Supplementary Materials for

**• Experimental evidence for short term directional selection of epigenetic trait variation**

Benoit Pujol *et al.*

\*Corresponding author. Email: [benoit.pujol@univ-perp.fr](mailto:benoit.pujol@univ-perp.fr)

**This PDF file includes:**

Figs. S1 to S2

Tables S1 to S6

Une image contenant diagramme

Description générée automatiquement

Fig. S1. Partitioning of the methylation status of the 126 DMRs established by the overlapping probe classification approach based on EM-seq data from 24 EpiRILs. U: unmethylated or M: methylated bisulfite sequence signal HMM: overlapping probe classification approach developed by Colomé-Tatché et al. (25). Rectangles represent the interquartile range (IQR), the white square with a cross represents the mean value, whiskers extend to ± 1.5 IQR to show variability outside the upper and lower quantiles, black dots beyond the end of the whiskers are outliers.

Une image contenant graphique

Description générée automatiquement

Fig. S2. Hierarchical clustering analysis supports molecular correspondence between reference epiRILs and the same epiRILs used in our selection experiments.

Col-wt and eight epiRILs used in our selection experiments sequenced by using an EM-seq approach are on the left and reference epiRILs for which WGBS data have been published are on the right. The cophenetic correlation coefficient between the two hierarchical clusters is 0.89. The slight modification that we found was in the control wt rather than the epiRILs, likely because the sequence coverage was higher in our data.

Table S1. Characteristics of the 126 DMRs used as markers in the epiRILs in the CpG context.

For each epiRIL (column one) the number of published DMR markers 22 are divided into those inherited from the ddm1 mutant (hypomethylated) (column two) and those inherited from the Columbia wild type (Col-wt) (methylated), which are in column three. In column four, we display the number of ddm1 markers found in our analysis of EM-seq data that were called “verified ddm1 markers” and in the column five, we show the number of verified Col-wt markers. Column six presents the sum of verified ddm1 and Col-wt markers, which we called “total verified markers”. Then, using this number we calculated the percentage similarity with the published data, which is shown in column seven. In column eight, we display the number of markers that changed. In column nine we detail the number of ddm1 markers that switched from hypomethylated back to methylated as in Col-wt (whose names are shown in column twelve); in column ten we show the Col-wt markers that switched to hypomethylated (whose names are shown in column thirteen); and in column eleven we show the markers that switched to hypermethylated and became different from Col-wt (whose names are shown in column fourteen). \*Markers shown in blue are those that contain a potentially mobile transposable element, according to Colomé-Tatché et al. (25).

Excel file (supplementary Table S1)

Table S2. Characteristics in the CHG context of the 126 DMRs used as markers in the epiRILs.

For each epiRIL (column one) the number of published DMR markers 22 are divided into those inherited from the ddm1 mutant (hypomethylated) (column two) and those inherited from the Columbia wild type (Col-wt) (methylated), which are in column three. In column four, we display the number of ddm1 markers found in our analysis of EMseq data that were called “verified ddm1 markers” and in the column five, we show the number of verified Col-wt markers. Column six presents the sum of verified ddm1 and Col-wt markers, which we called “total verified markers”. Then, using this number we calculated the percentage similarity with the published data, which is shown in column seven. In column eight, we display the number of markers that changed. In column nine we detail the number of ddm1 markers that switched from hypomethylated back to methylated as in Col-wt (whose names are shown in column twelve); in column ten we show the Col-wt markers that switched to hypomethylated (whose names are shown in column thirteen); and in column eleven we show the markers that switched to hypermethylated and became different from Col-wt (whose names are shown in column fourteen). \*Markers shown in blue are those that contain a potentially mobile transposable element, according to Colomé-Tatché et al. (25).

Excel file (supplementary Table S2)

Table S3. Whole genome bisulfite sequence mapping (BSMAP) percentage of aligned pairs.

BSMAP percentages of aligned pairs are presented for each epiRIL (identified in the left column) and the wild type genome (Col-wt) resulting from the enzymatic methyl sequencing (EM-seq) approach that we conducted and from available published data from whole genome sequencing bisulfite sequencing (WGBS).

|  |  |  |
| --- | --- | --- |
| epiRIL | EM-seq | WGBS |
| 11 | 73 .7 | - |
| 24 | 71.8 | - |
| 60 | 75.1 | 71.8 |
| 73 | 73.5 | - |
| 92 | 74.4 | - |
| 95 | 72.9 | - |
| 101 | 72 | - |
| 108 | 75.5 | - |
| 118 | 73.2 | - |
| 150 | 70 | 62.8 |
| 193 | 74.7 | 69.8 |
| 195 | 76 | - |
| 202 | 72.3 | 71.8 |
| 215 | 70.1 | - |
| 232 | 73.5 | 77.7 |
| 257 | 72.6 | - |
| 260 | 76.7 | 72.2 |
| 333 | 74.5 | - |
| 350 | 72.1 | - |
| 361 | 74.2 | - |
| 366 | 74 | - |
| 371 | 72.6 | - |
| 480 | 77.1 | 71.3 |
| 495 | 73 | - |
| Col-wt | 76 | 37.4 |

Table S4. Assessment of the correlation between the published WGBS data of reference epiRILs and the EM-seq data of the same epiRILs used in our selection experiments

Kendall rank correlation coefficients (R) and their associated p-values (p) between the Bisulfite Sequencing signal of the 126 DMRs for epiRILs used in our experiment sequenced by using an EM-seq approach and the published WGBS data of reference epiRILs. Correlations are shown for all contexts and then separately per methylation context. For all correlations, p-values were lower than 0.05.

|  |  |  |  |
| --- | --- | --- | --- |
| epiRIL | All contexts | CG context | CHG context |
| Col-wt | R = 0.79 | R = 0.46 | R = 0.64 |
| 60 | R = 0.79 | R = 0.72 | R = 0.77 |
| 92 | R = 0.75 | R = 0.74 | R = 0.74 |
| 150 | R = 0.83 | R = 0.69 | R = 0.76 |
| 193 | R = 0.81 | R = 0.70 | R = 0.78 |
| 202 | R = 0.79 | R = 0.71 | R =0.77 |
| 232 | R = 0.77 | R = 0.79 | R = 0.79 |
| 260 | R = 0.79 | R = 0.54 | R = 0.71 |
| 480 | R = 0.70 | R = 0.49 | R = 0.66 |

Table S5. Genomic annotation of the 126 DMR non redundant markers (Assembly version: AtGDB TAIR9/10 v171)

Excel file (supplementary Table S5)

Table S6. Trait values before and after selection.

Control treatment (CTRL): random selection of plants. Weak selection: selection by truncation of the upper and the lower third (n = 60 for each) of plants ranked by trait values to compose high and low selection lines (HSL and LSL, respectively). Strong selection: same method but selecting by truncation of the upper and lower ca.11% (n = 20 for each) of plants. G0: generation of plants before selection, G1: generation of plants after selection. Average trait values, their standard error and 95% Confidence intervals are given for each population, trait and selection intensity and line. Average values were estimated on the basis of 1000 bootstraps of random samples established without replacement, and the associated lines of selected individuals. This method avoids potential sampling bias of the results.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Population 1 | | | | Population 2 | | | |
| Trait | Selection | line | Gene-ration | Mean | SE | CImin | CImax | Mean | SE | CImin | CImax |
| Above ground dry biomass (g) | Weak | LSL | G0 | 0.57 | 0.01 | 0.55 | 0.58 | 0.49 | 0.01 | 0,47 | 0,51 |
| Weak | CTRL | G0 | 0.64 | 0.01 | 0.61 | 0.66 | 0.60 | 0.02 | 0,57 | 0,64 |
| Weak | HSL | G0 | 0.71 | 0.01 | 0.70 | 0.72 | 0.72 | 0.01 | 0,70 | 0,74 |
| Strong | LSL | G0 | 0.48 | 0.01 | 0.46 | 0.50 | 0.36 | 0.02 | 0,32 | 0,40 |
| Strong | CTRL | G0 | 0.64 | 0.02 | 0.60 | 0.68 | 0.60 | 0.03 | 0,54 | 0,67 |
| Strong | HSL | G0 | 0.77 | 0.01 | 0.76 | 0.79 | 0.83 | 0.01 | 0,81 | 0,86 |
| Weak | LSL | G1 | 0.69 | 0.01 | 0.67 | 0.71 | 0.72 | 0.01 | 0,70 | 0,75 |
| Weak | CTRL | G1 | 0.70 | 0.01 | 0.67 | 0.72 | 0.74 | 0.01 | 0,72 | 0,77 |
| Weak | HSL | G1 | 0.70 | 0.01 | 0.68 | 0.73 | 0.77 | 0.01 | 0,74 | 0,79 |
| Strong | LSL | G1 | 0.68 | 0.02 | 0.64 | 0.72 | 0.72 | 0.02 | 0,67 | 0,76 |
| Strong | CTRL | G1 | 0.70 | 0.02 | 0.66 | 0.73 | 0.74 | 0.02 | 0,70 | 0,79 |
| Strong | HSL | G1 | 0.70 | 0.02 | 0.67 | 0.74 | 0.79 | 0.02 | 0,75 | 0,84 |
| Rosette diameter (cm) | Weak | LSL | G0 | 8.80 | 0.09 | 8.62 | 8.99 | 6.73 | 0.08 | 6,58 | 6,88 |
| Weak | CTRL | G0 | 9.68 | 0.14 | 9.39 | 9.96 | 7.70 | 0.17 | 7,36 | 8,04 |
| Weak | HSL | G0 | 10.56 | 0.08 | 10.41 | 10.71 | 9.38 | 0.23 | 8,93 | 9,83 |
| Strong | LSL | G0 | 7.78 | 0.08 | 7.63 | 7.93 | 5.93 | 0.09 | 5,75 | 6,10 |
| Strong | CTRL | G0 | 9.68 | 0.25 | 9.20 | 10.17 | 7.69 | 0.30 | 7,11 | 8,27 |
| Strong | HSL | G0 | 11.42 | 0.09 | 11.25 | 11.60 | 11.59 | 0.32 | 10,96 | 12,23 |
| Weak | LSL | G1 | 8.35 | 0.08 | 8.19 | 8.51 | 8.26 | 0.11 | 8,05 | 8,46 |
| Weak | CTRL | G1 | 8.77 | 0.10 | 8.58 | 8.96 | 8.81 | 0.11 | 8,59 | 9,03 |
| Weak | HSL | G1 | 9.18 | 0.10 | 8.98 | 9.37 | 9.52 | 0.10 | 9,33 | 9,72 |
| Strong | LSL | G1 | 8.19 | 0.12 | 7.95 | 8.43 | 8.12 | 0.20 | 7,73 | 8,52 |
| Strong | CTRL | G1 | 8.78 | 0.16 | 8.46 | 9.11 | 8.80 | 0.19 | 8,42 | 9,17 |
| Strong | HSL | G1 | 9.44 | 0.18 | 9.09 | 9.79 | 9.92 | 0.18 | 9,57 | 10,27 |
| Flowering time (days) | Weak | LSL | G0 | 25.97 | 0.14 | 25.69 | 26.25 | 26.63 | 0.13 | 26,37 | 26,90 |
| Weak | CTRL | G0 | 27.33 | 0.25 | 26.85 | 27.82 | 28.26 | 0.29 | 27,69 | 28,83 |
| Weak | HSL | G0 | 28.67 | 0.19 | 28.29 | 29.05 | 29.98 | 0.25 | 29,48 | 30,48 |
| Strong | LSL | G0 | 24.67 | 0.34 | 24.00 | 25.34 | 25.60 | 0.23 | 25,14 | 26,05 |
| Strong | CTRL | G0 | 27.32 | 0.42 | 26.50 | 28.14 | 28.36 | 0.53 | 27,34 | 29,39 |
| Strong | HSL | G0 | 30.73 | 0.32 | 30.11 | 31.35 | 33.25 | 0.15 | 32,95 | 33,54 |
| Weak | LSL | G1 | 27.18 | 0.22 | 26.75 | 27.61 | 27.66 | 0.20 | 27,27 | 28,05 |
| Weak | CTRL | G1 | 28.03 | 0.24 | 27.56 | 28.50 | 27.94 | 0.18 | 27,59 | 28,28 |
| Weak | HSL | G1 | 28.83 | 0.25 | 28.34 | 29.31 | 28.16 | 0.15 | 27,86 | 28,45 |
| Strong | LSL | G1 | 26.77 | 0.41 | 25.96 | 27.57 | 27.02 | 0.27 | 26,48 | 27,55 |
| Strong | CTRL | G1 | 28.04 | 0.42 | 27.23 | 28.86 | 27.88 | 0.28 | 27,34 | 28,43 |
| Strong | HSL | G1 | 29.85 | 0.50 | 28.88 | 30.82 | 28.50 | 0.28 | 27,96 | 29,05 |
| Height at first silique (cm) | Weak | LSL | G0 | 10.29 | 0.18 | 9.93 | 10.65 | 9.49 | 0.18 | 9,14 | 9,84 |
| Weak | CTRL | G0 | 12.14 | 0.31 | 11.54 | 12.74 | 11.60 | 0.36 | 10,90 | 12,30 |
| Weak | HSL | G0 | 13.99 | 0.19 | 13.63 | 14.35 | 13.51 | 0.24 | 13,03 | 13,99 |
| Strong | LSL | G0 | 8.31 | 0.21 | 7.89 | 8.73 | 7.53 | 0.23 | 7,09 | 7,98 |
| Strong | CTRL | G0 | 12.11 | 0.53 | 11.08 | 13.14 | 11.58 | 0.61 | 10,38 | 12,78 |
| Strong | HSL | G0 | 16.05 | 0.23 | 15.59 | 16.51 | 16.18 | 0.24 | 15,71 | 16,65 |
| Weak | LSL | G1 | 10.89 | 0.17 | 10.55 | 11.22 | 12.20 | 0.21 | 11,79 | 12,60 |
| Weak | CTRL | G1 | 11.30 | 0.18 | 10.95 | 11.66 | 13.19 | 0.23 | 12,73 | 13,65 |
| Weak | HSL | G1 | 11.71 | 0.18 | 11.35 | 12.07 | 14.09 | 0.23 | 13,63 | 14,54 |
| Strong | LSL | G1 | 10.01 | 0.30 | 9.42 | 10.60 | 11.10 | 0.31 | 10,49 | 11,70 |
| Strong | CTRL | G1 | 11.29 | 0.31 | 10.68 | 11.90 | 13.13 | 0.39 | 12,38 | 13,89 |
| Strong | HSL | G1 | 12.78 | 0.31 | 12.18 | 13.39 | 14.61 | 0.58 | 13,48 | 15,73 |