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Floral glycerolipid profiles in homeotic mutants of *Arabidopsis thaliana*

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ABSTRACT

Flowers have distinct glycerolipid composition, yet its floral organ-specific profile remains elusive in Arabidopsis whose flowers are too tiny to dissect different floral organs. Here, we employed known floral homeotic mutants *agamous-1* (*ag-1*) and *apetala3-3* (*ap3-3*) to facilitate sample preparation enriched in different floral organs. The result of analysis on different polar glycerolipid classes and their fatty acid composition demonstrated that flowers of *ap3-3* and *ag-1* have distinct glycerolipid composition from that of wild type. Moreover, distinct set of glycerolipid biosynthetic genes is expressed in these mutants by qRT-PCR gene expression analysis. These data suggest that glycerolipid profile is distinct among different floral organs of *Arabidopsis thaliana*.

1. Introduction

Polar glycerolipids (hereafter glycerolipids) consist of a distinct set of lipid metabolites that function as membrane constituents and signaling molecules. Glycerolipids have distinct roles in multiple stages of flower development. Knockout studies in Arabidopsis have provided ample evidence of glycerolipid biosynthesis involved in flower development, such as defective pollen function in loss-of-function mutant of glycerol 3-phosphate acyltransferase 1 (GPAT1) [1] or phosphatidylserine synthase 1 (PSS1) [2].

Arabidopsis flowers are too tiny for dissecting and massive harvesting of specific floral organs, which prevented us from studying flower glycerolipids in Arabidopsis previously. Glycerolipid profiling of whole Arabidopsis flowers was performed [3], and distinct phospholipid composition with abundant phosphatidylserine (PS) and phosphatidylinositol (PI) were featured [2]. Moreover, developmental stage-specific profiles of glycerolipids were revealed by using a transgenic Arabidopsis plant whose flower development is synchronized [4]. However, these analyses did not provide glycerolipid profile at floral-organ resolution. We previously characterized floral organ-specific glycerolipids using Petunia flowers [5,6]. Petunia flowers show discrete biochemical features of glycerolipid metabolism with digalactosyldiacylglycerol (DGDG) as a major glycolipid and floral-organ-specific metabolism in diacylglycerol (DAG) production [5,6]. However, further studies were anticipated in Arabidopsis flowers which enable transcriptomic analysis and gene knockout studies in addition to the glycerolipid profiling.

Here, we took advantage of floral homeotic mutants whose flowers are enriched with specific floral organs. *AGAMOUS* (*AG*) is a master transcriptional regulator that initiates reproductive organ formation and determines floral meristem fate [7]. The mutant *ag-1*, with abolished *AG* function, therefore produces massive number of petals but not stamens or pistils (Fig. 1A). Likewise, we selected a mutant in *APETALA3* (*AP3*), *ap3-3*, whose flowers show homeotic conversions of petals to sepals and stamens to pistils (Fig. 1A) [8]. Glycerolipids enriched in different floral organs were discussed based on the combinatorial analyses of data in mature wild-type flowers (*Ler* background), *ag-1* and *ap3-3* [9]. Lipids abundant in petals are enriched in *ag-1* but lost in *ap3-3*, while lipids abundant in pistils are enriched in *ap3-3* but lost in *ag-1*. Lipids abundant in stamens are those deprived in *ap3-3* and *ag-1* but enriched in wild type. Sepals are increased both in *ap3-3* and *ag-1* compared to those in the wild-type flowers.

2. Materials and methods

2.1. Plant growth conditions

Plants were grown under continuous light (150 μmol/m^2 s) at 22 °C.

2.2. Lipid extraction and analysis

Mature flowers of wild type or homeotic mutants (*ap3-3* and *ag-1*) were harvested, immediately frozen in liquid nitrogen, and

Abbreviations: DAG, *sn*-1,2-diacylglycerol; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; 16:0, palmitic acid; 18:2, linoleic acid; 18:3, linolenic acid.

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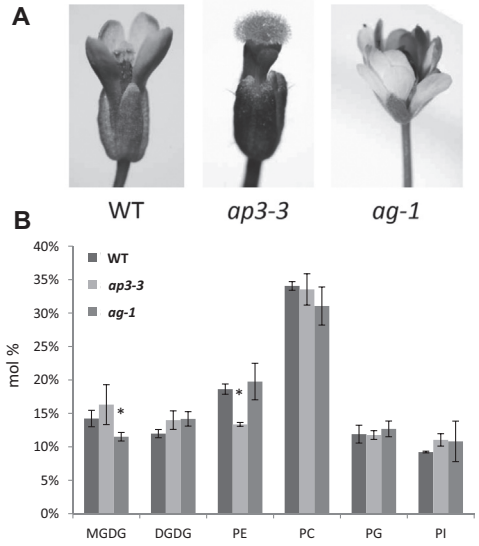

Fig. 1. Differential glycerolipid profiles in flowers of *ap3-3* and *ag-1* mutants in Arabidopsis. (A) Images of floral homeotic mutants used to enrich specific floral organs for total lipid extraction. (B) Glycerolipid profiles of the flowers of *ap3-3* and *ag-1* compared to the wild type shown by mol%. Lipids were analyzed by GC-FID. Data are mean ± SD of three biological replicates. Asterisks indicate significance ($p < 0.01$) from WT. DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol.

kept at −80 °C until lipid extraction. Prior to lipid extraction, frozen tissues were incubated in hot (75 °C) isopropanol containing 0.05% (v/v) butylated hydroxytoluene (Cat No. B1378, Sigma–Aldrich, St. Louis, MO) for 15 min to inhibit phospholipase D activity for PA production. Total lipid was extracted from ~500 µl (~10 mg dry weight) frozen tissue and analyzed by gas chromatography with FID detector (GC-2010, Shimadzu, Japan) as previously described [5]. Data were averaged by analysis of 3 biologically independent samples with standard deviation as error bars.

2.3. RNA extraction and qRT-PCR analysis

Total RNA was extracted from the samples using RNeasy plant mini kit (Qiagen), followed by reverse-transcription to cDNA by SuperScript III reverse-transcription kit (Invitrogen). qRT-PCR was performed with specific primers designed for each target genes [4] using 7500 Real Time PCR System (Life Technologies, Carlsbad, CA). Data were averaged by three biological replicates, each of which involves further three technical replicates. Actin was used as a reference, and primer specificity was examined previously [4].

3. Results and discussion

3.1. Floral glycerolipid profiling with use of floral homeotic mutants

We examined the glycerolipid profile in mature flowers of *ap3-3*, *ag-1* and the wild type (Fig. 1B). The result of the wild type

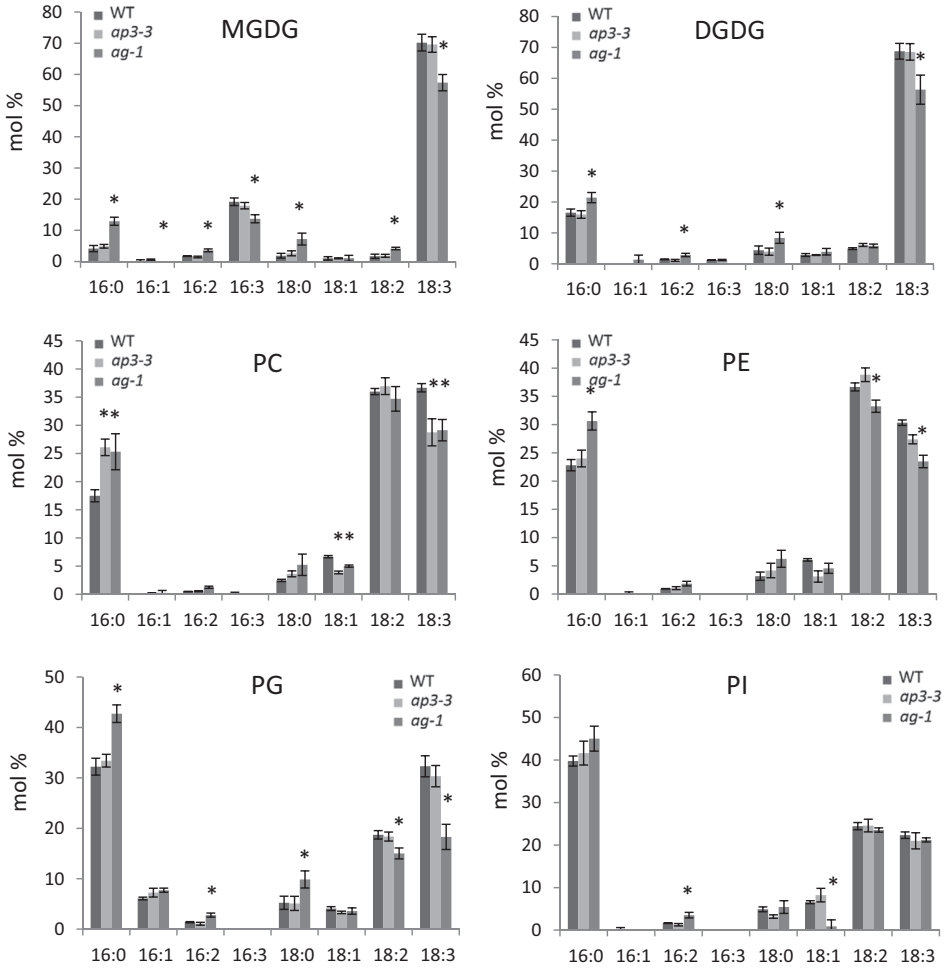

Fig. 2. Fatty acid composition of glycerolipids profiled in flowers of *ap3-3* and *ag-1* compared to the wild type. Lipids were analyzed by GC-FID. Data are mean ± SD of three biological replicates. Asterisks indicate significance ($p < 0.01$) from WT. Fatty acid species are shown as (No. of carbon atom):(No. of unsaturation), such as 16:0 for palmitic acid.

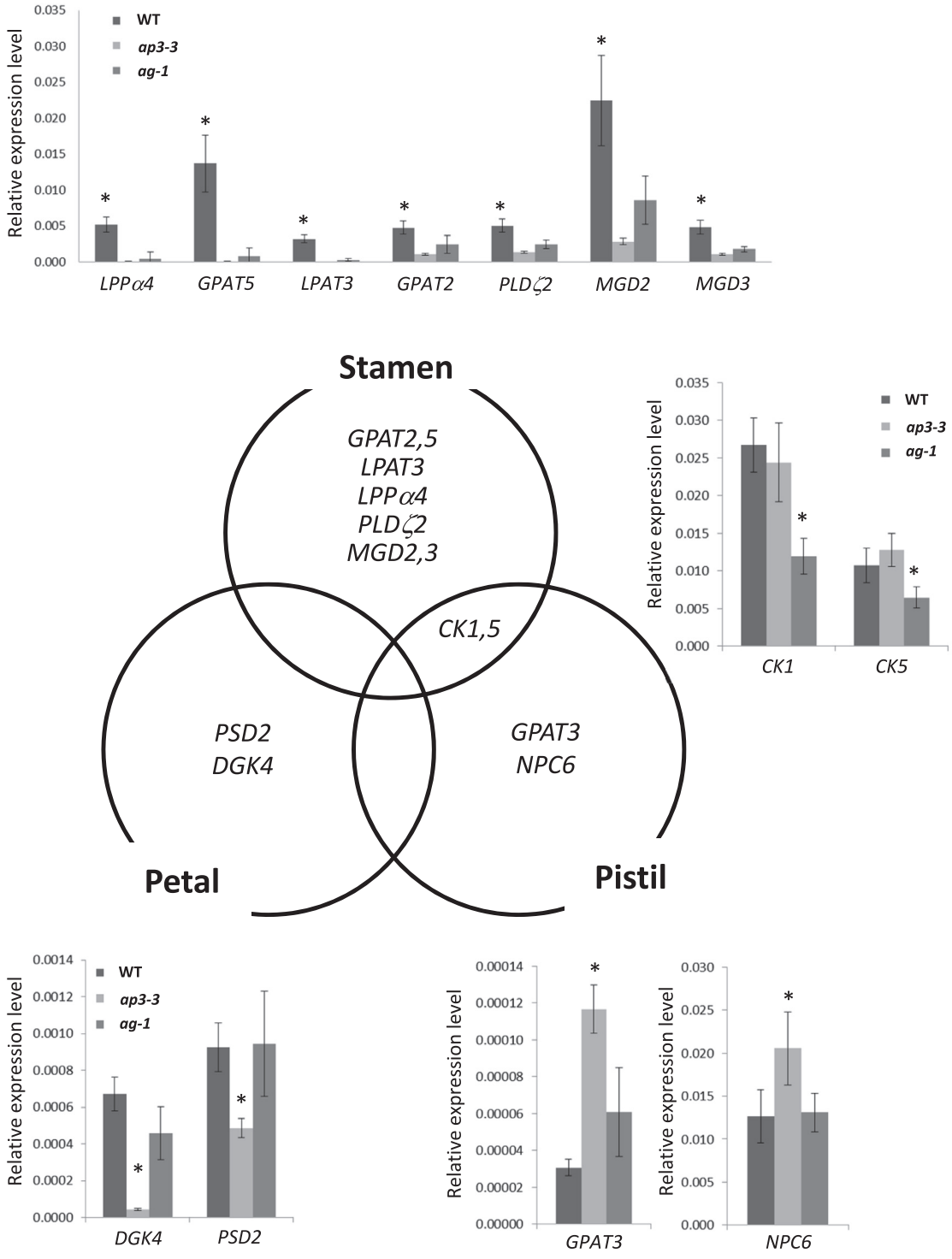

Fig. 3. Gene expression of glycerolipid metabolic genes analyzed by quantitative RT-PCR in the flowers of wild type, *ap3-3* and *ag-1* mutants. Data are mean ± SD of three biological replicates. Relative expression levels were shown using actin as a reference gene. Asterisks indicate significance ($p < 0.01$) from WT. CK, choline kinase; DGK, diacylglycerol kinase; GPAT, glycerol 3-phosphate acyltransferase; LPAT, lysophosphatidic acid acyltransferase; LPP, lipid phosphate phosphatase; MGD, monogalactosyldiacylglycerol synthase; NPC, non-specific phospholipase C; PLD, phospholipase D; PSD, phosphatidylserine decarboxylase.

flowers was largely consistent with the previous study with mass spectrometry analysis [3]. The *ap3-3* flowers had lower composition of phosphatidylethanolamine (PE) as compared to the wild type or *ag-1* (Fig. 1B). In addition, *ag-1* flowers had slightly lower composition of monogalactosyldiacylglycerol (MGDG) as compared to wild type or *ap3-3*. The ratio of MGDG to DGDG was almost 1:1 in three floral samples. This ratio is distinct from that in leaves, which maintain about a 2:1 ratio for functional photosynthetic membranes [10] but is in agreement with previous observations in flowers of Petunia [5] and Arabidopsis [3]. We next analyzed fatty acid composition of respective glycerolipid classes profiled in Fig. 1. Among different homeotic mutants, *ag-1* showed

high levels of saturated fatty acids, mainly palmitic acid (16:0) in most glycerolipids (Fig. 2). The increase in 16:0 level was observed at the expense of polyunsaturated fatty acids such as linoleic acid (18:2) and linolenic acid (18:3), and this feature is in good agreement with those in petals of Petunia [6]. These data suggest that pistils are unique in lower PE composition, and petals have lower MGDG and more saturated glycerolipid profiles than reproductive organs.

3.2. Expression of glycerolipid biosynthetic genes in different floral organs

To corroborate the floral glycerolipid profile with expression of relevant lipid biosynthetic genes, we used the Arabidopsis eFP browser [11] for a global search of the Affymetrix ATH1 Arabidopsis Genome Array data for polar glycerolipid biosynthetic genes expressed in different floral organs [12]. Among 76 genes which are annotated as (putative) glycerolipid biosynthetic genes, we first analyzed whether the expression of these genes was enriched in floral organs by dividing the maximum expression levels of petals, stamens and pistils by that of rosette leaves (Max{pe,st,pi}/rosette). The ratio of expression was >1 for 60 of the 76 genes (79%). Moreover, the ratio was >5 for 17 genes (22%) and >10 for 11 genes (14%). Therefore, most glycerolipid biosynthetic genes were highly expressed in floral organs. Next, we investigated the floral-organ specificity of glycerolipid biosynthetic gene expression. We calculated the ratio of Max{pe,st,pi}/Min{pe,st,pi} expression, and found that a considerable number of genes showed floral-organ specificity in expression.

We performed quantitative RT-PCR (qRT-PCR) analysis for 13 genes with Max{pe,st,pi}/Min{pe,st,pi} ratio >5. As shown in Fig. 3, 7 genes (*GPAT2*, *GPAT5*, *LPAT3*, *LPPα4*, *PLDζ2*, *MGD2* and *MGD3*) showed significantly reduced expression levels in both *ap3-3* and *ag-1* as compared to the wild type. Because stamens are commonly absent in these mutants, it is suggested that the expression level of these genes is enriched in the stamen. Expression of *GPAT3* and *NPC6* was higher in *ap3-3* as compared to the wild type or *ag-1*, suggesting that expression of these genes is enriched in the pistil. *DGK4* and *PSD2* showed reduced expression only in *ap3-3*, suggesting that petals are the major site for these genes to express. Expression of *CK1* and *CK5* was higher both in the wild type and *ap3-3* but not in *ag-1*, suggesting that these genes are preferentially expressed in reproductive organs. Stamen-specific expression of *GPAT5*, *LPAT3, MGD2* and *MGD3* is in agreement with previous GUS reporter assay [13–15]. However, expression level of *GPAT2* was too low to analyze the tissue specificity by RNA gel blotting [1] and other genes were not examined yet for floral-organ specific expression. These data suggest distinct expression pattern of glycerolipid biosynthetic genes among different floral organs of Arabidopsis, which may contribute to the establishment of floral lipid profiles revealed in Figs. 1 and 2.

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