

**The novel protein DELAYED PALE-GREENING1 is required
for early chloroplast biogenesis in *Arabidopsis thaliana***

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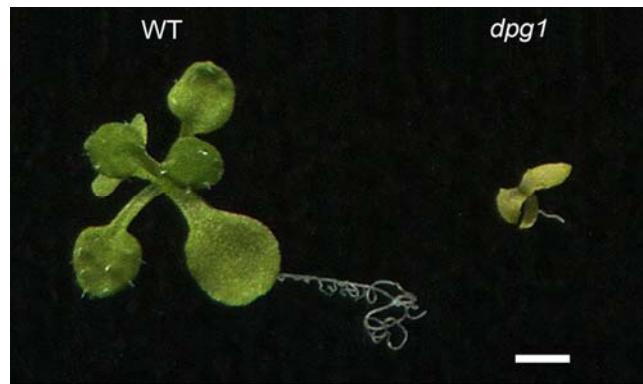
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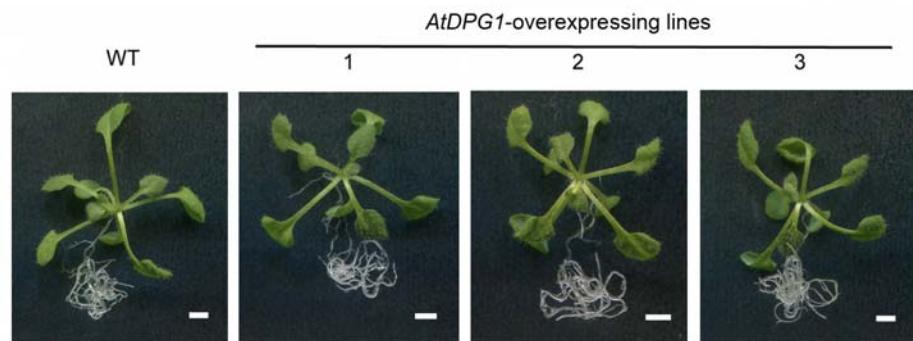
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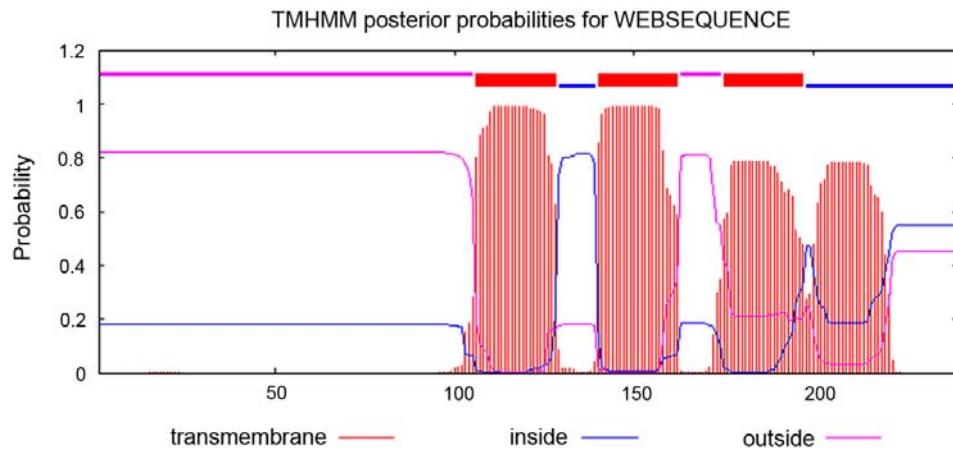
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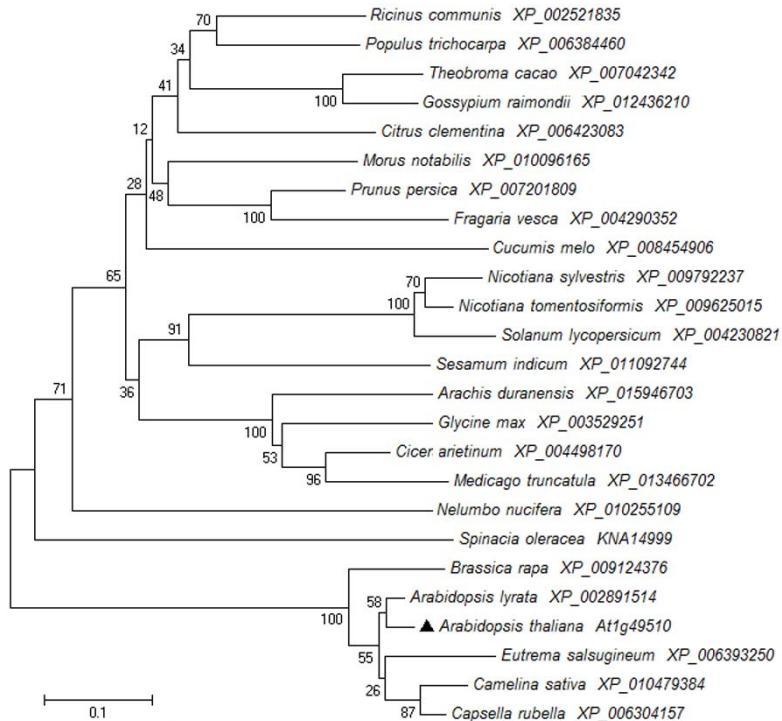
Supplementary Figure S1. Phenotypes of the wild type (WT) and *dpg1* mutant grown on 1/2 MS medium without sucrose for 28 days.



Supplementary Figure S2. Phenotypes of wild-type (WT) and *AtDPG1*-overexpressing lines grown on 1/2 MS medium for 21 days. Scale bar = 2 mm.



Supplementary Figure S3. Presence of transmembrane domain was calculated using TMHMM 2.0 Server. Red colour represents the transmembrane helices. The prediction indicates the presence of 3 domains in AtDPG1.



Supplementary Figure S4. A phylogenetic analysis of putative DPG1 homologues using MEGA 5.1 and a neighbor-joining method. A bootstrap analysis was performed with 1 000 replicates. Numbers in branches indicate bootstrap values (%).

Supplementary Table S1. Decreased fertility in the *dpg1* mutant

	WT	<i>dpg1</i>
Total number of siliques per plant	157.2±31.5	25.3±7.2*
Total number of seeds per silique	54.5±3.1	12.1±2.4*

Each value is the mean±SE of three independent determinations and asterisk indicates significant differences (Student's *t* test: *P<0.05)

Supplementary Table S2. List of primers used in this study

Primer name	Primer sequence (5'-3')	Function
<i>LBb1.3</i>	ATTTGCCGATTCCGGAAC	
<i>DPG1-LP</i>	ACGAGTCCGACGTTGTGATAC	Identifying <i>dpg1</i> mutant by PCR
<i>DPG1-RP</i>	TCCCAAACATGTTGGTTCTC	
<i>PDPG1-F</i>	CCCAAGCTTATTGGTAACTATCAAAGCG	Amplification of <i>AtDPG1</i> promoter
<i>PDPG1-R</i>	CATGCCATGGTTATTAAACTCTCTGT	
<i>OEDGY1-F</i>	CATGCCATGGCGATGTCTCATCTCTTCTT	Amplification of <i>AtDPG1</i> coding region
<i>OEDGY1-R</i>	GATGGGTAACCTCATTCTGTTCCAAACTG	
<i>AtDPG1-F1</i>	ACGATTCTCTCTCTCATTAGGC	Identifying <i>dpg1</i> mutant by semi-quantitative PCR
<i>AtDPG1-R1</i>	ACAATCATGGGTTGGTTCTTACTT	
<i>AtDPG1-F2</i>	TCTCTTCCTTCTTCGCCCTC	Identifying 35S:: <i>AtDPG1</i> transgenic lines by semi-quantitative PCR
<i>AtDPG1-R2</i>	CTTAAGACGAGGCCGAGATCTTCA	
<i>ACTIN2-F</i>	CAAACGAGGGCTGGAACAAGACT	Semi-quantitative PCR analysis of <i>ACTIN2</i> gene
<i>ACTIN2-R</i>	GCAACTGGGATGATATGGAAAAGA	
<i>rAtDPG1-F</i>	TCTCTTCCTTCTTCGCCCTC	Quantitative PCR analysis of <i>AtDPG1</i> gene
<i>rAtDPG1-R</i>	TCCCAAACATGTTGGTTCTC	
<i>rPSAA-F</i>	TCCTGAATGGAGATGTGGCG	Quantitative PCR analysis of <i>PSAA</i> gene
<i>rPSAA-R</i>	TAAGGCTCGAAGACCAATGC	
<i>rPSAB-F</i>	GTATTACCGCATCCCCAAGGG	Quantitative PCR analysis of <i>PSAB</i> gene
<i>rPSAB-R</i>	GGGTTAGAATGGCAGTCCGG	
<i>rPSBA-F</i>	TGAATGGCTATAAACCGGCG	Quantitative PCR analysis of <i>PSBA</i> gene
<i>rPSBA-R</i>	GAAAACAGCAGTCGCAGCTGC	
<i>rPSBB-F</i>	CGTTGGGTATATTAGCGGGCC	Quantitative PCR analysis of <i>PSBB</i> gene
<i>rPSBB-R</i>	AGGACGGTTCAATATTGCC	
<i>rRBCL-F</i>	CCTGGAGTTCCACCTGAAGAAC	Quantitative PCR analysis of <i>RBCL</i> gene
<i>rRBCL-R</i>	TGGTAGCATCGTCCTTGTAACG	
<i>rrpoA-F</i>	ACGCGCTTATTGTGTCCAAGG	Quantitative PCR analysis of <i>rpoA</i> gene
<i>rrpoA -R</i>	GACTATATCCCGCGATTCCCTCTC	
<i>rrpoB-F</i>	ATTGGATCATAATGGGATTTCG	Quantitative PCR analysis of <i>rpoB</i> gene
<i>rrpoB -R</i>	CTCGTAGATTCAAACCCATAGC	
<i>rrpoC1-F</i>	CTATAAAGAGTGGAAATTGCGC	Quantitative PCR analysis of <i>rpoC1</i> gene
<i>rrpoC1 -R</i>	CCCATTGATATCTCGTATCC	
<i>rrpoC2-F</i>	AACGATACCTCTAAGGGCTGG	Quantitative PCR analysis of <i>rpoC2</i> gene
<i>rrpoC2 -R</i>	ACCATATCTCAATAGATTGGCG	
<i>rHEMA1-F</i>	TAATGGGGTTCGTGTTCTCCG	Quantitative PCR analysis of <i>HEMA1</i> gene
<i>rHEMA1-R</i>	ATGCTAGCTGCATTAGACGCAG	
<i>rCHLH-F</i>	TGGTAGAGAGACAGAAAGCTCGAAA	Quantitative PCR analysis of <i>CHLH</i> gene
<i>rCHLH-R</i>	CCAAAGAACCTGCCAAGAG	
<i>rCHL27-F</i>	TCAAGACCGATTACAACCAGACA	Quantitative PCR analysis of <i>CHL27</i> gene
<i>rCHL27-R</i>	CGCTCAAGGAACTAACGAG	
<i>rCHLP-F</i>	ACCAGAAACAGAGCTAAGGACAAGA	Quantitative PCR analysis of

<i>rCHLP-R</i>	GCATCACCTACAAGAGGCCACAC	<i>CHLP</i> gene
<i>rPORB-F</i>	CAAACCGCTGCGACTTCAAGC	Quantitative PCR analysis of <i>PORB</i> gene
<i>rPORB-R</i>	TGCACGCCATTATCACGTTCC	Quantitative PCR analysis of <i>PORC</i> gene
<i>rPORC-F</i>	CAGACAGTTACAGGCCACGCCG	Quantitative PCR analysis of <i>CAO</i> gene
<i>rPORC-R</i>	TGTCTGCTAAAGCTTGGCCG	Quantitative PCR analysis of <i>CLA1</i> gene
<i>rCAO-F</i>	TAGGGGTGAAGACGGGAAACC	Quantitative PCR analysis of <i>OE23</i> gene
<i>rCAO-R</i>	CTCCATCGGTTGAGTATTCCC	Quantitative PCR analysis of <i>OE33</i> gene
<i>rCLA1-F</i>	GGTGGAACCGGGTTAAATCTC	Quantitative PCR analysis of <i>PSAN</i> gene
<i>rCLA1-R</i>	TGCACAGAAGGGTTAACGCC	Quantitative PCR analysis of <i>PSBW</i> gene
<i>rOE23-F</i>	GTATCTCCTGCTGATGCCGCC	Quantitative PCR analysis of <i>LHCB1</i> gene
<i>rOE23-R</i>	TGGAACCTGCACTTGAACCC	Quantitative PCR analysis of <i>LHCA4</i> gene
<i>rOE33-F</i>	GTATCTCCTGCTGATGCCGCC	Quantitative PCR analysis of <i>LHCA4</i> gene
<i>rOE33-R</i>	TGGAACCTGCACTTGAACCC	Quantitative PCR analysis of <i>AtGLK1</i> gene
<i>rPSAN-F</i>	CTGTGATCAAAGCTCAACGCG	Quantitative PCR analysis of <i>AtGLK2</i> gene
<i>rPSAN-R</i>	TTGGTTTGCTCCTCTCGAGG	Quantitative PCR analysis of <i>ACTIN2</i> gene
<i>rPSBW-F</i>	TCTTGGTTTGCCTCCAATGGG	
<i>rPSBW-R</i>	CTCATCAACCAAAGCCATCGC	
<i>rLHCB1-F</i>	GGTTTGTGTTGTGGTGGATGGTA	
<i>rLHCB1-R</i>	GTGAACCCAAGAACTGAAAATCCA	
<i>rLHCA4-F</i>	AACCCGCTTAACCTTGCTCTAC	
<i>rLHCA4-R</i>	CAAACCCCTAAGAATGCCAACATC	
<i>rLHCB4-F</i>	CAAGTTCTTGACCCGCTAGG	
<i>rLHCB4-R</i>	GATGATGGTGGTGTGGAGTGG	
<i>rRBCS-F</i>	GGTCGCTCCTTCAACGGACTT	
<i>rRBCS-R</i>	ATTCGGAATCGGTAAGGTCAAGG	
<i>rGLK1-F</i>	TATGACGGTGACAGTGACCGG	
<i>rGLK1-R</i>	AACTGTTCCACTGCCTCCACG	
<i>rGLK2-F</i>	TGTGTGTAAGCAAGAGGGTGG	
<i>rGLK2-R</i>	CTACCCCTAATTGCTCCACCG	
<i>rACTIN2-F</i>	CAAACGAGGGCTGGAACAAGACT	
<i>rACTIN2-R</i>	CTGTTGACTACGAGCAGGAGATGG	

Supplementary Table S3. The offspring segregated rates of the *dpg1* heterozygous carrying the 35S::*AtDPG1* construction

Lines	Morphology		Segregated rates
	Green	albino	
1	185	7	26:1
2	176	17	10:1
3	210	14	15:1
4	169	17	10:1
5	113	23	5:1
6	39	3	13:1
7	68	2	34:1
8	37	6	6:1

Supplementary Table S4. The prediction of subcellular localization of plant DPG1 proteins

Locus number	Gene name	Number of amino acid residues	Prediction of the subcellular localization by ChloroP 1.1 ^a			
			Score ^b	cTP ^c	CS-score ^d	cTP-length ^f
At1g49510	<i>AtDPG1</i>	240	0.561	Y	2.351	55
XP_002891514	<i>AIDPG1</i>	237	0.520	Y	4.081	36
XP_010479384	<i>CsDPG1</i>	237	0.520	Y	0.855	41
XP_006304157	<i>CrDPG1</i>	238	0.512	Y	2.351	54
XP_006393250	<i>EsDPG1</i>	239	0.575	Y	3.356	40
XP_009124376	<i>BrDPG1</i>	224	0.493	Y	1.581	26
XP_006423083	<i>CcDPG1</i>	249	0.519	Y	8.406	57
XP_007042342	<i>TcDPG1</i>	256	0.506	Y	4.303	49
XP_010096165	<i>MnDPG1</i>	247	0.538	Y	-0.817	54
XP_007201809	<i>PpDPG1</i>	237	0.499	-	4.207	25
XP_009792237	<i>NsDPG1</i>	253	0.568	Y	1.269	64
XP_006384460	<i>PtDPG1</i>	231	0.542	Y	0.318	19

^aSubcellular localization predicted by the ChloroP 1.1 program (<http://www.cbs.dtu.dk/services/ChloroP/>). Score^b: Output score from the second step network, the prediction cTP/no cTP is based solely on this score. cTP^c: Whether or not this is predicted as a cTP-containing sequence; "Y" means that the sequence is predicted to contain a cTP; "-" means that is predicted not to contain a cTP. CS-score^d: The MEME scoring matrix score for the suggested cleavage site. cTP-length^f: The predicted length of the presequence (Please note that the prediction of the transit peptide length is carried out and presented even if its presence is not predicted). Similar results were obtained when using the PREDOTAR V1.03 (<http://urgi.versailles.inra.fr/predotar/predotar.html>) and IPSORT (<http://hc.ims.u-tokyo.ac.jp/IPSORT/>) subcellular localization tools.