Evidence Supporting a Role of Jasmonic Acid in Arabidopsis Leaf Senescence

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In this work, the role of jasmonic acid (JA) in leaf senescence is examined. Exogenous application of JA caused premature senescence in attached and detached leaves in wild-type Arabidopsis but failed to induce precocious senescence of JA-insensitive mutant coil plants, suggesting that the JA-signaling pathway is required for JA to promote leaf senescence. JA levels in senescing leaves are 4-fold higher than in non-senescing ones. Concurrent with the increase in JA level in senescing leaves, genes encoding the enzymes that catalyze most of the reactions of the JA biosynthetic pathway are differentially activated during leaf senescence in Arabidopsis, except for allene oxide synthase, which is constitutively and highly expressed throughout leaf development. Arabidopsis lipoxygenase 1 (cytoplasmic) expression is greatly increased but lipoxygenase 2 (plastidial) expression is sharply reduced during leaf senescence. Similarly, AOC1 (allene oxide cyclase 1), AOC2, and AOC3 are all up-regulated, whereas AOC4 is down-regulated with the progression of leaf senescence. The transcript levels of 12-oxo-PDA reductase 1 and 12-oxo-PDA reductase 3 also increase in senescing leaves, as does PED1 (encoding a 3-keto-acyl-thiolase for β-oxidation). This represents the first report, to our knowledge, of an increase in JA levels and expression of oxylipin genes during leaf senescence, and indicates that JA may play a role in the senescence program.

Originally identified as a major component of fragrant oils, methyl jasmonate (MeJA) and its precursor jasmonic acid (JA) were first demonstrated to promote senescence in detached oat (Avena sativa) leaves (Ueda and Kato, 1980), and were subsequently shown to be a class of plant growth regulator that plays pervasive roles in several other aspects of plant development, including seed germination and pollen development, responses to mechanical and insect wounding, pathogen infection, and drought stress (for review, see Hildebrand et al., 1998; Schaller, 2001). Recent molecular genetic studies have confirmed the involvement of JA both in developmental (Xie et al., 1998; Sanders et al., 2000; Stintzi and Browse, 2000) and defense-related processes (Vijayan et al., 1998; Xie et al., 1998; Ryan, 2000). The role of JA in leaf senescence is not clear. Exogenously applied JA and MeJA led to decreased expression of photosynthesis-related genes encoding, for example, the small subunit of Rubisco, reduced translation and increased degradation of Rubisco, and rapid loss of chlorophyll in barley (Hordeum vulgare) leaves (Weidhase et al., 1987; Parthier, 1990). However, many questions remain unanswered, such as whether JA levels change in leaves undergoing senescence and whether specific genes of JA biosynthesis are induced during senescence.

The biosynthetic pathway of JA, starting with α-linolenic acid, has been elucidated (Fig. 1; Vick and Zimmerman, 1984; Schaller, 2001). There may exist two pathways for JA biosynthesis in plant tissues, a chloroplast-localized and a cytoplasm-localized pathway (Creelman and Mullet, 1995). The existence of a cytoplasmic pathway is suggested by a transgenic study involving overexpression of a cytosolic allene oxide synthase (AOS) in tobacco (Nicotiana tabacum; Wang et al., 1999). JA biosynthesis is tightly regulated and the concentrations of JA in uninduced plant tissues are generally very low in most plant species examined (Hildebrand et al., 2000; Wang et al., 2000). In addition, JA biosynthesis is subject to inductive control by various elicitors such as wounding (Hildebrand et al., 2000; Ryan, 2000; Wang et al., 2000). The expressions of several genes, including lipoxygenase (LOX) and AOS, were increased by exogenous application of JA and associated with an increased level of endogenous JA (Bell and Mullet, 1993; Melan et al., 1993; Laudert and Weiler, 1998; Maucher et al., 2000), indicating that JA biosynthesis is also subject to auto-induction (Schaller, 2001). However, the biosynthesis of JA during leaf senescence has not been characterized.

Here, we report that exogenous JA promotes typical senescence in both attached and detached Arabidopsis

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dopsis leaves but fails to induce precocious leaf senescence in the JA-insensitive mutant coi1, and that the endogenous JA levels in senescing Arabidopsis leaves are nearly 4-fold higher than that in fully expanded, non-senescing (NS) leaves. Consistent with an increased JA level in senescing leaves, genes encoding enzymes in the JA biosynthesis pathway are differentially activated during leaf senescence. These data suggest that JA has a role in leaf senescence in Arabidopsis.

RESULTS

JA Induces Precocious Leaf Senescence in Arabidopsis

To investigate the potential role of JA in leaf senescence, we treated Arabidopsis with JA. After growth on agar medium containing 30 μM JA for 12 d, expanding leaves (ELs) of Arabidopsis plants (ecotype Columbia-glabrous [Col-gl1]) displayed precocious senescence symptoms as indicated by visible yellowing (Fig. 2B). In contrast, leaves of Arabidopsis plants grown on the same medium without JA did not exhibit any senescence symptoms (Fig. 2A). Furthermore, coi1, a JA-insensitive mutant with the genetic background of Col-gl1 (Xie et al., 1998), did not undergo precocious senescence in the presence of JA (Fig. 2C), which demonstrated that the JA-responsive pathway is required for JA-promoted leaf senescence. It should be noted that natural leaf senescence in coi1 plants was not delayed compared with that of Col-gl1 (Y. He and S. Gan, unpublished data).

JA also promoted senescence in detached Arabidopsis leaves (Fig. 2D). Consistent with the visible yellowing, the photochemical quantum efficiency of photosystem II reaction center (Fv/Fm) in JA-treated leaves is much lower than that in control (Fig. 2E). To further investigate if the yellowing is a senescence process, we extracted total RNA from these leaves, and performed reverse transcription (RT)-PCR analysis of the expression of SAG12. SAG12 is a senescence-specific gene in Arabidopsis (Gan, 1995) that has been widely used as a molecular marker for leaf senescence (e.g. Weaver et al., 1998; Ludewig and...
Sonnewald, 2000; Morris et al., 2000; Hinderhofer and Zentgraf, 2001; Woo et al., 2001), but not for the hypersensitive reaction (Pontier et al., 1999). As shown in Figure 2F, SAG12 accumulated only in the leaves induced to become yellow by JA.

JA Levels Increase in Senescing Leaves

The fact that JA treatment promoted senescence in attached and detached Arabidopsis leaves prompted us to investigate whether the endogenous JA levels increase in senescing leaves. Total JA in fully expanded, NS leaves and in leaves at the early senescence stage (ES; these leaves showed up to 25% yellowing) of Arabidopsis was extracted and quantified using gas chromatography-mass spectrometry (GC-MS). As shown in Table I, the level of JA in ES leaves (130.0 pmol g fresh weight−1) is 4.7-fold higher than that in NS leaves (27.7 pmol g fresh weight−1). In contrast, the levels of OPDA in ES leaves (485.3 pmol g fresh weight−1) is less than one-half of that in NS leaves (1,143.0 pmol g fresh weight−1).

JA-Dependent Marker Gene PDF1.2 Is Up-Regulated during Leaf Senescence

PDF1.2 has been widely used as a JA-responsive marker gene (e.g. Penninckx et al., 1996; Moran and Thompson, 2001), which might be induced in response to an elevated JA level at senescing leaves. Therefore, we performed RT-PCR analysis using PDF1.2-specific primers (compare with Table II) to assess the transcript levels of this gene in leaves at the following four development stages: ELs (showing one-half size of fully expanded leaves); fully expanded, NS leaves; leaves at ES (up to 25% of a leaf shows yellowing); and at late-senescence stage (LS, more than 50% of a leaf shows yellowing). As shown in Figure 3, the level of PDF1.2 transcript increased 5.1- and 6.4-fold in ES and LS leaves, respectively, relative to that in NS leaves.

Genes Encoding Enzymes for JA Biosynthesis Are Differentially Activated during Leaf Senescence

We further investigated the molecular basis underlying the elevated JA level in senescing leaves. As shown in Figure 1, the biosynthesis of JA starts with α-linolenic acid, which is converted to its 13(S)-hydroperoxide by 13-LOX; the LOX product is subsequently converted to OPDA by sequential actions of AOS and allene oxide cyclase (AOC). OPDA is further reduced to form 3-oxo-2-cyclopentane-1-octanoic acid (OPC-8:0) by 12-oxo-phytodienoic acid reductase (OPR). After three cycles of β-oxidation, (±) 7-iso-JA is formed (Vick and Zimmerman, 1984; Schaller, 2001). We used RNA gel-blot and RT-PCR analyses to examine the steady-state mRNA levels of these JA biosynthesis-related genes in leaves at the four developmental stages described for the PDF1.2 analyses.

LOX

There are at least four LOX genes in the Arabidopsis genome. The nucleic acid sequence of LOX1 is divergent from the LOX2 sequence so that probes do not cross hybridize with each other (Bell and Mullet, 1993), whereas LOX3 (GenBank accession no. AJ249794) and LOX4 (GenBank accession no. AJ302042) share high homology. Thus, we used RNA gel-blot analysis to assess the expression of LOX1 and LOX2, and RT-PCR involving genespecific primers to analyze the transcript levels of LOX3 and LOX4. As shown in Figure 4A, LOX1, considered to be located in cytoplasm (Melan et al., 1993), was strongly up-regulated during leaf senescence. Consistent with previous reports (Bell and Mullet, 1993; Melan et al., 1993; Moran and Thompson, 2001), there were barely detectable signals in leaves before senescence (Fig. 4A). In contrast, LOX2, a plastidial stroma-localized LOX (Bell and Mullet, 1993; Creelman and Mullet, 1997), was sharply down-regulated (Fig. 4A). It is interesting that although LOX3 and LOX4, like LOX2, contain chloroplast transit peptide sequences and are believed to be plastidial, both genes (LOX3 and LOX4) were up-regulated with the progression of leaf senescence: The abundance of LOX3 transcript in ES and LS leaves is 3-fold of that in NS leaves, whereas the mRNA levels of LOX4 in ES and LS leaves are 7.3 and 5.4 times of that in NS leaves (Fig. 4).

AOS

The next gene in the JA biosynthesis pathway is AOS (Fig. 1). Unlike LOX, there exists only one AOS in the Arabidopsis genome. As shown in Figure 5, AOS was expressed at a relatively high level throughout leaf development, and was slightly up-regulated during leaf senescence.

AOC

AOC catalyzes the stereospecific cyclization of allene oxide to OPDA, thus establishing the stereochemistry of OPDA and JA (Vick and Zimmerman, 1984; Ziegler et al., 2000). AOC been has recently isolated from tomato (Lycopersicon esculentum; Ziegler et al., 2000). Using the amino acid sequence of tomato

| Table I. Levels of JA and 12-oxo-phytodienoic acid (OPDA) in Arabidopsis leaves |
|---------------------------------|-----------------|-----------------|
| Chemicals | NS (Four Replications) | ES (Three Replications) |
| JA       | 27.7 ± 4.5  | 130.0 ± 14.7  |
| OPDA     | 1,143.0 ± 292.1 | 485.3 ± 134.2 |

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AOC protein to search the Arabidopsis genome database, we found four annotated AOC genes in the genome. Furthermore, using these four annotated AOC nucleotide sequences to search the Arabidopsis expressed sequence tag database, three of four were found to match related ESTs. We refer to these AOCs as AtAOC1 (accession no. BAA95763), AtAOC2 (accession no. BAA95765), AtAOC3 (accession no. BAA95764), and AtAOC4 (accession no. AAG09557). Because of high sequence similarity among the cDNA regions of these AtAOCs, a pair of primers specific for each AtAOC (compare with Table II) were synthesized for RT-PCR analysis. The RT-PCR products were directly sequenced, and the results showed that each AtAOC was specifically amplified using the corresponding pair of primers (data not shown). Figure 6 shows that during leaf senescence, AtAOC1 is strongly up-regulated, and AtAOC2 is moderately up-regulated but its transcript abundance in leaves before senescence is very low. In contrast, AtAOC4 is down-regulated. It is interesting that AtAOC3 expression in ELs is relatively high, relatively low in NS leaves, and moderately up-regulated again during leaf senescence (Fig. 6).

OPR

Three OPR genes (OPR1, OPR2, and OPR3) have been isolated from Arabidopsis (Biesgen and Weiler, 1999; Sanders et al., 2000; Stintzi and Browse, 2000), and it has been reported that OPR3 is the major reductase converting OPDA to OPC-8:0 (Schaller et al., 2000). Because of the high sequence similarity between OPR1 and OPR2 (Biesgen and Weiler, 1999) as well as the sequence homology among OPR1, OPR2, and OPR3 (Sanders et al., 2000; Stintzi and Browse, 2000), the gene-specific primers for each OPR (compare with Table II) were used for RT-PCR analysis. As shown in Figure 7, both OPR3 and OPR1 are up-regulated during leaf senescence, especially in ES leaves: There are 2.9-fold (OPR1) and 2.3-fold (OPR3) increases relative to respective transcript abundance in NS leaves. In contrast, OPR2 appears to be constitutively expressed through these four stages of leaf development.

Table II. Primers used in this paper

<table>
<thead>
<tr>
<th>Primer No.</th>
<th>Nucleotide Sequences (from 5' to 3')</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CCACACGGATTACGCTGAG</td>
<td>Nos. 1 and 2 used for LOX1 northern-probe amplification</td>
</tr>
<tr>
<td>2</td>
<td>CCCCAGGCGGAGTACTCCG</td>
<td>Nos. 3 and 4 used for AOS northern-probe amplification</td>
</tr>
<tr>
<td>3</td>
<td>CCGAGTTTGCACGCTTCCG</td>
<td>Nos. 5 and 6 used for PTK1/2 northern-probe amplification</td>
</tr>
<tr>
<td>4</td>
<td>ACTTGTGACCGCCGCCC</td>
<td>Nos. 7 and 8 used for PDI northern-probe amplification</td>
</tr>
<tr>
<td>5</td>
<td>GAGGACCCCTGAGCCCTCTG</td>
<td>Nos. 9 and 10 used for LOX3 RT-PCR</td>
</tr>
<tr>
<td>6</td>
<td>GCCGGAGACAGACAGAAGAGCC</td>
<td>Nos. 11 and 12 used for LOX4 RT-PCR</td>
</tr>
<tr>
<td>7</td>
<td>CATGGGTTCCATCCCTGATAAA</td>
<td>Nos. 13 and 14 used for AtAOC1 RT-PCR</td>
</tr>
<tr>
<td>8</td>
<td>GACATATTTATATCTATAATTTTTGAGAAT</td>
<td>Nos. 15 and 16 used for AtAOC2 RT-PCR</td>
</tr>
<tr>
<td>9</td>
<td>AACAACATATAAACACATCTAAT</td>
<td>Nos. 17 and 18 used for AtAOC3 RT-PCR</td>
</tr>
<tr>
<td>10</td>
<td>CCACCCAGGACCGAAAGATCC</td>
<td>Nos. 19 and 20 used for AtAOC4 RT-PCR</td>
</tr>
<tr>
<td>11</td>
<td>GTAAGGACAGACAGAAGAGCC</td>
<td>Nos. 21 and 22 used for OPR1 RT-PCR</td>
</tr>
<tr>
<td>12</td>
<td>CATGGGTTCCATCCCTGATAAA</td>
<td>Nos. 23 and 24 used for OPR2 RT-PCR</td>
</tr>
<tr>
<td>13</td>
<td>CCGAGTTTGCACGCTTCCG</td>
<td>Nos. 25 and 26 used for OPR3 RT-PCR</td>
</tr>
<tr>
<td>14</td>
<td>GCCGGAGACAGACAGAAGAGCC</td>
<td>Nos. 27 and 28 used for SAG12 RT-PCR</td>
</tr>
</tbody>
</table>

Figure 3. RT-PCR analysis of the expression of JA-responsive marker gene PDF1.2 during leaf senescence. EL, About 50% of the fully expanded leaves; NS, fully expanded, NS leaves; ES, up to 25% of a leaf shows yellowing; LS, more than 50% of a leaf shows yellowing.
Thiolase

JA is believed to be synthesized from OPC-8:0 through three cycles of \( \beta \)-oxidation (Vick and Zimmerman, 1984; Schaller, 2001). Three 3-keto-acyl-thiolases, the enzyme responsible for \( \beta \)-oxidation, PED1, PKT1, and PKT2, have been identified from Arabidopsis (Rocha et al., 1996; Hayashi et al., 1998). PKT1 and PKT2 are encoded by the same genomic sequence (accession no. AF062589) and result from alternative splicing. PED1 (peroxisome defective) plays a key role in \( \beta \)-oxidation; a knockout of this thiolase causes defects in glyoxysomal fatty acid \( \beta \)-oxidation (Hayashi et al., 1998). PED1 has been shown to be involved in wounding-induced JA biosynthesis (M. Afitlhile and D.F. Hildebrand, unpublished data). RNA gel-blot analysis revealed that PED1 was strongly up-regulated during leaf senescence, whereas PKT1 and PKT2 were expressed at an extremely low level in these Arabidopsis leaves and...
could barely be detected in a 2-d exposed phospho-
image (Fig. 8).

**DISCUSSION**

A role for JA in leaf senescence has remained un-
clear. For example, transgenic potato (*Solanum tubero-
sum*) plants constitutively expressing a flax (*Linum usitatissimum*) AOS led to the overproduction of JA (Harms et al., 1995), but the authors did not report an ES phenotype in these transgenic plants. Also, the Arabidopsis triple mutant *fad3-2 fad7-2 fad8* (Vijayan et al., 1998) and the OPR3 knockout mutants because of T-DNA insertion (Schaller et al., 2000; Stintzi and Browse, 2000) produced little JA, but no significantly delayed leaf senescence phenotype was reported; similarly, in our work no obvious alteration in senes-
cence was observed in Arabidopsis mutants with impaired JA signaling (e.g. *coi1*). However, these facts may not necessarily contradict a senescence-
promoting role of JA and derivatives. Although the transgenic potato plants accumulated a much higher level of JA, they did not display characteristic pheno-
types such as the activation of JA-inducible genes. One plausible explanation is that the overproduced JA was sequestered so that it could not exert its biological action (Creelman and Mullet, 1997). On the other hand, it is not surprising that JA-
underproducing Arabidopsis mutants did not exhibit a significantly retarded leaf senescence phenotype because of the plasticity of leaf senescence (Gan and Amasino, 1997). It is known that many environmental stresses and endogenous factors can induce leaf senescence; these multiple pathways interconnect to form a regulatory network to control leaf senescence (Gan and Amasino, 1997; He et al., 2001). Thus, blocking a particular pathway (e.g. the JA-induced pathway) may not have an obvious effect on the progression of senescence (Bleecker and Patterson, 1997; Gan and Amasino, 1997).

How might JA induce leaf senescence? It has been suggested that JA promotes leaf senescence at the transcriptional level by activating a subset of SAGs (senescence-associated genes; Parthier, 1990). It is generally accepted that leaf senescence is driven by the expression of SAGs (Bleecker and Patterson, 1997; Buchanan-Wollaston, 1997; Gan and Amasino, 1997; Hajouj et al., 2000; Quirino et al., 2000). Recent studies have revealed that a number of SAGs in Arabi-
dopsis are up-regulated by JA or MeJA treatment. For example, MeJA induced expression of three SAGs (*SEN4, SEN5*, and *rVPE*; Park et al., 1998; Kinoshita et al., 1999). A micro-array analysis also revealed the induction of another six SAGs including *SEN1, SAG14*, and *SAG15* (Schenk et al., 2000). We have identified 125 Arabidopsis enhancer trap lines in which the reporter gene *GUS* displayed senescence-
associated expression in leaves, and we have found that *GUS* expression in 14 lines (14 of 125 lines or 11%) is induced upon JA treatment (He et al., 2001).

A senescence-promoting role might be associated with an elevated level of JA in senescing leaves. However, no quantitative analysis of JA pathway metabolite levels in senescing leaves has been performed (Creelman and Mullet, 1997). Our GC-MS analysis clearly showed that the JA level in early senescing leaves (up to 25% yellowing) is 5 times that in fully expanded, NS leaves in Arabidopsis (Table I).

The increase in JA level in senescing leaves is sup-
ported by our molecular findings that JA biosyn-
thesis-related genes are differentially activated dur-
ing leaf senescence (Figs. 4–8). It has been generally accepted that the initial steps of the JA biosynthesis pathway involving LOX, AOS, and AOC occur in chloroplasts (Schaller, 2001), which is well supported by studies involving the plastidial LOX2. LOX2 has been shown to play a role in wounding- and defense-
related responses in Arabidopsis plants (Bell and Mul-
et, 1993; Creelman and Mullet, 1997); it is un-
likely to be involved in JA biosynthesis during se-
nescence because its expression is turned off at the onset of leaf senescence (Fig. 4A). In contrast, the cytoplasmic *LOX1* is strongly activated during leaf senescence (Fig. 4A), and is likely to be responsible for the increased JA production in senescing Arabi-
dopsis leaves (Table I), although it is not known if the transcribed *LOX1* is translated to active enzyme. El-
levated LOX activities in senescing plant tissues have been repeatedly reported (for review, see Siedow, 1991). In addition to *LOX1*, the plastidial *LOX3* and *LOX4* are also up-regulated during leaf senescence in Arabidopsis (Fig. 4B) and thus may also contribute to JA biosynthesis in senescing leaves. Like *LOX*, there are four AOC genes in the Arabidopsis genome (this report), and three of them (*AOC1–AOC3*) are up-
regulated, whereas *AOC4* is down-regulated during leaf senescence (Fig. 6). To our knowledge, this is the first report on Arabidopsis AOCs, and their function-
ality needs to be further analyzed.

Recent studies have shown that OPR3 is involved in the biosynthesis of JA (Sanders et al., 2000; Schaller et al., 2000; Stintzi and Browse, 2000). This gene

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**Figure 8.** RNA gel blot analysis of thiolase genes during leaf senes-
cence. EL, NS, ES, and LS are as described in legend to Figure 3.
clearly is up-regulated during leaf senescence, especially at the early stage of leaf senescence (Fig. 7). In addition to OPR3, the transcript level of OPR1 also increases in senescing leaves, which is consistent with reporter gene studies that OPR1 promoter-directed reporter gene GUS accumulated in senescing Arabidopsis leaves (Biesgen and Weiler, 1999; Xie et al., 2001). Two cis elements are responsible for the accumulation of the OPR1 transcript during senescence (He and Gan, 2001). OPR2 appears to be constitutively expressed throughout the leaf development (Fig. 7). Whether OPR1 and OPR2 contribute to the accumulation of JA in senescing leaves remains unknown.

The final steps in the biosynthetic pathway of JA are believed to be three cycles of β-oxidation as supported by a study involving OPC derivatives (Miersch and Wasternack, 2000). Arabidopsis PED1 encodes a thiolase that is involved in the glyoxysomal fatty acid β-oxidation (Hayashi et al., 1998). PED1 is also involved in wound-induced JA biosynthesis (M. Afitlhile and D.F. Hildebrand, unpublished data). There are no reports as to whether other thiolase-encoding genes (e.g. PKT1 and PKT2) are involved in wounding- or defense-related JA biosynthesis. Our RNA gel-blot analysis shows that the abundance of PED1 transcripts is highly elevated in senescing leaves, whereas the mRNA of PKT1 and PKT2 is barely detectable throughout leaf development (Fig. 8), suggesting the involvement of PED1 in JA biosynthesis during leaf senescence.

In summary, our data support a role for JA in leaf senescence in Arabidopsis. This is based on the demonstration that exogenous application of JA induces leaf senescence, and this induction requires an intact JA signaling pathway. In addition, it was shown that the endogenous JA level in senescing leaves increased to nearly 500% of that in NS counterpart leaves, and this increase in JA level is apparently because of a subset of genes encoding isozymes for JA biosynthesis that are differentially activated during leaf senescence. The differential activation of these genes, especially the cytoplasmic LOX1, as discussed above indicates that the JA biosynthetic pathway in senescing leaves is mediated by different genes than those involved in the wounding and defense-related JA biosynthetic pathways involving the chloroplast-targeted LOX2.

**Plant Material and Growth Conditions**

The Arabidopsis mutant coi1 (Xie et al., 1998) was a gift from Dr. John G. Turner (John Innes Centre, Norwich, UK). The Arabidopsis ecotype Col-gl1 seeds were provided by Dr. Thomas Jack (Dartmouth College, Hanover, NH). Seeds were sterilized and grown in an Arabidopsis growth facility as previously described (He et al., 2001).

**RNA Analysis**

To minimize wounding- and dehydration-induced gene expression, leaf samples were quickly harvested and immediately frozen in liquid nitrogen. RNA extraction and northern-blot analysis were performed as described (He et al., 2001). RT-PCR was carried out by following the manufacturer’s instruction (CLONTECH Laboratories, Palo Alto, CA). The 18S rRNA primers and competitors of the QuantumRNA 18S Internal Standards Kit (Ambion Inc., Austin, TX) were used as an internal standard. The competitors were specially modified primers that anneal to the 18S rRNA templates but could not be extended, resulting in the production of an attenuated 315-bp internal fragment. Products of RT-PCR were recovered from agarose gels by using the GENECLEAN III kit (BIO101, Vista, CA) and directly sequenced by using an ABI PRISM 310 Genetic Analyzer (Perkin Elmer, Foster City, CA). The primers used for RT-PCR analysis and for amplification of gene-specific probes of genes involved in JA biosynthesis are listed in Table II.

**JA Treatments**

For in planta treatment, germinated seedlings were grown on 0.5× Murashige and Skoog agar medium containing 30 µM JA (Sigma, St. Louis) for 12 d under 16-h-light/8-h-dark cycle. JA treatment of detached leaves was performed as described (Xie et al., 2001). Detached young, NS rosette leaves were floated on 30 µM JA solution or water (control) for 4 d in the dark.

**Quantification of JA and OPDA**

JA and OPDA extraction and quantification were carried out according to a protocol modified from Albrecht et al. (1993). In brief, leaf material (about 1.0 g fresh weight) was collected from intact plants, quickly weighed, and immediately frozen in liquid nitrogen to minimize wound-induced JA accumulation. Samples were finely ground in a mortar while frozen. Dihydrojasmonic acid (a gift from Bedoukian Research Inc., Danbury, CT) was added to this sample at 0.2 nmol g fresh weight⁻¹. Extracted samples were analyzed by GC-MS (GC Plus, electron ionization mode, 30-m × 0.25-mm HP-5 column; Hewlett-Packard, Palo Alto, CA). The temperature gradient was 120°C for 1 min, 120°C to 270°C, at 6°C min⁻¹. Quantification was by selective ion monitoring (measuring ions m/z = 224 for JA methyl ester, m/z = 226 for methyl dihydrojasmonate, and m/z = 306 for OPDA methyl ester).

**MATERIALS AND METHODS**

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third-party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.
Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence in leaves was measured by using a portable modulated chlorophyll fluorometer (model OS1-FL, Opti-Sciences, Tyngsboro, MA) according to the manufacturer’s instructions. The $F_v$ and $F_m$ of each leaf were directly quantified by using module 6 of the OS1-FL.

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