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拟南芥角果衰老过程中膜脂的变化*

禹晓梅[1,2,4], 王 荣[1], 李唯奇[1,3**]

(1 中国科学院昆明植物研究所 中国西南野生生物种质资源库, 云南 昆明 650201; 2 中国科学院大学, 北京 100049; 3 红河学院生物系, 云南 蒙自 661100; 4 玉溪中烟种子有限责任公司, 云南 玉溪 653100)

摘要: 角果发育对某些物种的生殖发育具有重要的作用。拟南芥种子附着在角果里, 角果在早期发育时进行光合作用, 角果成熟后开裂散落种子之前, 其细胞会经历一个衰老的过程。一般植物细胞在衰老过程中要经历膜脂降解的过程, 但是角果细胞衰老过程仍未知。通过比较角果衰老过程中拟南芥野生型 (WS) 及与膜脂代谢密切相关的磷脂酶 Dδ 缺失突变体 (PLDδ-KO) 中膜脂分子的组成情况、膜脂含量、相对含量及双键指数值, 结果发现, 在拟南芥角果衰老过程中: (i) 质体膜脂和质体外膜脂显著下降; (ii) 不同膜脂降解速率不一样, 质体膜脂的降解比质体外膜脂的降解快; (iii) 总的双键指数 DBI 下降; (iv) 磷脂酶 Dδ 缺失突变体 (PLDδ-KO) 的角果膜脂组成的基本水平和变化样式与野生型 (WS) 非常相似。结果说明, 角果在衰老过程中发生了膜脂的激烈降解。据此推测: (i) 膜脂水解产物可能转移到种子中用于储藏脂三酰甘油的合成; (ii) 质体膜脂相对含量下降和质体外膜脂相对含量上升导致了总的 DBI 下降; (iii) PLDδ 参与了角果衰老中的膜脂代谢。

关键词: 拟南芥; 角果衰老; 膜脂; 脂类组学

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Changes in Membrane Lipids during Silique Senescence in *Arabidopsis*

YU Xiao-Mei[1,2,4], Wang Rong[1], LI Wei-Qi[1,3**]

(1 *Germplasm Bank of Wild Species in Southwest China*, *Kunming Institute of Botany*, *Chinese Academy of Sciences*, Kunming 650201, China; 2 *University of Chinese Academy of Sciences*, Beijing 100049, China; 3 *Department of Biology*, *Honghe University*, Mengzi 661100, China; 4 *Yuxi Zhongyan Tobacco Seed Co.*, *Ltd*, Yuxi 653100, China)

Abstract: Silique development plays an important role in the reproductive development of many species. In *Arabidopsis*, the seeds are contained within a silique, which is to photosynthesize in the early stages of development and the cell undergoes a programmed of senescence prior to dehiscence after silique riping. In general, degradation of membrane lipids is an essential process during plant cell senescence, however, the senescence of silique has not been reported. In the present study, the changes of molecular species in membrane lipids, the contents of membrane lipids, relative levels (%), and the double-bond index (DBI) during the senescence of siliques were examined in wild-type Arabidopsis (ecotype Wassilewskija; WS) and an *Arabidopsis* mutant deficient in phospholipase Dδ (PLDδ-KO). PLDδ is correlated closely with lipid metabolism in *Arabidopsis*. The present study revealed that during the senescence of siliques of *Arabidopsis*: (i) levels of both extraplastidic and plastidic lipids decreased significantly; (ii) the degradation of lipids did not occur at the same rate for different lipid species, the rate of decline in levels of plastidic lipids was more rapid than that of extraplastidic lipids; (iii) the DBI of total membrane lipids decreased; and (iv) in siliques of the PLDδ mutant plants, the levels and variations in the levels of membrane lipids were similar to those observed in siliques of WS. These results suggest that severe degradation of lipid molecular spe-

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** Author for correspondence; E-mail: weiqili@mail.kib.ac.cn

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作者简介: 禹晓梅 (1984-) 女, 博士, 主要从事种子生物学研究。

cies occurred during *Arabidopsis* silique senescence. Our findings suggest that (i) the products of hydrolysis of membrane lipids may be transferred to seeds for the synthesis of triacylglycerols for lipid storage; (ii) the decline of DBI of total membrane lipids might be caused by the dramatic degradation of plastidic lipids and the relative increase of extraplastidic lipids; and (iii) PLDδ is involved in the metabolism of membrane lipids during silique senescence.

Key words: *Arabidopsis*; Silique senescence; Membrane lipids; Lipidomics

Abbreviations: ESI-MS/MS, electrospray ionisation tandem mass spectrometry; DAF, days after flowering; DBI, double-bond index; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; WS, Wassilewskija; PLD, phospholipase D; PLDδ-KO, phospholipase Dδ knock out; WS, Wassilewskija; TAG, triacylglycerol; DAF, days after flowering

The last phase of flower development is fertilisation of the ovules and the formation of fruit (Folter *et al.*, 2004). *Arabidopsis* is a model system to study the ripening and senescence of fruit (Giovannoni, 2004; Kou *et al.*, 2012). *Arabidopsis* fruits, which are called siliques, are both biologically and economically important because they produce seeds and their dehiscence enables seed dispersal (Kou *et al.*, 2012). Senescence is a highly organized process regulating tissue ageing and death to enable nutrient recycling to occur. Silique senescence is a complex process that manifests at the levels of morphology (e. g., greening and yellowing of siliques), physiology (e. g., photosynthesis), cell composition (e. g., regulated changes in the levels of sucrose, chlorophyll), biochemistry (e. g., changes in the concentrations of and sensitivities to hormones), and molecular genetics, with all of these processes affecting cell growth and differentiation (Folter *et al.*, 2004; Louvet *et al.*, 2006; Fallahi *et al.*, 2008; Louvet *et al.*, 2011; Walton *et al.*, 2012). However, there has been only limited research on cell membrane lipids during silique senescence.

Cellular lipids play pivotal roles in the structures and metabolic regulation of cell membranes (Devaiah *et al.*, 2006). In plants, increasing evidence has also demonstrated roles for lipids in cell senescence. Loss of membrane phospholipids was observed during natural and ethylene-induced senescence of cut carnation flowers (Thompson *et al.*, 1982; Brown *et al.*, 1987). Changes in the metabolic relationships among phospholipids, and between galactolipids and phospholipids, occur during senescence-associated lipid breakdown. Both the natural senescence of rose petals and dark-induced senescence of cabbage leaves are associated with an overall decrease in the levels of membrane phospholipids (Borochov *et al.*, 1982; Buchanan, 1997). In addition, levels of both extraplastidic and plastidic lipids decrease during the natural senescence of tobacco leaves (Koiwai *et al.*, 1981). However, many questions related to the lipid metabolism of *Arabidopsis* during silique senescing remain unanswered. For example, how does the profile of membrane lipids change during this process? Do changes in lipid metabolism during the senescence of siliques resemble those during the senescence of leaves, and what are the mechanisms involved?

In plants, the activities of several lipolytic enzymes have been described, including phospholipase D (PLD), PLA, PLC, nonspecific acyl hydrolase, and galactolipases (Wang, 2004; Li *et al.*, 2008). PLD hydrolyses phospholipids to generate phosphatidic acid (PA), which is the most active phospholipase in plants. Recently, studies have shown that PLDα1 and PLDδ, the two most abundant types of the 12-member *Arabidopsis* PLD family, play important roles in plant growth and resistance to stress (Zien *et al.*, 2001; Wang, 2002, 2004; Uraji *et al.*, 2012). Phospholipase Dδ knock out (PLDδ-KO) plants are more sensitive to H_2O_2-induced cell death and less tolerant of freezing injuries than their wild-type counterparts. PLDα1 contributes significantly to PA levels in roots, seeds, flowers, and

flower stalks, but little to the very low PA levels found in siliques and leaves (Devaiah *et al.*, 2006). However, it is unknown whether knock out of the gene that encodes PLDδ affects the composition of basal membrane lipids in *Arabidopsis* during silique senescence.

Membranes, particularly plasma and chloroplast membranes, are very important during plant growth. Cell membranes can adjust the level of unsaturation of their lipids, as well as their lipid composition and structure, in response to stress (Navari-Izzo *et al.*, 1993). The degree of unsaturation of membrane glycerolipids (measured as the double-bond index, DBI) is the major factor that determines membrane fluidity. A high DBI indicates the presence of more unsaturated membrane lipids and higher fluidity than those of membranes with lower DBI values. When membranes exhibit a decrease of membrane fluidity, this leads to leakiness and loss of selective permeability, which are early and ubiquitous features of leaf senescence (Fan *et al.*, 1997; Lim and Nam, 2005; Espinoza *et al.*, 2007; Martinez *et al.*, 2008). However, the changes in the degree of unsaturation of membrane lipids during silique senescence have not been investigated.

To deepen our understanding of the metabolism and functions of lipids in cells, increasing attention has focused on the development of comprehensive strategies for the analysis of these factors in recent years (Brugger *et al.*, 1997; Han and Gross, 2005; Wenk, 2005). This has promoted the emergence of lipidomics, which is becoming an integral part of functional genomics. Plant lipidomics is based on electrospray ionisation tandem mass spectrometry (ESI-MS/MS) analysis, which makes it possible to measure hundreds of lipid molecules in vivo with small samples and in a short time; and this enables accurate calculation of the DBI of all measured membrane glycerolipids (Welti *et al.*, 2002). Several studies have employed lipidomics to profile changes in molecular species and DBI at low temperatures, and to characterise the function of genes that encode lipolytic enzymes, in combination with genetic approaches (Devaiah *et al.*, 2007; Hong *et al.*, 2009; Zhang *et al.*, 2009; Scherer *et al.*, 2011).

This study involved the use of lipidomics to: (i) determine how membrane glycerolipid species and DBI change during *Arabidopsis* silique senescence; and (ii) compare changes in the glycerolipid profiles during silique senescence between Wassilewskija (WS) and PLDδ-KO plants in order to determine the effect of PLDδ on changes in the lipid compositions of siliques.

1 Methods and materials

1.1 Plant materials

PLDδ-KO plants were generated by T-DNA insertion (PLDδ-KO) into the ecotype WS, there were no differences phenotype between WS and PLDδ-KO plants during normal growth (Zhang *et al.*, 2003). The loss of PLDδ was confirmed by the absence of its transcript, protein, and activity (Zhang *et al.*, 2003; Li *et al.*, 2008).

1.2 Plant growth and sampling

Both of the *Arabidopsis* genotypes studied were grown in Scott's Metro-Mix soil. Pots containing the seeds were kept at 4 ℃ for two days and then moved to a greenhouse with natural lighting during springtime. Individual flowers were tagged at the day of flowering, and developing and senescence siliques were sampled 15, 20, 25, and 30 days after flowering (DAF). In order to follow embryo development of the seeds, intact silique has been used (Folter *et al.*, 2004; Louvet *et al.*, 2006; Wagstaff *et al.*, 2009). Siliques were harvested for lipid analysis at each time point.

1.3 Lipid extraction and ESI/MS-MS analysis

The processes of lipid extraction, ESI-MS/MS analysis, and quantification were performed as described previously with minor modification (Welti *et al.*, 2002; Li *et al.*, 2008; Zheng *et al.*, 2011). Each sample contained dry weight of 10 to 20 mg. To inhibit lipolytic activity, the siliques were transferred immediately into 3 mL of isopropanol containing 0.01%

butylated hydroxytoluene in a 75 ℃ water bath. The tissue was extracted three times with chloroform/methanol (2:1) containing 0.01% butylated hydroxytoluene, with continuous agitation over for 7 days. The remaining plant tissue was dried overnight at 105 ℃ and weighed to give the dry weight of the tissue. Lipid samples were analysed on a triple quadrupole MS/MS equipped for ESI. Automated ESI-MS/MS analysis was performed in the Analytical Loaboratory of the Kansas Lipidomics Research Centre, Manhattan, USA. ESI-MS/MS analysis and quantification were performed as described previously (Kansas Lipidomics Research Centre, http://www.k-state.edu/lipid/lipidomics). The lipids of each head-group class were quantified by comparison with two internal standards for the specific class.

1.4 Data analysis

Data processing was performed as previously described (Welti *et al.*, 2002; Devaiah *et al.*, 2006; Zheng *et al.*, 2012). DBI was calculated using the following formula: DBI = [∑(N × mol% lipid)]/100, where N is the total number of double bonds in the two fatty acid chains of each glycerolipid molecule (Osmond *et al.*, 1982; Rawyler *et al.*, 1999; Bakht *et al.*, 2006; Zheng *et al.*, 2011). Five replicates of each treatment were analysed. The Q-test was performed on the total amount of lipid in each class, and data from discordant samples were removed. The data were subjected to one-way analysis of variance (ANOVA) using SPSS 13.0. Statistical significance was tested by Fisher's least significant difference (LSD) method.

2 Results and discussion

2.1 Profiling changes in the molecular species of membrane lipids during silique senescence in *Arabidopsis*

Lipidomic analysis was applied to each stage of silique senescence (Fig. 1). Silique senescence in *Arabidopsis* is developmentally regulated and is far less responsive to environmentally induced variability than leaves, and, unlike petals, neither organ undergoes abscission in *Arabidopsis* (Wagstaff *et al.*, 2009). The *Arabidopsis* silique elongates and developed immediately after pollination and reaches its final length approximately 15 DAF, at which point the silique is most green, thereafter the siliques became a little yellow at 20 DAF is associated with the senescence, but the yellowing of siliques that is very evident at 25 DAF is associated with the senescence and desiccation. By 30 DAF, the silique is fully yellow and becomes shattered. Using a lipidomic approach based on ESI-MS/MS (Welti *et al.*, 2002; Li *et al.*, 2008), we identified more than 140 molecular species of polar glycerolipids in *Arabidopsis* siliques of different ages in the absence of environmental stress (Tables 1-2 and Fig. 2-3). These molecular species included phospholipids of six head-group classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), PA, and phosphatidylglycerol (PG), as well as galactolipids of two head-group classes: monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) (Welti *et al.*, 2002). Each molecular species was identified in relation to the total number of acyl carbon atoms and double bonds (Welti *et al.*, 2002). An overview of the findings (Tables 1-2 and Figs. 2-3) reveals that the levels of most lipid species changed dramatically during silique senescence.

2.2 Dramatic decreases in the levels of lipid molecular species during the senescence of *Arabidopsis* siliques

Hierarchal clustering of the lipid profiles was used to obtain an overall appreciation of the changes in identities and concentrations of lipids during the senescence of siliques (Fig. 2). The total amounts of lipid and the average levels of molecular species in each head-group class are shown in Table 1. Levels of both extraplastidic and plastidic lipids decreased during silique senescence of *Arabidopsis*. The amount of total lipids had declined by 11.2% at 20 DAF (from 123.7 to 109.96 nmol·mg^{-1}), which was significantly less than the decreases of 51.74% (at 25 DAF)

and 64.91% (at 30 DAF) observed in WS. These data indicate that the largest decline occurred during silique senescence, although lipid levels had started to decline even before distinct yellowing became apparent. The decreases in the levels of lipid molecular species during the senescence of *Arabidopsis* siliques is resemble to leaf senescence (Wagstaff *et al.*, 2009).

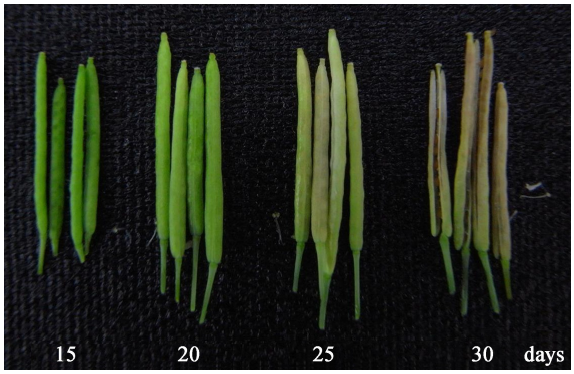

Fig. 1　The senescence of siliques at 15, 20, 25, and 30 days after flowering in WS

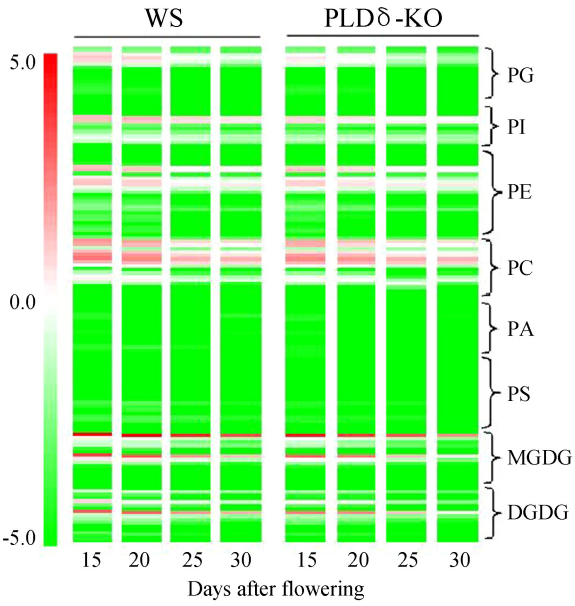

Fig. 2　Hierarchal clustering analysis of *Arabidopsis* lipid molecular species during the senescence of siliques in WS and PLDδ-KO plants. Each coloured bar within a column represents a lipid molecular species in the indicated cultivars and treatments. The colour of each bar represents the level of corresponding lipid species, with expression shown as log2 of the lipid amount (nmol/mg dry weight). A total of 140 lipid species in the indicated lipid classes were organised according to their class (as indicated), total acyl carbons (in ascending order within a class), and total double bonds (in ascending order with class and total acyl carbons). The dry weight is dry weight minus lipid (*i.e.* dry weight after lipid extraction)

In this context, an interesting question arises: why do the levels of lipids decrease substantially during the silique senescence that occurs naturally as part of the plant life cycle? It seems that the most likely answer to this question is that terminal events in the life cycle of a plant organ initially provide a mechanism for the mobilisation of nutrients from the ageing tissue to support the development of younger tissues or seeds. This is followed by a cell-death phase during which unwanted structures are discarded (Wagstaff *et al.*, 2009).

Unlike leaves, the seeds contained within siliques contain not only membrane phospholipids, but also storage lipids, 90% or more of which are present only in triacylglycerols (TAGs). During seed maturation, TAGs are biosynthesised (Ting *et al.*, 1997; Siloto *et al.*, 2006) and accumulate in discrete subcellular organelles called oil bodies. TAGs in the oil bodies are hydrolysed by lipases to provide energy and carbon during germination. The process of TAG synthesis for the storage of lipids shares similar pathways and common chemical intermediates (such as PA) with that of membrane lipids. PA is a secondary product of PLC activity, and it is the primary product of PLD signalling. PLD hydrolyses structural lipids such as PC to produce PA (Hartog *et al.*, 2001; Munnik, 2001). The synthesised PA is dephosphorylated to DAG by a PA phosphatase. DAG is further acylated to form TAGs by DAG acyltransferase (Parthibane *et al.*, 2012). Moreover, the senescence of the silique walls is very closely linked to seed maturation, the process is far less responsive to environmental stimuli than the senescence of most organs (Wagstaff *et al.*, 2009). This suggests that the products of the hydrolysis of silique membrane lipids may be transferred to seeds. As in seeds, assimilates such as PA are transferred from silique walls and redistributed to the developing seeds for the synthesis of TAGs. However, there remains a need to verify what happens to those polar lipids and TAGs during silique senescence and seed maturation and germination.

2.3 Levels of different lipid species do not decrease at the same rate during silique senescence

We analysed the changes in the relative levels of the various lipid species (mol %), which can reflect interconversion among lipids (Fig. 3 and Table 2). Whereas the relative levels of some lipids declined, those of others increased. This suggests that different lipids were degraded at different rates. Specifically,

Table 1 Changes in lipid classes during *Arabidopsis* silique senescence in WS and PLDδ-KO plants (nmol·mg^{-1} DW)

Values are means±*SE* (n=4 or 5). Values in the same row with different letters are significantly different (P<0.05). An asterisk indicates that the value is significantly different from that of WS under the same conditions (P<0.05)

Lipid class	Plant species	Lipids/nmol·mg^{-1} DW			
		15 d	20 d	25 d	30 d
PG	WS	7.03 ± 1.04^{a}	6.20 ± 0.97^{a}	3.07 ± 0.46^{b}	1.96 ± 0.25^{c}
	PLDδ-KO	5.00 ± 0.54^{a*}	3.29 ± 0.46^{b*}	1.66 ± 0.10^{c*}	1.03 ± 0.13^{d*}
PI	WS	7.20 ± 1.01^{a}	7.13 ± 0.71^{a}	5.14 ± 0.56^{b}	4.16 ± 0.62^{b}
	PLDδ-KO	4.84 ± 0.36^{a*}	4.23 ± 0.54^{ab*}	3.64 ± 0.49^{bc*}	3.24 ± 0.38^{c}
PE	WS	13.38 ± 1.73^{a}	11.57 ± 1.36^{a}	5.79 ± 0.72^{b}	6.09 ± 0.52^{b}
	PLDδ-KO	11.33 ± 1.12^{a}	8.34 ± 1.21^{b*}	6.02 ± 0.81^{c}	5.50 ± 0.55^{c}
PC	WS	29.20 ± 2.83^{a}	25.35 ± 4.83^{a}	13.03 ± 0.23^{b}	12.42 ± 1.09^{b}
	PLDδ-KO	23.21 ± 1.83^{a*}	19.02 ± 1.93^{b}	13.06 ± 1.31^{c}	11.38 ± 0.80^{c}
PA	WS	0.22 ± 0.04^{a}	0.11 ± 0.03^{b}	0.12 ± 0.03^{b}	0.15 ± 0.04^{a}
	PLDδ-KO	0.22 ± 0.05^{a}	0.08 ± 0.04^{b}	0.05 ± 0.02^{b*}	0.10 ± 0.03^{b}
PS	WS	0.62 ± 0.10^{ab}	0.70 ± 0.11^{a}	0.46 ± 0.07^{bc}	0.32 ± 0.07^{c}
	PLDδ-KO	0.43 ± 0.03^{a*}	0.36 ± 0.07^{ab*}	0.29 ± 0.07^{bc*}	0.21 ± 0.05^{c}
MGDG	WS	52.79 ± 6.97^{a}	47.07 ± 6.51^{a}	25.39 ± 4.07^{b}	14.02 ± 1.31^{c}
	PLDδ-KO	36.71 ± 4.39^{a*}	24.71 ± 3.41^{b*}	12.57 ± 1.25^{c*}	6.42 ± 1.01^{d*}
DGDG	WS	13.01 ± 2.02^{a}	11.61 ± 1.55^{a}	6.60 ± 0.93^{b}	3.99 ± 0.62^{c}
	PLDδ-KO	9.22 ± 1.13^{a*}	6.56 ± 1.06^{b*}	3.49 ± 0.48^{c*}	1.91 ± 0.21^{d*}
Total lipids	WS	123.70 ± 19.78^{a}	109.96 ± 14.78^{a}	59.70 ± 6.79^{b}	43.41 ± 5.72^{c}
	PLDδ-KO	91.21 ± 13.99^{a}	66.77 ± 10.07^{b*}	40.95 ± 3.97^{c*}	29.97 ± 3.22^{d*}

Table 2 Mol% changes in lipid classes during *Arabidopsis* silique senescence in WS and PLDδ-KO plants

Values are means±*SE* (n=4 or 5). Values in the same row with different letters are significantly different (P<0.05). An asterisk indicates that the value is significantly different from that of WS under the same conditions (P<0.05)

Lipid class	Plant species	Lipids/mol%			
		15 d	20 d	25 d	30 d
PG	WS	5.67 ± 0.63^{a}	5.62 ± 0.75^{a}	5.27 ± 0.56^{a}	4.48 ± 0.61^{a}
	PLDδ-KO	5.50 ± 0.32^{a}	4.95 ± 0.44^{a}	4.07 ± 0.25^{b*}	3.43 ± 0.33^{c*}
PI	WS	5.86 ± 0.61^{b}	6.52 ± 0.56^{b}	9.00 ± 1.32^{a}	9.59 ± 0.81^{a}
	PLDδ-KO	5.40 ± 0.73^{c}	6.32 ± 0.65^{c}	8.86 ± 0.50^{b}	10.84 ± 0.16^{a*}
PE	WS	10.88 ± 0.84^{b}	10.46 ± 0.82^{b}	8.97 ± 1.09^{b}	14.14 ± 1.28^{a}
	PLDδ-KO	12.48 ± 0.75^{c}	12.57 ± 0.74^{c*}	14.66 ± 0.86^{b*}	18.38 ± 0.60^{a*}
PC	WS	23.84 ± 2.42^{a}	23.00 ± 2.09^{a}	20.20 ± 2.50^{b}	28.77 ± 1.61^{b}
	PLDδ-KO	25.63 ± 1.93^{a*}	28.79 ± 2.16^{b*}	31.91 ± 1.32^{c*}	38.12 ± 2.10^{c*}
PA	WS	0.18 ± 0.03^{b}	0.10 ± 0.01^{c}	0.20 ± 0.04^{b}	0.43 ± 0.07^{a}
	PLDδ-KO	0.25 ± 0.04^{a}	0.12 ± 0.04^{b}	0.13 ± 0.03^{b*}	0.32 ± 0.05^{a}
PS	WS	0.50 ± 0.03^{b}	0.63 ± 0.08^{a}	0.76 ± 0.11^{a}	0.75 ± 0.12^{a}
	PLDδ-KO	0.48 ± 0.07^{c}	0.55 ± 0.06^{bc}	0.71 ± 0.09^{a}	0.68 ± 0.10^{ab}
MGDG	WS	42.36 ± 3.13^{a}	42.88 ± 3.24^{a}	44.15 ± 7.07^{a}	32.17 ± 1.87^{b}
	PLDδ-KO	39.91 ± 3.28^{a}	36.69 ± 2.84^{a*}	30.74 ± 1.92^{b*}	21.29 ± 2.13^{c*}
DGDG	WS	10.53 ± 0.89^{a}	10.59 ± 0.86^{a}	11.23 ± 1.62^{a}	9.57 ± 0.55^{a}
	PLDδ-KO	10.05 ± 0.67^{a}	9.73 ± 0.58^{a}	8.51 ± 0.55^{b*}	6.36 ± 0.66^{c*}

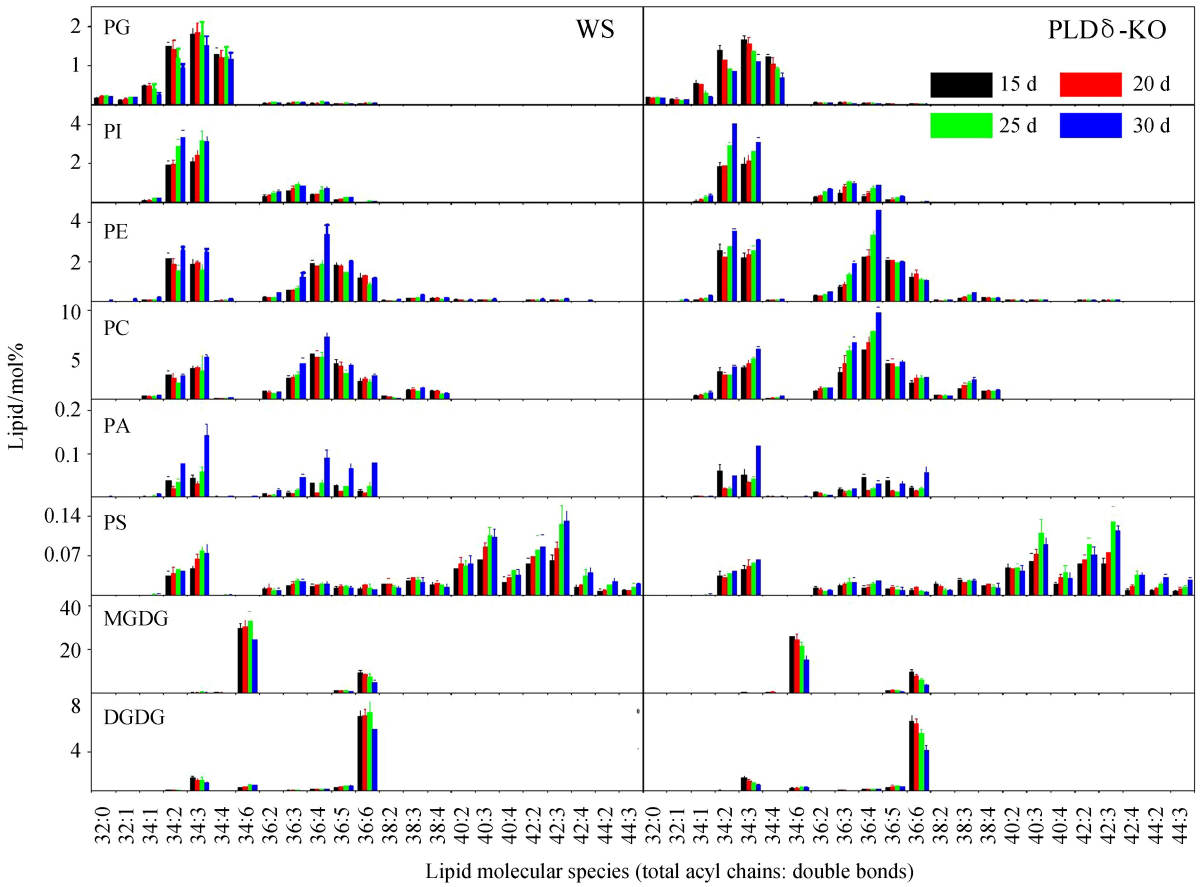

Fig. 3　The changes of relative levels (mol%) of lipid molecular species during *Arabidopsis* silique senescence in WS and PLDδ-KO plants. Values are means±*SE* (n=4 or 5)

differences in the rates of degradation of extraplastidic and plastidic lipids occurred in terms of their relative abundances during silique senescence in *Arabidopsis*. During silique senescence in both WS and PLDδ-KO plants, the relative levels of plastidic lipids (PG, MGDG, DGDG) decreased, whereas those of extraplastidic lipids (PI, PE, PC) increased. This suggested that plastidic lipids, including MGDG, DGDG and PG are degraded more rapidly than non-plastidic lipids, such as PE, PC, PI, and PS, which are found primarily in the membranes of non-photosynthetic organelles, such as the endoplasmic reticulum and mitochondria. Shortly after the onset of silique senescence, the catabolism of plastidic lipids began before that of lipids in the plasma membrane. In this study, levels of PA decreased after the beginning of silique senescence. Membrane lipids normally contain less than 1% PA; however, the content of PA was shown to increase dramatically upon exposure to biotic and abiotic stresses (Wang, 2005; Li *et al.*, 2008). This suggests that silique senescence is natural event, which occurs as plants age.

2.4　The DBI of total lipids decreased, with no change of the DBIs of different lipid classes

Maintaining the integrity and optimal fluidity of membranes is very important for organisms. In this study, DBI was employed to reflect the membrane fluidity. We determined the mol% content of each lipid molecule species based on the data of nmol/mg dry weight, and calculated the DBI of each lipid species.

We found that the DBI of total membrane lipids decreased significantly during silique senescence, especially at 30 DAF (Table 3), from 4.67 to 4.27. However, no significant changes in DBI of each membrane lipid class except of total DBI in WS

plants during silique senescence. The decrease in the DBI of total membrane lipids might have been caused by the dramatic decrease in the levels of plastidic lipids and an increase in the relative levels of extraplastidic lipids. Given that the DBI was calculated based on the mol% content of each lipid molecule species, it is conceivable that the dramatic decrease in the levels of galactolipids might have contributed to the decrease of the DBI of total membrane lipids. This suggested that silique senescence were associated with the decrease in DBI of total lipids, which may have influenced the fluidity and integrity of cellular membranes.

2.5 The variation in levels of polar lipids in PLDδ-KO plants was difference to that observed in WS plants

The effect of PLD on senescence of siliques might be related to its structural role or the effects of its product, PA (Hong *et al.*, 2008). To assess the role of PLDδ in the senescence of *Arabidopsis* siliques, we employed compared the lipid profiles, especially the level of PA between the *Arabidopsis* PLDδ-KO mutant and wild-type WS plants. Although growth conditions can affect PA levels, but the PA content was very low and decreased during silique senescence in WS plants. We also found that the level and variation of PA and other membrane lipid components were different between WS and PLDδ-KO plants (Tables 1-3 and Fig. 2-3). For example, table1 indicated that rates of decline in levels of PE and PC were more rapid in WS than in PLDδ-KO, especially during the senescence of siliques (from 20 d to 25 d). More importantly, less PA was detected in senesced-siliques of PLDδ-KO than that of WS. Table 2 also showed that relative levels of both PE and PC were higher in PLDδ-KO than in WS. Total lipids at each stage are significantly lower in PLDδ-KO mutant than in WS. Lipid profiling data indicate that PLDδ was involved in the metabolism of membrane lipids during silique senescence.

In plants, the PLD family comprises 12 members, whereas only two PLDs have been identified in animals (Qin and Wang, 2002). It has been hypothesised that the basis for the differences between

Table 3 Changes in the double-bond index (DBI) of membrane lipids during *Arabidopsis* silique senescence in WS and PLDδ-KO plants

Values are means±*SE* (n=4 or 5). Values in the same row with different letters are significantly different ($P<0.05$). An asterisk indicates that the value is significantly different from that of WS under the same conditions ($P<0.05$)

Lipid class	Plant species	Double-bond index (DBI)			
		15 d	20 d	25 d	30 d
PG	WS	2.64 ± 0.05^{b}	2.62 ± 0.05^{b}	2.68 ± 0.05^{ab}	2.74 ± 0.03^{a}
	PLDδ-KO	2.60 ± 0.04^{b}	2.59 ± 0.2^{b}	2.67 ± 0.01^{a}	2.60 ± 0.07^{ab}
PI	WS	2.72 ± 0.04^{ab}	2.75 ± 0.02^{a}	2.73 ± 0.02^{a}	2.68 ± 0.02^{b}
	PLDδ-KO	2.70 ± 0.02^{ab}	2.74 ± 0.04^{a}	2.68 ± 0.01^{b*}	2.63 ± 0.03^{c}
PE	WS	3.65 ± 0.09^{ab}	3.72 ± 0.05^{a}	3.63 ± 0.02^{b}	3.52 ± 0.02^{c}
	PLDδ-KO	3.60 ± 0.06^{a}	3.66 ± 0.06^{a}	3.50 ± 0.02^{b*}	3.40 ± 0.02^{c*}
PC	WS	3.69 ± 0.05^{a}	3.72 ± 0.01^{a}	3.70 ± 0.03^{a}	3.68 ± 0.06^{a}
	PLDδ-KO	3.61 ± 0.02^{a*}	3.63 ± 0.07^{ab*}	3.55 ± 0.02^{b*}	3.53 ± 0.03^{b*}
PA	WS	3.44 ± 0.12^{a}	3.46 ± 0.16^{a}	3.58 ± 0.13^{a}	3.69 ± 0.14^{a}
	PLDδ-KO	3.44 ± 0.11^{a}	3.38 ± 0.20^{a}	3.57 ± 0.14^{a}	3.63 ± 0.14^{a}
PS	WS	2.92 ± 0.09^{a}	2.98 ± 0.07^{a}	2.95 ± 0.06^{a}	2.91 ± 0.06^{a}
	PLDδ-KO	2.86 ± 0.09^{a}	2.96 ± 0.07^{a}	2.91 ± 0.06^{a}	2.88 ± 0.07^{a}
MGDG	WS	5.87 ± 0.02^{a}	5.87 ± 0.02^{a}	5.87 ± 0.01^{a}	5.88 ± 0.01^{a}
	PLDδ-KO	5.84 ± 0.02^{a}	5.84 ± 0.01^{a}	5.86 ± 0.01^{a}	5.85 ± 0.01^{a}
DGDG	WS	5.41 ± 0.02^{b}	5.45 ± 0.05^{ab}	5.47 ± 0.03^{a}	5.47 ± 0.02^{a}
	PLDδ-KO	5.36 ± 0.03^{b}	5.42 ± 0.02^{a}	5.44 ± 0.02^{a}	5.39 ± 0.04^{ab*}
Total lipids	WS	4.67 ± 0.06^{a}	4.69 ± 0.10^{a}	4.60 ± 0.12^{a}	4.27 ± 0.08^{b}
	PLDδ-KO	4.56 ± 0.09^{a}	4.50 ± 0.07^{a*}	4.29 ± 0.05^{b*}	3.97 ± 0.06^{c*}

plant and animal PLDs is that they might play a more diverse and important role in plants than in other organisms (Wang, 2002). This makes it especially interesting to elucidate the unique and redundant functions of plant PLDs. This paper shows that PLDδ is involved in changes in the levels of PLDs during silique senescence because other PLDs can compensate for the loss of PLDδ function in PLDδ-deficient plants.

3 Conclusion

The metabolism of organelle membranes plays a crucial role during silique senescence. This paper has revealed complex and considerable changes in lipid molecular species during silique senescence. The degradation of membrane lipids and the decrease of the DBI of total membrane lipids in siliques were similar to those found in leaf senescence. The results of this study also suggest that PLDδ is involved in the metabolism of membrane lipids during silique senescence. The terminal events in the life cycle of a plant organ initially provide a mechanism for the mobilisation of nutrients from ageing tissue to support the development of younger tissue or seeds. From the obtained findings, we inferred that the products of hydrolysis of membrane lipids may be transferred to seeds for the synthesis of storage lipids, namely, TAGs; however, further studies are needed to confirm this hypothesis.

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