Translatome and metabolome effects triggered by gibberellins during rosette growth in Arabidopsis

Dimas M Ribeiro, Wagner L Araújo, Alisdair R Fernie, Jos H M Schippers, and Bernd Mueller-Roeber

Supplementary Material

Supplementary Tables S2-S5, Figures S1-S3

Supplementary Table S1 – see separate file

Table S2 Expression of GA-related genes in plants treated with PAC and/or GA_3. Values set in bold type were determined by the Student's *t*-test to be significantly different from the control. Data are means ± SE of three independent replicates (each replicate is a pool of five plants).

AGI ID	Name	Relative value						Total mRNA vs. polysomal mRNA		
		Total mRNA			Polysomal mRNA					
		GA	PAC	PAC+GA	GA	PAC	PAC+GA	GA	PAC	PAC+GA
At5g25900	*KO*	**-1.75±0.15**	**1.95±0.21**	**-1.88±0.25**	**-1.60±0.12**	1.05±0.09	**-1.63±0.18**	1.09	1.86	1.15
At1g05160	*KAO1*	**-1.74±0.09**	**2.15±0.29**	**-2.19±0.35**	-1.16±0.12	1.26±0.17	-1.12±0.15	1.30	1.49	1.70
At2g32440	*KAO2*	-1.43±0.11	1.54±0.24	-1.62±0.26	-1.50±0.12	1.45±0.06	-1.33±0.10	0.95	1.06	1.22
At4g25420	*GA20ox1*	**-6.78±0.36**	**6.75±0.84**	**-9.29±0.51**	**-4.58±0.68**	**3.08±0.36**	**-7.16±1.07**	1.48	2.19	1.30
At5g51810	*GA20ox2*	**-10.88±1.03**	**27.20±1.04**	**-3.34±1.05**	**-5.81±1.14**	**17.15±1.74**	**-2.46±0.26**	1.87	1.59	1.36
At5g07200	*GA20ox3*	**-9.36±1.19**	**37.08±1.21**	**-28.77±2.11**	**-8.47±1.04**	**24.36±3.88**	**-21.41±2.17**	1.10	1.52	1.34
At1g15550	*GA3ox1*	**-4.95±1.11**	**8.84±1.18**	**-15.31±1.11**	**-4.18±1.69**	**4.66±1.03**	**-4.35±1.12**	1.18	1.90	3.52
At1g80340	*GA3ox2*	**-4.59±0.96**	**4.75±0.28**	**-3.80±0.73**	-1.43±0.09	1.34±0.14	-1.59±0.10	3.21	3.54	2.39
At1g78440	*GA2ox1*	1.44±0.14	**-5.99±1.02**	1.36±0.15	1.23±0.12	**-3.25±0.47**	1.16±0.09	1.17	1.84	1.17
At1g30040	*GA2ox2*	**1.99±0.15**	**-3.17±0.52**	**2.57±0.44**	**2.71±0.51**	**-2.98±0.29**	**2.49±0.35**	0.73	1.06	1.03
At2g34555	*GA2ox3*	**2.92±0.47**	**-2.12±0.15**	**3.33±0.39**	**1.96±0.06**	-1.17±0.03	**1.98±0.22**	1.49	1.81	1.68
At1g47990	*GA2ox4*	1.18±0.03	**-2.80±0.24**	1.29±0.05	**1.92±0.08**	**-1.62±0.11**	1.27±0.04	0.61	1.73	1.01
At1g50960	*GA2ox7*	**1.70±0.10**	**-1.82±0.16**	**1.81±0.12**	1.08±0.02	-1.57±0.15	1.50±0.11	1.57	1.16	1.21
At1g21200	*GA2ox8*	**2.91±0.46**	**-5.61±0.26**	**3.74±0.62**	1.80±0.09	-1.40±0.13	1.34±0.06	1.62	4.01	2.79
At3g63010	*GID1B*	**-3.02±0.61**	**3.37±1.16**	**-2.32±1.12**	**-2.65±0.82**	**2.01±0.16**	**-2.11±0.53**	1.14	1.67	1.09
At1g66350	*RGL1*	1.19±0.53	-1.95±0.54	1.07±0.31	**1.65±0.13**	**-1.63±0.33**	**1.65±0.05**	0.72	1.19	0.65
At3g03450	*RGL2*	**1.54±0.17**	**-2.72±0.15**	**1.64±0.13**	1.26±0.06	**-1.81±0.09**	1.37±0.24	1.22	1.50	1.19
At5g17490	*RGL3*	1.27±0.55	**-2.25±0.53**	1.05±0.89	**1.57±0.13**	**-1.48±0.20**	1.25±0.12	0.81	1.52	0.84

Table S3 Relative metabolite content of rosettes of plants treated with PAC and/or GA_3. Values are means ± SE of six replicates (each replicate is a pool of five plants). Values set in bold type were determined by the Student's *t*-test to be significantly different from the control ($P < 0.05$).

Metabolite	Control	GA_3	PAC	PAC+GA_3
Amino acids				
Alanine	1.00±0.10	**1.69±0.14**	**6.69±0.95**	**3.42±0.11**
Arginine	1.00±0.07	1.09±0.15	**1.44±0.11**	1.04±0.14
Asparagine	1.00±0.10	**0.53±0.11**	0.89±0.10	**0.36±0.04**
Aspartate	1.00±0.08	0.98±0.09	**1.54±0.16**	1.01±0.10
Cysteine	1.00±0.06	1.06±0.15	**2.37±0.25**	1.18±0.03
GABA	1.00±0.05	1.11±0.04	1.04±0.03	1.02±0.04
Glutamate	1.00±0.02	**0.70±0.08**	0.93±0.04	**0.59±0.05**
Glutamine	1.00±0.10	0.73±0.15	1.04±0.04	**0.58±0.07**
Glycine	1.00±0.07	1.23±0.36	**0.45±0.03**	**1.68±0.17**
Histidine	1.00±0.13	1.27±0.15	0.70±0.09	1.00±0.07
Homoserine	1.00±0.12	1.13±0.24	0.98±0.08	**1.51±0.17**
Isoleucine	1.00±0.11	0.90±0.13	**1.20±0.05**	0.80±0.04
Leucine	1.00±0.06	1.04±0.04	**1.29±0.04**	1.02±0.02
Lysine	1.00±0.13	1.11±0.15	1.04±0.06	0.93±0.06
Methionine	1.00±0.04	1.03±0.12	**1.26±0.06**	1.02±0.06
Ornithine	1.00±0.11	1.21±0.54	1.02±0.20	1.17±0.19
Phenylalanine	1.00±0.06	1.11±0.03	1.14±0.11	1.03±0.05
Proline	1.00±0.07	1.21±0.31	1.09±0.04	**0.53±0.05**
Serine	1.00±0.03	1.02±0.08	0.95±0.03	1.04±0.04
Threonine	1.00±0.04	0.90±0.12	**1.28±0.08**	0.87±0.05
Tryptophan	1.00±0.25	**1.78±0.28**	**0.62±0.11**	**1.64±0.12**
Tyrosine	1.00±0.17	1.18±0.22	0.96±0.04	0.92±0.08
Valine	1.00±0.06	1.04±0.04	1.10±0.09	1.12±0.03
Organic acids				
Ascorbate	1.00±0.12	1.03±0.13	0.95±0.02	0.82±0.03
Aconitate	1.00±0.13	1.04±0.18	0.71±0.09	0.70±0.08
Citrate	1.00±0.22	**1.9±0.24**	1.32±0.11	1.22±0.19
Fumarate	1.00±0.14	0.95±0.11	**2.04±0.20**	0.92±0.12
Isocitate	1.00±0.11	0.72±0.17	1.03±0.11	**0.55±0.06**
α-Ketoglutarate	1.00±0.20	0.72±0.12	0.66±0.09	0.79±0.12
Lactate	1.00±0.26	**0.38±0.03**	**0.27±0.02**	0.69±0.08
Malate	1.00±0.16	0.85±0.09	**1.75±0.12**	0.80±0.09
Pyruvate	1.00±0.10	**0.80±0.03**	0.89±0.07	1.05±0.05
Quinic acid	1.00±0.05	0.84±0.07	**1.44±0.11**	0.89±0.08
Succinato	1.00±0.12	1.08±0.07	1.13±0.10	1.02±0.07
Sugars and sugar derivatives				
Erythritol	1.00±0.02	**1.50±0.03**	**0.84±0.06**	**1.42±0.05**
Fructose	1.00±0.15	1.02±0.19	0.98±0.09	0.89±0.03
Fructose-6-P	1.00±0.05	1.03±0.05	1.01±0.11	1.12±0.11
Glucose	1.00±0.08	1.18±0.08	0.97±0.05	1.03±0.02
Glucose-6-P	1.00±0.09	1.12±0.06	1.05±0.08	0.98±0.05
3-P-Glycerate	1.00±0.12	**2.49±0.56**	0.82±0.19	**2.14±0.39**
Mannose	1.00±0.12	1.03±0.15	0.93±0.04	0.79±0.05
Rhamnose	1.00±0.06	1.04±0.03	**1.30±0.06**	0.97±0.03
Sucrose	1.00±0.07	0.98±0.05	1.15±0.06	0.95±0.04
Myo-Inositol	1.00±0.07	1.01±0.08	0.95±0.01	0.89±0.03
Trehalose	1.00±0.05	1.20±0.08	0.91±0.04	1.04±0.04

Table S4 Paclobutrazol increases protein and chlorophyll concentration in fully expanded leaves. Twenty days after sowing, when the first pair of true leaves was completely expanded, plants grown singly in pots were watered with 10 mL PAC (0.17 mM). For GA treatment, each plant was sprayed every second day with 1 mL of 50 µM GA_3. Leaf discs (12.5 mm^2) were harvested from the first pair of true leaves five days after treatment. Area of the first pair of true leaves at 20 days after sowing (start of treatments) was 92.7 ± 7.5 mm^2; protein and chlorophyll concentrations were 14.0 ± 1.1 (79.2 ± 5.9) and 1.6 ± 0.07 (8.9 ± 0.8) mg g^{-1} FW [µg leaf $disc^{-1}$]. Comparisons were made in each column by Tukey test at the 5 % level. Mean ± SE of six replicates. Each replicate consisted of 10 leaf discs.

Treatment	Area	Protein		Total chlorophyll	
	[mm^2]	[mg g^{-1} FW]	[µg leaf $disc^{-1}$]	[mg g^{-1} FW]	[µg leaf $disc^{-1}$]
Control	92.1±8.3 a	14.3±1.0 b	81.5±7.2 b	1.5±0.03 b	8.6±0.2 b
GA_3	93.6±8.1 a	13.8±1.2 b	78.7±8.1 b	1.4±0.05 b	8.2±0.3 b
PAC	91.8±9.8 a	20.0±0.8 a	110.0±7.4 a	2.2±0.07 a	12.0±0.5 a
PAC+GA_3	92.4±5.3 a	14.5±0.9 b	82.6±6.6 b	1.6±0.04 b	8.3±0.3 b

Table S5. Sequence of primers used for qRT-PCR.

AGI ID	Name	Primer sequences	
		Forward	Reverse
At5g25900	*KO*	TCGGTGTAGCATTGAAGC	TCGACAAAGTCACACCGA
At1g05160	*KAO1*	TGAGGTTCTACAAAGAGCAAAGGC	ACTCGAAGTGTCTCATCGACAACC
At2g32440	*KAO2*	AAGAGTGATGTCCAAATGGATGGC	GGTCCAAATGGACGTTCCTAAACC
At4g25420	*GA20ox1*	AGATTACTTCTGCGATGCGTTGG	TCTTGATACACCTTCCCAAATGGC
At5g51810	*GA20ox2*	CAAGAGTTCGAGCAGTTTGGGAAG	TCGGAAATAGTCTCGGTTTACGC
At5g07200	*GA20ox3*	ACATAGGCGACACCTTCATGGC	TCCTTTCTCTCTCGCTGTTCACC
At1g60980	*GA20ox4*	GAACATTGGCGACACTTTAATGGC	TGGTGGCTTCACCACTTTGTCC
At1g44090	*GA20ox5*	AACGTTGGAGACACCTTCATGGC	ACTGCCCTGTGGTAACAACTCC
At1g15550	*GA3ox1*	CCCAACATCACCTCAACTACTGC	GGCCCATTCAATGTCTTCTTCGC
At1g80340	*GA3ox2*	CCAGCCACCACCTCAAATACTGTG	ATTAGGCCCGGCCCATTGTATG
At4g21690	*GA3ox3*	TCATGCCGAGTTCTGCAATGTG	CCTAACGAGCCCATCAACATGC
At1g80330	*GA3ox4*	CCTCATGATCACACCAAGTACTGC	TCTTCCACGGTGACACCAAGTG
At1g78440	*GA2ox1*	AACGTTGGTGACTCTCTCCAGGTG	AACCCTATGCCTCACGCTCTTG
At1g30040	*GA2ox2*	AGATGGAAGTTGGGTCGCTGTC	CCCGTTAGTCATAACCTGAAGAGC
At2g34555	*GA2ox3*	TCCACCTGACCCGTTTGGTTAC	TACTCAAGCCAGCCAAGGTCAC
At1g47990	*GA2ox4*	TTTCGCTCTCAGCTCTCCTTCAGC	TCACGAGCCAACTGCTTAACTGC
At1g50960	*GA2ox7*	TGATGATCGCAACAACCTCAGAAC	CGAACTCACGTTCACTTGTTTCCC
At1g21200	*GA2ox8*	TGGTGACTTATTTCAGGCATGGAG	CCTTTGGGTTCGTCATCACACG
At3g63010	*GID1B*	TGGAGACTATGGCTGGTGGTAAC	AGTGGGACAATTCTCTTGCATTCG
At1g66350	*RGL1*	TTGTTGCTTGGATGGCAAACGC	ACGATTGATTCGCCACGCAGATG
At3g03450	*RGL2*	TTCGAAACCCGACCCGAATCTG	AACCGGTGGAGCTCAAATACCG
At5g17490	*RGL3*	AGCTGTTAGCGACGGTTAAGGC	ACGTCACCGTTATGGTTCGCTTC

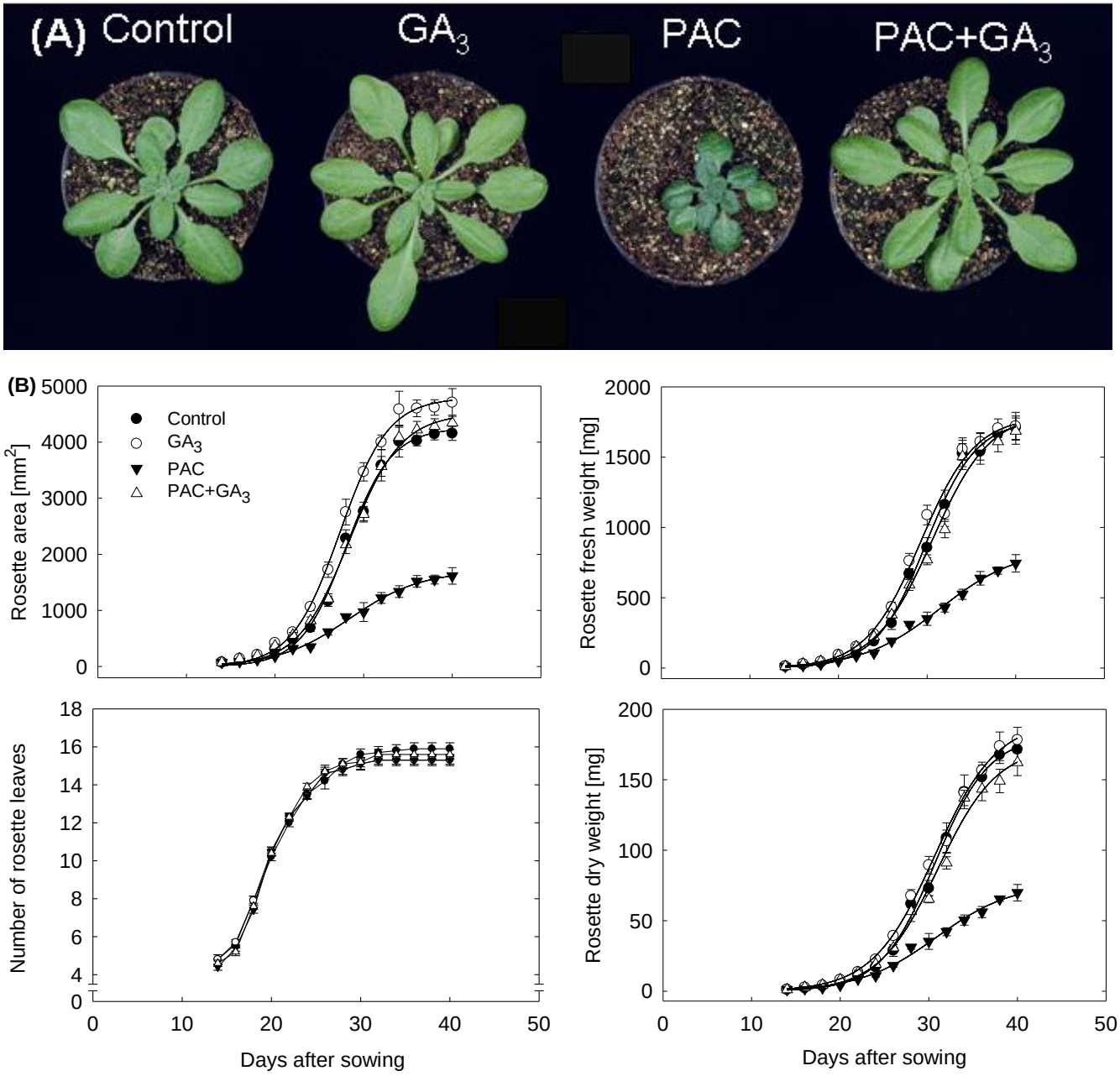


Figure S1 Phenotypic changes of *Arabidopsis* wild-type plants caused by PAC and/or GA_3 treatment. (A) Shoots of 27-day-old plants. (B) Time course of rosette growth of plants treated with PAC and/or GA_3. Rosette growth was described by the sigmoidal function $y = A / (1 + \exp \{ - [(x - x_0) / b]\})$. Values are presented as means $\pm$ SE of ten individual determinations.

Figure S2: MapMan representations of differentially expressed genes.
(A) PAC versus control - Metabolism overview.

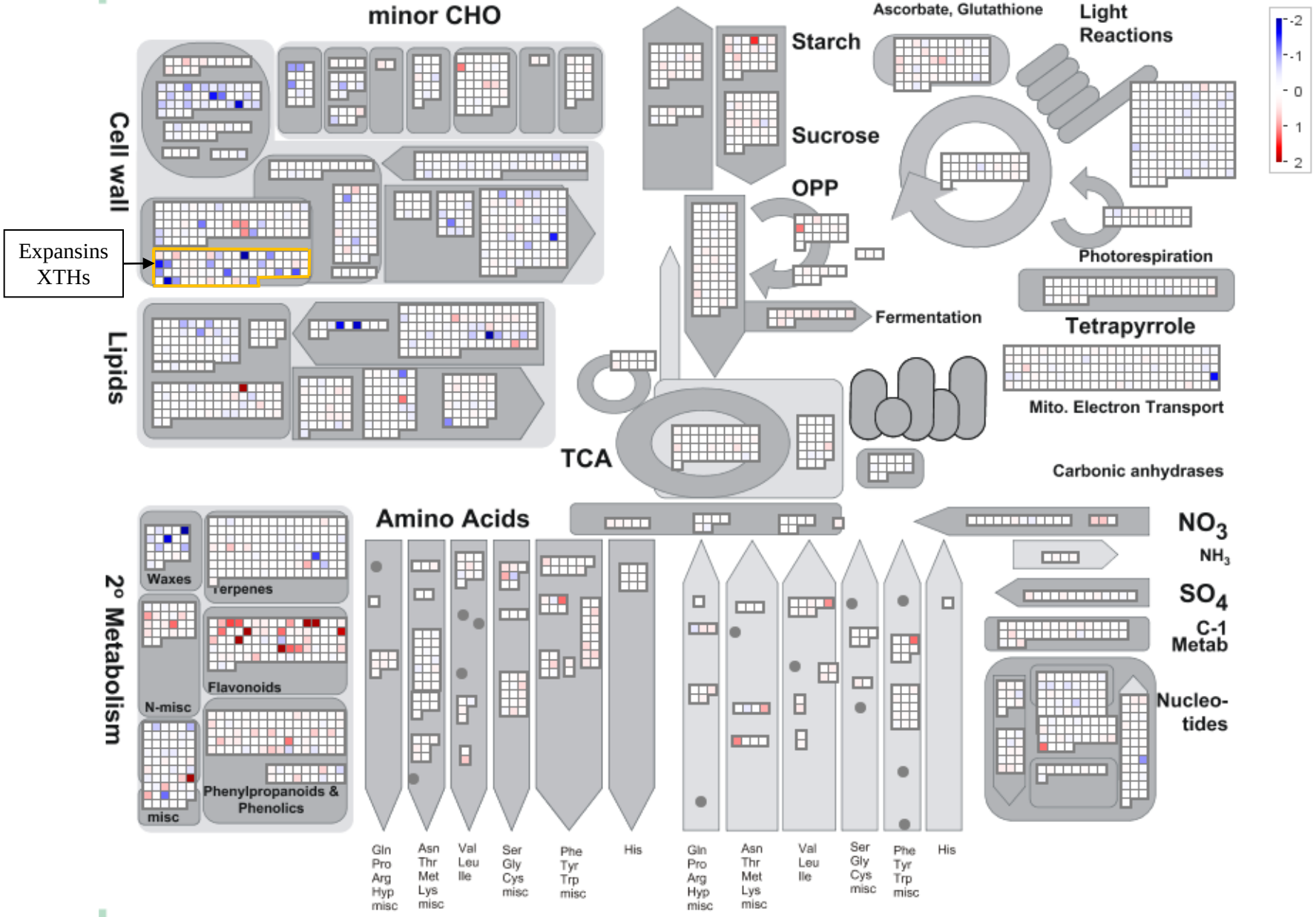


Figure S2: MapMan representations of differentially expressed genes.
(B) GA versus control - Metabolism overview.

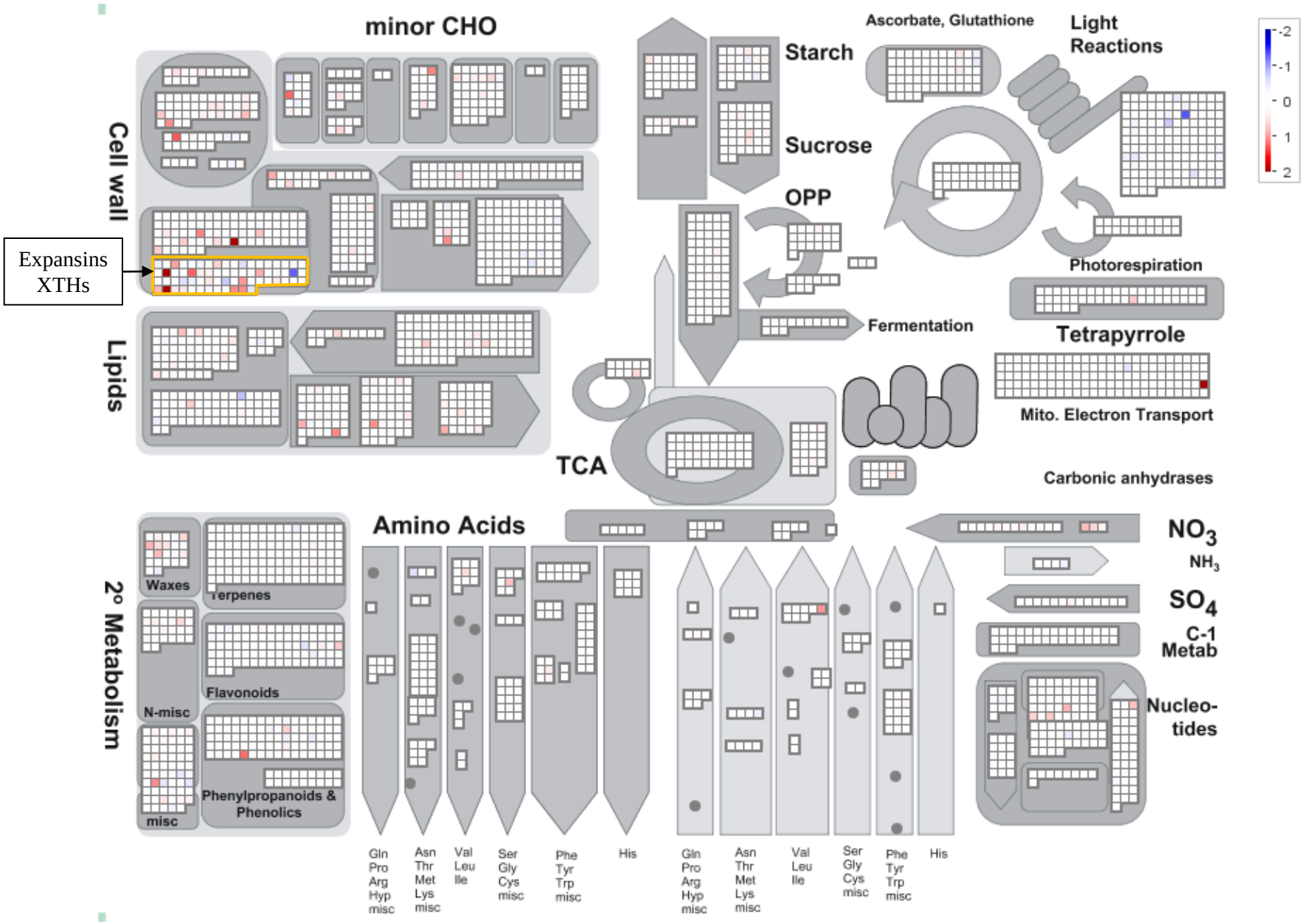


Figure S2: MapMan representations of differentially expressed genes.
(C) PAC versus control - Secondary metabolism.

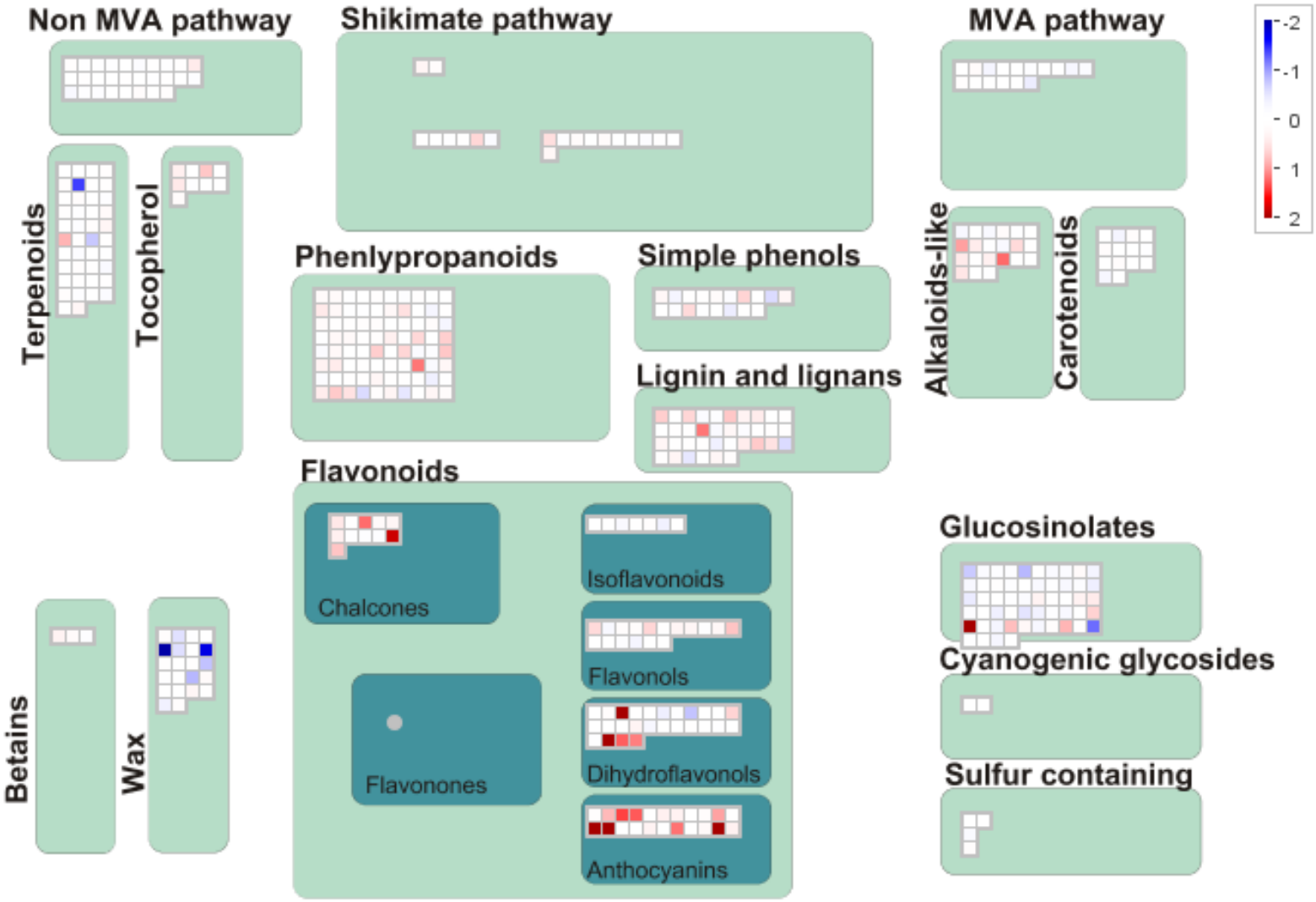


Figure S2: MapMan representations of differentially expressed genes.
(D) GA versus control - Secondary metabolism.

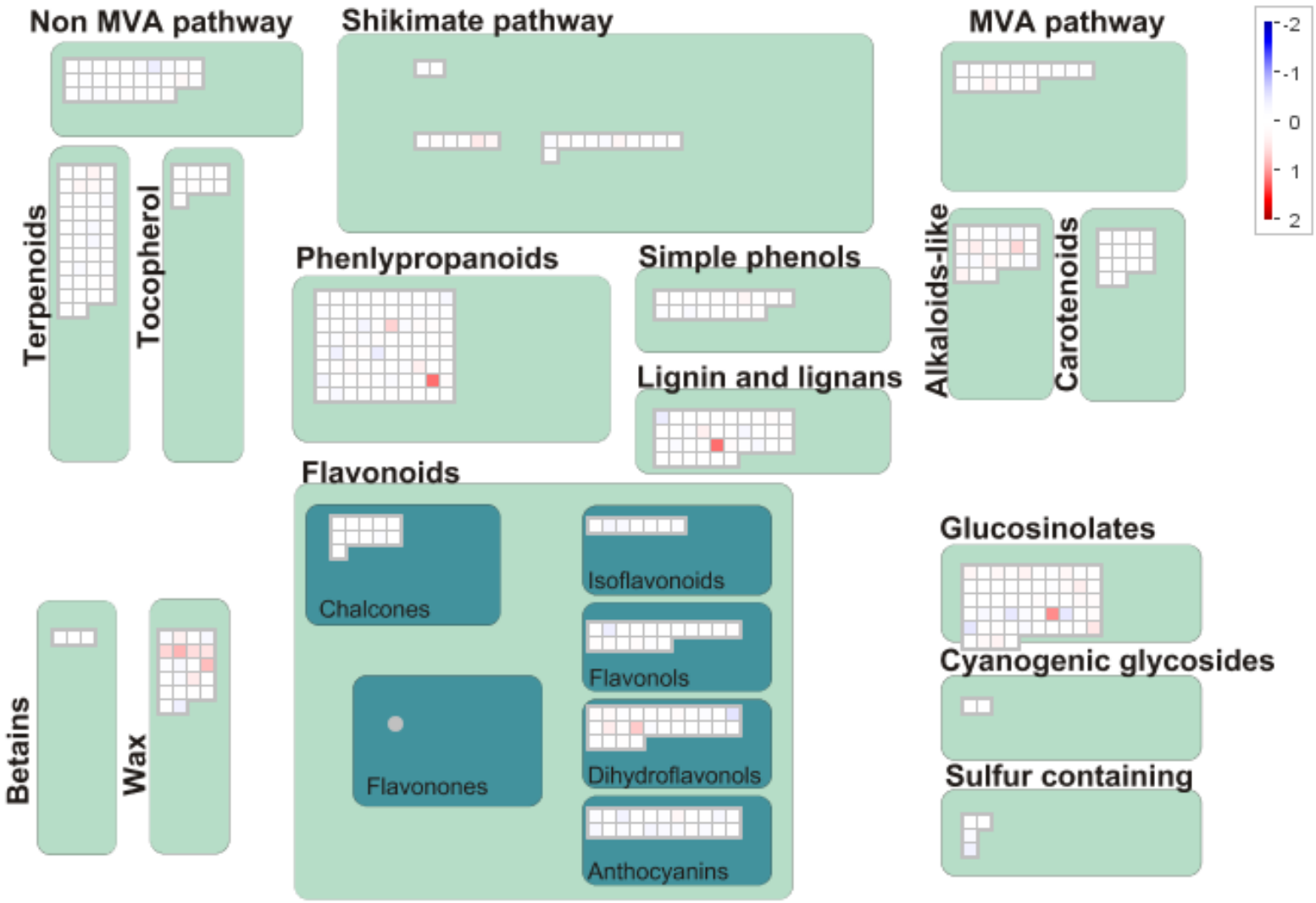


Figure S2: MapMan representations of differentially expressed genes.
(E) PAC versus control - Cellular response.

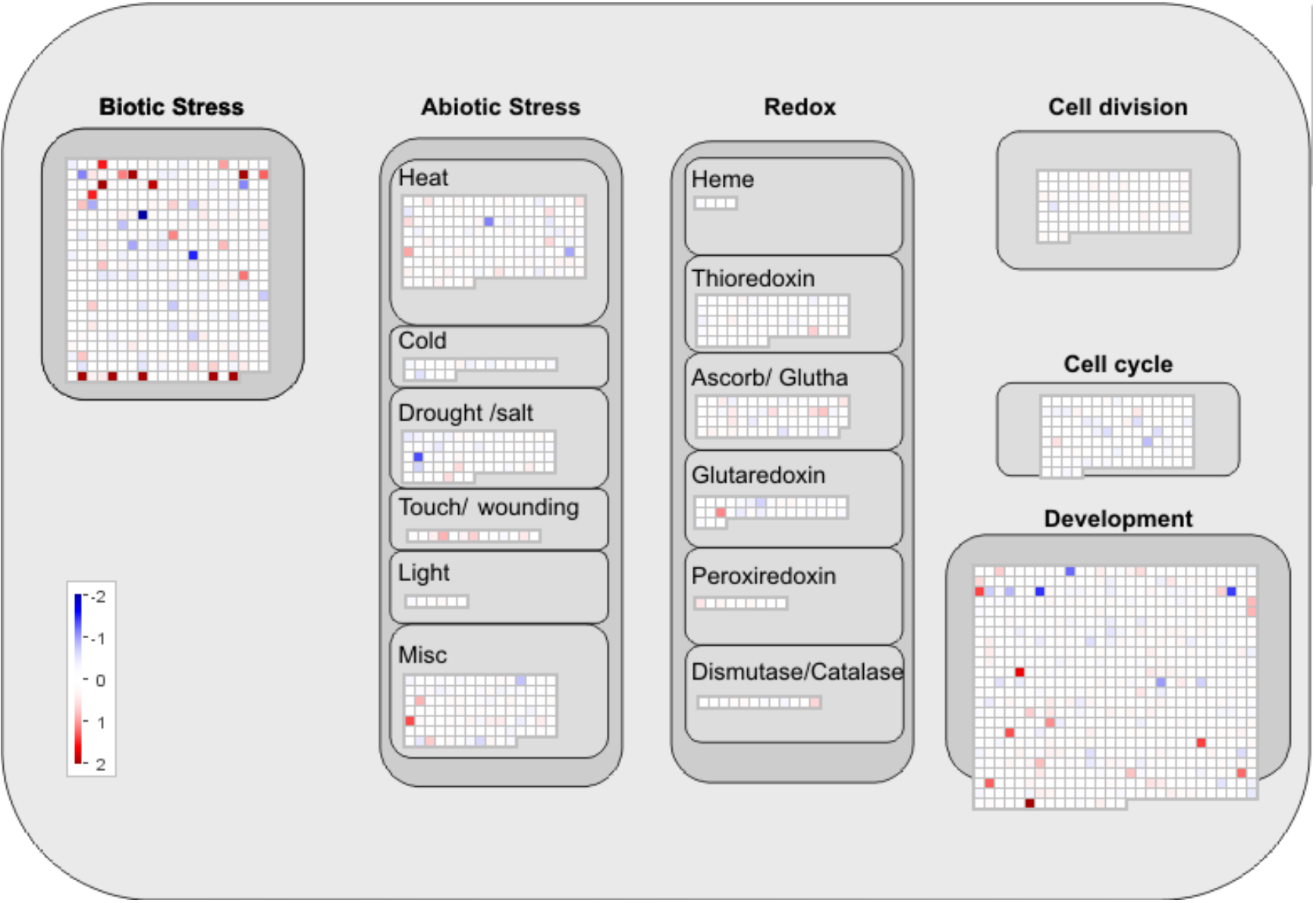
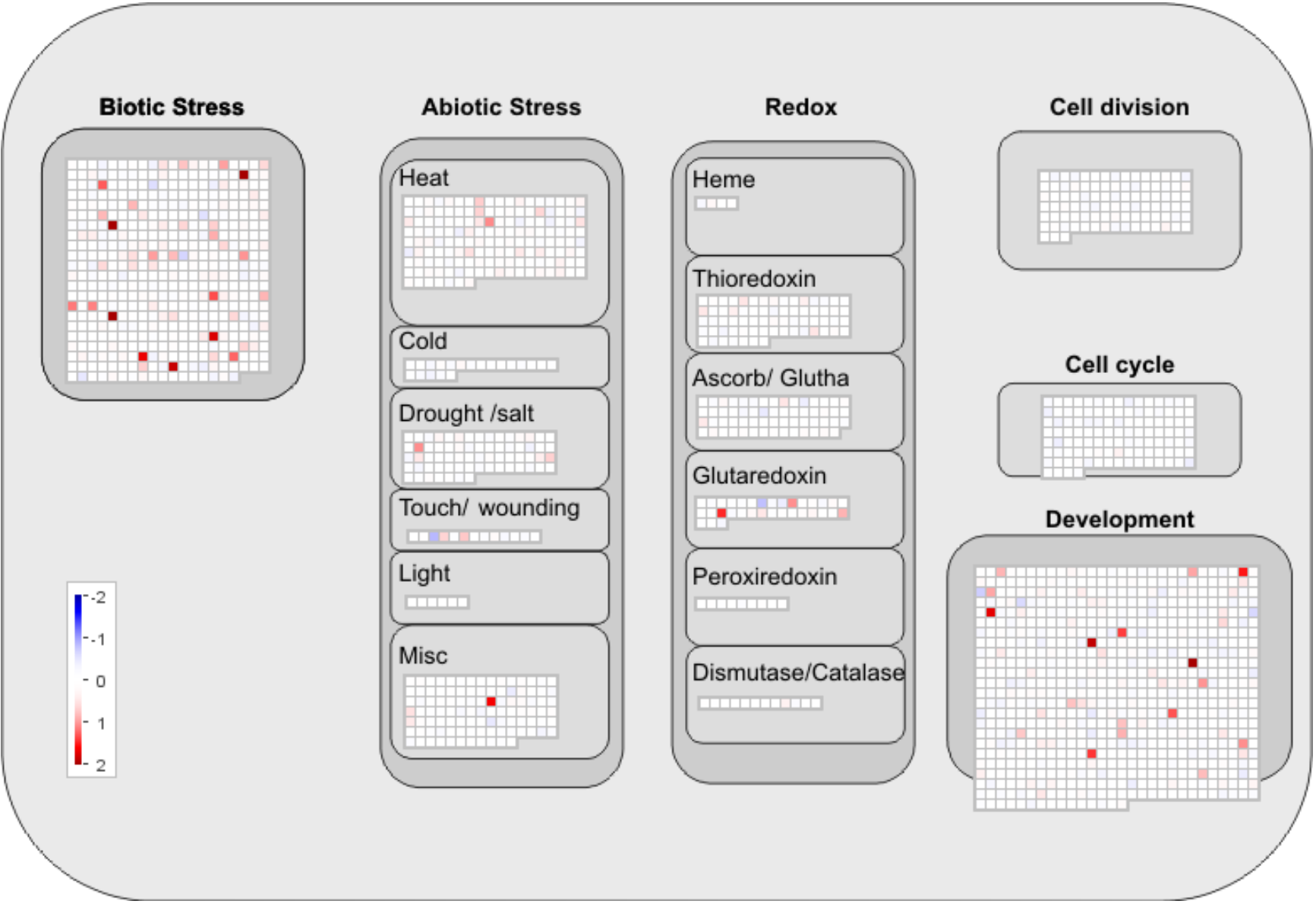


Figure S2: MapMan representations of differentially expressed genes.
(F) GA versus control - Cellular response.



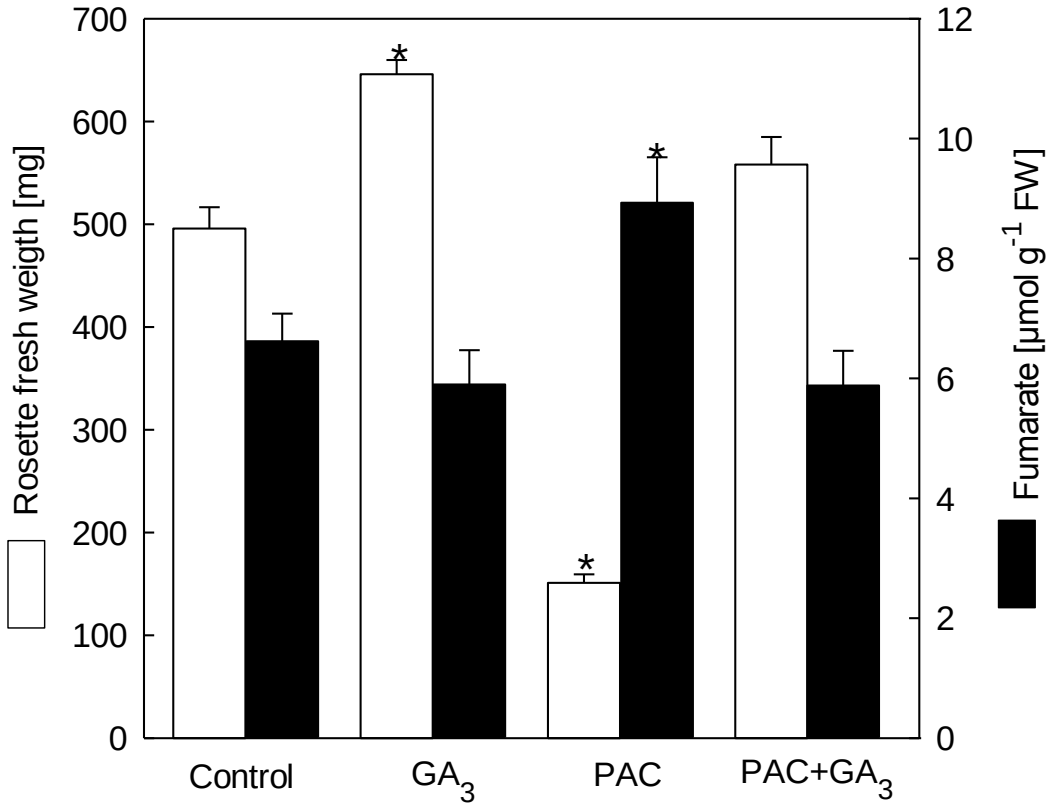


Figure S3 Effect of treatment with PAC and/or GA_3 on shoot biomass of *Arabidopsis* plants (27 days after sowing) and fumarate levels. Fourteen days after sowing, plants grown individually in pots were watered with 10 mL PAC (0.17 mM). To revert the growth inhibition, three days after PAC treatment plants were sprayed every second day with 1 mL of 50 µM GA_3. Asterisks indicate values determined by the Student's *t*-test to be significantly different from control ($P < 0.05$). Data are means $\pm$ SE of six replicates (each replicate is a pool of five plants).