Plant Physiology and Biochemistry 47 (2009) 535–539

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Activation of galactolipid biosynthesis in development of pistils and pollen tubes

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ARTICLE INFO

Article history:
Received 17 November 2008
Accepted 30 December 2008
Available online 9 January 2009

Keywords:
Galactolipids
Monogalactosyldiacylglycerol
Digalactosyldiacylglycerol
Pistils
Pollen tubes
Petunia hybrida
Lilium longiflorum

ABSTRACT

Galactolipids such as monogalactosyldiacylglycerol and digalactosyldiacylglycerol are essential lipids for the proper functioning of photosynthetic membranes. However, the function of galactolipids in flowers is unknown. Previously, we reported that pistils have higher galactolipid-producing activity than leaves. The present study investigated galactolipid biosynthesis in pistils in more detail using *Petunia hybrida* and *Lilium longiflorum*. The results showed that digalactosyldiacylglycerol levels increased during flower development. In addition, the galactose incorporation activity into galactolipids was induced, suggesting that the pathway for the production of digalactosyldiacylglycerol was stimulated. Interestingly, a significant increase in galactolipids was also observed in elongated pollen tubes. Therefore, pistils are the main site of galactolipid biosynthesis and whose galactolipid biosynthesis activity is induced during flower development, and this induction includes considerable galactolipid biosynthesis in pollen tubes.

1. Introduction

Galactolipids, such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), are a unique lipid class found ubiquitously in photosynthetic organisms from cyanobacteria to land plants. In particular, galactolipids comprise 80% of the photosynthetic membranes in seed plants [7]. The most abundant polar lipid, MGDG, comprises 50% of the plastid membrane lipids, and analyses of the crystal structure of cyanobacterial photosystems revealed that one and six molecules of MGDG are found in the Photosystem I [17] and Photosystem II [24] complexes, respectively. Furthermore, analyses of Arabidopsis mutants *mgd1-1* and *mgd1-2* suggested that MGDG is an essential component for photosynthesis [3,14,22]. DGDG also plays role in photosynthesis: the Arabidopsis *dgd1* mutant has decreased DGDG content, which results in a pale-green phenotype and decreased photosynthetic activity [8,11]. In addition, DGDG is known to replace a major portion of the extraplastidic membrane phospholipids upon phosphate starvation, presumably to preserve the internal phosphate source and maintain the function of cellular membranes [1,2,10,15,20].

MGDG is synthesized in the plastid envelope by UDP-galactose:1,2-*sn*-diacylglycerol (DAG)-3-β-galactosyltransferase (MGDG synthase, EC 2.4.1.46) [29]. This enzyme catalyzes the transfer of a galactose moiety from UDP-galactose to DAG. On the other hand, DGDG is predominantly synthesized by DGDG synthase, which transfers an additional galactose moiety from UDP-galactose to MGDG, whereas an unidentified galactolipid:galactolipid galactosyltransferase is also capable of synthesizing DGDG from MGDG at a very low level in Arabidopsis [20]. In Arabidopsis, two sets of galactolipid biosynthetic pathways are known that localize to the inner and outer envelopes of chloroplast, respectively [5]. The pathway consisted of Type A MGDG synthase (MGD1) and a DGDG synthase (DGD1) is considered to account for the bulk of galactolipid synthesis. The outer-envelope pathway consists of Type B MGDG synthases (MGD2/3) and DGD2 and seems to make no significant contribution to DGDG synthesis under normal

Abbreviations: DAG, 1,2-*sn*-diacylglycerol; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLC, phospholipase C; PLD, phospholipase D.

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doi:10.1016/j.plaphy.2008.12.018

conditions [19,23]. However, this pathway contributes to DGDG production during phosphate starvation [23].

Until recently, the function of galactolipids was studied intensively in photosynthetic organs because it was suggested that the *in vivo* functions of galactolipids were related to photosynthesis. However, we previously showed that Type B MGDG synthase genes (MGD2 and MGD3) were expressed highly in floral organs and elongating pollen tubes [21]. Furthermore, a separate study showed that floral organs have a distinct galactolipid composition and pistils have higher galactolipid biosynthetic activity than leaves [25], suggesting the importance of galactolipids and their biosynthesis in floral organs.

To understand the unique galactolipid biosynthetic process in floral organs, we analyzed galactolipids in pistils in which the highest galactolipid biosynthetic activity was observed previously [25]. The present study showed that not only pistils but also pollen tubes produced considerable amounts of galactolipids. These results suggest a new function of galactolipids in pistils and pollen tubes.

2. Materials and methods

2.1. Materials

Petunia hybrida plants were grown in soil and Lily plants (*Lilium longiflorum*) were grown hydroponically. Categorization of flower developmental stage was performed as defined previously [25]. For lipid analysis of pollen tubes, cut flowers of *L. longiflorum* were purchased from a florist shop and used to sample pollens.

2.2. Lipid analyses

Membrane lipids were extracted according to the method of Bligh and Dyer [6]. Quantification of lipid contents and fatty acid composition were conducted as described previously [25].

2.3. Galactose incorporation activity assay

Galactose incorporation activity was assayed according to the method described previously [30] with some modifications as described subsequently [25].

2.4. In vitro elongation of pollen tubes

Flowers of *L. longiflorum* were incubated hydroponically until anthesis. For *in vitro* pollen tube elongation, conditions were optimized as described below based on previous reports [12,13]. Mature pollen grains of dehydrated anthers were incubated for 12 h on the surface of liquid germination medium containing 1.0 mM KCl, 0.1 mM $CaCl_2$, 1.6 mM H_3BO_3, 1.0 mM 2-(*N*-morpholino) ethanesulfonic acid buffer adjusted to pH 5.5 with KOH, and 7% sucrose. Elongated pollen tubes were collected with a small spatula and then frozen immediately in liquid nitrogen. For the non-germinated control, mature pollen grains were collected directly in liquid nitrogen and used for lipid extraction.

3. Results

3.1. Pistils showed highest DGDG production during flower development

Previously, we reported that the ratio of DGDG to MGDG in the flowers of *P. hybrida* increased during development, and the highest ratio was obtained in mature pistils (stage 3) [25]. In addition, the highest galactolipid synthetic activity was observed in the pistils, suggesting that pistils are the most active organs for galactolipid biosynthesis. To investigate how such characteristic features of galactolipid biosynthesis are established during flower development, the contents of MGDG and DGDG among three floral organs of Petunia (petals, stamens, and pistils) were compared between flowering buds (stage 2; [25]) and completely opened flowers (stage 3). As shown in Fig. 1, significant changes were observed in the pistils. Whereas the pistils contained about four times as much MGDG as DGDG in stage 2, the amount of DGDG became comparable to that of MGDG in stage 3, giving a prominent increase in the DGDG/MGDG ratio among the three floral organs. In stamens, there was no change in the ratio of MGDG to DGDG although the overall galactolipid content decreased in stage 3. Petals showed no significant changes between stage 2 and stage 3. This suggests that DGDG biosynthesis may be highly induced in pistils between stage 2 and stage 3.

3.2. Galactose incorporation activity into MGDG, but not DGDG, was induced in maturing pistils

To investigate how such intense accumulation of DGDG occurs in pistils between stage 2 and stage 3, galactose incorporation activities into MGDG and DGDG in three floral organs were compared between stage 2 and stage 3. As shown in Fig. 2, the galactose incorporation activity to MGDG and DGDG increased in petals but decreased in stamens between stage 2 and stage 3. However, in pistils, galactose incorporation into MGDG increased in stage 3, even though that into DGDG was not changed. Because the content of DGDG, but not MGDG, increased significantly in this stage (Fig. 1), this increase in galactose incorporation into MGDG suggests that the MGDG produced may serve as a substrate for DGDG biosynthesis, that is, MGDG synthesis may be a limiting step for DGDG accumulation in pistils at this stage.

3.3. Galactolipids accumulated in elongated pollen tubes of Lily

As shown in Figs. 1 and 2, pistils showed the highest increase in DGDG level and galactose incorporation activity between stage 2 and stage 3. Because pollen tube elongation occurs in pistils between stage 2 and stage 3, we investigated whether elongated pollen tubes contain galactolipids. For this purpose, Lily (*L. longiflorum*) was selected, as *in vitro* pollen tube elongation conditions are well established [12,13]. The membrane lipid composition of elongated pollen tubes (12 h incubation on *in vitro* pollen tube elongation media) was compared to that of pollen grains before

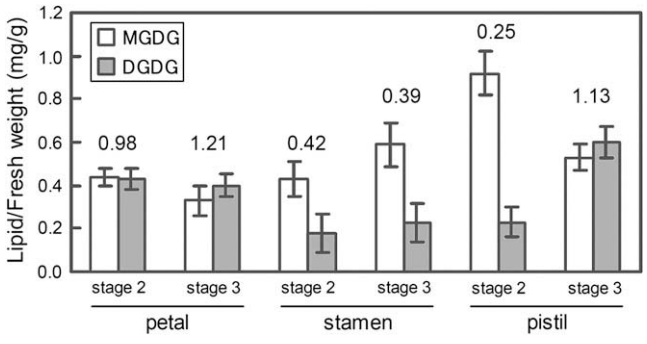

Fig. 1. Galactolipid contents (lipids/fresh weight, mg/g) of three floral organs (petals, stamens, and pistils) of *Petunia hybrida*. Lipids were extracted by the method of Bligh and Dyer [6], separated by two-dimensional thin layer chromatography, and the fatty acyl moieties were analyzed by gas chromatography. Values are the means ± SD from three independent measurements.