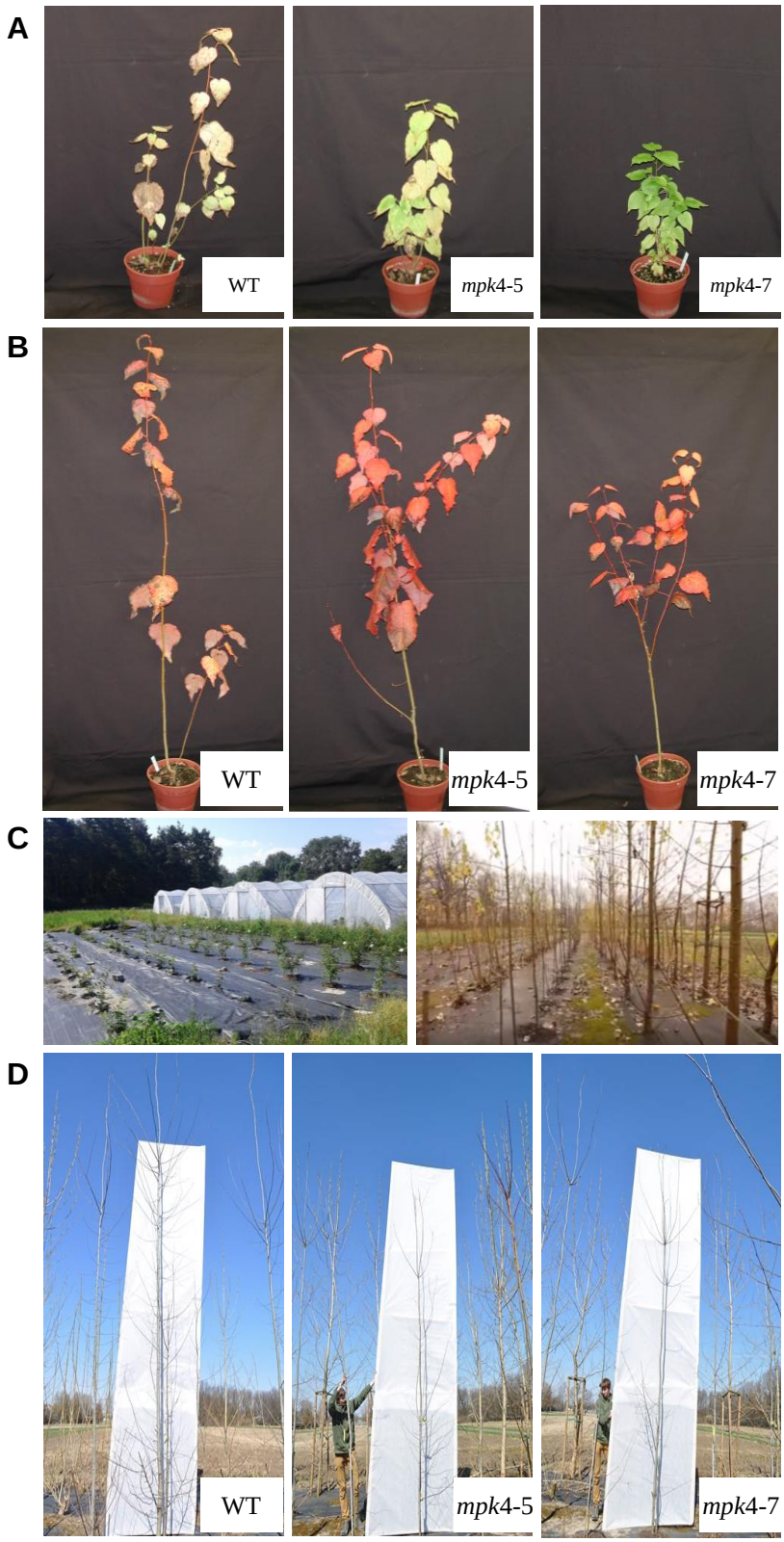
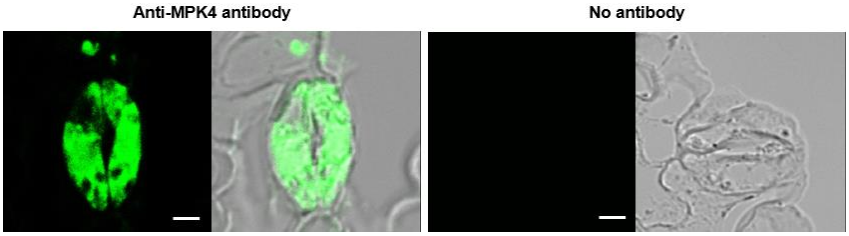
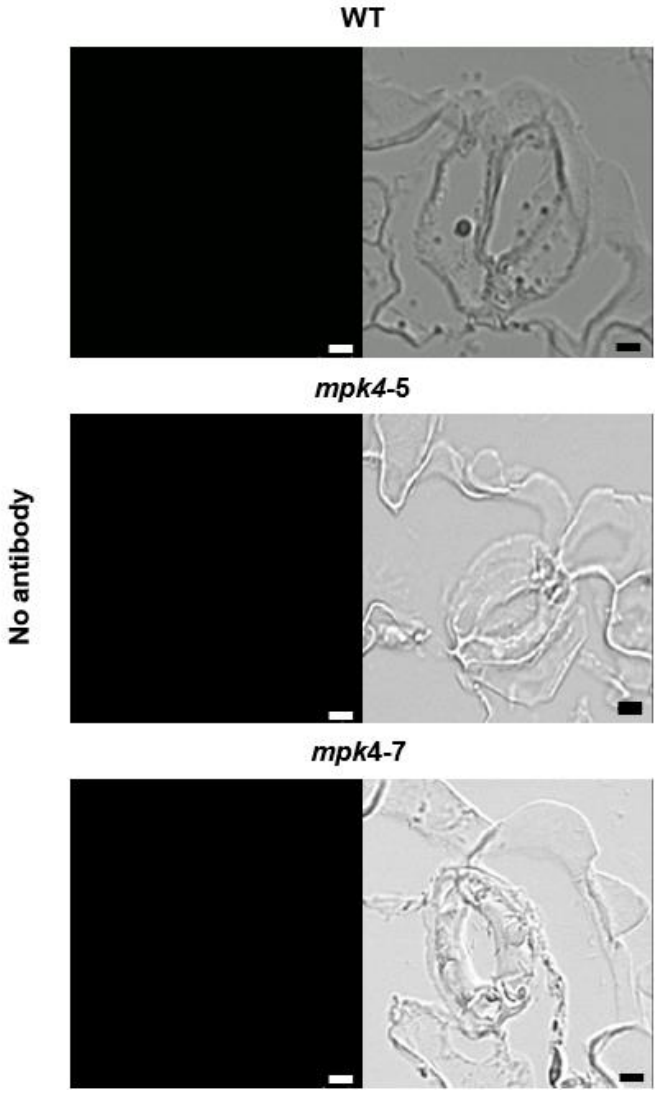


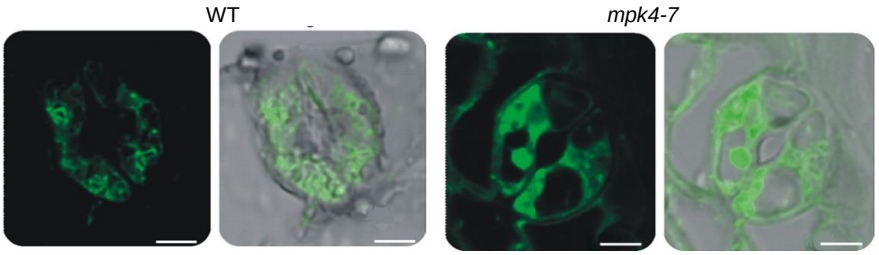
Supplemental Figure S1. WT and *mpk*4 phenotype. (**A**) Six-month-old plants; and (**B**) one-year-old plants in greenhouse conditions; (**C**) first and second season; and (**D**) third season of tree growth in field conditions.



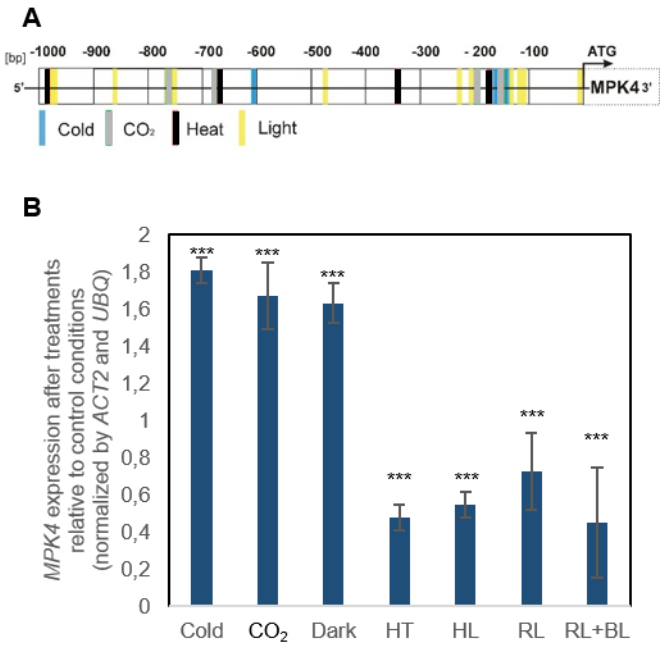
Supplemental Figure S2. MPK4 localization in the cytoplasm of guard cells. A control was prepared without primary antibody. Scale bars: 10 μm. Presented pictures are representative for 10 independent analysis.



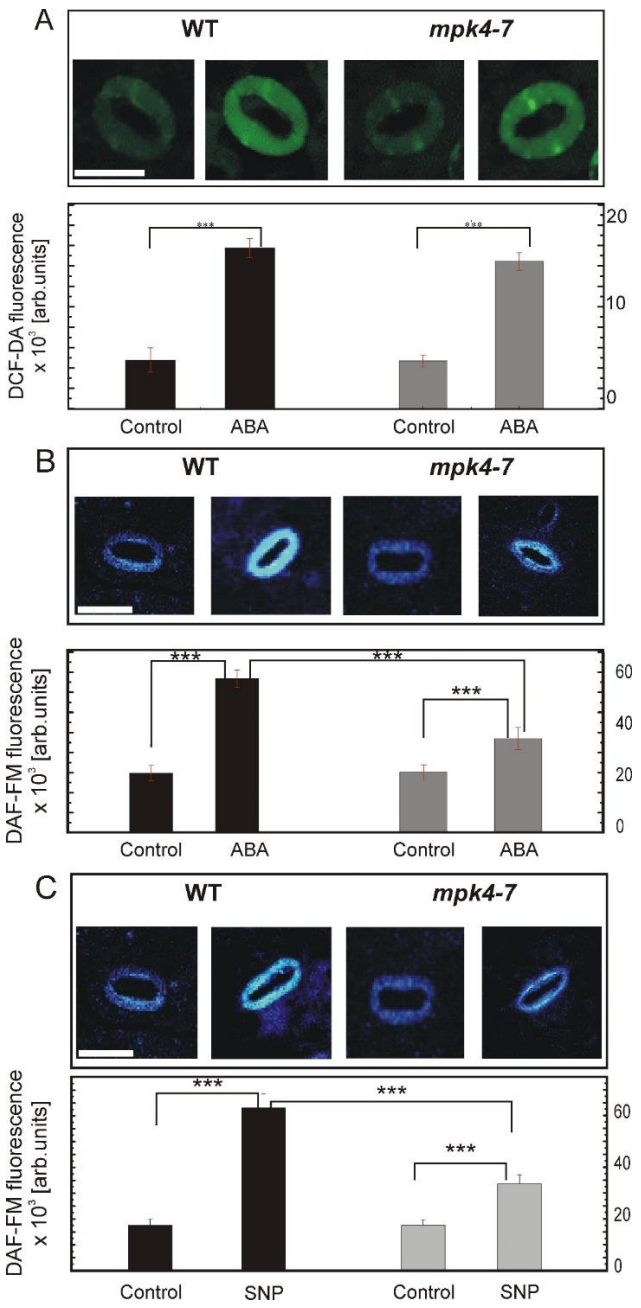
Supplemental Figure S3. No antibody control for Fig. 2. A control was prepared without primary antibody. Scale bars: 5 μm. Presented pictures are representative for 10 analysis.



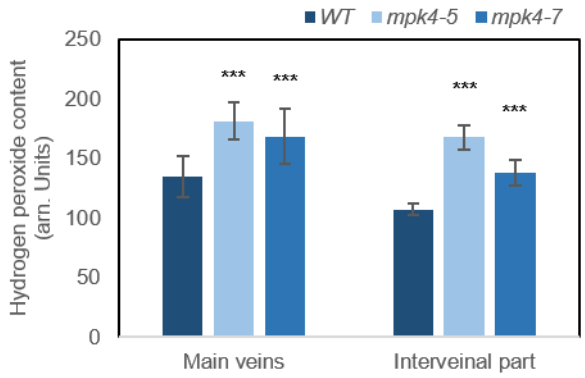
Supplemental Figure S4. Anti-tubulin antibody for microtubule distribution in wild type (WT) and transgenic *mpk4-7* guard cell wall. Scale bars: 5 μm. Presented pictures are representative for 10 analysis.



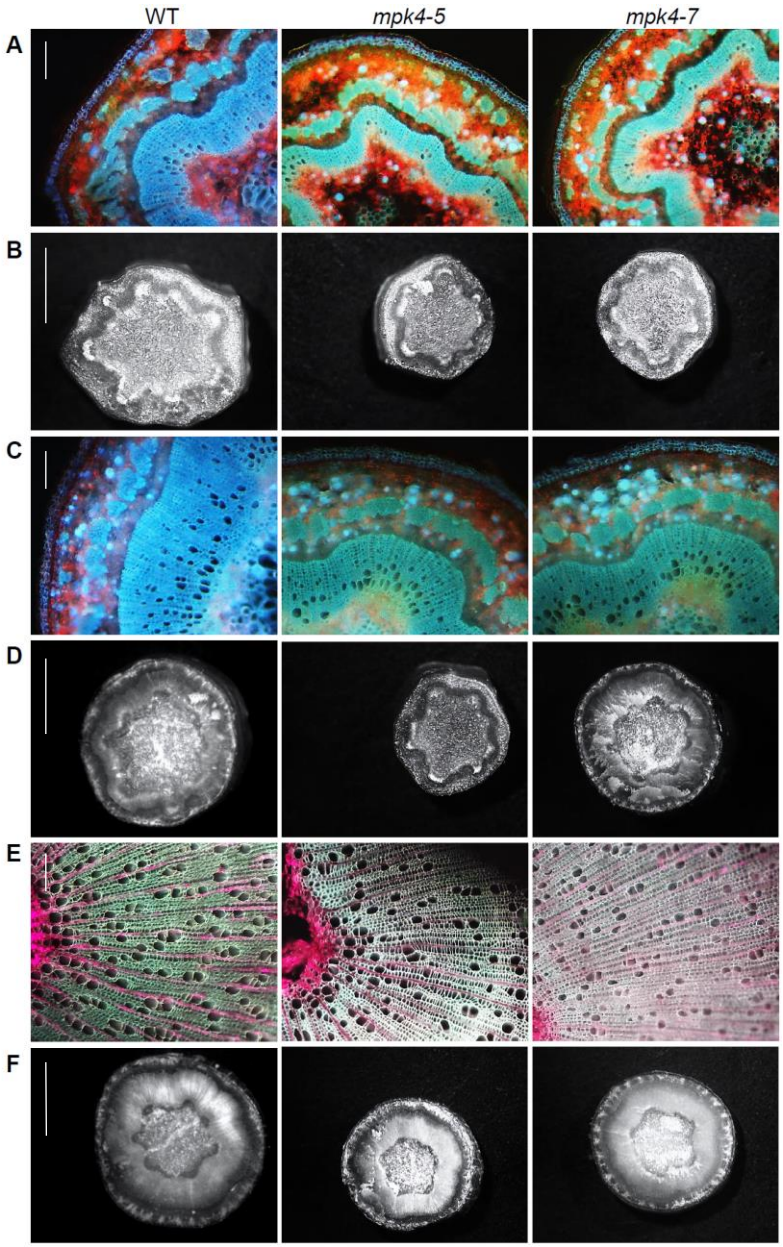
Supplemental Figure S5. *MPK*4 expression under environmental control. (A) The distribution of cis-regulatory elements (CREs, specific non-coding DNA motifs) in the 1 kb of 5' promoter region of *MPK*4 from *P. trichocarpa*. The CREs are located in the promoter region upstream of a coding sequence, positions are with respect to the first base of the translation start site (ATG). The presence of various motifs responsible for the regulation of gene expression under different environmental stimuli was predicted and their positions are marked on the figure. Analysis of *MPK*4 promoter sequence allowed to identify CREs composed of 5–20 nucleotides that were localized in the proximal promoter i.e. several hundred nucleotides upstream of a transcription start site (TSS) of *MPK*4 gene. The distribution of CREs was random, however, CREs frequency was highly enriched at 100-250 bp upstream of the TSS (high and low temperature, CO_2, and light). **(B)** *MPK*4 expression pattern in response to different environmental stimuli in WT plants after 2 h-treatment of leaves with different environmental factors: cold (4 °C), high CO_2 (CO_2; 2000 ppm), dark, high temperature (HT, 42 °C), red-orange light (RL, 617 nm, Light 2), and red-orange supplemented with blue royal light (RL+BL, 447 nm, Light 3). Moreover, *MPK4* transcript abundance was also measured in leaves exposed by 1 h to high light intensity (HL, 2000 µmol of photons m^{-2} s^{-1}, Light 1). 21 °C, and ambient CO_2 were used as control conditions. Expression level is presented relatively to WT control plants, referred to 1. Bars represent means ± SD (n = 6). The asterisks indicate significant differences from the WT revealed by the Tukey HSD test (***) $P < 0.001$.



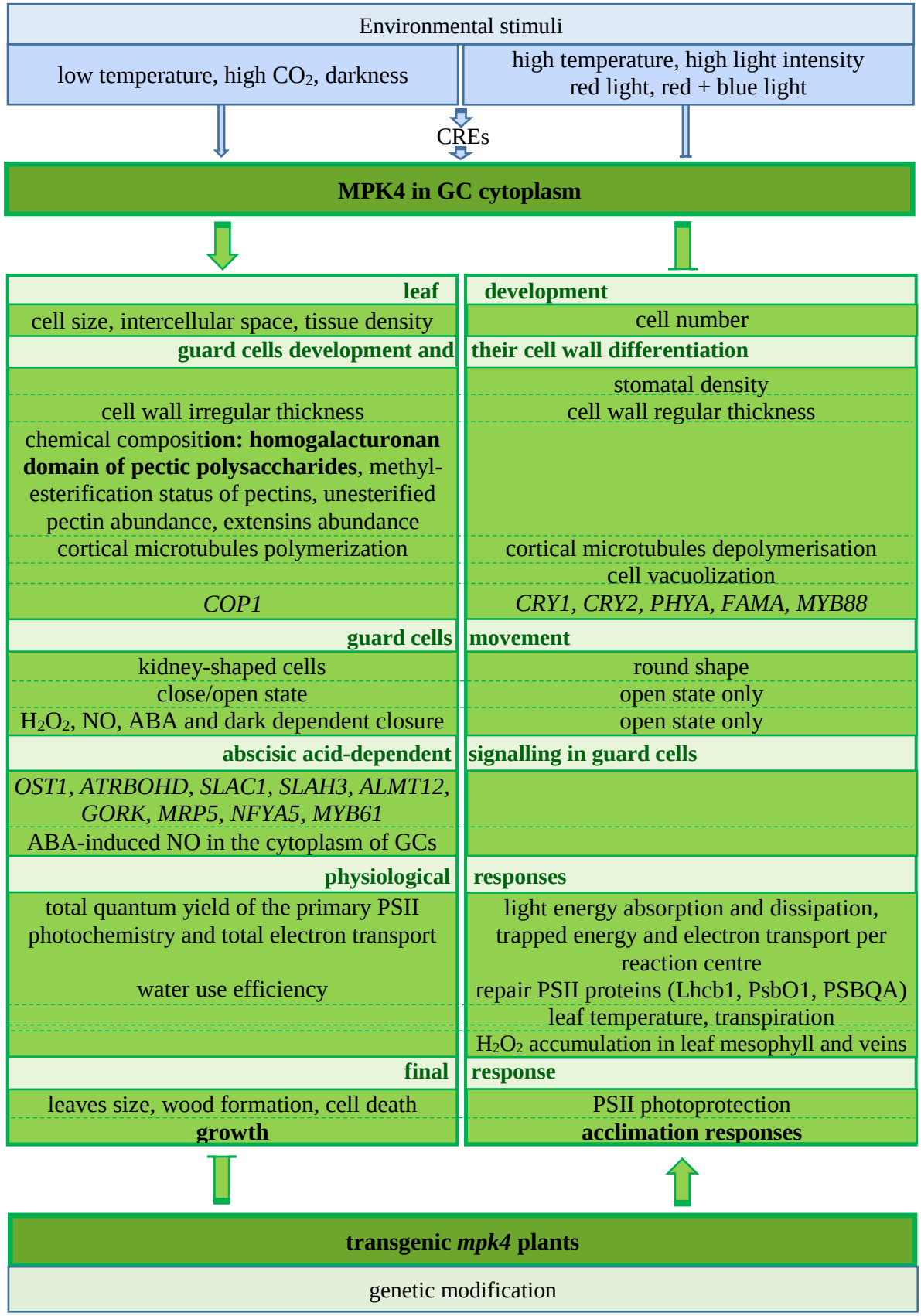
Supplemental Figure S6. Unaffected ABA-induced H_2O_2 accumulation in transgenic *mpk4-7* guard cells, and impaired ABA- and SNP-induced NO production in *mpk4-7* guard cells, when compared to wild type (WT) guard cells. To examine whether MPK4 acts upstream or downstream in H_2O_2- and NO-dependent signal transduction pathways and ABA-triggered H_2O_2 and NO production, fluorescent dye 2′,7′-dichlorofluorescin diacetate (DCF-DA) to detect H_2O_2 level and diaminofluorescein (DAF-FM) to detect NO level in GCs were used. **(A)** H_2O_2 production in response to ABA treatment examined with DCF-DA analyzed microscopically. Samples treated with ABA-free solution were used as control. (**B** and **C**) NO production in response to ABA and SNP (NO donor) treatments examined with DAF-FM. Samples treated with ABA- and SNP-free solution were used as a control. Scale bars: 30 μm. Presented values are means ± SD (n = 6). The asterisks indicate significant differences from the WT revealed by the Tukey HSD test (***) $P < 0.001$.



Supplemental Figure S7. Hydrogen peroxide content in main veins and interveinal part of the leaf. Content was determined with 3,3'-diaminobenzidine (DAB) staining and ImageJ software (v. 1.52a, 32 bits photo, greyscale from 0 (black) to 256 (white), NIH USA) ± SD (n = 18-30). The value of hydrogen peroxide content is calculated as function 256 minus the read value to express proportional relationship. The asterisks indicate significant differences from the WT revealed by the Tukey HSD test (***) $P < 0.001$.



Supplemental Figure S8. Growth of *mpk4* plants. Cross-section of stem was performed in wild type (WT), and transgenic *mpk4-5, mpk4-7* plants. (**A, C, E**) Cross-sections were made at the distance from the apex of the plant: 1 cm, 10 cm, 30 cm, respectively, and photographed under fluorescent microscopy (UV-2A, EX330-380) at magnitude: 100x, scale bar: 100 μm. (**B, D, F**) Cross-sections were made at the distance from the tip of plant: 10 cm (magnitude 15x, scale bar: 0.6 cm), 30 cm (magnitude 15x, scale bar: 0.6 cm), 50 cm (magnitude 10x, scale bar: 1cm), respectively, and photographed under lupa binocular.



Supplemental figure S9. List of acclimation and growth responses / mechanisms affected by MPK4.

Supplemental Table S1. Cell number, area, and the circularity index in wild type (WT), and transgenic *mpk4-5,* and *mpk4-7* plants. The parameters were measured with ImageJ v.1.52a and presented relatively to WT, referred as 100. The asterisks indicate significant differences from the WT revealed by the Tukey HSD test (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$.

		Area		**Cell number**		**Circularity index**	
	WT	*mpk*4-5	*mpk*4-7	*mpk*4-5	*mpk*4-7	*mpk*4-5	*mpk*4-7
Adaxial epidemis	100.00	46.88 *	79.13	150.00 *	133.33	120.17 *	111.40
Palisade parenchyma	100.00	41.88 ***	69.69 *	150.00	121.43	102.36	98.99
Spongy parenchyma	100.00	78.59 *	55.01	140.00	160.00	126.23	112.86
Abaxial epidermis	100.00	50.74 *	38.86 **	144.44	133.33	116.79	118.22

Supplemental Table S2. *In silico* analysis of the cis-regulatory elements (CREs) distribution in the *MPK*4 promoter of *P. trichocarpa* under different environmental stimuli. The 1 kb promoter region was analyzed for the presence of motifs responsible for the regulation of gene expression under low temperature, high CO_2, drought, high temperature, high light. *ID/IUPAC – Identification code in International Union of Pure and Applied Chemistry.

Related function	ID/IUPAC*	Organism described	Position	Strand
Low temperature responsive element	MYCCONSENSUSAT	*A. thaliana*	602	+
			602	-
			155	+
			155	-
	LTREATLTI78	*A. thaliana; H. vulgare*	146	+
	CBFHV	*H. vulgare*	146	+
	LTRECOREATCOR15	*A. thaliana; B. napus*	145	+
CO_2 responsive element	EECCRCAH1	*Ch. reinhardtii*	677	-
			191	-
Drought responsive element	MYBATRD22	*A. thaliana*	756	+
	MYB1AT		755	+
	DRE2COREZMRAB17	*Z. mays*	146	+
High temperature responsive element	HSE	*B. oleracea*	169	-
			331	-
			673	-
			975	-
Light responsive element	Sp1	*O. sativa*	137	-
		Z. mays	111	+
		Z. mays	116	+
	-10PEHVPSBD	*H. vulgare*	981	-
	MRE	*P. crispum*	760	+
	Box-I	*P. sativum*	865	+
	GT-1 CONSENSUS	*O. sativa; N. tabacum; A. thaliana; S. oleracea*	970	-
			650	-
			475	-
			233	+
			206	+
			4	+

Supplemental Table S3. *In silico* analysis of the *cis*-regulatory elements (CREs) distribution in the *MPK*4 promoter of *P. trichocarpa* under different hormonal stimuli. The 1 kb promoter region was analysed for the presence of motifs responsible for the regulation of gene expression under gibberellin, auxin, salicylic acid stimuli. *ID/IUPAC – Identification code in International Union of Pure and Applied Chemistry.

Related function	ID/IUPAC	Organism described	Position	Strand
Gibberellin responsive element	GARE-motif	*B. oleracea*	415	+
Auxin responsive element	TGA	*B. oleracea*	959	-
Salicylic acid responsive element	TCA	*N. tabacum*	850	+

Supplemental Table S4. The promoter sequence upstream of the coding sequence of *MPK*4. (*XM_002302563.2*).

> POPTR_0002s16400| Chr02:12265644..12270287 reverse

AAGTCTAGAGTTTGTATTAGAATAATTAAATTATCATGATTAAAGATGT
CGAGGTTATTTATTTTTGTGTTTTAATTTTTTTTTTAAATTAAATTTTATT
TATTTATTTATTTGCTTAAAATATTTTTTTGTTGTTTTCAAATATTTTAAT
ATATCTATGTTAAAAATAAAATTTTAAAAATAAAAAATATTTTTTTAAA
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ATAAAAACAATATAAAAAAGGTCAGCAATTCTCGAGGCACGAGGGCA
AAGCTTTACCTGTTCAAAGGTTCACCCTACACATAAACGGTTCAAAGA
CGTTCAAATGTTATTTATTTATTTATTAAAATAATATATTTTTTAAAATA
TTTATTTTTAATATTAGTATATAAAAAGCAATCCAAATATATATATATA
TATATATATAAATAAAATATAAAGCAAGTTTTTTTATCAAATTTTAAAA
AAAATTGCTGGGAACAAAAAATAAATTTTATGACCTGTTTAAAAATAT
AATAATTTTTTTTAATTTTTTTTTTAAAATATATTAAAATAAAATAATTTT
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TTAATTTAAAAATAAAAAATTTAAATTTCTTTAAAAATATGGTTGAATC
CTATAATATACTAGATATATGGTAACAGTGGTAATAAATAAATTAATT
AATAAAAAGAAAAACTCCCACGCGCAAATCAAAAGAAATGTCAAAAC
GACAAAACTTTACACCTGCCTACCGACACGCCCGCCCCAAATATTAGC
CCTTCCTCCCCTCCCCTCTGCTTTCTACTTCATCAACAACTTTAGTACTG
GACGGACTGCTTAATCACCTCTCTCTCTCTAGCTTGTAATCGGATCCTT
ACTAGTTGCTGCTACTGAAAA

Supplemental Table S5. List of primers used in RT-qPCR.

Gene name	Accession	Primer sequence (5'→ 3')	Reaction efficiency
UBQ	POPTR_001G418500	F: GTTGATTTTTGCTGGGAAGC R: GATCTTGGCCTTCACGTTGT	0,856
ACT2	POPTR_0001s31700	F:TTCTGGTGATGGTGTGTCTCA R:GACCAGCAAGATCCAAACGAA	0,965
COP1	POPTR_0014s15740	F:GCCCTTGCTGTAGCCATTAC R:GACATGAGGGTGTCCAGGTC	0,898
CRY1	POPTR_0002s09730	F:CTCCAATTGCCTTCCCTCAAG R: CCTCATAGCGGCGATTTGTAG	0,864
CRY2	POPTR_0005s17100	F:GGAAAACCATGAGCCTGTGAG R:AGGAAGATGTCATGCTTGGGA	0,870
PHYA	POPTR_0013s00220	F:ACATCCTGTTCTTGGCATTGG R:GAAACATCCCCGAATCCCATG	0,856
FAMA	POPTR_0017s08160	F:TGGCTGATGTTGAAGTGAAGC R:GTGCTGCAATGGTCTTACTCA	0,890
MYB88	POPTR_0010s10310	F:GGAGACAGATCACACCCACAA R:GGGAAGCCACGTTAACATTGT	0,932
MYB61	POPTR_0005s00340	F: TTTGCAGAGGTGTGGAAAGA R: CCTGCTGTGAAAATGCTCCT	0,892
NFYA5	POPTR_0006s14740	F: GGGACCAATTCACAAGGTCA R: GAGAACTGGCCTCATTTTGC	0,880
ALMT12	POPTR_0001s02700	F: CGTCATGACTGTTGTTGTTGTG R: CCCTGCTAAGAGGGTTCCTAAG	0,936
MRP5	POPTR_0014s18670	F: ATCAAGCTTTCTCTCCTGCATC R: TGACTGTGAGACATAAGCAGCA	0,925
OST1	POPTR_0004s15270	F:GGAGACCGGTATGAGTTAGTGAAA R: CTGTTTATCCCTCATCAACCTAGC	0,886
SLAC1	POPTR_0005s20240	F: CGTTCGCAAGAAGAACAGC R: TTGCTTCTCAATTGTGCCTTT	0,892
SLAH3	Potri.015G026700	F: GCTGCCTTTGAGAACCAAAC R: CAAGGTGGCAAGCTGATGTA	0,884
RBOH D	POPTR_0003s15810	F: GCTTCTACCAGTGGCCTACTTC R: TAATTCTCCGGCAAATTCCTTA	0,912
GORK	POPTR_0017s02430	F: CTGAGTCGTGAATCGGTCATTA R: AATTTTGTCCATGCTCGATACC	0,911
HSP70	POPTR_0010s21350	F: AGCCCTGATCAAATCAATCG R: TCAACAAAACCCCGAACAAT	0,889
HSP90	POPTR_0017s01160	F: CTGATTTGCTCCGGTACCAT R: TTCCACTGCCTTCTTGCTTT	0,931
HSFA1	POPTR_0001s02140	F: CCCCATGTTTCTCAAATGCT R:CCATCATTGCATGTCTCTGG	0,965
LHCB1.2	POPTR_0005s26080	F: CCGCATCATCAGCTAAACAA R: AGAGCTTCACTGCCTTTCCA	0,919
LHCB2.3	POPTR_0014s16300	F: CTCTGAGCAAACCCCATCAT R: GATCTGCAGACAAACCAGCA	0,908
LHCB5	POPTR_0019s09140	F: AAATTTGGAGCCAACTGTGG R: GGTTGATGGGGATGTTCTTG	0,908
PSBO1	POPTR_0005s13860	F: AGTGCTGAAGGAGTCCCAAA R: GGCTTGAAGGCAAATGACTC	0,916
PSBQA	POPTR_0004s03160	F: GAATGTGCCTAGCAACACCA R: AAGAACCAGAAGCCAAACCA	0,906
MPK4	POPTR_0002s16400	F: AAGAAGATTGGTAATGCATTTGA R:TAGCAATAACATTTTCATGATCCA	0,881