Developmental Cell
Article

Cooperation and Functional Diversification of Two Closely Related Galactolipase Genes for Jasmonate Biosynthesis

Youbong Hyun,[1] Sungwook Choi,[1] Hyun-Ju Hwang,[1] Jihyeon Yu,[1] Sang-Jip Nam,[2] Jaeyoung Ko,[2] Ju-Young Park,[3] Young Sam Seo,[4] Eun Yu Kim,[4] Stephen Beungtae Ryu,[5] Woo Taek Kim,[4] Yong-Hwan Lee,[3] Heonjoong Kang,[2] and Ilha Lee[1,6,*]
[1]National Research Laboratory of Plant Developmental Genetics, Department of Biological Sciences, Seoul National University, Seoul 151-742, Korea
[2]School of Earth and Environmental Sciences, Seoul National University, Seoul 151-747, Korea
[3]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea
[4]Department of Biology, College of Science, Yonsei University, Seoul 120-749, Korea
[5]Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Ochang, Chungbuk 363-883, Korea
[6]Global Research Laboratory for Flowering at SNU and UW, Seoul 151-742, Korea
*Correspondence: ilhalee@snu.ac.kr
DOI 10.1016/j.devcel.2007.11.010

SUMMARY

Jasmonic acid (JA) plays pivotal roles in diverse plant biological processes, including wound response. Chloroplast lipid hydrolysis is a critical step for JA biosynthesis, but the mechanism of this process remains elusive. We report here that *DONGLE* (*DGL*), a homolog of *DEFECTIVE IN ANTHER DEHISCENCE1* (*DAD1*), encodes a chloroplast-targeted lipase with strong galactolipase and weak phospholipase A_1 activity. *DGL* is expressed in the leaves and has a specific role in maintaining basal JA content under normal conditions, and this expression regulates vegetative growth and is required for a rapid JA burst after wounding. During wounding, *DGL* and *DAD1* have partially redundant functions for JA production, but they show different induction kinetics, indicating temporally separated roles: *DGL* plays a role in the early phase of JA production, and *DAD1* plays a role in the late phase of JA production. Whereas *DGL* and *DAD1* are necessary and sufficient for JA production, phospholipase D appears to modulate wound response by stimulating *DGL* and *DAD1* expression.

INTRODUCTION

Jasmonic acid (JA) and its derivatives, collectively referred to as the jasmonates, are lipid-derived plant hormones that are ubiquitous throughout the plant kingdom. These compounds play pivotal roles during plant developmental processes, such as seed maturation, viable pollen production, root growth, and tendril coiling, and they also function as important signaling molecules in plant defense responses to biotic and abiotic stress (Creelman and Mullet, 1997; Li et al., 2004). JA is synthesized from α-linolenic acid, which is released from membrane lipids, via the so-called octadecanoid pathway that involves enzymes located in two different subcellular compartments—the chloroplast and the peroxisome (Turner et al., 2002). In the chloroplasts, released α-linolenic acid is transformed to OPDA (12-oxo-phytodienoic acid) by a series of enzymatic reactions involving chloroplast-localized lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) (Bell et al., 1995; Feussner et al., 1995; Maucher et al., 2000; Ziegler et al., 2000; Froehlich et al., 2001; Stenzel et al., 2003). The resulting OPDA is then transferred to the peroxisome, where it is converted to JA by OPDA reductase 3 (OPR3), an acyl-activating enzyme (OPCL1), and three cycles of β-oxidation (Stintzi and Browse, 2000; Li et al., 2005; Koo et al., 2006). The F-box protein CORONATIN INSENSITIVE 1 (COI1) is a JA signaling component, and most JA-dependent gene regulation is also dependent on the activities of COI1 (Xie et al., 1998). Recent reports showed that SCF^{COI1} ubiquitin ligase binds to and degrades members of the jasmonate ZIM domain (JAZ) protein family, which are repressors of JA signaling, in response to JA, and that the binding of SCF^{COI1}-JAZ is promoted by a JA-isoleucine (JA-Ile) conjugate (Thines et al., 2007; Chini et al., 2007). This indicates that COI1 is a JA receptor and that JA-Ile is the active signal in the JA signaling pathway.

A proportional response to wounding is critical for plants to cope with their biotic/abiotic environments. Various responses related to defense and wound healing are induced by mechanical wounding and insect herbivory (Reymond et al., 2000; Kessler and Baldwin, 2002; Arimura et al., 2005). To evoke such a wide variety of responses, global changes in gene-expression profiles are regulated by wound-induced JA and its cyclopentenone precursor, OPDA (Reymond et al., 2000; Taki et al., 2005). To date, however, the precise mechanism by which JA is produced as a response to wounding is still unclear. Laudert et al. (2000) reported that overexpression of the *Arabidopsis AOS* gene in tobacco or *Arabidopsis* does not alter the basal level of JA; after wounding, however, these transgenic plants produce a higher level of JA than the wild-type, suggesting that wound-induced JA production is determined by the supply of substrate to AOS rather than by the amount of AOS. Consequently, the

release of α-linolenic acid has been regarded as a committed step in JA production in response to wounding. Other studies in *Arabidopsis* and tomato plants suggest that lipid-hydrolyzing enzymes, including phospholipase A (PLA) and phospholipase D (PLD), act as crucial components for JA production during wound response (Ryu and Wang, 1998; Wang et al., 2000; Ishiguro et al., 2001).

The *Arabidopsis* DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1) protein is a chloroplastic phospholipase that catalyzes the production of α-linolenic acid from chloroplast membrane lipid (Ishiguro et al., 2001). The mutant *dad1* is defective in anther dehiscence, pollen maturation, and flower opening, but the overall morphology of the *dad1* mutant is almost the same as that of the wild-type. The expression of *DAD1* is normally restricted to flowers, and the *dad1* mutant has reduced levels of JA in flower bud clusters. However, wounding causes a rapid induction of *DAD1* expression in the leaves, and the kinetics of this expression correlates well with the kinetics of JA accumulation (McConn et al., 1997; Wang et al., 2000; Ishiguro et al., 2001; Stintzi et al., 2001). Based on these studies, it has been proposed that *DAD1* contributes to the wound induction of JA biosynthesis. However, the levels of JA accumulation after wounding in *dad1* mutants and the wild-type were similar (Ishiguro et al., 2001), thereby leaving open the question of which components provide α-linolenic acid for JA production after wounding. Two possible candidates have been proposed. First, the presence of other chloroplast-localized lipases (*At*PLA$_1$-I family) homologous to DAD1 in the *Arabidopsis* genome (Ishiguro et al., 2001; Ryu, 2004) suggests that functional redundancy may exist among members of this family for wound-inducible JA accumulation. However, there is as yet no empirical evidence supporting this hypothesis. Second, phosphatidic acid (PA) produced by PLD may function as a precursor of α-linolenic acid. Supportive of this hypothesis, PA levels are rapidly increased by wounding prior to the accumulation of α-linolenic acid and JA (Ryu and Wang, 1998; Lee et al., 1997; Wang et al., 2000), and the suppression of *PLDα1* by antisense RNA in *Arabidopsis* results in the reduced accumulation of α-linolenic acid and JA during the wound response (Wang et al., 2000). Consistently, although the transcription of *PLDα1* is not changed, the translocalization of PLDα1 from the cytoplasm to the plasma membrane is stimulated by wounding; as such, the latter can be regarded as a mechanism of enzyme activation. However, the actual participation of PA as a precursor of α-linolenic acid has not been confirmed experimentally, and the identities of enzymes catalyzing the conversion of PA to α-linolenic acid have been elusive. In addition, several recent studies have suggested that PA acts as a secondary signaling molecule in various biological processes (Wang, 2002; Bargmann and Munnik, 2006).

In the present study, we identified a new, to our knowledge, component of the chloroplast-targeted lipase required for JA biosynthesis, *DONGLE* (*DGL*). *DGL* is a member of the *AtPLA$_1$-I* family and is expressed in vegetative tissues in the absence of wounding. We show that DGL has galactolipase as well as PLA$_1$ enzymatic activity. DGL is required for the production of basal levels of endogenous JA, which regulates seedling growth and is involved in the rapid JA burst after wounding. We also show that wound-induced JA production is dependent on the function of *DGL* and *DAD1*, and that PLDα1 may provide a secondary signaling molecule for the wound-inducible expression of *DGL* and *DAD1*.

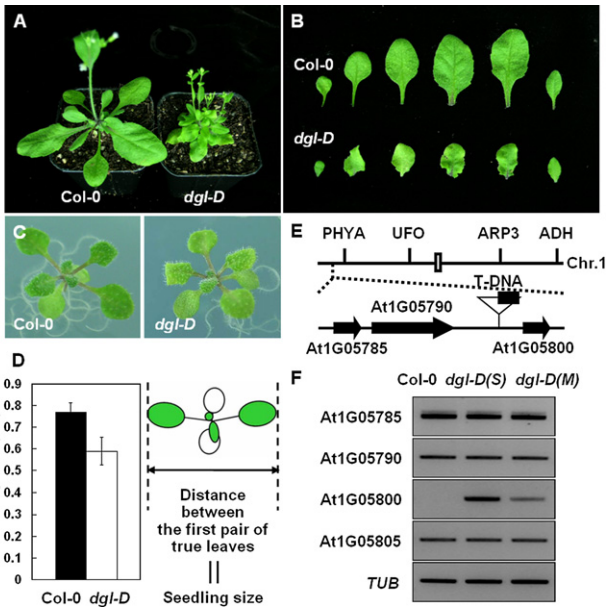

Figure 1. Phenotypes and Molecular Characterization of *dgl-D*

(A) Morphological phenotypes of wild-type and *dgl-D* mutants grown for 25 d.

(B) Leaf morphologies of 25-day-old plants.

(C) Phenotypes of 9-day-old seedlings. A short petiole and round-shaped leaves are visible in *dgl-D* mutants.

(D) Comparison of size between 9-day-old seedlings of the wild-type and the *dgl-D* mutant. A schematic diagram of a 9-day-old seedling is depicted in the right panel. White and green circles represent cotyledons and true leaves, respectively. Values are mean ± SD from three experiments. n > 30/experiments.

(E) T-DNA insertion locus in *dgl-D*. The white box in the chromosome and the black box in T-DNA are the centromere and four copies of *35S* enhancers, respectively.

(F) Overexpression of At1G05800 in *dgl-D*. Expressions of genes adjacent to the T-DNA insertion locus were analyzed by RT-PCR. *dgl-D*(S) and *dgl-D*(M) refers to plants showing the strong and mild phenotype, respectively. Expression of *tubulin* (*TUB*) is used as a quantitative control.

RESULTS

Overexpression of *DONGLE* Causes the Dwarf Phenotype

Mutant plant lines showing developmental and growth defects were obtained by activation-tagging mutagenesis on a Columbia-0 (Col) ecotype of *Arabidopsis thaliana* (Weigel et al., 2000). One mutant exhibiting a dominantly inherited dwarf phenotype was isolated among 40,000 T1 transformants and was designated as *dgl-D* on the basis of its round-leaf morphology (*dgl-D*; "*dongle*" means "round-shaped" in Korean) (Figure 1A). The *dgl-D* mutant was smaller than the wild-type; in particular, the leaves were smaller due to the reduced expansion, with the reduction being more severe in the length than in the width (Figure 1B). The reduction in overall size was detectable in 9-day-old seedlings as a shorter distance between the first pair of two true leaves (Figures 1C and 1D). The *dgl-D* mutant also showed a defect in apical dominance that resulted in the simultaneous generation of multiple inflorescences (Figure 1A). The mutant

flowers produced short anthers, which often resulted in pollination failure, but the pollen grains were viable and fertile, thus producing viable seeds (data not shown).

The segregation ratio of 3:1 for Basta resistance (308:90, resistant:sensitive progenies; $\chi^2 = 0.27$, $p > 0.60$) in the F_2 population and the detection of a single-copy T-DNA in genomic Southern blot hybridization analysis (Figure S1B, see the Supplemental Data available with this article online) showed that a T-DNA insertion into a single locus caused the *dgl-D* phenotype. Plants with this mutant phenotype were observed only among Basta-resistant progenies, although the strength of the mutant phenotype decreased or disappeared in some Basta-resistant progenies in successive generations (Figure S1A). This suppression of the phenotype was due to gene silencing (Figure 1F; Figure S1). Thermal asymmetric interlaced (TAIL)-PCR analysis identified the T-DNA insertion in an intergenic region between At1G05790 and At1G05800 on chromosome I (Figure 1E). Reverse transcription-PCR (RT-PCR) analysis revealed that only At1G05800 was overexpressed in *dgl-D* (Figure 1F). In addition, the mutants showing a weak phenotype, due to gene silencing (*dgl-D(M)*), showed a reduced expression of At1G05800. Transgenic plants overexpressing At1G05800 under the control of the *35S* promoter of the cauliflower mosaic virus (*35S* promoter) resembled that of the *dgl-D* mutant (Figures S2A and S2B), thereby demonstrating that At1G05800 is the *DGL* gene.

DGL is an intron-less gene that encodes a 417 amino acid polypeptide with 2 functional domains—a plastid-targeting transit peptide and a lipase domain (Figure S3A). A search of databases identified DGL (*At*PLA$_1$-Iα1) as a member of the *At*PLA$_1$-I family and to be closely related to DAD1 (*At*PLA$_1$-Iβ1), an enzyme catalyzing the initial step of JA biosynthesis (Ishiguro et al., 2001; Ryu, 2004; Figure S3).

DGL, a Chloroplast-Localized Galactolipase, Mediates an Initial Step of JA Biosynthesis

The cellular localization of DGL was determined by a protoplast transient assay by using constructs encoding *DGL:GFP* (Green Fluorescent Protein) and *DAD1:GFP* (as a control). As shown in Figure 2A, the fluorescence of both DGL:GFP and DAD1:GFP was detected in the chloroplasts as punctate dots. Because DAD1 is targeted to the chloroplast (Ishiguro et al., 2001) and such a punctate dot pattern was previously reported in chloroplast-localized NDPK2 (Sharma et al., 2007), we conclude that DGL is localized in the chloroplast.

To determine if DGL protein has lipase activity, we fused the coding sequence of DGL, except the transit peptide, to the C terminus of the maltose-binding protein (MBP). The MBP:DGL fusion protein efficiently hydrolyzed phosphatidylcholine (PC) to lysophosphatidylcholine (LPC) and free fatty acid (FFA) in an sn-1-specific manner, with the highest activity at pH 7.0, demonstrating that DGL has PLA$_1$ activity (Figures 2B and 2C; Figure S4). Because galactolipids are the dominant lipids (close to 80%) of the thylakoid membranes (Douce and Joyard, 1990), we determined whether MBP:DGL and MBP:DAD1 use galactolipids as substrate (Figure 2D). Commercially available lipases, *R. miehei* lipase, and *C. rugosa* lipase, the known galactolipase and phospholipase, respectively, were used as controls (Lo et al., 2004). Surprisingly, MBP:DGL showed approximately three times higher galactolipase activity than PLA$_1$ activity

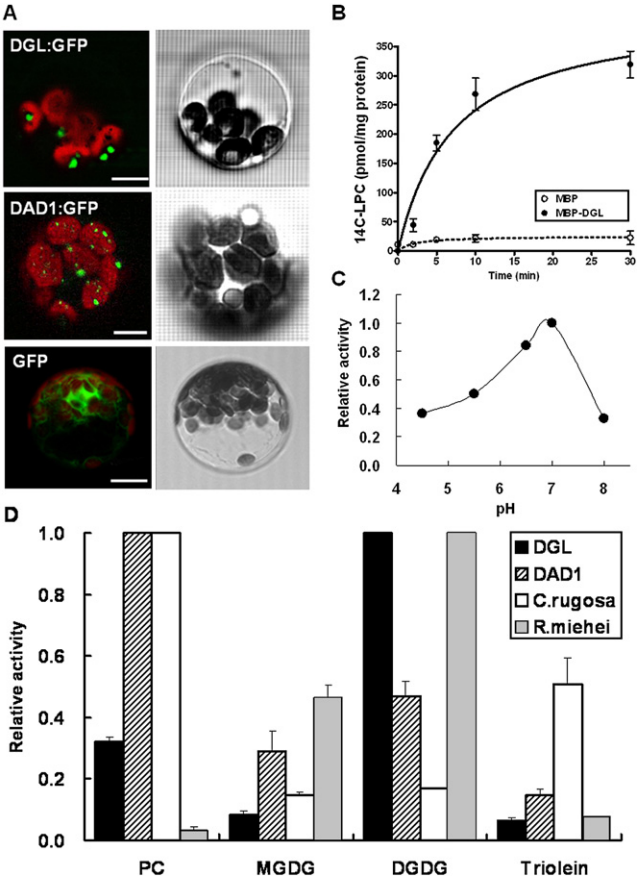

Figure 2. *DGL* Encodes a Chloroplast-Targeted Phospho-/Galactolipase A

(A) Subcellular localization of DGL:GFP in *Arabidopsis* protoplasts. Red fluorescence is autofluorescence emitted by the chloroplast. DAD1:GFP and GFP are used as positive and negative control respectively.

(B) Enzyme kinetics of MBP:DGL for PC hydrolysis. Radioactive-labeled 1-palmitoyl-2-^{14}C-linoleoyl-PC was used as a substrate. Released LysoPC was quantified. Values are mean ± SD from three experiments.

(C) pH dependence of DGL activity. The relative value compared with the activity at pH 7.0 is presented after a 30 min reaction.

(D) Substrate specificity of MBP:DGL and MBP:DAD1. The release of free fatty acids from equal concentrations of PC, MGDG, DGDG, and triolein was quantified. Data are presented as relative activities compared to the maximum activity (assigned the value as 1) for each lipase. Values are mean ± SD from five experiments.

when digalactosyldiacylglycerol (DGDG) was used as substrate. In contrast, MBP:DAD1 showed ~50% lower lipolytic activity for DGDG compared to PC. When monogalactosyldiacylglycerol (MGDG) was used as a substrate, the lipolytic activity of both MBP:DGL and MBP:DAD1 was decreased to 20%–30% compared to PC, which is consistent with the previous report (Ishiguro et al., 2001). The results show that DGL has stronger galactolipase activity than DAD1 with DGDG as a substrate.

The possible role of DGL in JA biosynthesis was studied by analyzing the *dgl-D* mutant both genetically and biochemically. The *dgl-D* mutation was introduced into the *opr3* and *coi1* backgrounds by genetic crossing to determine whether the *dgl-D* phenotype is caused by JA overproduction. Because OPR3 is

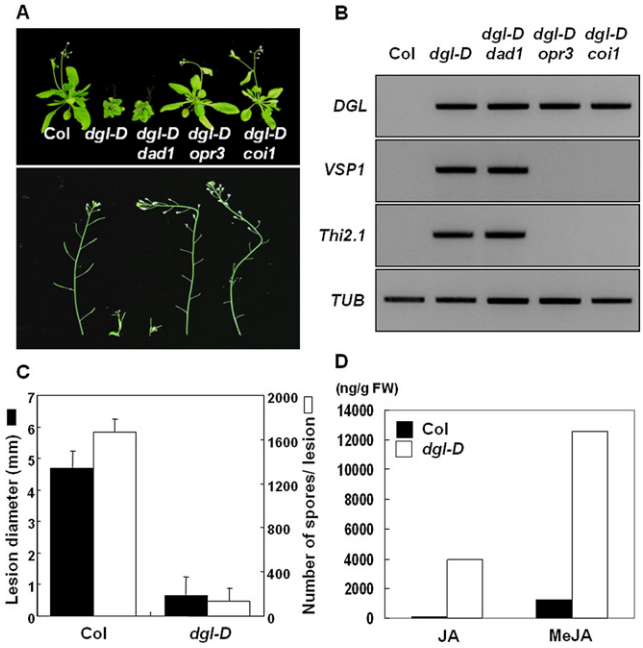

Figure 3. *dgl-D* Overproduces JA

(A) Genetic analysis for the biological function of *DGL*. Overall morphologies are shown for 35-day-old plants (top). The inflorescences from 45-day-old plants (bottom) are presented for the male-sterile phenotype. The double mutants *dgl-D opr3* and *dgl-D coi1* show undeveloped siliques due to sterility.

(B) Expressions of the *DGL* and JA-responsive genes in each genotype. The expression of *TUB* is used as a quantitative control.

(C) Resistance to *A. brassicicola* is quantified with lesion size and spore numbers per each lesion. The results were obtained 3 days after inoculation. Values are mean ± SD from three experiments.

(D) JA and MeJA contents in *dgl-D*. The rosette leaves from 35-day-old plants were used.

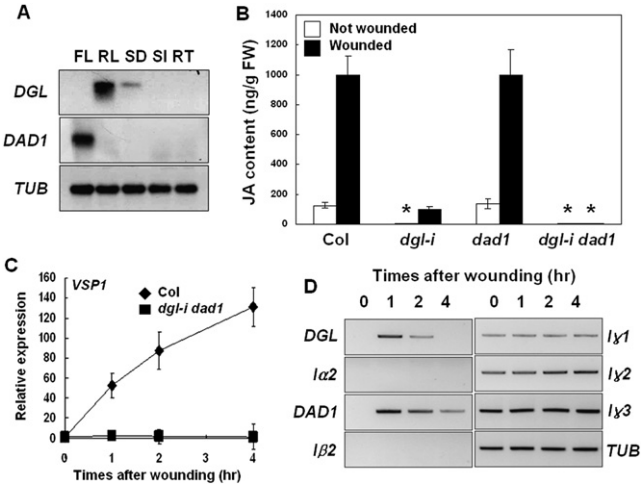

Figure 4. Only *DGL* and *DAD1* Participate in JA Production

(A) Tissue-specific expression of *DGL* and *DAD1*. Tissues were harvested from 35-day-old wild-type plants and, in the case of seedlings, from 9-day-old plants. FL, flowers; RL, rosette leaves; SD, seedlings; SI, siliques; RT, roots. Expression of *TUB* is used as the quantitative control.

(B) JA levels in *dgl-i*, *dad1*, and *dgl-i dad1*. The rosette leaves of 35-day-old plants were used. For wounded tissues, the JA level was measured 1 hr after wounding. *, the JA level was below the detection limit of our experimental instrument. Values are mean ± SD from three experiments.

(C) Wound responses in *dgl-i dad1*. Real-time-qPCR analysis was performed to analyze *VSP1* expression after wounding. Fold induction compared to unwounded wild-type is given as a value on the vertical axis. *Actin* expression of each sample is used as a quantitative control. Values are mean ± SD from three experiments.

(D) Expression patterns of *AtPLA$_1$-I*-family members after wounding.

located downstream of DAD1 in the JA biosynthetic pathway (Stintzi and Browse, 2000) and COI1 is a JA signaling component (Xie et al., 1998), the expectation was that the *dgl-D* phenotype would disappear in *dgl-D opr3* and *dgl-D coi1* double mutants, as was indeed the case (Figure 3A). In addition, the male-sterile phenotype, which is caused by a JA deficiency, appeared in the double mutants (Figure 3A). In contrast, *dgl-D* was epistatic to *dad1* (Figure 3A). These results suggest that DGL acts upstream of OPR3 in the JA biosynthetic pathway and is capable of replacing DAD1 activity. The mutant *dgl-D* showed an increased expression of JA-responsive genes, such as *VEGETATIVE STORAGE PROTEIN1* (*VSP1*) and *Thionin2.1* (*Thi2.1*) (Figure 3B). However, the expression of *VSP1* and *Thi2.1* was suppressed in *dgl-D opr3* and *dgl-D coi1* (Figure 3B). As overexpression of JA causes enhanced resistance to pathogens (Thaler et al., 2004), *dgl-D* plants showed resistance to a necrotrophic fungal pathogen, *Alternaria brassicicola* (Figure 3C; Figure S5).

The levels of JA and methyl jasmonate (MeJA) in the *dgl-D* mutant and the wild-type were measured by gas chromatography/mass spectrometry (GC/MS). In triplicate analyses, the *dgl-D* mutant exhibited much higher amounts than the wild-type. However, there were large variations in the levels of JA and MeJA between independent samples of the *dgl-D* mutant, possibly indicating variations in the levels of gene silencing in *dgl-D*. The most representative result is presented in Figure 3D. Taken together, our results suggest that DGL is a chloroplast-localized phospho-/galactolipase A participating in an initial step of JA biosynthesis.

DGL Is Expressed in Vegetative Tissues and Maintains an Endogenous Level of JA

The expression pattern of *DGL* was analyzed to determine whether the function of *DGL* differs from that of *DAD1* (Figure 4A). *DGL* was weakly expressed in whole 9-day-old seedlings and was relatively highly expressed in the rosette leaves of 35-day-old adult plants, although at a level much lower than that found in wounded plants. *DGL* was not detected in the flowers nor in the siliques and roots (Figure 4A). In contrast, *DAD1* is expressed only in the flowers as reported (Ishiguro et al., 2001). This result may indicate that *DGL* has a unique function in vegetative tissues, in contrast to *DAD1*, which functions in reproductive tissues.

Because a T-DNA insertion allele was not available, an RNAi-mediated knockdown allele, *dgl-i*, was generated and used to elucidate the biological function of *DGL*. Ten independent transgenic lines were generated, and two representative lines in which the specific suppression of the *DGL* gene had been confirmed were studied (Figure S6). Without wounding, JA in leaves of 35-day-old *dgl-i* plants was undetectable, whereas wild-type and *dad1* plants showed basal levels of JA in a GC/MS assay (Figure 4B). This suggests that *DGL*, but not *DAD1*, is necessary

for the biosynthesis of basal-level endogenous JA in vegetative tissues.

Among the *AtPLA$_1$-I* Family, Only *DGL* and *DAD1* Participate in JA Biosynthesis

Because JA production is strongly induced by wounding, we measured the JA level in *dgl-i* and *dad1* mutants after wounding (Figure 4B). At 1 hr after wounding, *dad1* showed wild-type levels of JA induction as previously reported (Ishiguro et al., 2001), whereas *dgl-i* showed weak induction. However, *dgl-i* recovered to almost wild-type level of JA at 4 hr after wounding (Figure 6C), suggesting that only the early phase of JA production is defective in *dgl-i* (discussed further below). The JA level in a *dgl-i dad1* double mutant was below the detection limit of our experimental instruments, irrespective of wound treatment (Figure 4B). This indicates that only *DGL* and *DAD1* among the seven members of the *AtPLA$_1$-I* family have roles in JA biosynthesis, although they are closely related in amino acid sequence (Figure S3) (Ryu, 2004). An expression analysis of *VSP1*, an indicator of JA response, by real-time quantitative PCR (real-time-qPCR) revealed that the induction by wounding was completely blocked in *dgl-i dad1*, confirming the GC/MS results (Figure 4C).

The seven members of the *AtPLA$_1$-I* family with chloroplast-targeted transit peptides have been categorized into three subclasses based on phylogenetic analyses (Ryu, 2004). To investigate the potential involvement of other members of this family in JA biosynthesis, we analyzed the expression patterns after wounding and the phenotypes of transgenic plants overexpressing each family member. *DGL* and *DAD1* expression was rapidly induced by wounding (Figure 4D). In contrast, *AtPLA$_1$-Iα2* and *AtPLA$_1$-Iβ2* expression was not detected even at 4 hr after wounding. The remaining members, *AtPLA$_1$-Iγ1*, *AtPLA$_1$-Iγ2*, and *AtPLA$_1$-Iγ3*, which belong to a separate subclass, were constitutively expressed at a high level and did not show any significant induction by wounding (Figure 4D). Consistent with this, the transgenic plants overexpressing *AtPLA$_1$-Iα2*, *AtPLA$_1$-Iβ2*, *AtPLA$_1$-Iγ1*, *AtPLA$_1$-Iγ2*, or *AtPLA$_1$-Iγ3* did not exhibit the JA overproduction phenotype observed in *35S::DGL* (data not shown). Therefore, these results confirm that only *DGL* and *DAD1* participate in JA biosynthesis. In addition, because *dad1* shows normal JA induction and *dgl-i* recovers the wild-type level of JA at a late phase of the wound response, our data suggest that cooperation between two functionally redundant lipase enzymes, DGL and DAD1, is necessary and sufficient for wound-inducible JA production.

DGL Regulates Vegetative Organ Growth

The *dgl-i* mutant did not exhibit the male-sterile phenotype observed in *dad1* plants; rather, *dgl-i* has a larger seedling size, which is an opposite phenotype to *dgl-D* (Figure 5A). The rosette leaves and stems of adult *dgl-i* plants showed enhanced elongation, but these phenotypes were highly variable and depended on the growth conditions (data not shown). As expected, *dad1* seedlings were of normal size, whereas *dgl-i dad1* seedlings were the same size as those of *dgl-i* (Figure 5A). To determine whether the increased size of *dgl-i* seedlings was caused by an endogenous JA deficiency, the size of *opr3* seedlings was compared with that of the wild-type (WS). As expected, the *opr3* seedlings were also larger (Figure 5A). In addition, adult

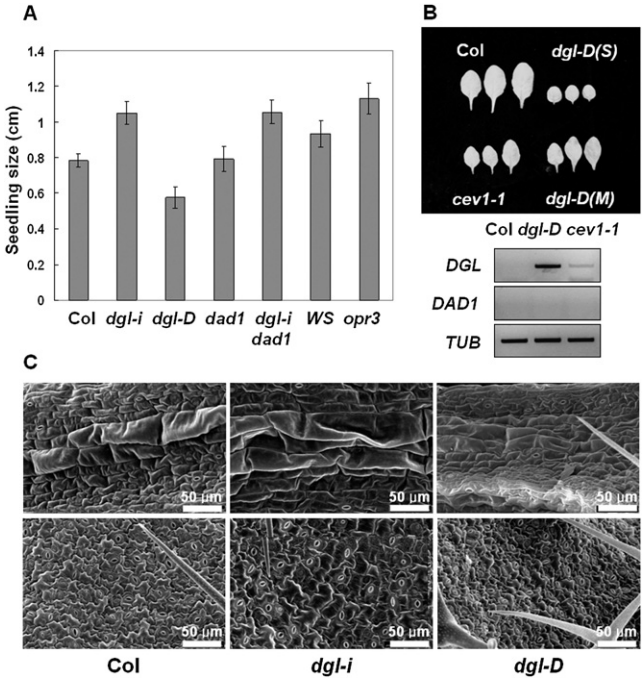

Figure 5. *DGL* Regulates Vegetative Tissue Growth

(A) Seedling size of *dgl-i* and *dad1*. The distances between the first pair of two true leaves are used as parameters of the size of 9-day-old seedlings, as described in Figure 1D. Values are mean ± SD from three experiments. n > 30/experiments.

(B) Phenotype comparison between *dgl-D* and *cev1-1*. The largest rosette leaves from 20-day-old plants are presented (top). *DGL* and *DAD1* expressions are analyzed by RT-PCR (bottom).

(C) Comparison of cell size in *dgl-i* and *dgl-D* seedlings. Scanning electron microscopy was performed on 9-day-old seedlings. Photos from petioles (top) and the adaxial surface of rosette leaves (bottom) are presented. The scale bar is 50 µm.

opr3 and *coi1* plants showed an enhanced elongation of the rosette leaves and stems that was similar to *dgl-i* (data not shown). The phenotype of the *constitutive expression of VSP1* (*cev1*) mutant, which constitutively produces JA (Ellis et al., 2002), further supported the function of *DGL* for vegetative organ growth. The *cev1* mutant has small leaves and increased expression of *DGL*, although the expression level was much lower than *dgl-D* (Figure 5B). Consistent with this, the leaf morphology of *cev1* resembled that of *dgl-D(M)*, which has partial gene silencing. In contrast to *DGL*, *DAD1* expression was not changed in *cev1* (Figure 5B), suggesting that JA overproduction in *cev1* is caused by ectopic expression of *DGL*.

The microscopic structure of 9-day-old *dgl-i*, *dgl-D*, and wild-type plants was examined by scanning electron microscopy to determine whether the enhanced size of *dgl-i* seedlings is caused by an increase of cell expansion (Figure 5C). Most of the cells of both the petiole and the adaxial surface of the rosette leaf of *dgl-i* plants were larger than those of the same tissues in the wild-type. Conversely, these cells of *dgl-D* plants were smaller than those of the wild-type, indicating that *DGL* affects cell expansion (Figure 5C). These results suggest that *DGL* regulates leaf growth by maintaining the endogenous JA contents in vegetative tissues.

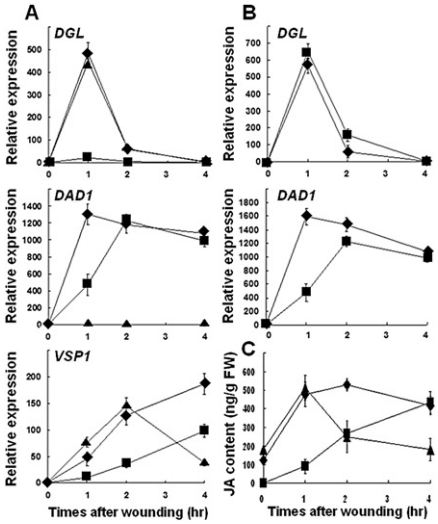

Figure 6. Wound Responses in *dgl-i* and *dad1*

(A) Expression of *DGL*, *DAD1*, and *VSP1* in wild-type, the *dgl-i* mutant, and the *dad1* mutant after wounding. Real-time-qPCR was performed on 35-day-old plants after wounding. Col, diamonds; *dgl-i* , squares; *dad1*, triangles.

(B) Wound responses in *opr3*. WS, diamonds; *opr3*, squares. Fold induction compared to unwounded wild-type is given as a value on the vertical axis. The expression of *Actin* in each sample is used as a quantitative control.

(C) JA induction kinetics in wild-type, the *dgl-i* mutant, and the *dad1* mutant after wounding. Col , diamonds; *dgl-i*, squares; *dad1*, triangles.

Values are mean ± SD from three experiments.

Roles of *DGL* and *DAD1* in Wound-Inducible JA Biosynthesis Are Temporally Separated

The expression patterns of *DGL* and *DAD1* were compared during the wound response (Figure 6A). In the wild-type, both *DGL* and *DAD1* exhibited peak expression 1 hr after wounding. However, whereas the expression of *DGL* decreased rapidly after 1 hr, that of *DAD1* decreased slowly and remained at a relatively high level until 4 hr after wounding. To assess whether the higher expression level of *DAD1* in the late phase has any function, we compared the expression of *VSP1* in wild-type and *dad1* plants (Figure 6A). The expression of *VSP1* in both plant lines showed a similar increase up to 1–2 hr after wounding; thereafter, expression started to decrease in *dad1* plants but showed a continuous increase in the wild-type. This result suggests that a relatively longer duration of *DAD1* expression after wounding is required for the continuous increase of *VSP1* in the late phase. In contrast, *VSP1* expression in *dgl-i* plants decreased in the early phase (Figure 6A), indicating that *DGL* is necessary for the early induction of *VSP1* expression.

To confirm such temporally diversified functions of *DGL* and *DAD1* in wound-inducible JA production, we compared the JA induction kinetics between *dgl-i* and *dad1* mutants (Figure 6C). At 1 hr after wounding, the level of JA was very low in the *dgl-i* mutant, but JA accumulation was normal in the *dad1* mutant, indicating that the early phase of wound-induced JA production requires *DGL*. Conversely, the level of JA fell rapidly in the *dad1* mutant at 2 hr after wounding compared to the wild-type, thereby showing that *DAD1* is necessary for the accumulation of JA during the late phase. The JA level in *dgl-i* recovered to the wild-type level 4 hr after wounding.

The induction kinetics of *DGL* and *DAD1* in different genetic backgrounds was also analyzed. In *dgl-i*, the induction kinetics of *DAD1* after wounding was delayed by 1 hr, and the maximal expression level of *DAD1* decreased slightly (Figure 6A). The same expression pattern of *DAD1* was found in the *opr3* genetic background (Figure 6B). In contrast, the expression of *DGL* was unaffected by either *dad1* or *opr3*. Because *DGL* maintains a basal level of endogenous JA in the absence of wounding, such a delay in the expression of *DAD1* in *dgl-i* or *opr3* mutants suggests that a basal level of JA is required for a rapid induction of *DAD1* by wounding, and that the induction mechanisms of *DGL* and *DAD1* by wounding are different. It also suggests that the functions of *DGL* and *DAD1* are temporally separate in wound-inducible JA production.

PLDα1 Induces Wound Response through the Activation of *DGL* and *DAD1*

Previously, several authors have proposed that *PLDα1* provides α-linolenic acid to the JA biosynthetic pathway (Wang et al., 2000; Arimura et al., 2005; Bargmann and Munnik, 2006). However, our results, showing that the lack of both *DGL* and *DAD1* causes the complete suppression of JA induction, are not consistent with the proposed role of *PLDα1*. A possible alternative is that PA synthesized by *PLDα1* acts as a signaling molecule in wound response and that the reduced accumulation of JA in *pldα1* is caused by decreased wound signaling, which, in turn, results in a decrease in the induction of *DGL* and *DAD1*. To examine this possibility, we performed real-time-qPCR analysis of the expression patterns of *DGL* and *DAD1* in wounded leaves after treatment with *1*-butanol, a well-known inhibitor of PLD. In the presence of *1*-butanol, PLDα1 attaches *1*-butanol instead of the hydroxyl group onto the polar head of PA during the hydrolyzation process, and the resulting product loses the biological activity of PA, although the α-linolenic acid moiety remains intact (Bargmann and Munnik, 2006). In an aqueous solution containing 0.2% *1*-butanol, the induction of *DGL* and *DAD1* by wounding was significantly blocked (Figure 7A). In contrast, the induction of both genes in mock solution or in a solution containing 0.2% *tert*-butanol occurred normally (Figure 7A). This result suggests a potential role of PA as a wound-signaling molecule. The role of PLD in vivo was examined by analyzing the expression of *DGL* and *DAD1* in wounded *pldα1* mutants (Figure 7B). In this mutant, the induction of *DGL* and *DAD1* by wounding was significantly suppressed. However, the expression of both genes was not fully suppressed in *pldα1*, possibly due to the presence of functionally redundant genes with *PLDα1. Arabidopsis* has three additional genes homologous to *PLDα1*, any or all of which may have a role in wound-inducible PA production (Bargmann and Munnik, 2006).

To genetically prove this hypothesis, we generated *35S::PLDα1* transgenic lines and introduced them into a *dgl-i dad1* double mutant background. In the wild-type background, *35S::PLDα1* exhibited neither JA overproducer phenotypes (data not shown) nor induction of *DGL* and *DAD1* without wounding (Figure 7C). However, wounding caused higher expression of *DGL*, *DAD1*, and *VSP1* in *35S::PLDα1* than in the wild-type (Figure 7C). Although the ectopic expression of *PLDα1* is not sufficient to induce the wound response, our results indicate that it strengthens the response; the wound-stimulated translocalization of PLDα1

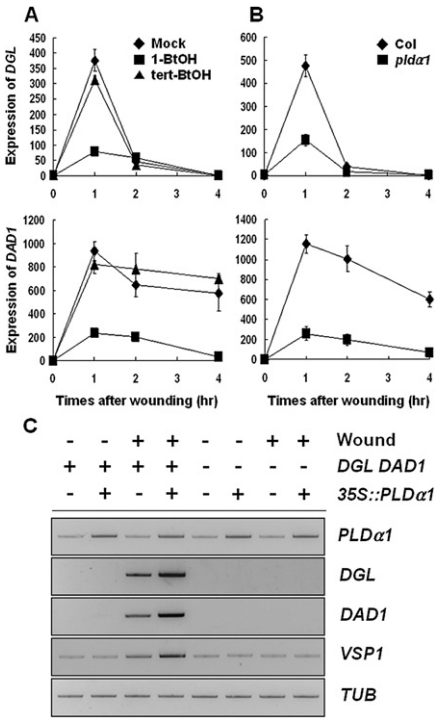

Figure 7. PLD Regulates the Transcriptional Activation of *DGL* and *DAD1*

(A) The PLD inhibitor reduces wound-induced *DGL* and *DAD1* expression. Wounded leaves were soaked in solutions containing water (Mock), 0.2% *1*-butanol (*1*-BtOH), and 0.2% *tert*-butanol (*tert*-BtOH). Values are mean ± SD from three experiments.

(B) Suppression of wound-induced *DGL* and *DAD1* expression in *pldα1*. Real-time-qPCR was performed on 35-day-old plants after wounding. Analysis of gene expression was performed by real-time-qPCR. Fold induction compared to the unwounded wild-type is given as a value on the vertical axis. *Actin* expression of each sample is used as a quantitative control. Values are mean ± SD from three experiments.

(C) Wound response in *35S::PLDα1*. RT-PCR was performed on 25-day-old plants after 1 hr of wounding. Genetic background and wounding are presented as plus (+) and minus (−) symbols. *TUB* expression is used as a quantitative control.

from cytoplasm to plasma membrane may be required for the wound response as reported previously (Wang et al., 2000). In contrast to this, wound-induced *VSP1* expression was completely blocked in *35S::PLDα1 dgl-i dad1* (Figure 7C). Taken together, our results suggest that the in vivo function of *PLDα1* is to amplify or reinforce, by an as yet unknown mechanism, the expression of *DGL* and *DAD1* during the wound response.

DISCUSSION

Through activation-tagging mutagenesis, we have isolated a dwarf mutant, *dgl-D*, that overexpresses a gene encoding a member of the $AtPLA_1$-*I* family. Similar to DAD1, which catalyzes a critical step in the JA biosynthetic pathway, DGL has a function in JA production. Here, we show that DGL has strong, but that DAD1 has weak, galactolipase activity for DGDG, and that the two closely related lipases are necessary and sufficient for wound-inducible JA production. We also show that *DGL* and *DAD1* have temporally and spatially separate functions. Finally, our results suggest that PLD induces the wound response by enhancing the expression of *DGL* and *DAD1*, rather than by providing the JA precursors.

Novel Functions of Endogenous JA in *Arabidopsis*

In contrast to *DAD1*, *DGL* is expressed in the leaves and is required for basal-level production of JA under normal conditions (Figure 4). The *dgl-D* mutant, a JA overproducer, exhibits a severe dwarf phenotype, whereas *dgl-i* and *opr3,* the JA-deficient mutants, exhibit larger seedling sizes than wild-type through increased cell expansion (Figure 5). These results strongly support the finding that the endogenous JA in the vegetative tissues has a growth-inhibiting effect on organ size, which is consistent with the previous reports showing that exogenous treatment of JA inhibits seedling growth (Xie et al., 1998).

In wound responses, both *dgl-i* and *opr3* exhibite a defect in *DAD1* induction kinetics (Figure 6), suggesting that the appropriate induction of *DAD1* by wounding is dependent on the basal level of endogenous JA. In contrast, the induction kinetics of *DGL* was not affected by *opr3*, indicating that the induction mechanisms of *DGL* and *DAD1* are different. In various studies, a positive-feedback regulation of JA in its biosynthetic pathway has been proposed. In agreement with this, we have identified increased expression of *LOX*, *AOS*, *AOC*, and *OPR3* in *dgl-D* and JA-treated plants (data not shown). However, the expression of *DAD1* was not increased under the same experimental conditions, suggesting that endogenous JA does not directly participate in *DAD1* induction, but that JA makes plants more competent to mount a wound response, possibly by preparing the components required for the perception or transduction of wound signals.

Functional Diversification of *DGL* and *DAD1* in JA Biosynthesis

DGL and *DAD1* are members of the $AtPLA_1$-*I* family that are predicted to be localized to the chloroplast based on sequence analysis (Ryu, 2004). Seven homologous genes of this family, including *DGL* and *DAD1*, are present in the *Arabidopsis* genome. Among them, only *DGL* and *DAD1* show wound-inducible expression and the JA overproducer phenotype when overexpressed. These results are somewhat surprising because, based on amino acid sequence analysis, the closest homolog of *DGL* is $AtPLA_1$-*Iα2*, and that of *DAD1* is $AtPLA_1$-*Iβ2* (Ryu, 2004). However, when the sequences of the lipase domain were excluded from the phylogenetic analysis, *DGL* and *DAD1* showed the highest homology (Figure S3C). This indicates that the sequences outside the lipase domain may be critical in the determination of lipolytic activity for JA biosynthesis.

Albeit functionally redundant, DGL and DAD1 show slightly different substrate specificity: the preferential substrate for DGL is DGDG, but that for DAD1 is PC (Figure 2). The two also have spatially and temporally separate functions. Whereas *DGL* is expressed in vegetative tissues, *DAD1* is expressed in reproductive tissues. Thus, in normal conditions, *DGL* is involved in the regulation of leaf growth, but *DAD1* is involved in stamen development. In the wound response, *DGL* shows strong and transient expression during the early phase, whereas *DAD1* expression remains strong for a relatively longer period of time until

the late phase (Figure 6). In addition, we observed specific induction of *DGL* in *cev1* mutants (Figure 5B). *CEV1* encodes a cellulose synthase, and a *cev1* mutation causes a constitutive wound response (Ellis et al., 2002), suggesting that the wound signal is derived from the cell wall. Our results showing that *DGL*, but not *DAD1* expression, is induced by the *cev1* mutation further support that *DGL* and *DAD1* respond to wound signals differently. Therefore, our results reveal functional diversification between *DGL* and *DAD1* through differential gene regulation.

PLD Activates the Expression of *DGL* and *DAD1*

Our results show that the cooperation of *DGL* and *DAD1* is critical in wound-inducible JA accumulation. The induction of *VSP1* by wounding disappeared in the *dgl-i dad1* double mutant, and the actual amount of JA was almost negligible (Figure 4), indicating that JA production upon wounding is totally dependent on the functions of two related galactolipase enzymes, DGL and DAD1. This result contradicts the previous suggestion that PA produced by *PLDα1* acts as a JA precursor (Wang et al., 2000; Arimura et al., 2005; Bargmann and Munnik, 2006). Our genetic analyses with *pldα1* and *35S::PLDα1* support the finding that *PLDα1* does not provide a JA precursor directly, but instead provides a secondary signaling molecule for the activation of *DGL* and *DAD1* in the wound signaling cascade. Consistent with this proposal is the observation that PA induces a wound-activated MAPK in soybean (Lee et al., 2001), and that the tobacco homolog salicylic acid-induced protein kinase (SIPK) functions as an upstream signaling factor for JA induction by wounding (Seo et al., 2007). It is therefore highly probable that PA activates *DGL* and *DAD1* through the MAPK signaling cascade.

DGL Has Galactolipase Activity

Both DGL and DAD1 proteins are localized in the chloroplast, where OPDA, a JA precursor, is produced from lipid. Thylakoid lipid composition is unique in that the major components are galactolipids instead of the commonly encountered phospholipids: ~50% of the total lipid content is MGDG, ~30% is DGDG, and ~10% is diacylphosphatidylglycerol (Douce and Joyard, 1990). Although galactolipase activity has been detected in many plants, the genes for chloroplast-associated galactolipase have not been isolated thus far (Lo et al., 2004). Here, we show that DGL has strong galactolipase activity for DGDG (Figure 2). Consistent with the biochemical role of DGL, the *dgd1* mutant, which has a 90% reduction in DGDG content, exhibits a dwarf phenotype, as does *dgl-D* (Dörmann et al., 1999). In addition to DGL, DAD1 has galactolipase activity for DGDG, although it is weak. Thus, our results suggest that the major source of JA is the acyl chains derived from DGDG. We propose changing the name of the gene family including *DGL* and *DAD1* to galactolipase instead of *AtPLA$_1$*.

Recently, it was shown that wounding stimulates the rapid accumulation of galactolipids containing OPDA; thus, it has been suggested that most of the OPDA is produced not from FFAs, but from acyl groups remaining esterified to the galactolipids (Buseman et al., 2006). If this hypothesis is correct, DGL and DAD1 must have enzyme activity to release OPDA from galactolipids because our genetic data suggest that DGL and DAD1 are necessary for JA biosynthesis. In addition, although α-linolenic acid (18:3 fatty acid) is the major acyl group in DGDG, JA may also be produced from dinor-OPDA (converted from a 16:3 fatty acid). Therefore, the substrate specificity for DGL and DAD1 needs to be further analyzed.

A rice genome database search revealed the presence of homologs of both *DGL* and *DAD1*, suggesting the conservation of a similar mechanism for JA production in other plant species. In contrast to higher plants, an algal genome (*Chlamydomonas reinhardtii*) database search revealed the presence of only a *DGL* homolog: we failed to find any *DAD1* homolog in the algal genome. When a moss (*Physcomitrella patens*) genome database was searched, the sequences of both *DGL* and *DAD1* hit the same gene as a homolog. Combined with the recent result showing that the algal oxylipin pathway is mediated by galacto/phospholipase activity (Lion et al., 2006), these in silico analyses suggest that *DGL* is likely the primitive form of galactolipase for the oxylipin pathway, and that the DAD1-type lipase has diverged from the same ancestral gene during evolution.

EXPERIMENTAL PROCEDURES

Plant Materials and Growth Conditions

All of the plants used in this study, except *opr3*, have the Col-0 ecotype background (*opr3* has the WS background). All of the plants were grown under a long-day photoperiod (16 hr:8 hr, light:dark) at 22°C and 60% relative humidity. The activation-tagging mutant pool was generated by the transformation of Col-0 with *Agrobacteria* containing pSKI015 (accession number AF187951) by using the floral dip method (Weigel et al., 2000). For measurement of seedling size, progenies of candidate mutants were sown on plates containing MS media (Sigma). The distance between the first pair of two true leaves on 9-day-old seedlings was measured as parameters of seedling size. The analyses were carried out in triplicate; each data set consisted of measurements from ~30 seedlings.

Analysis of Gene Expression

RT-PCR was performed to analyze the expressions of individual genes. Total RNA was extracted with TRI-Reagent (Sigma) by following the manufacturer's protocol. A total of 2 μg RNA was reverse transcribed by using RevertAid M-MuLV reverse transcriptase (Fermentas). PCR amplification was performed by using gene-specific primers. Real-time-qPCR analysis was performed by using an Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems). SYBR Green qPCR master mixes (Bio-Rad) were used in PCR amplification.

Lipase Assay

Phospholipase A_1 activity was examined by using radiolabeled PC with asymmetric fatty acids. Fifteen pmol 1-palmitoyl-2-^{14}C-linoleoyl-PC (1.96 GBq/mmol; Amersham Bioscience) was incubated with 20 μg MBP-DGL for 30 min at 30°C. The lipids were extracted and separated by thin-layer chromatography (Silica Gel 60; Merck). Radioactive bands of ^{14}C-PC, ^{14}C-lysoPC, and ^{14}C-FFA product were detected and quantified with a Bio-Imaging Analyzer (BAS2500; Fuji Photo Film). To determine the substrate specificity of DGL, the level of FFA released from substrates was analyzed. A total of 20 μg MBP-DGL and MBP-DAD1 were incubated with various lipid substrates, then the released FFAs were measured by using an NEFA colorimetric kit (Wako Pure Chemicals) as described by Ishiguro et al. (2001). More precise information for the lipase assay is given in Supplemental Experimental Procedures.

Measurement of Endogenous JA and MeJA Contents

All of the endogenous jasmonic acids were extracted from 500 mg fresh rosette leaves by using a mixture of dichloromethane:methanol (1:1, v/v) as described in Weber et al. (1997). The concentrated samples were separated into JA and MeJA fractions by HPLC (isocratic reversed high-performance liquid chromatography; polar RP C-18, 250 × 100 mm, flow rate = 2.0 ml/min, UV = 228 nm, H_2O:acetonitrile = 45:55). MeJA fractions of plant extracts and

standard MeJA (Sigma) were directly analyzed by GC/MS as described in Creelman et al. (1992). JA fractions of plant extracts were analyzed after methylation with diazomethane. The quantification of jasmonic acids in each genotype was repeated three times.

Supplemental Data

Supplemental Data include detailed Supplemental Experimental Procedures, six figures, and one table and are available at http://www.developmentalcell.com/cgi/content/full/14/2/183/DC1/.

ACKNOWLEDGMENTS

We thank X. Wang and J.G. Turner for kindly providing the *pldα1* and *cev1-1* seeds, R. Amasino for critical reading of the manuscript, and anonymous reviewers for critical comments. This work was supported partially by the Biogreen 21 Program (20050401-034-706-141-01-00); the Korea Ministry of Science and Technology under the National Research Laboratory Program (2006-01952); the Global Research Laboratory Program (2006-03870); and a grant from the Korea Science and Engineering Foundation through the Plant Metabolism Research Center, Kyung Hee University to I.L. S.-J.N. and H.K. were supported by MarineBio 21, the Ministry of Maritime Affairs & Fisheries, and Y.H., S.C., and J.Y. were supported by the Brain Korea 21 program.

Received: May 15, 2007
Revised: September 18, 2007
Accepted: November 13, 2007
Published: February 11, 2008

REFERENCES

Arimura, G., Kost, C., and Boland, W. (2005). Herbivore-induced, indirect plant defences. Biochim. Biophys. Acta *1734*, 91–111.

Bargmann, B.O., and Munnik, T. (2006). The role of phospholipase D in plant stress responses. Curr. Opin. Plant Biol. *9*, 515–522.

Bell, E., Creelman, R.A., and Mullet, J.E. (1995). A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. Proc. Natl. Acad. Sci. USA *92*, 8675–8679.

Buseman, C.M., Tamura, P., Sparks, A.A., Baughman, E.J., Maatta, S., Zhao, J., Roth, M.R., Esch, S.W., Shah, J., Williams, T.D., et al. (2006). Wounding stimulates the accumulation of glycerolipids containing oxophytodienoic acid and dinor-oxophytodienoic acid in *Arabidopsis* leaves. Plant Physiol. *142*, 28–39.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozazo, F.M., Ponce, M.R., et al. (2007). The JAZ family of repressors is the missing link in jasmonate signaling. Nature *448*, 666–673.

Creelman, R.A., and Mullet, J.A. (1997). Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. *48*, 355–381.

Creelman, R.A., Tierney, M.L., and Mullet, J.E. (1992). Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc. Natl. Acad. Sci. USA *89*, 4938–4941.

Douce, R., and Joyard, J. (1990). Biochemistry and function of the plastid envelope. Annu. Rev. Cell Biol. *6*, 173–216.

Dörmann, P., Balbo, I., and Benning, C. (1999). *Arabidopsis* galactolipid biosynthesis and lipid trafficking mediated by DGD1. Science *284*, 2181–2184.

Ellis, C., Karafyllidis, I., Wasternack, C., and Turner, J.G. (2002). The *Arabidopsis* mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. Plant Cell *14*, 1557–1566.

Feussner, I., Hause, B., Vörös, K., Parthier, B., and Wasternack, C. (1995). Jasmonate-induced lipoxygenase forms are localized in chloroplast of barley leaves (*Hordeum vulgare* cv. Salome). Plant J. *7*, 949–957.

Froehlich, J.E., Itoh, A., and Howe, G.A. (2001). Tomato allene oxide synthase and fatty acid hydroperoxide lyase, two cytochrome P450s involved in oxylipin metabolism, are targeted to different membranes of chloroplast envelope. Plant Physiol. *125*, 306–317.

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

Kessler, A., and Baldwin, I.T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. Annu. Rev. Plant Biol. *53*, 299–328.

Koo, A.J.K., Chung, H.S., Kobayashi, Y., and Howe, G.A. (2006). Identification of a peroxisomal acyl-activating enzyme involved in the biosynthesis of jasmonic acid in *Arabidopsis*. J. Biol. Chem. *281*, 33511–33520.

Laudert, D., Schaller, F., and Weiler, E.W. (2000). Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. Planta *211*, 163–165.

Lee, S., Suh, S., Kim, S., Crain, R.C., Kwak, J.M., Nam, H.-G., and Lee, Y. (1997). Systemic elevation of phosphatidic acid and lysophospholipid levels in wounded plants. Plant J. *12*, 547–556.

Lee, S., Hirt, H., and Lee, Y. (2001). Phosphatidic acid activates a wound-activated MAPK in *Glycine max*. Plant J. *26*, 479–486.

Li, C., Schilmiller, A.L., Liu, G., Lee, G.I., Jayanty, S., Sageman, C., Vrebalov, J., Giovannoni, J.J., Yagi, K., Kobayashi, Y., et al. (2005). Role of β-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. Plant Cell *17*, 971–986.

Li, L., Zhao, Y., McCaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E., and Howe, G.A. (2004). The tomato homolog of *CORONATINE-INSENSITIVE1* is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell *16*, 126–143.

Lion, U., Wiesemeier, T., Weinberger, F., Beltrán, J., Flores, V., Faugeron, S., Correa, J., and Pohnert, G. (2006). Phospholipases and galactolipases trigger oxylipin-mediated wound-activated defence in the red alga *Gracilaria chilensis* against epiphytes. ChemBioChem *7*, 457–462.

Lo, M., Taylor, C., Wang, L., Nowack, L., Wang, T.-W., and Thompson, J. (2004). Characterization of an ultraviolet B-induced lipase in *Arabidopsis*. Plant Physiol. *135*, 947–958.

Maucher, H., Hause, B., Feussner, I., Ziegler, J., and Wasternack, C. (2000). Allene oxide synthase of barley (*Hordeum vulgare* cv. Salome): tissue specific regulation in seedling development. Plant J. *21*, 199–213.

McConn, M., Creelman, R.A., Bell, E., Mullet, J.E., and Browse, J. (1997). Jasmonate is essential for insect defense in *Arabidopsis*. Proc. Natl. Acad. Sci. USA *94*, 5473–5477.

Reymond, P., Weber, H., Damond, M., and Farmer, E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell *12*, 707–719.

Ryu, S.B. (2004). Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci. *9*, 229–235.

Ryu, S.B., and Wang, X. (1998). Increase in free linolenic and linoleic acids associated with phospholipase D-mediated hydrolysis of phospholipids in wounded castor bean leaves. Biochim. Biophys. Acta *1393*, 193–202.

Seo, S., Katou, S., Seto, H., Gomi, K., and Ohashi, Y. (2007). The mitogen-activated protein kinases WIPK and SIPK regulate the levels of jasmonic and salicylic acids in wounded tobacco plants. Plant J. *49*, 899–909.

Sharma, R., Soll, J., and Bölter, B. (2007). Import and localization of nucleoside diphosphate kinase 2 in chloroplast. J. Plant Res. *120*, 451–456.

Stenzel, I., Hause, B., Miersch, O., Kurz, T., Maucher, H., Weichert, H., Ziegler, J., Feussner, I., and Wasternack, C. (2003). Jasmonate biosynthesis and the allene oxide cyclase family of *Arabidopsis thaliana*. Plant Mol. Biol. *51*, 895–911.

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

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

Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., et al. (2005). 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. Plant Physiol. *139*, 1268–1283.

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

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signaling. Nature *448*, 661–666.

Turner, J.G., Ellis, C., and Devoto, A. (2002). The jasmonate signal pathway. Plant Cell *14*, S153–S164.

Wang, C., Zien, C.A., Afitlhile, M., Welti, R., Hildebrand, D.F., and Wang, X. (2000). Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. Plant Cell *12*, 2237–2246.

Wang, X. (2002). Phospholipase D in hormonal and stress signaling. Curr. Opin. Plant Biol. *5*, 408–414.

Weber, H., Vick, B.A., and Farmer, E.E. (1997). Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate family. Proc. Natl. Acad. Sci. USA *94*, 10473–10478.

Weigel, D., Ahn, J.H., Blázquez, M.A., Borevitz, J.O., Christensen, S.K., Fankhauser, C., Ferrándiz, C., Kardailsky, I., Malancharuvil, E.J., Neff, M.M., et al. (2000). Activation tagging in *Arabidopsis*. Plant Physiol. *122*, 1003–1013.

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

Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Hamberg, M., Grimm, R., Ganal, M., and Wasternack, C. (2000). Molecular cloning of allene oxide cyclase. J. Biol. Chem. *275*, 19132–19138.

Accession Numbers

Coordinates of the genomic sequence of *DGL* have been deposited in GenBank at NCBI with the accession code EU411040.