

New Phytologist Supporting Information

Article title: Evidence of chromatin and transcriptional dynamics for cold development in peach flower bud Authors: Monica Canton, Cristian Forestan, Gianpiero Marconi, Esther Carrera, Claudio Bonghi and Serena Varotto Article acceptance date: 15 July 2022

The following Supporting Information is available for this article:



Fig. S1 Peach flower bud development during cold season. Images were photographed at 0 chilling units (CU), 200CU, 475CU, and 770CU from branches located at the median portion of the plant.

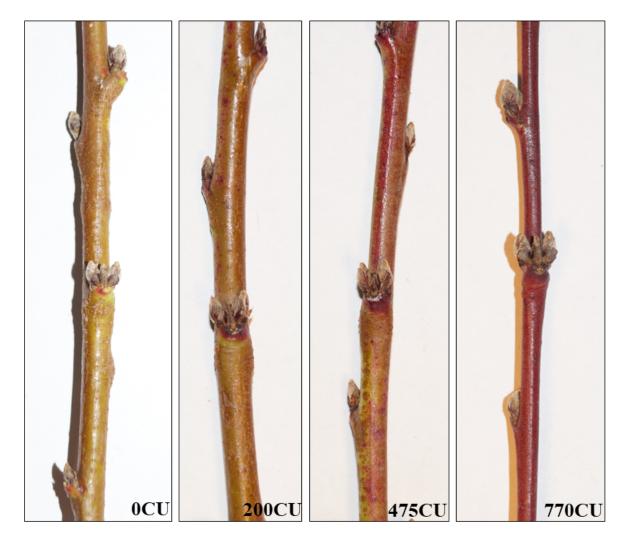




Fig. S2 Comparison between *PpDAM* loci gene annotation using the refence *Prunus persica* genome annotation (blue rectangles) and Reference Annotation Based Transcript (RABT) annotation (red rectangles).

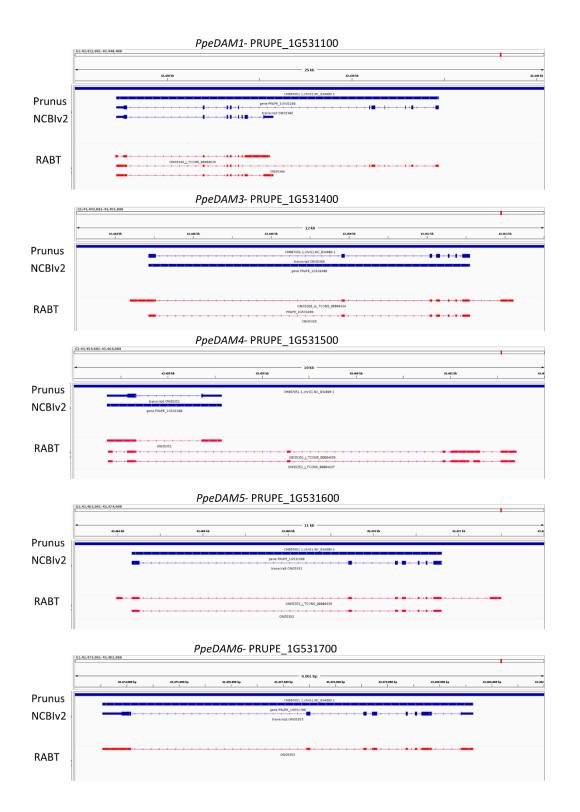




Fig. S3 Prunus GO terms of reference and newly annotated transcripts were de-novo annotated using Trinotate (Bryant *et al.*, 2017) and compared with the prunus GO annotation available at EnsemblPlants/Biomart database on July 2020 using WEGO GO plotting tool categorized using level 2 of the GO lineage.

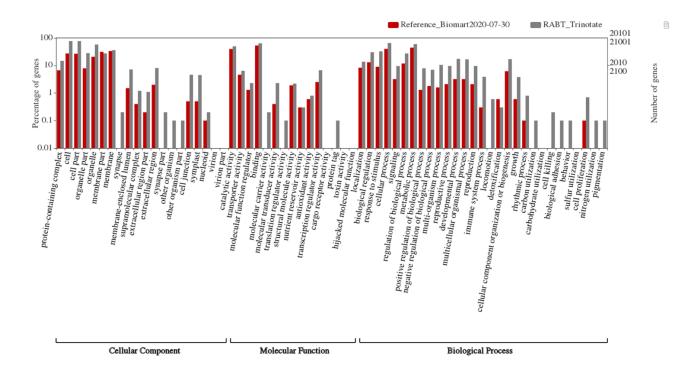
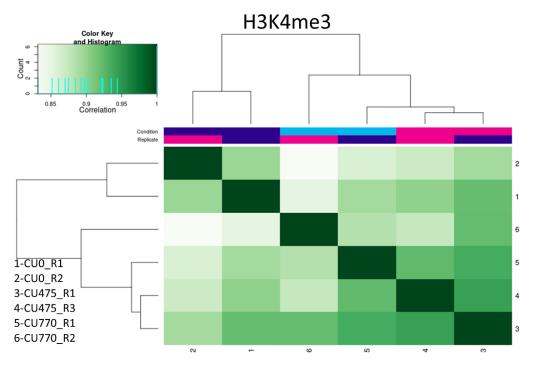




Fig. S4 Correlation heatmaps between replicates, using read count data were produced using DiffBind R package version 4.2.





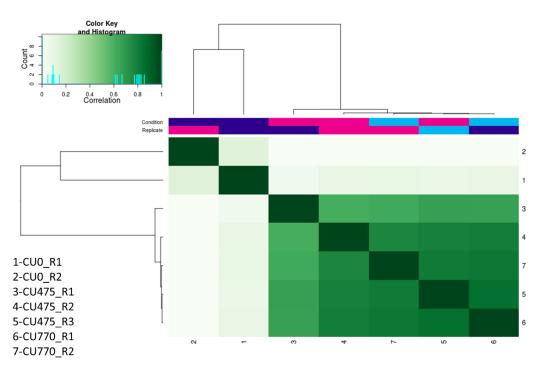




Fig. S5 Quantification of Gibberellin 1 (GA1), Indol-3-Acetic acid (IAA), isopentenyl adenine (iP), dihydrozeatin (DHZ) and t-zeatine (tZ) during chilling accumulation. Error bars indicate standard deviation (+/- SD).

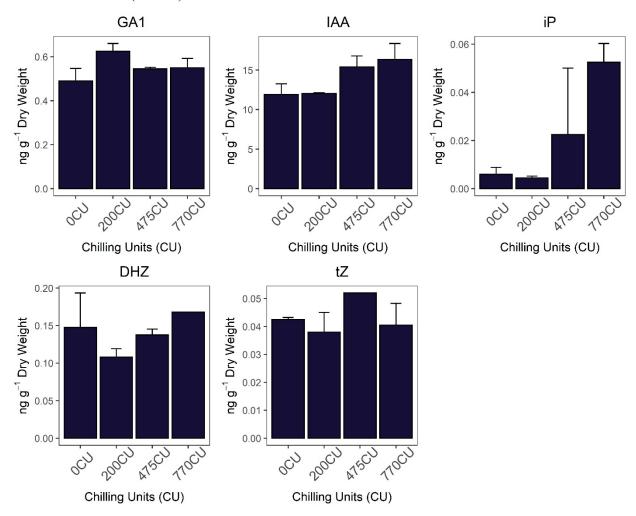




Fig. S6 Principal component analysis (PCA) of samples by transcriptome profile. PC1 and PC2 represent the first two largest sample variances from overall gene expression (a). Identification and classification of novel genes using RABT approach (b).

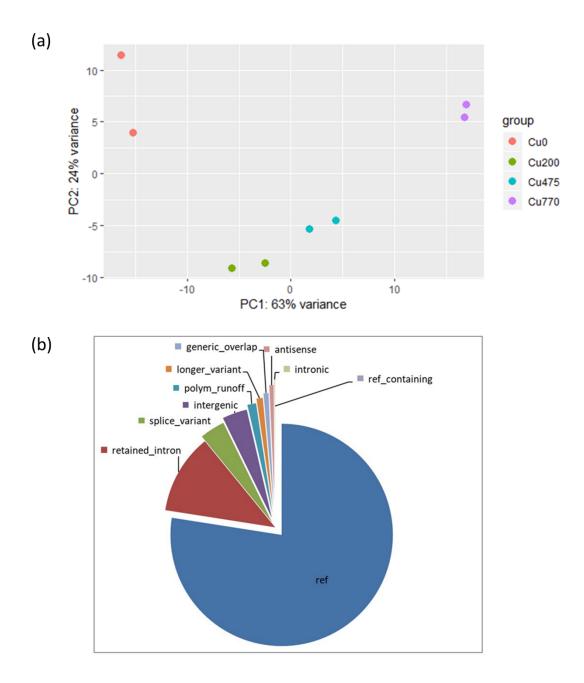




Fig. S7 Gene expression validation in RT-qPCR of *PpeDAM6, PpeDAM5, PpeDAM4, PpeDAM3, PpeDREB1D, PpeCYP707A4, PpeNCED5* and *PpeGA20ox.* A correlation analysis was performed for each gene using the RNAseq-FPKM values and qPCR expression normalized to *PpeUBQ.* A Pearson correlation is reported for each gene. Error bars indicate standard error (+/- SE).

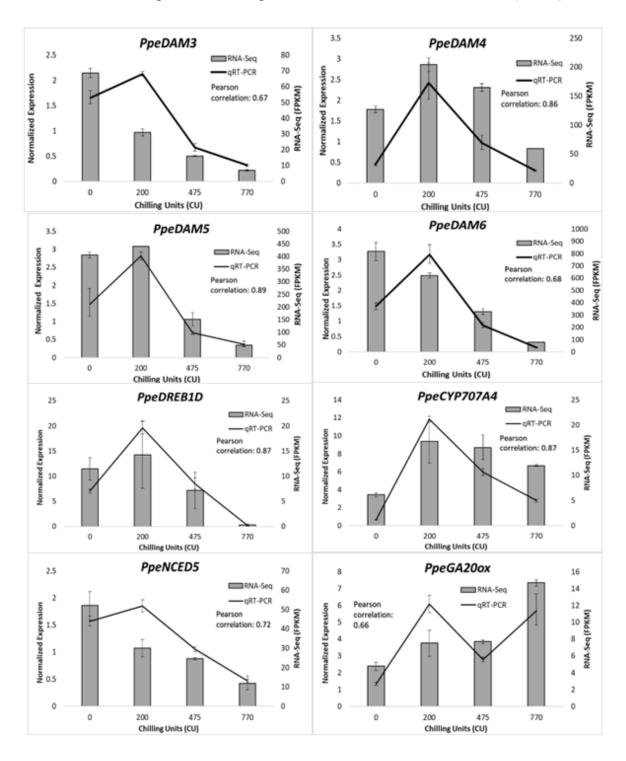




Fig. S8 In situ hybridization of *PpeDAM4* mRNAs in peach floral buds during chilling accumulation. The image represents a longitudinal section of a floral bud labeled with a sense mRNA probe. Bars = 500μ m.

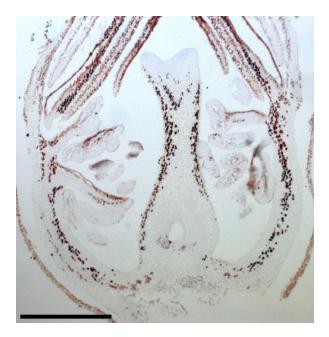




Fig. S9 Principal component analysis (PCA) of the differentially methylated regions (DMR) in CG, CHG and CHH at 200 (ff9), 475 (ff11) and 770CU (ff13) against 0CU (ff8). All three biological replicates are present.

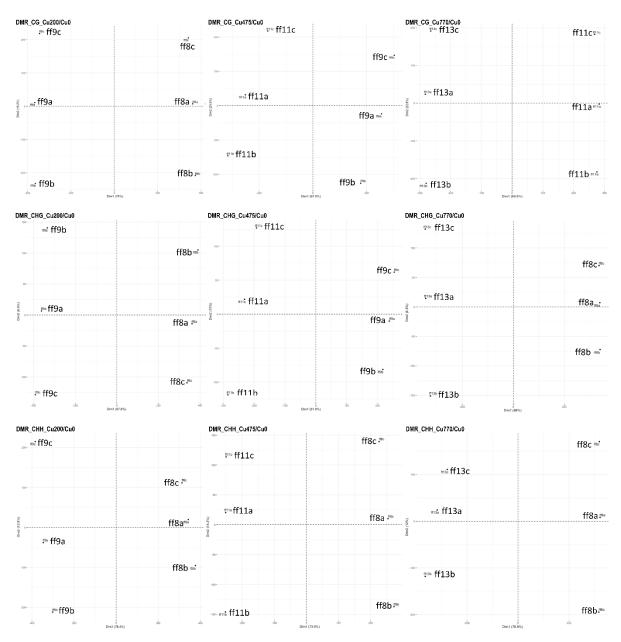




Fig. S10 Enrichment analysis of DMRs in different genomic regions. Enrichment analysis was performed using the binomial distribution of all of the MCSeEd loci as expected and the differentially methylated regions (CG, CHG, CHH contexts; note that scales for each context differ), as the observed datasets. Light gray = expected number of DMRs, Dark gray = observed number of DMRs. Asterisks indicate significant (*p < 0.05), highly significant (*p < 0.001), and extremely significant differences (***p < 0.0001) and ns means non-significant, calculated using the t test.

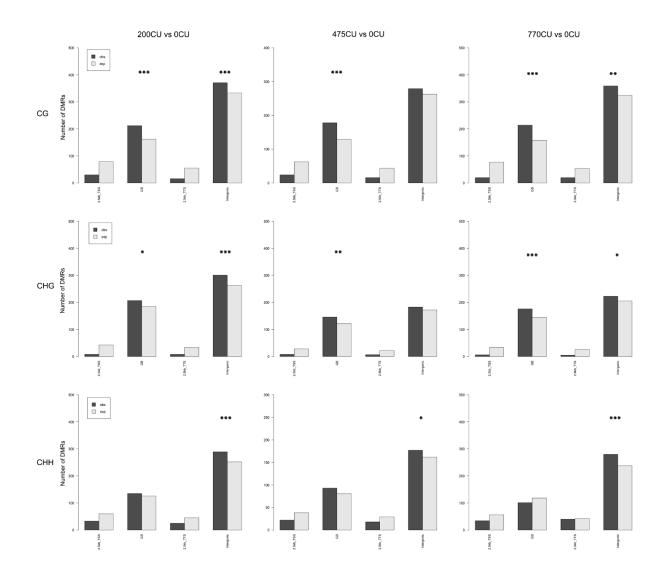




Fig. S11 DMRs distribution by pairs (200CU *vs* 0CU, 475CU *vs* 0CU and 770CU *vs* 0CU) across the transcribed genic regions extended by 2.5 kb at both ends (EGBs) at the differentially methylated regions (CG, CHG, CHH contexts).

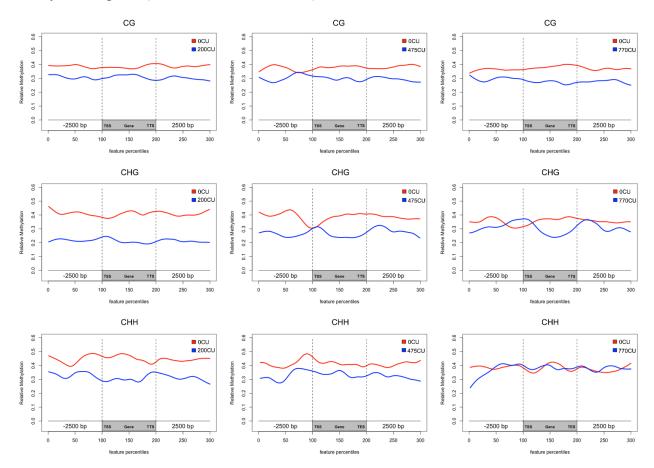


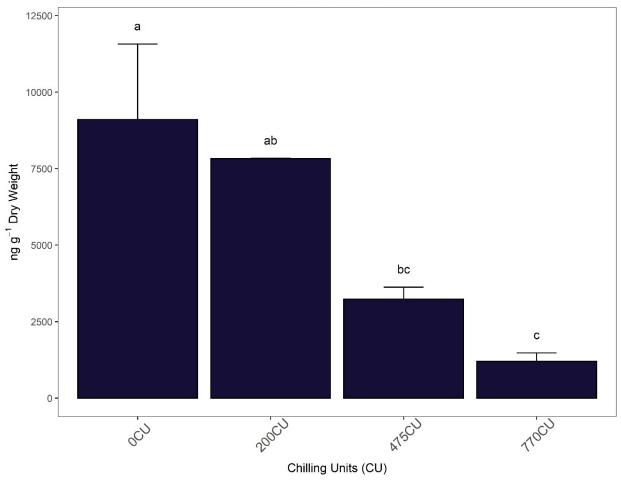


Fig. S12 Gene Ontology analysis (GO) of Differentially methylated genes (DMG) in all three different methylation contexts.

Term Name		Term ID	200CU x 0CU	p_adj	770CU x 00
response to drug		GO:0042493	1.000	7.424×10 ⁻³	1.000
sepal giant cell differentiation		GO:0090392	1.000	1.913×10 ⁻²	1.000
plant epidermal cell fate specification		GO:0090628	1.000	1.913×10 ⁻²	1.000
self proteolysis		GO:0097264	1.000	2.074×10 ⁻²	1.000
regulation of abscisic acid biosynthetic proce	ess	GO:0010115	6.070×10 ⁻²	3.003×10 ⁻²	1.000
response to water dipeptide transport		GO:0009415 GO:0042938	3.681×10 ⁻² 1.000	1.000	1.000 4.119×1
negative regulation of seed germination		GO:0010187	2.584×10 ⁻¹	1.000	4.567×1
(
				1 to 8 of 8	< Page 1 of 1
ppersica.GO_CC.RABT11E10			200CU x 0CU	475CU x 0CU	770CU x 0
Term Name clathrin-coated pit		Term ID GO:0005905	p_adj 4.121×10 ⁻²	p_adj 7.668×10 ⁻²	p_adj
				1 to 1 of 1 K	< Page 1 of 1
ppersica.GO_MF.RABT11E10			200CU x 0CU	475CU x 0CU	770CU x 00
Term Name		Term ID	p_adj	p_adj	p_adj
ubiquinol-cytochrome-c reductase activity		GO:0008121	1.000	1.109×10 ⁻³	1.439×1
calcium-dependent cysteine-type endopepti		GO:0004198	1.000	1.160×10 ⁻²	1.000
tripeptide transmembrane transporter activit	ty	GO:0042937	7.426×10 ⁻¹	1.000	1.923×1
peroxiredoxin activity mannitol transmembrane transporter activity	v	GO:0051920 GO:0015575	1.000	1.000	2.694×1 3.295×1
mannitol transmembrane transporter activity myo-inositol transmembrane transporter act		GO:0015575 GO:0005365	1.000	1.000	3.295×1 3.295×1
D-ribose transmembrane transporter activity		GO:0015591	1.000	1.000	3.295×1
sorbitol transmembrane transporter activity		GO:0015576	1.000	1.000	3.295×1
D-xylose transmembrane transporter activity	1	GO:0015148	1.000	1.000	3.295×1
phospholipase A1 activity		GO:0008970	1.607×10 ⁻¹	1.000	3.979×1
oligopeptide transmembrane transporter act	tivity	GO:0035673	1.000	1.000	4.274×1
				1 to 11 of 11	< Page 1 of 1
ppersica.GO_BP.RABT11E10			200CU x 0CU	475CU x 0CU	770CU x 0
Term Name		Term ID	p_adj	p_adj	p_adj
negative regulation of anoikis		GO:2000811	4.806×10 ⁻³	1.000	1.000
positive regulation of anoikis		GO:2000210	4.806×10 ⁻³	1.000	1.000
negative regulation of gene expression, epig	jenetic	GO:0045814	5.753×10 ⁻¹	1.000	9.109×
maintenance of chromatin silencing		GO:0006344 GO:0006662	2.159×10 ⁻² 2.527×10 ⁻²	1.000	5.863× 1.000
glycerol ether metabolic process cilium assembly		GO:00060271	6.675×10 ⁻²	2.555×10 ⁻²	1.000
photosystem I assembly		GO:0048564	4.400×10 ⁻²	1.000	1.000
centrosome cycle		GO:0007098	4.851×10 ⁻²	7.005×10 ⁻¹	3.609×
				1 to 8 of 8	C Page 1 of
ppersica.GO_CC.RABT11E10			200CU x 0CU	475CU x 0CU	770CU x 0
Term Name		Term ID	p_adj	p_adj	p_adj
clathrin-coated vesicle		GO:0030136	6.173×10 ⁻²	1.670×10 ⁻²	1.757×
nuclear microtubule		GO:0005880	9.814×10 ⁻¹	1.000	1.803×
cell cortical microtubule cytoskeleton		GO:0005623 GO:0030981	1.952×10 ⁻² 3.616×10 ⁻²	1.000	1.000
containintotabale cylosiceton		00.00.000	3,010×10	1.000	1.000
PROVIDE CO. MERADINESS			200CU x 0CU	1 to 4 of 4	Page 1 of 770CU x 0
ppersica.GO_MF.RABT11E10		Term ID			
Term Name dethiobiotin synthase activity		GO:0004141	p_adj 8.759×10 ⁻²	p_adj	p_adj 3.354×
AMP deaminase activity		GO:0003876	4.229×10 ⁻²	1.000	1.000
				1 to 2 of 2	C C Page 1 of
			200CU x 0CU	1 to 2 of 2	
ppersica.GO_BPRABT11E10 Term Name		Term ID	200CU x 0CU p_adj		770CU x
ppersica.GO_BRRABT11E10 Term Name sugar mediated signaling pathway		GO:0010182	p_adj 9.006×10 ⁻³	475CU x 0CU p_adj 1.000	770CU x p_adj 9.737×
ppersica.GO_BPRABT11E10 Term Name sugar mediated signaling pathway palant-type cell wall organization	ative to reword	GO:0010182 GO:0009664	p_adj 9.006×10 ⁻³ 5.138×10 ⁻¹	475CU x 0CU p_adj 1.000 1.000	770CU x p_adj 9.737× 2.135×
ppersica.GO_BRRABT11E10 Term Name sugar mediated signaling pathway	ative to reprod	GO:0010182	p_adj 9.006×10 ⁻³	475CU x 0CU p_adj 1.000	770CU x p_adj 9.737×
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ppersica.GO_BPRABT11E10 Term Name sugar mediated signaling pathway palant-type cell wall organization	alive to reprod	GO:0010182 GO:0009664	p_adj 9.006×10 ⁻³ 5.138×10 ⁻¹	475CU x 0CU p_adj 1.000 1.000 1.000	770CU x l p_adj 9.737× 2.135× 1.000
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Fig. S13 Histograms graphs representing the ABA/GA4 ratio at $p \le 0.05$ level at 0 Chilling Units (CU), 200CU, 475CU and 770CU. Error bars indicate standard deviation (+/- SD) and letters indicate differences between time points determined by Tukey's test.



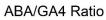




Fig. S14 Longitudinal sections of peach buds during flower development. Image (a) represent floral buds at 0CU stained with 0.1% Aniline blue. (b) is negative control of aniline signal. Bars $= 500 \mu m$

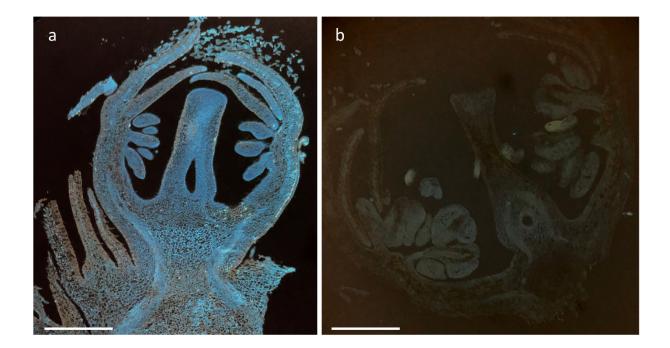




 Table S1 List of primers employed in this work.

PpeDAM6_Reverse5'- CAGCTGGTGGAGGTGGCAATTATG- 3'PpeDAM5_Forward5'- CCACATCAAACTGAGTAAGGAACTC- 3'PpeDAM5_Reverse5'- GCTAACAACCAGCTAAGGCAGACG- 3'PpeDAM4_Forward5'- GAAGAGCTGGATCTGGATGAGTTGC- 3'PpeDAM4_Reverse5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGAGAGAGACTGAGAGCA- 3'PpeGA20ox_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA20ox_Reverse5'- CAAACACCTCAAGCCTCCAACT- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'	Table S1 Sequences of primers used in this	study
PpeDAM6_Reverse5'- CAGCTGGTGGAGGTGGCAATTATG- 3'PpeDAM5_Forward5'- CCACATCAAACTGAGTAAGGAACTC- 3'PpeDAM5_Reverse5'- GCTAACAACCAGCTAAGGCAGACG- 3'PpeDAM4_Forward5'- GAAGAGCTGGATCTGGATGAGTTGC- 3'PpeDAM4_Reverse5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'PpeDAM4_Reverse5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Forward5'- GCTGAATTACTACCACCGTGCC- 3'PpeGA200x_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA200x_Forward5'- CAAACACCTCAAGGCAGAGGATTTGGGGG- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Forward5'-ATCTCATCTCACGCACCTTTTTGGCGG- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-CAAGGTAGCTACCACATTAAGGAGAACC- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCAATCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUREB1D_Forward5'-AAGGCTAAGATCCAAGACAAAGAG- 3'	Gene Name	Sequence
PpeDAM5_Forward5'- CCACATCAAACTGAGTAAGGAACTC- 3'PpeDAM5_Reverse5'- GCTAACAACCAGCTAAGGCAGACG- 3'PpeDAM4_Forward5'- GAAGAGCTGGATCTGGATGAGTTGC- 3'PpeDAM4_Reverse5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGAGAGAGAGAGAGA- 3'PpeGA200x_Forward5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeGA200x_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'-ATCTCATCTCACGCACCTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-TAAGGGGGTGGTGAATGAGGAGA- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCAATCCATCATCCATCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCATCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDAM6_Forward	5'- GGTACAAAGCGCACACAAATGATCTCG- 3'
PpeDAM5_Reverse5'- GCTAACAACCAGCTAAGGCAGACG- 3'PpeDAM4_Forward5'- GAAGAGCTGGATCTGGATGAGTTGC- 3'PpeDAM4_Reverse5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGAGAGAGAGAGAGAGAPpeGA200x_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA200x_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATGCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATGAGAGAACC- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDAM6_Reverse	5'- CAGCTGGTGGAGGTGGCAATTATG- 3'
PpeDAM4_Forward5'- GAAGAGCTGGATCTGGATGAGTTGC- 3'PpeDAM4_Reverse5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGAGAGAGAGACGAPpeGA200x_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA200x_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeDREB1D_Forward5'-CAAGTCCCACGTCGTGAATGAGGAGAACC- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCAATCCATCAATCCAT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDAM5_Forward	5'- CCACATCAAACTGAGTAAGGAACTC- 3'
PpeDAM4_Reverse5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGAGAGAGAGACAPpeGA20ox_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA20ox_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'- ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeDREB1D_Forward5'-CAAGGAGAGCTACCACAATAGCCT- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-CAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDAM5_Reverse	5'- GCTAACAACCAGCTAAGGCAGACG- 3'
PpeDAM3_Forward5'- ACCAGCTAAGGCAGACGATGA- 3'PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGACTGAGAGCA- 3'PpeGA20ox_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA20ox_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeDREB1D_Forward5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDAM4_Forward	5'- GAAGAGCTGGATCTGGATGAGTTGC- 3'
PpeDAM3_Reverse5'- GAGGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	PpeDAM4_Reverse	5'- TCTGATTGTTGGCTTCTACCAGCTCAGT- 3'
PpeGA20ox_Forward5'- GCTGAATTACTACCCACCGTGCC- 3'PpeGA20ox_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeDREB1D_Forward5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-CAAGGCTAAGATCCAAGACAAGAG- 3'	PpeDAM3_Forward	5'- ACCAGCTAAGGCAGACGATGA- 3'
PpeGA20ox_Reverse5'- CAAACACCTCAAGCCCTCCAACT- 3'PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeCYP707A4_Reverse5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-TAAGGGGGTGGTGAATGAGGAGA- 3'PpeUBQ_Forward5'-CAAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDAM3_Reverse	5'- GAGGGAGAGAGAGACTGAGAGCA- 3'
PpeNCED5_Forward5'-ATTTTAGGGTGAGAGGTTTTGGGGG- 3'PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeCYP707A4_Reverse5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-TAAGGGGGTGGTGAATGAGGAGA- 3'PpeDREB1D_Reverse5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'-AAGGCTAAGATCCAAGACAAGAG- 3'	PpeGA20ox_Forward	5'- GCTGAATTACTACCCACCGTGCC- 3'
PpeNCED5_Reverse5'-ATCTCATCTCACGCACCTTTTTGGC- 3'PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT- 3'PpeCYP707A4_Reverse5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'PpeDREB1D_Forward5'-TAAGGGGGTGGTGAATGAGGAGA- 3'PpeDREB1D_Reverse5'-CAGTTCCCACGTCATTCCAATCCAT- 3'PpeUBQ_Forward5'- AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeGA20ox_Reverse	5'- CAAACACCTCAAGCCCTCCAACT- 3'
PpeCYP707A4_Forward5'-TCACCAAGGAGACTACCACAATAGCCT-3'PpeCYP707A4_Reverse5'-CAAGGAAGCCAACATCAAAGGAGAACC-3'PpeDREB1D_Forward5'-TAAGGGGGTGGTGAATGAGGAGA-3'PpeDREB1D_Reverse5'-CAGTTCCCACGTCATTCCAATCCAT-3'PpeUBQ_Forward5'-AAGGCTAAGATCCAAGACAAAGAG-3'	PpeNCED5_Forward	5'-ATTTTAGGGTGAGAGGTTTTGGGGGG-3'
PpeCYP707A4_Reverse 5'-CAAGGAAGCCAACATCAAAGGAGAACC-3' PpeDREB1D_Forward 5'-TAAGGGGGTGGTGAATGAGGAGA-3' PpeDREB1D_Reverse 5'-CAGTTCCCACGTCATTCCAATCCAT-3' PpeUBQ_Forward 5'- AAGGCTAAGATCCAAGACAAAGAG-3'	PpeNCED5_Reverse	5'-ATCTCATCTCACGCACCTTTTTGGC- 3'
PpeDREB1D_Forward 5'-TAAGGGGGTGGTGAATGAGGAGA- 3' PpeDREB1D_Reverse 5'-CAGTTCCCACGTCATTCCAATCCAT- 3' PpeUBQ_Forward 5'-AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeCYP707A4_Forward	5'-TCACCAAGGAGACTACCACAATAGCCT- 3'
PpeDREB1D_Reverse 5'-CAGTTCCCACGTCATTCCAATCCAT- 3' PpeUBQ_Forward 5'- AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeCYP707A4_Reverse	5'-CAAGGAAGCCAACATCAAAGGAGAACC- 3'
PpeUBQ_Forward 5'- AAGGCTAAGATCCAAGACAAAGAG- 3'	PpeDREB1D_Forward	5'-TAAGGGGGTGGTGAATGAGGAGA-3'
	PpeDREB1D_Reverse	5'-CAGTTCCCACGTCATTCCAATCCAT- 3'
PpeUBQ_Reverse 5'- CCACGAAGACGAAGCACTAAG- 3'	PpeUBQ_Forward	5'- AAGGCTAAGATCCAAGACAAAGAG- 3'
	PpeUBQ_Reverse	5'- CCACGAAGACGAAGCACTAAG- 3'



Table S2 Prunus GO terms of reference and newly annotated transcripts were de-novo annotated using Trinotate (Bryant *et al.*, 2017) and compared with the prunus GO annotation available at EnsemblPlants/Biomart database on July 2020 using WEGO GO plotting tool categorized using level 2 of the GO lineage.

		Reference_Biomart	
		2020-07-30	RABT_ Trinotate
Gene		20.101	21.001
Annotated Genes		20.101	21.001
GO Terms	Biological	12.019	17.668
	Cellular	11.05	17.645
	Function	15.641	18.18
	Total	38.71	53.493



	AciI	EcoT22I	PstI		Name	Oligo sequence
1	FF8_R1_	FF8_R1_	FF8_R1	index_	PCR2_Idx_7_CG	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGG
	P25	P10	_P1	7	ATGT	AGTTCAGACGTGTGC
2	FF8_R2_	FF8_R2_	FF8_R2			
	P26	P11	_P2			
3	FF8_R3_	FF8_R3_	FF8_R3			
	P27	P12	_P3			
4	FF11_R1	FF11_R1	FF11_R	index_	PCR2_Idx_12_C	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTG
	_P25	_P10	1_P1	12	TTGTA	GAGTTCAGACGTGTGC
5	FF11_R2	FF11_R2	FF11_R			
	_P26	_P11	2_P2			
6	FF11_R3	FF11_R3	FF11_R			
	_P27	_P12	3_P3			
7	FF13_R1	FF13_R1	FF13_R	index_	PCR2_Idx_14_A	CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTG
	_P25	_P10	1_P1	14	GTTCC	GAGTTCAGACGTGTGC
8	FF13_R2	FF13_R2	FF13_R			
	_P26	_P11	2_P2			
9	FF13_R3	FF13_R3	FF13_R			
	_P27	_P12	3_P3			
10	FF9_R1_	FF9_R1_	FF9_R1	index_	PCR2_Idx_15_A	CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGG
	P25	P10	_P1	15	TGTCA	AGTTCAGACGTGTGC

 Table S3 List of codes, index adaptors and oligonucleotides.



11	FF9_R1_	FF9_R1_	FF9_R1
	P26	P11	_P2
12	FF9_R1_	FF9_R1_	FF9_R1
	P27	P12	_P3



Enzyme	Recognition	Cleavage site	Methyl	Cleavage blocked	Methyl
	site		sensitive	by	Context
AciI	CCGC /	C'CGC /	Yes	C 5mCGC / G	CG
	GCGG	G'CGG		5mCGG	
PstI	CTGCAG	CTGCA'G	Yes	CTG 5mCAG	CHG
EcoT22I	ATGCAT	ATGCA'T	Yes	ATG 5mCAT	СНН
MseI	TTAA	T'TAA	No	Not sensitive	-

Table S4 Characteristics of the restriction enzymes used for the MCSeEd technique.

 Table S5 Sequencing data summary of DNA methylation sequencing.

		Total	Unique	
Enzyme	Sample ID	sample	mapped	Useful reads
		reads	reads	(%)
	ff11_a_aci_P25	7954662	5474988	68.83
	ff11_b_aci_P26	7241206	5151317	71.14
	ff11_c_aci_P27	7507888	5000475	66.60
	ff13_a_aci_P25	7373074	5113836	69.36
	ff13_b_aci_P26	6185175	4286994	69.31
	ff13_c_aci_P27	8509766	5903144	69.37
Acil	ff8_a_aci_P25	6730952	4387580	65.19
	ff8_b_aci_P26	5345257	3535845	66.15
	ff8_c_aci_P27	6077477	3909964	64.34
	ff9_a_aci_P25	7202216	4914323	68.23
	ff9_b_aci_P26	6746382	4716060	69.91
	ff9_c_aci_P27	8680212	5809155	66.92
	ff11_a_pstl_P1	4867693	4046797	83.14
	ff11_b_pstI_P2	3960294	3307900	83.53
	ff11_c_pstI_P3	5531513	4584420	82.88



	ff13_a_pstl_P1	3869289	3263086	84.33
	ff13_b_pstl_P2	3539286	2958903	83.60
	ff13_c_pstI_P3	4581077	3889422	84.90
Pstl	ff8_a_pstl_P1	6772054	5404083	79.80
	ff8_b_pstI_P2	3934093	3098230	78.75
	ff8_c_pstl_P3	4708474	3797767	80.66
	ff9_a_pstl_P1	4825129	4013298	83.17
	ff9_b_pstI_P2	4408025	3653957	82.89
	ff9_c_pstI_P3	4482585	3741011	83.46
	ff11_a_eco_P10	4589178	2770709	60.37
	ff11_b_eco_P11	3291022	1968646	59.82
	ff11_c_eco_P12	5738414	3482576	60.69
	ff13_a_eco_P10	4903715	2743805	55.95
	ff13_b_eco_P11	3697103	2003721	54.20
	ff13_c_eco_P12	5913241	3373410	57.05
EcoT22I	ff8_a_eco_P10	4487305	2658309	59.24
	ff8_b_eco_P11	3078440	1763084	57.27
	ff8_c_eco_P12	3568308	2174719	60.95
	ff9_a_eco_P10	3719620	2220525	59.70
	ff9_b_eco_P11	3296702	1932096	58.61
	ff9_c_eco_P12	2253555	1381590	61.31



Chilling Units	GA4	GA1	ABA	İAA	DHZ	iP	tZ
0CU	0.15	0.62	1104.6	12.87	0.18	0.008	0.042
0CU	0.16	0.45	1735.8	10.98	0.086	0.013	0.032
0CU	0.15	0.53	355.8	9.2	0.115	0.004	0.043
Mean	0.153333	0.533333	1065.4	11.01667	0.127	0.008333	0.039
Standard Dev.	0.005774	0.085049	690.8346	1.835275	0.048135	0.004509	0.006083
200CU	0.13	0.6	1157.5	11.99	0.116	0.004	0.096
200CU	0.18	0.65	1410	12.1	0.1	0.005	0.033
200CU	0.09	0.54	704.2	9.92	0.092	0.036	0.043
Mean	0.133333	0.596667	1090.567	11.33667	0.102667	0.015	0.057333
Standard Dev.	0.045092	0.055076	357.6289	1.228102	0.01222	0.018193	0.033858
475CU	0.17	0.54	596.6	16.38	0.132	0.007	0.052
475CU	0.2	0.55	214.9	14.41	0.143	0.003	0.052
475CU	0.21	0.67	621.3	10.93	0.1	0.042	0.043
Mean	0.193333	0.586667	477.6	13.90667	0.125	0.017333	0.049
Standard Dev.	0.020817	0.072342	227.8398	2.759644	0.022338	0.021455	0.005196
770CU	0.16	0.52	223.2	17.77	0.168	0.007	0.057
770CU	0.22	0.58	220.4	14.94	0.168	0.058	0.035
770CU	0.17	0.78	502.8	11.13	0.156	0.047	0.046
Mean	0.183333	0.626667	315.4667	14.61333	0.164	0.037333	0.046
Standard Dev.	0.032146	0.136137	162.2415	3.332031	0.006928	0.026839	0.011



Sample	Raw	Filter rRNA	A Filter Viroid Trimmon		Mapped	Assigned
	Reads				reads	Reads
0CU_R1	35137978	34810465	33153296	31705652	23615797	22427388
OCU_R2	30607014	29975719	28796787	27461165	20482604	18823160
200CU_R1	35398354	35241638	34231915	33070401	24534230	23162635
200CU_R2	38936842	37908325	36963825	35435382	27126737	25423199
475CU_R1	35558527	34654478	33493265	32121978	24497568	22988354
475CU_R2	32985237	32516190	31400257	30097235	22344678	20883271
770CU_R1	37207522	36050286	34888822	33539838	25793818	24321151
770CU_R2	31035155	30260528	29433691	28163737	21546440	20425390

 Table S7 RNA-Seq summary statistics.



			FPKM Values					
Gene ID	Gene ID Ppe	0CU	200CU	475CU	770CU	AT	Cluster	Class
AT						Gene_name		
AT1G69120	PRUPE_3G249300	1.114243	0.408766	-0.29696	-1.22604	AP1	2	А
AT4G36920	PRUPE_6G231700	0.241227	1.038683	0.082492	-1.3624	AP2	2	А
AT3G54340	PRUPE_1G371300	-1.43871	0.140035	0.465982	0.83269	AP3	1	В
AT5G20240	PRUPE_1G489400	-0.89018	-0.81231	0.629327	1.073163	PI	1	В
AT3G54340	PRUPE_7G164100	-1.19168	-0.45973	0.775973	0.875443	AP3	1	В
AT4G18960	PRUPE_4G070500	-1.1175	-0.49667	0.489497	1.124676	AG	1	С
AT3G02310	PRUPE_3G249400	-1.14836	-0.45915	0.505075	1.102433	SEP2	1	Е
AT2G45650	PRUPE_2G151000	-1.31741	-0.08032	0.323927	1.073801	AGL6	1	Е

Table S8 Genes belonging to ABCDE model.



Methods S1

in situ Hybridization

In situ hybridization experiment was performed to localize the *DAM4* expression domains and was conducted as previously described by (Varotto *et al.*, 2003).

Slides were deparaffinized and treated with 10 μ g mL-1 proteinase K. Transcript amplification of DAM4 was performed using the primers present in the Table S1 and designed on coding DNA sequence (CDS). Then, probes were cloned using TOPO® Cloning (Thermo Fisher). In vitro transcription of the DIG-UTP (Roche) labeled RNA sense and antisense probes was obtained using T7 and SP6 polymerases. The hybridization was performed in a 50% formamide buffer at 48°C overnight. Digoxigenin (DIG) detection and signal visualization were done using Anti-Digoxigenin-AP antibody (Roche) and NBT plus BCIP (Roche), following the manufacturer's instructions. Slides were air-dried and mounted with DPX mounting medium (Fluka Biochemika).

Hormone Quantification

Frozen flower buds (200 mg) were grinded and suspended in 80% methanol-1% acetic acid containing internal standards and mixed by shaking during one hour at 4°C. The extract was kept a -20°C overnight and then centrifuged and the supernatant dried in a vacuum evaporator. The dry residue was dissolved in 1% acetic acid and passed through a reverse phase column (HLB Oasis 30 mg, Waters), as described in (Seo et al., 2011). For CKs, the extracts were additionally passed through an Oasis MCX (cationic exchange) and eluted with 60% methanol- 5% NH4OH to obtain the basic fraction containing cytokinins. To recover the acid fraction, the MCX cartridge was eluted with methanol. The final residues were dried and dissolved in 5% acetonitrile-1% acetic acid and the hormones were separated by UHPLC with a reverse Accucore C18 column (2.6 μ m, 100 mm length; Thermo Fisher Scientific) with an acetonitrile gradient containing 0.05% acetic acid, at 400 µL/min. For GAs and ABA, the gradient was 2 to 55% acetonitrile over 21 min. The hormones were analyzed with a Q-Exactive mass spectrometer (Orbitrap detector; ThermoFisher Scientific) by targeted Selected Ion Monitoring (tSIM; capillary temperature 300°C, S-lens RF level 70, resolution 70.000) and electrospray ionization (spray voltage 3.0 kV, heater temperature 150°C, sheath gas flow rate 40 μ L/min, auxiliary gas flow rate 10 μ L/min) in negative mode for acidic hormones or positive mode for CKs. The concentrations of hormones in the extracts were



determined using embedded calibration curves and the Xcalibur 4.0 and TraceFinder 4.1 SP1 programs. The internal standards for quantification of each of the different plant hormones were the deuterium-labelled hormones, (purchased from OlChemim Ltd, Olomouc, Czech Republic).

RNA Sequencing (RNA-Seq) and differentially expressed genes (DEG) identification

DNase digestion using the RNAse-Free DNase Set (Qiagen) was included during RNA isolation. RNA concentration and quality were determined by measuring OD260/230 and OD260/280 ratio on a NanoDrop 2000c spectrophotometer (Thermo Scientific).

For each sample x replicate combination, 30 M paired-end reads of 150 nucleotides were generated. The quality of reads using FastQC was assessed (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Initial read quality assessment revealed the peach RNA-Seq libraries contained residual contaminations of rRNA and RNA virus from Peach latent mosaic viroid. The sequenced reads were pre-processed for rRNA and viroid contaminant reads filtering with ERNEFILTER 2.1.1 (Del Fabbro et al., 2013), and then Trimmomatic (Bolger et al., 2014) was applied for adapter clipping and low quality sequence filter and trimming. High quality reads were finally mapped to the P. persica genome v.2.0 (Verde et al., 2017) obtained from the Ensembl (http://plants.ensembl.org/index.html; release 43) using the spliced aligner HISAT2 (Kim et al., 2015). Mapped reads were used for Reference Annotation Based Transcript (RABT) assembly of each individual RNA-Seq sample using Stringtie v2.0.4 (Pertea et al., 2015; Kovaka et al., 2019). Reassembled transcriptomes were merged using Stringtie v2.0.4 and then compared and integrated into the reference Prunus persica transcriptome annotation using Gffcompare (Pertea & Pertea, 2020) and through a customized Perl script. Final gene annotation allowed the correction of PpDAM loci annotation (Supplementary Figure S2) and the identification of 2,846 new bud-expressed loci not included in the reference transcriptome.

Gene expression counts were generate for reference and newly annotated genes using featureCounts software program (Liao *et al.*, 2014) and principal component analysis (PCA) was first used to assessing the biological replicates quality.

The differential expression analysis was carried out using DESeq2 (Love *et al.*, 2014): after estimation of size factors and dispersion between samples and genes, differentially expressed genes were identified applying the likelihood ratio test (LRT). Differently to the default Wald test,



LRT is used to identify any genes that show change in expression across the different levels (CU accumulation), resulting particularly useful in analyzing time course experiments. Genes with a LRT adjusted p-value ≤ 0.01 , and showing a fold change in expression of at least 1.5 (up or down) in the comparison of each stage with the remaining three, were considered as significantly differentially expressed genes (DEGs). Gene clusters exhibiting particular patterns across samples were identified and plotted using the DEGreport R package (Pantano, 2020) using variance stabilizing transformation (VST) expression values as input.

Gene Ontology (GO) enrichment was determined by comparing the number of DEGs included in each cluster to the number of expressed genes in each GO term with gProfiler web-software (Raudvere *et al.*, 2019): the hypergeometric statistic for every term was used to estimate the significance of enriched pathways and processes in the gene lists and the default ontology-focused g:SCS correction method for multiple testing was applied. Prunus GO terms of reference and newly annotated transcripts were *de-novo* annotated using Trinotate (Bryant *et al.*, 2017) and compared with the *Prunus persica* GO annotation available at EnsemblPlants/Biomart database on July 2020 (Supplementary Figure S3 and table S2) using WEGO GO plotting tool (Ye *et al.*, 2018), categorized using level 2 of the GO lineage.

RNA-Seq Validation

Total RNA was extracted following the previously cited protocol. cDNA synthesis was performed with the SuperScript III reverse transcriptase kit (Invitrogen) according to the manufacturer's instructions. Quantitative Real-Time PCR expression analysis was performed using a StepOnePlusTM Real-Time PCR System (Applied Biosystems) and the FAST SYBR® GREEN PCR Master Mix (Thermo Fisher Scientific), following the manufacturer's guidelines. Melting curves analysis revealed a single amplification product in each reaction. Three technical replicates were carried out for each primer combination in each sample and an absolute quantification of gene expression (normalized to UBIQUITIN –UBQ- transcript quantities) was performed with the StepOne Software 2.3 (Thermo Fisher Scientific). Primer sequences, specifically designed on each target gene (*PpeDAM6, PpeDAM5, PpeDAM4, PpeDAM3, PpeDREB1D, PpeCYP707A4, PpeNCED5, PpeGA200x*, and *PpeUBQ*), are reported in Table S1.



Chromatin Immunoprecipitation Sequencing (ChIP-Seq) analysis

FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to assess the quality of the reads. The ChIP-Seq raw reads were processed for adapter clipping and quality score trimming using Trimmomatic v 0.39 (Bolger et al., 2014). Clean reads were mapped to the P. v.2.0 al., 2017) persica genome (Verde obtained from Ensembl et (http://plants.ensembl.org/index.html) with bowtie2 v2.4.1 (Langmead & Salzberg, 2012); softtrimming (5 bp at 5' and 10 bp at 3') was enabled. Reads with MAPQ > 10 were used for the subsequent analysis. Aligned reads were sorted using SAMtools v.1.3 and duplicated reads were removed using Picard v.2.16.0 (http://broadinstitute.github.io/picard/). ChIP-Seq peak calling and differential binding analysis were performed using Model-based Analysis of ChIP-Seq (MACS2) (Zhang et al., 2008; Feng et al., 2012). Uniquely mapped and not duplicated reads were used for peaks calling with the "callpeak" subcommand for each immunoprecipitated sample/replicate with respect to the input control, replicate signals were combined with the "cmbreps" subcommand using the Fisher's combined probability test prior to differential peak enrichment analysis using the "bdgdiff" tool. Identified peaks and differentially enriched peaks were associated with nearby genes using HOMER v4.11 software (Heinz et al., 2010). Correlation heatmaps between replicates, using read count data were produced using DiffBind R package version 4.2 (Fig. S4; Stark & Brown, 2011; Ross-Innes et al., 2012). Library corresponding to replicate R2 of H3K4me3 was not included into analysis pipeline due to it low quality.

Library preparation and sequencing for DNA methylation analysis

On-Column RNase Digestion was performed. DNA concentration and quality were determined by measuring OD260/230 and OD260/280 ratio, respectively, on a NanoDrop 2000c spectrophotometer (Thermo Scientific) and using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA). Illumina sequencing was performed at Novogene (HK) Company Limited according to the standard operation. Since a methylation-sensitive enzyme cannot digest methylated site, the read count at a specific locus is expected to anticorrelate to genomic methylation level, this is efficiently used for estimating differential methylation changes over different genomic regions between two samples (Marconi *et al.*, 2019). The raw reads were checked by quality analysis using the FastQC (www.bioinformatics.babraham.aC.uk/projects/fastqc/, accessed on 30 May 2020) program and ambiguous and poor-quality reads (with a base count of Phred value <20), were removed using the



TrimGalore program (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore, accessed on 30 May 2020).

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