

Supplementary Material

Genetic manipulation of protein phosphatase 2A affects multiple agronomic traits and physiological parameters in potato (*Solanum tuberosum*)

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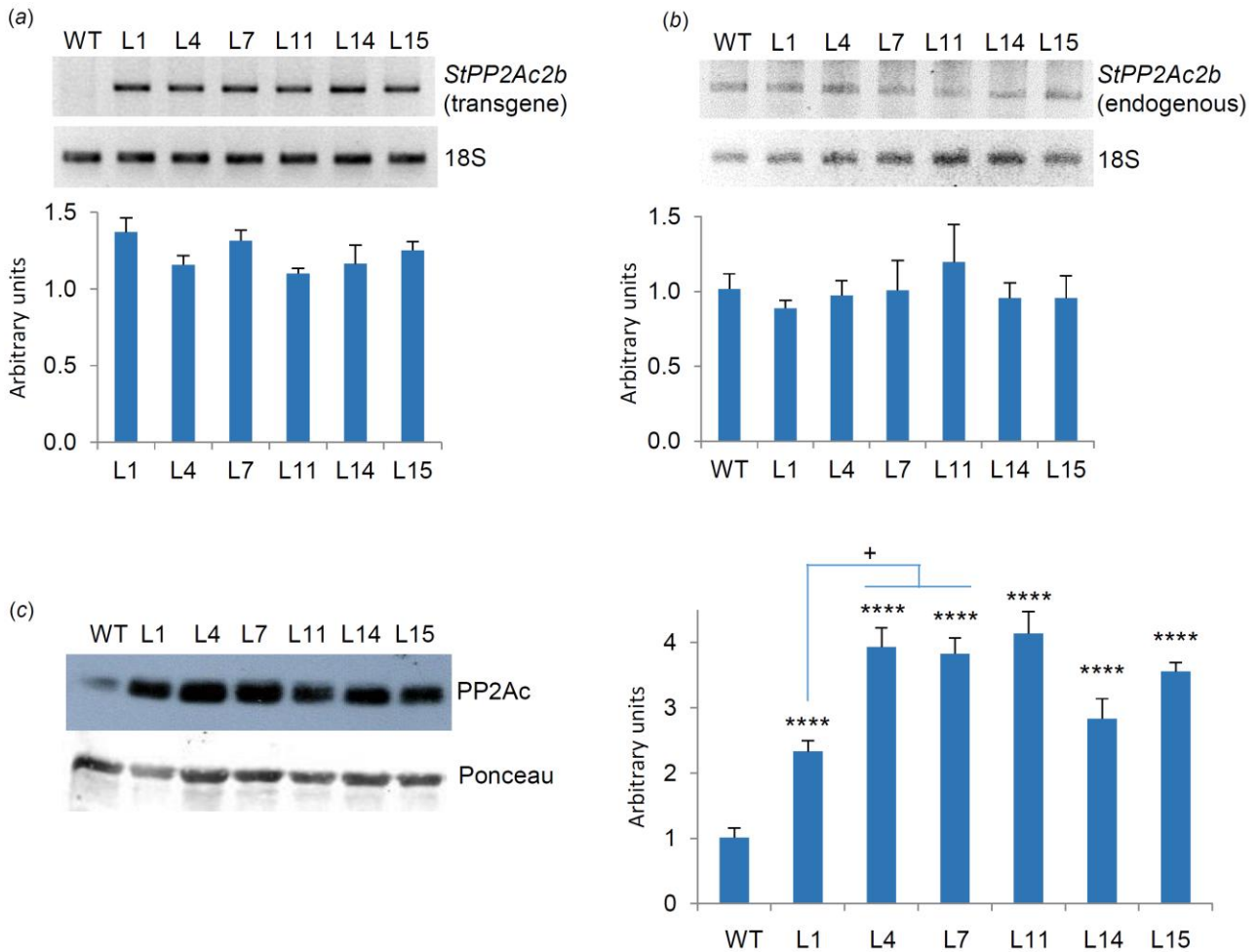


Fig. S1. Molecular characterization of *StPP2Ac2b*-OE lines. (a and b) Semi-quantitative RT-PCR analysis of transgene and endogenous *StPP2Ac2b*, respectively, of wild type (WT) and *StPP2Ac2b*-OE lines (L). Representative results of four independent experiments are shown. Quantitative data of RT-PCR bands (mean \pm s.e.m.; n=4) are displayed in the bar graphs. (c) Western blot analysis for PP2Ac. A representative blot of six independent experiments is shown. Quantitative data of western blot bands (mean \pm s.e.m.; n=6) are displayed in the bar graph. All measurements were performed on detached leaves of plants cultured in vitro. The asterisks and crosses in the bar graphs indicate statistical significance: **** P < 0.001, compared with the WT; + P < 0.05, L4 vs. L7.

Primers and reaction conditions used for semi-quantitative RT-PCR: transgene FW: 5'-CACTGGATAACATACGATCATTGG-3' and transgene RV: 5'-TGATAATCATCGCAAGACCG-3' (annealing temperature: 50.5°C; 28 cycles); endogenous *StPP2Ac2b* FW: 5'-GAGAGTTGAGAAGAGGCACTG-3' and endogenous *StPP2Ac2b* RV: 5'-CTATGGAATACCAAATATACAGAC-3' (annealing temperature: 52.0°C; 34 cycles); 18S FW: 5'-GGCATTTCGTATTCATAGTCAGAG-3' and 18S RV: 5'-CGGTTCTTGATTAATGAAAACATCCT-3' (annealing temperature: 58.0°C; 17 cycles). Bands were quantified relative to the internal control 18S rRNA using the ImageJ software).

Western blot analysis was carried out using the commercial anti-PP2Ac 1D6 monoclonal antibody (Upstate Biotechnology, Lake Placid, NY), and a horseradish peroxidase-conjugated secondary antibody: Bands were visualized using the ECL technique (GE Healthcare). PP2Ac bands were quantified relative to the Ponceau S staining of the Rubisco large subunit band, using the ImageJ software.

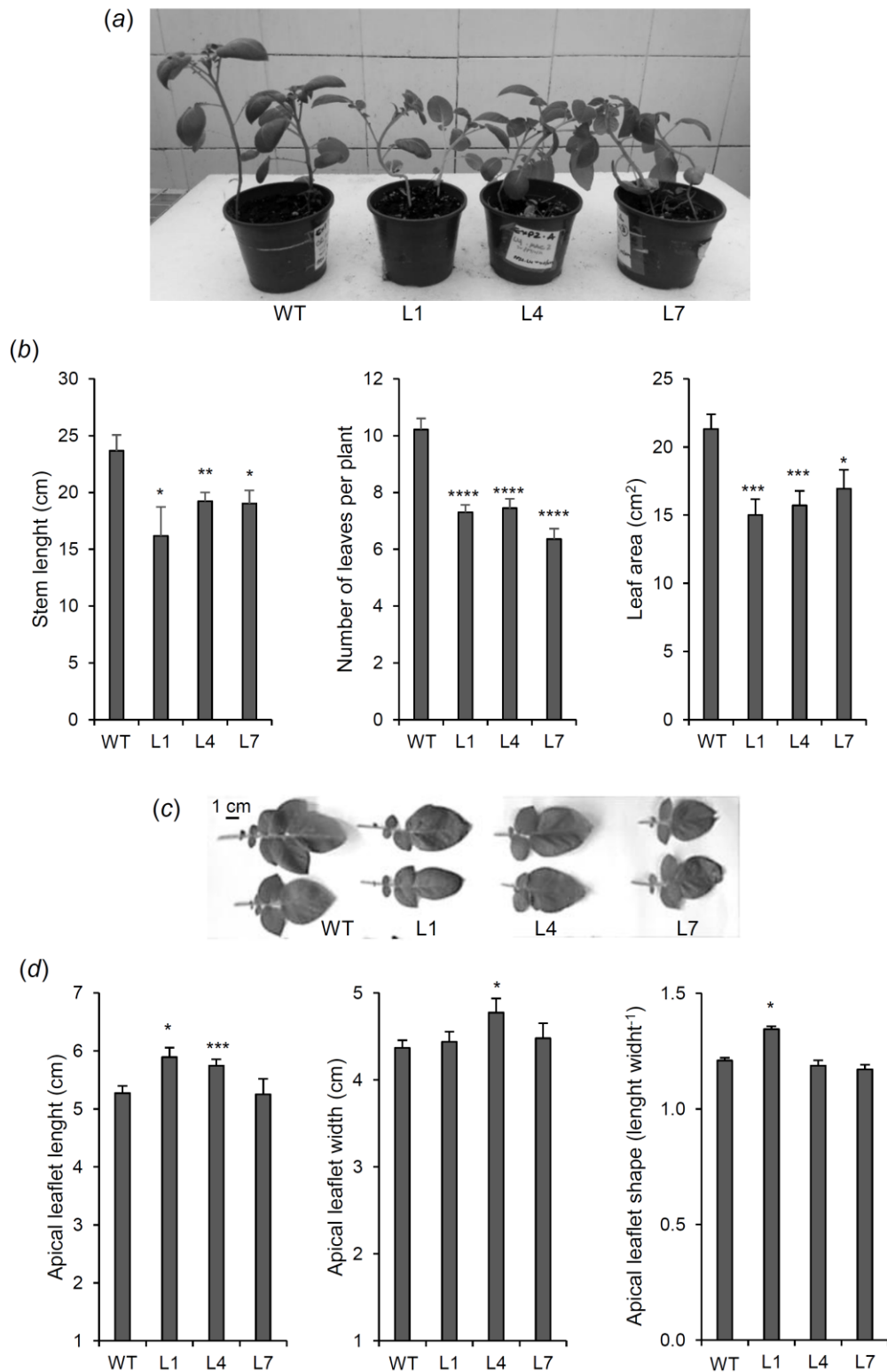


Fig. S2. Growth and morphological parameters. Wild type and StPP2Ac2b-OE plants obtained from seed tubers were cultivated in greenhouse. Four weeks after planting, plants were photographed (a), and growth and morphology parameters were evaluated. (b) Stem length, number of leaves per plant and leaf area. (c) Representative image of the youngest fully expanded leaf. (d) Apical leaflet length and width, and apical leaflet shape. Data are the mean \pm s.e.m. of 10 plants per condition. The experiment was repeated three times with similar results. The asterisks indicate statistical significance (Student's *t*-test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$, compared with the WT.

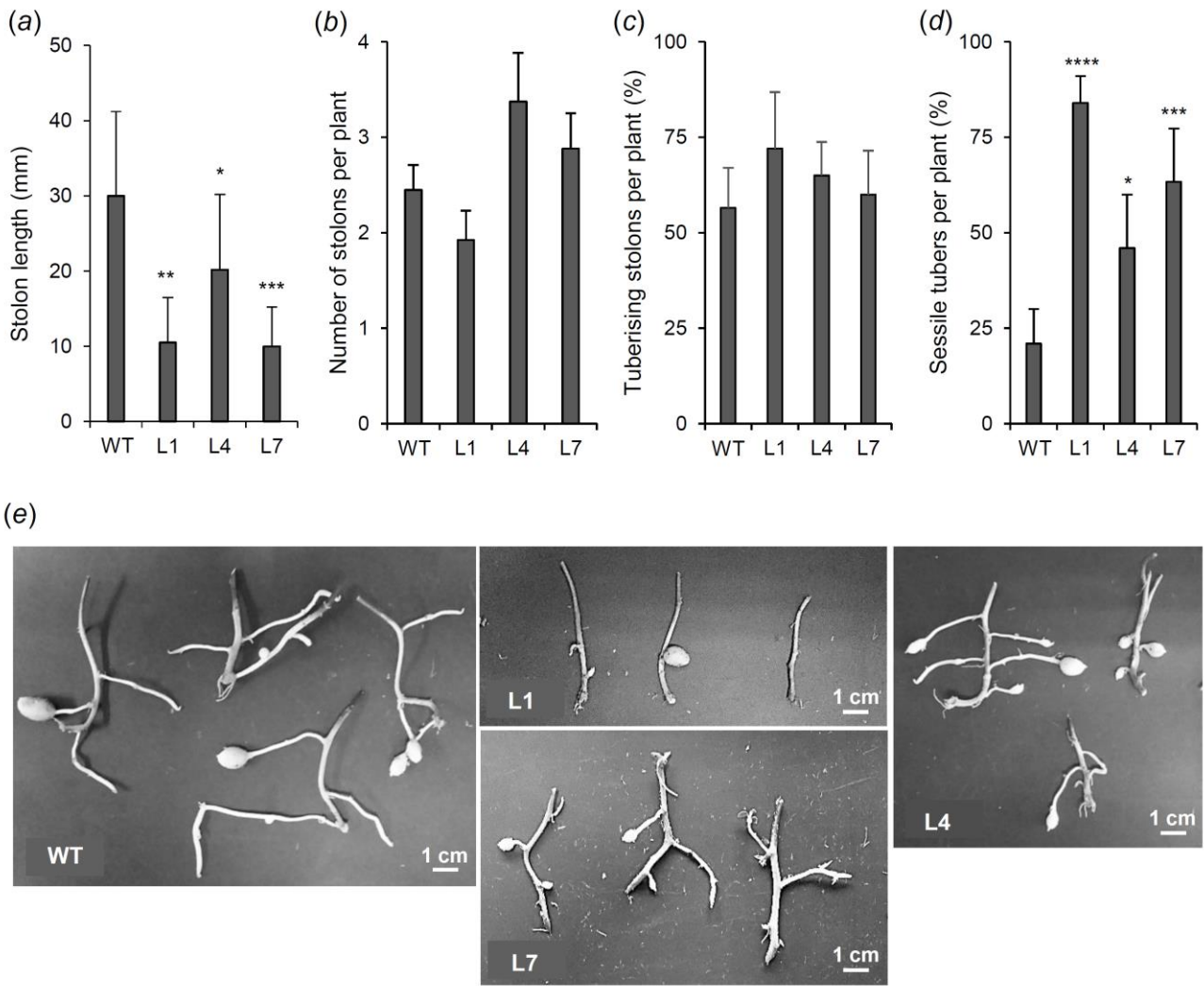


Fig. S3. Stolon characteristics. Wild type and StPP2Ac2b-OE plants produced ex vitro were cultivated in greenhouse. (a-d) Stolon length, number of stolons per plant, percentage of tuberizing stolons and percentage of sessile tubers per plant, determined four weeks after transfer to soil. (e) Representative image of the stolons. Data are the mean \pm s.e.m. of 10 plants per condition. The experiment was repeated three times with similar results. The asterisks indicate statistical significance (Student's *t*-test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$, compared with the WT.

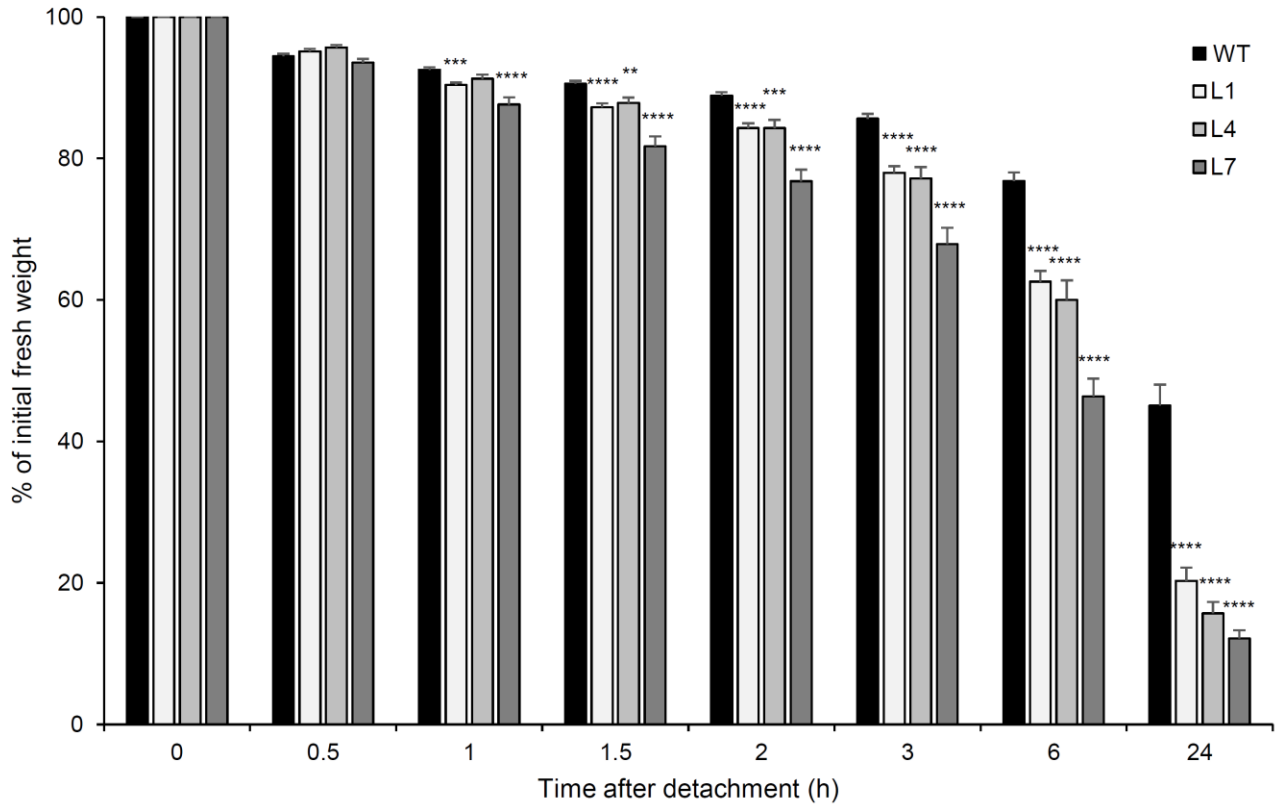


Fig. S4. Statistical analysis of Fig. 3a. Data are the mean \pm s.e.m. of seven independent experiments, each consisting of five leaves per line. The asterisks indicate statistical significance (Student's *t*-test): ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$, compared with the WT.

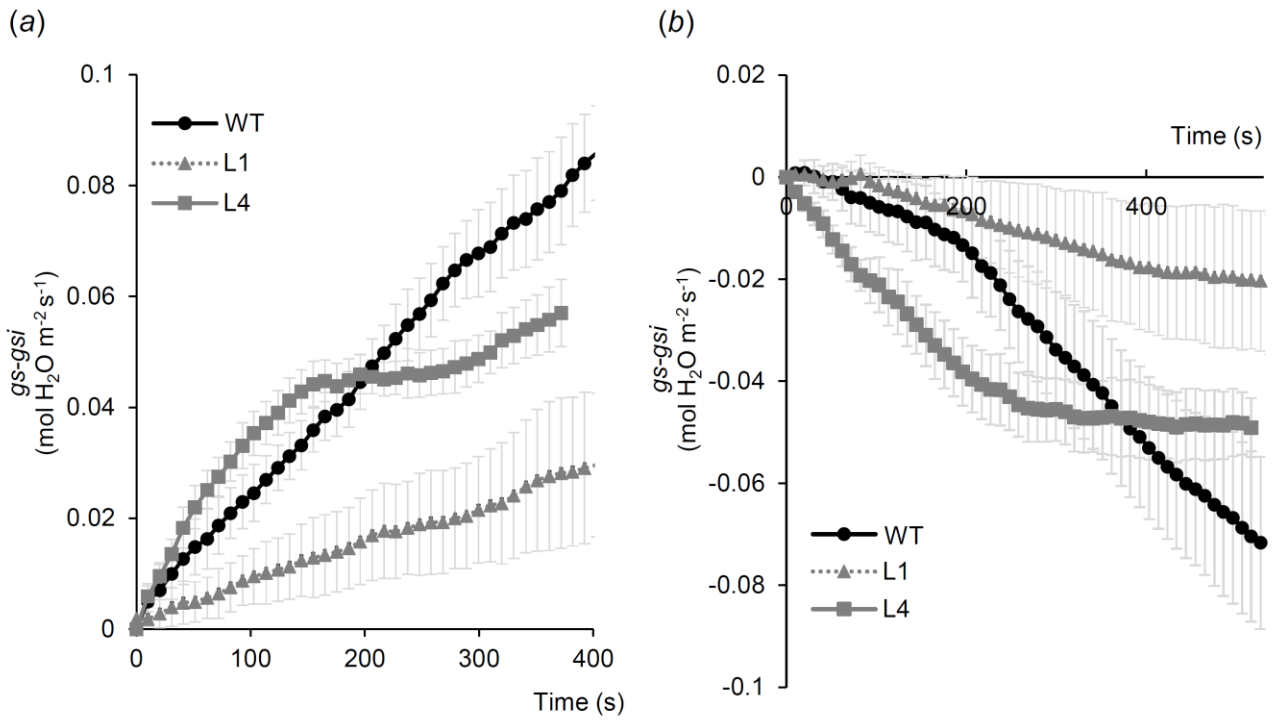


Fig. S5. Stomatal kinetics of StPP2Ac2b-OE plants. Temporal responses of stomatal conductance to a step change increase in (a) irradiance from $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$, and (b) CO_2 concentration from $400 \mu\text{mol mol}^{-1}$ to $1000 \mu\text{mol mol}^{-1}$. Stomatal conductance was measured with a portable photosynthesis system, as described in Materials and methods. Each point represents the mean \pm s.e.m. of four replicates, taken in different plants. The initial (g_{si}) values in a were similar for all the plants analyzed (0.138 ± 0.008 for WT, 0.109 ± 0.007 for L1 and 0.135 ± 0.04 for L4).

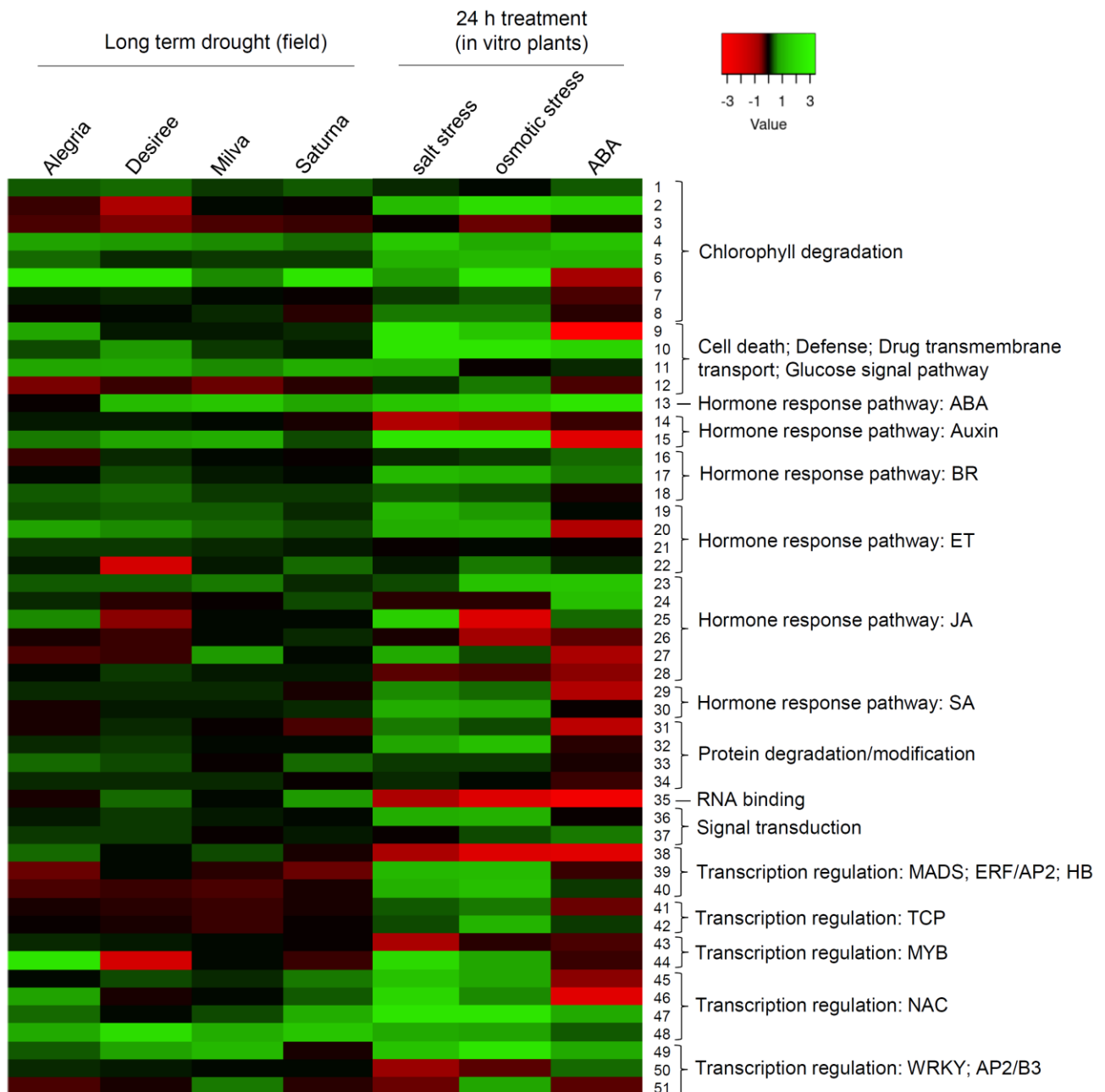


Fig. S6: Expression profiles of senescence-promoting genes in response to abiotic stresses. Heat map visualization of RNA-seq data available at <http://spuddb.uga.edu/>. Transcript IDs are shown in Supplementary Table S1. Differential expression was determined as log₂ fold change. The heat map was constructed using the Heatmapper web server (<http://www.heatmapper.ca/>). The RNA-seq datasets information is provided in Supplementary Table S2.

Table S1 Transcript IDs of senescence-promoting genes used to construct the heat map of Fig. S5.

	ID (<i>S. tuberosum</i> database (http://spuddb.uga.edu/)	Arabidopsis homolog	Functional annotation in <i>S. tuberosum</i> database
1	Soltu.DM.11G020880.1	AT3G44880 PAO/ACD1 Chlorophyll degradation	Pheophorbide a oxygenase family protein with Rieske [2Fe-2S] domain
2	Soltu.DM.12G007710.1	AT4G11910 NYE2 Chlorophyll degradation	non-yellowing
3	Soltu.DM.07G010570.1	AT4G13250 NYC1 Chlorophyll degradation	NAD(P)-binding Rossmann-fold superfamily protein
4	Soltu.DM.08G026750.1	AT4G22920 AtNYE1 Chlorophyll degradation	non-yellowing
5	Soltu.DM.01G026560.2	AT5G13800 PPH Chlorophyll degradation	pheophytinase
6	Soltu.DM.02G021370.1	AT5G15250 AtFtsH6 Chlorophyll degradation	FTSH protease
7	Soltu.DM.04G037460.1	AT5G42270 FTSH5 Chlorophyll degradation	FtsH extracellular protease family
8	Soltu.DM.08G009290.1	AT5G04900 NOL Chlorophyll degradation	NYC1-like
9	Soltu.DM.08G022080.2	AT1G32080 AtLrgB Cell death (MP prot)	membrane protein, putative
10	Soltu.DM.01G005860.8	AT2G41060 UBA2b Defense	RNA-binding (RRM/RBD/RNP motifs) family protein
11	Soltu.DM.04G031660.1	AT1G58340 BCD1 Drug transmembrane transport	MATE efflux family protein
12	Soltu.DM.12G025470.1	AT4G29130 HXK1/GIN2 Glucose signal pathway	hexokinase
13	Soltu.DM.05G023010.3	AT5G59220 SAG113 Hormone response pathway: ABA	protein phosphatase 2CA
14	Soltu.DM.07G013020.1	AT1G19220 ARF19 Hormone response pathway: Auxin	Transcriptional factor B3 family protein / auxin-responsive factor AUX/IAA-related
15	Soltu.DM.01G042050.2	AT1G59750 ARF1 Hormone response pathway: Auxin	auxin response factor
16	Soltu.DM.10G017720.1	AT1G20330 SMT2/CVP1 Hormone response pathway: BR	sterol methyltransferase
17	Soltu.DM.10G020520.1	AT2G38050 DET2/DWF6 Hormone response pathway: BR	3-oxo-5-alpha-steroid 4-dehydrogenase family protein
18	Soltu.DM.04G023990.1	AT4G39400 BRI Hormone response pathway: BR	Leucine-rich receptor-like protein kinase family protein
19	Soltu.DM.07G022640.1	AT1G04310 ERS2 Hormone response pathway: ET	Signal transduction histidine kinase, hybrid-type, ethylene sensor
20	Soltu.DM.11G002410.1	AT1G66330 AAF Hormone response pathway: ET	senescence-associated family protein

21	Soltu.DM.01G006210.1	AT3G20770 EIN3 Hormone response pathway: ET	Ethylene insensitive 3 family protein
22	Soltu.DM.09G005730.2	AT5G03280 EIN2 Hormone response pathway: ET	NRAMP metal ion transporter family protein
23	Soltu.DM.03G037120.1	AT1G72520 LOX4 Hormone response pathway: JA	lipoxygenase
24	Soltu.DM.09G024180.1	AT1G55020 LOX1 Hormone response pathway: JA	PLAT/LH2 domain-containing lipoxygenase family protein
25	Soltu.DM.09G028490.2	AT2G33150 PED1/KAT2 Hormone response pathway: JA	peroxisomal 3-ketoacyl-CoA thiolase
26	Soltu.DM.08G006230.1	AT2G44050 COS1 Hormone response pathway: JA	6,7-dimethyl-8-ribityllumazine synthase / DMRL synthase / lumazine synthase / riboflavin synthase
27	Soltu.DM.01G002140.1	AT3G45140 LOX2 Hormone response pathway: JA	lipoxygenase
28	Soltu.DM.06G026830.1	AT5G63110 HDA6 Hormone response pathway: JA	histone deacetylase
29	Soltu.DM.06G026140.1	AT1G74710 SID2 Hormone response pathway: SA	isochorismate synthase
30	Soltu.DM.04G032870.1	AT4G24230 ACPB3 Hormone response pathway: SA	acyl-CoA-binding domain
31	Soltu.DM.07G001220.1	AT4G30520 AtSARK Protein degradation/modification	Leucine-rich repeat protein kinase family protein
32	Soltu.DM.06G009100.1	AT5G05700 DLS1/ATE1 Protein degradation/modification	arginine-tRNA protein transferase
33	Soltu.DM.12G028490.1	AT2G42620 ORE9/MAX2 Protein degradation/modification	RNI-like superfamily protein
34	Soltu.DM.03G007830.1	AT1G69270 RPK1 Protein degradation/modification	receptor-like protein kinase
35	Soltu.DM.01G049440.1	AT2G21660 ATGRP7 RNA binding	cold, circadian rhythm, and rna binding
36	Soltu.DM.06G023430.1	AT1G80350 BOT1 Signal transduction	P-loop containing nucleoside triphosphate hydrolases superfamily protein
37	Soltu.DM.12G019640.1	AT2G43790 MPK6 Signal transduction	MAP kinase
38	Soltu.DM.06G012820.1	AT2G45660 SOC1 Transcription regulation: MADS	AGAMOUS-like
79	Soltu.DM.07G019630.1	AT4G28140 Rap2.4f Transcription regulation: ERF/AP2	Integrase-type DNA-binding superfamily protein
40	Soltu.DM.08G028150.1	AT5G41410 BEL1 Transcription regulation: HB	POX (plant homeobox) family protein
41	Soltu.DM.07G023850.1	AT3G15030 TCP4 Transcription regulation: TCP	TCP family transcription factor

42	Soltu.DM.08G007360.2	AT4G18390 TCP2 Transcription regulation: TCP	TEOSINTE BRANCHED 1, cycloidea and PCF transcription factor
43	Soltu.DM.03G034270.1	AT1G49010 AtMYBL Transcription regulation: MYB	Duplicated homeodomain-like superfamily protein
44	Soltu.DM.12G001810.1	AT2G47190 ATMYB2 Transcription regulation: MYB	myb domain protein
45	Soltu.DM.05G005300.1	AT1G69490 NAP Transcription regulation: NAC	NAC-like, activated by AP3/PI
56	Soltu.DM.11G004630.3	AT3G10500 NTL4/NAC053 Transcription regulation: NAC	NAC domain containing protein
57	Soltu.DM.03G029980.1	AT3G29035 AtNAC3 Transcription regulation: NAC	NAC domain containing protein
58	Soltu.DM.06G025800.1	AT5G39610 ORE1/NAC2 Transcription regulation: NAC	NAC domain containing protein
49	Soltu.DM.08G028850.1	AT4G23810 WRKY53 Transcription regulation: WRKY	WRKY family transcription factor
50	Soltu.DM.10G025140.1	AT1G13260 RAV1/EDF4 Transcription regulation: AP2/B3	AP2/B3-like transcriptional factor family protein
51	Soltu.DM.02G004200.1	AT1G62300 WRKY6 Transcription regulation: WRKY	WRKY family transcription factor

Table S2: RNA-seq datasets used in Fig. S5.

Sample	Cultivar	Library ID
long-term drought (field)	Alegria	Control: SRR3161990; SRR3162030; SRR3162022 Drought: SRR3161994; SRR3162036; SRR3162028
long-term drought (field)	Desiree	Control: SRR3161991; SRR3162031; SRR3162023 Drought: SRR3161995; SRR3162035; SRR3162027
long-term drought (field)	Milva	Control: SRR3161992; SRR3162032; SRR3162024 Drought: SRR3161996; SRR3162036; SRR3162028
long-term drought (field)	Saturna	Control: SRR3161993; SRR3162033; SRR3162025 Drought: SRR3161997; SRR3162037; SRR3162029
salt stress - 150 mM NaCl, 24 h (whole in vitro plant)	DM 1-3 R44	Control: SRR122131 NaCl: SRR122120
osmotic stress - 260 μ M mannitol, 24 h (whole in vitro plant)	DM 1-3 R44	Control: SRR122131 Mannitol: SRR122128
ABA - 50 μ M, 24 h (whole in vitro plant)	DM 1-3 R44	Control: SRR122126 ABA: SRR122138

Table S3: Parameters estimated from the light-response curves shown in Fig. 8.

Parameter	units	WT	L1	L4	L7
Maximum gross CO₂ assimilation rate (Pgmax)	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	17.49 ± 0.17	16.88 ± 3.64	15.41 ± 0.30 **	8.53 ± 1.32 *
Maximum net CO₂ assimilation rate [PN(I_{max})]	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	12.38 ± 0.89	12.24 ± 2.89	7.45 ± 1.18 *	5.47 ± 1.14 **
Quantum yield [$\Phi(\text{I}_0)$]	$\mu\text{mol CO}_2 \mu\text{mol}^{-1}$ (photons)	0.06 ± 0.01	0.09 ± 0.01	0.02 ± 0.01 *	0.06 ± 0.01
Dark respiration (RD)	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	0.90 ± 0.17	1.04 ± 0.23	0.5 ± 0.24	0.73 ± 0.09
Light compensation point (I_{comp})	$\mu\text{mol (photons) m}^{-2} \text{ s}^{-1}$	17.17 ± 2.90	11.53 ± 1.19	29.71 ± 11.77	14.46 ± 2.10
Light saturation (I_{max})	$\mu\text{mol (photons) m}^{-2} \text{ s}^{-1}$	1098 ± 238	667 ± 131	893 ± 29	383 ± 38

Data are the mean value \pm s.e.m. (n=3). The asterisks indicate statistical significance (Student's *t*-test): **P* < 0.05, ***P* < 0.01, compared with the WT.