

Supplemental Figure S1. Complementation of *rao7EMS* with wild-type *MYB29* restoring normal antimycin A induction of *PromAOX1a*-driven *LUC*. Luminescence image of a Petri dish containing *myb29* T-DNA knock-out line (*rao7KO*, see Supplemental Fig. S2) (top left), Col:*LUC* (top right), *rao7EMS* (bottom left), and *rao7EMS* complemented with the expression of the wild-type *MYB29*-coding sequence under the constitutive Cauliflower Mosaic Virus 35S promoter (P35S) (*rao7EMS+P35S:MYB29*) (bottom right). Whereas both wild type (Col:*LUC*) and *rao7EMS+P35S:MYB29* show induced luminescence upon treatment with antimycin A, *rao7EMS* luminescence is enhanced.



Supplemental Figure S2. Confirmation of T-DNA knock-out line for the MYB29/RA07-encoding gene. PCR with gene (At5g07690)-specific primers (LP+RP) and a T-DNA left border (LB) primer. MWM, molecular weight marker.



Supplemental Figure S3. Phenotypic analysis of *rao7* mutants under nonstress conditions. A, Plate-based growth progression analysis of Col:*LUC, rao7EMS*, and *rao7KO*. Arrows define the time (days after sowing) that Col:*LUC* plants have reached the growth stages as defined by Boyes et al. (2001). Stage 0.1, imbibition; stage 0.5, radical emergence; stage 0.7, hypocotyl emergence from seed coat; stage 1.0, cotyledons fully opened; stage 1.02, two rosette leaves >1 mm in length; stage 1.04, four rosette leaves >1 mm in length. Representative images of seedlings at 5, 7, 9, 12 and 15 days after sowing are shown. B, Soil-based growth progression as in (A): stage 1.10, 10 rosette leaves >1 mm; stage 5.10, first flower buds visible; stage 6.00, first flower opens. Representative images of seedlings at 26, 36, and 46 days after sowing are shown. C-E, Representative growth parameters of Col:*LUC* and *rao7* mutants: number of rosette leaves >1 mm, maximum rosette radius (cm), and plant height (cm) over time. Days are relative to the days after sowing after a 3-day stratification at 4°C. Data are given as averages for 15 plants \pm SE. Asterisk indicates significant differences in mutant compared to the wild type (*, *P* <0.05; **, *P* <0.01, Student's *t* test).



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Supplemental Figure S4. Yeast one-hybrid analysis for MYB29 binding to the *AOX1a* **1.85-kb promoter.** The *AOX1a* promoter was divided into 25 regions of approximately 100 bp with 25 bp overlapping with the previous and next regions. Yeast Y182 cells were cotransformed with the *MYB29* pGADT7-rec2 prev vector and pHIS2 *AOX1a* bait vector. Cells were spotted on double dropout media (DDO; transformation control) and triple dropout media for binding assay (TDO supplemented with 3-aminotriazole [3-AT] to control background growth). Binding of p53 to its p53-binding site or empty pHIS2 vector were used as positive (+) and negative (-) controls, respectively. TSS, transcriptional start site.

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	Ţ				GO:0010363 regulation of plant-type hypersensitive response	
	-		4	-	GO:0012501 programmed cell death	-
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					GO:0009751 response to salicylic acid	
					GO:0048583 regulation of response to stimulus	-
					GO:0009627 systemic acquired resistance	-
					GO:0009725 response to normone	H
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					GO:0035556 intracellular signal transduction	
			H		GO:0009862 systemic acquired resistance, SA signaling pathway	
					GO:0009723 response to ethylene	_
					GO:0032870 cellular response to hormone stimulus	
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					GO:0009738 abscisic acid-activated signaling pathway	
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					GO:0009595 detection of biotic stimulus	
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					GO:0006468 protein phosphorylation	
					GO:0009414 response to water deprivation	-
					GO:0043168 anion binding	
-					GO:0044283 small molecule biosynthetic process	
			H		GO:0051606 detection of stimulus	
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					GO:0044711 single-organism biosynthetic process	
					GO:0009651 response to salt stress	
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					GO:0042538 hyperosmotic salinity response	
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	-	-	GO:0005507 copper ion binding
	-		GO:0019760 glucosinolate metabolic process
			CO:0080167 response to karrikin
			GO:0005199 structural constituent of cell wall
			GO:0010337 regulation of salicylic acid metabolic process
			GO:0047893 flavonol 3-O-olucosyltransferase activity
			GO:0016209 antioxidant activity
			GO:0042991 transcription factor import into nucleus
			GO:0032101 regulation of response to external stimulus
			GO:0006096 glycolytic process
	4	-	GO:0002832 negative regulation of response to biotic stimulus
	-	-	GO:0032102 negative regulation of response to external stimulus
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			GO:0048767 root hair elongation
			GO:0016630 protochlorophyllide reductase activity
			GO:0009543 chloroplast thylakoid lumen
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			G0:001934 expetine biosynthetic process G0:0019344 cysteine biosynthetic process G0:0010218 response to far red light G0:0010117 carotenoid biosynthetic process G0:0005515 protein binding G0:0005576 extracellular region G0:0004534 chloroplast part G0:0004583 positive regulation of transcription, DNA-templated G0:0045833 positive regulation of transcription, DNA-templated G0:0014583 positive regulation of transcription, DNA-templated G0:0014583 positive regulation of transcription, DNA-templated G0:001555 cell wall organization G0:0004522 external encapsulating structure organization G0:001555 cell wall organization G0:0019288 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathway G0:004221 transcription regulatory region DNA binding G0:0004212 transcription regulatory region DNA binding G0:0006088 pentose-phosphate shunt G0:0009605 leaf morphogenesis G0:0003003 cellular cation homeostasis G0:0007623 circadian rhythm G0:0007838 divalent metal ion transport G0:007838 divalent metal ion transport G0:0007838 divalent metal ion transport G0:0009826 multidimensional cell growth G0:0009826 auxin polar transport G0:0010027 thylakoid membrane organization G0:001155 regulation of proton transport G0:0010025 was biosynthetic process G0:0010025 was biosynthetic process
			G0:001934 expative regulation of developmental process G0:0019344 cysteine biosynthetic process G0:0010218 response to far red light G0:001717 carotenoid biosynthetic process G0:0005515 protein binding G0:0005576 extracellular region G0:004434 chloroplast part G0:0004583 protein dimerization activity G0:004583 protein dimerization activity G0:004583 postive regulation of transcription, DNA-templated G0:004583 postive regulation of transcription, DNA-templated G0:001555 cell wall organization G0:00171555 cell wall organization G0:00171555 cell wall organization G0:0019684 photosynthesis, light reaction G0:0019684 sispoenteryl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathwar G0:0042212 transcription regulatory region DNA binding G0:0006088 pentose-phosphate shunt G0:0007638 divalent metal ion transport G0:0007638 divalent metal ion transport G0:0007638 divalent metal on transport G0:0007623 circadian rhythm G0:000926 auxin polar transport G0:000926 auxin polar transport G0:0010027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:00101027 thylakoid membrane organization G0:0010055080 cation homeostasis G0:0010027 thylakoid membrane organization G0:00100576 polysaccharide metabolic process G0:0010027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:00101027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:00101027 thylakoid membrane organization G0:00101027 thylakoid membrane organization G0:00101027 thylakoid membrane organization G0:00101027 thylakoid membrane organization G0:00
			G0:001934 cystene biosynthetic process G0:0019344 cystene biosynthetic process G0:0010218 response to far red light G0:001576 extracellular region G0:001576 extracellular region G0:000576 extracellular region G0:0004434 chloroplast part G0:000636 unsaturated fatty acid biosynthetic process G0:0046983 protein dimerization activity G0:0045893 positive regulation of transcription, DNA-templated G0:00143 cutin biosynthetic process G0:000526 extranal encapsulating structure organization G0:004522 external encapsulating structure organization G0:0019288 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathwar G0:000638 photoropism G0:00019288 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathwar G0:00019288 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathwar G0:00019288 isopentenyl diphosphate biosynthetic pr. G0:0006088 pentose-phosphate shunt G0:00019288 divalent metal ion transport G0:00070838 divalent metal ion transport G0:00070838 divalent metal ion transport G0:00070825 multidimensional cell growth G0:0009826 auxin polar transport G0:0010027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:0010027 thylakoid membrane organization G0:0005976 polysaccharide metabolic process G0:00005977 polysaccharide metabolic process G0:00009637 response to blue light G0:0004878 chemical homeostasis
			G0:001934 cysteine biosynthetic process G0:0019344 cysteine biosynthetic process G0:0010218 response to far red light G0:0052689 carboxylic ester hydrolase activity G0:0005515 protein binding G0:0005576 extracellular region G0:0004434 chloroplast part G0:0004638 unsaturated fatty acid biosynthetic process G0:0046983 protein dimerization activity G0:0046983 protein dimerization activity G0:0045893 positive regulation of transcription, DNA-templated G0:0045893 positive regulation of transcription, DNA-templated G0:00143 cutin biosynthetic process G0:0004529 external encapsulating structure organization G0:0017555 cell wall organization G0:0019268 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathway G0:0045229 external encapsulating structure organization G0:00019268 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathway G0:004634 photosynthesis, light reaction G0:00019268 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathway G0:004212 transcription regulatory region DNA binding G0:000526 auxin plate ration homeostasis G0:0007623 circadian rhythm G0:0007623 divalent metal ion transport G0:0004841 anthocyanin accumulation in tissues in response to UV light G0:000926 auxin plater transport G0:0004844 applast G0:0010027 thylakoid membrane organization G0:0010155 regulation of proton transport G0:0005976 polysaccharide metabolic process G0:00005976 polysaccharide metabolic process G0:00005976 polysaccharide metabolic process G0:0000577 response to blue light G0:0004878 chemical homeostasis G0:0003700 sequence-specific DNA binding transcription factor activity G0:0004878 chemical homeostasis
			G0:001934 cysteine biosynthetic process G0:0019344 cysteine biosynthetic process G0:0010218 response to far red light G0:0010117 carotenoid biosynthetic process G0:0005515 protein binding G0:0005515 protein binding G0:0004534 choroplast part G0:0004583 positive regulation of transcription, DNA-templated G0:0014583 positive regulation of transcription, DNA-templated G0:0014583 positive regulation of transcription, DNA-templated G0:0010143 cutin biosynthetic process G0:0004529 external encapsulating structure organization G0:0004522 external encapsulating structure organization G0:0019228 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathway G0:004522 external encapsulating structure organization G0:0019288 isopentenyl diphosphate biosynthetic pr, methylerythritol 4-phosphate pathway G0:004212 transcription regulatory region DNA binding G0:00042212 circadian rhythm G0:0007623 divalent metal ion transport G0:0007623 divalent metal ion transport G0:0007638 divalent metal ion transport G0:0009326 auxin polar transport G0:0009326 auxin polar transport G0:0010155 regulation of proton transport G0:0010157 regulation of proton transport G0:0005680 cation homeostasis G0:0010157 regulation of proton transport G0:0005677 response to blue light G0:0009878 chemical homeostasis G0:0000978 chemical homeostasis

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Supplemental Figure S5. Gene Ontology enrichment analysis of genes regulated by RAO7/MYB29. RAO7 positively (RAO7_P) and negatively (RAO7_N) regulated genes and their respective coexpression clusters (c1, c2, c3, c4 and c5) were analyzed for enriched Gene Ontology (GO) terms. GO terms enriched with FDR < E-03 (-log₁₀(FDR) > 3) in at least one of the gene lists are displayed after trimming for redundant GO terms. FDR values smaller and larger than E-03 are displayed in red-yellow and black, respectively.



Supplemental Figure S6. Transcriptional response to antimycin A (AA) regulated through the RAO7/MYB29-EMS function. A, Heatmap representation of the expression of genes of which the AA response is either negatively or positively regulated specifically through the RAO7/MYB29-EMS function. Genes were classified as negatively and positively regulated by MYB29-EMS only, when their AA fold change was increased (*RAO7-EMS_N*) and decreased (*RAO7-EMS_P*) in the *rao7EMS* and not in the *rao7KO* mutants, respectively. Genes were further classified in clusters (c1, c2, c3, c4, and c5) according to their expression characteristics in response to AA in the different genotypes by means of K-means clustering. Colors represent \log_2 fold changes of AA treatment compared to mock treated with blue and red/yellow representing transcripts that are down-regulated and up-regulated by the AA treatment, respectively. B, Proportion of AA up- and down-regulated genes through MYB29 or through the MYB29-EMS function. C, Gene Ontology Slim enrichment analysis of the *RAO7-EMS_N* and *RAO7-EMS_P* genes and their respective coexpression subclusters. Color codes represent the negative logarithm (base 10) of the FDR adjusted *P* value. Significantly enriched GO terms (FDR < 0.05) are indicated with a red-yellow color.





Supplemental Figure S7. Gene Ontology enrichment analysis of genes regulated by RAO7/MYB29-EMS. RAO7-EMS positively (*RAO7-EMS_P*) and negatively (*RAO7-EMS_N*) regulated genes and their respective coexpression clusters (c1, c2, c3, c4, and c5) were analyzed for enriched Gene Ontology (GO) terms. GO terms enriched with FDR < E-03 ($-\log_{10}(FDR) > 3$) in at least one of the gene lists are displayed after trimming for redundant GO terms. FDR values smaller and larger than E-03 are displayed in red-yellow and black, respectively.



Supplemental Figure S8. Correlation of the RAO7-EMS-regulated gene sets with functional annotations. The RAO7 and RAO7-EMS positively and negatively regulated genes (*RAO7_P, RAO7_N RAO7-EMS_P, and RAO7-EMS_N*) were used for gene set enrichment analysis with GO biological processes (BP) (FDR < 3.00E-05; see Supplemental Fig. S5 and S7) and differentially expressed (DE) genes (UP, up-regulated; DN, down-regulated) after hormone or stress treatments, the latter obtained from the literature through the Plant Gene Set Enrichment Analysis tool (FDR < 3.00E-03) (Yi et al., 2013). Afterwards, all gene lists were compared to each other in a pairwise manner. Only gene list pairs with significant overlaps (FDR < E-03) and containing at least 10% of genes from either of both gene lists were used for network construction and only gene sets directly connected to at least one of the four RAO7-regulated gene lists were retained. Edge thickness and distance in the network correspond to the Jaccard Index of similarity. Node shape refers to the type of gene list (square, GO-BP; circle, DE gene lists from literature) and node color corresponds to nodes connected only with *RAO7_P* or *RAO7-EMS_P* (red), with *RAO7_N* or *RAO7-EMS_N* (blue) or with both positively and negatively regulated gene sets (grey). ABA, abscisic acid; BRZ, brassinozole; BR, brassinosteroid; CK, cyto-kinin; ET, ethylene; ER, endoplasmic reticulun; GA, gibberellic acid; HL, high light; HR, hypersensitive response; IAA, indole-3-acetic acid; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; PCD, programmed cell death; SA, salicylic acid; SAR, systemic acquired resistance.

A. RAO7/MYB29-negatively regulated (RAO7_N)

	Amadeus <i>de</i>	Pavaluo	STAMP motifs							
	<i>novo</i> motif	(HG)	Name	E-value	Sequence	Name	E-value	Sequence		
(1)		5.3E-35	WBBOXPCWR KY1	3.9E-07		WRKY6_ oneSite	5.8E-07	ATAGTCAAC		
(2)	TTGAC_A	1.0E-30	WRKY6_ oneSite	2.8E-09	GTTGACTAT	WRKY40_ oneSite	1.1E-08	CTTTGACCAA		
(3)	CAGTCARE	4.3E-21	WRKY6_ oneSite	3.2E-08	ATAGTCAAC	WBBOXPC- WRKY1	3.9E-06			
(4)	ACCCC	3.4E-23	CE3OSOSEM	5.0E-06		ABREMOTIFIII- OSRAB16B	5.7E-06	GCCACGCGGC		
(5)	GAATAGIC	2.1E-20	SP8BFIB- SP8BIB	1.6E-06						
(6)	^A _cAAGT_G	9.4E-19	RBENTGA3	6.9E-07	TCCAAGTTGGA	O2F1BE2S1 ABREDISTB BNNAPA	1.2E-06 4.7E-06	TCCACGTCGA Gacaagtggc		
(7)	GTccc_A	7.3E-16	DRECRT- COREAT	2.3E-06	GTCGG ₂	AtERF-1_twoSite AtERF-2_twoSite AtERF-5_twoSite	2.9E-06	TGGCGGCT		
(8)	AGerGCCA	2.3E-12	AtERF-3 AtERF-4	2.8E-08 4.5E-08	GAGCCGCCA	AtERF-1_twoSite AtERF-2_twoSite AtERF-5_twoSite	1.6E-07	AGCCGCCA		
(9)		9.6E-16	2SSEEDPROT BANAPA	2.4E-05		WBBOXPCWR KY1	2.6E-05			
(10)		1.9E-15	ACIPVPAL2	1.0E-11 9.2E-11	CCCACCTACC	AtMYB- 84_M00970	8.6E-11			
(11)		2.3E-15	UPRE1AT	1.0E-05	ATTGGTCCACG	GCBP2ZM- GAPC4	2.4E-05	CGGGCCCAC		
(12)		1.6E-14	ERELEE4	5.8E-05	TTTGAA _? T					
(13)		2.8E-14	MADS- A_M00408* MADS- B_M00404*	2.1E-07 9.6E-07	* 	CArG1 CArG3	1.0E-06 1.0E-06	-GTTTACATAAATGGAAAA -GTTACTAAAAATGGAAAA		
(14)		3.1E-14	CBF1*	9.9E-09	ETCACGTG	LS5ATPR1 LS7	8.3E-08	GTGACGTAGA		
(15)		3.1E-14	TATABOX1 TATABOXOSP AL	1.7E-08 2.4E-07	CGTATTTA TATTTAA	CONSERVED- 11NTZMATP1	1.2E-06	ACGTATTAAAA		
(16)		7.6E-14	PIATGAPB PI	9.0E-05	GTGATCAC	RNFG2OS	1.1E-04	CCAGTGTGCCCCTGG		
(17)	GACGAATA	9.0E-14	AS1LIKECSHP- RA	1.6E-06	AAATGACGAAAATGC	27BPDRCON- SENSUSPS25S	1.8E-06			

Supplemental Figure S9. De novo promoter motif discovery in RAO7/MYB29-regulated genes. Enriched degenerate 8mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the $RAO7_N$ (A) and $RAO7_P$ (B) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus large run delivered 20 enriched motifs as output. De novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publically available were copied from the STAMP website and indicated with asterisks. HG, hypergeometric.

A. RAO7/MYB29-negatively regulated (RAO7_N) (continued)

	Amadeus de	P-value	STAMP motifs							
	novo motif	(HG)	Name	E-value	Sequence	Name	E-value	Sequence		
(18)		1.3E-13	GLUTAACAOS	5.1E-04		DRE2COREZ MRAB17	5.1E-04	GTCGGT		
(19)	TCGATGCC	2.0E-13	TGA1A- NTPR1A	3.6E-06	CGTCATCTCGATGACG					
(20)	ATAATCG	3.9E-12	ATHB6	1.3E-07	TAATAATT	ATHB1	8.5E-07	`CAAT ∩ ATT <u>G</u>		

B. RAO7/MYB29-positively regulated (RAO7_P)

	Amadeus de	P-value	STAMP motifs							
	<i>novo</i> motif	(HG)	Name	E-value	Sequence	Name	E-value	Sequence		
(1)		4.5E-24	SORLIP2	4.1E-06	GGGCC	GCBP2ZM- GAPC4	1.8E-05	CGGGCCCAC		
		ary site.				SITEIIAOSPCNA	1.8E-05	ACGGGCCCA		
(2)		6.8E-22	ABREDISTB- BNNAPA	3.3E-09	GACAAGTGGC	ABREAZM- RAB28	6.0E-08	GCCACGTGGG		
(3)		7.5E-19	RYREPEAT- BNNAPA	1.3E-04		AUXRETGA1- GMGH3	2.3E-04	TGACGTAA		
(4)		4.5E-16	TATCCAC- HVAL21	1.9E-11		SREATMSD	4.5E-09			
(5)		5.7E-16	MYB80_ M01052*	1.4E-07	BEGATAT C	TaMYB80	3.7E-07 💈			
(6)				3.25-08		AtMYB15	3.7E-07 🖁			
	AVUASYIA	1.46-14	AGIE VEALZ	0.2L-00		AtMYB84	7.4E-07 #			
(7)		4.4E-14	JASE2ATOPR1	1.3E-05	TTGACGACGTAT <u>G</u>	LS5ATPR1 LS7	2.3E-05	GTGACGTAGA		
	GAVELAIA		bZIP911_M003 59*	3.8E-05	TGACGTGTAC	QELEMENTZ MZM13	7.8E-05	TGACCT		
(8)	ΛΤΛΛΑΤ ΟΑ	205 14	ΤΔΤΔΒΟΥ1	4.1F-07		CARG1ATAP3	1.1E-06	GTTTACATAAATGGAAAA		
(0)		2.0E-14		4.1E-07		ATHB6	1.5E-06	TAATAATTG		
(9)		7.0E-14	SPL1_oneSite	9.2E-07	ATTGTACGG	ACGTOSGLUB1	1.5E-06	CACGTAC		
(10)		1 6F-13	TGTCACACM-			SEBFCONSSTP R10A	9.2E-07	₽ <mark>₽TGTC≁C</mark>		
		1.02 10	CUCUMISIN	1.7 E-07		BIHD1OS	1.9E-06	TGTCA		

Supplemental Figure S9. De novo promoter motif discovery in RAO7/MYB29-regulated genes. Enriched degenerate 8mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the $RAO7_N$ (A) and $RAO7_P$ (N) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus large run delivered 20 enriched motifs as output. De novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publically available were copied from the STAMP website and indicated with asterisks. HG, hypergeometric. (*Continued*)

	Amadeus <i>de</i>	P-value	STAMP motifs					
	<i>novo</i> motif	(HG)	Name	E-value	Sequence	Name	E-value	Sequence
(11)		5.3E-13	REBETALGLHC B21	7.5E-07	TATCCG	MYBST1	2.8E-06	TATCC
(12)		1.0E-12	MYBST1	1.9E-06	TATCC	CATATGGMSAU	R1.9E-05	
(13)	ATACGAAC	6.9E-13	PEND*	1.1E-05		ABAREG2	4.9E-05	ATGTACGAAGC
(14)		9.6E-13	ATHB5_ M00503*	7.1E-06		ATHB6	7.4E-06	TAATAATT G
(15)		1.1E-12	TATABOX1	1.3E-08	CTATAAATAC	ATML1_oneSite PDF2_oneSite	4.6E-08	GTAAATGCAC
(16)		2.1E-12	TATABOX3 TATABOX2	4.9E-07 6.7E-06	ATTAATA ATTTATA	SORLIP4	9.1E-06	CCATCATAC
(17)		2.4E-12	SP8BFIBSP8BIB SPF1_M00702*	6.4E-07 1.6E-06	AATAGTA	SORLIP4	3.9E-05	CCATCATAC
(18)	C_TTATAG	2.8E-12	TATABOX1	1.5E-05 🗤	GTATTTATA <mark>g</mark>	ATHB- 5_M00503*	1.6E-05	
(19)	AC_CCA_A	3.2E-12	LREBOX2- PSRBCS3	4.0E-05	CATATTAACCACACA	SE1PVGRP183	5.1E-05	* ATCCCCCACAGTGTGGCCCATTAT
(20)		8.6E-12	DRE2COREZM-	2.2E-05		AtWER*	2.9E-05	ACTETCTCTAAA
(20)		5.01 .L	NADI/		,nvvvn <u>v</u>	GARE1OSREP1	6.4E-05	TAACAGA

B. RAO7/MYB29-positively regulated (RAO7_P) (Continued)

Supplemental Figure S9. De novo promoter motif discovery in RAO7/MYB29-regulated genes. Enriched degenerate 8mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the *RAO7_N* (A) and *RAO7_P* (B) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus large run delivered 20 enriched motifs as output. De novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publically available were copied from the STAMP website and indicated with asterisks. HG, hypergeometric. (*Continued*)

	Amadeus de P-value		STAMP motifs							
	novo motif	(HG)	Name	E-value	Sequence	Name	E-value	Sequence		
(1)		5.5E-25	ABREMOTIFIII OSRAB16B	2.8E-06	GCCGCGTGGC	OCETYPEIINT HISTONE	5.3E-06	GATCCGCGTGA		
(2)		9.1E-19	ABREMOTIFIII OSRAB16B	2.2E-06	TTGAC	MYBCORE ATCYCB1	4.6E-06	CCGTT		
(3)	AAcGCc A	3.7E-19	AtSR1	1.0E-06		APOLYA_ M00310	5.7E-06			
(4)	AAAGTCs	1.1E-18	CEREGLUBO X3PSLEGA	6.8E-06	TGTAAAAGT	Т-ВОХ	2.3E-05	AAAAGTCG		
(5)	GGGCATAA	5.9E-17	TEFBOXATEE F1AA1	9.9E-10	ACCCCCATAATCCTAA					
(6)	CG G CA	2.2E-16	LRENPCABE	1.2E-08		ACGTABREM OTIFA2OSEM	2.7E-07	ACGTG		
(7)	CGI_TA_A	9.3E-16								
(8)		4.6E-14	ABASEED1	1.2E-04	TGTTACGTGCC					
(9)		5.0E-14	AS1LIKE CSHPRA	5.7E-11	GCATTITCGTCATT	RBCSBO X3PS	6.1E-08	ATCATTITCACT		
(10)		9.4E-14	ACGTSEED2	1.5E-05	ACACACGTCAA	PREATPRODH	7.5E-05	ACTCAT		
	B. RAO7/MYB29-	EMS pos	sitively regu	lated ((RAO7-EMS_P)					
(1)		6.0E-34	SREATMSD	9.7E-10	C TATCC	IBOX	1.2E-09			
(2)	ATGTGGC	1.3E-20	LRENPCABE	1.5E-09	ACGTGGCA	HY5AT	1.9E-07	TGACACGTGGCA		
(3)	CCAC_CAA	6.0E-17	GLUTEBP1OS	1.4E-07	AAGCAACACACAAC	LREBOX2PSR BCS3	2.3E-07	*CATATTAACCACACA		
(4)		3.8E-16	GGTCCCATG MSAUR	9.5E-13	ATGGGACC	SITEIIBOS PCNA	7.1E-08			
			AUXREPSIAA4	1.2E-11	AIGGGAC e					
(5)		2.5E-15	E2FBNTRNR	2.2E-07		ANAERO2CO NSENSUS	3.2E-05			
(6)		4.8E-15	SEBFCONSSTF R10A TGTCACACM	7.0E-06 6.1E-05		GATA	2.8E-05	₽ <mark>₽ТАТС</mark> ₽		
(7)		1.1E-14	CUCUMISIN NAM_oneSite	1.7E-05	TCATCCCTT	S2FSORPL21	2.2E-04			
						UP2ATMSD	3.1E-05			
(8)		3.1E-14	DCRNIA1	5.2E-07	ACCCIECC	TELO-box	9.6E-05			

A. RAO7/MYB29-EMS negatively regulated (RAO7-EMS_N)

Supplemental Figure S10. De novo promoter motif discovery in RAO7/MYB29-EMS--regulated genes. Enriched degenerate 8-mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the *RAO7-EMS_N* (A) and *RAO7-EMS_P* (B) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus normal run provided 10 enriched motifs as output. The de novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publicly available were copied from the STAMP website and indicated with an asterisk (*). HG, hypergeometric.

	Amadeus de	P-value	STAMP motifs						
	novo motif	r-value	Name	E-value	Sequence	Name	E-value	Sequence	
(6)		4.8E-15	SEBFCONSST R10A	7.0E-06	ਙ <mark>TGTC</mark> ≏C	GATA	2.8E-05	¦⊊TATC _全	
			TGTCACACM CUCUMISIN	6.1E-05	TGTCACA				
(7)		1.1E-14	NAM_oneSite	1.7E-05	TCATCCCTT	S2FSORPL21	2.2E-04		
			AMMORESIIU	5 2E 07		UP2ATMSD	3.1E-05	AAACCCTA	
(8)		3.1E-14	DCRNIA1	0.22 07		TELO-box	9.6E-05	AAACCCTAA	
(8)		3.1E-14	AMMORESIIU DCRNIA1	5 2E-07		UP2ATMSD	3.1E-05	AAACCCTA	
				0.22 07		TELO-box	9.6E-05	AAACCCTAA	
(9)	STCcGetA	7.3E-14							
(10)		6.4E-13	CDA1ATCAB2	4.2E-06	GCGTTTTG	PROXBBNNAPA	4.7E-05	GGTGTTTG	

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0.1

Supplemental Figure S11. Sequence conservation of potential MYB29 homologs. A, Phylogenetic tree of MYB29 sequence-similar proteins from dicots obtained through the Ensembl Plants release 33 platform with BLAST in Ensembl (E-value < 1.00E-65). *Glycine max* (GLYMA), *Populus trichocarpa* (POPTR), and *Vitis vinifera* (VIT) protein sequences similar (E-value < 1.00E-62) to MYB29 were included as an outgroup of the tree. MYB29 was identified in a Brassicaceae-specific clade, including *Arabidopsis thaliana* (At) MYB28 and MYB76 and *Arabidopsis lyrata* (AL), *Brassica napa* (BN), *Brassica oleracea* (Bo), and *Brassica rapa* (Bra) homologs. These results indicate that the *Arabidopsis thaliana MYB29, MYB28*, and *MYB76* genes and their respective orthologous genes were obtained from a common ancestral gene. The phylogenetic tree was constructed in Mega 5 with the maximum likelihood method and bootstrap values were estimated with 1,000 replicates (Tamura et al. 2011). B, Analysis of the conservation of MYB29 arginine (R) 178 (highlighted with asterisk) in the homologous proteins. Conservation was found in all included species in the clade: *A. lyrata* (3/3), *B. napa* (1/4), *B. oleracea* (1/5), *B. rapa* (2/4). Sequences were aligned by means of the ClustalW algorithm in BioEdit (version 7.2.5) with default alignment parameters (Hall, 1999). Residues common or similar in at least half of the sequences are marked with colored boxes.



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Supplemental Figure S12. Comparison of genes regulated by RAO7, KIN10, and sucrose. Venn diagrams depicting overlap between genes regulated by RAO7 and SNF1 KINASE HOMOLOG 10 (KIN10) (A), RAO7-EMS and KIN10 (B), RAO7 and sucrose (C), RAO7-EMS and sucrose (D), RAO7, KIN10, and sucrose (E), and RAO7-EMS, KIN10, and sucrose (F). KIN10-regulated genes were obtained from Baena-Gonzalez et al. (2007) and differential genes upon sucrose treatment from Gonzali et al. (2006). The probability (*P* value) of having an overlap size equal or greater than observed was calculated with a cumulative hypergeometric test in R (v.3.2.3). N.s., not significant.



Supplemental Figure S13. Plot of the total within-group sum of squares against the number of clusters in K-means solutions. For each gene list ($RAO7_N$, $RAO7_P$, $RAO7-EMS_N$, and $RAO7-EMS_P$), genes with similar expression patterns (log_2 AA-fold changes in Col:*LUC*, *rao7EMS*, and *rao7KO*) were grouped by K-means clustering (with k = 2 to 20 number of clusters) with the *kmeans* function of R (Hartigan and Wong, 1979; R Core Team, 2014) and total within-group sum of squares were calculated. An appropriate number of clusters (k) was chosen for which the decrease of the within-group sum of squares dropped off.