Guard cells control hypocotyl elongation through HXK, HY5 and PIF4

Gilor Kelly[1], Danja Brandsma[1], Aiman Egbaria[1,2], Ofer Stein[1], Adi Doron-Faigenboim[1], Nitsan Lugassi[1], Eduard Belausov[1], Hanita Zemach[1], Felix Shaya[1], Nir Carmi[1], Nir Sade[2] and David Granot[1*]

[1]*Institute of Plant Sciences, Agricultural Research Organization, The Volcani Center, Rishon LeZion 7505101, Israel*

[2]*School of Plant Science and Food Security, Tel Aviv University, Tel Aviv, Israel*

***Corresponding author:** David Granot, granot@agri.gov.il

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Supplementary Table 1. qPCR Primers used in this study

Gene	Primer sequence	Accession No.
TUB2 f (a)	AAACTCACTACCCCCAGCTTTG	At5g62690
TUB2 r	CACCAGACATAGTAGCAGAAATCAAGT	
HXK1 f (a)	GCCTTTGAAGAGGATTGTGC	At4g29130
HXK1 r	CATGACACGGAAGTTTGTCC	
HY5 f	TCAGAACGAGAACCAGATGC	AT5G11260
HY5 r	GAAGGAGATCAAAGGCTTGC	
HY5 f	TCAGAACGAGAACCAGATGCT	AT5G11260
HY5 r	CAGCATTAGAACCACCACCA	
PIF1 f	GGAGCTTTTATGGCAGAACG	AT2G20180
PIF1 r	AAATCATCGTCACGGAGAGG	
PIF3 f	ACGGACCACAGTTCCAAGTC	AT1G09530
PIF3 r	ATTGCCAATCGTCGAAGAAC	
PIF4 f	TCCTCAAGTCATGCCTCCTC	AT2G43010
PIF4 r	TAGGTCCAACGGTCGTTACC	
PIF5 f	TCAAAACCCGGTACAGTTGC	AT3G59060
PIF5 r	ACGGTCTGCATCTGATTTCC	
SAUR50 f (b)	CACTTCCCTGTCTATGTCGGA	AT4G34760
SAUR50 r	TCTTCCTCGGCTCGTTGTAA	
SAUR65 f (b)	AACAAAGAGCTGCCCTCAAGA	AT1G29460
SAUR65 r	AAACAGCCCTTCTCCACAGC	
AtEXP8 f (c)	CTCTTTCCGAAGAGTACCATGT	AT2G40610
AtEXP8 r	GTGTACGTCTCCTGCTCCTC	
YUC8 f (c)	CGATGAGACCAGTGGCTTGT	AT4G28720
YUC8 r	TTTTCTCCCGTAGCCACCAC	
XTR7 f (c)	CGGCTTGCACAGCCTCTT	AT4G14130
XTR7 r	TCGGTTGCCACTTGCAATT	
PHYB f	ACCGTCGTCAAGTCACACTC	AT2G18790
PHYB r	TGATTCGCCGGATTGTTCGA	
COP1 f	GGTACTCGCTGCGTGATTC	AT2G32950
COP1 r	GAGGAGTGTTCCAAGGCAAG	
DET1 f	TTCCACAACCACCCTACCTC	AT4G10180
DET1 r	GTAGCGAAAAGACCGAAACG	

(a) Primers adapted from Kelly *et al.* (2012)

(b) Primers adapted from Sun *et al.* (2016)

(c) Primers adapted from Gangappa *et al.* (2017)

Supplementary Table 2. Primers used for mutant genotyping

Gene	Accession No.	Primer sequence	Method
***pif4-101* (d)**	AT2G43010	LP-AATACATTTTGCAGGCAATCG RP-CGTAATGAAGTTGCACGTTTACTC LB3-TAGCATCTGAATTTCATAACCAATCTCGATACAC	PCR
Cop1-4 (d)	AT2G32950	GCCACATGAGAAGAACCAGATTG TGAGTAGACAAGGAGGAACAAACC	Sequencing
hy5	AT5G11260	LP-ATTCCTTCCCAAAATGTCTCG RP-ATGCGAGTGAATGACCATTTC LBaI TGGTTCACGTAGTGGGCCATCG	PCR

(d) Primers adapted from Pacin *et al.* (2016)

Supplementary Table 3. Primers used for cloning and transgenic plants identification

Promoter/gene	Primer sequence
GCHXK	TCTCAACAAATTCCCCTTGC CATGACACGGAAGTTTGTCC
GCHXK2 hemi/homo zygoute	ACCAACCACCAACTAACCTCA GCTCCCTGGATACAACGACA TGCCTGCTTGCCGAATATCA
35SHXK	CAACCACGTCTTCAAAGCAA CATGACACGGAAGTTTGTCC
35SFRK1	CAACCACGTCTTCAAAGCAA AACATACGGCCGAACTCATC
35SFRK2	CAACCACGTCTTCAAAGCAA ACGATGTTTCTATGCTCCTCCCT
GCHY5	TCTCAACAAATTCCCCTTGC ACCACCTCCTCTCTTGTTTCC
35SHY5	CAACCACGTCTTCAAAGCAA ACCACCTCCTCTCTTGTTTCC
GCPIF4	TCTCAACAAATTCCCCTTGC ACCGGGATTGTTCTGAATTG
GCHY5-GFP	TCTCAACAAATTCCCCTTGC ACCACCTCCTCTCTTGTTTCC ACTGGGTGCTCAGGTAGTGG

Supplementary Table 4. Accession numbers and probe ID numbers of *AtHY5*, *AtHYH* used in the microarray database analysis

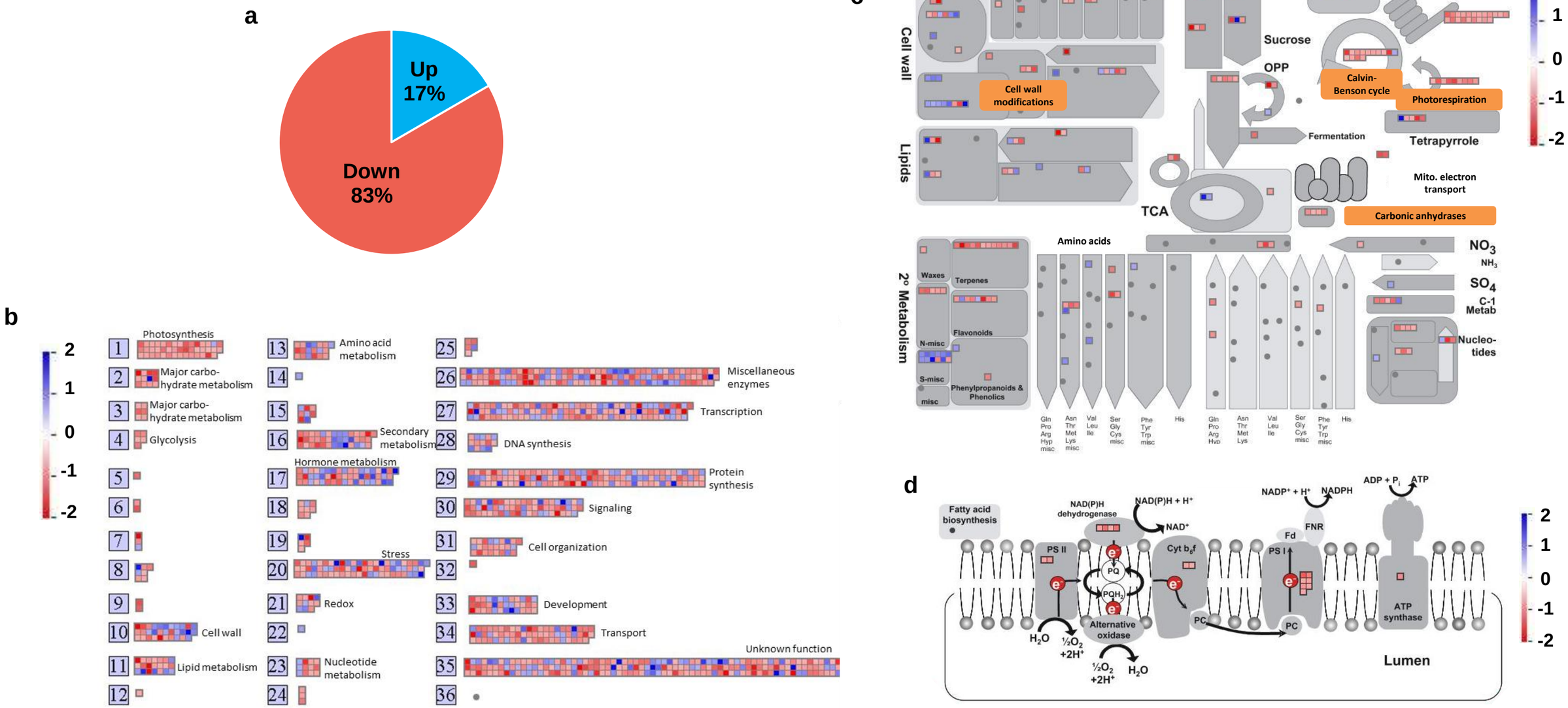
Gene name	Accession	Probe set ID
AtHY5	AT5G11260	250420_AT
AtHYH	AT3G1769	258349_AT

Supplementary Table 5. Optimized LC-MS-MS parameters for quantification of plants hormone.

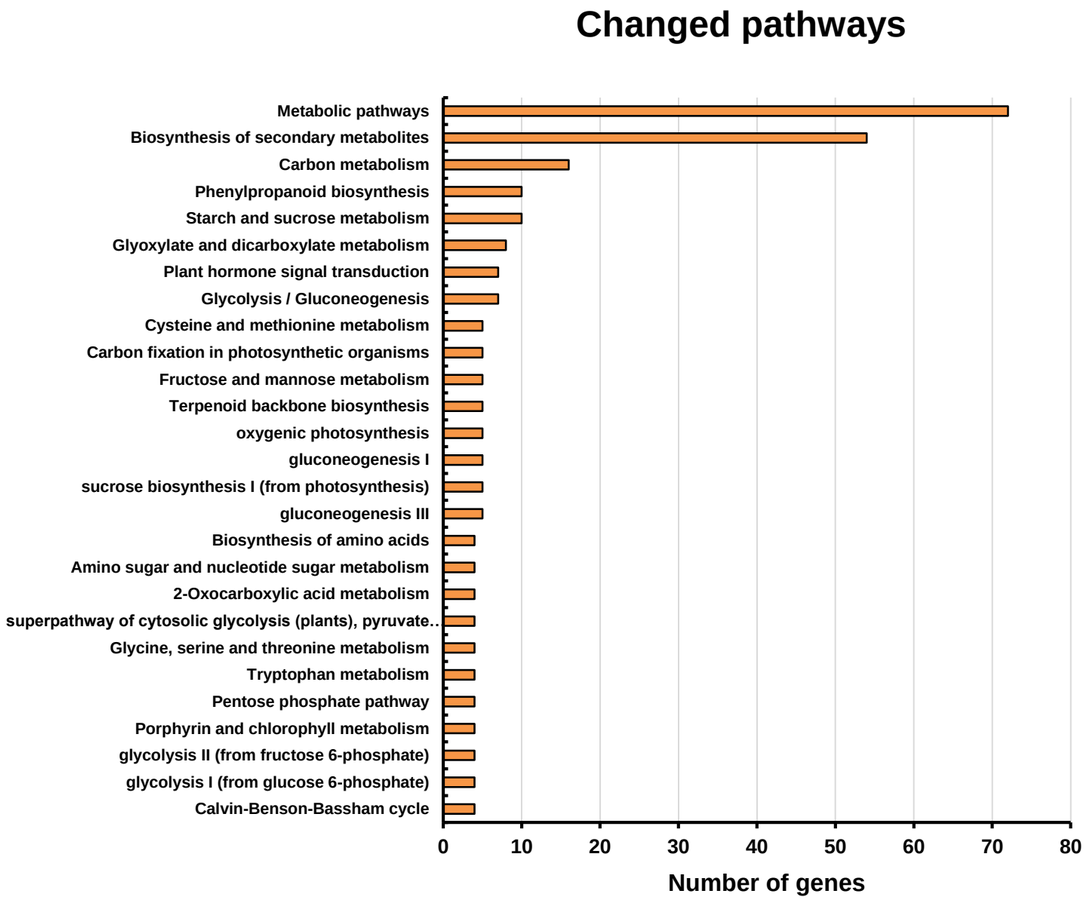
Analite and IS	Retention time (min)	Ionization mode	MRM transition (m/z)	Dwell time (msec)	Cone (V)	Collision (V)
IAA	7.50	+	176>130	70	18	26
			176>103			28
D5-IAA	7.42	+	181>134	70	20	16
			181>106			30
IAAsp	5.50	+	291>134	100	20	12
			291>130			20
DN-IAAsp	5.40	+	297>136	100	20	12
			297>134			22
IAGlu	5.77	+	305>148	100	22	12
			305>130			16
DN-IAGlu	5.81	+	311>150	100	20	12
			311>134			20
OxIAA	5.18	+	192>146	50	14	14
			192>128			24

Supplementary Table 6. Solvent gradient program for auxins

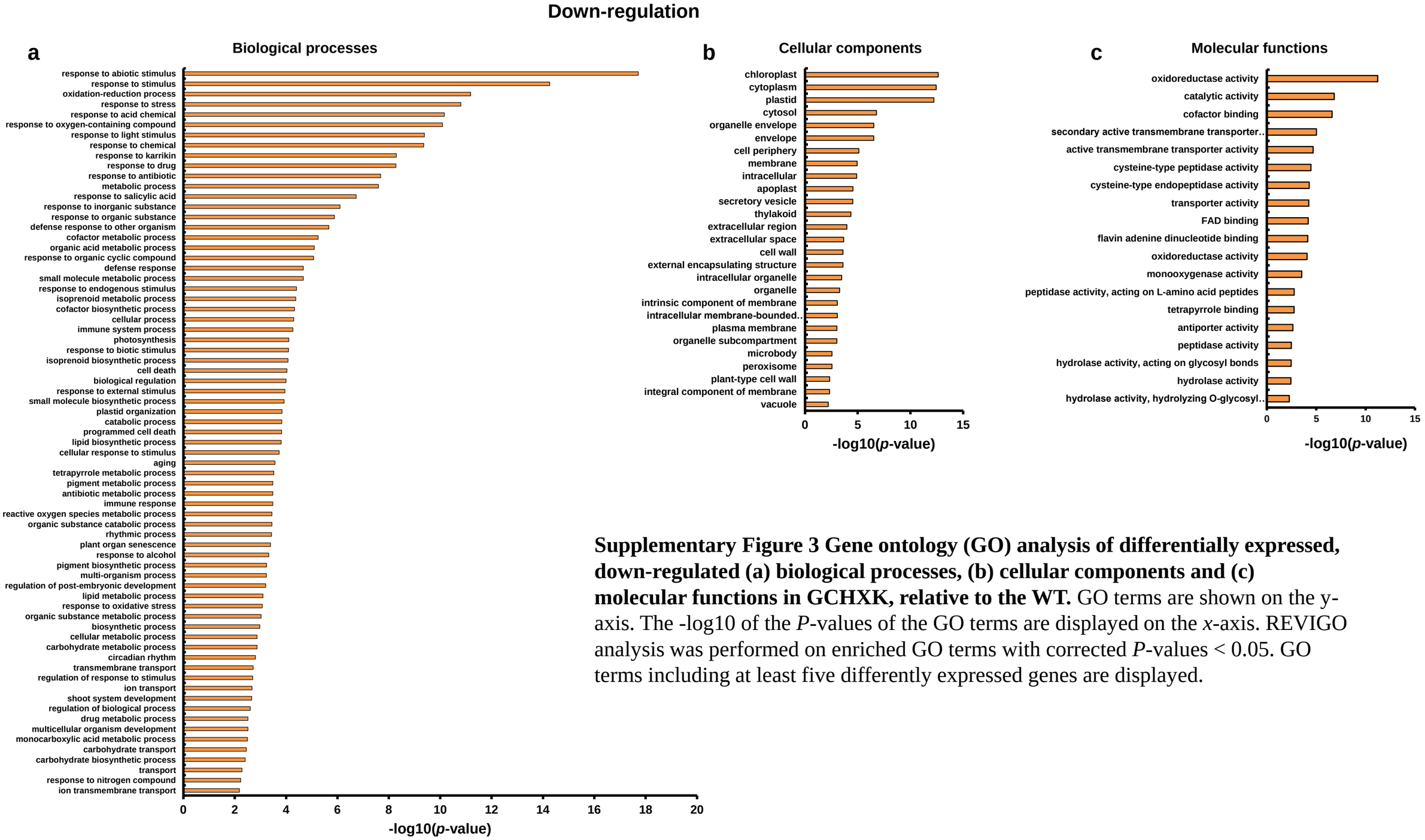
Time (min)	Phase A %	Phase B %
Initial	95	5
0.5	95	5
14	50	50
15	5	95
18	5	95
19	95	5
22	95	5



Supplementary Figure 1 Genes differentially expressed in GCHXK transgenic seedlings relative to the WT. Transcriptome analysis of 4-day-old GCHXK and WT seedlings. **a** The total number of transcripts that were up-regulated (blue) and down-regulated (red) in GCHXK relative to WT. **b** MapMan functional overview of transcript level changes in GCHXK seedlings relative to the WT. Genes are grouped into functional categories. **c** MapMan metabolism overview showing transcript level changes in GCHXK seedlings relative to WT. Light reactions, Calvin cycle, carbonic anhydrases and cell-wall groups are highlighted in orange. **d** GCHXK-regulated genes in the photosynthetic light reaction, as visualized by MapMan. **b-d** Genes up-regulated by GCHXK are stained blue and genes down-regulated by GCHXK are stained red.

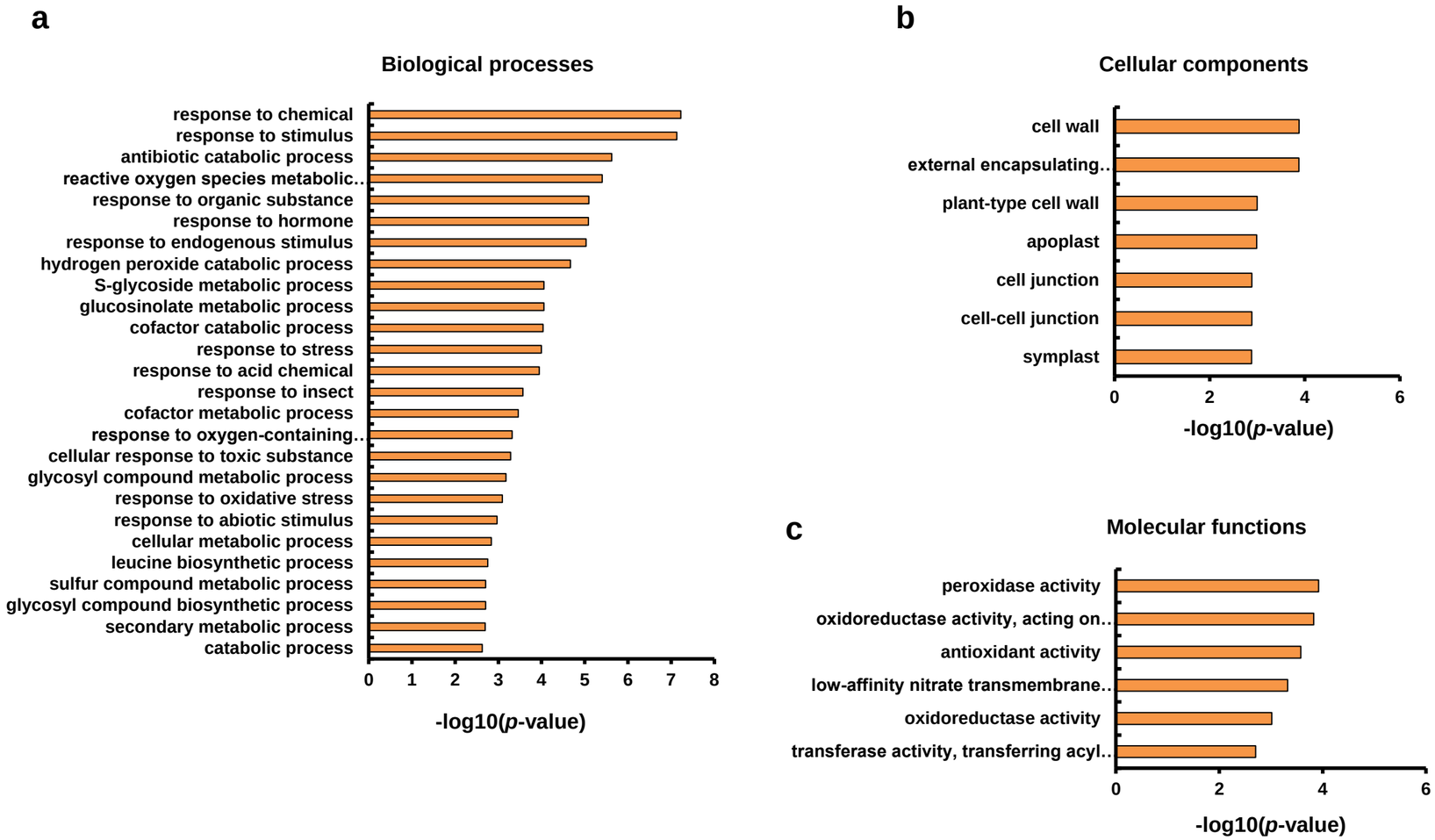


Supplementary Figure 2 Pathways changed by GCHXK. Pathways changed in GCHXK, relative to the WT, displayed by the number of genes significantly changed for each pathway. Changed pathways were analyzed by enrichment analysis using KOBAS (based on the KEGG PATHWAY and BioCyc databases). Pathways with more than three changed genes are displayed.

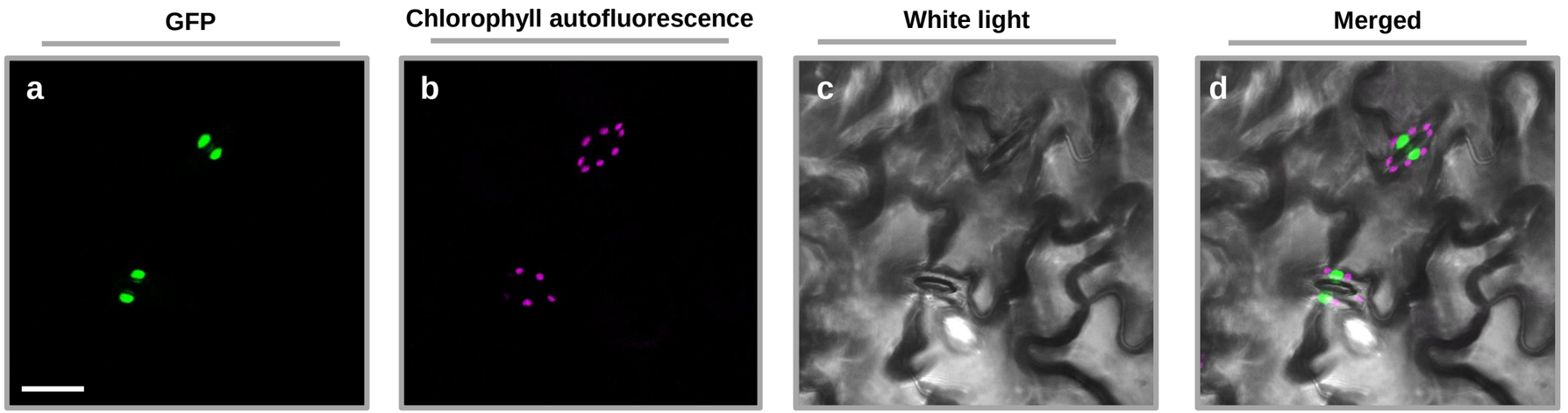


Supplementary Figure 3 Gene ontology (GO) analysis of differentially expressed, down-regulated (a) biological processes, (b) cellular components and (c) molecular functions in GCHXK, relative to the WT. GO terms are shown on the y-axis. The -log10 of the *P*-values of the GO terms are displayed on the *x*-axis. REVIGO analysis was performed on enriched GO terms with corrected *P*-values < 0.05. GO terms including at least five differently expressed genes are displayed.

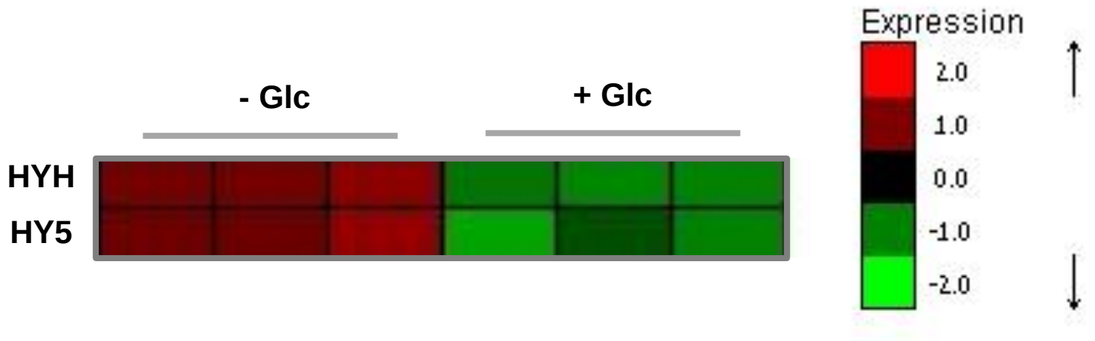
Up-regulation



Supplementary Figure 4 Gene ontology (GO) analysis of differentially expressed, up-regulated (a) biological processes, (b) cellular components and (c) molecular functions in GCHXK, relative to the WT. GO terms are shown on the y-axis. The -log10 of the *P*-values of the GO terms are displayed on the x-axis. REVIGO analysis was performed on enriched GO terms with corrected *P*-values < 0.05.



Supplementary Figure 5 Distribution of GCHY5-GFP and chlorophyll autofluorescence in the epidermis of young seedlings. Representative confocal images of GCHY5-GFP epidermis taken from the cotyledon of a 7-day-old seedling. **a** GFP fluorescence (stained green). **b** Chlorophyll autofluorescence (stained magenta). **c** White light. **d** Merged image of **(a-c)**. Bar = 20 µM.



Supplementary Figure 6 HY5 and HYH expression in response to glucose. Data were obtained from the NASCArrays microarray database, Experiment No. 593, in which 7-day-old seedlings were exposed to 3% glucose for 6 h, followed by microarray analysis. The colors red and green represent induced or reduced expression, respectively, and the intensity of the color indicates the strength of expression. The accession number and the Affimetrix probe ID number for each gene are listed in Table S4.