA and ET defense pathways although a number of JA-specific or ET-specific genes were found in wounding and defense responses (Lorenzo et al., 2003). Crosstalk between JA and ET was found mediated through the physical interaction of JAZ proteins with ETHYLENE

INSENSITIVE3 (EIN3) and EIN3-LIKE1 (EIL1), two central positive transcription factors for the ET responses (Zhu et al., 2011). At least JAZ1, JAZ3, and JAZ9 can bind EIN3 and EIL1. Therefore, the JAZ proteins can repress the function of EIN3/EIL1, possibly by suppressing the DNA binding of EIN3 (Zhu et al., 2011). In the current model, ET is needed for EIN3/EIL1 stabilization and JA for EIN3/EIL1 release from the JAZ protein repression due to ubiquitin-mediated proteolysis, providing a reasonable explanation for the synergy in many ET/JA-regulated processes (Zhu et al., 2011). As we described above, MYC2, MYC3, and MYC4 are the key signaling players for JA signaling downstream JAZs are also required for ET signaling (Lorenzo et al., 2004). On the other hand, Overexpression of ET-responsive transcription factors ERF1 and ORA59 significantly activates JA responses (Lorenzo et al., 2003; Pré et al., 2008).

Very limited information for the interaction between JA and ABA is available so far. ABA and MeJA were reported to induce stomatal closure, most likely by triggering the production of reactive oxygen species (ROS) in stomatal guard cells (Munemasa et al., 2007). The *coi1* mutation suppresses only MeJA-mediated ROS production without influencing ABA-mediated ROS production, suggesting that *COI1*-dependent JA signaling acts through ABA pathway for stomatal closure (Munemasa et al., 2007). Anderson et al. (2004) showed that interaction between ABA and ethylene signaling is mutually antagonistic in vegetative tissues. Exogenous ABA suppressed both basal and JA/ethylene–activated transcription of defense genes. By contrast, ABA deficiency as conditioned by mutations in the *ABA1* and *ABA2* genes, which encode enzymes involved in ABA biosynthesis, resulted in up-regulation of basal and induced transcription from JA-ethylene responsive defense genes (Anderson et al., 2004).

4. THE physiological roles of JA in plant development and defense

4.1. JA is an essential signal for plant defense against insect herbivory

JA is one of the major defense hormones in plants (Browse, 2009), and it provides a major mechanism of induced defenses against insects herbivores and a wide spectrum of pathogen species, especially necrotrophic fungi. The defense property of jasmonates to various insect herbivores has been extensively studied in the past decades. To the best of our knowledge, there is no report supporting a negative role of jasmonates in defense against insect species. This topic has been covered by a number of excellent reviews (Farmer et al., 2003; Felton and Tumlinson, 2008; Browse, 2009; Howe and Jander, 2008). Here, we describe the major lines of evidence that point to the evolution, action and significance of JA as a defense hormone *in planta*. (1) Mechanical wounding or damage caused by herbivore feeding results in rapid accumulation of JAs at the site of wounding (Glauser et al., 2008). Successive feeding on the leaves causes steady increase of JA content throughout the entire plant (Reymond et al., 2004). (2) Wounding or exogenous application of JA/MeJA generally up-regulates the genes involved in JA biosynthesis (Mueller, 1997; Leon and Sanchez-Serrano, 1999). Most JA biosynthesis genes such as *LOX2*, *LOX*3, *LOX4*, *AOS*, *OPR3*, and *AOC3* and signaling genes such as *MYC2*, *JAZ1*, *JAZ2*, *JAZ5*, *JAZ6*, *JAZ7*, *JAZ8*, *JAZ9*, and *JAZ10* were found highly inducible in response to wounding, MeJA treatment, and herbivore feeding (Chung et al.,

2008). (3) Insect feeding or wounding induces hundreds of defense-related genes in JA-dependent manner, including genes involved in pathogenesis, indole glucosinolate metabolism, and detoxification (Reymond et al., 2004). (4) Insect feeding, wounding, or MeJA treatment activates synthesis of anti-insect substance, e.g., proteinase inhibitors (PIs) in *Arabidopsis* (Farmer et al., 1992), nicotine in tobacco, papain inhibitor(s) in tomato (Bolter, 1993), vinblastine in rose periwinkle (*Catharanthus roseus*), artemisinin in annual wormwood (*Artemisia annua*) (De Geyter et al., 2012), and poisonous secondary metabolites such as glucosinates and camalexin in *Arabidopsis*. (5) JA biosynthesis or perception mutants of *Arabidopsis* such as, *fad3-2 fad7-2 fad8*, *aos*, *opr3*, *jar1,* and *coi1,* as well as those from other species such as tomato *jar1*, and maize *opr7 opr8* are highly susceptible to insect attack (McConn et al. 1997; Laudert and Weiler, 1998; Stintzi et al., 2001; Staswick et al., 1998; Xie et al., 1998; Li et al., 2004; Yan et al., 2012). These JA mutants are shown to be compromised in resistance to a wide range of arthropod herbivores including caterpillars (*Lepidoptera*), beetles (*Coleoptera*), thrips (*Thysanoptera*), leafhoppers (*Homoptera*), spider mites (*Acari*), fungal gnats (*Diptera*), and mirid bugs (*Heteroptera*) (Howe and Jander, 2008). On the other hand, JA-pathway overexpression mutants such as *cev1*, cex1, and *fou2* are highly resistant to insect and pathogen attacks (Ellis and Turner, 2001; Xu et al., 2001; Bonaventure et al., 2007). (6) Exogenous application of JA or MeJA can elevate resistance of a number of plant species to insects attack (Avdiushko et al., 1997). The JA precursor OPDA also contributes to plant defense against insect attacks (Stintzi et al., 2001). (7) When attacted by herbivores, plants can rapidly release volatile organic compounds (VOC, consisting mainly of fatty acid-derived products and terpenes) and green leafy volatiles (GLV, including mainly of (*Z*)-3-hexenal, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate). These can effectively induce direct defense response — activation of JA biosynthesis pathway in the attacked and neighboring plants, and indirect defense response — attraction of insect enemies that parasitize or prey on feeding insects (Paré and Tumlinson, 1999; Engelberth et al., 2004).

4.2. The roles of JA in induced systemic resistance (ISR) against microbial pathogens

JA is an essential phytohormone for defense response against a wide spectrum of pathogens, alone or in combination with other hormones, such as ET, SA, and ABA (Browse, 2009; Adie et al., 2007). Although all plant hormones including GA, auxin (IAA), and brassinosteroids (BR) may be involved in plant defense responses against pathogens (Smith et al., 2009), numerous studies have shown that SA, JA, and ET are the major players in induced resistance of plants (Dong, 1998; Kunkel and Brooks, 2002). The SA-mediated pathway is typically activated in response to pathogens and mediates the initiation of a hypersensitive response (HR) and induction of pathogenesis-related proteins (PRs) that confer systemic acquired resistance (SAR) against a broad array of pathogens (Smith et al., 2009). Rhizobacteria-mediated induced systemic resistance (ISR) in plants primarily depends on JA and ET (Pieterse et al., 1998). Regarding the relationship of JA with ET, the widely held belief is that ET acts synergistically with JA in the activation of responses to pathogens (Lorenzo and Solano, 2005). Several defense-related genes including *PR1*, *PR3*, *PR4*, *PR5,* and *PDF1.2* are synergistically induced by JA and ET (Lorenzo et al., 2003). Exogenous

application of JA and ET can activate expression of genes in both JA biosynthesis and signaling pathway (Chung et al., 2008; Pré et al., 2008). As to SA interaction with JA/ET, either to biotrophic or necrotrophic pathogens, SA was suggested to act mutually antagonistically with JA/ET pathways (Lorenzo and Solano, 2005).

Considering only the role of JA, it has been frequently demonstrated to be an indispensible phytohormone signal for resistance/susceptibility to several diseases caused by fungal, bacterial, and viral pathogens.

1. **JA mediates resistance of plant to necrotrophic pathogens such as *Botrytis cenerea* and *Alternaria brassicicola***. JA perception mutant *coi1* display enhanced susceptibility to *B. cenerea* and *A. brassicicola* (Thomma et al., 1998) and JA biosynthesis mutant *aos* as well as signaling mutant *coi1* is also highly susceptible to *B. cenerea* (Rowe et al., 2010). Interestingly, there are exceptions that JA negatively regulates resistance to necrotrophic fungi. For example, JA signaling mutant *jin1/jai1*, which acts downstream of *COI1* in JA signaling pathway, showed higher resistance to necrotrophic fungi such as *B. cinerea* and *Plectosphaerella cucumerina* (Lorenzo et al., 2004). JA signaling mutant *coi1* displayed enhanced resistance to necrotrophic fungus *Fusarium oxysporium* (Thatcher et al., 2009).
2. **JA biosynthesis mutants show extremely high susceptibility to soil-borne oomycete *Pythium* spp.** *Arabidopsis* fatty acid desaturase triple mutant *fad3-2 fad7-2 fad8*, deficient in biosynthesis of the JA precursor linolenic acid, is more susceptible to the root pathogen *Pythium mastophorum*; 90% of the triple mutant plants did not survive the infection as compared to only 10% of wild-type plants (Vijayan et al., 1998). Exogenously applied MeJA reduced death rate of *fad3-2 fad7-2 fad8*. Another *Arabidopsis* JA mutany *jar1* (*jasmonic-acid resistant 1*), shows reduced sensitivity to jasmonates and deficient JA signaling (Staswick et al., 1998). Both *fad3-2 fad7-2 fad8* and *jar1* plants exhibit enhanced susceptibility to *Pythium irregular*e (Staswick et al., 1998). In maize, the double mutant *opr7 opr8*, deficient in JA biosynthesis, showed extreme susceptibility to *Pythium aristosporium* (Yan et al., 2012). When tarnsfered to a field from sterile soil *opr7 opr8* plants displayed "wilting" phenotype due to root rots 6 d after the transfer (Figure 10H and 10I), and 11 days after transplanting, all the *opr7 opr8* plants died, while wild-type plants continued to display normal growth (Yan et al., 2012).
3. **JA signaling promotes pathogenesis of biotrophic pathogens**. Resistance of host plants against fungal and bacterial biotrophic pathogens is associated with activation of SA-dependent signaling and SAR. As SA and JA/ET signaling tend to be mutually inhibitory, JA/ET signaling is expected to have negative effects on resistance to these pathogens. Results from studies of *Peronospora parasitica*, *Erysiphe* spp., and P*seudomonas syringae* support the idea that SA signaling is important for resistance against biotrophs. In the cases of *P. parasitica* and *Erisyphe* spp., JA/ET-dependent responses do no seem to play a major role because infection does not induce JA/ET pathways. However, JA/ET signaling may also be effective if activated artificially (Glazebrook, 2005). In the case of *bacterial biotroph P.syringae,* SA-dependent defense responses clearly play an important role in limiting *P.syringae* growth. Mutants with defects in SA signaling, including *eds1*, *pad4*, *eds5*, *sid2*, and *npr1*, show enhanced susceptibility to virulent strains and in some

cases, avirulent strains. *P. syringae* DC3000 inhibits SA signaling by producing a toxin called coronatine, which imitates JA-Ile (a bioactive JA-amino acid conjugate). The coronamic acid moiety of coronatine structurally resembles ACC (the ET precursor, aminocyclopropane carboxylic acid). Resistance of JA insensitive mutant *coi1* to *P. syringae* DC3000 is associated with elevated levels of SA and enhanced expression of SA-regulated genes, suggesting that coronatine contributes to virulence by activating JA signaling, thereby repressing SA-dependent defense mechanisms that limit *P. syringae* growth (Glazebrook, 2005).

4. **JAs may have positive roles in plant resistance against viruses.** Members of the geminivirus family are plant viruses with circular, single-stranded DNA genomes that infect a wide range of plant species and cause extensive yield losses in important crops such as tomato, maize, and cotton. In *Arabidopsis*, exogenous application of jasmonates reduces susceptibility to geminivirus infection (Lozano-Durán et al., 2011). In a case of turnip crinkle virus (TCV), SA, but not JA/ET, is required for the development of hypersensitive reaction (HR) and systemic resistance in *Arabidopsis* (Kachroo et al., 2000). However, applying 60 μM JA and then 100 μM SA 24 h later, enhanced resistance to *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), and TCV in *Arabidopsis*, tobacco, tomato, and hot pepper (Shang et al., 2011), indicating JA and SA have additive positive effects to resistance against RNA viruses.
5. **JA positively regulates resistance against parasitic plants.** In plant-parasitic plants interaction, both JA and SA were found to positively regulate host-plant defense responses to parasitic plants (Bar-Nun et al., 2008; Runyon et al., 2010). The holoparasitic plant, *Orobanche aegyptiaca*, is capable of infecting many host plants, including *Arabidopsis thaliana*. Low dose exposure to MeJA or methyl salicylic acid (MeSA) effectively induced resistance of *Arabidopsis* seedlings to *O. aegyptiaca* (Bar-Nun et al., 2008). Runyon et al. (2010) reported that the parasitic plant *Cuscuta pentagona* grew larger on mutant tomato plants, in which the SA (*NahG*) or JA (*jin1*) pathways were disrupted, suggesting that these hormones can act independently to reduce parasite growth. Large increases of both JA and SA were detected in host plant tomato after parasitism was established (*i.e.*, haustoria formation) (Runyon et al., 2010). Host production of JA was transitory and reached a maximum at 36 hr, whereas SA peaked 12 hr later and remained elevated 5 d later (Runyon et al., 2010).
6. **JA is a key player in induced systemic resistance against root knot nematodes (RKN)**. Foliar application of JA induces a systemic defense response that reduces avirulent nematode reproduction on susceptible tomato plants. JA enhances *Mi*-mediated resistance (*Mi* is a resistant gene in tomato) of resistant lines at high temperature (Cooper et al., 2005). However, using JA-signaling mutant *jar1* (equal to *coi1* of *Arabidopsis*) and JA biosynthesis mutant *def1*, it was found that endogenous JA signaling pathway is required for tomato susceptibility to RKNs (Bhattarai et al., 2008).

4.3. JAs serve an important role against abiotic stresses

Salinity is one better studied abiotic stress in plants. Foliar application of MeJA can effectively alleviate salinity stress symptoms in soybean seedlings (Yoon et al., 2009). In

grapevine, exogenous jasmonate can rescue growth in the salt-sensitive cell line and salt stress response is modulated by JA-signaling components such as JAZ proteins (Ismail et al., 2012).

JA plays import role of plant resistance against ozone (O_3) stress. JA biosynthesis mutant *fad3/7/8* and JA-signaling mutant *jar1* have greater sensitivity to O_3 (Rao et al., 2000). Furthermore, MeJA pretreatment decreased O_3-induced H_2O_2 content and SA concentrations and completely abolished O_3-induced cell death (Rao et al., 2000).

4.4. JAs are required signals for pollen development in dicots and for sex determination in monocots

In addition to defense function, jasmonate was frequently shown to serve essential roles in reproductive processes of plants. In *Arabidopsis*, JA biosynthesis mutants such as *fad3/7/8*, *aos, opr3,* and JA signaling mutant *coi1* are male sterile, strongly supporting JA as an essential signal for development of male organ of bisexual flowers (Browse, 2009). This JA-dependent male sterility phenotype consists of three characteristics: 1) the anthers of mutants lost dehiscence to shed pollen at flowering time; 2) the pollen grains in the anthers are predominantly (>97%) inviable; 3) the stamen filaments are substantially shorter in mutant, that is, the anthers do not elongate sufficiently to the stigma level (Browse, 2009). The fertility defective phenotype of JA biosynthetic mutants is rescued by exogenous JA treatment; however, the signaling mutant *coi1* cannot be rescued by JA application (Browse, 2009). Some JA signaling components have also been implicated in stamen development. *MYB21* and *MYB24* are JA-responsive transcription factors in *opr3* stamens and were isolated as JA-dependent transcription factors for flower development (Mandaokar et al., 2006). A *myb21* mutants exhibited shorter anther filaments, delayed anther dehiscence, and greatly reduced male fertility. A *myb24* mutants was phenotypically wild type, but creation of a *myb21myb24* double mutant indicated that introduction of the *myb21* mutation exacerbated all three aspects of the *myb24* phenotype. Exogenous jasmonate could not restore fertility to *myb21* or *myb21myb24* mutant plants. All these results indicate that *MYB21* and *MYB24* are JA signaling components mediating JA response during stamen development (Mandaokar et al., 2006).

In addition, overexpression of Jas domain-defective JAZ proteins such as JAZ1-ΔJas and JAZ10.4 which are resistant to SCF^{COI1}/26S proteolysis complex resultes male sterile phenotypes, further demonstrating that JA is essential for male fertility in *Arabidopsis* (Thines et al., 2007; Chung and Howe, 2009). However, some JA signaling mutants are male fertile instead of sterile. For example, *myc2* mutant does not show a male-sterile phenotype (Lorenzo et al., 2004); *jar1* mutant is male fertile (Staswick and Tiryaki, 2004). In these mutants, it may be possible that an alternative component or ligand for JA signaling exists. Surprisingly, in contrast to *Arabidopsis*, JA perception mutant *jar1* of tomato (*Solanum lycopersicum*) are male-fertile but female-sterile (Li et al., 2004), suggesting that JA roles in reproductive differentiation of plants largely depend on the species.

Like *Arabidopsis*, the monocotyledonous plant rice bears bisexual flowers. The JA-deficient mutant of rice *hebiba* showed male sterility (Riemann et al., 2003), supporting that JA is

required for male organ formation of bisexual flower plants. Maize is another monocot and belongs to monoecious plants, which bears distinct male inflorescence (called tassel) and female inflorescence (called ear) on the same plant. The monosexual florets in the tassel or the ears develop from bisexual floret primordia of top or axillary meristems though a sex determination program mediated by a number of sex-determining genes (Bortiri and Hake, 2007). Recent study on *ts1* (*tasselseed1*), a mutant in which male inflorescence (tassel) becomes female-fertile structure that can be pollinated to bear seeds, showed that jasmonates is an essential phytohormone that initiates sex determination program of tassel (Acosta et al., 2009). In our recent study, the JA-deficient mutant *opr7 opr8* showed 100%-feminized tassel, strongly supporting the JA signal requirement for tassel formation in maize (Figure 10A and 10B) (Yan et al., 2012). *TS1* encodes a 13-lipoxygenase (i.e. *LOX8*), disruption of which causes JA-deficiency locally in the tassel meristem. *opr7 opr8* is a double mutant of OPR isoforms required for JA biosynthesis, mutation of which results in JA depletion systemically in the plant. Several studies have showed that gibberellin (GA) is involved in ear formation. GA biosynthesis mutants such as *an1*, *d1*, *d2*, *d3,* and *d5* and GA perception mutants *D8* and *D9*, all showed dwarfism and masculinized ears (i.e. male florets are produced in ears), indicating GA is another important phytohormone for sex determination in maize (Chuck, 2010). Putting the studies of JA and GA together, we may hypothesize that JA and GA act antagonistically in male and female flowers, respectively, in maize sex determination process.

4.5. JAs has a role in female organgenesis in some plant species

In tomato, JA signaling mutant *jar1* (the ortholog of *coi1*) showed seed-bearing sterility: 1) the size and mass of mature ripened *jai1-1* fruit were significantly less than those of mature wild-type fruit; 2) vast majority (>99%) of fertilize ova of the mutant fruit were not viable during the fruit development and only a few viable seeds were recovered from the fruit. It is estimated that the number of viable seeds produced by *jai1-1* plants was <0.1% of the viable seed yield from wild-type plants grown under identical conditions (Li et al., 2004). In maize, JA-deficient mutant *opr7 opr8* showed outgrowth of multiple female reproductive buds and extreme elongation of ear shanks, indicating JA is a crucial signal for female organ growth (Figure 10C and 10D) (Yan et al., 2012).

4.6. JAs regulate vegetative growth

Activation of JA defense signaling against biotic and abiotic stresses depletes available resources and severely restricts plant growth. It is well known that JAs act in plant as growth inhibitors in root and shoots (Staswick et al., 1992). Wound-induced accumulation of endogenous JAs strongly suppresses plant growth of roots and shoots by inhibiting cell mitosis (Zhang and Turner, 2008). The inhibition role of JAs depends on JA signaling pathway. JA perception mutant *coi1* relieved JA inhibition to roots and shoots (Xie et al., 1998). JA-signaling mutants such as *jin1*/*myc2*, *jin4*/*jar1*, and *jai3* have largely reduced growth inhibition to roots and leaves by JA application (Lorenzo et al., 2004). JA signal

integrates other plant hormones including CK (cytokinins), GA, IAA, ABA, and ET to regulate growth processes and defense responses (Sano et al., 1996; Cheng et al., 2009; Nagpal et al., 2005; Anderson et al., 2004; Lorenzo et al., 2003).

4.7. JA involved in trichome development

Trichomes are branching structures or hair-like appendages differentiated from epidermal cells in the aerial part of plant, which function as barriers to protect plants against herbivores, insects, abiotic damage, UV irradiation, and excessive transpiration (Ishida et al., 2008). Trichome formation is initiated by various environmental cues, such as wounding and insect attack (Yoshida et al., 2009), and by different endogenous developmental signals, including phytohormones, such as jasmonate (Traw and Bergelson, 2003; Li et al., 2004; Yoshida et al., 2009), gibberellin (Perazza et al., 1998), ethylene (Plett et al., 2009), and salicylic acid (Traw and Bergelson, 2003). Tomato JA perception mutant *jar1* has no trichomes on the surface of young fruit and significantly less on the leaf and stem surfaces (Li et al., 2004). JA biosynthesis mutant *aos* and perception mutant *coi1* produced fewer trichomes than the wild type and MeJA treatment increases trichome density in *aos* but not *coi1*, indicating JA signal is a positive regulator of trichome development in *Arabidopsis* (Yoshida et al., 2009). JA signal controls trichome patterning in *Arabidopsis* via a key transcription factor GRABRA3 of which JA treatment enhanced expression prior to trichome initiation (Yoshida et al., 2009). GRABRA3 interacts with other transcription factors such as TRANSPARENT TESTA GLABRA1 (TTG1) and GLABRA1 (GL1) to control trichome initiation (Yoshida et al., 2009). Furthermore, a recent study showed that JAZ proteins interact with these transcription factors to regulate trichome development (Qi et al., 2011).

4.8. JAs promote fruit/seed ripening

JA perception mutant *jar1* of tomato bears much smaller fruits compared with wild type, and the young seeds of the mutant fruits suffer from high rate of seed abortion (>99%), indicating that JA signal is an essential signal during the early stage of fruit development and seed maturation in tomato (Li et al., 2004). In apples (*Malus sylvestris*) and sweet cherries (*Prunus avium*), endogenous JA accumulated in the early ripening stage of the fruit and seeds, also indicating that JA plays an important role in fruit/seed development (Kondo et al., 2000).

4.9. JAs act as an internal signal facilitating leaf senescence

Leaf senescence involves senescence-associated cell death (PCD), which is controlled by age under the influence of endogenous and environmental factors (Lim et al., 2007). Several phytohormones including JA, cytokinins, ethylene, ABA, and SA were implicated in leaf senescence program (Lim et al., 2007). Regarding the role of JA in leaf senescence, most studies support that JA positively regulates leaf senescence process (Ueda and Kato, 1980; He et al., 2002; Schenk et al., 2000; Castillo and León, 2008). Senescence-like phenotypes are induced by exogenous application of MeJA or JA in *Artemisia absinthium* or *Arabidopsis* (Ueda and Kato, 1980; He et al., 2002); and some senescence-up-regulated genes such as

SEN1, *SEN4*, *SEN5*, *SAG12*, *SAG14*, and *SAG15* are responsive to JA treatment (He et al., 2002; Schenk et al., 2000). Delayed yellowing phenotype during natural senescence and upon dark incubation of detached leaves was observed in JA biosynthesis mutant *kat2* and signaling mutant *coi1* (Castillo and León, 2008). Casting doubt about the role of JA in senescence, JA-defective mutants *aos* and *opr3* senesced similar ro wild type under natural senescence conditions or upon dark treatment (He et al., 2002; Schommer et al., 2008). In maize, strong genetic evidence was obtained for JA involvement in the leaf senescence (Yan et al., 2012). The leaves of JA-deficient mutant *opr7 opr8* displayed senesced substantially later than wild type (Figure 10G).

4.10. JAs activate secondary metabolism beneficial to development and defense

Secondary metabolites play diverse roles in plants. For example, flowers synthesize and accumulate anthocyanin pigments in petals to attract pollinating insects. In addition, anthocyanins absorb visible as well as UV radiation and are effective antioxidants and scavengers of reactive oxygen species, protecting plant tissues from the effects of excess incidental visible or UV-B radiation and oxidative stress (Quina et al., 2009). Other secondary metabolites such as polyamines, quinones, terpenoids, alkaloids, phenylpropanoids, and glucosinolates act as phytoalexins to protect plants against microorganisms or herbivores (Chen et al., 2006).

Gaseous MeJA enhance production of anthocyanins in soybean seedlings (Franceschi and Grimes, 1991). Wounding or MeJA treatment activates rapidly the expression of anthocyanin biosynthesis genes and increase anthocyanin level in the detached corolla of *Petunia hybrida* (Moalem-Beno *et al*., 1997). In *Arabidopsis*, JA or MeJA treatment strongly enhances anthocyanin accumulation in the shoots, especially in the petiole of the seedling (Lorenzo et al., 2004) and this JAs-activating anthocyanin accumulation depends on COI1-mediated JA signaling pathway (Shan et al., 2009). JA induces anthocyanin biosynthesis via up-regulation of the 'late' anthocyanin biosynthetic genes *DFR*, *LDOX*, and *UF3GT* (Shan et al., 2009). JA coincidently activates anthocyanin biosynthetic regulators such as transcription factors *PAP1*, *PAP2*, and *GL3* (Shan et al., 2009). Either these biosynthetic genes or transcription factors are *COI1*-dependent (Shan et al., 2009). In the monocot plant maize, *opr7opr8* double mutant lack anthocyanin pigmentation in brace roots and auricles (Figure 10E and 10F), but not in leaf blade or sheath, indicating that endogenous JA controls anthocyanins pigmentation in specific tissues of maize (Yan et al., 2012).

JAs also effectively activate defensive metabolites against insects or pathogens. Early studies concluded that MeJA application strongly induced anti-insect protein accumulation such as proteinase inhibitors I and II (PI-I, II) (Farmer et al., 1992) and vegetative storage protein (VSP) (Liu et al., 2005). Nicotine, an alkaloid toxic to most insects by interfering with the transmitter substance between nerves and muscles, widely exists in tobacco (*Nicotiana tabacum*) and related species. Exogenous application of JA or wounding of leaves activate nicotine biosynthesis in a *COI1*- and *MYC2*-dependent manner, indicating JA signal is required in tobacco to control nicotine metabolism (Shoji et al., 2008; Shoji and Hashimoto,

2011). In *Arabidopsis*, camalexin (3-thiazol-2'yl-indole) is the main phytoalexin induced by a variety of microorganisms including bacteria, fungi, and oomycetes. JA signaling is required for the activation of camalexin synthesis in response to infection by *P. syringae* pv. *maculicola* ES4326 (Zhou et al., 1999). Glucosinolates are a group of thioglucosides found in all cruciferous plants such as *Arabidopsis* and *Brassica napus*. The hydrolysed products of glucosinolates contribute to plant defense against microorganisms. MeJA treatment increases total glucosinolate content in leaves of *B. napus* up to 20 fold (Doughty et al., 1995). In *Arabidopsis*, accumulation of camalexin and indole glucosinolates can be trigged by elicitors from the plant pathogen *Erwinia carotovora*, and this induction effect is *COI1*-dependent (Brader et al., 2001). There are also a number of examples that jasmonates effectively activate secondary metabolites in medicinal plant species such as artemisinin synthesis in *Artemisia annua* and vinblastine (an alkaloid) in *Catharanthus roseus* (De Geyter et al., 2012). All pathways of the above metabolites, including nicotine, camalexin, glucosinolates, artemisinin, and vinblastine belong to JA-elicited plant secondary metabolism, which is regulated by JA-signaling components such as *COI1*, *MYC2*, *ERF1* and *JAZs* (De Geyter et al., 2012).

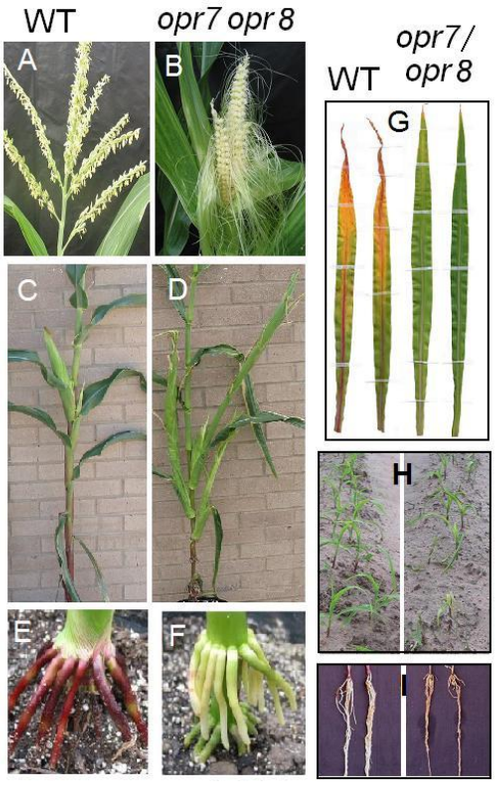

Figure 10. Genetic morphological phenotypes of JA-deficient mutant in maize
(A) Tassel of wild type. (B) Feminized tassel structure of JA deficient mutant *opr7 opr8*. (C) The ear of wild type. (D) Multiple elongated ears of *opr7 opr8*.(E) Anthocyanins accumulation in the brace roots of wildtype. (F) Lack anthocyanins pigmentation of *opr7 opr8* brace roots. (G) The senescence phenotype of third leaves of wild type (left) and *opr7 opr8* (right). (H) & (I) *opr7 opr8* (right) is highly susceptible to *Pythium aristosporum* compared with wild type (left).

5. Conclusion

Our understanding of the biosynthesis, regulation, and signaling mechanisms of jasmonates has increased substantially in the last few years. JA biosynthesis enzymes showed 'self-activation', in which the final product, JA, positively regulate the enzyme activity of this pathway. Currently, only (+)-7-iso-jasmonoyl-L-Ile has been conclusively shown to function as the bioactive ligand to JA signaling machinery SCF^{COI1}/JAZs complex. The molecular mechanism of JA signal perception and transduction was found to mimic many aspects of the auxin signaling process. In the presence of low levels of JA, JAZ proteins repress the expression of JA-responsive genes by interacting directly with the bHLH (basic helix-loop-helix) transcription factors MYC2, MYC3, and MYC4, which are positive regulators of JA responses. When JA levels increased, the bioactive form of ligand JA-Ile promotes binding of JAZs to SCF^{COI1} to form SCF^{COI1}-JAZ-JA-Ile reception complex and subsequent degradation of JAZ repressors via the ubiquitin/26S proteasome pathway, resulting in derepression of primary response genes. A number of recent studies found a wide spectrum of JA functions in plant including the regulation of developmental and defense processes, such as, resistance against insects and pathogens, root growth, fruit/seed maturation, leaf senescence, anthocyanin pigmentation, sex determination (of monoecious plant), female or reproductive organ formation.

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