

Supplementary Materials

Divergent DNA Methylation Signatures of Juvenile Seedlings Grafts and Adult Apple Trees

Authors: Adrien Perrin¹, Nicolas Daccord¹, David Roquis^{1,2}, Jean-Marc Celton¹, Emilie Vergne¹ and Etienne Bucher^{1,2}

Accession numbers:

GSE138492: global depository accession number comprising methylome and transcriptome data

GSE138377: bisulfite sequence data and methylation calling files + Supplemental table S3

GSE138491: microarray data

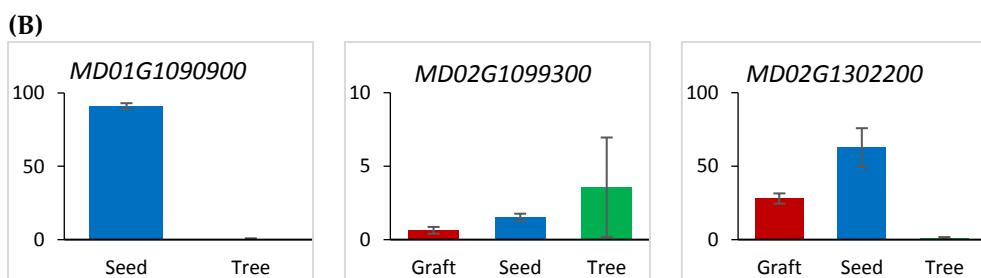
Large datasets:

Supplemental table S3: DMRs list of OGvsSD and OGvsYG comparisons, including Gene- and TE-DMRs. File deposited on GEO: GSE138377

Supplemental table S4: DETs list of OGvsSD and OGvsYG comparison

Supplemental table S5: DTG- and DTTE-DMRs list of OGvsSD and OGvsYG comparisons

(A)	Gene_ID	Ratio OGvsSD OGvsYG	TAIR name	Short description	Forward primer	Reverse primer
	MD01G1090900	4.51	AT1G01060	Homeodomain-like superfamily protein	CAGAGTTTCACGCCCTTC	CTCGTGCTGGTACACACTT
	MD02G1302200	3.17	AT3G63490	Ribosomal protein L1p/L10e family	CAGAATTCAAGCAGGGAAA	CACCTGGTGGCTTATTGCT
	MD03G1128600	3.09	AT3G44890	ribosomal protein L9 chloroplast	GCTGCTCAAGGAAATGAAGG	TCAACGAGATCTGGGTGT
	MD12G1237700	3.01	ATCG01120	ribosomal protein S15	GAAGAAAAAGACGAAAAACAGAGG	TGACTCCGAATATCCAACC
	MD16G1267700	3.01	AT5G14460	Pseudo-uridine synthase family protein	CCTGCAAAGAAAAGCAAAGG	CTCTTATCAGCCTCGCCATC
	MD17G1271400	3.2	AT5G17300	Homeodomain-like superfamily protein	GAACAGCCAGTGATGACGAA	TACTTCCGGTGCTTGCTCT
	MD02G1099300	-1.75	AT5G20790	Transmembrane protein	CATTGAGTTCCGCTCACAA	CCAGGTCAACGTCACTCTCA
	MD09G1006500	-3.81	AT3G02040	Senescence-related gene 3	CCGTCTCCAAGTGGTGT	GCCTTAACCTCCGACACAA



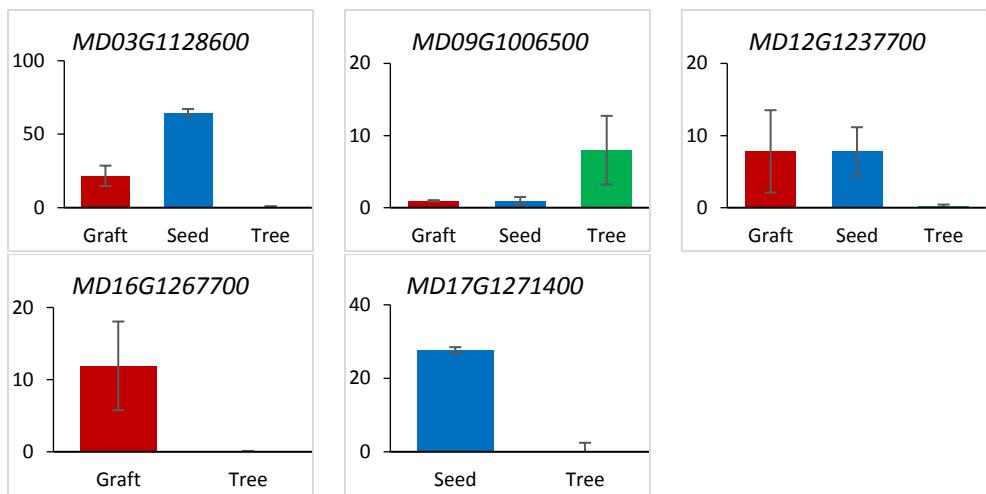


Figure S1. Microarray validation. (A) Q-PCR primers for micro array data validation. Indicated ratios came from micro array data in both comparisons. (B) Q-PCR validation of micro array data.

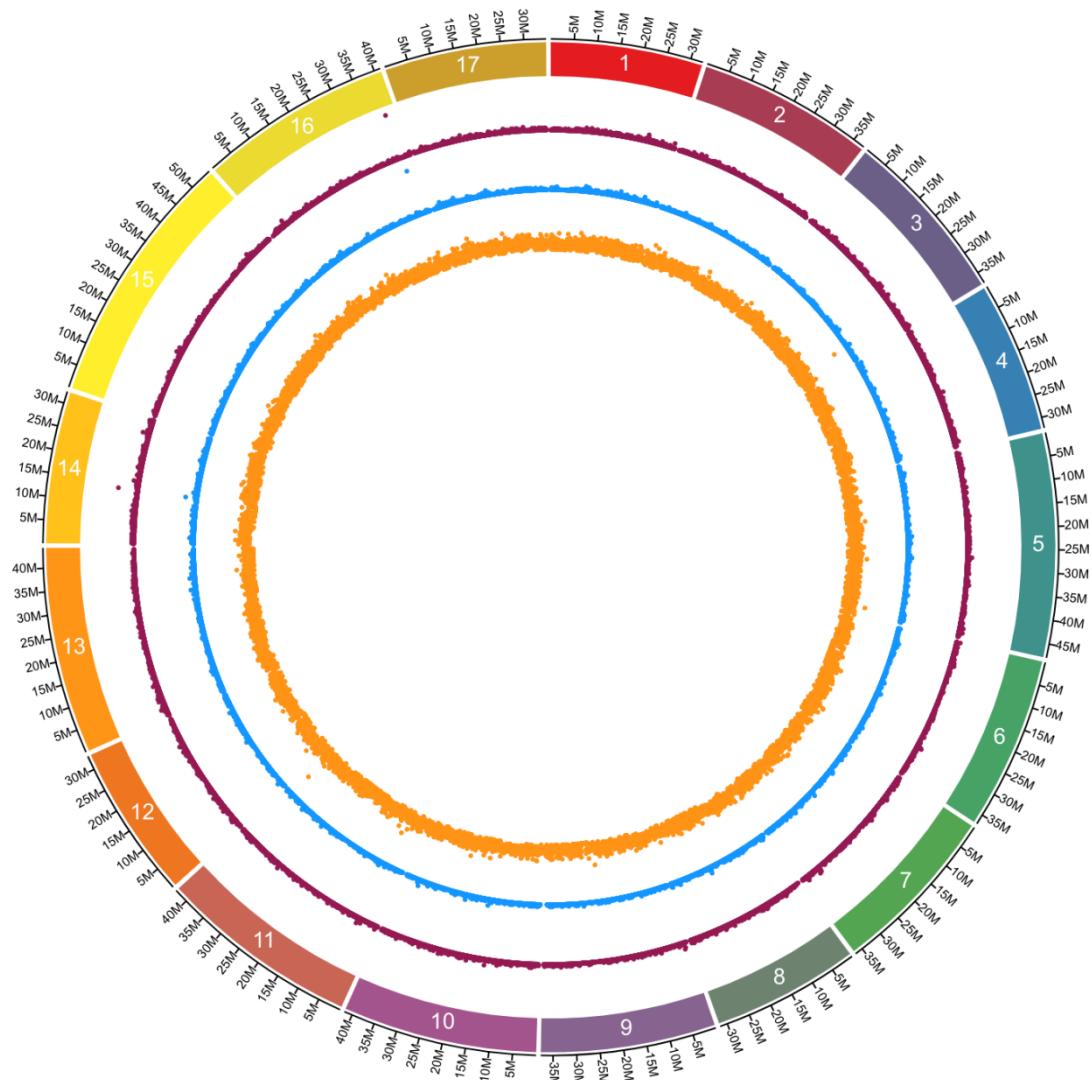


Figure S2. Methylation overview in GDDH13. Density plot of DMRs on all GDDH13 genome in OGvYG. In red, DMRs in CG context, in blue CHG and in orange CHH. Each point represent number of DMRs in 50kb windows of genome.

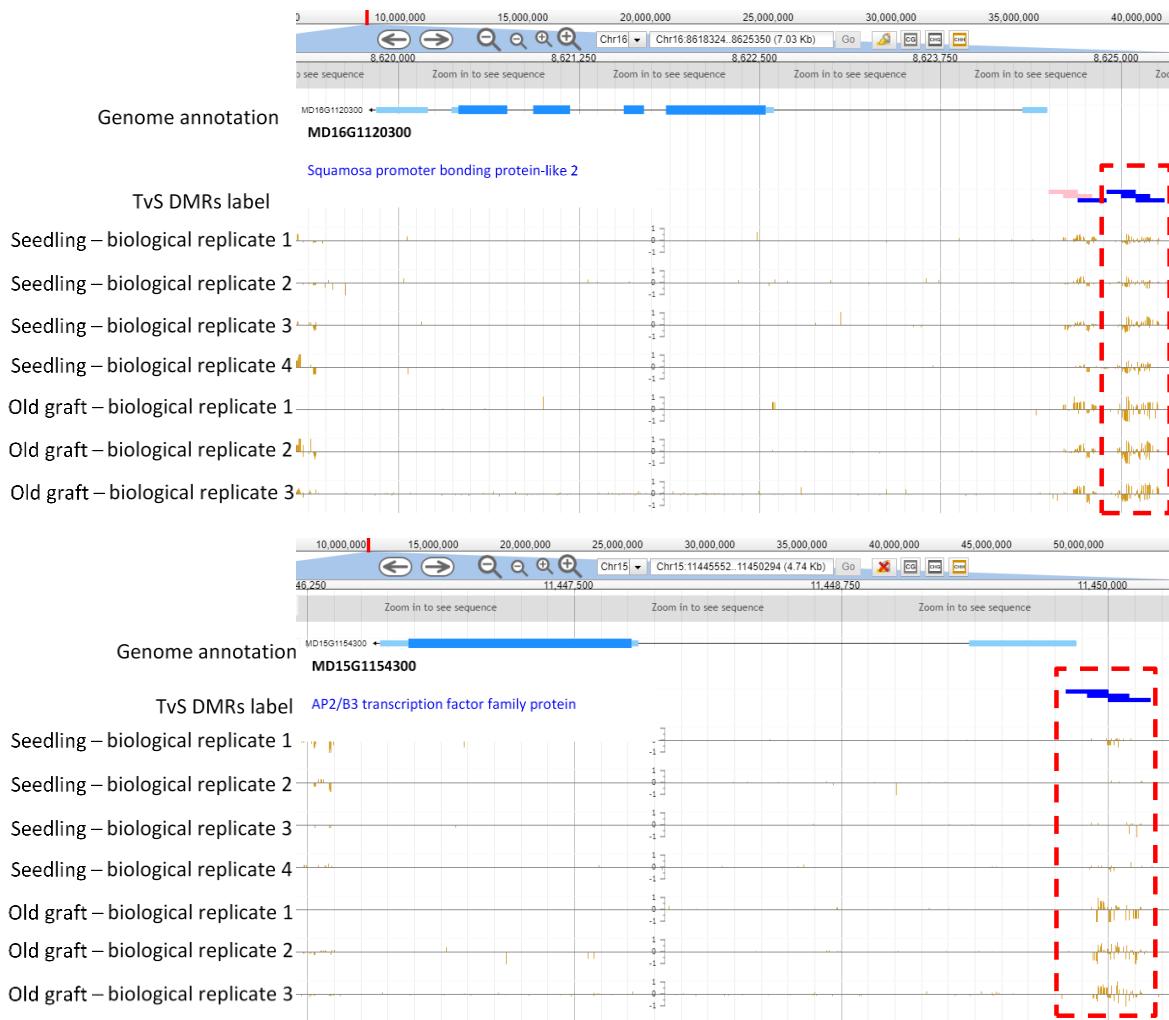


Figure S3. Jbrowse screenshot of two DTG-DMRs present in scatterplot (fig 7A) with DMRs in the promotor of genes highlight by a red dashed box.

Table S1. Resume of defined samples and details of sampling.

Sample	Way of multiplication	Years of obtention	Years sampling	Numbers sampled plants	Numbers leaves sampled per plant	Pooled	Number of biological replicates
Old graft	Grafting (Asexual)	2005	2016	1	4	Yes	3
Seedling	Seedling (Sexual)	2016	2016	4	1	No	4
Young graft	Grafting (Asexual)	2016	2016	20	1	Yes	2

Table S2. Fixed threshold to filter DMRs calculated between each comparison. Threshold were empirically fixed by observation of methylation calling file in the Jbrowse software.

DMRs	Standard deviation average threshold			p-value
	Old graft	Seedling	Young graft	
CG -CHG	<0.07	<0.11	<0.07	<1%
CHH	<0.05	<0.05	<0.05	

Table S3. DMRs list of OGvsSD and OGvsYG comparisons, including Gene- and TE-DMRs. File deposited on GEO with the following accession number GSE138377.**Table S4. DETs list of OGvsSD and OGvsYG comparison.** Excel file available in additional information (named: "Supp_Tab_S4").**Table S5. DTG- and DTTE-DMRs list of OGvsSD and OGvsYG comparisons.** Excel file available in additional information (named: "Supp_Tab_S5").**Table S6. Functional classes found in enrichment analysis on Mapman software.** In blue are indicated the number of DTGs up-regulated in SD or YG compare to OG, down-regulated ones are in red. "p-value" corresponds to the p-value obtained in the enrichment analysis and corrected by the BH method.

Category name	Bincode	OGvsSD			OGvsYG			Commons		
		Down	Up	p-value	Down	Up	p-value	Down	Up	other
Coenzyme metabolism		7	0	19	2.56E-05			1	3	1.59E-02
Secondary metabolism/terpenoids		9.1	2	11	5.43E-03					
Secondary metabolism/phenolics/ p-coumaroyl-coa synthesis		9.2.1				0	2	7.50E-03		
Chromatin organization		12	19	3	2.48E-05	1	14	6.03E-03		
Cell cycle		13				5	39	9.05E-04		
Rna biosynthesis/transcriptional activation/SBP transcription factor	15.7.18	8	0	3.37E-03						
Rna biosynthesis/organelle machineries		15.9						1	5	1.50E-02
RNA processing		16				5	30	3.53E-03		
Protein biosynthesis		17	9	40	3.17E-03	5	29	2.90E-04	5	53
Protein modification/phosphorylation/ TKL kinase superfamily		18.8.1						1	9	1.655E-03
Protein degradation/peptide tagging		19.4	18	15	7.94E-03	16	7	5.10E-04		
Protein degradation/peptidase families/serine-type peptidase activities		19.5.2						1	9	1.833E-04
Cytoskeleton		20				1	20	1.04E-06		
Cell wall		21				13	0	1.58E-05		
Protein translocation/chloroplast/thylakoid membrane SRP insertion system		23.1.7						1	7	1.50E-02
Solute transport		24				16	1	3.12E-06		
Enzyme classification		50	30	84	4.24E-03	31	25	7.32E-03		
Not assigned		35				235	617	7.50E-03	122	252
								14		1.17E-02

Table S7. Count of DTG-DMRs in OGvsSD and OGvsYG comparisons. Here we included the unclassified gene class “35” not present in Figure 6. Hypo- or hypermethylated in SD or YG compared to OG.

		OG vs SD			OG vs YG		
		Hypomethylated	Hypermethylated	Σ	Hypomethylated	Hypermethylated	Σ
CHH	head	1394	55	1449	406	261	667
	body	295	21	316	137	70	207
	tail	684	31	715	228	137	365
CHG	head	0	2	2	0	3	3
	body	1	5	6	0	2	2
	tail	0	4	4	0	3	3
CG	head	1	5	6	0	7	7
	body	3	4	7	0	4	4
	tail	1	4	5	0	10	10
Σ		2379	131	2510	771	497	1268

Table S8. Count of DTTE-DMRs in CHH context in OGvsSD and OGvsYG comparisons. Hypo- or hyper-methylated in SD or YG compared to OG.

		OG vs SD			OG vs YG		
		Hypomethylated	Hypermethylated	Σ	Hypomethylated	Hypermethylated	Σ
CHH	head	0	0	0	0	0	0
	body	115	22	137	153	113	266
	tail	10	1	11	5	6	11
CHG	head	0	0	0	0	0	0
	body	0	3	3	1	28	29
	tail	0	0	0	1	0	1
CG	head	0	0	0	0	0	0
	body	1	6	7	1	22	23
	Tail	0	0	0	0	1	1
Σ		126	32	158	161	170	331