

Involvement of PpDof1 transcriptional repressor in the nutrient condition-dependent growth control of protonemal filaments in *Physcomitrella patens*

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Supplementary Table S1. Upregulated genes in the *ppdof1 ppdof2* disruptants

Locus tag	Annotation	log ₂ change
PHYPADRAFT_68138	unknown	3.20 ± 0.47
PHYPADRAFT_165889	Iron/manganese superoxide dismutases, alpha-hairpin domain	2.48 ± 0.84
PHYPADRAFT_69453	Chlorophyll a-b binding protein	2.95 ± 1.04
PHYPADRAFT_202843	Peroxiredoxin	2.94 ± 0.14
PHYPADRAFT_173587	unknown	2.90 ± 0.61
PHYPADRAFT_160804	Glycosyl hydrolases family 17	2.86 ± 0.24
PHYPADRAFT_167726	Cupin domain	2.90 ± 0.37
PHYPADRAFT_64376	unknown	2.87 ± 0.30
PHYPADRAFT_163308	Ferric reductase-like transmembrane component	2.82 ± 0.11
PHYPADRAFT_25927	Glycoside hydrolase family 19 chitinase domain	2.70 ± 0.15
PHYPADRAFT_125261	Ferredoxin. DnaJ domain or J-domain.	2.58 ± 0.21
PHYPADRAFT_73142	unknown	2.58 ± 0.82
PHYPADRAFT_122188	PQ loop repeat	2.54 ± 0.13
PHYPADRAFT_90820	Esterase and lipase	2.31 ± 0.57
PHYPADRAFT_37064	Cu-Zn superoxide dismutase	2.30 ± 1.11
PHYPADRAFT_232139	unknown	2.29 ± 0.72
PHYPADRAFT_145447	DnaJ domain or J-domain	2.25 ± 0.51
PHYPADRAFT_114029	Secretory peroxidase	2.13 ± 0.45
PHYPADRAFT_145680	Alpha-amylase inhibitors (AAI), lipid transfer (LT) and seed storage (SS) protein family	2.10 ± 0.59
PHYPADRAFT_173687	unknown	2.08 ± 0.64
PHYPADRAFT_95604	unknown	1.98 ± 0.17
PHYPADRAFT_170107	Mitochondrial carrier protein	1.92 ± 0.05
PHYPADRAFT_69013	Pollen proteins Ole e I like	1.91 ± 0.60
PHYPADRAFT_30880	TIR domain. P-loop containing nucleoside triphosphate hydrolases.	1.84 ± 0.30
PHYPADRAFT_89542	unknown	1.84 ± 0.16
PHYPADRAFT_146384	Proline dehydrogenase	1.82 ± 1.02
		1.79 ± 0.31
		1.79 ± 0.33

PHYPADRAFT_94476	unknown	1.79 ± 0.12
PHYPADRAFT_117974	Alpha-amylase inhibitors (AAI), lipid transfer (LT) and seed storage (SS) Protein family	1.74 ± 0.06
PHYPADRAFT_89773	unknown	1.72 ± 0.17
PHYPADRAFT_158136	unknown	1.69 ± 0.10
PHYPADRAFT_87007	unknown	1.65 ± 0.46
PHYPADRAFT_71631	Helix-loop-helix domain	1.65 ± 0.11
PHYPADRAFT_107294	Scramblase	1.64 ± 0.26
PHYPADRAFT_122405	Alpha-amylase inhibitors (AAI), lipid transfer (LT) and seed storage (SS) Protein family	1.62 ± 0.10

Supplementary Table S2. Downregulated genes in the *ppdof ppdof2* disruptants

Locus tag	Annotation	log ₂ change
PHYPADRAFT_100880	SGNH_hydrolase	-3.66 ± 0.56
PHYPADRAFT_194870	SGNH_plant_lipase_like	-2.86 ± 0.29
PHYPADRAFT_68163	unknown	-3.59 ± 0.63
PHYPADRAFT_233344	unknown	-3.18 ± 0.18
PHYPADRAFT_86373	unknown	-2.87 ± 0.20
PHYPADRAFT_23582	Leucine-rich repeat (LRR) protein	-3.13 ± 0.42
PHYPADRAFT_74713	unknown	-3.07 ± 0.29
PHYPADRAFT_173117	Ribosomal_S5	-3.01 ± 0.21
PHYPADRAFT_161936	Late embryogenesis abundant protein	-2.87 ± 0.61
PHYPADRAFT_223469	Catalase	-2.87 ± 0.31
PHYPADRAFT_149648	GT1_Glycogen_synthase_DULL1_like	-2.82 ± 0.41
PHYPADRAFT_118942	Secretory_peroxidase	-2.76 ± 1.23
PHYPADRAFT_86110	unknown	-2.74 ± 0.75
PHYPADRAFT_90070	unknown	-2.70 ± 0.53
PHYPADRAFT_100451	unknown	-2.69 ± 0.77
PHYPADRAFT_181837	Glyoxal oxidase N-terminus	-2.67 ± 0.40
PHYPADRAFT_153753	The Major facilitator superfamily	-2.66 ± 0.56
PHYPADRAFT_159273	Ligand-binding bet_v_1 domain of major pollen allergen of white birch	-2.66 ± 0.29
PHYPADRAFT_66753	High affinity sulphate transporter 1	-2.29 ± 0.79
PHYPADRAFT_153324	PpDof1	-2.63 ± 0.49
PHYPADRAFT_96498	unknown	-2.63 ± 0.61
PHYPADRAFT_76126	unknown	-2.62 ± 1.00
PHYPADRAFT_229808	Helix-loop-helix domain	-2.61 ± 1.06
		-2.06 ± 0.72
		-2.58 ± 0.96
		-2.57 ± 0.83
		-2.54 ± 0.37
		-2.56 ± 0.42
		-2.55 ± 0.26

PHYPADRAFT_97018	ATP-binding cassette transporter, subfamily G, member 8, group WBC protein PpABCG8	-2.49 ± 0.55
PHYPADRAFT_77295	Alpha-amylase inhibitors (AAI), lipid transfer (LT) and seed storage (SS) Protein family	-2.45 ± 0.42
PHYPADRAFT_168333	unknown	-2.45 ± 0.12 -2.44 ± 1.61
PHYPADRAFT_135849	Nuclear transport factor 2 (NTF2-like) superfamily	-2.42 ± 0.17 -2.31 ± 0.14
PHYPADRAFT_96658	unknown	-2.36 ± 0.73 -1.97 ± 0.99
PHYPADRAFT_110746	Glyoxal oxidase N-terminus	-2.35 ± 0.37 -2.30 ± 0.43
PHYPADRAFT_64365	Von Willebrand factor type A (vWA) domain. Cache domain.	-2.34 ± 0.53 -2.14 ± 0.44
PHYPADRAFT_217084	Alpha-tubulin suppressor and related RCC1 domain-containing proteins	-2.32 ± 0.38 -1.98 ± 0.15
PHYPADRAFT_162067	Xyloglucan fucosyltransferase	-2.30 ± 0.44
PHYPADRAFT_169433	unknown	-2.29 ± 0.51 -2.19 ± 0.16
PHYPADRAFT_134960	Exostosin family	-2.26 ± 0.61
PHYPADRAFT_91246	unknown	-2.25 ± 0.29
PHYPADRAFT_80479	unknown	-2.25 ± 0.46
PHYPADRAFT_229071	F-box domain	-2.22 ± 0.70 -1.90 ± 0.30
PHYPADRAFT_188562	Glycosyltransferase_GTB_type	-2.22 ± 0.12 -2.14 ± 0.21
PHYPADRAFT_231894	BTB/POZ domain	-2.20 ± 0.35
PHYPADRAFT_219649	Cytochrome P450	-2.19 ± 0.22 -2.13 ± 0.32
PHYPADRAFT_135133	DNA recombination-mediator protein A	-2.15 ± 0.63
PHYPADRAFT_90046	unknown	-2.12 ± 0.82
PHYPADRAFT_91983	unknown	-2.08 ± 0.13

PHYPADRAFT_234128	unknown	-2.08 ± 0.21
PHYPADRAFT_86159	unknown	-2.07 ± 0.62
PHYPADRAFT_56368	Chalcone and stilbene synthases	-2.07 ± 0.11
PHYPADRAFT_80897	unknown	-2.06 ± 0.50
PHYPADRAFT_115513	Major intrinsic protein (MIP) superfamily	-2.06 ± 0.76
PHYPADRAFT_138651	PpDof2	-2.53 ± 0.57
PHYPADRAFT_34885	MAEBL; Provisional	-2.03 ± 0.38
PHYPADRAFT_66006	Agenet domain	-2.02 ± 0.68
PHYPADRAFT_169177	P-loop NTPase	-2.01 ± 0.26
PHYPADRAFT_161598	unknown	-2.01 ± 0.17
PHYPADRAFT_84239	Glycosyl hydrolase family 79, N-terminal domain	-2.00 ± 0.37
PHYPADRAFT_169496	unknown	-1.96 ± 0.86
PHYPADRAFT_91770	unknown	-1.96 ± 0.26
PHYPADRAFT_29408	Root cap	-1.95 ± 0.16
PHYPADRAFT_102974	unknown	-1.93 ± 0.77
PHYPADRAFT_166762	unknown	-1.92 ± 0.09
PHYPADRAFT_7641	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.92 ± 0.40
PHYPADRAFT_182167	Late embryogenesis abundant protein, group 4	-1.92 ± 0.27
PHYPADRAFT_91962	unknown	-1.91 ± 0.62
PHYPADRAFT_169169	PpDof14	-1.90 ± 0.48
PHYPADRAFT_92203	unknown	-1.90 ± 0.72
PHYPADRAFT_127405	Fatty acid hydroxylase superfamily	-1.89 ± 0.17
PHYPADRAFT_78176	Fatty acid hydroxylase superfamily	-1.89 ± 0.15
PHYPADRAFT_64198	SGNH_hydrolase	-1.87 ± 0.45
PHYPADRAFT_126819	Chalcone and stilbene synthases	-1.87 ± 0.34
PHYPADRAFT_62169	Major intrinsic protein (MIP) superfamily	-1.86 ± 0.12
PHYPADRAFT_113110	Exo70 exocyst complex subunit	-1.86 ± 0.84
PHYPADRAFT_126580	Rhamnogalacturonate lyase family	-1.85 ± 0.29
PHYPADRAFT_166880	Uncharacterized conserved protein (DUF2235)	-1.85 ± 0.17
PHYPADRAFT_230875	C2H2-type zinc finger protein	-1.83 ± 0.79
PHYPADRAFT_225082	Xyloglucan endotransglycosylase, member of glycosyl	-1.82 ± 0.24

	hydrolase family 16	-1.76 ± 0.21
PHYPADRAFT_77701	unknown	-1.82 ± 0.48
PHYPADRAFT_163142	Fasciclin domain	-1.80 ± 0.07
PHYPADRAFT_135547	SGNH_hydrolase	-1.76 ± 0.15
PHYPADRAFT_152778	GDP-fucose protein O-fucosyltransferase	-1.75 ± 0.51
PHYPADRAFT_89752	Plant protein of unknown function (DUF869)	-1.75 ± 0.33
PHYPADRAFT_84406	F-box domain, cyclin-like	-1.74 ± 0.59
PHYPADRAFT_147950	E1-E2 ATPase	-1.73 ± 0.66
PHYPADRAFT_82584	GT1_Glycogen_synthase_DULL1_like	-1.73 ± 0.14
PHYPADRAFT_99425	unknown	-1.72 ± 0.49
PHYPADRAFT_102734	Carbonic anhydrase alpha, prokaryotic-like subfamily	-1.72 ± 0.29
PHYPADRAFT_19854	homeobox domain, ZF-HD class	-1.71 ± 0.04
PHYPADRAFT_195732	GH31_MGAM_SI_GAA,maltase-glucoamylase (MGAM), sucrase-isomaltase (SI), and lysosomal acid alpha-glucosidase	-1.71 ± 0.11
PHYPADRAFT_233161	unknown	-1.67 ± 0.13
PHYPADRAFT_60268	homeobox domain, ZF-HD class. ZF-HD protein dimerisation region.	-1.65 ± 0.13 -1.64 ± 0.33
PHYPADRAFT_211389	Esterase_lipase	-1.64 ± 0.27
PHYPADRAFT_21889	Leucine-rich repeats (LRRs), ribonuclease inhibitor (RI)-like subfamily. Catalytic domain of Protein Kinases.	-1.64 ± 0.27
PHYPADRAFT_19707	Stigma-specific protein, Stig1	-1.63 ± 0.33

Supplementary Table S3. Primers used for semi-quantitative and quantitative RT-PCR analysis

Target	Forward (5' > 3')	Reverse (5' > 3')
For RT-PCR and semi-quantitative RT-PCR		
<i>PpDof1</i> (Fig. 1)	CGCCATTAAGCTGTTTGGTAAGA	CGTTATTACTCTCCTTGCCACATGA
<i>PpDof1</i> (Fig. S1)	CGCCATTAAGCTGTTTGGTAAGA	TGTCAAGGCTATCGCACCTA
<i>PpDof2</i> (Fig. 1)	AAGGCCGCTACCTCGATGAAC	CGGCCTTACAAGAGGTTGTCACG
<i>PpDof2</i> (Fig. S1)	TCAGTAGGTGGCAAGACGGATAG	GGAAGATTCATTGCAAGTGCTC
<i>PpDof3</i>	TGGTCACACCTTTTCCTCTTTCTTC	GTACTIONGGCTGCTGGAACCTT
<i>PpDof4</i>	AGAGATGTTGAAGGGCGAGTAGC	GATTACTGGGACTGCTGGCATT
<i>PpDof5</i>	TAACTGCTCGATTCAAAGTGATTCC	TGCAGTCTTTGTCCATTCCATTT
<i>PpDof6</i>	TCAGAAAGCAGGTTTCATCCCTAAAG	AACGCTGATGAATTGTAAGCAGA
<i>PpTUA2</i> (PHYPADRAFT_29741)	TGTGCTGCTGGATAATGAAGCG	CTCGTGCTGTTTCGAAATCATGC
For quantitative RT-PCR		
PHYPADRAFT_69453	CATTACCGGCTGCCTGACCCC	ACCCGGGGACGCCTAGGTAGT
PHYPADRAFT_167726	GGGCAGTAAGCCTGTGGTGGC	CGCTGCCGAACAAGGTCGCT
PHYPADRAFT_149648	GCCGGACGACTTCAAGCGCT	TGGAGGTAAGACCCGCACTGGA
PHYPADRAFT_181837	GCCTGAGGCAAGTTCGTGCT	CCTTGCGCTTCCACCACCGA
<i>PpABC8</i> (PHYPADRAFT_97018)	ATCGGCGCTTCCGTACAGGC	GCGTGGCCAGCCTTTGAGGT
<i>PpTUB1</i> (PHYPADRAFT_186458)	GACTGCTTGCAAGGTTTCCAAG	GTTCAAGTCGCCAAACGAAGGA

Supplementary Table S4. PCR primers used for the isolation of cDNA and genomic clones and preparation of DNA probes for genomic Southern blot analysis

Target	Forward (5' > 3')	Reverse (5' > 3')
The 5' region of PpDof5 cDNA	ACATCTCCTCTCAGCTATGGACCTC	ACTGAACGACATCGTGCTCCTA
The 3' region of PpDof5 cDNA	TAGGAGCACGATGTCGTTTCAGT	
PpDof1 genomic clone	ATGAGGAGGTTGTTGAGCACGTA	CCCATAAACCCAGTCCACACATTC
PpDof2 genomic clone	TCAGTAGGTGGCAAGACGGATAG	CACATACCCGTCCAGACATTAC
PpDof1-specific probe	GATACCCATTCATTCATCCAAACAA	ATTGCTCCTTTTTCTCCTCAACAGT
PpDof2-specific probe	GGCAGTGTATTTTGCTTTGCTTTG	CAATAACCCATTTCCATTCTGATTGC

Legends for supplementary figures

Supplementary Fig. S1. Targeted disruption of the *PpDof1* and *PpDof2* genes. (A) Genomic organization of the wild type (WT) and disrupted *PpDof1* and *PpDof2* gene loci and gene targeting vectors, depicted schematically. The white, hatched and black boxes indicate the 5' and 3' untranslated regions, the coding regions and the sequence encoding the Dof domain, respectively. Regions corresponding to the DNA probes used for genomic Southern blot analysis, which were prepared by PCR using the primers listed in Supplementary Table S4, are indicated by black bars. E, *EcoRI* site; P35S, cauliflower mosaic virus 35S RNA promoter; *HPT*, hygromycin phosphotransferase gene; Tnos, terminator sequence of nopaline synthase gene; Pm35S, a modified 35S promoter; *NPTII*, neomycin phosphotransferase II gene; 35SPA, the polyadenylation signal of cauliflower mosaic virus 35S RNA gene. (B) Genomic Southern blot analysis using *EcoRI*-digested genomic DNA. PpDof1- (PpDof1) and PpDof2-specific (PpDof2) probes were labeled using the AlkPhos Direct Labeling System and signals were detected using the CDP-star kit (GE Healthcare, Tokyo, Japan). Positions of the DNA fragments originating from the wild-type (w) and disrupted (d) *PpDof1* or *PpDof2* genes are indicated by arrowheads. Only bands of the expected sizes were detected using genomic DNA of all transformants, with the exception of the *ppdof1*-#2 line and the PpDof1-specific probe. (C) Semi-quantitative RT-PCR analysis of *PpDof1* and *PpDof2* mRNA in the wild type and disruptant lines. Transcripts of *PpTUA2* (accession number AB096719) were detected as a control.

Supplementary Fig. S2. Nuclear localization of PpDof2. Fluorescence (G) and corresponding bright (BF) images of maize protoplasts transfected with expression vectors for GFP alone, the EIN3(1-449)-GFP fusion protein as a nuclear localization control (Yanagisawa *et al.*, 2003), or the GFP-PpDof2 fusion protein.

Supplementary Fig. S3. Expression levels of *PpDof1* and *PpDof2* in protonemal colonies grown under different nutrient conditions. RNA was prepared from protonemal colonies that were initially grown in regeneration medium for three days after protoplasting and then with BCD medium, BCD medium supplemented with ammonium (BCD+A) or BCD medium supplemented with ammonium and glucose

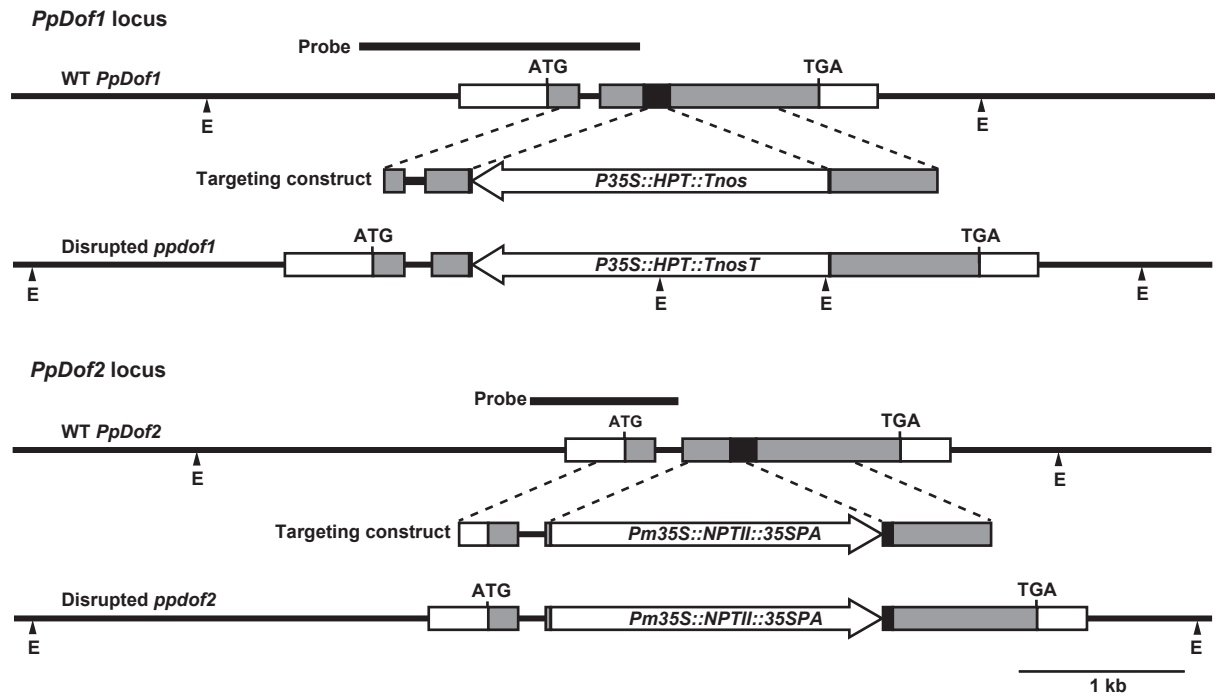
(BCD+A+G) for 10 days. Values are the means \pm SD of three replicates relative to the transcript levels of the β -tubulin 1 gene (*PpTUB1*) (Holm *et al.*, 2010). The expression levels of these genes in the wild type plants grown on BCD medium supplemented with ammonium and glucose were assigned a reference value of 1 unit.

References

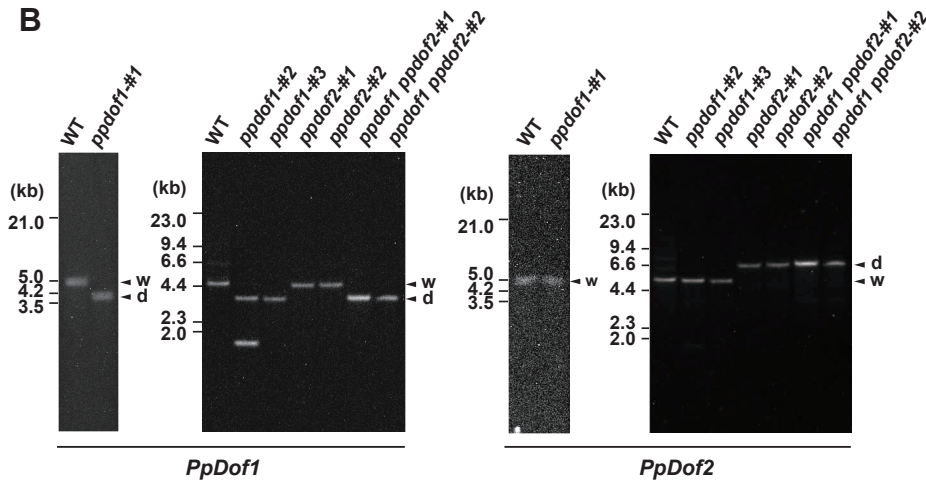
Holm K, Källmann T, Gyllenstrand N, Hedman H, Lagercrantz U. 2010. Does the core circadian clock in the moss *Physcomitrella patens* (Bryophyta) comprise a single loop? *BMC Plant Biology* **10**, 109.

Yanagisawa S, Yoo S-D, Sheen J. 2003. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. *Nature* **425**, 521-525.

A



B



C

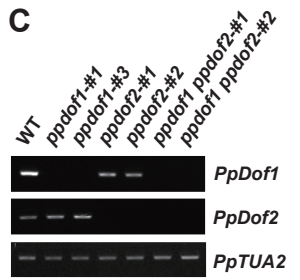


Figure S1. Sugiyama et al.

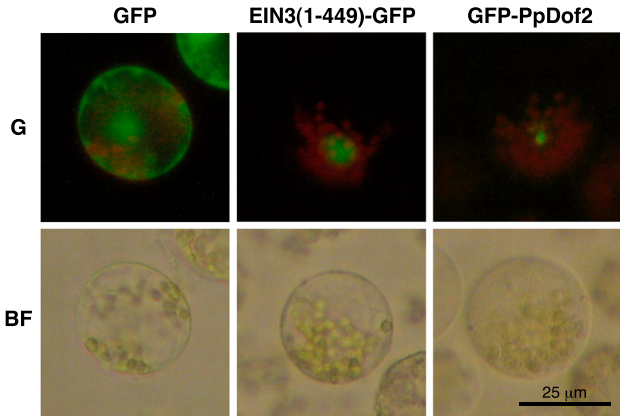


Figure S2. Sugiyama et al

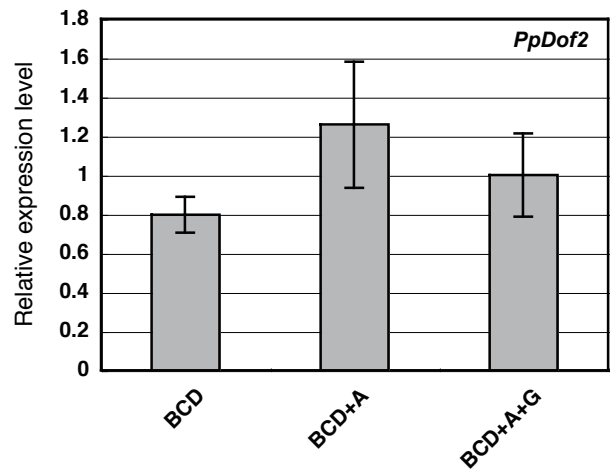
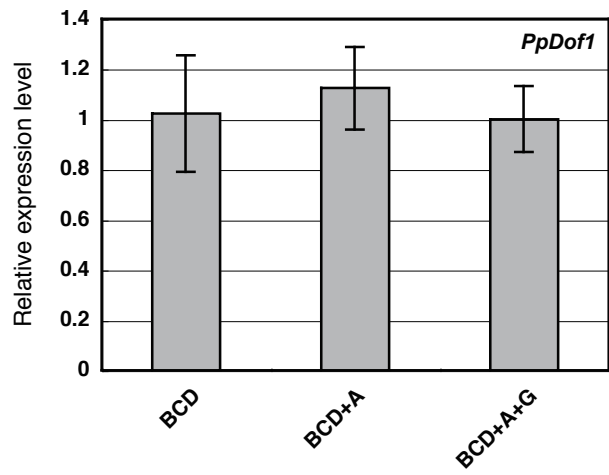


Figure S3. Sugiyama et al.