

Supplementary Materials

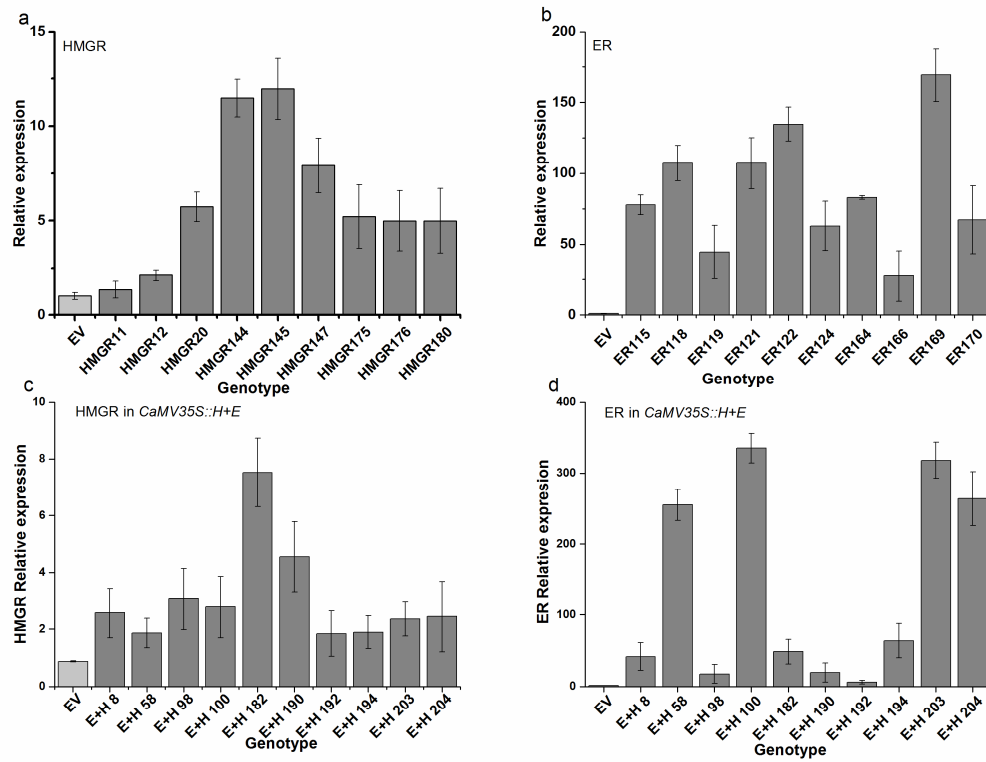


Figure S1 The real time RT-PCR transcript levels in primarily screening of the transgenic lines. The relative expression levels of (A) HMGR, (B) ER and (C) HMGR and (D) ER in H+E in 10-week-old *P. ternata* transgenic lines overexpressing *HMGR*, *ER* or *H+E* under the *CaMV35S* promoter. The *Actin* gene was used as a control to normalize internal expression level.

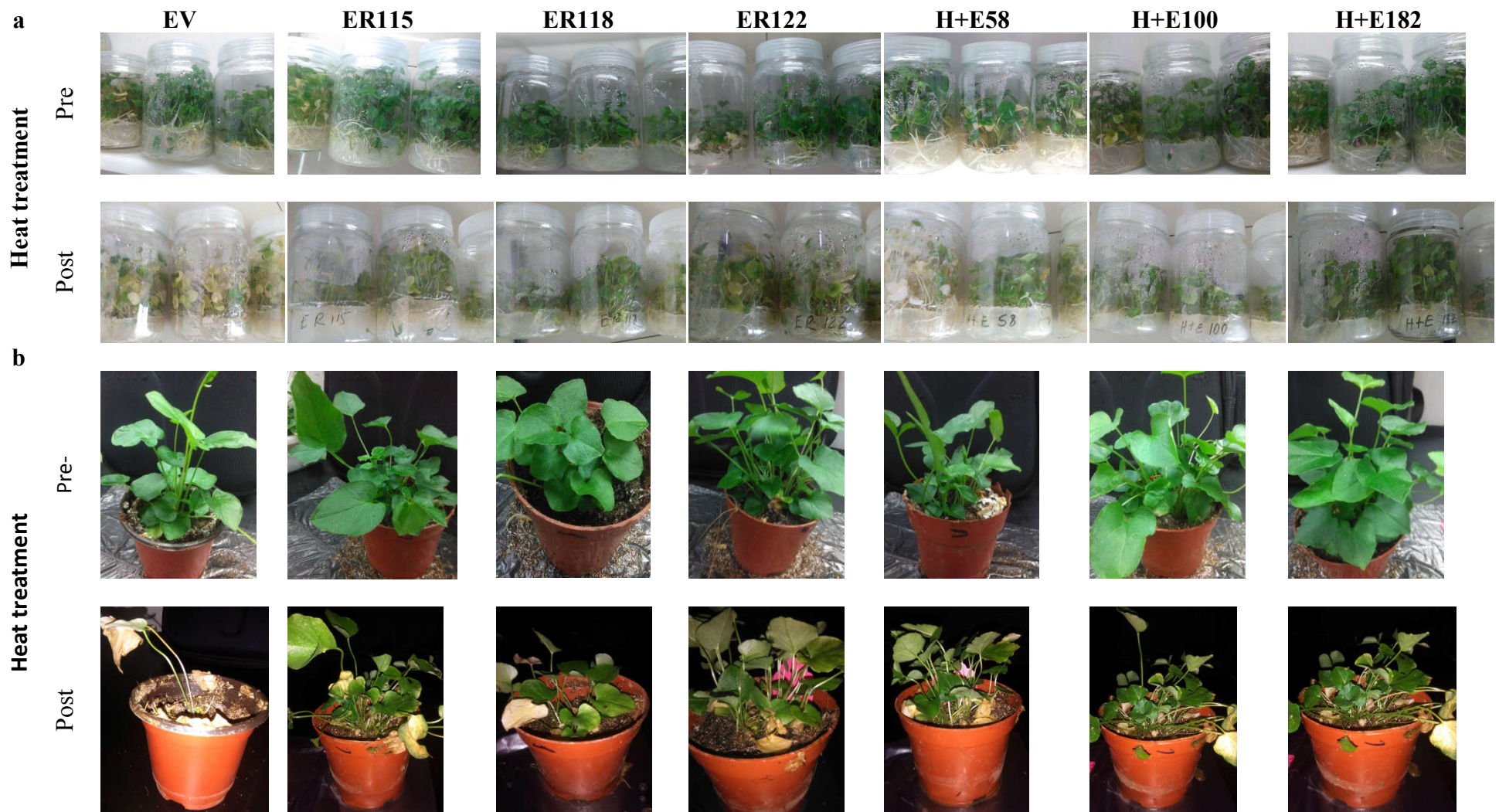


Figure S2 Preliminary heat treatment of *P. ternata* seedlings for selection of thermo-tolerant lines. (A) seven-week-old seedling grown on MS medium (n=10), (B) Ten-week-old seedlings transplanted to soil. The seedlings were treated with a high temperature (40°C, 7-d) (n=20).

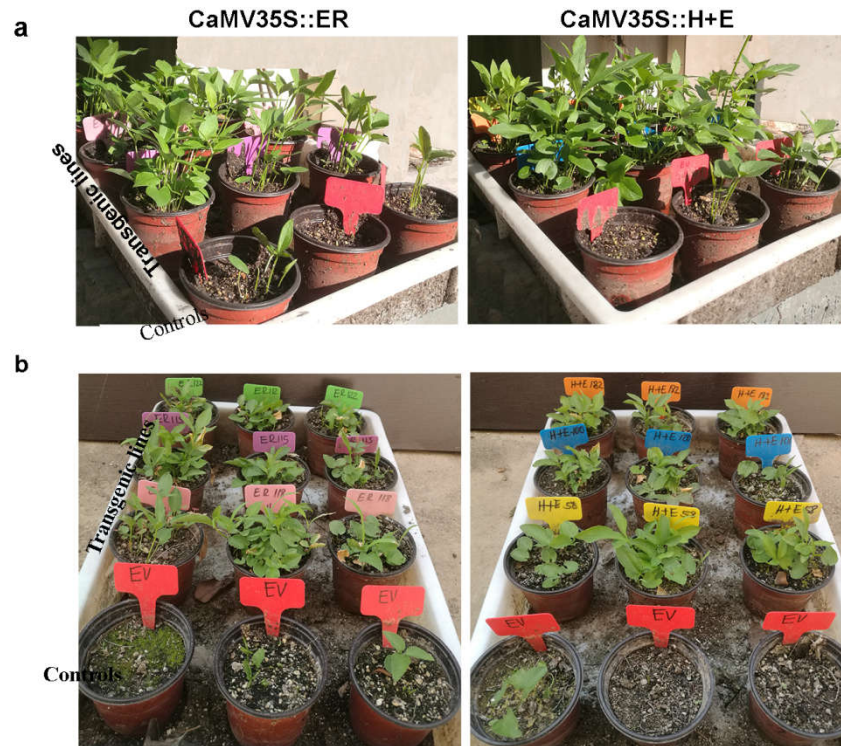
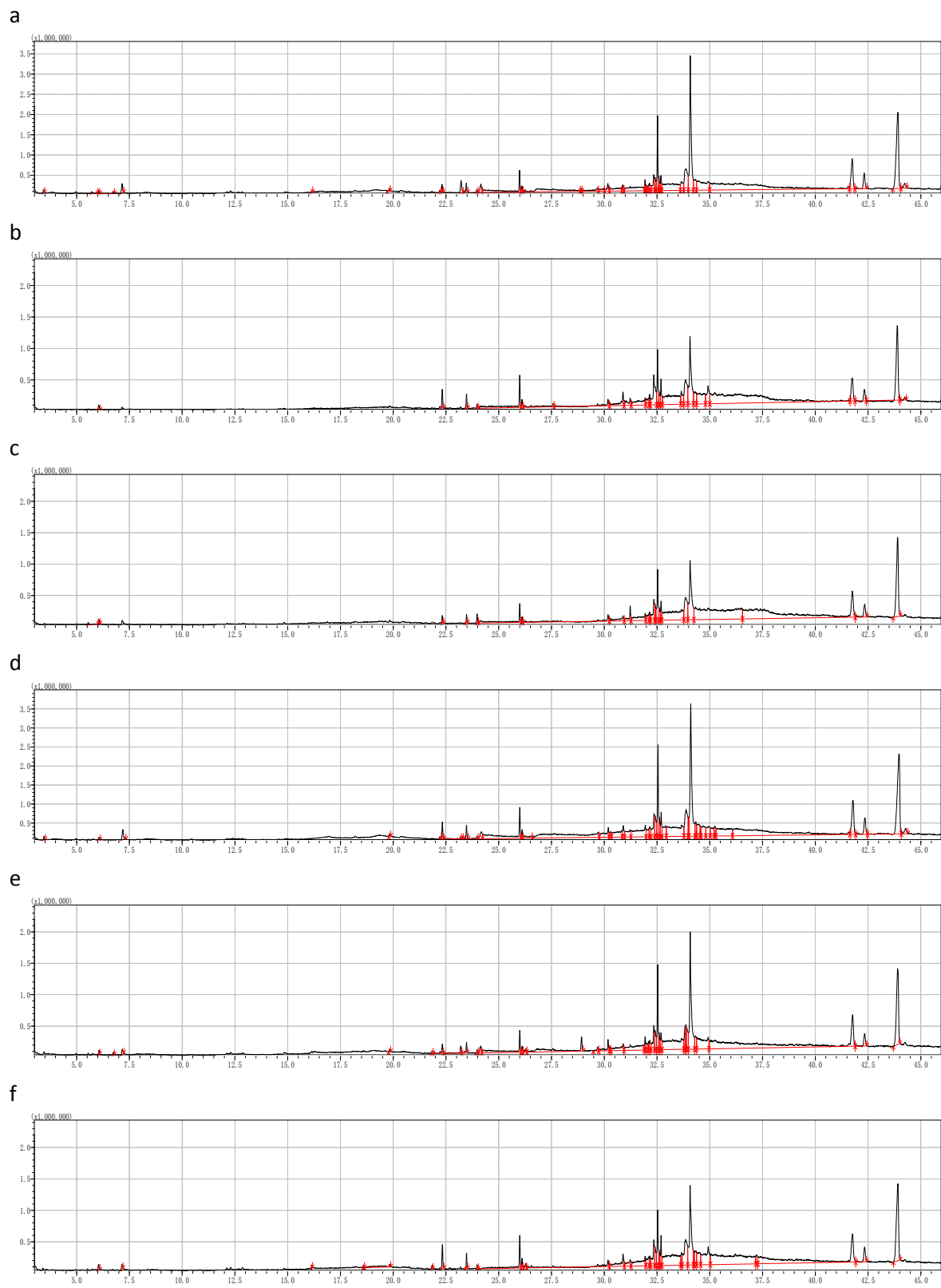


Figure S3 Pictures of transgenic *Pinellia* plants during open field trials. The 16-week-old plants that grown during spring (A) and summer (B) seasons. The seedlings were grown in a growth chamber for 8 weeks and acclimatized in growth culture room for 2 weeks then transferred to open natural field. The study was conducted for two consecutive year, 2018 and 2019 (n=30, for each season)



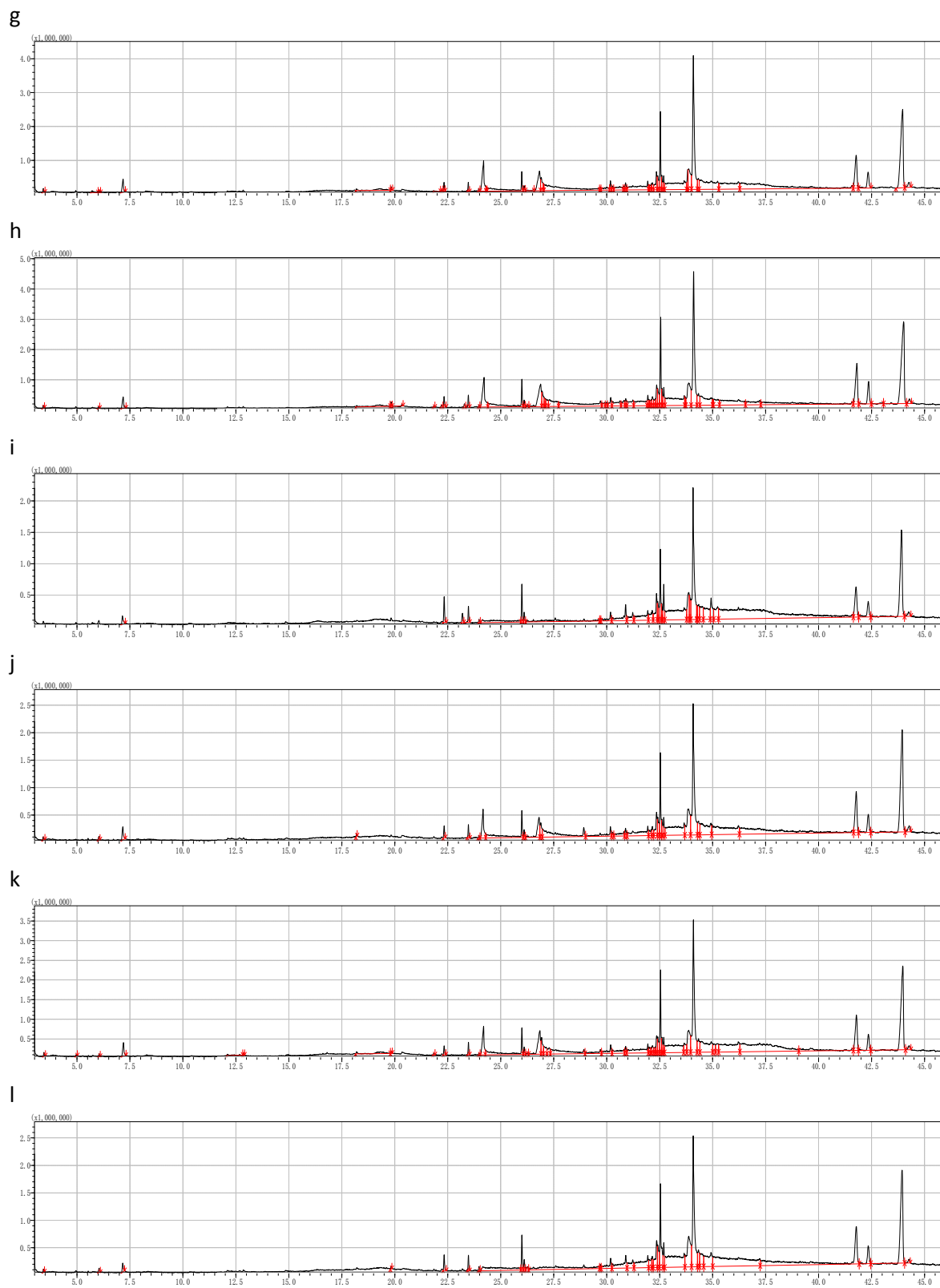


Figure S4 Total ion chromatograms of the control and transgenic *P. ternata* methanolic tuber extracts. Scanning was performed from 60 to 665 atomic mass units and the identified individual compound is displayed together with its retention time in Table S6. (A-C) *EV*, (D-F) *ER*, (G-I) *HMGR* and (J-L) H+E genotypes

Table S1 Primers used for amplifying genes and quantifying gene expression

Gene	Primers	Sequences (5'-3')
Releasing <i>HMGR</i> from a vector	HMGR forward	CGGGGGATCCTCATTGTTGGCTTCTTAAATCTTGT
	HMGR reverse	GCTTATCGATTTAAGACTGTGTGTGAGAAAGTTCC
<i>HMGR</i> cloning	<i>Nco</i> I-HMGR forward	TTGACCATGGTACAAAGTTTAGATAAGAATTTCC
	<i>Bst</i> EII-HMGR reverse	GAGCTGGTCACCTCATTGTTGGCTTCTTAAAT TTG
<i>pCA1301-HMGR-ER</i> construction		
NcoI and BSTEII cleavage sites	<i>Nco</i> I-HMGR forward	TTGACCATGGTACAAAGTTTAGATAAGAATTTCC
	<i>delBst</i> II reverse	GTTGGTAGCCACCACCACGCGCTT
BSTEII cleavage site and part of NcoI cleavage site	<i>delBst</i> II forward	GTGGTGGTGGCTACCAACGTATAGCT
	BSTEII-HMGR reverse	GAGCTGGTCACCTCATTGTTGGCTTCTTAAATCTTG
NcoI, BSTEII cleavage site on HMGR fragment	NcoI-HMGR forward	TTGACCATGGTACAAAGTTTAGATAAGAATTTCC
	BSTEII-HMGR reverse	GAGCTGGTCACCTCATTGTTGGCTTCTTAAATCTTG
Recombinant plasmids identification	Forward	TTGACCATGGTACAAAGTTTAGATAAGAATTTCC
	Reverse	GAGCTGGTCACCTCATTGTTGGCTTCTTAAATCTTG
RT-qPCR		
<i>HMGR</i>	Forward	GTTATGAATGGCATACATGCCG
	Reverse	GTCATAGGCACTTCTATTGTACC
<i>ER</i>	Forward	CCCCGAGCATTTCAAAAGCT
	Reverse	TGCTCCAAATCACCAAGGGA
<i>Actin</i>	Forward	GGTATTGGGCTGGGTTTTGG
	Reverse	AGATATCACCTCAGCCGCTG

Table S2 The phenotypic measures of the *HMGR* transgenic lines challenged with high temperatures

Genotype	Chlorophyll content				Surviving and recovering rate (40°C, 15-d)								Long-term (35°C, 90-d)
	0 h	24 h	24 h	72 h	0-d	1-d	3-d	5-d	10-d	15-d (Rd-1)	7-d	15-d	
EV	47.08±	41.45±	35.19±	11.28±	100	65.03±	34.68±	38.56±	39.61±	36.08±3.93	31.28±	52.03±	12.63±0.92
	0.93	1.14	0.48	0.71		4.9	3.02	3.5	3.35		2.62	4.05	
HMGR144	46.38±	40.87±	33.24±	13.35±	100	64.71±	28.23±	33.14±	34.68±	31.44±3.9	29.89±	52.17±	13.65±1.95
	0.79	0.98	0.48	0.73		3.3	3.4	4	3.4		2.41	4.66	
HMGR145	45.58±	40.16±	33.79±	12.49±	100	66.65±	18.26±	23.86±	22.87±	24.80±2.68	29.12±	50.37±	15.27±3.47
	0.93	1.19	0.47	0.69		4.2	2.62	2.85	1.91		2.05	3.97	
HMGR147	47.49±	42.25±	34.60±	12.50±	100	63.61±	12.37±	16.91±	17.41±	18.21±2.59	30.74±	54.17±	13.42±1.66
	0.92	1.19	0.48	0.75		3.9	2.74	2.99	2.05		2.33	4.86	

EV, empty vector; *HMGR*, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; d, day; Rd, recovery days

Table S3 Physio-biochemical assays for *HMGR* transgenic lines treated with a high temperature (40°C-15-d)

Genotype	Relative water content			Ion leakage				Lipid peroxidation		H ₂ O ₂ concentration		Total protein content		SOD activity	
	0 h	24 h	48 h	0 h	24 h	48 h	72 h	0 h	48 h	0 h	72 h	0 h	48 h	0 h	48 h
EV	95.34±	87.58±	87.33±	14.8±	16.42±	21.6±	31.6±	0.25±	0.44±	3.26±	5.73±	1.38±	0.610±	0.07±	0.18±
	0.4	1	0.84	0.92	1.4	4.1	4.5	0.07	0.11	0.16	1.34	0.11	0.12	0.01	0.01
HMGR144	97.42±	87.34±	86.35±	14.8±	17.90±	22.2±	35.4±	0.21±	0.28±	3.45±	5.29±	1.64±	0.650±	0.07±	0.13±
	0.29	1.47	1.4	1.6	1.4	5.4	4.6	0.05	0.08	0.33	0.24	0.13	0.08	0.011	0.06
HMGR145	96.82±	87.47±	83.71±	14.2±	18.70±	23.2±	36.4±	0.26±	0.26±	3.41±	5.07±	1.90±	0.710±	0.06±	0.11±
	0.5	1.6	1.3	1.3	1.3	3.4	4.25	0.02	0.02	0.15	1.03	0.26	0.05	0.01	0.06
HMGR147	96.18±	86.19±	85.35±	15.4±	18.70±	23.2±	36.4±	0.29±	0.29±	3.06±	4.59±	1.80±	0.710±	0.08±	0.12±
	1.9	1.4	1.02	1.7	1.4	3.5	4.4	0.03	0.03	0.21	0.41	0.09	0.06	0.01	0.06

EV, empty vector; *HMGR*, 3-hydroxy-3-methyl glutaryl coenzyme A reductase

Table S4 Monthly average temperatures, humidity and rainfall in Wuhan, China, 2018 & 2019

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature													
2018	Max	6	11	18	24	28	31	34	34	29	25	19	10
	Ave	4	8	14	20	24	28	31	31	25	23	17	8
	Min	1	5	11	15	21	24	27	27	22	19	14	6
2019	Max	10	9	20	26	29	32	34	37	31	27	18	11
	Ave	8	7	17	23	27	30	32	34	28	24	16	9
	Min	5	5	12	1	21	24	27	28	22	18	13	8
Humidity	2018	71	57	67	63	65	63	67	65	62	45	59	62
	2019	63	72	57	60	52	64	66	60	53	49	76	66
Rainfall	2018	48.74	21.9	64.85	58.77	84.27	59.14	90.32	49.86	30.04	17.9	87.7	95.6
	2019	60.6	77	134.3	113.3	117.8	142.5	136.4	74.4	21.3	8.6	52	91.2

Table S5 Plant growth performance evaluation during spring season

Genotype	Root length (cm)	Fresh biomass (g)	Dry biomass (g)	Tuber fresh wt (g)	Tuber dry wt (g)
EV	12.21±1.26	8.27±0.91	1.85±0.41	5.23±0.89	3.95±1.36
ER115	12.04±1.11	8.88±0.69	1.88±0.57	6.69±1.21	6.05±1.14
ER118	12±1.26	10.97±0.69	2.71±0.35	4.82±0.82	3.66±0.94
ER122	12.42±1.6	10.52±0.87	2.53±0.66	6.91±2.35	5.78±0.78
H+E58	12.05±1.22	9.38±0.94	2.13±0.35	7.81±2.17	6.1±0.53
H+E100	12.13±1.11	10.54±0.79	2.22±0.41	7.78±1.75	6.08±0.36
H+E182	12.03±1.53	9.95±0.91	2.21±0.65	8.72±1.72	6.22±0.79

Table S6 Relative intensity of abundantly detected compounds in controls and *HMGR* overexpressing transgenic lines

No.	RT	Name of the compound	M.F	M.W (gmol ⁻¹)	Peak area (%)			
					Genotype			
					<i>EV</i>	<i>ER</i>	<i>HMGR</i>	<i>H+E</i>
1	3.44	Aceturic acid, 2TMS derivative	C ₇ H ₁₅ NO ₃ Si	189	0.4±0.12	ND	0.4±0.1	0.33±0.02
2	22.22	3-(Prop-2-enoyloxy) dodecane	C ₁₅ H ₂₈ O ₂	240	1.6±0.1	ND	0.3±0.12	Tr
3	22.33	2-Hydroxy-4,6-dimethylbenzaldehyde	C ₉ H ₁₀ O ₂	150	1±0.5	1.5±0.7	0.63±0.2	1.1±0.4
4	23.97	Benzoic acid, 3-formyl-	C ₈ H ₆ O ₃	150	1.17±0.5	0.33±0.1	0.9±0.12	ND
5	24.12	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	279	0.3±0.2	0.4±0.04	5.9±0.1	4±0.8
6	26.0	10-Methyl-E-11-tridecen-1-ol propionate	C ₁₇ H ₃₂ O ₂	268	ND	2.1±0.2	0.4±0.08	2.4±0.25
7	26.11	7-Hexadecenoic acid, methyl ester (Z)-	C ₁₇ H ₃₂ O ₂	268	0.94±0.1	1.1±1.03	3.23±0.9	3±1.2
8	27.28	1-β -d-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazole	C ₈ H ₁₁ N ₇ O ₄	269	1.23±0.3	Tr	0.4±0.1	0.11±0.03
9	30.18	Ethylene brassylate	C ₁₅ H ₂₆ O ₄	270	0.8±0.07	Tr	0.66±0.01	0.6±0.05
10	32.16	1,3,5-Trisilacyclohexane	C ₃ H ₁₂ Si ₃	132	Tr	0.32±0.12	0.3±0.02	0.84±0.44
11	32.53	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	C ₁₁ H ₁₆ O ₄	212	6.8±0.1	0.23±0.11	3.1±2.3	6.7±0.7
12	32.69	Bis (2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390	1.2±0.8	ND	1.1±0.4	0.9±0.2
13	34.08	7-Hexadecyn-1-ol	C ₁₆ H ₃₀ O	238	17.12±3.8	23.7±6.3	19.9±0.13	21±1.2
14	41.75	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C ₂₀ H ₃₄ O ₂	306	3.6±0.3	2.8±1.1	8±0.5	6.26±1.3
15	42.36	Cholestan-3-ol, 2-methylene-, (3β. 5α)-	C ₂₈ H ₄₈ O	400	3.3±0.2	2.2±0.12	1.2±0.11	Tr
16	43.75	(-)-Globulol	C ₁₅ H ₂₆ O	222	21.1±3.1	18.6±2.9	2.6±1.13	7.3±0.35
17	43.98	Aromadendrene-4, 10-diol	C ₁₅ H ₂₆ O ₂	238	8.7±1.6	7.3±0.1	24.3±0.74	23.5±1.7

The intensity of peak is the mean of three *HMGR* overexpressing genotypes or the mean of three control plants. RT, Retention time; M.F, Molecular formula; M.W, Molecular weight; Tr, trace ≤ 0.1 ; ND, not detected.