

Supplementary Materials

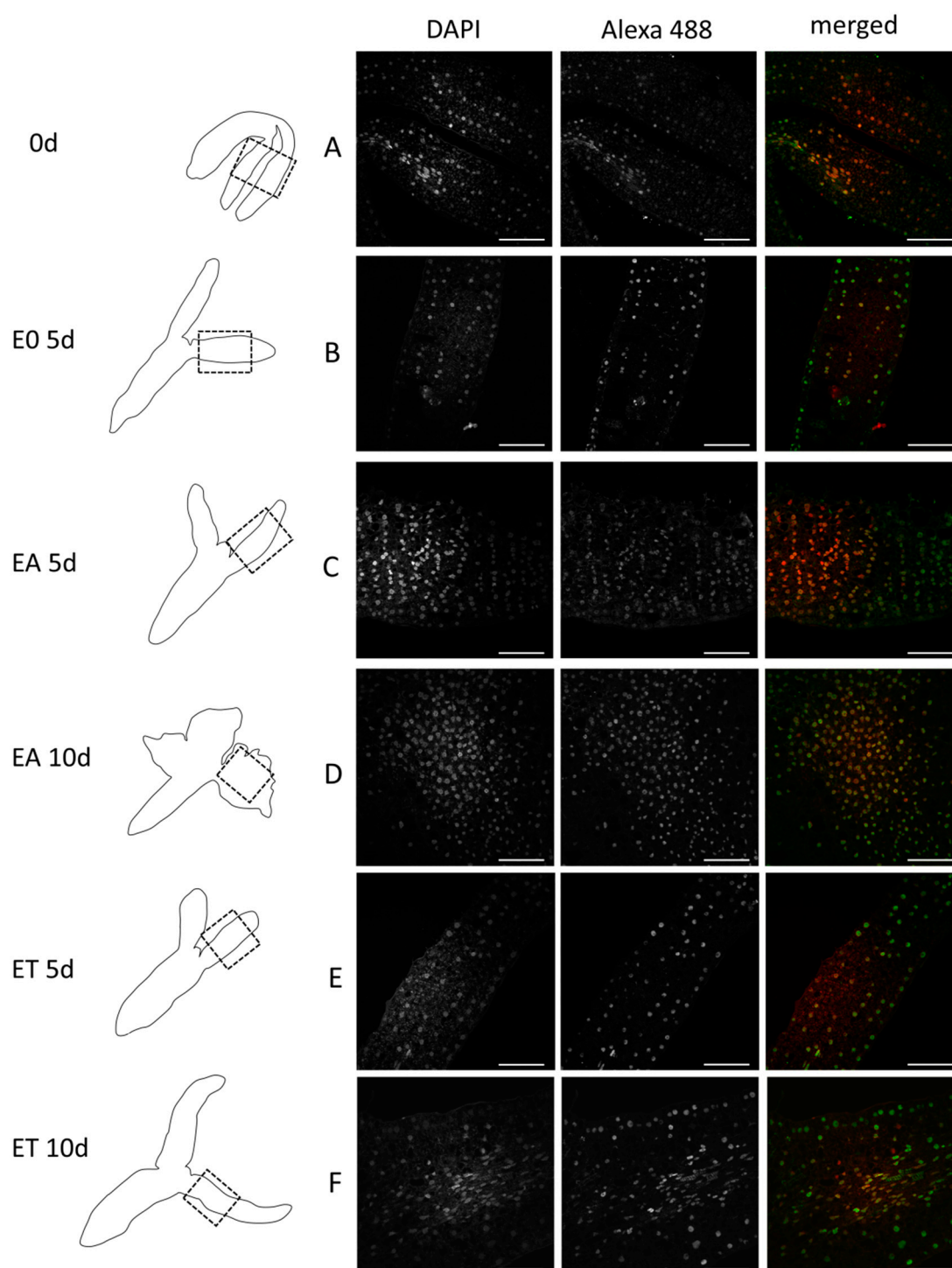


Figure S1. The immunodetected spatio-temporal pattern of H3ac in the cotyledons of the Col-0 explants. The explants were freshly isolated (0 d) (A) and *in vitro* cultured for 5 and 10 days on different media, including E0 (B), EA (C, D) and ET (E, F). Dashed line squares indicate explant areas in which Alexa 488 (H3ac) and DAPI (DNA) fluorescence intensity were measured. Red (computer altered)—DAPI; green (computer altered)—Alexa 488 (immunostaining of H3ac). Scale bar represents 50 μm.

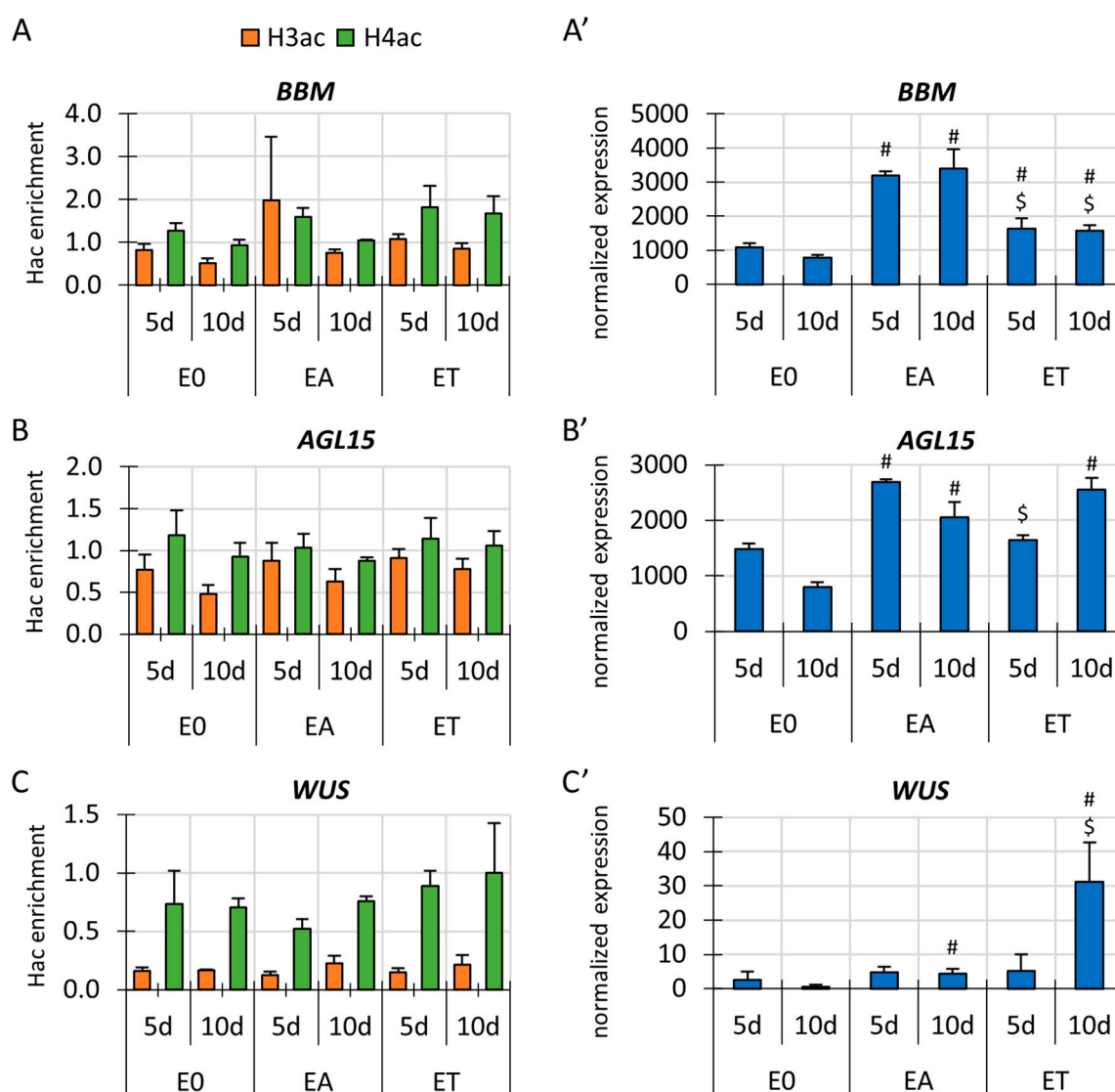


Figure S2. The H3ac and H4ac levels and expression profiles of *BBM* (A,A'), *AGL15* (B,B') and *WUS* (C,C') in the Col-0 explants that had been cultured on the E0, EA and ET media for 5 and 10 days. Hac enrichment indicates the amount of DNA after ChIP that was normalised to the internal control (*ACTIN7*). ChIP-qPCR: a two-way ANOVA analysis ($p < 0.05$) followed by Tukey's HSD test ($p < 0.05$) were used to determine the values that were significantly different from the E0 culture at the same age (#); the EA culture at the same age (\$) ($n = 3$; means are given \pm SD). RNA-seq: Wald's exact test was used to identify any differentially expressed genes (DEGs) under the p -value adjustment ($p < 0.05$) for multiple comparisons with the Benjamini-Hochberg False Discovery Rate (FDR) correction. Values that were significantly different from the E0 culture at the same age (#); the EA culture at the same age (\$) ($n = 3$; means are given \pm SD).

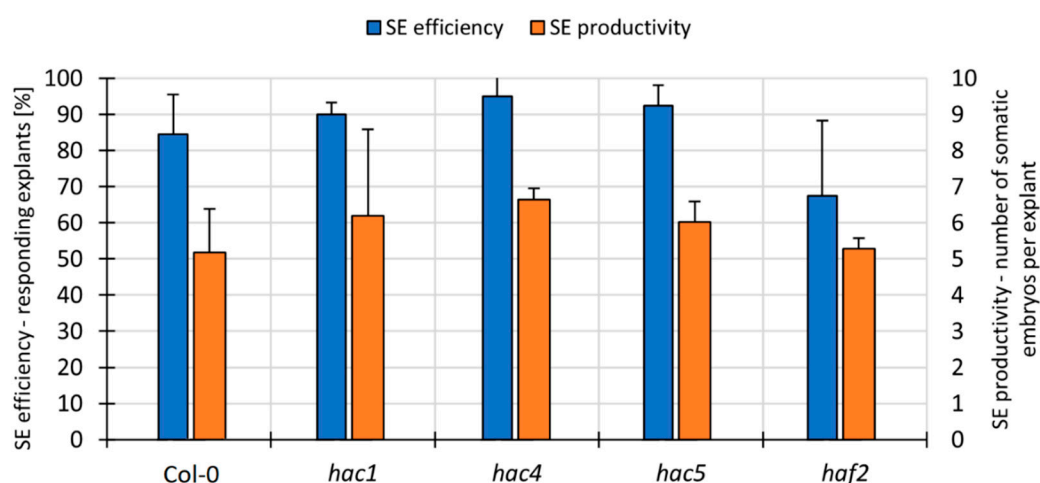


Figure S3. The embryogenic capacity of the *hat* mutants on the ET medium, evaluated in the 21-day-old culture. No significantly different values from the control WT culture (Col-0) were observed ($n \geq 3$; means \pm SD are given) (Student's t-test, $p < 0.05$).

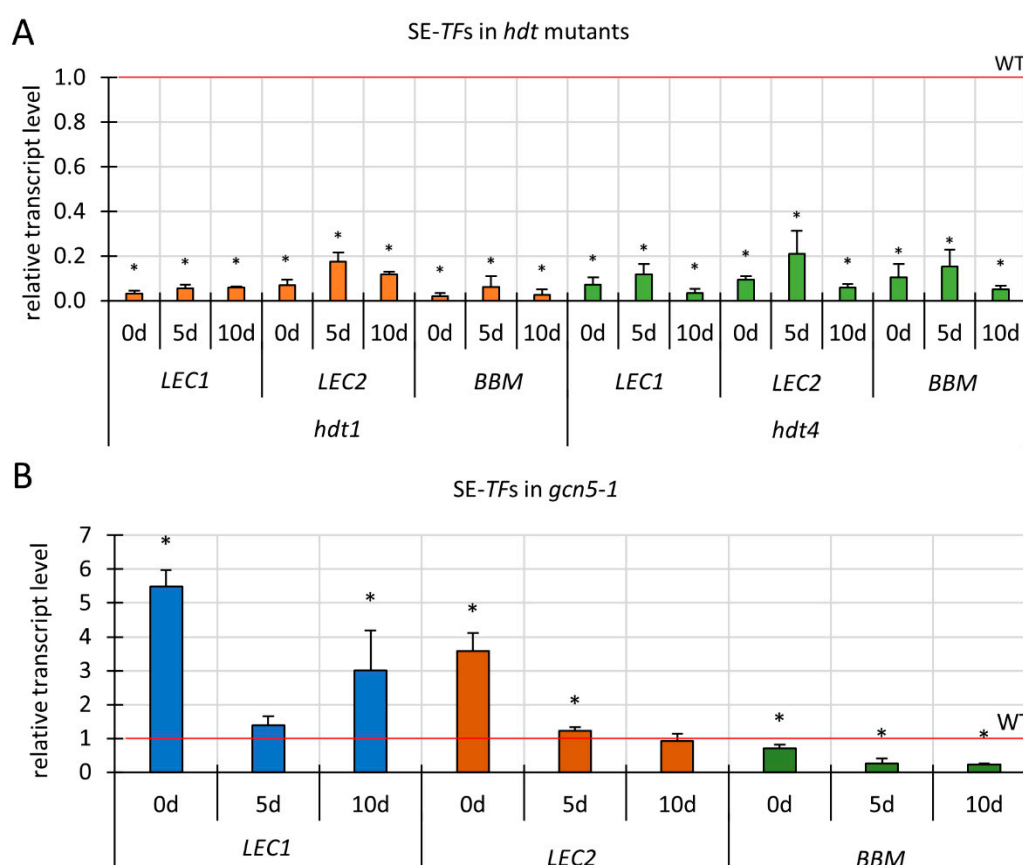


Figure S4. The expression profiles of the SE-TFs in the *hdt1*, *hdt4* (A) and *gcn5-1* (B) mutants in the freshly isolated (0 d) and explants that had been cultured on the EA medium (5 and 10 d). The relative transcript level was normalized to the internal control (*TIN* gene) and calibrated to the control WT cultures of Col-0 (A) and Ws-2 (B). Graph represents the data from the RT-qPCR analysis. An asterisk indicates an expression level that was significantly different from the one observed in WT ($n = 3$; means \pm SD are given) (Student's t-test, $p < 0.05$).

Table S1. The *hat* mutants used in the study.

Gene	AGI	ID	Mutant	Insertion site	Source of seeds
<i>HAG1/GCN5</i>	AT3G54610	SALK_048427C	<i>hag1-5</i>	9 th intron	NASC
		-	<i>gcn5-1</i> (Ws-2 back-ground)	13 th exon	¹
<i>HAG2</i>	AT5G56740	SALK_051832C	<i>hag2-1</i>	promoter/exon	NASC
<i>HAG3</i>	AT5G50320	SALK_104121	<i>hag3</i>	3'UTR	NASC
<i>HAG4</i>	AT5G64610	SALK_027726C	<i>hag4</i>	1 st exon	NASC
<i>HAG5</i>	AT5G09740	SALK_106046	<i>hag5</i>	2 nd intron	NASC
<i>HAC1</i>	AT1G79000	SALK_122894	<i>hac1</i>	14 st exon	NASC
<i>HAC2</i>	AT1G67220	SALK_049434C	<i>hac2-1</i>	promoter	NASC
<i>HAC4</i>	AT1G55970	SALK_006923	<i>hac4</i>	8 th exon	NASC
<i>HAC5</i>	AT3G12980	SALK_152684	<i>hac5</i>	5'UTR/exon	NASC
<i>HAC12</i>	AT1G16710	SALK_052490	<i>hac12-1</i>	15 th intron	NASC
<i>HAF1</i>	AT1G32750	SALK_110848C	<i>haf1-1</i>	20 th exon	NASC
<i>HAF2</i>	AT3G19040	SALK_088103C	<i>haf2</i>	10 th intron	NASC

¹ Seeds kindly provided by Prof. Konstantinos Vlachonasios, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Table S2. The *hdac* mutants and RNAi transgenic lines used in the study.

Gene	AGI	ID	Mutant/transgenic line	Insertion site	Source of seeds
<i>HDA2</i>	AT5G26040	SALK_041074C	<i>hda2</i>	1 st exon	¹
<i>HDA5</i>	AT5G61060	SALK_007503	<i>hda5-1</i>	12 th intron	²
<i>HDA6</i>	AT5G63110	CS24039	<i>HDA6:RNAi</i> (Ws-2 background)	-	NASC
<i>HDA7</i>	AT5G35600	SALK_002912C	<i>hda7</i>	promoter	¹
<i>HDA8</i>	AT1G08460	GK_100A06	<i>hda8</i>	1 st exon	¹
<i>HDA9</i>	AT3G44680	SALK_007123	<i>hda9-1</i>	5 th exon	²
<i>HDA14</i>	AT4G33470	SALK_097005C	<i>hda14</i>	4 th intron	NASC
<i>HDA15</i>	AT3G18520	SALK_004027	<i>hda15-1</i>	2 nd exon	²
<i>HDA18</i>	AT5G61070	SALK_006938	<i>hda18-1</i>	7 th exon	NASC
<i>HDA19</i>	AT4G38130	CS30925	<i>HDA19:RNAi</i> (Ws-2 background)	-	NASC
<i>HDT1</i>	AT3G44750	GK_355H03	<i>hdt1</i>	3 rd exon	¹
<i>HDT2</i>	AT5G22650	SAIL_1247_A02	<i>hdt2</i>	promoter	¹
<i>HDT3</i>	AT5G03740	SALK_129799C	<i>hdt3-1</i>	5 th intron	NASC
<i>HDT4</i>	AT2G27840	GABI_836B08	<i>hdt4</i>	5'UTR	¹
<i>SRT1</i>	AT5G55760	SALK_001493	<i>srt1</i>	14 th exon	NASC
<i>SRT2</i>	AT5G09230	SALK_149295	<i>srt2</i>	2 nd exon	NASC

Seeds kindly provided by:

¹ Dr. Kim Boutilier, Bioscience, Wageningen University and Research, Wageningen, Netherlands;

² Prof. Keqiang Wu, Institute of Plant Biology, National Taiwan University, Taipei, Taiwan.

Table S3. Summary of the culture combinations and methods used in the study.

Method	0d	Analysed samples							
		E0			EA			ET	
		5d	10d	21d	5d	10d	21d	5d	10d
ELISA total H3ac	x	x	x		x	x		x	x
Immunodetection of H3ac	x	x			x	x		x	x
RNA-seq	x	x	x		x	x		x	x
SE response				x			x		
<i>hag1-5, gcn5-1</i> and <i>HDA19:RNAi</i>									
other <i>hat/hdac</i> mutants							x		
ChIP-qPCR		x	x		x	x		x	x
RT-qPCR	x				x	x			
ChIP-qPCR in <i>HDA19:RNAi</i> line					x				

Table S4. ChIP-qPCR analysis: sequence of the gene-specific primers, size of the amplicons and localisation of the analysed gene regions.

Gene	AGI	pF [5' - 3']	pR [5' - 3']	Amplico n size [bp]	Analyzed region
<i>LEC1</i>	AT1G21970	TGAGACTTTGCAACAA CGAACT	AAAATCGTCTTCTCCG GGCC	95	
		CGGCAGAGAAACAAT GGAAC ¹	CAGAGATGGATTTTGG TAGCTG ¹	65	
<i>LEC2</i>	AT1G28300	CCGTTTTTCATACCCGG TGGA	TTGCGTAACCGGGATC TGAG	93	
<i>FUS3</i>	AT3G26790	TTCGATCTGCACAACA CACA	TGGTGGATCTCTTTGG GTTTCA	119	
<i>AGL15</i>	AT5G13790	TTTGGGGGAAAATGG GTCGT	CCCAGAACGCCTCTTG GAAA	92	
<i>MYB118</i>	AT3G27785	AGGGTTTCATGCTTCA CTCA	TGAACACTGACTCGA ACTCCA	110	
<i>BBM</i>	AT5G17430	AGGCTTCTCTCTCTCT CCTCA	AACATCTACGGCGGTT CTGG	82	
<i>WUS</i>	AT2G17950	GCCACAGCATCAGCA TCATC	CGACACGTGTAACCA CCAGA	90	
<i>ACTIN7</i> ²	AT5G09810	CGTTTCGCTTTCCTTAG TGTTAGCT	AGCGAACGGATCTAG AGACTCACCTTG	134	

¹ – used for ChIP-qPCR in Ws-2 and *HDA19:RNAi* line.² – according to Luo et al. [63].**Table S5.** The gene-specific primers used for the RT-qPCR.

Gene	AGI	pF [5' - 3']	pR [5' - 3']
<i>LEC1</i>	AT1G21970	GTGGAGCTCCCTTCTCTCACT	CTGGACCACGATACCATTTGTT
<i>LEC2</i>	AT1G28300	AGGGAAAGGAACCACTACGAA	CAGTGGTGAGGTCCATGAGAT
<i>BBM</i>	AT5G17430	AATGCTAATCATCAAGACAAT	ATCTACCTGTCCACCGATGC
<i>TIN</i>	AT4G27090	GTCGTTATCGTCGACGTTGTT	CCTCGATCAAAGCCTTCTTCT

Table S6. Comparison of the mean Alexa 488 and DAPI fluorescence intensity in the longitudinal sections across cotyledons of the Col-0 explants that had been cultured on the E0, EA and ET media for 0, 5 and 10 days.

		Replicate	Alexa 488 fluo- rescence		DAPI fluo- rescence		Alexa 488/DAPI fluorescence
			Mean	No. of nuclei	Mean	No. of nuclei	
0d		1	336	154	1301	254	0.26
		2	533	132	851	404	0.63
		3	246	27	579	359	0.42
E0	5d	1	804	85	1926	154	0.42
		2	598	266	1495	207	0.40
		3	719	137	1319	85	0.54
		4	666	322	881	261	0.76
EA	5d	1	467	378	1344	402	0.35
		2	707	441	1238	651	0.57
		3	948	190	1382	316	0.69
		4	463	629	796	642	0.58
	10d	1	472	923	877	742	0.54
		2	634	1983	666	1917	0.95
ET	5d	1	973	554	1977	464	0.49
		2	1209	322	1913	278	0.63
		3	533	1029	996	811	0.54
	10d	1	1215	255	1694	100	0.72
		2	1039	606	1469	443	0.71
		3	710	1930	948	2055	0.75

Table S7. The *HAT* gene expression patterns in the Col-0 explants that had been cultured on the E0, EA and ET media for 5 and 10 days.

Gene family	Gene name	Gene ID	A				B			
			EA vs. E0				ET vs. E0			
			5d		10d		5d		10d	
			FC	p-value	FC	p-value	FC	p-value	FC	p-value
<i>GNAT</i>	<i>HAG1/GCN5</i>	AT3G54610	1.2	1E-01	1.3	✓3E-02	1.0	1E+00	1.2	2E-01
	<i>HAG2</i>	AT5G56740	1.8	✓5E-05	1.5	✓5E-03	2.2	✓4E-08	1.8	✓6E-05
	<i>HAG3</i>	AT5G50320	1.1	1E-01	1.3	✓3E-03	1.1	6E-01	1.1	2E-01
<i>MYST</i>	<i>HAG4</i>	AT5G64610	1.6	✓4E-03	1.2	5E-01	1.2	5E-01	0.9	5E-01
	<i>HAG5</i>	AT5G09740	1.3	1E-01	1.4	✓4E-02	1.0	1E+00	1.2	2E-01
<i>CBP</i>	<i>HAC1</i>	AT1G79000	1.0	9E-01	1.0	1E+00	0.8	3E-01	0.8	2E-01
	<i>HAC2</i>	AT1G67220	1.8	3E-01	5.9	✓9E-04	2.4	1E-01	12.6	✓1E-06
	<i>HAC4</i>	AT1G55970	1.2	4E-01	1.0	1E+00	0.7	1E-01	1.0	9E-01
	<i>HAC5</i>	AT3G12980	1.3	✓1E-02	1.1	4E-01	0.9	4E-01	0.9	2E-01
	<i>HAC12</i>	AT1G16710	1.1	4E-01	1.1	4E-01	0.9	6E-01	0.9	4E-01
<i>TAF_{II}250</i>	<i>HAF1</i>	AT1G32750	1.0	9E-01	1.3	3E-01	1.3	5E-01	1.2	5E-01
	<i>HAF2</i>	AT3G19040	1.0	1E+00	1.1	8E-01	3.8	✓1E-03	2.0	1E-01

Values represent the relative expression level (fold change, FC) in EA vs. E0 (A) and ET vs. E0 (B) media at the 5th and 10th day of the culture. Data from the RNA-seq analysis are given. Wald's exact test was used to identify any differentially expressed genes (DEGs) under a *p*-value adjustment (*p* < 0.05) for multiple comparisons with the Benjamini-Hochberg False Discovery Rate (FDR) correction. Significantly different values are indicated with a checkmark.

Table S8. The HDAC gene expression patterns in the Col-0 explants that had been cultured on the E0, EA and ET media for 5 and 10 days.

Gene family	Gene name	Gene ID	A				B			
			EA vs. E0				ET vs. E0			
			5d		10d		5d		10d	
			FC	p-value	FC	p-value	FC	p-value	FC	p-value
RPD3/ HDA1	HDA2	AT5G26040	1.3	2E-01	0.7	9E-02	1.1	1E+00	0.7	1E-01
	HDA5	AT5G61060	1.6	✓ 8E-05	1.5	✓ 2E-03	1.8	✓ 6E-06	1.3	✓ 5E-02
	HDA6	AT5G63110	1.2	1E-01	1.1	6E-01	1.7	✓ 7E-09	1.3	✓ 1E-03
	HDA7	AT5G35600	1.4	NA	1.0	NA	0.6	8E-01	1.7	NA
	HDA8	AT1G08460	0.9	7E-01	1.1	8E-01	0.7	✓ 2E-02	1.1	5E-01
	HDA9	AT3G44680	1.4	✓ 3E-04	1.4	✓ 3E-04	1.4	✓ 5E-03	1.1	7E-01
	HDA10	AT3G44660	1.0	NA	1.0	NA	1.0	1E+00	0.9	NA
	HDA14	AT4G33470	1.3	3E-01	0.9	4E-01	1.0	1E+00	0.7	7E-02
	HDA15	AT3G18520	1.3	✓ 4E-02	1.4	✓ 7E-03	1.3	6E-02	1.2	2E-01
	HDA17	AT3G44490	1.5	NA	0.9	NA	0.8	1E+00	1.0	1E+00
	HDA18	AT5G61070	1.4	5E-01	1.7	2E-01	4.7	✓ 2E-04	7.5	✓ 2E-07
	HDA19	AT4G38130	1.2	3E-01	1.6	✓ 7E-03	1.4	9E-02	1.5	✓ 2E-02
	HDT1	AT3G44750	1.9	✓ 2E-10	1.6	✓ 4E-06	2.1	✓ 6E-12	1.0	8E-01
	HDT2	AT5G22650	1.5	✓ 5E-02	1.5	✓ 2E-02	2.5	✓ 7E-07	1.3	2E-01
HD2	HDT3	AT5G03740	1.6	✓ 1E-03	1.3	7E-02	2.2	✓ 7E-09	2.0	✓ 1E-07
	HDT4	AT2G27840	1.4	1E-01	1.1	8E-01	1.8	✓ 1E-02	0.7	1E-01
SIR2	SRT1	AT5G55760	1.5	✓ 4E-03	1.4	✓ 2E-02	1.6	✓ 8E-04	1.5	✓ 1E-03
	SRT2	AT5G09230	1.1	6E-01	1.0	1E+00	0.9	5E-01	1.1	6E-01

Values represent the relative expression level (fold change, FC) in the EA vs. E0 (A) and ET vs. E0 (B) media at the 5th and 10th day of culture. Data from the RNA-seq analysis are given. Wald's exact test was used to identify any differentially expressed genes (DEGs) under a *p*-value adjustment (*p* < 0.05) for multiple comparisons with the Benjamini-Hochberg False Discovery Rate (FDR) correction. Significantly different values are indicated with a checkmark.

Table S9. The promoter and co-expression analysis of the *HAT* genes.

HAT	AuxRE ¹	TFBS for SE-TFs ^{2,3}	Co-expression with ARFs		Co-expression with SE-TFs ³	
			Environmental stress	Hormone treatment	Environmental stress	Hormone treatment
HAG1/ GCN5	YES	LEC1, FUS3, MYB118, WUS	-	ARF4, ARF6, ARF8	-	-
HAG2	NO	LEC1, AGL15	-	-	LEC1, AGL15	AGL15
HAG3	YES	LEC1, AGL15, WUS	ARF4	ARF4, ARF8	AGL15	AGL15
HAG4	YES	LEC1, AGL15	ARF1	ARF1, ARF4, ARF8, ARF11, ARF16, ARF17, ARF18	-	AGL15
HAG5	YES	LEC1, MYB118, WUS	ARF1, ARF4, ARF6, ARF8, ARF13, ARF17, ARF21	-	LEC1, WUS	-

HAC1	YES	LEC1, FUS3	-	ARF12	-	-
HAC2	YES	LEC1, AGL15, WUS	ARF4, ARF6, ARF8, ARF11	-	LEC1, AGL15, WUS	-
HAC4	YES	LEC1, AGL15	-	ARF16	LEC1, AGL15	AGL15
HAC5	YES	LEC1, LEC2, FUS3, AGL15, MYB118, WUS	-	ARF4, ARF8	AGL15	AGL15
HAC12	YES	LEC1, MYB118	ARF1, ARF2, ARF4, ARF6, ARF8, ARF13, ARF17, ARF21, ARF23	ARF1, ARF4, ARF8, ARF11, ARF16, ARF17, ARF18	LEC1	-
HAF1	YES	LEC1, AGL15, WUS	-	ARF4, ARF8, ARF16	-	-
HAF2	YES	LEC1, MYB118, WUS	-	-	LEC1, WUS	-

¹ – AuxRE – auxin-response elements.

² – TFBS – transcription factor binding site.

³ – SE-TFs analysed in the study.

Table S10. The promoter and co-expression analysis of the HDAC genes.

HDAC	AuxRE ¹	TFBS for SE-TFs ^{2,3}	Co-expression with ARFs		Co-expression with SE-TFs ³	
			Environmental stress	Hormone treatment	Environmental stress	Hormone treatment
HDA2	YES	LEC1	ARF1, ARF4, ARF8, ARF10, ARF17	ARF1, ARF4, ARF8, ARF10, ARF16, ARF18	-	-
HDA5	YES	LEC1, LEC2, FUS3, AGL15	ARF4, ARF6, ARF8, ARF13, ARF17, ARF21, ARF23	ARF1, ARF8, ARF16, ARF18, ARF48	LEC1, LEC2, AGL15	-
HDA6	YES	LEC1, WUS	ARF4, ARF6, ARF8, ARF11	-	LEC1, WUS	-
HDA7	YES	LEC1, LEC2, FUS3, WUS	-	-	LEC1, LEC2, WUS	-
HDA8	YES	LEC1, AGL15	-	-	LEC1, AGL15	-
HDA9	YES	LEC1	ARF1, ARF4, ARF17	ARF1, ARF4, ARF6, ARF8,	-	-

				ARF11, ARF16, ARF17, ARF18			
HDA10	NO	LEC1, MYB118, WUS	-	-	-	-	
HDA14	NO	LEC1, LEC2, MYB118, WUS	-	-	-	-	
HDA15	YES	LEC1, LEC2, FUS3, MYB118, WUS	ARF4, ARF6, ARF8, ARF11	-	LEC1, LEC2, FUS3, WUS	-	
HDA17	NO	LEC1, MYB118, WUS	-	-	-	-	
HDA18	YES	LEC1, WUS	ARF2, ARF4, ARF6, ARF8, ARF12, ARF13, ARF21, ARF23	-	LEC1, WUS	-	
HDA19	YES	LEC1, MYB118, WUS	ARF4, ARF6, ARF8, ARF11, ARF12, ARF13, ARF17, ARF21, ARF23	ARF6, ARF11, ARF17	LEC1, WUS	-	
HDT1	YES	LEC1	ARF1	ARF1, ARF4, ARF6, ARF8, ARF11, ARF16, ARF17, ARF18	-	-	
HDT2	YES	LEC1, AGL15, MYB118	ARF1, ARF4, ARF6, ARF8, ARF10, ARF17	ARF1, ARF4, ARF6, ARF8, ARF11, ARF16, ARF17, ARF18	AGL15	AGL15	
HDT3	NO	LEC1, MYB118	-	-	-	-	
HDT4	YES	LEC1, AGL15	-	-	-	-	
SRT1	NO	LEC1, FUS3	-	-	LEC1, LEC2, FUS3	-	
SRT2	NO	LEC1, WUS	-	-	LEC1, WUS	-	

¹ – AuxRE – auxin-response elements.

² – TFBS – transcription factor binding site.

³ – SE-TFs analysed in the study.