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Research article

The TOC159 mutant of *Arabidopsis thaliana* accumulates altered levels of saturated and polyunsaturated fatty acids

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ABSTRACT

We evaluated whether the TOC159 mutant of Arabidopsis called *plastid protein import* 2-2 (*ppi*2-2) accumulates normal levels of fatty acids, and transcripts of fatty acid desaturases and galactolipid synthesis enzymes. The *ppi*2-2 mutant accumulates decreased pigments and total fatty acid content. The MGD1 gene was downregulated and the mutant accumulates decreased levels of monogalactosyldiacylglycerol (MGDG) and 16:3, which suggests that the prokaryotic pathway was impaired in the mutant. The HY5 gene, which encodes long hypocotyl5 transcription factor, was upregulated in the mutant. The DGD1 gene, an HY5 target was marginally increased and the mutant accumulates digalactosyldiacylglycerol at the control level. The mutant had increased expression of 3-ketoacyl-ACP synthase II gene, which encodes a plastid enzyme that elongates 16:0 to 18:0. Interestingly, glycerolipids in the mutant accumulate increased levels of 18:0. A gene that encodes stearoyl-ACP desaturase (SAD) was expressed at the control level and 18:1 was increased, which suggest that SAD may be strongly regulated at the post-transcriptional level. The molar ratio of MGDG to bilayer forming plastid lipids was decreased in the cold-acclimated wild type but not in the *ppi*2-2 mutant. This indicates that the mutant was unresponsive to cold-stress, and is consistent with increased levels of 18:0, and decreased 16:3 and 18:3 in the *ppi*2-2 mutant. Overall, these data indicate that a defective Toc159 receptor impaired the synthesis of MGDG, and affected desaturation of 16 and 18-carbon fatty acids. We conclude that expression of the MGD1 gene and synthesis of MGDG are tightly linked to plastid biogenesis.

1. Introduction

Chloroplasts are the site of many biochemical processes including photosynthesis and fatty acid synthesis. The light reactions of photosynthesis take place in the thylakoid membranes. The biogenesis of thylakoid membranes is tightly linked to the development of the chloroplasts from proplastids, and this requires monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), which accounts for 75% of total thylakoid lipids (Kobayashi et al., 2007). During photomorphogenesis, the synthesis of photosystem proteins, lipids and pigments is coupled with their coordinated assembly into functional photosystems (Pogson et al., 1998). Both chlorophylls and carotenoids are required for the assembly and stability of light harvesting complex (LHC) apoproteins (Pogson et al., 1998).

Fatty acid synthesis takes place in the stroma yielding palmitate (16:0)-acyl carrier protein (ACP), which is elongated to stearate (18:0)-ACP by 3-ketoacyl-ACP synthase II (KAS II). Therefore, KAS II activity determines the ratio of 16 to 18-carbon fatty acids. The 18:0-ACP is desaturated to oleate (18:1)-ACP by a soluble stearoyl-ACP desaturase (Ohlrogge and Browse, 1995). Thus, 16:0-ACP and 18:1-ACP are the main products of fatty acid synthesis in the chloroplast stroma. In the plastids, acyltransferases transfer 16:0 and 18:1 from ACP to glycerol-3-phosphate, which result in the synthesis of phosphatidylglycerol (PG), MGDG, DGDG, and sulfoquinovosyldiacylglycerol (SQDG) (Ohlrogge and Browse, 1995). The photosynthetic protein-pigment complexes are embedded in the thylakoid membranes, and these membranes also contain MGDG, DGDG, SQDG and PG (Benning, 2008). Therefore, chloroplast lipids are major components of thylakoid membranes and they stabilize photosystems and the light-harvesting complex II (Dörmann, 2007).

The 16:0 and 18:1 are also exported as CoA thioesters from the plastids to the endoplasmic reticulum where these fatty acids are used in the synthesis of extrachloroplast lipids such as

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Abbreviations

MGD1	monogalactosyldiacylglycerol synthase 1
HY5	long hypocotyl5
KAS II	3-ketoacyl-ACP synthase II
*ppi*2-2	plastid protein import 2-2
18:0	stearic acid
16:3	hexadecatrienoic acid
18:3	α-linolenic acid

phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) (Ohlrogge and Browse, 1995; Wallis and Browse, 2010). The diacylglycerol (DAG) that is derived from PC is returned to the plastids, where it is incorporated into MGDG and DGDG by MGD1 and DGD1 synthases, respectively (Benning, 2008). MGDG that is synthesized by the prokaryotic pathway contains hexadecatrienoic acid (16:3) at the sn-2 position. However, MGDG that is synthesized from eukaryotic DAG contains unsaturated 18-carbon fatty acids, and a small amount of 16:0 may be present at the sn-1 position (Wallis and Browse, 2010).

The desaturation of 16:0 and 18:1 that are esterified on the glycerol backbone is carried out by membrane-bound fatty acid desaturases (fad). In *Arabidopsis*, MGDG that is synthesized by the chloroplast pathway is a substrate for FAD5 desaturase (Heilmann et al., 2004), which yields 16:1Δ^7 at the sn-2 position of MGDG. The 16:1Δ^7 is desaturated by FAD6 and FAD7/8 enzymes to yield 16:3$\Delta^{7,10,13}$. Therefore, the accumulation of 16:3 serves as a relative measure of FAD5 desaturase activity (Heilmann et al., 2004).

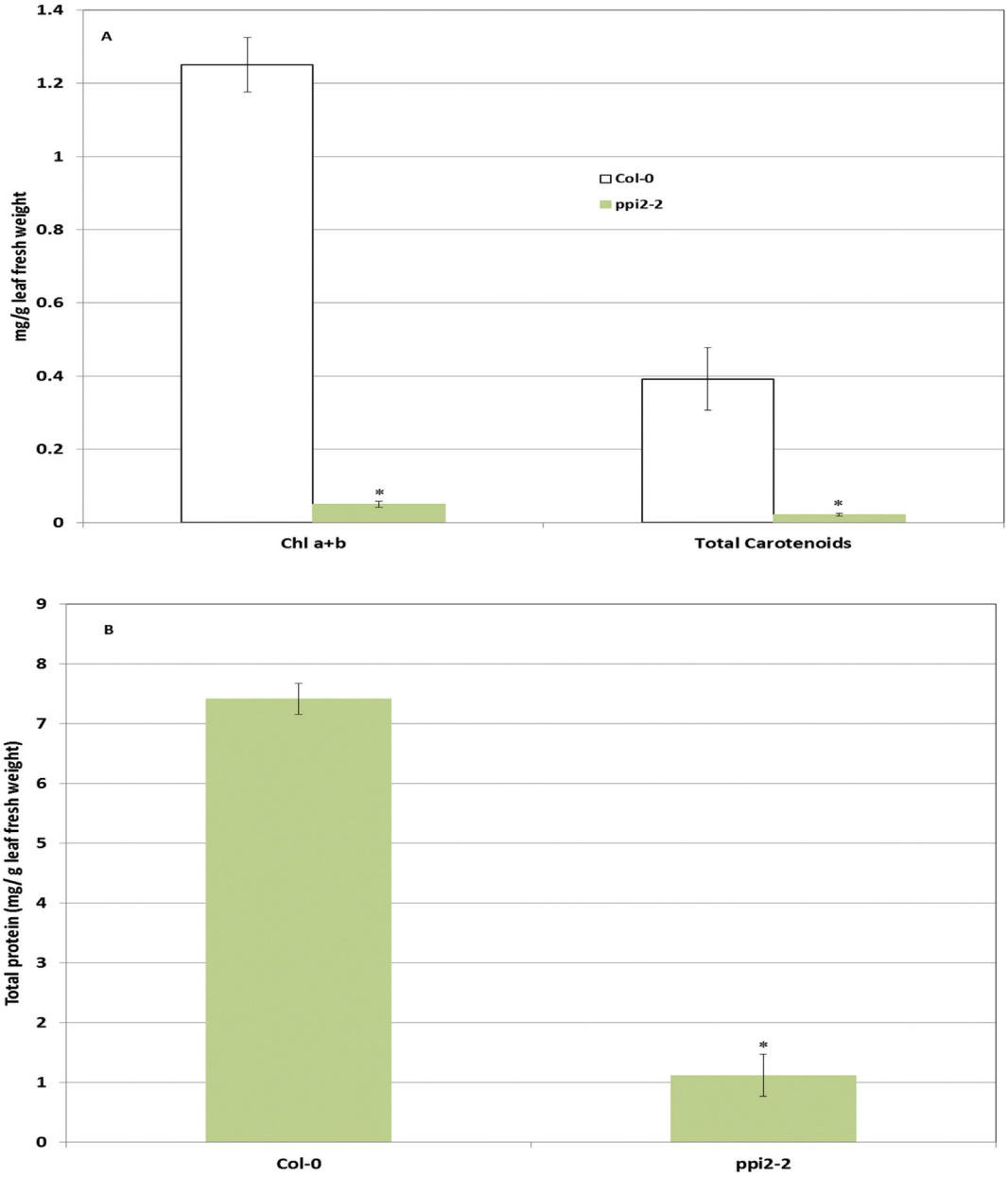

Fig. 1. The accumulation of photosynthetic pigments and total protein in the wild type and *ppi*2-2 mutant of Arabidopsis. Values are means ± SE of 3 or 4 biological replicates. Asterisks above the error bars indicate significant difference ($P < 0.05$) of each treatment compared to the wild type (Col-0) as determined by Student's *t* test.