****

**Figure S1. Rice epiline selection scheme for EUE and pedigree generated by consecutive selfings**.

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**Figure S2. Field grain yield of LR1, LR2 and LR3 epilines, and the control line in selfing generation S6.** \* p < 0.05 (ANOVA with Dunnett's post-hoc test)

****

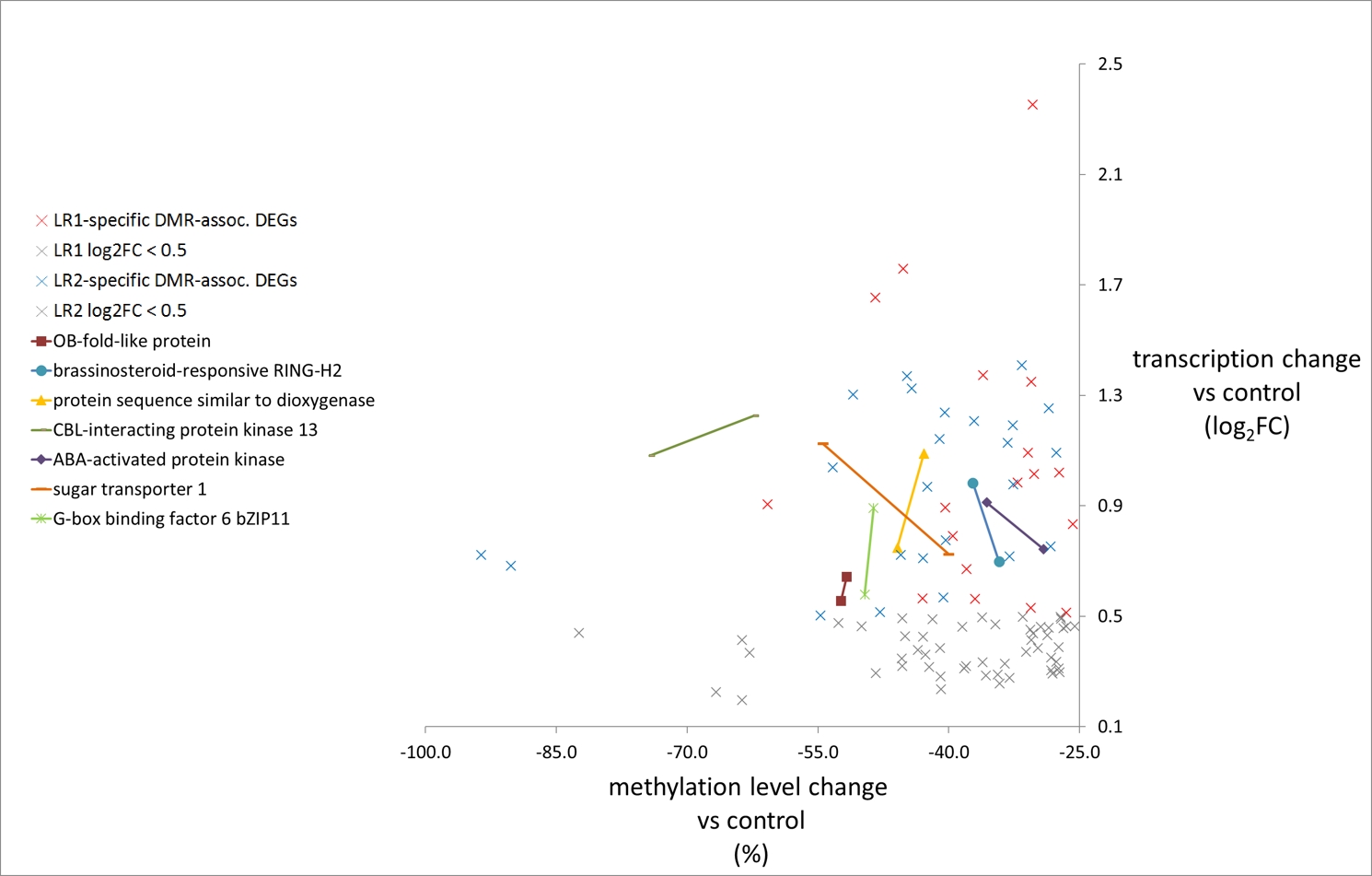
**Figure S3. Up- and down-regulated genes in LR1, LR2 and LR3 epiline transcriptomes.** Venn diagrams show common and epiline-specific numbers of up-regulated (A) and down-regulated (B) differentially expressed genes.

****

**Figure S4. Quantitative real-time PCR validation of up- and down-regulated differentially expressed genes from the RNA-seq dataset.** Means ± SD (n = 3), \* p < 0.05 (Student’s *t*-test).

****

**Figure S5. Genome-wide regions with highly differential methylation (hDMRs) in LR1, LR2 and LR3 rice epilines compared with the control line.** Chromosomes sizes are unified. Following bar code patterns are used for hDMRs: CG‑hDMR, bar; CHG‑hDMR, non-filled rectangle; CHH‑hDMR, dashed line; hyper-methylated hDMR, orange; hypo-methylated hDMR, blue; similar methylation or moderately changed in epiline compared with control line, black.

****

**Figure S6. Expression and cytosine methylation changes of up-regulated DEGs associated with upstream hypo-methylated DMRs in epilines.** Values for epilines LR1 and LR2 are indicated.Each DMR-associated differentially expressed gene (DEG) is shown with transcription and methylation change versus the control line. Epiline-specific DMR-associated DEGs are colored (red/blue crosses) when reaching the threshold of log2FC ≥ 0.5. Epiline-common DMR-associated DEGs are emphasized by connected data points with specific symbols depicted in the legend.

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**Figure S7. Verification of differential methylated regions (DMR) methylation upstream of a differential expressed gene (DEG) that are commonly measured by whole-genome bisulfite sequencing (WGBS) and targeted bisulfite sequencing (targeted BS).** (A) For three DMR-associated DEGs the geneID is indicated, followed by the BS technique used for the detection and verification of cytosine methylation levels in their upstream DMR in the control line and commonly or specific for epilines LR1 and /or LR2, below the chromosome position, the cytosine site (CG, CHG and CHH), and finally the methylation levels intervals in the control line and epilines are represented by circle (> 0 %: empty, > 30 %: quarter-filled, > 40 %: half-filled, > 60 %: three quarter-filled, and > 70 %: filled circle) (B) Compilation of the DMR analysis by WGBS and targeted BS and its position upstream of the coding region of the DEG.

**Table S1. Primers used for targeted BS**

|  |  |
| --- | --- |
| **Name (OSINDICA gene ID)** | **Sequence** |
| B1-A (01G43010) | GGAAATTTCAAACTAAAATTTCAAACCC |
| B2-B (01G43010) | TATCTACAAAAATAAAAATACTCTCACC |
| E1-A (06G27040) | TGTATTTTTTTAAGTATGAAATGTGTTAT |
| E1-B (06G27040) | TTAATACTTCTAATTAATATCCAATCATC |
| F1-A (11G27950) | TTAGTTAAAGAATTTTAATGGGAGA |
| F1-B (11G27950) | AATAAAACAATTTAATTACACCAAAAC |

**Table S2. Cellular respiration (%) in the rice epilines upon selection in three consecutive selfings (S1, S2 and S3) and non-selectively in S4 and S5.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Line** | **Relative cellular respiration** | | | | |
| **Selection** | | | **No selection** | | |
| **S1a** | **S2a** | **S3b** | **S4b** | **S5b** | |
| **control** | 100 | 100 | 100 | 100 | 100 | |
| **LR1** | 95 | 86 | 88\*\* | 89\*\*\* | 88\*\*\* | |
| **LR2** | 95 | 87 | 86\*\*\* | 85\*\*\* | 86\*\*\* | |
| **LR3** | 96 | 94 | 95\*\* | 95\*\* | 94\*\* | |

a No statistics done: only one repetition (limited seed availability).

b Three repetitions/assay; 35 plants/repetition, ANOVA with Dunnett’s post-hoc test, \* p < 0.05, \*\* p< 0.01, \*\*\* p < 0.001, versus control line (%).

**Table S3. RNA-seq clean read mapping.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Line - BR** | **Clean reads** | **Uniquely mapped**  **paired reads** | **Uniquely mapped paired reads (%)** | **CDS covered**  **(total number)** |
| **Control - BR1** | 20,696,700 | 11,586,326 | 56.0 | 26,386 |
| **Control - BR2** | 23,053,068 | 12,498,034 | 54.2 | 26,595 |
| **Control - BR3** | 23,704,964 | 12,783,984 | 53.9 | 26,742 |
| **LR1 - BR1** | 36,573,006 | 17,124,186 | 46.8 | 27,825 |
| **LR1 - BR2** | 35,026,478 | 20,398,270 | 58.2 | 28,624 |
| **LR1 - BR3** | 41,685,212 | 23,671,850 | 56.8 | 29,275 |
| **LR2 - BR1** | 48,894,674 | 27,822,660 | 56.9 | 29,945 |
| **LR2 - BR2** | 55,664,104 | 30,495,500 | 54.8 | 30,394 |
| **LR2 - BR3** | 62,977,664 | 36,393,862 | 57.8 | 30,742 |
| **LR3 - BR1** | 45,399,480 | 22,897,348 | 50.4 | 28,731 |
| **LR3 - BR2** | 33,163,660 | 18,993,710 | 57.3 | 28,180 |
| **LR3 - BR3** | 33,761,456 | 19,366,434 | 57.4 | 27,921 |

BR: biological replicate

CDS: coding sequence

**Table S4. Differentially expressed genes.**

See separate Table S4.xlxs file.

**Table S5. List of primer sequences used for qRT-PCR analysis.**

Fwd: forward; Rev: reverse primer

**Table S6. Functional categorization of LR1 and LR2 common up-regulated DEGs (PLAZA 2.5; p <0.05)**

See separate Table S6.xlxs file.

**Table S7. Quality control of the raw BS-seq read.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Line** | **Raw reads** | **Reads after DEREP** | **Reads after CLIPPING** | **Reads after FINAL TRIMMING and FILTERING** | **Clean bases** |
| **Control** | 383,993,330 | 177,416,147 | 133,096,956 | 109,589,964 | 10,244,701,748 |
| **LR1** | 415,027,717 | 327,126,008 | 217,648,342 | 181,509,214 | 17,245,419,867 |
| **LR2** | 337,468,167 | 181,122,330 | 135,485,383 | 119,976,903 | 11,344,771,148 |
| **LR3** | 382,821,715 | 237,597,775 | 179,314,054 | 158,728,234 | 15,056,652,802 |

**Table S8. Mapping the BS-seq reads (Bismark).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Line** | **Single-end alignments with a unique best hit** | **Mapping efficiency (%)** | **Sequences without alignments under any condition** | **Sequences that did not map uniquely** | **Genomic sequence context not extractable (edges of chromosomes)** |
| **control** | 67,448,272 | 61.5 | 18,993,944 | 23,147,748 | 825 |
| **LR1** | 115,957,809 | 63.9 | 29,884,804 | 35,666,601 | 1,337 |
| **LR2** | 77,004,904 | 64.2 | 18,603,158 | 24,368,841 | 898 |
| **LR3** | 104,214,730 | 65.7 | 23,643,089 | 30,870,415 | 1,183 |

**Table S9. DMRs upstream of DEGs.**

See separate Table S9.xlxs file.

**Table S10. Validation of cytosine methylation levels for a set of DMR-associated DEGs.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **OSINDICA gene ID (DMR-associated DEG)** | **Chromosome** | **Common cytosine position** | **Cytosine site** | **Methylation level detected by targeted BS** | | | **Methylation level detected by WGBS** | | | **Pearson correlation coefficient** | | |
| **Control line** | **LR1** | **LR2** | **Control line** | **LR1** | **LR2** | **Control line** | **LR1** | **LR2** |
| 01G43010 | Chr01 | 28980782 | CHH | 1.67 | 3.95 | 4.27 | 0.00 | 0.00 | 0.00 | 0.79 | 0.98 | 0.99 |
| 01G43010 | Chr01 | 28980791 | CHH | 1.61 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980795 | CpG | 75.73 | 98.06 | 75.52 | 41.18 | 81.82 | 94.44 |  |  |  |
| 01G43010 | Chr01 | 28980798 | CHG | 1.95 | 8.57 | 7.06 | 26.32 | 10.00 | 12.50 |  |  |  |
| 01G43010 | Chr01 | 28980812 | CHH | 0.94 | 1.51 | 1.45 | 0.00 | 0.00 | 5.26 |  |  |  |
| 01G43010 | Chr01 | 28980814 | CHH | 4.33 | 8.95 | 6.86 | 0.00 | 4.35 | 5.00 |  |  |  |
| 01G43010 | Chr01 | 28980816 | CHH | 6.93 | 3.19 | 6.06 | 0.00 | 17.39 | 4.55 |  |  |  |
| 01G43010 | Chr01 | 28980817 | CHH | 8.31 | 10.76 | 7.76 | 0.00 | 8.70 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980819 | CHG | 5.67 | 16.39 | 21.55 | 22.73 | 33.33 | 30.43 |  |  |  |
| 01G43010 | Chr01 | 28980820 | CpG | 30.23 | 93.74 | 88.86 | 30.43 | 87.50 | 95.65 |  |  |  |
| 01G43010 | Chr01 | 28980828 | CHH | 2.07 | 1.64 | 2.47 | 4.35 | 0.00 | 4.35 |  |  |  |
| 01G43010 | Chr01 | 28980841 | CHH | 0.15 | 1.16 | 0.21 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980849 | CHH | 1.40 | 0.63 | 1.87 | 0.00 | 3.70 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980859 | CHH | 0.03 | 0.05 | 0.02 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980860 | CHH | 0.40 | 0.06 | 0.44 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980861 | CHH | 1.25 | 0.02 | 1.04 | 0.00 | 0.00 | 5.88 |  |  |  |
| 01G43010 | Chr01 | 28980864 | CHH | 0.12 | 0.86 | 0.15 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980865 | CHH | 0.04 | 1.64 | 1.37 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980871 | CHH | 0.84 | 0.17 | 0.65 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980873 | CHH | 0.33 | 1.21 | 3.49 | 0.00 | 4.55 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980877 | CHH | 0.50 | 1.27 | 1.41 | 0.00 | 0.00 | 0.00 |  |  |  |
| 01G43010 | Chr01 | 28980878 | CHH | 2.71 | 0.04 | 0.70 | 5.88 | 0.00 | 0.00 |  |  |  |
| 06G27040 | Chr06 | 18595378 | CHH | 2.20 | 0.86 |  | 0.00 | 11.11 |  | 0.98 | 0.99 |  |
| 06G27040 | Chr06 | 18595380 | CHH | 0.59 | 0.94 |  | 16.67 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595382 | CHH | 3.54 | 0.77 |  | 16.67 | 3.33 |  |  |  |  |
| 06G27040 | Chr06 | 18595383 | CHH | 3.80 | 4.72 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595386 | CHH | 0.39 | 0.13 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595396 | CpG | 74.29 | 5.88 |  | 83.33 | 8.33 |  |  |  |  |
| 06G27040 | Chr06 | 18595398 | CHH | 0.08 | 2.98 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595405 | CHH | 0.02 | 0.03 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595406 | CHH | 1.06 | 0.05 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595418 | CHH | 0.41 | 0.08 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595425 | CpG | 96.26 | 87.59 |  | 89.47 | 93.10 |  |  |  |  |
| 06G27040 | Chr06 | 18595429 | CHH | 1.76 | 0.05 |  | 11.11 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595432 | CHH | 0.16 | 0.10 |  | 0.00 | 3.33 |  |  |  |  |
| 06G27040 | Chr06 | 18595444 | CHH | 0.04 | 0.03 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595449 | CHH | 1.12 | 0.07 |  | 0.00 | 0.00 |  |  |  |  |
| 06G27040 | Chr06 | 18595458 | CHH | 1.10 | 0.07 |  | 0.00 | 0.00 |  |  |  |  |
| 11G27950 | Chr11 | 18711943 | CHH | 1.17 |  | 0.58 | 0.00 |  | 0.00 | 0.93 |  |  |
| 11G27950 | Chr11 | 18711944 | CHH | 1.66 |  | 0.66 | 8.33 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18711946 | CHH | 0.60 |  | 0.20 | 8.33 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18711948 | CHH | 1.92 |  | 0.39 | 0.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18711949 | CHG | 19.36 |  | 1.05 | 14.29 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18711955 | CHG | 16.55 |  | 1.09 | 5.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18711960 | CpG | 48.46 |  | 0.86 | 57.14 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18711977 | CHH | 1.70 |  | 0.15 | 0.00 |  | 3.23 |  |  |  |
| 11G27950 | Chr11 | 18712003 | CHH | 0.14 |  | 0.23 | 0.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18712004 | CHH | 0.36 |  | 0.41 | 0.00 |  | 4.00 |  |  |  |
| 11G27950 | Chr11 | 18712005 | CHG | 10.33 |  | 2.57 | 3.45 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18712010 | CHH | 0.28 |  | 0.27 | 0.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18712016 | CHH | 0.11 |  | 0.37 | 0.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18712024 | CHH | 0.28 |  | 0.17 | 0.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18712026 | CHH | 0.51 |  | 0.17 | 0.00 |  | 0.00 |  |  |  |
| 11G27950 | Chr11 | 18712036 | CHG | 5.73 |  | 1.47 | 10.00 |  | 4.35 |  |  |  |

Color code shows extent of methylation level, i.e. low (blue), medium (white), high (red) at specific cytosine site (CG, CHG and CHH) and extent of methylation level (blue –white - red)

**Table S11. H3K4me3- and PolII-enriched and depleted regions in LR2 compared with those in the control line.**

See separate Table S11.xlxs file.

**Table S12. GO term enrichment analyses of transcriptional up-regulated genes, associated with H3K4me3- and PolII-enrichments in LR2 compared with the control line.**

See separate Table S12.xlxs file.