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Expression of an *Oncidium* Gene Encoding a Patatin-Like Protein Delays Flowering in Arabidopsis by Reducing Gibberellin Synthesis

Chih-Ching Lin[1,3], Chung-Fu Chu[1,3], Pu-Huan Liu[1,3], Hsin-Hung Lin[1,3], Shih-Chien Liang[1], Wei-En Hsu[1], Jeng-Shane Lin[1], Hsin-Mei Wang[1], Ling-Lan Chang[1], Ching-Te Chien[2] and Shih-Tong Jeng[1,*]

[1]Institute of Plant Biology and Department of Life Science, National Taiwan University, Taipei 106, Taiwan
[2]Division of Silviculture, Taiwan Forestry Research Institute, Taipei 100, Taiwan
[3]These authors contributed equally to this work
*Corresponding author: E-mail, stjeng@ntu.edu.tw; Fax, +886-2-23918940
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The involvement of lipase in flowering is seldom studied, and this research provides evidence that fatty acids produced by lipase affect flowering. *OSAG78* encoding a patatin-like protein was isolated from *Oncidium* Gower Ramsey. OSAG78 fused with green fluorescent protein was found to localize at the cell membrane. Transgenic Arabidopsis overexpressing *OSAG78* demonstrated higher lipase activity than the wild-type control. In addition, the amount of free linoleic acid and linolenic acid in transgenic Arabidopsis was found to be higher than that in the wild type. Transgenics overexpressing *OSAG78* exhibited altered phenotypes, including smaller leaves and rounder flowers, and also demonstrated a late flowering phenotype that could be rescued by gibberellin A_3 (GA_3) application. Several flowering-related genes were analyzed, indicating that the expression of gibberellin-stimulated genes was decreased in the plants overexpressing *OSAG78*. Also, the expression of *AtGA2ox1*, *AtGA3ox1* and *AtGA20ox1* genes encoding GA2-, GA3- and GA20-oxidases, respectively, which are mainly responsible for gibberellin metabolism, was decreased, and the level of GA_4, a bioactive gibberellin, measured by gas chromatography–mass spectrometry was also reduced in the overexpressing lines. Furthermore, the expression levels of *AtGA3ox1* and *AtGA20ox1* were significantly decreased in wild-type Arabidopsis treated with linoleic acid, linolenic acid or methyl jasmonate. The membrane-bound OSAG78 might hydrolyze phospholipids to release linoleic acid and linolenic acid, and then depress the expression of genes encoding GA3- and GA20-oxidase. These changes reduced the bioactive gibberellin level, and, finally, late flowering occurred. Our results indicate that a patatin-like membrane protein with lipase activity affects flowering through the regulation of gibberellin metabolism.

Keywords: Flowering • Gibberellin • Lipase • Methyl jasmonate • *Oncidium* • Patatin.

Abbreviations: DAG, days after germination; FA, fatty acid; GA2ox, GA2-oxidase; GA3ox, GA3-oxidase; GA20ox, GA20-oxidase; GC-MS-SIM, gas chromatography–mass spectrometry–selected ion monitoring; GFP, green fluorescent protein; JA, jasmonic acid; LA, linoleic acid; LNA, linolenic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MJ, methyl jasmonate; PLA, phospholipase A; pNPP, *p*-nitrophenyl palmitate; RT–PCR, reverse transcription–PCR; SEM, scanning electron microscopy.

Introduction

The transition from a vegetative stage to a flowering stage is an important decision for plants. In Arabidopsis, the initiation of flowering is regulated by photoperiod, vernalization and autonomous pathways. It is further controlled by the hormone gibberellin (He and Amasino 2005). Mutation at the *SPINDLY* locus of Arabidopsis activates gibberellin signal transduction constitutively, and causes plants to flower early (Jacobsen and Olszewski 1993). In contrast, the Arabidopsis mutants *gai* and *ga1-3* with a blockage of gibberellin signaling and biosynthesis, respectively, exhibit a late flowering phenotype (Wilson et al. 1992). GA thus profoundly affects the flowering time of plants.

The activities of GA20-oxidase (GA20ox), GA3-oxidase (GA3ox) and GA2-oxidase (GA2ox) are the main regulatory steps of gibberellin biosynthesis (Hedden and Phillips 2000). Overexpression of *GA20ox* in rice and citrus increases bioactive gibberellins (Oikawa et al. 2004, Fagoaga et al. 2007). Early flowering under both long- and short-day environments was also observed in Arabidopsis overexpressing *GA20ox* (Huang et al. 1998, Coles et al. 1999). While antisense expression of *GA20ox1* reduces bioactive gibberellin levels (Fagoaga et al. 2007), it delays flowering in short days (Coles et al. 1999). Arabidopsis with mutated *GA3ox1* contains reduced bioactive gibberellins and exhibits a semi-dwarf phenotype (Talon et al. 1990). In

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contrast, overexpression of a bean *GA2ox* in Arabidopsis results in extreme dwarf and semi-dwarf phenotypes (Hedden and Phillips 2000). The treatment of gibberellin-deficient mutants of Arabidopsis with gibberellin results in an increase in mRNA content of *GA2ox*. This finding points to the existence of positive feedback of *GA2ox* genes (Thomas et al. 1999). Therefore, GA20ox and GA3ox positively regulate and GA2ox negatively regulates bioactive gibberellin levels within plants.

Genes encoding proteins with papatin domains were first found in potato, and they belong to a gene family with 10–18 members per haploid genome (Mignery et al. 1988, Twell and Ooms 1988). Patatin-like proteins have also been reported in tobacco and rubber tree, and their activities include lipase and phospholipase A (PLA) (Dhondt et al. 2002, Jekel et al. 2003). Based on the genomic sequence, 10 patatin-related *PLA* genes were discovered in Arabidopsis, and they are divided into three groups (Holk et al. 2002). Of these 10 genes, *AtPLA I* (AC004392), *AtPLA IIA* (AC002505) and *AtPLA IVA* (GI:4006869), and *AtPLA IVC* (GI:4006871) were preferentially found in shoots, roots and flowers, respectively. They are located primarily in the cytosol (Holk et al. 2002).

Patatin-like proteins are involved in the development of tuber in potato (Liu et al. 1991, Peña-Cortés et al. 1991, Perl et al. 1991). They were also found in other plants with various functions. *SUGAR-DEPENDENT1* encodes a protein with a patatin-like acyl hydrolase that initiates storage oil breakdown in germinating Arabidopsis seeds (Eastmond 2006). In addition, two of the Arabidopsis patatin-like genes, *PLP2/AtPLA IIA* and *PLP7/AtPLA IIIA*, are strongly induced in leaves challenged with fungal and bacterial pathogens (Narusaka et al. 2003, Camera et al. 2005). Plants silenced for *PLP2/AtPLA IIA* expression display enhanced resistance to *Botrytis cinerea*, whereas plants that overexpress *PLP2/AtPLA IIA* are much more sensitive to pathogen infection (Camera et al. 2005). Also, the induction of *AtPLA IIA* is regulated by abiotic stresses, including cold, high salinity, ABA, salicylic acid, methyl jasmonate (MJ), ethephon, ultraviolet C light, wounding and iron deficiency (Narusaka et al. 2003, Rietz et al. 2004). However, the relationship between the expression of lipase encoded by *PLP2/AtPLA IIA* and the sensitivity of plants to pathogens is not clear at the molecular level.

Patatin-like proteins have lipase and PLA activities and are capable of hydrolyzing membrane glycerophospholipids to become lysophospholipids and free fatty acids (FAs) (Yang et al. 2007). Lysophospholipids, such as lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE), are believed to be active components in cellular signaling pathways (Drissner et al. 2007). LPC is capable of inducing the phosphate transporter genes *StPT3* and *StPT4* of potatoes and *LePT4* of tomatoes. It is also a signal involved in the arbuscular mycorrhizal symbiosis (Drissner et al. 2007). Free FAs, including linoleic acid (LA) and linolenic acid (LNA), can be converted into bioactive oxylipin compounds such as jasmonic acid (JA) (Weber 2002). JA and 12-oxophytodienoic acid are accumulated after the activation of PLA2 encoded by a patatin-like gene in tobacco cells infected by the tobacco mosaic virus. This finding indicates that patatin-like proteins may mediate the production of JA and defense responses in plants (Dhondt et al. 2000, Dhondt et al. 2002). An Arabidopsis acyl hydrolase encoded by *At*PLAI hydrolyzes phospholipid at the *sn*-1 and *sn*-2 position. This may result in the production of free FAs that can be converted into JA and its derivatives by downstream metabolic enzymes (Yang et al. 2007). Hence, both lysophospholipids and free FAs are involved in the signal transduction of plants.

In this study, *OSAG78* encoding a patatin-like protein was isolated from *Oncidium*. The localization of OSAG78 in plant cells was studied by the expression of OSAG78 fused to green fluorescent protein (GFP). Also, in order to analyze the function of *OSAG78* further, Arabidopsis overexpressing *OSAG78* were obtained, and their phenotypes and flowering time were investigated. Expression levels of flowering-related genes, including *FLOWERING LOCUS T* (*FT*), *CONSTANT* (*CO*), *FLOWERING LOCUS C* (*FLC*), *SUPPRESSOR OF CO OVEREXPRESSION 1* (*SOC1*), *GLABROUS1* (*GL1*) and gibberellin metabolism genes, encoding GA2ox, GA3ox and GA20ox, in wild-type and transgenic Arabidopsis were analyzed by real-time PCR. Finally, the amounts of GA_4, LA and LNA in plants were measured by gas chromatography to elucidate the relationship between free FAs and gibberellin contents in cells.

Results

OSAG78 is a membrane protein with a patatin-like domain

OSAG78 was isolated from suppression subtractive hybridization between fully opened and partially senescent flowers of *Oncidium*. Reverse transcription–PCR (RT–PCR) was used to confirm *OSAG78* expression of flowers in the different senescent stages. The result indicates that *OSAG78* is a senescence-repressed gene in *Oncidium* flowers (**Fig. 1a**). Amino acid sequence analysis by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) reveals that OSAG78 contains 407 amino acids and is homologous to lipid acyl hydrolase-like proteins that bear one patatin domain, such as those from *Oryza sativa* and *Arabidopsis thaliana* (**Fig. 1b**), and their identities are 45–51%. The phylogenetic analysis shows that OSAG78 unroots from a phylum composed of rice lipid acyl hydrolase protein (**Fig. 1c**). The hydrophobicity of OSAG78 was predicted by Vector NTI (Invitrogen) whose scanning window was set as 50 amino acids. The hydropathy plot indicates that amino acids 38–296 of OSAG78 are mainly hydrophobic with a small hydrophilic region, whereas amino acids 297–407 are hydrophilic (**Fig. 2a**). The long stretch of the hydrophobic domain gave us a hint that OSAG78 is a membrane protein with transmembrane domains. OSAG78 was further fused to the N-terminus of GFP to form OSAG78–GFP. This construct was then delivered into onion epidermal cells via bombardment. Fluorescence signals of OSAG78–GFP appeared

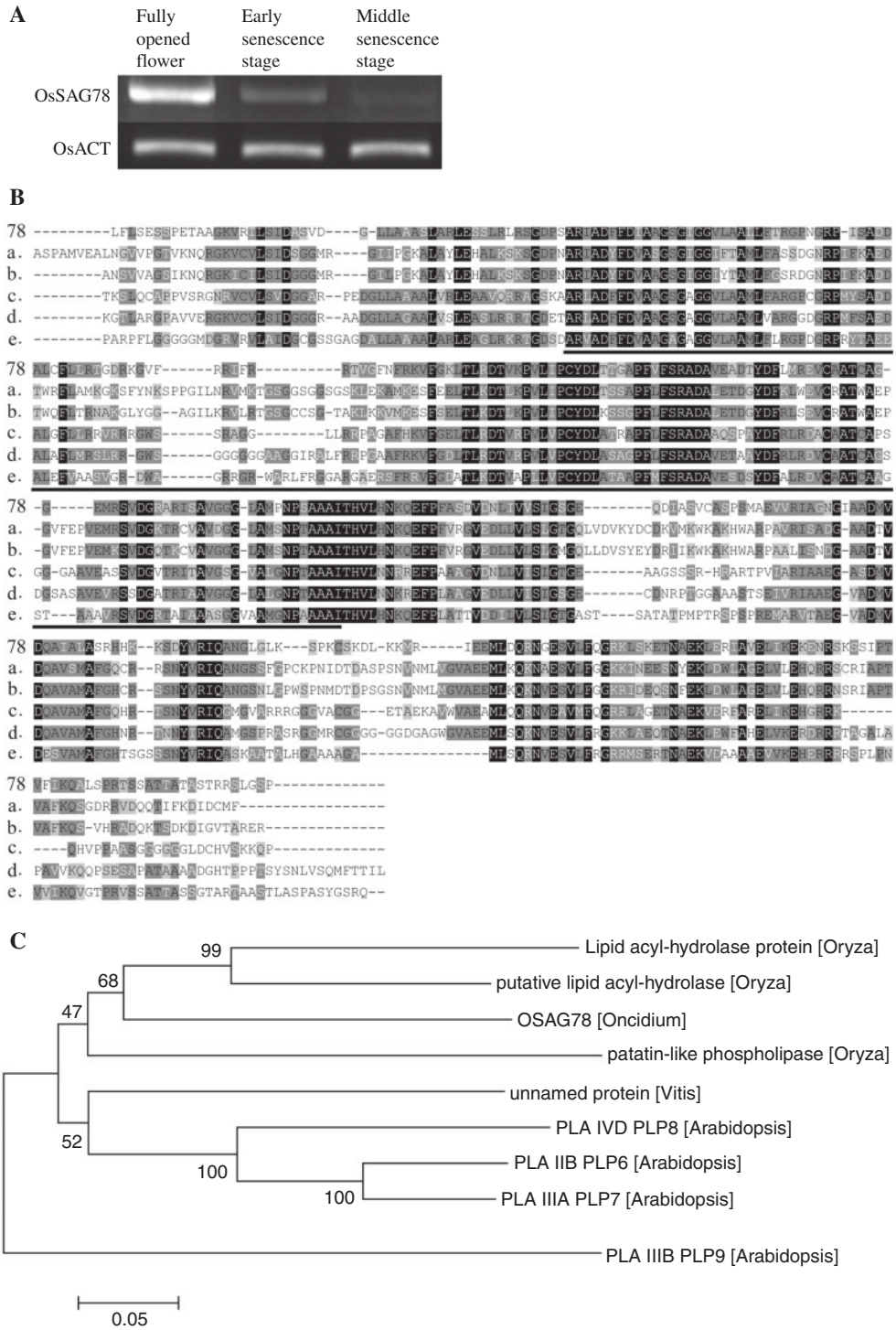

Fig. 1 *OSAG78* expression in *Oncidium* and comparison of OSAG78 with other patatin-like proteins. (a) Analyses of *OSAG78* expression in the fully opened flowers and the early and middle senescence flowers of *Oncidium* by RT–PCR. The expression of the *OnACT* gene was used as an internal control. (b) Alignment of OSAG78 with several plant proteins bearing a patatin-like domain. The patatin domain is indicated by black underlining. The letters with a black background indicate identical amino acids, and those with a gray background indicate conserved amino acids. The black letters on the gray background are the major conserved amino acids, and the white letters on the gray background are the second major conserved amino acids in these proteins for comparison. The number 78 represents the deduced amino acid sequence of *OSAG78*; a, *A. thaliana*, NP_181455; b, *A. thaliana*, NP_191055; c, *O. sativa*, NP_911167; d, *O. sativa*, NP_909908; e, *O. sativa*, NP_001050740. (c) Phylogenetic tree of OSAG78 and several plant proteins with a patatin-like domain. The tree was built using MEGA 4.0 software upon protein sequence alignment. Lipid acyl hydrolase protein [Oryza], BAC19963; putative lipid acyl-hydrolase [Oryza], AAK72885; patatin-like phospholipase [Oryza], BAD45518; unnamed protein [Vitis], CAO46768; PLA IVD, PLP8 [Arabidopsis], AT4G29800; PLA IIB, PLP6 [Arabidopsis], AT2G39220; PLA IIIA, PLP7 [Arabidopsis], AT3G54950.1; PLA IIIB, PLP9 (*STURDY*) [Arabidopsis], AT3G63200.

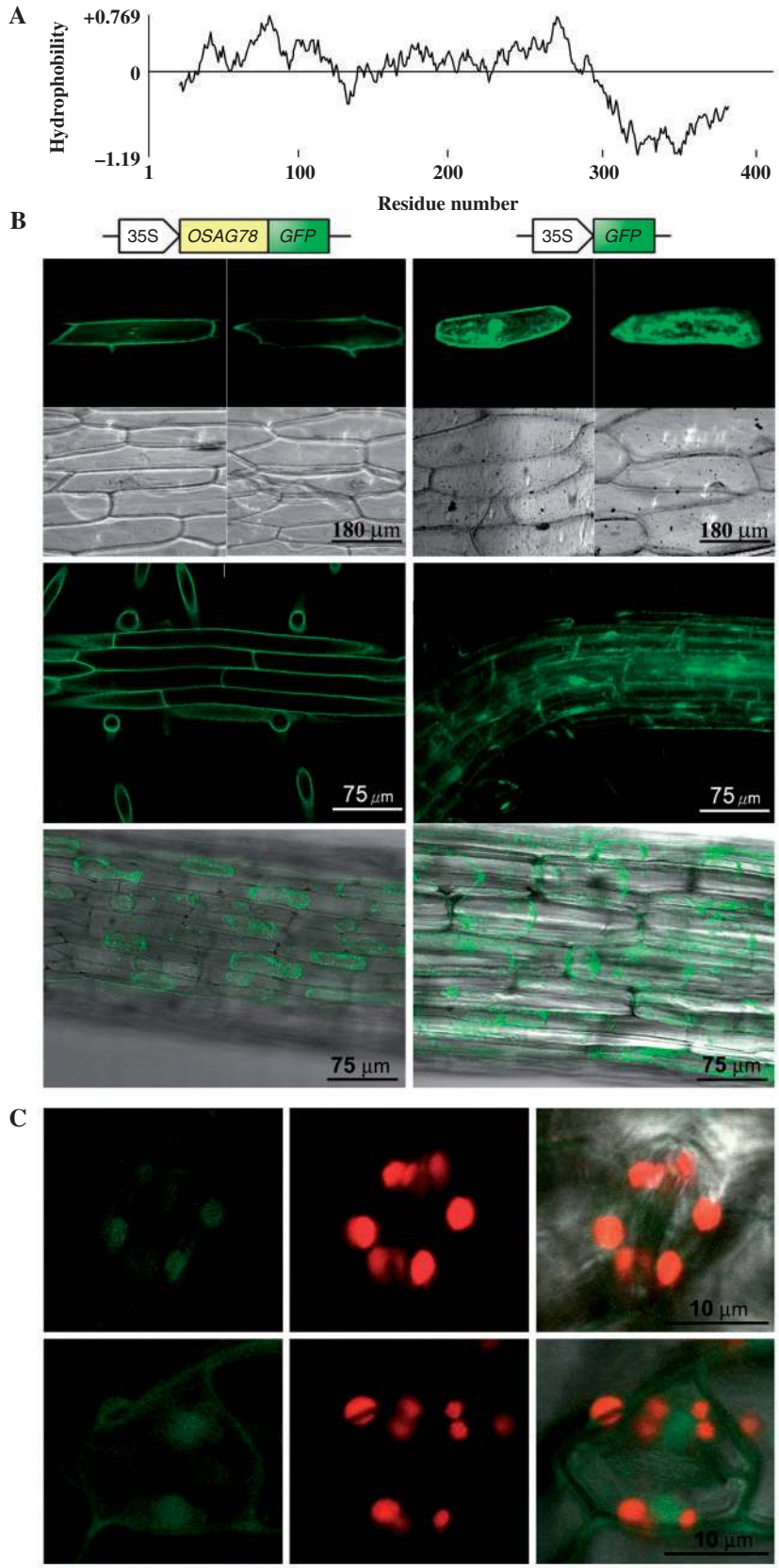

Fig. 2 Hydropathy plot and cellular localization of OSAG78. (a) The hydropathy plot of OSAG78 was predicted by Vector NTI (Invitrogen) whose scanning window was set as 50 amino acids. (b) The cellular localization of OSAG78 fused to the N-terminus of green fluorescent protein

(continued)

at the peripheral region of cells when observed under a confocal microscope (Fig. 2b, top of the left panel). Fluorescence of GFP alone was scattered through the entire cell, as shown in the GFP control image (Fig. 2b, top of the right panel). These results indicate that OSAG78 tends to associate with the plasma membrane. Furthermore, the roots of the transgenic Arabidopsis overexpressing OSAG78–GFP or GFP were harvested and observed using a confocal microscope. The OSAG78–GFP fusion protein also localized at the plasma membrane of the root cells. Meanwhile, GFP alone was spread throughout the cells (Fig. 2b, center panels). These results from transgenic plants are consistent with those from the onion cells by bombardment. However, we were not sure whether the localization of OSAG78–GFP is at the plasma membrane or at the apoplast. The roots of transgenic plants were additionally treated with hypertonic solution to produce plasmolysis. OSAG78–GFP separated from the cell wall, indicating that OSAG78 is localized at the plasma membrane (Fig. 2b, bottom of the left panel). The distribution of OSAG78–GFP was also observed in chloroplasts (Fig. 2c), indicating that OSAG78–GFP may also occur in the membrane of chloroplast. All these data strongly suggest that OSAG78 is indeed a membrane protein.

OSAG78 has lipase activity

The BLAST result of the OSAG78 peptide sequence indicates that OSAG78 contains a patatin domain that is similar to that of acyl hydrolases (Fig. 1b), so OSAG78 might demonstrate lipase activity. Hence, crude proteins from the leaves of Arabidopsis overexpressing *OSAG78* driven by the *35S* promoter were isolated for lipase assays via their ability to hydrolyze *p*-nitrophenyl palmitate (pNPP), a substrate used in lipase assays (Pencreac'h and Baratti 1996). Lipase activities of transgenic lines *35S::OSAG78-8*, *35S::OSAG78-9* and *35S::OSAG78-11* were much higher than that of wild-type controls (Fig. 3a). Since *35S::OSAG78-9* plants showed the highest lipase activity among these transgenics, it was used for further analysis in this study. Similarly, gas chromatography analyses revealed that the amount of FAs, LA and LNA in *35S::OSAG78* plants was also significantly more elevated than that in wild-type controls (Fig. 3b). These results indicate that OSAG78 has lipase activity, and also that OSAG78 can hydrolyze phospholipids to produce free FAs.

Phenotypes of Arabidopsis overexpressing *OSAG78*

To dissect the detailed functions of OSAG78, transgenic Arabidopsis with *OSAG78* driven by the *35S* or *AGAMOUS LIKE5* (*AGL5*) promoter were created. *35S::OSAG78* Arabidopsis grew more slowly than wild-type controls. Typically, wild-type plants already flowered at 30 days after germination (DAG) (Fig. 4a), while the *35S::OSAG78* plants still remained in the vegetative state (Fig. 4b). Also, the flowers, stamens and pistils of *35S::OSAG78* plants were shorter and the rosette and cauline leaves were much smaller than those of wild-type plants (Fig. 4c–f). Interestingly, the stable transgenic Arabidopsis overexpressing OSAG78–GFP, which was used for localization assay (Fig. 2b, c), also showed the same phenotypes as *35S::OSAG78* plants. The *AGL5* promoter is a flower-specific promoter (Savidge et al. 1995). Similarly to *35S::OSAG78* transgenics, flowers, stamens and pistils in the *AGL5::OSAG78* plants were shorter than those in wild-type plants (Fig. 4c–e). However, the leaves of *AGL5::OSAG78* plants were the same size as those of the wild type (Fig. 4f). The expression of *OSAG78* in transgenic plants was also analyzed by RT–PCR. The expression of *OSAG78* in *35S::OSAG78* plants could be detected in both flowers and leaves, while the expression of *OSAG78* could only be detected in the flowers of *AGL5::OSAG78* plants (Fig. 4g). In addition, the expression level of *OSAG78* driven by the *AGL5* promoter was lower than in transgenics harboring the *35S* promoter (Fig. 4g). Therefore, the phenotypic changes in the plants with *AGL5::OSAG78* are less severe than those in *35S::OSAG78* plants. Microscopic analysis revealed that transgenic plants overexpressing *OSAG78* exhibit smoother and smaller cell surfaces in leaf, petal and seed coat cells than the wild-type plants (Fig. 5a–g). Abnormal cell shapes may result from the overexpression of OSAG78, which, as a lipase, is located in the membrane and may cause the membrane to function abnormally.

Overexpression of OSAG78 causes plants to flower late

A standard indicator for flowering time is the number of rosette leaves produced on the primary stem before the first flower is initiated, and plants flowering late produce more leaves (Koornneef et al. 1991). Under the same growth conditions, wild-type Arabidopsis flowered at about 27 DAG, whereas *35S::OSAG78* plants flowered at about 35 DAG (Table 1). This indicates that transgenic plants with *35S::OSAG78* demonstrated a delayed flowering phenotype. Expression levels of several flowering-related genes, including *FT*, *CO*, *FLC* and *SOC1*, were examined using real-time PCR (Fig. 6a). *FLC* is the central floral repressor that inhibits the expression of the floral integrator genes *FT* and *SOC1*, and *CO* is a positive regulator that

Fig. 2 Continued
(OSAG78–GFP) was examined by confocal microscopy. The expression of OSAG78–GFP (the left panels) and the expression of GFP alone (the right panels) through particle bombardment were visualized in onion cells (the upper panels). The root cells of transgenic Arabidopsis overexpressing OSAG78–GFP and plants overexpressing GFP alone were visualized (the center panels), and they were also treated with 0.4 M mannitol to produce plasmolysis before examination (the lower panels). (c) The localization of OSAG78–GFP in guard cells was examined by confocal microscopy. The expression of OSAG78–GFP is indicated as green, and the localization of chloroplasts is shown as red. Guard cells from *35S::OSAG78-GFP* plants are present in the upper panels, and those from *35S::GFP* plants is in the lower panels. The last pictures of each upper and lower panels are the images overlapping bright field and fluorescence images of GFP and chloroplast.

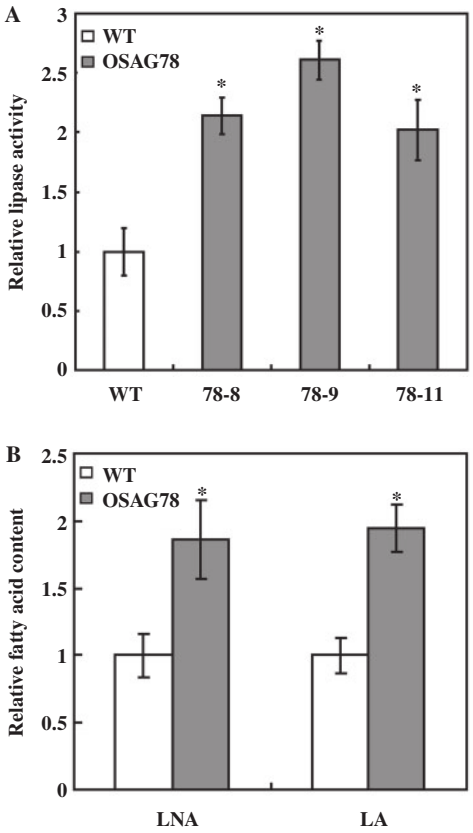

Fig. 3 Relative lipase activity and relative linoleic acid and linolenic acid contents. (a) The lipase activities of 21-day-old wild-type plants and *35S::OSAG78* Arabidopsis (OSAG 78) lines 8, 9 and 11 are shown as WT, 78-8, 78-9 and 78-11, respectively. Crude protein extracts from plants were treated with pNPP, and lipase activities were measured by 410 nm absorbance. The lipase activity of wild-type plants was treated as the normalized reference, with a value of 1, for determination of the relative lipase activity. (b) The relative amounts of linoleic acid (LA) and linolenic acid (LNA) in 21-day-old wild-type (WT) and *35S::OSAG78* Arabidopsis (OSAG78) were measured by gas chromatography. These data are normalized by the levels of the internal control, pentadecanoic acid, and their ratios relative to each fatty acid in wild-type plants are shown as relative contents. Each bar represents the average of three biological assays. Error bars indicate the standard deviation of the mean. Statistical differences between wild-type and transgenic plants are marked with asterisks when $P < 0.05$ according to Student's test.

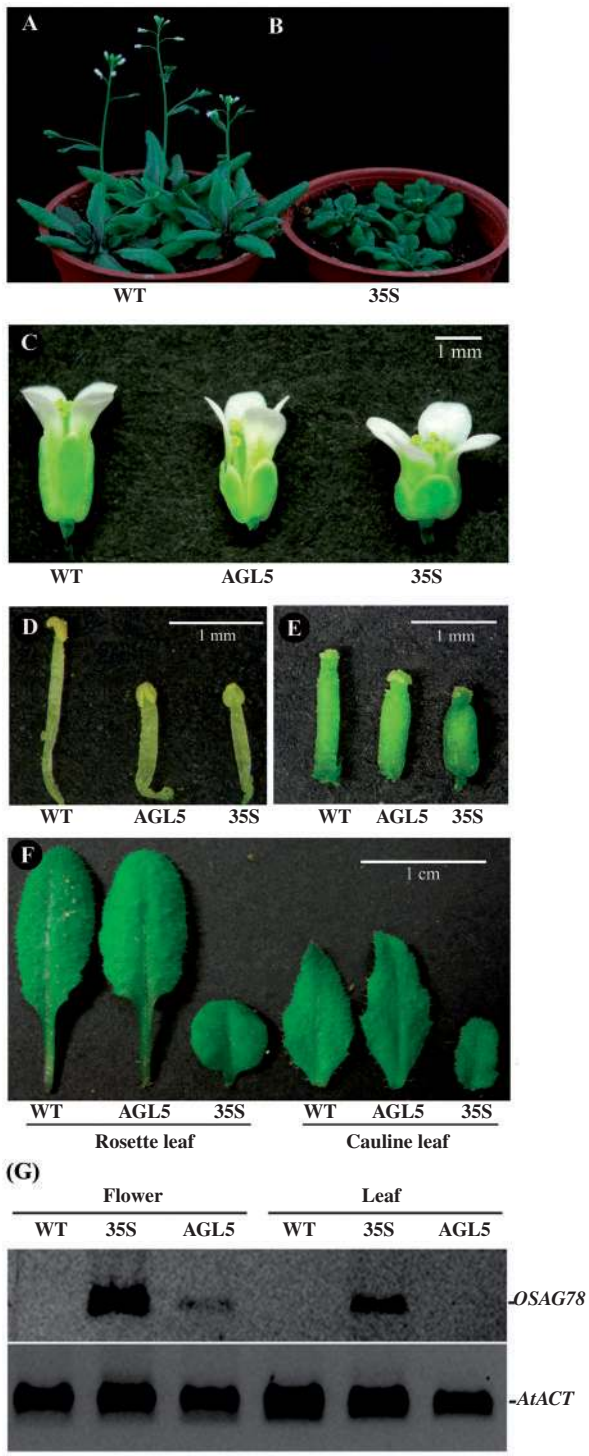

Fig. 4 Phenotypes of Arabidopsis overexpressing *OSAG78*. Wild-type (a) and *35S::OSAG78* (b) plants at 30 days after germination are shown. Flowers (c), stamens (d), pistils (e) and leaves (f) of wild-type (WT) and transgenic Arabidopsis overexpressing *OSAG78* driven by the *35S* (35S) and *AGL5* (AGL5) promoters are indicated. (g) Expression of *OSAG78* in Arabidopsis. The expression levels of *OSAG78* driven by *35S* and *AGL5* promoters in the flowers and leaves of wild-type and transgenic Arabidopsis were analyzed by RT–PCR. The expression of the *actin* (*AtACT*) gene was used as an internal control. The black and white colors of this ethidium bromide-stained gel were reversed.

stimulates *FT* and *SOC1*. The expression levels of *FLC*, *CO* and *FT* were similar in *35S::OSAG78* plants and the wild-type controls, whereas *SOC1* was significantly down-regulated in *35S::OSAG78* plants (**Fig. 6a**). A low *SOC1* expression level may explain the late flowering phenotype of *35S::OSAG78* plants. *SOC1* is inhibited by *FLC* but activated by *CO* and gibberellin (He and Amasino, 2005). Since the expression levels of *FLC* and *CO* were about the same in wild-type and *35S::OSAG78* plants, the decrease in *SOC1* expression may be due to a decline in gibberellin concentration. There was evidence that the expression of the gibberellin-inducible gene *GL1* was also decreased in

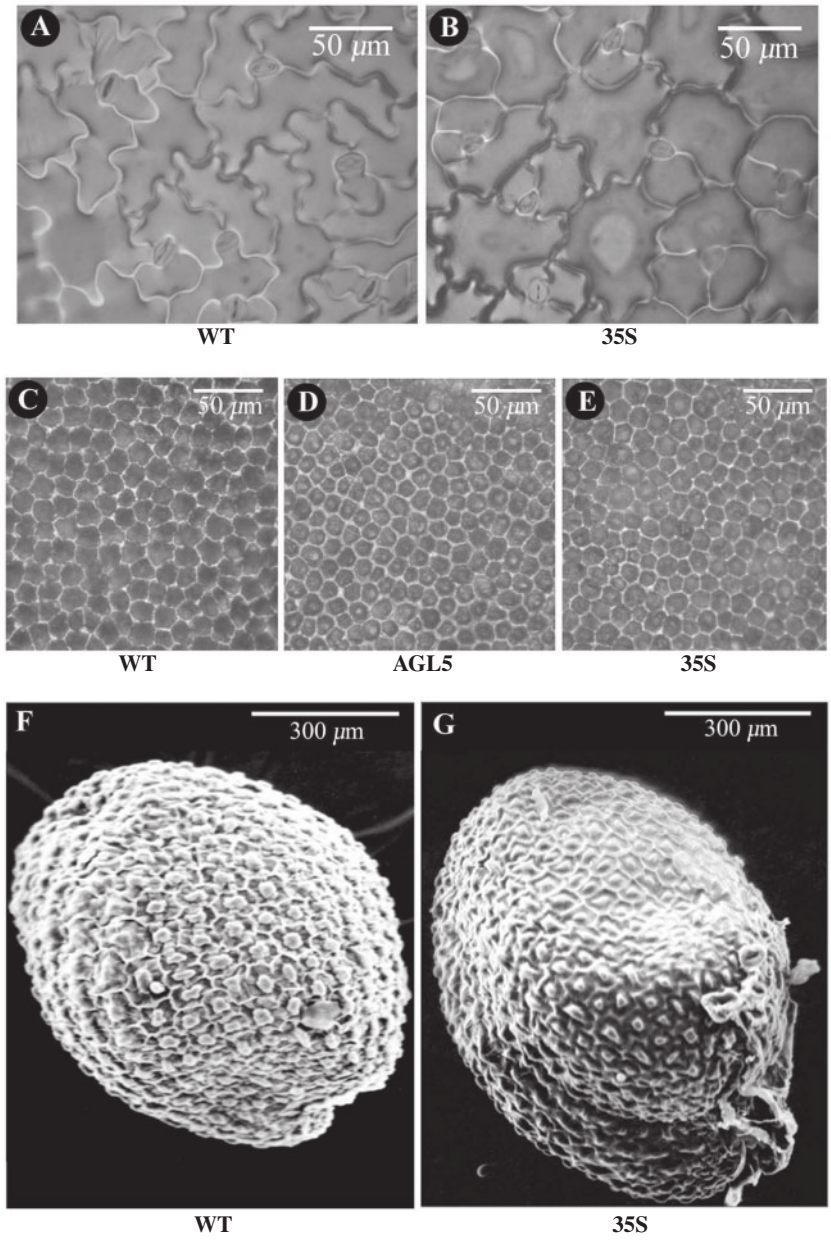

Fig. 5 Cell morphology of Arabidopsis overexpressing *OSAG78*. Cells in the leaf epidermis (a and b) and petals (c, d, and e) of wild-type (WT) and transgenic Arabidopsis overexpressing *OSAG78* driven by the *35S* (35S) and *AGL5* (AGL5) promoters were observed using light microscopy. The seeds from wild-type and *35S::OSAG78* Arabidopsis were observed using a scanning electron microscope (f and g).

35S::OSAG78 plants (**Fig. 6a**). This finding indicates that the amount of gibberellin may be lower in *35S::OSAG78* plants than in wild-type controls. Furthermore, when *35S::OSAG78* transgenics were treated with 10 μM GA_3, the late-flowering phenotype was rescued (**Table 1**). Without GA_3 treatment, wild-type plants flowered at 27.1 DAG with 11.5 rosette leaves (**Table 1**), and the late flowering *35S::OSAG78* plants flowered at 35.4 DAG with 15.6 rosette leaves. After GA_3 treatment, wild-type plants flowered slightly earlier at 25.1 DAG with 9.2 rosette leaves. However, the flowering profile of *35S::OSAG78* plants under GA_3 treatment was 28.3 DAG with 11.4 rosette leaves. This profile is almost the same as that of wild-type plants without GA_3 treatment. In addition, *SOC1* expression in the plants treated with GA_3 was also measured by real-time RT–PCR. The *SOC1* expression level in the *35S::OSAG78* plants treated with GA_3 was similar to that in the wild-type plants (**Fig. 6b**). These results strongly suggest that the late flowering phenotype of *35S::OSAG78* plants is caused by a shortage of gibberellin.

35S::OSAG78 plants have less bioactive gibberellin

Since gibberellin treatment can rescue the late flowering phenotype of *35S::OSAG78* plants, this phenotype may be

Table 1 Number of rosette leaves and number of days after germination when flowering

	Without gibberellin treatment		With gibberellin treatment	
	Wild type	*OSAG78*	Wild type	*OSAG78*
Rosette leaves	11.5 ± 0.5	15.6 ± 2.0	9.2 ± 0.4	11.4 ± 0.6
Days	27.1 ± 0.5	35.4 ± 3.2	25.1 ± 0.9	28.3 ± 1.2

The number of rosette leaves and number of days after germination are indicated when wild-type and *35S::OSAG78* Arabidopsis initiated flowering with and without gibberellin treatment. Data are from 25 plants, and are presented as the mean ± SD.

caused by a lack of gibberellin rather than gibberellin insensitivity. The amounts of GA_1 and GA_4, the biologically active gibberellins, were further measured with their internal controls using gas chromatography–mass spectrometry–selected ion monitoring (GC-MS-SIM). The presence of GA_1 was not detected in either wild-type or *35S::OSAG78* plants, probably due to its low abundance in the 21-day-old Arabidopsis under 100 $\mu mol\ m^{-2}\ s^{-1}$ light. However, the level of GA_4 in *35S::OSAG78* plants was significantly lower than that in wild-type plants (**Fig. 7**). Gibberellin levels within Arabidopsis can be variable depending on their growth conditions (Fei et al. 2004, Magome et al. 2008, Rieu et al. 2008), but this result indicates that reduced GA_4 in plants may make *35S::OSAG78* plants flower late.

Furthermore, the expression levels of *GA2ox1*, *GA2ox2*, *GA3ox1* and *GA20ox1* from the 21-day-old leaves of the wild type and three independent transgenics were examined by real-time RT–PCR. The reasons for studying these specific gibberellin oxidase genes are described in the Discussion. GA20ox and GA3ox are enzymes that catalyze bioactive gibberellin synthesis, whereas GA2ox is responsible for converting bioactive gibberellins into an inactive form. In terms of genes encoding gibberellin oxidases responsible for bioactive gibberellin synthesis, the expression levels of *GA3ox1* and *GA20ox1* were reduced in *35S::OSAG78* plants compared with those in wild-type plants (**Fig. 8**). In relation to GA2ox, the expression level of *GA2ox1* is decreased, and the expression level of *GA2ox2* was not significantly affected (**Fig. 8**). The decrease in bioactive GA_4 caused by the reduced expression of the *GA3ox1* and *GA20ox1* genes in *35S::OSAG78* plants may further diminish the expression of *GA2ox1* due to the positive feedback of *GA2ox* genes by bioactive gibberellins (Thomas et al. 1999). However, the expression of *GA2ox2* is not influenced by bioactive gibberellins, and this may be due to the different regulatory mechanism of *GA2ox1* and *GA2ox2*. These results indicate that the major genes encoding gibberellin oxidases for gibberellin biosynthesis in Arabidopsis are down-regulated in *35S::OSAG78* plants, and consequently *35S::OSAG78* plants have less bioactive GA_4.

Effects of free FAs and lysophospholipids on wild-type plants

Phospholipids on the membrane can be converted into free FAs, such as LA and LNA, and lysophospholipids, such as LPC and LPE, in the presence of OSAG78 as a lipase (**Fig. 3a**). Wild-type plants were treated with LA, LNA, LPC or LPE to mimic the physiological status of *35S::OSAG78* plants. The expression levels of *GA2ox1*, *GA2ox2*, *GA3ox1* and *GA20ox1* were evaluated using real-time RT–PCR (**Fig. 9**). In terms of genes encoding gibberellin oxidases responsible for bioactive gibberellin synthesis, the addition of LPC and LPE either enhanced or did not affect the expression levels of *GA3ox1* and *GA20ox1*, and thus LPC and LPE may not be the factors reducing GA_4 in *35S::OSAG78* plants. However, the expression levels of *GA3ox1* and *GA20ox1* were significantly decreased by LNA or LA (**Fig. 9**). In terms of genes encoding gibberellin oxidases for converting bioactive gibberellins to inactive forms, the addition of LPC did not affect the expression of *GA2ox1* and *GA2ox2*. Whereas the addition of LPE increased *GA2ox1* expression, it decreased *GA2ox2* expression. However, the expression level of *GA2ox1* was increased by the presence of LNA, but the expression of *GA2ox2* was decreased by LA. These results indicate that the lysophospholipids LPC or LPE may not reduce bioactive GA_4 in cells, but that the presence of the free FAs LA and LNA is able to decrease the expression of genes encoding enzymes responsible for bioactive gibberellin synthesis.

MJ reduces the expression of GA3ox and GA20ox

Free FAs released by PLA2 from phospholipids are precursors of JA (Yang et al. 2007). Therefore, the expression levels of the MJ-inducible genes *PDF 1.2* and *pathogenesis-related 4* (*PR4*) in wild-type and *35S::OSAG78* plants were evaluated by real-time PCR. These genes were found to be up-regulated in *35S::OSAG78* plants (**Fig. 6c**). This suggests that *35S::OSAG78* transgenics have higher MJ levels. The increased MJ concentration may be explained by elevated concentrations of free FAs in *35S::OSAG78* plants.

Wild-type plants were further treated with MJ, and the expression levels of *GA2ox1*, *GA2ox2*, *GA3ox1* and *GA20ox1* were analyzed using real-time RT–PCR (**Fig. 9**). Interestingly, mRNAs of *GA3ox1* and *GA20ox1* were down-regulated in plants treated with MJ. These results indicate that MJ may decrease the bioactive gibberellin level by altering gibberellin metabolism, and this results in a delay in flowering. This interpretation is further supported by the Arabidopsis mutant *JASMONATE RESISTANCE 1* (*jar-1*), which is insensitive to MJ. *jar-1* flowers at 24 DAG, and it shows earlier flowering than wild-type plants (**Supplementary Fig. S1**). Therefore, MJ may negatively regulate the function of gibberellin in flowering.

Discussion

The induction of genes encoding lipase may have roles in signaling (Major and Constabel 2006). Lipase from OSAG78 may convert phospholipids into free FAs and lysophospholipids. Free FAs and lysophospholipids are also produced by cytosolic PLA2 when a suspension cell culture of parsley is induced by auxin. Hence, both FAs and lysophospholipids have been

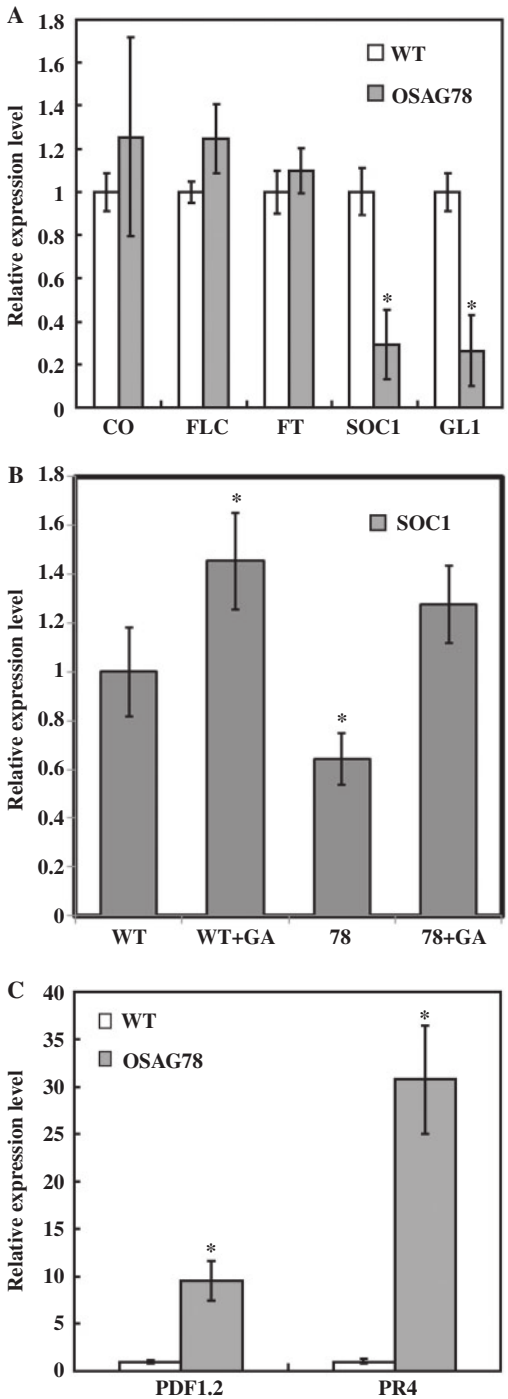

Fig. 6 Expression levels of flowering-related and MJ-inducible genes in Arabidopsis. (a) Expression levels of *CONSTANT* (*CO*), *FLOWERING LOCUS C* (*FLC*), *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF CO OVEREXPRESSION 1* (*SOC1*) and *GALABAR1* (*GL1*) genes in 21-day-old plants were analyzed by real-time RT–PCR. *SOC1* and *GL1* are gibberellin-inducible genes. (b) The expression level of the *SOC1* gene was analyzed by real-time RT–PCR. When plants were 7 d old, they were sprayed with GA_3 every 2 d. RNAs of plants with or without GA_3 treatment were then harvested for RT–PCR assays when plant were 14 d old. The number 78 is the number of plants overexpressing *OSAG78*. (c) Expression levels of MJ-inducible *PDF 1.2* and *pathogenesis-related 4* (*PR4*) genes were analyzed by real-time RT–PCR. These data are normalized by the expression levels of the *actin* (*AtACT*) gene, and their ratios relative to each gene expressed in wild-type plants are shown as relative expression levels. Each bar represents the average of three biological assays with the standard deviation of the mean. Statistical differences between wild-type and transgenic plants are marked with asterisks when $P < 0.05$ according to Student's test.

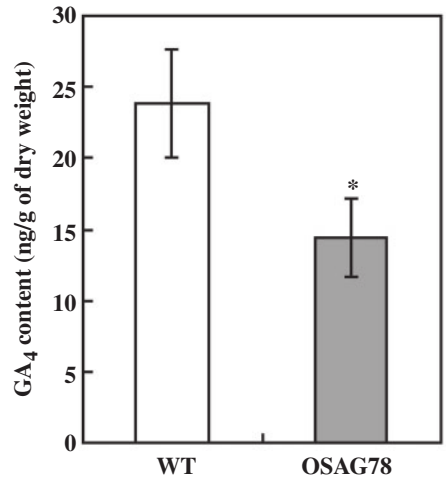

Fig. 7 GA_4 levels in wild-type and *35S::OSAG78* Arabidopsis. Triplicate biological assays of gibberellin levels (ng g^{-1} DW) in Arabidopsis using GC-MS-SIM are shown. Data come from at least 100 plants for each biological measurement.

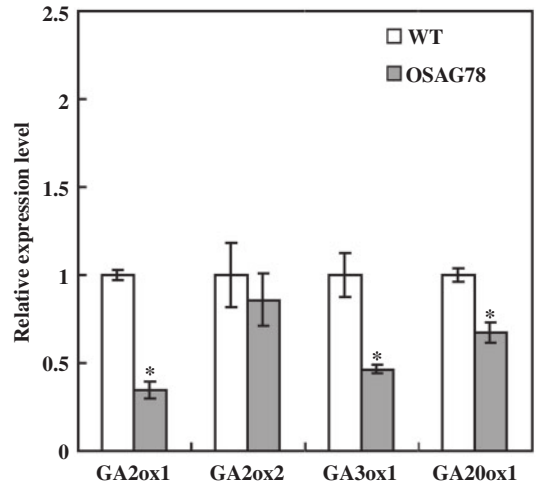

Fig. 8 Analyses of *GA2ox1*, *GA2ox2*, *GA3ox1* and *GA20ox1* expression in the 21-day-old *35S::OSAG78* and wild-type Arabidopsis by real-time RT–PCR. These data are normalized by the expression levels of the *actin* (*AtACT*) gene, and their ratios relative to each gene expressed in wild-type plants are shown as relative expression levels. Each bar represents the average of three biological assays with standard deviation of the mean. Statistical differences between wild-type and transgenic plants are marked with asterisks when $P < 0.05$ according to Student's test.

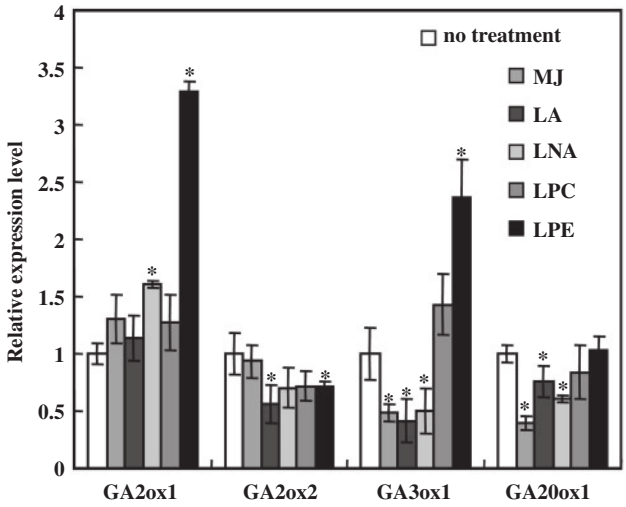

Fig. 9 Expression levels of *GA2ox1*, *GA2ox2*, *GA3ox1* and *GA20ox1* in 21-day-old wild-type Arabidopsis treated with methyl jasmonate (MJ), free fatty acids and lysophospholipids. Wild-type Arabidopsis were treated with MJ, linoleic acid (LA), linolenic acid (LNA), lysophosphatidylcholine (LPC) or lysophosphatidylethanolamine (LPE) for 30 min. Expression levels of *GA2ox1*, *GA2ox2*, *GA3ox1* and *GA20ox1* in wild-type Arabidopsis were analyzed by real-time RT–PCR. These data are normalized by the expression levels of the *actin* (*AtACT*) gene, and their ratios relative to each gene without treatment are shown as relative expression levels. Each bar represents the average of at least three biological assays with the standard deviation of the mean. Statistic differences among plants with and without treatments are marked with asterisks when $P < 0.05$ according to Student's test.

suggested to be secondary signals or indicators of auxin (Paul et al. 1998). In addition, Arabidopsis *AtPLA I* encoding an acyl hydrolase hydrolyzes phospholipids at the *sn*-1 and *sn*-2 positions to produce free FAs (Yang et al. 2007). However, how a cytosolic PLA2 is able to react with a membrane-bound phospholipid to produce free FAs and lysophospholipids has not been investigated. In this study, OSAG78 was identified to be a membrane protein (**Fig. 2b**). It is reasonable to conclude that OSAG78 reacts with its phospholipid substrates in the membrane in order to release FAs and lysophospholipids as signals.

Up to now, eight *GA2ox*, four *GA3ox* and five *GA20ox* genes have been found in Arabidopsis (Yamaguchi 2008), but only several specific *GAox* genes were analyzed in this study (**Figs. 8**, **9**). In terms of *GA2ox* genes, both *GA2ox1* and *GA2ox2* take part in the homeostasis of bioactive gibberellins (Thomas et al. 1999). Compared with those from *GA2ox1* and *GA2ox2*, the transcript level of *GA2ox3* in Arabidopsis is low (Thomas et al. 1999). The study of *GA2ox4* is limited, while *GA2ox5* is presumed to be a pseudogene (Hedden et al. 2002) and *GA2ox6* is mainly involved in seed germination and somatic embryo production (Wang et al. 2004). The expression levels of *GA2ox7* and *GA2ox8* in Arabidopsis are low and undetectable by Northern analysis (Schomburg et al. 2003), and also *GA2ox7* transcripts were not detected in a microarray analysis due to their low abundance (Magome et al. 2008). Hence, only the expression of *GA2ox1* and *GA2ox2* was assayed in this study. In terms of *GA3ox* genes, *GA3ox1* is expressed throughout all stages of plant development, and *GA3ox2* is mainly expressed in germinating seeds and very young seedlings (Yamaguchi et al. 1998). In contrast, *GA3ox3* and *GA3ox4* are only expressed in developing flowers and siliques (Sun 2008). As predicted by their expression patterns and levels, *GA3ox1* has a predominant role in plant development. Therefore, the expression of *GA3ox1* was also analyzed in this study. In terms of *GA20ox* genes, five genes, *GA20ox1*–*GA20ox5*, encoding GA20 oxidases were found in Arabidopsis. Northern assay indicated that *GA20ox1* is mainly expressed in stems, *GA20ox2* is only expressed in flowers and siliques, and *GA20ox3* is only expressed in siliques (Phillips et al. 1995). Quantitative RT–PCR indicated that among the five *GA20ox* genes, *GA20ox1* is the major gene expressed in 24-day-old leaves and stems (Rieu et al. 2008). Therefore, the expression of *GA20ox1* in 21-day-old leaves and stems was also studied here.

The addition of LPE or LPC to wild-type Arabidopsis either enhanced or did not affect the expression levels of bioactive gibberellin synthesis genes, including *GA3ox1* and *GA20ox1* (**Fig. 9**). Hence, the presence of lysophospholipids may not be the cause of the reduction in bioactive gibberellin in plants. Thus lysophospholipids may not be the signals responsible for the reduction of gibberellins in *35S::OSAG78* plants. However, the expression of *GA3ox1* and *GA20ox1* genes was reduced in wild-type Arabidopsis treated with LA or LNA (**Fig. 9**). Therefore, the addition of free FAs was assumed to reduce bioactive gibberellin in plants. Free FAs released by the hydrolyzing phospholipids of OSAG78 may be the signals for decreasing bioactive gibberellin in plant cells.

An increase in free LA and LNA was also found in tomato leaves upon wounding (Conconi et al. 1996), and free FAs were also associated with phospholipase D-mediated hydrolysis of phospholipids in wounded castor bean leaves (Ryu and Wang 1998). LNA was identified as the precursor for JA formation (Farmer and Ryan, 1992). Furthermore, LA can be oxidized to LNA by desaturase (Banilas et al. 2007, Yang et al. 2007), and then increases the level of JA in cells. Hence, both LA and LNA were reported to be precursors of JA (Vick and Zimmerman 1984, Hung and Kao 1998). In this study, we showed that *35S::OSAG78* transgenics contained an increased amount of free LA and LNA (**Fig. 3b**). Also, the addition of LA, LNA or MJ to Arabidopsis reduced the levels of *GA3ox1* and *GA20ox1* mRNAs in wild-type plants (**Fig. 9**). Therefore, LA and LNA may regulate the expression of *GA3ox1* and *GA20ox1* through JA. However, LA is also the precursor of dihydrojasmonic acid, which is highly active in growth inhibition and senescence induction (Gundlach and Zenk 1998). It would be very interesting to know whether dihydrojasmonic acid is also involved in the regulation of gibberellin oxidase genes.

The dramatic phenotypes of *35S::OSAG78* plants included small and thick leaves and round flowers (**Fig. 4c, f**). Overexpression of *POTH1*, a class 1 homeobox gene, also

produces dwarf plants and reduces leaf size in potatoes. It alters the levels of intermediates in the gibberellin biosynthesis pathway, and hence the bioactive gibberellin is decreased by 50%. Further studies showed that *POTH1* overexpression in plants reduces the accumulation of *GA20ox* mRNA (Rosin et al. 2003). The fact that the levels of both *GA20ox* mRNA (**Fig. 8**) and bioactive gibberellin (**Fig. 7**) were decreased in *35S::OSAG78* plants implies that a relationship between leaf size and gibberellin levels may exist. Arabidopsis overexpressing *STURDY*, which encodes a papatin-like protein, exhibits phenotypes similar to those of *35S::OSAG78* plants, including a stiff inflorescence stem, thicker leaves, shorter siliques and round flowers. However, the molecular effect of *STURDY* on these phenotype changes has not been studied (Huang et al. 2001). Our work indicates that OSAG78, a membrane protein, contains lipase activity that can hydrolyze the phospholipids in the membrane. The phenotypes of *35S::OSAG78* plants may result from the further downstream reactions induced by the hydrolysis products of phospholipids by OSAG78.

The interactions between gibberellin and MJ have been little investigated. In this study, the addition of MJ significantly decreased the accumulation of *GA3ox1* and *GA20ox1* mRNAs (**Fig. 9**), whose protein products are the major enzymes responsible for active gibberellin biosynthesis (Hedden and Phillips 2000). Gibberellin has been reported to promote the degradation of DELLA proteins, which are plant growth repressors. In the quadruple-DELLA mutant Arabidopsis, which lacks four of five DELLA genes, MJ-inducible genes, including *PDF 1.2*, *LOX2* and *TAT1*, become insensitive to MJ treatment (Navarro et al. 2008). This may indicate that certain MJ-inducible genes are repressed and regulated by gibberellin. In addition, MJ inhibits the germination of *Amaranthus caudatus* seeds in darkness, but GA_3 and GA_{4+7} rescue this inhibition (Bialecka and Kgpczynski 2003). Furthermore, MJ inhibits the floral bud formation of the short-day plant *Pharbitis nil*, and, again, GA_3 partially rescues this inhibitory effect (Maciejewska and Kopcewicz 2002). These results may indicate that an antagonist effect may occur between gibberellin and MJ. However, JA treatment does not affect the expression levels of *GA2ox1*, *GA3ox1* and *GA20ox2* in Arabidopsis *gal-3 gai-t6 rga-t2 rgli-1* mutants, which cause the plant to become gibberellin deficient and malfunctional in terms of DELLA (Cheng et al. 2009). It would be interesting to investigate further the molecular mechanism of the interaction between MJ and gibberellin in regulating cellular functions.

Materials and Methods

Plant materials and growth conditions

Oncidium Gower Ramsey (*Oncidium Goldiana* × *Oncidium Guinea Gold*) was obtained from Xu Dong Oncidium Farm, Yangmei, Taiwan. The seedlings of *Oncidium* were grown in a greenhouse at 20–28°C. *Arabidopsis thaliana* (Col-0) wild-type and transgenic plants were grown at 22°C under a 16 h light/8 h dark photoperiod with cool fluorescent light at 100 μmol m^{-2} s^{-1}, and 21-day-old plants were used in this study.

Plasmid construction

For GFP assays, *35S::OSAG78-GFP* and *35S::GFP* were obtained as follows. *OSAG78* from *Oncidium* Gower Ramsey was isolated from a suppression subtractive library between fully open and senescent flowers using a Clontech PCR-select cDNA subtraction kit. The full-length cDNA of *OSAG78* was cloned via PCR using *Oncidium* cDNA as template and OSAG78-F and OSAG78-R as primers (**Supplementary Table S1**). The *GFP* fragment was also obtained by PCR using pRTL2-S65TGFP as template (von Arnim et al. 1998) and GFP-F and GFP-R as primers (**Supplementary Table S1**). The amplified *OSAG78* and *GFP* fragments were, respectively, digested and cloned into *Xba*I/*Spe*I and *Spe*I/*Sac*I sites of pBI22178in downstream of the *35S* promoter. pBI22178in modified from pBI221 (Clontech) contains the intron 2 from *OSAG78* and possesses an extra *Spe*I site for cloning operation. Finally, OSAG78-fused GFP and GFP alone in pBI22178in was digested with *Hin*dIII and *Eco*RI, and cloned into binary vector pPZP2GB (Chu et al. 2005) to become pPZP35S/OSAG78/GFP and pPZP35S/GFP, respectively.

Creation of transgenic plants

Transgenics were generated using the floral dip method (Clough and Bent 1998) with *Agrobacterium* LBA4404 carrying pPZP derivatives. About 10 independent transgenics were obtained for each construct, and three independent T_3 plants generated by self-fertilization were chosen for further study. Flowering time was reported as the number of days and the number of rosette leaves from sowing to the appearance of the first flowering buds.

Particle bombardment

Onion epidermal cell layers were peeled and placed inside up on MS plates. Plasmid DNAs, pPZP35S/GFP and pPZP35S/OSAG78/GFP (10 μg), were introduced into onion cells using a pneumatic particle gun (PDS-1000/He; Bio-Rad). The precipitation mixture contained 0.5 mg of gold particles in 10 μl of sterile water, 10 μl of plasmid DNA (1 μg $μl^{-1}$), 50 μl of 2.5 mM calcium chloride, 10 μl of 0.1 M spermidine and 100 μl of 70% ethanol. After 15 min incubation at −20°C, the supernatant was removed. Coated particles were collected and re-suspended in 20 μl of 95% ethanol, and they were loaded onto the screen holder of the inflow gun. The condition of bombardment was a vacuum of 26.5 inches Hg, a helium pressure of 1,100 p.s.i. and a 6 cm target distance. Tissues were then incubated on the MS plates for 24 h at 25°C before they were investigated by confocal microscopy.

Optical, confocal and scanning electron microscopy (SEM)

Epidermal impressions of leaves and petals were made using Loctite Superglue on a microscope slide, and observed by

optical microscopy. A Leica TCS-SP2 confocal laser scanning microscope (Leica Lasertechnik GmbH) with an excitation/emission wavelength at 488/500–535 nm was used to investigate the plant tissues expressing GFP. For SEM studies, seed samples were fixed for 24 h in FAA (3.7% formalin, 5% acetic acid and 70% ethyl alcohol) and dehydrated in an ethyl alcohol series, after which the ethyl alcohol was replaced by isoamyl acetate. The samples were dried using a critical point dryer (HCP-2, Hitachi) and coated with platinum/palladium using a sputter coater (Ion Sputter E-102, Hitachi). The seeds processed for SEM studies were prepared following the methods of Sanchez et al. (1990) at 16 kV.

Protein extraction and lipase assays

The entire aerial parts of the 21-day-old plants were homogenized into powder with liquid nitrogen, and then the soluble proteins were extracted with an extraction buffer [140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.2% Tween-20, 40 mM L-ascorbic acid, 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 mM Na_2EDTA, pH 7.2 at 25°C]. Protein concentrations were measured by the Coomassive blue binding method (Sedmak and Grossberg 1977). Protein aliquots (10 μg) were mixed with 1.65 mM pNPP (Sigma) and reaction buffer [50 mM Tris–HCl, 0.4% (w/v) Triton X-100 and 0.1% (w/v) arabic gum, pH 8.0] at 28°C to start the reaction (Pencreac'h and Baratti 1996). The hydrolysis of pNPP was examined by spectrophotometry (DU 640B spectrophotometer, Beckman Coulter) at 410 nm absorbance every 30 min for 3 h. Lipase activity was represented as absorbance units at 410 nm min^{-1}.

Lipid analysis

The entire aerial parts of the 21-day-old plants were detached and soaked in 3 ml of transmethylation reagent (1 N methanolic HCl, 10 μg ml^{-1} butylated hydroxytoluene and 0.15 mg ml^{-1} pentadecanoic acid). The reaction mixture was heated to 50°C overnight and then cooled down to room temperature. Then, 3 ml of 0.9% NaCl and 0.15 ml of hexane were added to the reaction mixture, which was then mixed thoroughly by vortex for 16 s. After centrifugation at 2,400 r.p.m. for 5 min at 4°C, the hexane layer, containing FA methyl ester, was collected and analyzed by gas chromatography. Gas chromatography was carried out on Agilent 6980 with a HP-5 capillary column (30 m × 0.32 mm × 0.25 μm) and a flame ionization detector. The detector temperature was maintained at 260°C, and the flame was set at 40 ml min^{-1} H_2. Helium was used as the carrier gas and the flow rate was 1.0 ml min^{-1}. Sample extract in 1 μl of hexane was injected with a 20 : 1 split ratio. The oven temperature was programmed from 50 to 170°C at a rate of 50°C min^{-1}, 170 to 300°C at a rate of 4°C min^{-1}, 300 to 320°C at a rate of 40°C min^{-1}, and then held at 320°C for 3.6 min. The total analysis time of one sample was 40.00 min. The retention times for LA and LNA were 16.623 and 16.488 min, respectively, which were determined by running pure LA and LNA through the gas chromatograph. The relative amount of FAs was presented after normalization using the internal control, pentadecanoic acid.

Real-time RT-PCR

Total RNAs from the aerial parts of 21-day-old plants were isolated using Trizol reagent (Invitrogen) according to the procedure published previously (Chen et al. 2008). RNAs from plants were treated with DNase I (Ambion) before the reverse transcription. Total RNAs (3 μg) were reverse transcribed with M-MLV reverse transcriptase (Promega) with oligo(dT) primer. The final cDNA products were stored at −20°C.

SYBR Green Supermix from Bio-Rad was used as the real-time PCR pre-mix. The amplification reagents contain 10 μl of SYBR Green Supermix, 125 nM of forward and reverse primers (**Supplementary Table S1**), 100 ng of cDNA and H_2O to 20 μl. The program was an initial 10 min at 95°C followed by 40 cycles of denaturation at 95°C for 20 s, and annealing/extension at 60°C for 25 s. The target genes for *FT*, *CO*, *FLC*, *SOC1*, *GL1*, *GA2ox1*, *GA2ox2*, *GA3ox1*, *GA20ox1*, *PDF 1.2* and *PR4* are *AT1G65480*, *AT5G15840*, *AT5G10140*, *AT5G62165*, *AT3G27920*, *AT1G78440*, *AT1G30040*, *AT1G15550*, *AT4G25420*, *AT5G44420* and *AT3G04720*, respectively. Detection of fluorescent signals and data analyses were performed on an ABI PRISM 7500 thermocycler (Applied Biosystems). The expression of the *actin* gene was also measured, and data were normalized by the expression levels of the *actin* gene, and their ratios relative to that of wild-type or untreated plants are shown as the relative expression level. The error bars in the figures indicate standard deviations from at least three biological assays.

GA_3 treatment and gibberellin quantification

Arabidopsis seedlings were sprayed with 10 μM GA_3 in 0.02% (v/v) Tween-20 once per week from when they were 3 days old. Then, the number of rosette leaves and the number of days until initiating flowering were measured as described by Koornneef et al. (1991).

Endogenous GA_1 and GA_4 were analyzed by GC-MS-SIM. Fresh samples (about 5–7 g of leaves) from the entire aerial parts of the 21-day-old plants were lyophilized, and then ground in liquid N_2 using a mortar and pestle. The internal standards of 50 ng of [17, 17-$^2H_2$]gibberellins were added to each sample after grinding. Sample extraction was carried out overnight with 15 ml of 80% (v/v) methanol containing 0.4 mg of butylated hydroxytoluene and 2 mg of ascorbate at 5°C, and the residue was re-extracted in the same solvent for another 2 h at 5°C. The methanol extracts were combined and reduced to about 4–5 ml of water residue using a rotary vacuum evaporator and SpeedVac (Savant Instruments). The residue was adjusted to pH 8.5 with 0.05 M potassium phosphate buffer and partitioned with 20 ml of hexane three times. The aqueous fraction was then adjusted to pH 3.0 with 0.5 M potassium phosphate buffer (pH 2.0) and partitioned with 20 ml of ethyl acetate (EtOAc) three times again. The pooled EtOAc fraction was taken to dryness by the SpeedVac.

Following drying in vacuo using the SpeedVac, the sample was dissolved in 30% aqueous methanol containing 0.1% acetic acid and passed through a Millipore 0.45 μm polyvinylidene fluoride membrane, and then injected into a Beckman System Gold HPLC with a LiChrosphere RP-18 column (250 × 4 mm i.d. × 5 μm particle size; Merck). The HPLC fractions of gibberellins were dried using the SpeedVac. The fractions of GA_1 and GA_4 were injected into a Waters HPLC with a Nucleosil 100 N(CH3)2 column (250 × 4.6 mm i.d. × 5 μm particle size; Cronus) for further purification and the gibberellin fractions from this column were dried in vacuo. Gibberellins were derivatized by adding ethereal diazomethane, then dried with N_2, and were further trimethylsilated. The derivatized samples were analyzed using an Agilent Technologies 6890N GC and 5975 MSD with a DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific). Operating conditions for HPLC and GC-MS-SIM were described by Chen et al. (2007). The *m/z* ratios of GA_1 and GA_4 are 506/508 and 284/286, respectively, and were used for quantification.

Methyl jasmonate, fatty acid and lysophospholipid treatments

MJ, FAs and lysophospholipids were purchased from Sigma. MJ was diluted to 50 mM in 9% ethanol as stock solution. The FAs, LA and LNA, and the lysophospholipids, LPC and LPE, were first dissolved in $CHCl_3$, and dried by vacuum centrifugation. The appropriate amount of 10 mM Tris–HCl (pH 7.5) was added to FAs and lysophospholipids to obtain 5 mM stock solutions, which were sonicated for 30 s before being used. The entire aerial parts of 21-day-old Arabidopsis were floated in 5 mM MES, 1 mM KCl and 0.005% L-77 solution with 50 μM MJ, 5 μM FAs or 5 μM lysophospholipids for 30 min, and mRNAs of the leaves after treatment were then analyzed by RT–PCR.

Supplementary data

Supplementary data are available at PCP online.

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