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REGULAR PAPER

Over-expression of *Arabidopsis thaliana SFD1/GLY1*, the gene encoding plastid localized glycerol-3-phosphate dehydrogenase, increases plastidic lipid content in transgenic rice plants

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Abstract Lipids are the major constituents of all membranous structures in plants. Plants possess two pathways for lipid biosynthesis: the prokaryotic pathway (i.e., plastidic pathway) and the eukaryotic pathway (i.e., endoplasmic-reticulum (ER) pathway). Whereas some plants synthesize galactolipids from diacylglycerol assembled in the plastid, others, including rice, derive their galactolipids from diacylglycerols assembled by the eukaryotic pathway. *Arabidopsis thaliana* glycerol-3-phosphate dehydrogenase (G3pDH), coded by *SUPPRESSOR OF FATTY ACID DESATURASE 1* (*SFD1; alias GLY1*) gene, catalyzes the formation of glycerol 3-phosphate (G3p), the backbone of many membrane lipids. Here *SFD1* was introduced to rice as a transgene. *Arabidopsis* SFD1 localizes in rice plastids and its over-expression increases plastidic membrane lipid content in transgenic rice plants without any major impact on ER lipids. The results suggest that over-expression of plastidic G3pDH enhances biosynthesis of plastid-localized lipids in rice. Lipid composition in the transgenic plants is consistent with increased phosphatidylglycerol synthesis in the plastid and increased galactolipid synthesis from diacylglycerol produced via the ER pathway. The transgenic plants show a higher photosynthetic assimilation rate, suggesting a possible application of this finding in crop improvement.

Keywords *Arabidopsis* · SFD1 · G3pDH · Transgenic rice · Lipids · ESI–MS/MS

Abbreviations

DAG	Diacylglycerol
DGDG	Digalactosyldiacylglycerol
ER	Endoplasmic reticulum
FA	Fatty acid
GLC	Gas–liquid chromatography
G3p	Glycerol 3-phosphate
G3pDH	Glycerol-3-phosphate dehydrogenase
IRGA	Infrared gas analyzer
MGDG	Monogalactosyldiacylglycerol
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
SFD1	*SUPPRESSOR OF FATTY ACID DESATURASE 1*
TAG	Triacylglycerol

Introduction

Lipids contribute from 5 to 10 % of dry weight in plants, and plastid membranes make up a major portion of plant membrane lipids (Ohlrogge and Browse 1995). The abundant thylakoid membranes in the plant chloroplast are the site of the light-dependent reactions of photosynthesis (Browse 2010).

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Plants contain mostly either 16 or 18 carbon fatty acids (FA) with 0–3 carbon–carbon double bonds (Ohlrogge and Browse 1995; Stumpf 1984; Mukherjee 1983). FAs in plants are synthesized in plastids. Plants possess two distinct pathways for membrane lipid biosynthesis, referred as prokaryotic (plastidic) and eukaryotic (ER) pathways. In the prokaryotic pathway, phosphatidic acid (PA) is made by incorporating acyl chains into the sn-1 and sn-2 positions of a G3p molecule with the preference for 18:1 and 16:0 FAs, respectively (Ohlrogge and Browse 1995). PA made in the prokaryotic pathway is the starting molecule for other plastidic membrane lipids including diacylglycerol (DAG), which is converted to monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). PA is also converted to phosphatidylglycerol phosphate, which is then converted to phosphatidylglycerol (PG) (Ohlrogge and Browse 1995). C18 and C16 FAs of MGDG undergo desaturation to generate 18:3 and 16:3 molecules respectively in plastids. In contrast, in the eukaryotic pathway, PA is made in the ER with the incorporation of 18:1 or 16:0 FAs in the sn-1, and 18:1 FA in the sn-2 position of G3p (Ohlrogge and Browse 1995). This ER-derived PA is the starting material for other extraplastidic lipids including phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC) and phosphatidylethanolamine (PE). However, unlike prokaryotic pathway lipids, in eukaryotic pathway lipids the 16:0 FAs remain mostly saturated.

In many plants, the prokaryotic pathway described above for galactolipid formation in the plastid does not occur. Instead, the prokaryotic pathway contributes only PG in plastidic lipids while the rest of the plastidic lipids are generated from diacylglycerol formed by the eukaryotic (ER) pathway and transported into plastids (Ohlrogge and Browse 1995). These plants contain mostly 18:3 acyl chains in their galactolipids and are referred as “18:3 plants”. Lack of plastidial phosphatidate phosphatase and acyl desaturase activities are considered to be the primary reasons for differences between 18:3 and 16:3 plants (Heinz and Roughan 1983; Mongrand et al. 1998; Ohlrogge and Browse 1995). Compared to 16:3 plants, isolated chloroplast of 18:3 plants show severely reduced PA-dephosphorylation, and desaturation of 18:1 (at sn-1) and 16:0 (at sn-2) FAs (Heinz and Roughan 1983). The striking features of lipid composition of the 18:3 plants are the absence or a negligible presence of “34:6 DGDG” and “34:6 MGDG” species, i.e. those composed of 18:3 and 16:3 FAs in sn-1 and sn-2 positions respectively (Browse et al. 1986; Ohlrogge and Browse 1995). This contrasts with plant species in which plastidic lipids are made largely by the prokaryotic pathway and, as a result, contain high levels of 16: 3 FA in galactolipids. The plants of the latter class are referred as “16:3 plants”. These plants contain a high level of 34:6 MGDG and some 34:6 DGDG species, due to plastidic lipid biosynthesis and desaturation of C16-carbon FAs (Ohlrogge and Browse 1995; Stumpf 1984).

The two alternative pathways for lipid biosynthesis in plants are associated with different pools of G3p (Chanda et al. 2011; Shen et al. 2006, 2010). G3p is derived from dihydroxyacetone phosphate (DHAP) by glycerol-3-phosphate dehydrogenase (G3pDH) (i.e., DHAP reductase), using the reduction potential of NAD(P)H (Miquel et al. 1998; Shen et al. 2010). In *Arabidopsis*, the plastid-localized G3pDH enzyme encoded by the gene *SFD1*/*GLY1* contributes to the plastidic G3p pool (Chanda et al. 2011). Consequently, the *sfd1/gly1* mutants of *Arabidopsis* harbor altered lipid composition in their plastids without any major effect in ER lipid composition (Miquel et al. 1998; Shen et al. 2010; Nandi et al. 2004; Chanda et al. 2011; Lorenc-Kukula et al. 2012). Expression of SFD1 in *sfd1* mutant background partially restores plastidic lipid composition (Lorenc-Kukula et al. 2012). In addition, there is an alternate pathway ubiquitously present in the cytosol of all plants; this pathway uses glycerol as a substrate to generate G3p via glycerol kinase (Lu et al. 2001; Eastmond 2004; Chanda et al. 2011). G3p metabolism has been proven to be of immense importance in plant defense and lipid metabolism (Mandal et al. 2011; Chanda et al. 2011; Yu et al. 2013). A lesion in *SFD1/GLY1* leads to loss of systemic acquired resistance (SAR) (Nandi et al. 2004; Chanda et al. 2011; Yu et al. 2013). A mutation in glycerol kinase coding gene *NON-HOST RESISTANCE 1* (*NHO1, also known as GLI1*), leads to loss of general resistance, and as well as SAR in *Arabidopsis* (Lu et al. 2001; Kang et al. 2003; Chanda et al. 2011). In addition, G3pDH also appears to be a promising candidate gene for enhancement of lipid content in oilseeds. Over-expression of yeast cytosolic G3pDH in *Brassica napus* through seed specific napin promoter yielded increased FA and lipid contents in transgenic plants (Vigeolas et al. 2007).

Rice is an 18:3 plant, which contains high levels of 18:3 with negligible amounts of 16:3 (Toriyama et al. 1988; Roughan and Batt 1969). This study was undertaken to investigate the possibility of increasing rice lipid content by increasing plastidic G3pDH by over-expressing Arabidopsis *SFD1/GLY1* gene. Our results show substantial enhancement in lipid content in transgenic rice plants mostly due to the enhancement of plastidic lipids in transgenic plants.

Materials and methods

Vector construction and generation of transgenic plants

The *SFD1* cDNA was obtained from the *Arabidopsis* stock centre. The cDNA was cloned as *BamHI-SacI* fragment in the pTCK303 vector (Wang et al. 2004) under maize ubiquitin promoter. Embryogenic calli of Japonica rice cultivar TP309 (*Oryza sativa* L. ssp. *japonica* cv.Taipei 309) were transformed with the construct by using *Agrobacterium*

tumefaciens strain *LBA4404* as described previously (Singh et al. 2013). The transgenic plants were regenerated in the presence of hygromycin 50 mg/L and were further kept for 3 weeks under selection pressure in the liquid MS medium containing hygromycin (50 mg/L). The plantlets were transferred to soil and after acclimatization about a week in the culture room, were transferred to the glass house.

RNA isolation, northern blot hybridization and RT-PCR

Extraction of total RNA from rice leaves was carried out as described previously (Singh et al. 2013). From each transgenic plant 10 µg of total RNA was transferred to nylon membrane and probed with radio-labeled SFD1 cDNA probe. cDNA preparation and RT-PCR for hptII gene was carried out as described earlier (Singh et al. 2013). The primers used are listed in the supplementary material.

Southern blot analysis

Total genomic DNA was isolated from leaf samples obtained from about a month-old soil-grown plants using the cetyltrimethylammonium bromide (CTAB) method (Maguire et al. 1991).Genomic DNA (10 µg) was digested with *BamHI* and hybridized with radio-labeled *SFD1* cDNA probe.

Fatty acid and lipid profile analysis

Leaf samples were harvested from 60-day-old greenhouse grown transgenic and WT plants. Each sample consisted of randomly taken upper 6–7 cm portion from approximately 10 leaves. The samples were quickly immersed in 3 ml of isopropanol containing 0.01 % butylated hydroxytoluene at 75 °C and mixed thoroughly. Extraction of total lipids and determination of lipid profile was carried out exactly as described previously (Nandi et al. 2003; Welti et al. 2002). After completion of the extraction procedure the remaining tissue was left in the hot-air oven over night to dry completely. The weight of the dried leaves was used to calculate the lipid content of the tissues. An automated electrospray ionization–tandem mass spectrometry at Kansas Lipidomic Research Center (https://www.k-state.edu/lipid/lipidomics) was used to profile lipid composition in rice leaves. Sample preparation, processing, data acquisition and analysis and acyl group identification were carried out as described previously (Welti et al. 2002).

GFP-SFD1 localization in rice protoplast

pCXDG-GFP-SFD1 construct was generated after PCR amplification of SFD1 cDNA (primers mentioned under supplementary material) in the *XcmI* site of pCXDG vector (Chen et al. 2009). Rice protoplasts were isolated from the leaves of 10 days-old seedlings and transformed with the vector construct, as described earlier (Zhang et al. 2011). To a suspension of 100 µL protoplast (~10^5 cells), 25 µg of plasmid DNA was added and PEG mediated transfection method was followed (Zhang et al. 2011). The protoplasts were cultured in a multi-well plates in dark at 26 °C for 15 h, before observing under a fluorescent microscope (Nikon Eclipse Ti and visualized at 60× magnification). For GFP, excitation filter was of 515–555 nm, and the emission filter was of 465–495 nm. Autofluorescence due to chlorophyll was observed at 580–660 nm with excitation wavelength of 515–565 nm.

SFD1-GFP localization in rice leaf sheath cells

Leaf sheaths from young rice seedlings at the 4–6 leaf stage were sectioned with a double folded razor blade, exactly as described in Wang et al. (2013). With a scalpel blade, the greenish regions were removed from the sections. The selected white portions of the sections were placed on half-strength MS plates and bombarded with pCXDG-GFP-SFD1 or only pCXDG plasmid DNA coated tungsten particles at 1,100 psi, with Biolistic PDS-1000/He (Bio-Rad) as per manufacturer's instructions. The bombarded sheath-sections were incubated at 28 °C for 2 days in the dark. The sections were stained with ER-Tracker Red dye (Cat # E34250, Life Technologies, USA) and observed under a fluorescent microscope, Nikon Eclipse Ti, at 60× magnification.

Photosynthetic efficiency measurements

Fully grown leaves of 60-day-old rice plants were used for measuring photosynthetic rate with the GFS-3000 gas exchange system (Walz, Effeltrich, Germany). The photosynthetic rate was determined at the ambient CO_2 concentration of ~380 ppm, with a reference area of 2.5 cm^2, by sensing the photosynthetic active radiation (PAR; 1500 µmole m^{-2} s^{-1}). The photosynthetic assimilation rate (*A*) was calculated by the instrument (von Caemmerer and Farquhar 1981). Maximal photosystem II quantum yield, Fv/Fm, was measured by PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The plants were dark adapted for 2 h before application of saturation pulse at maximum intensity and data recorded as per the instrument manufacturer's instructions.

Chlorophyll estimation

Chlorophyll was extracted from leaf samples of 75-day-old plants in 100 % methanol in six replicates. *Chla* and *Chlb* contents were determined after taking absorbance at 665

and 652 nm respectively, and relative contents were calculated as described previously (Ritchie 2006). Summation of *Chla* and *Chlb* is represented as total chlorophyll content.

Results

Generation of transgenic rice plants with Arabidopsis *SFD1/GLY1*

Transgenic rice plants with *SFD1* cDNA under the control of the maize ubiquitin promoter were generated by transforming embryogenic calli obtained from rice cultivar TP309. Five transgenic T0 plants, obtained from independent calli were selected for further study. Integration of the transgene was confirmed by Southern hybridization (Fig. 1a) and expression of the *SFD1* transcript was confirmed by northern hybridization using radio-labeled *SFD1* cDNA probe (Fig. 1b). The T1 seedlings carrying the transgene were selected on the basis of PCR with hygromycin resistance gene (Fig. 1c). Only the confirmed transgenic T1 lines were transferred to the soil for further analyses. At the T2 generation, we identified two homozygous single copy insertion transgenic lines, originating from the T0 lines #1 and #4. The T3 progeny plants from these lines showed equivalent expression of *SFD1* mRNA (Fig. 1d). The growth and development of all transgenic plants were similar to the untransformed WT plants (Supplementary Fig. S1A). In spite of having increased plastidic lipid content (discussed later), the transgenic plants harbored levels of chlorophyll in their leaves equivalent to those of untransformed control plants (Supplementary Fig. S1B).

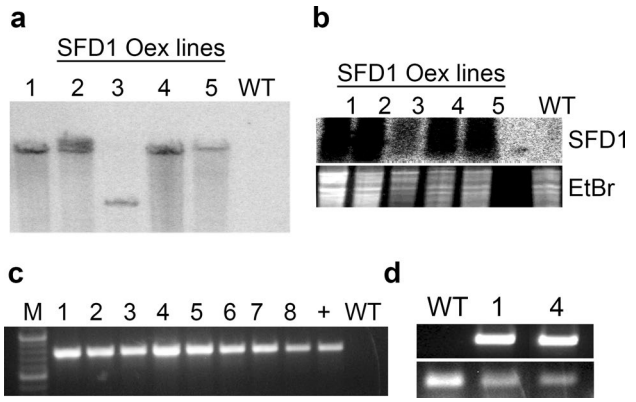

Fig. 1 Confirmation for generation of transgenic rice plants and expression of transgenes. **a** Southern blot with radio-labeled *SFD1* cDNA as probe to confirm the integration of the transgene in the rice genome. **b** Northern blot analysis to detect the level of *SFD1* transcript accumulation in the transgenic lines. Ethidium bromide (EtBr) stained gel indicates the relative amount of total RNA loaded in each *lane*. **c** PCR for detection of hygromycin resistance marker in the transgenic lines. The *lane numbers* indicate individual T2 plants of line number #1. **d** Reverse-transcription PCR of two homozygous T3 progeny taking one each originating from T0 line #1 and 4. In A, B and D the *numbers* on the *lane* indicate the T0 transgenic line number

SFD1/GLY1 over-expression leads to an increase in C18 FAs in transgenic rice plants

To examine, whether SFD1 over-expression in rice influences lipid composition, we determined the FA profile from five independent T0 transgenic plants as well as from five untransformed WT plants, by gas-liquid chromatography. Total FA content of the transgenic plants was significantly higher (30–40 %) than that of untransformed plants (Fig. 2a). Analysis of FA composition showed that the increment in the total FA is mostly contributed by 18:3 followed by 18:2 (Fig. 2b). However, in contrast, 16-carbon FAs were almost unchanged between transgenic and control plants (Fig. 2b). The results were surprising to us, as the *sfd1* mutation in *Arabidopsis* largely affects 16-carbon containing lipid species (Nandi et al. 2004). Thus, we looked into the details of the lipid composition alterations in the homozygous transgenic plants.

SFD1/GLY1 over-expression enhances plastidic lipid content without much impact on extraplastidic lipids

To compare the lipid content and profile of *SFD1* transgenic plants to the untransformed TP309 plants (WT), T3 progeny from 2 independent homozygous transgenic lines, originated from T0#1 and T0#4 were selected. Plants were grown in a greenhouse for 60 days before collecting samples. For each transgenic line, we selected five T3 plants,

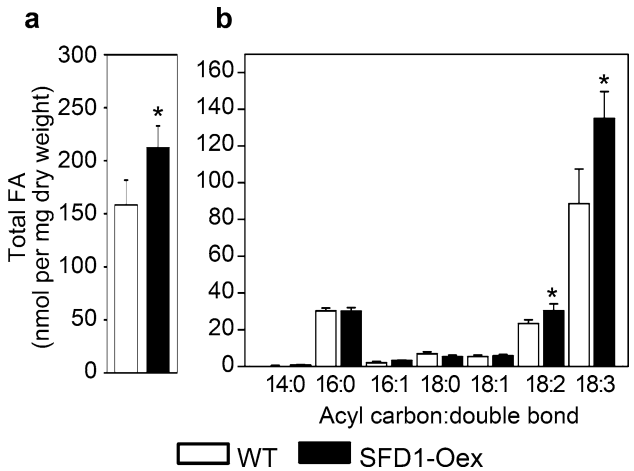

Fig. 2 FA composition WT and T0 transgenic plants. **a** Total FA content and **b** FA composition of WT and T0 transgenic plants. Each *bar* represents mean and standard deviation of five plants. * indicates statistically different (*t* test, $P < 0.05$) FA content between transgenic and WT plants

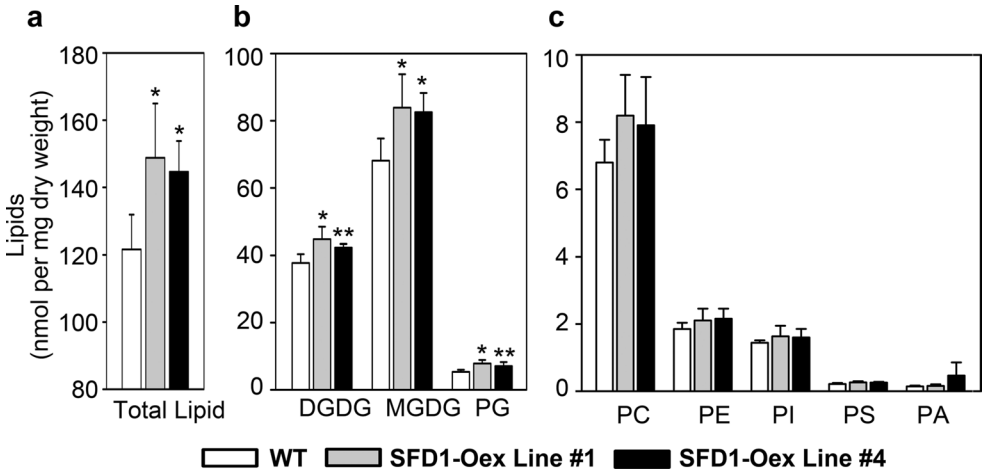

Fig. 3 Lipid class analysis by electrospray ionization–tandem mass spectrometry. **a** Total membrane lipids; **b** plastidic glycerolipids DGDG, MGDG and PG; **c** and ER lipids PC, PE, PI, PS and PA in wild-type (WT) and two transgenic rice lines with over-expression of *SFD1*. Total lipids indicate the summation of all detected lipids by ESI–MS–MS analysis, which include both di-acyl and lyso-lipids species. *Each bar* indicates the average and standard deviations of five plants. * and ** *above* the *bars* indicate the significant difference (*t* test) with *P* values <0.05 and <0.1, respectively

and one sample was collected from each plant. Similarly five samples were collected from untransformed plants. Each sample consisted approximately 1.0 g of leaf tissues. The total lipids were identified and quantified on the basis of head group and molecular mass through an automated electrospray ionization–tandem mass spectrometry approach (Welti et al. 2002). Both transgenic lines containing the *SFD1* over-expression construct contained approximately 20 % higher lipid than the untransformed WT plants (Fig. 3a). Further, the analyses indicated that the excess lipid content was mostly attributable to enhanced plastidic lipid content (Fig. 3b). Monogalactosyldiacyl glycerol (MGDG) is the major constituent of plastidic lipid both for rice and *Arabidopsis,* followed by digalactosyldiacyl glycerol (DGDG) and phosphatidylglycerol (PG) (Nandi et al. 2003, 2004; Roughan and Batt 1969; Toriyama et al. 1988). We found that all the plastidic lipids were increased in the transgenic plants compared to the WT plants (Fig. 3b). Level of sulfoquinovosyldiacyl glycerol (SQDG) remained below our detection limit, both in WT and transgenic plants. However, in contrast to the plastidic lipids, the ER lipids were only marginally impacted by *SFD1* over-expression (Fig. 3c). Phosphatidylcholine (PC) is the major ER-synthesized lipid in plants. Even though there appeared to be a trend toward a modest increase in PC content in the transgenic plants, the difference between transgenic and WT plants was not statistically significant. Other ER lipids such as phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA) were also not affected by the expression of the *SFD1* (Fig. 3c).

To compare the influence of SFD1/GLY1 overexpression between plastidic and ER lipids, we calculated the mol % of lipids species in WT and transgenic plants (Supplementary Fig. S2). Since plastidic lipids constitute more than 90 % of the total lipids, and enhancement in the lipid content was mostly in the plastidic lipids, we did not expect much alternation in the mol percentage. However, as anticipated, we observed a trend towards higher mol % of MGDG and PG, and reduced mol % of PC, PE and PI, in the transgenic plants compared to the WT plants.

Over-expression of *SFD1/GLY1* results in an increase in PG and 36-carbon galactolipids

Lipid profiling of MGDG and DGDG showed that the lipids increased in the transgenic plants were mostly 36:6 galactolipids (Fig. 4). 36:5 MGDG was also increased. Total 34:6 galactolipids (18:3 + 16:3) in rice occur very low compared to levels in *Arabidopsis* (Nandi et al. 2003, 2004; Roughan and Batt 1969), and we did not observe any significant increase in the transgenic plants (Fig. 4). *SFD1* over-expression also resulted in increased levels of PG with total acyl carbons of 34 and 32 (Fig. 4). PG is present in both plastids and extra-plastidic membranes. An increase in formation of G3p in the plastid has the potential to increase the formation of PG in an 18:3 plant, such as rice, due to potentially increased availability of the PA precursor. In accordance with the observation of non-significant changes in overall ER lipid content (Fig. 3c), the distribution of ER lipid molecular species was unaffected in the transgenic plants (Supplementary

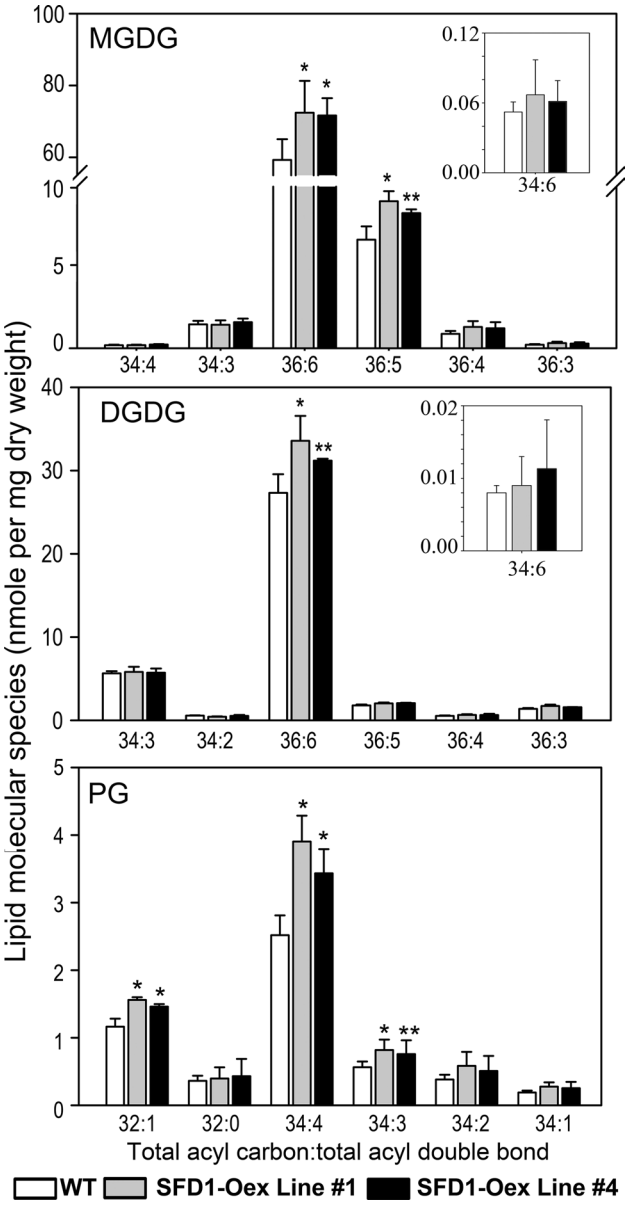

Fig. 4 Analysis of major plastidic glycerolipids by electrospray ionization–tandem mass spectrometry of wild-type (WT) and two transgenic rice lines with over-expression of *SFD1*. *Each bar* indicates the average and standard deviations of 5 plants. * and ** *above* the *bars* indicate the significant difference (t-test) with *P* values < 0.05 and < 0.1, respectively. *Insets* in the MGDG and DGDG panels are of the corresponding lipids at different scale

Fig. S3). We also did not observe any significant difference the ratio of 34C-PA or 36C-PA to total PA between WT and transgenic plants (Supplementary Fig. S4). Thus, the direct effect of enhanced G3pDH in plastidic lipids by prokaryotic pathway was limited only for PG species. These data indicate an enhancement of galactolipids biosynthesis via the eukaryotic pathway in the transgenic plants.

GFP-SFD1 is localized in plastids of rice protoplasts

SFD1 -GFP is shown to localize in plastids of a transgenic *Arabidopsis* plant (Chanda et al. 2011; Lorenc-Kukula et al. 2012). Allelic *sfd1/gly1* mutations mostly affect carbon flux of plastidic lipid biosynthesis (Chanda et al. 2011; Miquel et al. 1998; Nandi et al. 2004). Because transgenic rice plants show enhanced levels of galactolipids that appear to be made from DAG synthesized by the ER pathway, it was important to observe SFD1 localization in rice cells. The SFD1 coding sequence was fused to that of the C-terminal of GFP in the pCXDG vector, to encode a single peptide (GFP-SFD1) and transiently expressed in rice protoplast. As a control we expressed free GFP (empty vector). As expected, the free GFP was distributed throughout the cell (Fig. 5). In contrast, GFP-SFD1was localized exclusively in the plastids of rice cells (Fig. 5). To rule out the possibility that GFP1-SFD1 was localized in plastid-associated ER, we examined the sub-cellular localization of GFP-SFD1 and an ER-tracker dye. In order to minimize autofluorescence of chlorophyll, we selected non-green tissues from rice leaf sheaths, and transiently expressed GFP-SFD1 or free GFP by the particle bombardment method. We observed a non-overlapping fluorescence of ER-tracker and GFP-SFD1 (Supplementary Fig. S5), suggesting that SFD1-GFP is not localized in ER of rice cells.

Photosynthetic efficiency is enhanced in the transgenic plants

The transgenic plants showed significant enhancement of plastidic lipid content. We hypothesized that increased lipid content might influence photosynthesis efficiency in transgenic plants. We analyzed photosynthetic efficiency of WT and transgenic plants in terms of carbon assimilation rate (von Caemmerer and Farquhar 1981) using an infrared gas analyzer (IRGA). As shown in the Fig. 6a, the photosynthetic efficiency of the transgenic plants was 32–40 % higher than that of the untransformed WT plants. Further, to investigate whether over-expression of SFD1/GLY1 caused any photosynthetic stress in rice, we measured fluorescence-based maximum quantum yield in transgenic plants. Increase in the fluorescence yield from dark adapted (F_0) to maximum fluorescence (F_m) by saturating light is a parameter of nutritional stress in plants (Parkhill et al. 2001). The ratio of F_v (F_m–F_0) to F_m (F_v/F_m) as determined by a pulse amplitude modulated (PAM) fluorometer, showed no difference between WT and transgenic plants (Fig. 6b), suggesting that the over-expression of SFD1/GLY1 or the hygromycin resistance gene did not cause photosynthetic stress in the transgenic plants.

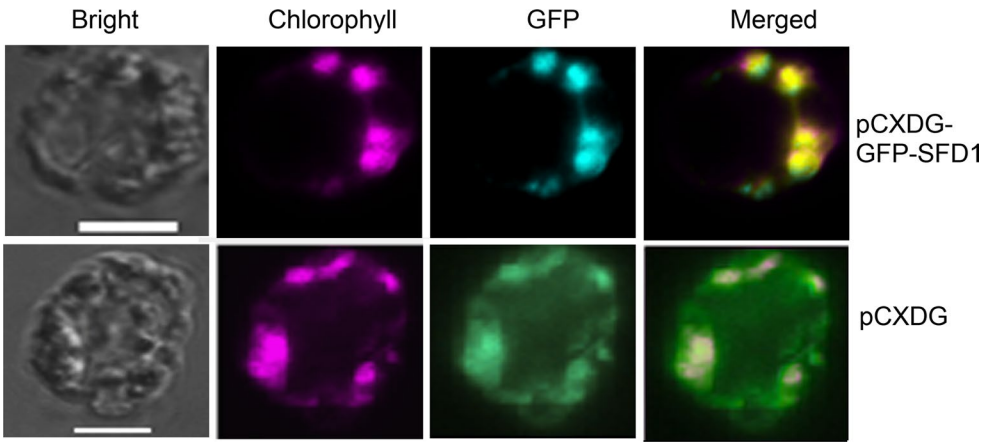

Fig. 5 Subcellular localization of GFP-SFD1 in rice protoplast. *Upper panel* represents protoplasts transfected with pCXDG-GFP-SFD1 construct, and the *lower panel* with the empty vector. The *scale bar* is of 5 µm length

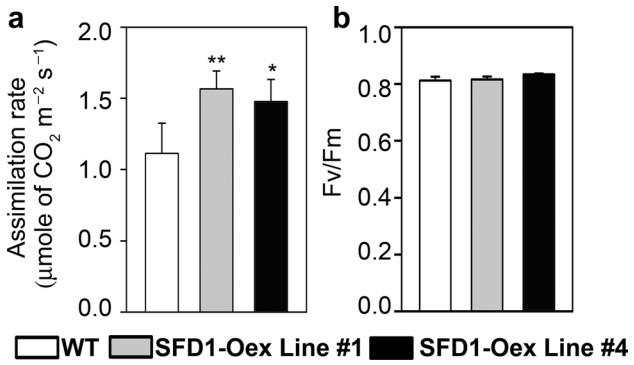

Fig. 6 Photosynthetic efficiency studies in WT and transgenic rice lines. **a** Photosynthetic efficiency as determined by IRGA. **b** Fv/Fm ratios as obtained by PAM fluorometer. *Each bar* indicates the average and standard deviations of 5 samples. * and ** *above* the *bars* indicate the significant difference (*t* test) with *P* values <0.05 and <0.1, respectively

Discussion

Over-expression of SFD1 increases total FA and lipid contents in rice. Influence of over-expression is mostly observed in the plastidic membrane lipids. Our findings suggest the G3pDH content poses a limitation to the overall lipid content in rice leaves. FA biosynthesis takes place exclusively in the plastids both in 16:3 and 18:3 plants (Browse et al. 1986; Ohlrogge and Browse 1995). In 18:3 plants like rice, only a small fraction of total FA gets incorporated into plastid lipids and the majority are exported out for ER route (Supplementary Fig. S6). Thus, in the 18:3 plants, plastidic FAs may result in a feedback inhibition unless transported out or consumed immediately. Having increased G3pDH in the plastid may reduce this feedback inhibition thereby increase the overall lipid content. The possible mechanism of such inhibition may be the conversion of FAs into PA during peak hours of FA biosynthesis, which eventually may convert back for transportation to ER. This hypothesis is supported by the observation of an overall increase in FA content in transgenic rice plants (Fig. 2a). The transgenic plants showed enhanced MGDG and DGDG with 18:3 FAs. Since, 18:3 plants like rice, lack PA-dephosphorylation activity, are unable to form DAG via the plastidic pathway (Heinz and Roughan 1983). Thus, increased levels of PA in the plastid will not directly convert to MGDG and DGDG. It is possible that in transgenic plants, the increased plastidic G3P is exported to ER, which in turn enhances the ER-pathway. The plastidic MGDG and DGDG are produced from DAG synthesized by the ER pathway. This hypothesis is further supported by the observation of FA compositions of galactolipids. Being an 18:3 plant, rice contains little amount of 16:0 FA-containing MGDG/DGDG, and is defective in desaturation of those16:0 FAs (Heinz and Roughan 1983; Ohlrogge and Browse 1995; Mongrand et al. 1998). Enhancement of plastidic pathway would have reflected in the enhancement of 34:6 MGDG/DGDG and overall 34-C lipids. In contrast, we did not observe any such enhancement in 34:6-MGDG/DGDG in transgenic plants (Fig. 4a, b). However, there was an increase in 34-C PG species (Fig. 4c). Thus, in the transgenic plants prokaryotic pathway contributed only the enhancement of PG molecules, whereas the eukaryotic pathway contributed MGDG and DGDG lipid species (Supplementary Fig. S6). Increased level of acidic lipids such as PA and PG is also likely to enhance MGDG biosynthesis, via activation of MGDG synthase enzyme MGD1 (Shimojima et al. 2013; Dubots et al. 2010). Enhancement of ER pathway is also likely to influence ER-resident lipids. However, in plants, plastidic lipids constitute more than 90 % of total cellular lipids (Holzl et al. 2009; Nandi et al. 2003, 2004). Thus, the effect of SFD1 over-expression on ER-lipids remained mostly at an inappreciable level, except for PC, which is the most abundant ER-lipid (Fig. 3c).

Our results in transgenic rice reflect a gain of function phenotype for *SFD1/GLY1*. In transgenic rice, there was a significant increase in the plastidic lipid content. Since plastidic lipids constitute the major fraction of all lipids, an increase in the plastidic component also led to an increase in the overall lipid content in the transgenic plants. Photosynthesis takes place in the thylakoid membrane of chloroplasts. About 75 mol % of thylakoid is composed of MGDG and DGDG (Benson 1971; Allen et al. 1970; Dormann and Benning 2002). PG and SQDG are the other components of the thylakoid membrane. Thylakoid assembly and maintenance depend on the availability of these lipid species. Besides providing a lipid matrix for photosynthesis, these lipids are also part of the photosynthetic complexes that take part in the process of conversion of light energy into chemical energy (Domonkos et al. 2008; Jordan et al. 2001; Jones 2007; Holzl et al. 2009). Thus, lipid composition greatly influences photosynthetic efficiency in plants. Our results suggest that enhancement of plastidic lipids positively influences photosynthesis efficiency in rice. Availability of G3p is also attributed towards restricting accumulation of triacylglycerols (TAGs) in oilseeds (Baud and Lepiniec 2010; Perry et al. 1999), which is supported by the observation of increased seed oil in transgenic rapeseed expressing yeast G3pDH. Enhancement of 3–4 fold G3pDH activity in developing seeds of transgenic *Brassica napus* leads to an increase of 40 % seed-oil content (Vigeolas et al. 2007). Altogether, our observations may find application in increasing the yield and quality of feed and fuel. Since the rice leaf tissues are major sources of fodder crops, increased lipid content in the leaves may be a source of increased energy for farm animals fed on haystacks. Similarly, the additional lipid content may lead to value-addition as a biofuel in the transgenic plants.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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