Synthesis and transfer of galactolipids in the chloroplast envelope membranes of *Arabidopsis thaliana*

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Supplemental Methods

Expression of DGDG Synthases in *E. coli.* The plasmid pACYC-31-DGD1 harbors the full-length DGD1 open reading frame (ORF) ligated into the *Bam*HI, *Pst*I sites of pACYC-31, a derivative of pACYC184 containing the expression cassette from pQE31 (Qiagen) (1). The vectors pQE31-DGD2 and pACYC-31-DGD2 harbor the DGD2 ORF in the *Bam*HI, *Kpn*I sites of pQE31 and pACYC-31, respectively (2). The NDGD1 sequence (M^{1} to E^{338}) was amplified from the 22-1 cDNA (primers Ben239, Ben294 introducing *Bam*HI, *Pst*I sites; Table S3) and the PCR fragment ligated into pACYC-31. The CDGD1 sequence (T^{339} to W^{808}) was amplified by PCR from cDNA 22-1 (primers Ben293, Ben241, adding *Bam*HI, *Pst*I sites) and the fragment ligated into pACYC-31. NDGD1 without stop codon was amplified by PCR from clone 22-1 (primers D12F, D12R introducing *Bam*HI sites) and ligated into the *Bam*HI site of the pQE31-DGD2 construct, 5' to and in frame with the DGD2 sequence (2). The fusion construct NDGD1DGD2 was released with *Bam*HI (partial digestion), *Kpn*I and ligated into pQE31 and pACYC-31. DGDG synthases in pQE31 were expressed in *E. coli* M15(pREP4) (Qiagen). Furthermore, *E. coli* XL1-Blue cells harboring the cucumber MGD1 cDNA in pGEX-3X (3) were transformed with pACYC-31 constructs containing the different DGDG synthase cDNAs (1). After induction of protein production with IPTG, lipids were extracted, separated by TLC and stained with α-naphthol.

Lipid Overlay, Liposome Binding and Liposome Fusion/Aggregation Assays

After expression in *E. coli*, DGD1, NDGD1 or CDGD1 proteins carrying Nus and His tags (pET43b vector) were extracted in the presence of 6 M guanidinium chloride and purified on a Ni^{2+} affinity column. Proteins in a soluble form were eluted with guanidinium-free imidazol buffer. Lipid binding with purified DGD1 and NDGD1 proteins was tested as described (4). For lipid overlay assays, nitrocellulose membranes containing spots of 5 µmol lipid were prepared using cardiolipin (from bovine heart, Sigma), PA, PC, PG (from egg yolk, Sigma), MGDG, DGDG (from

spinach, Larodan), di16:0-DAG (Sigma) and di18:3-DAG (Larodan). Protein binding was monitored by immunoblot analysis.

Multilamellar liposomes for liposome binding assays were generated from mixtures of PC (from soybean, Avanti) and PA (from egg yolk, Sigma) (4). Recombinant protein was added to the liposomes in a total volume of 50 µl, and bound and soluble protein separated by centrifugation. Proteins were observed by SDS polyacrylamide gel electrophoresis and Coomassie staining. The Nus protein (empty pET43b vector) was used as control.

Liposome aggregation assays were performed as described (5). Unilamellar vesicles containing 100 % PC or 75 % PC/25 % PA were prepared using an extruder (Avanti) with 100 nm mesh size. The vesicles were incubated with recombinant Nus (control), NDGD1 or CDGD1 proteins and changes in absorption (turbidity) at 350 nm recorded. For Fig. 4A, the initial absorption was set to 0, and final absorption after 300 sec was calculated as 100 % (NDGD1) and 0 % (CDGD1). For Fig. 4B, the turbidity measured after 300 sec with NDGD1 and 20 % and 0 % PA were set to 100 % and 0 %, respectively.

Nus or NDGD1 proteins were added to 200 nmol of liposomes (prepared by extrusion, see above) in a protein-to-lipid molar ratio of 1:500. After mixing and incubation on ice for 1 min, liposome fusion was monitored by differential interference contrast (DIC) light microscopy (Leica DMI4000 B) with a 20 x, 0.50 NA objective (Leica HCX PL FLUOTAR). The LAS V4.3 software (Leica) was used for imaging.

Overexpression of glycosyltransferases in *Arabidopsis*. Complementation of the *dgd1-1* mutant with the full-length *DGD1* cDNA (line R376) was described previously (1, 6). The NDGD1 sequence was amplified from the DGD1 cDNA 22-1 (primers PD1, PD3) and ligated into the *Bam*HI, *Pst*I sites of pBinAR (7). The CDGD1 part was amplified by PCR (primers PD2, Ben241, introducing a new start codon) and ligated into the *Bam*HI, *Pst*I sites of pBinAR. The coding sequence of the *DGD2* cDNA clone 16 (2) was released with *Bam*HI and *Xho*I (partial digestion) and ligated into the *Bam*HI, *Sal*I sites of pBinAR. The fusion sequence NDGD1DGD2 was released from pQE31-NDGD1DGD2 (see above) with *Bam*HI (partial digestion) and *Sal*I and ligated into pBINAR. Complementation of the *dgd1-1* mutant with the NM1GlcT construct in pCAMB35SOCS12 containing the *Chloroflexus* glucosyltransferase GlcT (chlo02003783, ZP_00356752, Caur_0652,

YP_001634281) fused behind the N-terminal signal sequence of the tobacco MGD1(amino acids 1 - 148) (8) was described previously (9). The CDGD1 sequence was amplified by PCR from the DGD1 cDNA 22-1 (primers PD835, PD836, introducing *Avr*II, *Asc*I sites) and ligated into pGEMTeasy. The chloroplast targeting sequence of tobacco MGD1 was obtained by *Bgl*II/*Avr*II digestion from the NM1GlcT vector (9). The NM1 fragment and the CDGD1 *Avr*II/*Asc*I fragment were ligated in one step into the *Mlu*I/*Bam*HI sites of p35OCS-BM (DNA Cloning Service, Hamburg). The entire cassette harboring the 35S promoter, the NMGD1CDGD1 sequence and the OCS terminator was released with *Sfi*I and ligated into pLH6000 (DNA Cloning Service). The DGD2 ORF was amplified from pQE31-DGD2 (2) by PCR (primers PD538, PD539, introducing *Avr*II, *Bam*HI sites) and cloned into pGEMTeasy. The *Chloroflexus* GlcT sequence was deleted from NM1GlcT in pCAMB35SOCS12 (9) and replaced with the DGD2 sequence released from pGEMTeasy with *Bln*I, *Bam*HI. The GlcT sequence (9) was amplified (primers PD367, PD368) and subcloned into the *BamH*I, *Xba*I sites of pBlueScriptSKII+ ("pBlueScript-GlcT"). The NDGD1 sequence was amplified from clone 22-1 (1) (primers D12F, D12R introducing *Bam*HI sites). This fragment was ligated into the *Bam*HI site, 5' to GlcT in pBlueScript-GlcT. The NDGD1GlcT sequence was released with *Bam*HI (partial digestion), *Xba*I, and cloned into the *Bam*HI, *Xba*I sites of pBinAR-Hyg (7). The N-terminal region of the DGD2 cDNA (ND2, amino acids 1 - 125) was amplified (primers PD369, PD370, introducing *Bam*HI sites). The ND2 fragment was ligated into the *Bam*HI site of pBlueScript-GlcT (see above) and the ND2GlcT sequence ligated into the *Kpn*I, *Xba*I sites of pBinAR-Hyg.

RNA-Extraction and semiquantitative RT-PCR

Arabidopsis thaliana (Col2) was germinated in Petri dishes containing solidified medium (MS salts, 2% w/v sucrose, 0.9% w/v agar) for 2 weeks before transfer to phosphate-deficient medium (10, 11). Plants were transferred to Petri dishes containing 1 mM or no phosphate and grown for an additional period of 8 days as described. Total RNA was extracted from 50-100 mg of leaf tissue, DNase treated, and employed for cDNA synthesis. RT-PCR was performed using the following primer pairs: bn2670/bn2671 (full length DGD1, At3g11670.1), bn2670/bn2672 (DGD1 splicing variant, At3g11670.2), bn2673/bn2674 (genomic DNA of DGD1) and bn2557/bn2558 (actin).

Tables S1-S3

Table S1. Galactolipid content and fatty acid composition of *Arabidopsis dgd1-1* plants expressing different DGDG synthase fusion proteins

	WT	*dgd1-1*	*dgd1-1* DGD1	*dgd1-1* CDGD1	*dgd1-1* DGD2	*dgd1-1* NDGD1 DGD2	*dgd1-1* NM1 CDGD1	*dgd1-1* NM1 DGD2
					MGDG			
16:0	0.7	5.1	1.8	3.7	3.1	1.6	1.4	0.9
16:1	0.4	3.7	1.2	1.2	1.0	0.7	0.5	0.5
16:2	0.9	0.6	0.0	0.3	1.0	0.6	0.5	0.7
16:3	37.3	15.6	34.0	12.2	14.1	21.8	14.1	23.1
18:0	0.2	0.6	0.3	0.2	0.2	0.1	0.2	0.1
18:1	0.1	1.0	0.0	0.0	0.0	0.0	0.2	0.4
18:2	1.7	2.6	2.1	1.8	2.1	1.6	0.9	0.9
18:3	58.6	70.6	60.7	80.6	78.5	73.3	82.2	73.5
					DGDG			
16:0	12.1	25.5	12.0	24.0	24.3	2.8	11.1	4.4
16:1	0.3	4.5	1.8	8.4	5.8	1.6	3.7	0.6
16:2	0.6	1.7	0.2	0.0	2.0	0.4	0.7	0.5
16:3	3.4	3.5	2.6	5.8	2.8	12.5	5.7	12.6
18:0	0.8	3.8	0.8	1.8	5.3	0.3	1.5	0.0
18:1	0.6	5.0	1.1	1.9	9.7	0.6	1.3	0.7
18:2	4.1	9.0	3.5	5.1	6.9	1.1	6.3	2.2
18:3	77.8	47.2	78.0	52.9	43.2	80.3	69.6	79.0

Lipid composition was measured in leaves after separation by TLC and quantification of fatty acid methyl esters by GC. Data represent mean of three measurements and are given in mol%. SD was always < 3 mol%.

Table S2. Fatty acid composition of glycolipids isolated from *Arabidopsis dgd1* plants expressing different GlcT (*Chloroflexus)* fusion proteins.

	WT	*dgd1-1*	*dgd1-1* NDGD1GlcT	*dgd1-1* ND2GlcT	*dgd1-1* NM1GlcT
			MGDG		
16:0	0.7	6.9	2.2	1.3	3.6
16:1	0.4	5.9	0.7	1.5	1.0
16:2	0.9	0.5	0.9	0.5	0.1
16:3	37.3	14.9	20.1	17.2	22.2
18:0	0.2	2.2	1.1	0.2	0.3
18:1	0.1	0.9	0.3	0.4	0.8
18:2	1.7	0.5	1.4	1.2	1.9
18:3	58.6	68.2	73.7	77.6	70.1
			DGDG		
16:0	12.1	25.5	29.7	24.7	24.4
16:1	0.3	4.5	4.0	9.5	0.8
16:2	0.6	1.7	1.7	1.4	0.1
16:3	3.4	3.5	9.1	4.4	5.7
18:0	0.8	3.8	3.8	5.3	3.7
18:1	0.6	5.0	3.4	3.3	1.6
18:2	4.1	9.0	10.6	6.8	12.5
18:3	77.8	47.2	37.6	44.7	51.0
			GlcGalDG		
16:0	-	-	2.8	2.8	3.7
16:1	-	-	0.9	0.9	0.5
16:2	-	-	0.9	0.9	0.1
16:3	-	-	16.9	16.9	19.0
18:0	-	-	0.7	0.7	0.4
18:1	-	-	0.3	0.3	0.4
18:2	-	-	1.0	1.0	1.6
18:3	-	-	76.1	76.1	74.3

Lipid composition was measured in leaves after separation by TLC and quantification of fatty acid methyl esters by GC. Data are mean of three measurements and are given in mol%. SD was always < 3 mol%. Data for *dgd1-1*-NM1GlcT are from (9).

Table S3: Oligonucleotides used in this study.

Primer	Function		Sequence (5'-3')
Ben239	NDGD1 expression in	BamHI	GCGGATCCGGTAAAGGAAACTCTAATT
Ben294	pACYC-31	PstI	TCCTGCAGTAGGCTTCACAAAATCAGT
Ben293	CDGD1 expression in	BamHI	ATGGATCCGGAGTACACCGGAAAACAAA
Ben241	pACYC-31	PstI	TTCTGCAGTCTACCAGCCGAAGATTGG
D12F	NDGD1DGD2 expression	BamHI	CACGGATCCCATGGTAAAGGAAACTC
D12R	in pACYC-31, pBINAR	BamHI	CACGGATCCACAGGCTTCACAAAATC
PD1	NDGD1 expression	BamHI	CCGGATCCCATGGTAAAGGAAACTCTA
PD3	in pBINAR	PstI	GGCTGCAGCTAAGGCTTCACAAAATCAGT
PD2	CDGD1 expression	BamHI	TCGGATCCATGGAGACACCGGAAAACAAA
Ben241	in pBINAR	PstI	see above
PD835	NMGD1CDGD1 expression	AvrII	– ATCCTAGGTGAGACACCGGAAAACAAAAGG
PD836	in pLH6000	AscI	GGCGCGCCCTACCAGCCGAAGATTGGCT
PD538	NMGD1DGD2 expression	AvrII	ATCCTAGGTATGACGAATCAGCAGGAGCA
PD539	in pCAMB35SOCS12	BamHI	CGCGGATCCTCAATCTTGCTTGCGAGTAT
PD367	NDGD1GlcT expresssion	BamHI	AGTGGATCCGATGCCGGTGTTAATCTTG
PD368	in pBINARHyg	XbaI	ACT TCTAGACTTAGTCATGGCGGTGACTCT
PD369	NDGD2GlcT expression	BamHI	CACGGATCCCATGACGAATCAGCAGGAGCA
PD370	in pBINARHyg	BamHI	CACGGATCCACCTCGAGGACAGCAATG TC
PAK62	Cloning of Nus-Tag	NdeI	GGAATTGTGAGCGGATAACAATTCC
PAK63	into pET43b-DGD1	SacII	CTCCGCGGAACCACTAGTCGCTTCGTCACCGAAC
PAK84	Cloning of 3' His-Tag	NdeI	CAATCTTCGGCTGGAGACTGCAGGAATTC G
PAK85	into pET43b-DGD1	SacII	C GAATTCCTGCAGTCTCCAGCCGAAGATTG
PAK15	Cloning of NDGD1	BamHI	CATACGGATCCGGTAAAGGAAACTC
PAK82	into pET43b-NDGD1	PstI	GCCTGCAGAGCCTTCACAAAATCAGTCC
bn1310	Cloning of CDGD1	BamHI	AGGATCCGACACCGGAAAACAAAAGGC
bn1311	into pET43b-CDGD1	PstI	TTCTGCAGCCAGCCGAAGATTGGC
bn2670	DGD1 exon 6 forward		GAAGAGAGATCCCGTGGTG
bn2671	DGD1 exon 7 reverse		AAACTTCCCCATGGCTAGTG
bn2672	DGD1 intron 6 forward		CAAGATGTGGGAAAGACAATC
bn2673	DGD1 intron 5 forward		TTGTTCTGTTGCTTGAATCCTC
bn2674	DGD1 exon 6 reverse		AGCGTGGTCCCTTCCTTTG
bn2557	Actin forward		GCCATCCAAGCTGTTCTCTC
bn2558	Actin reverse		GAACCACCGATCCAGACACT

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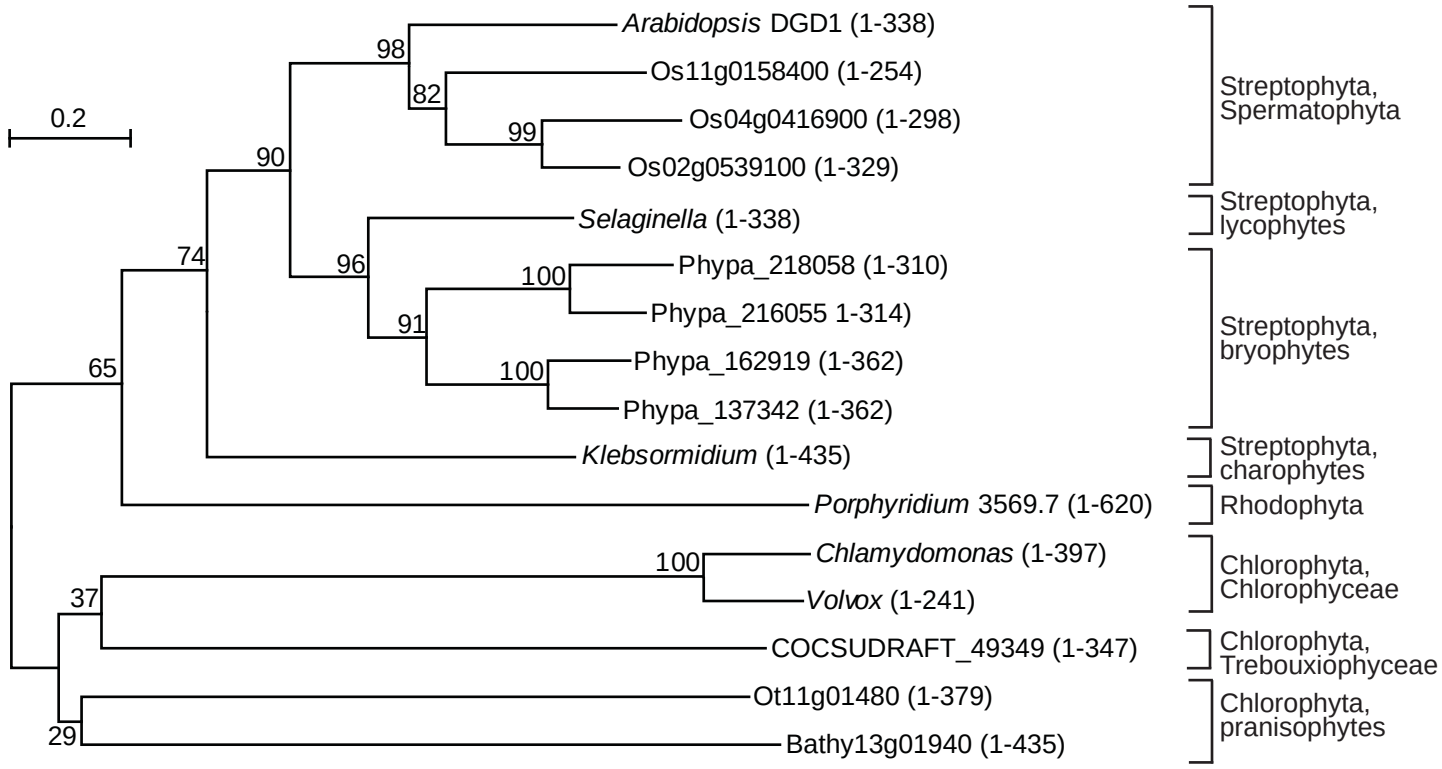

Fig. S1. Phylogenetic analysis of NDGD1 amino acid sequences.
An unrooted phylogenetic tree was constructed employing the neighbor-joining method with NDGD1 amino acid sequences of DGDG synthases from *Arabidopsis thaliana* (DGD1, At3g11670), *Oryza sativa* (DGD1, Os02g0539100, Os04g0416900, Os04g0416900), *Selaginella moellendorffii* (SELMODRAFT_130585, XP_002989787), *Physcomitrella patens* (Phypa_218058, XP_001771288; Phypa_216055, XP_001770278; Phypa_162919, XP_001761588; Phypa_137342, XP_001772591), *Klebsormidium flaccidum* (kfl00531_0060), *Chlamydomonas reinhardtii* (Cre13.g583600), *Volvox carteri f nagariensis* (VOLCADRAFT_116955, XP_002948730), *Coccomyxa subellipsoidea* (COCSUDRAFT_49349, XP_005642814), *Ostreococcus tauri* (Ot11g01480, XP_003081950), *Bathycoccus prasinos* (Bathy13g01940, XP_007509487), *Porphyridium purpureum* (evm.model.contig_3569.7). Numbers in brackets indicate amino acid positions of the sequence parts used for alignment. In the x-dimension, branch length represents evolutionary distance based on the number of amino acid differences per site.

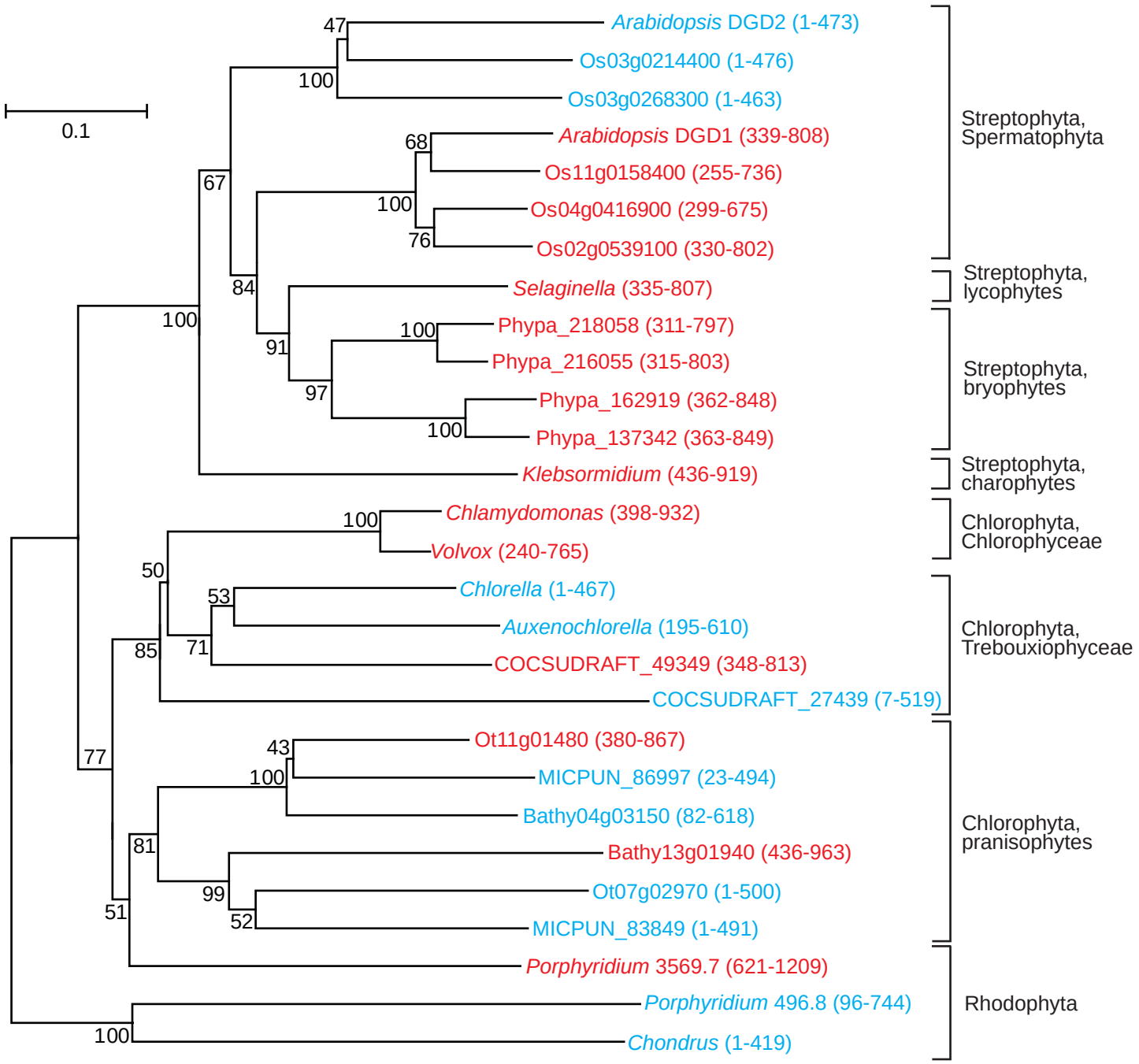

Fig. S2. Phylogenetic analysis of CDGD1 and DGD2 amino acid sequences.
An unrooted phylogenetic tree was constructed employing the neighbor-joining method with CDGD1 and DGD2 amino acid sequences. Sequences are from *Arabidopsis thaliana* (DGD1, At3g11670; DGD2, At4g00550), *Oryza sativa* (DGD1, Os02g0539100, Os04g0416900, Os04g0416900; DGD2, Os03g0214400, Os03g0268300), *Selaginella moellendorffii* (SELMODRAFT_130585, XP_002989787), Physcomitrella patens (Phypa_218058, XP_001771288; Phypa_216055, XP_001770278; Phypa_162919, XP_001761588; Phypa_137342, XP_001772591), *Klebsormidium flaccidum* (kfl00531_0060), *Chlamydomonas reinhardtii* (Cre13.g583600), *Volvox carteri f nagariensis* (VOLCADRAFT_116955, XP_002948730), *Coccomyxa subellipsoidea* (COCSUDRAFT_49349, XP_005642814; COCSUDRAFT_27439, XP_005650356), *Ostreococcus tauri* (Ot11g01480, XP_003081950; Ot07g02970, XP_003080403), *Bathycoccus prasinos* (Bathy13g01940, XP_007509487; Bathy04g03150, XP_007513661), *Chlorella variabilis* (CHLNCDRAFT_31413, XP_005846865), Auxenochlorella protothecoides (F751_0628, KFM26760), Micromonas sp. (MICPUN_86997, XP_002509215; MICPUN_83849, XP_002503614), *Porphyridium purpureum* (evm.model.contig_3569.7; evm.model.contig_496.8) and *Chondrus crispus* (CHC_T00008344001, XP_005719327). Numbers in brackets indicate amino acid positions of the sequence parts used for alignment. In the x-dimension, branch length represents evolutionary distance based on the number of amino acid differences per site. Numbers in brackets indicate amino acid positions of the sequence parts used for alignment. In the x-dimension, branch length represents evolutionary distance based on the number of amino acid differences per site. Species names depicted in red carry a long NDGD1 extension ("DGD1 type"), and blue indicates sequences lacking a long extension ("DGD2 type").

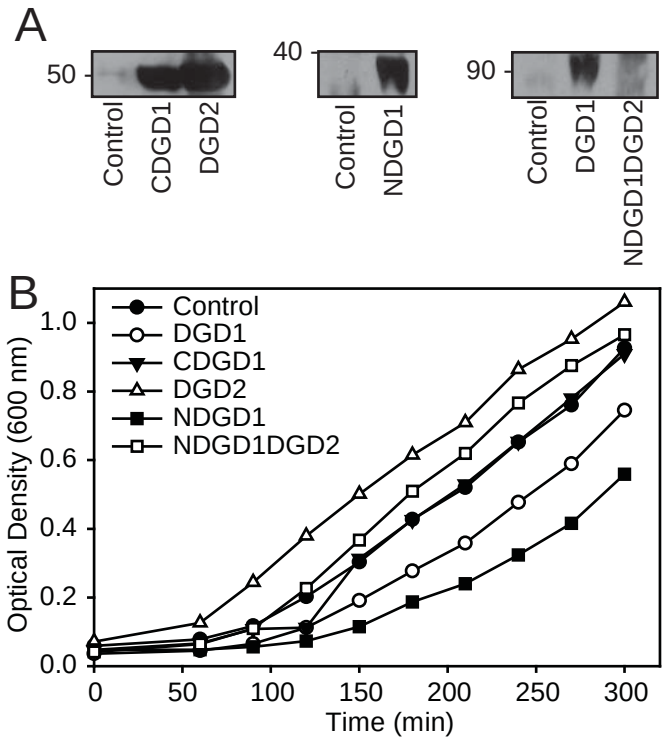

Fig. S3. DGDG synthesis in *E. coli* expressing cucumber MGDG synthase (MGD1) and different DGDG synthases.
(A) Accumulation of DGD1 and DGD2 polypeptides after expression of pQE31 constructs in *E. coli*. DGD1 and DGD2 cDNAs in pQE31 were expressed in *E. coli* and protein extracts used for immunoblot analysis with the His detector peroxidase kit. Because of the very strong expression of CDGD1 and DGD2, and the lower expression of NDGD1, DGD1, NDGD1DGD2, the blots with CDGD1 and DGD2 were exposed to X-ray films for a shorter time than those with NDGD1, DGD1 and NDGD1DGD2. Numbers indicate protein sizes in kDa (marker proteins).
(B) Growth curves of *E. coli* cells expressing cucumber MGD1 (in pGEX-3X) along with different *Arabidopsis* DGDG synthase constructs (in pACYC-31). One representative experiment is shown.

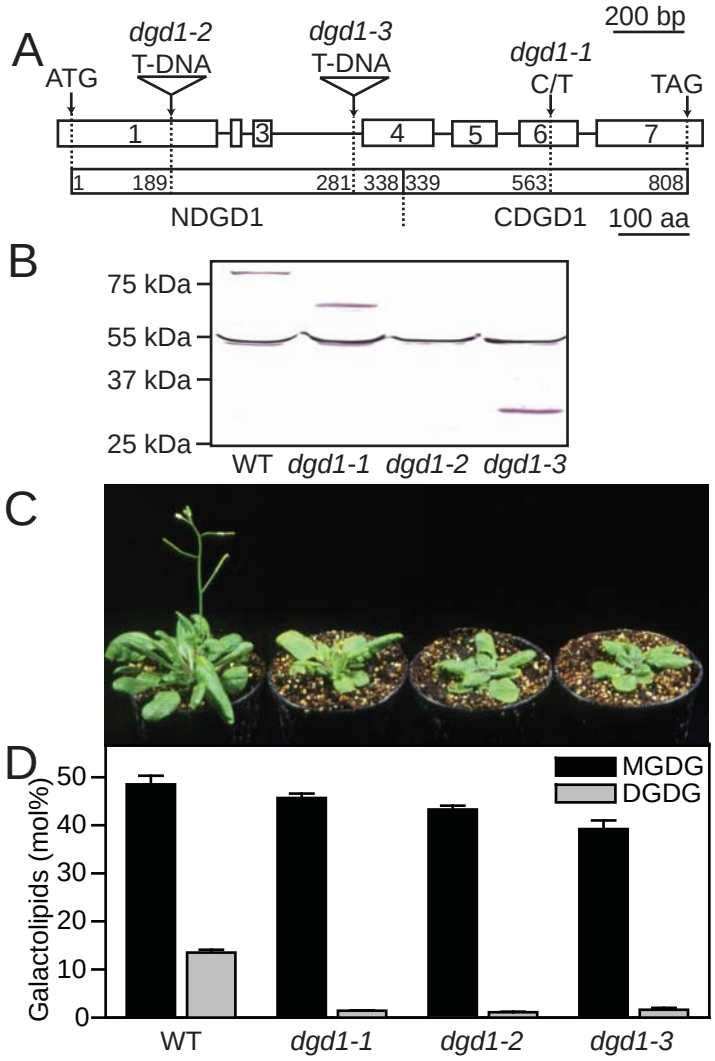

Fig. S4. Loss-of-function mutant alleles of *dgd1*.
(A) Exon-intron structure of the DGD1 gene. The point mutation C/T introducing a premature stop codon in *dgd1-1* (Dörmann et al., 1995), and the positions of T-DNA insertions in the mutants *dgd1-2* and *dgd1-3*, are indicated by arrows and amino acid positions.
(B) Accumulation of DGD1 protein in *Arabidopsis dgd1* plants as revealed by immunoblots using anti-NDGD1 antibodies (against a synthetic peptide of amino acids 159 - 172 in exon 1). DGD1, amino acids 1 - 808, 91.8 kDa; *dgd1-1*, 1 - 563, 64.1 kDa; *dgd1-2*, 1 - 189, 21.1 kDa (not detectable); *dgd1-3*, 1 - 273, 30.9 kDa. Note the unspecific crossreaction with a protein at 55 kDa.
(C) Growth of the Arabidopsis mutants *dgd1-1*, *dgd1-2* and *dgd1-3*.
(D) MGDG and DGDG contents in *Arabidopsis dgd1* mutant plants. Lipids were isolated by TLC and quantified by GC of fatty acid methyl esters. Values indicate mean and SD of three measurements from separate plants.

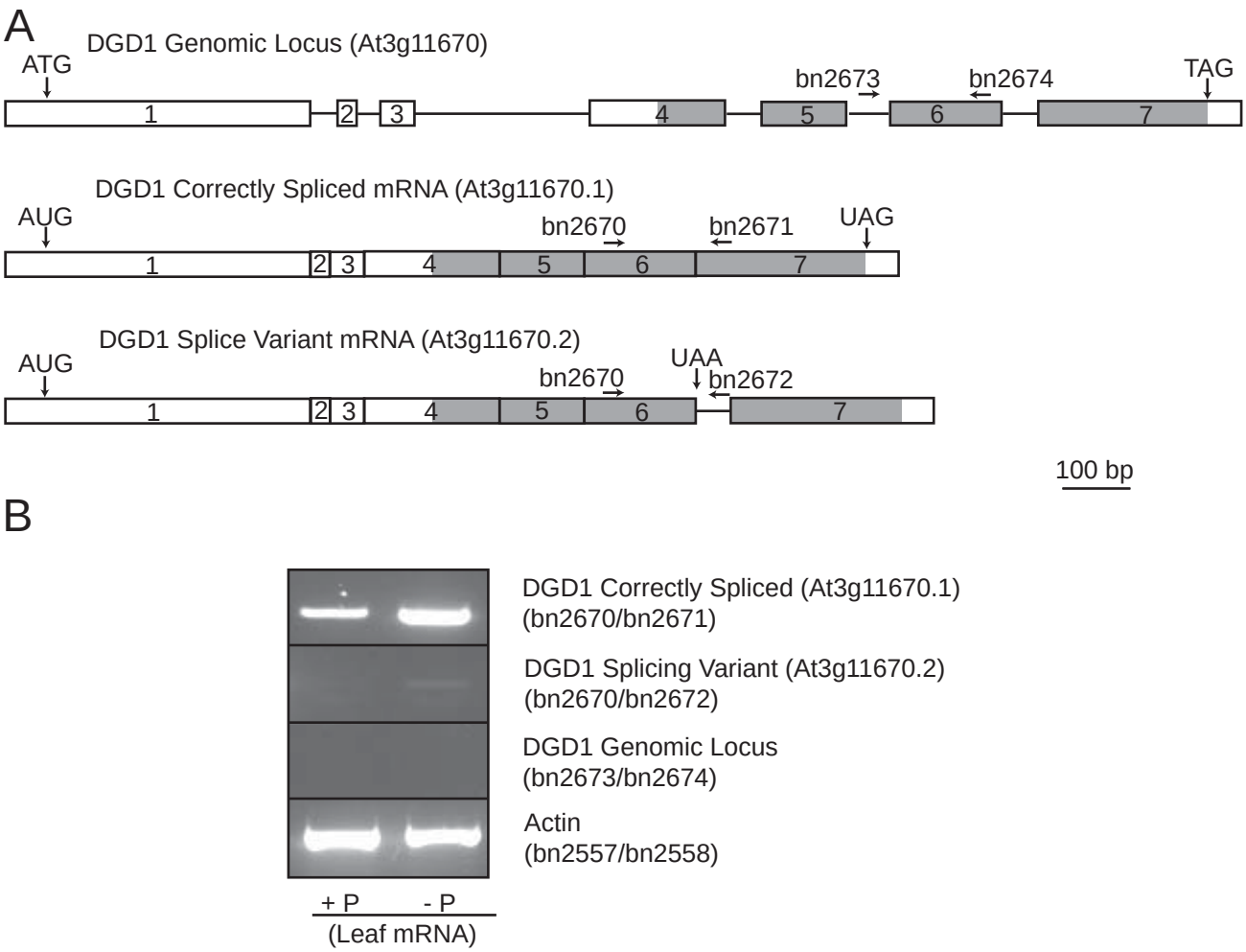

Fig. S5. The splice variant of DGD1.
(A) In addition to the correctly spliced DGD1 mRNA (At3g11670.1), a splice variant containing intron 6 (At3g11670.1) was annotated in the Genbank database. The lack of splicing at the exon 6/intron 6 boarder results in a longer DGD1 mRNA containing a premature UAA stop codon in the middle of the glycosyltransferase domain. Therefore, the corresponding polypeptide presumably is inactive. Open boxes, NDGD1 coding sequence; grey boxes, CDGD1 coding sequence.
(B) DGD1 transcript abundance of DGD1 is upregulated under phosphate deprivation. Semi-quantitative rt-PCR using primers for the amplification of At3g11670.1 shows a strong induction in leaves of plants grown without phosphate. RT-PCR with primers specific for the splice variant At3g11670.2 reveals much weaker bands, as the mRNA is not detectable under +P conditions, and only very weak at -P conditions.

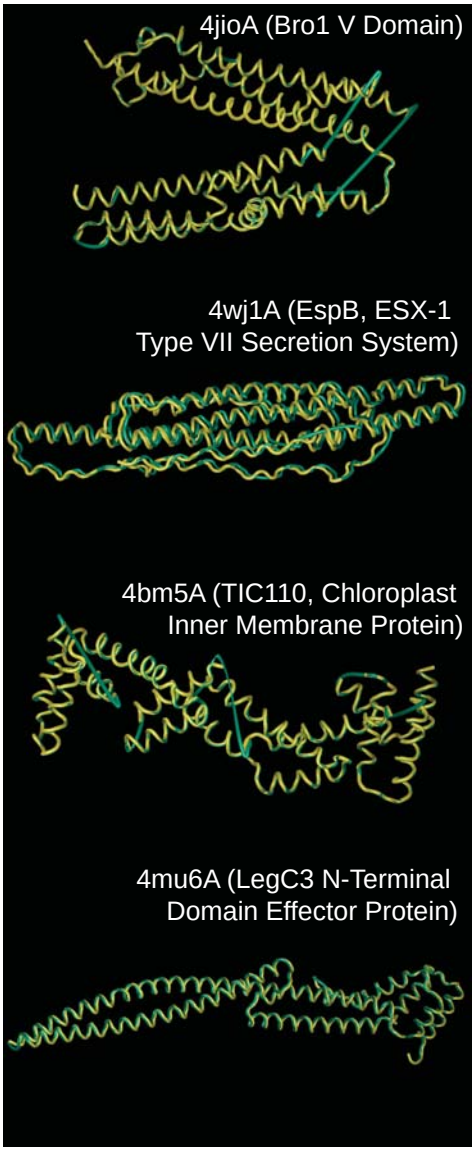

Fig. S6. Threading templates used for NDGD1.
The Arabidopsis NDGD1 sequence (green) was used for structure prediction following the I-TASSER algorithm. The top 4 threading templates are shown (yellow); Bro1 V domain (4jioA); EspB from the ESX-1 type VII secretion system (4wj1A) from Mycobacterium tuberculosis; chloroplast inner membrane protein TIC110 (4bm5A); N-terminal domain of effector protein LegC3 (4mu6A) from Legionella pneumophila. The Z-scores (Z-score > 1 indicates good alignment) with 4jioA, 4wj1A, 4bm5A and 4mu6A are 1.21, 1.39, 1.18 and 1.38, respectively.

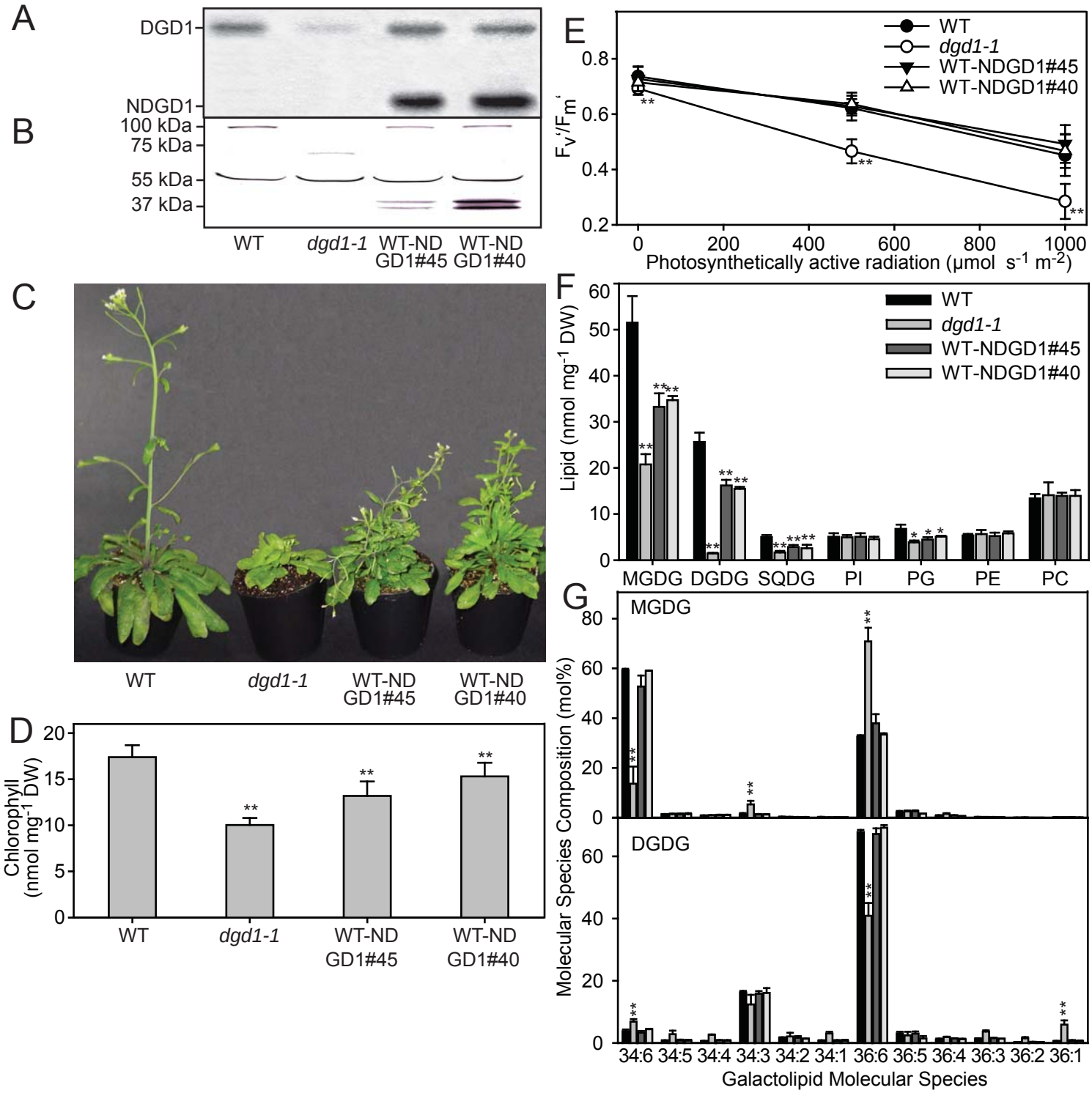

Fig. S7. Overexpression of NDGD1 in *Arabidopsis* wild type affects galactolipid accumulation and morphology of transgenic plants.
NDGD1 was overexpressed under control of the CaMV 35S promoter.
(A) Expression of NDGD1 recorded by Northern blot hybridization.
(B) Immunoblot analysis of NDGD1 overexpressing plants with anti-NDGD1 antibodies. DGD1, amino acids 1 - 808, 91.8 kDa; dgd1-1, 1 - 563, 64.1 kDa; NDGD1, 1 - 338, 38.3 kDa. Note the unspecific crossreaction with a 55 kDa protein.
(C) Growth of *Arabidopsis* plants overexpressing NDGD1. WT, dgd1-1 and two independent lines (WT-NDGD1#45, WT-NDGD1#40) were grown on soil for 35 days.
(D) Chlorophyll content in transgenic NDGD1 overexpressing lines. Chlorophyll in leaf extracts was measured photometrically.
(E) Photosynthetic quantum yield Fv'/Fm' in NDGD1 overexpressing plants measured by chlorophyll fluorescence.
(F) Glycerolipid content and (G) molecular species composition of MGDG and DGDG in WT, *dgd1-1* and plants overexpressing NDGD1. Lipids were quantified by Q-TOF MS/MS.
Data in (D)-(G) show mean and SD of the measurements of 5 different plants. Values significantly different to WT (*, $p < 0.05$; **, $p < 0.01$, Student t-test).

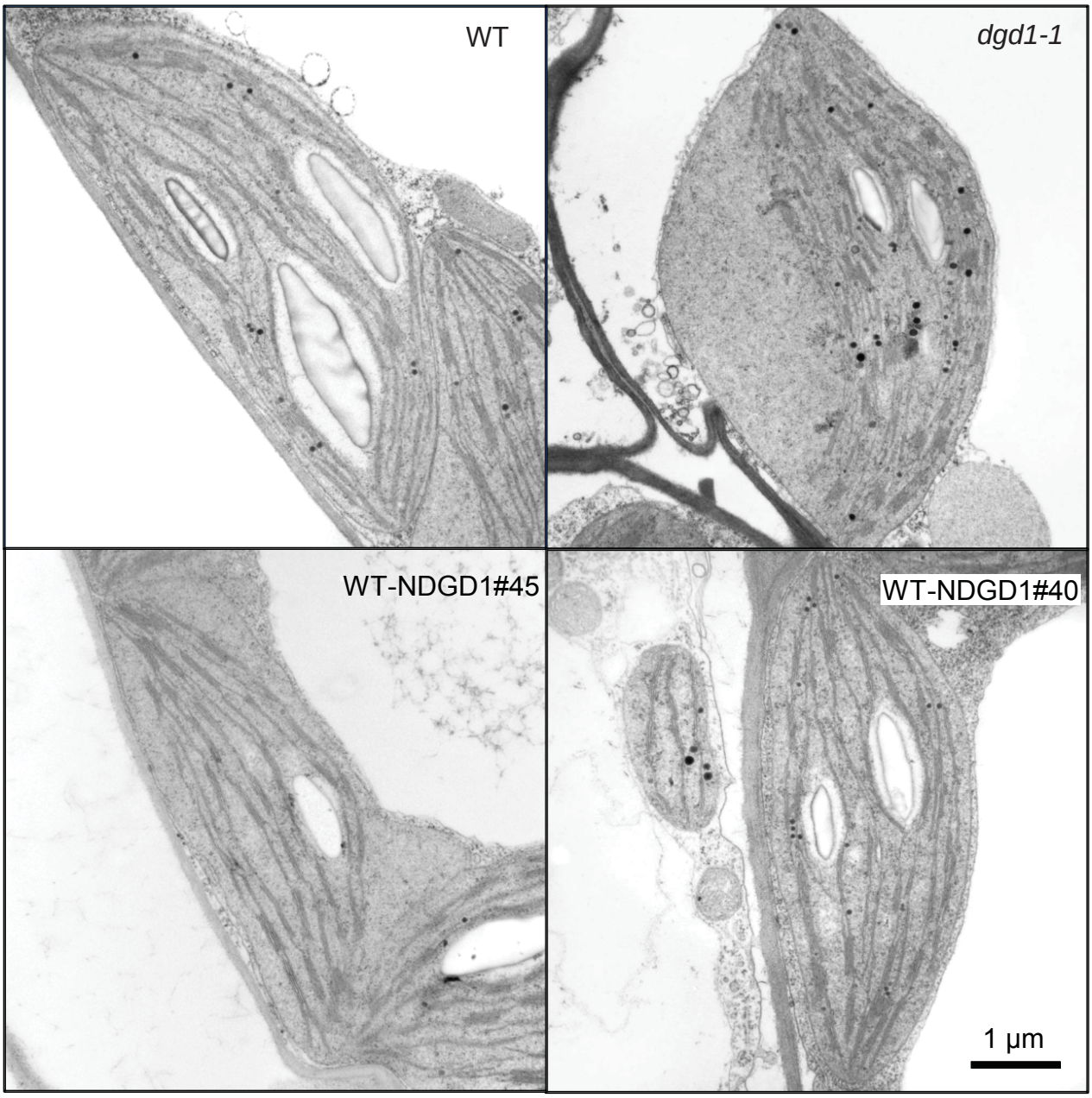

Fig. S8. Chloroplast ultrastructure of Arabidopsis WT plants overexpressing NDGD1 under control of the CamV35S promoter.
Two transgenic plants, WT-NDGD1#45 and WT-NDGD1#40 were selected by Northern hybridization. Chloroplasts of WT, *dgd1-1*, WT-NDGD1#45 and WT-NDGD1#40 were analyzed by electron microscopy of leaf ultrathin sections. While *dgd1-1* chloroplasts show large thylakoid-free stroma areas and differences in thylakoid structure, the thylakoid and envelope structures of WT-NDGD1#45 and WT-NDGD1#40 are very similar as WT.