Molecular Sciences





Figure 1. DNA methylation levels at the promoter of selected TaABCCs/TaHMA2 transporters in Pirsabak 2004 (PS) and Fakhr-e-sarhad (FS). For total DNA methylation levels, McrBC was used that specially digested methylated DNA, therefore, bands represent the non-DNA methylation levels. Bands intensity was calculated with ImageJ for the bar graphs. The values from the control were divided by the values from the metal stress that represent the DNA methylation levels.


Figure 2. CHG/CHH DNA methylation levels at the promoter of TaABCC3 transporter. Basal levels (A), and in response to $\mathrm{Pb}, \mathrm{Cd}$ and Zn metals (B) in the roots of Pirsabak 2004 (PS) and Fakhr-e-sarhad (FS) varieties are presented. DNA was digested with AluI and haeIII for CHH/CHG methylation. Equal amount of digested and undigested DNA were used as template for $\mathrm{pPCR}, \%$ to non-digested DNA was calculated and relative to control is presented.

Table 1. Primers used in this study.

| Gene |  | Sequence 5'-3' |
| :---: | :---: | :---: |
| qRT-PCR |  |  |
| TaMET3 | F | ACGATAGCATATGTACTACAGCG |
|  | R | GCGAGCACTCATCTTTGTTCTAC |
| TaCMT | F | TCCTCCGTTGGGACCCCT |
|  | R | GACTAGACTCGAACCCCACAC |
| TaMET2b | F | TTAACCAATGCTTGACTGTGGTG |
|  | R | AGCACCGTTTTGATTCAGGGTAT |
| TaMET1 | F | AAGCTGCTGTGTTTGTGGATAAC |
|  | R | TCAGCAAGTTTAGCTGCTTGTTC |
| TaMET2a | F | GGTGTCTGATACACATGACCAGT |
|  | R | CCAAACAGAAGTAGCGTGGTCTA |
| TaHMA2 | F | GGGCATCCGCTTATTTGG |
|  | R | TTCCACTGCCTTTCTCCCTC |
| TaABCC2 | F | GGGAGTGCATGATTTGCGGTGTAGG |
|  | R | TA CCC TGG CCA AGC ACA CAA GTT GCC |
| TaABCC3 | F | TTGCTAGCAATTACTGGATGGCGTG |
|  | R | TGGAATCGAAGAAAGACATAGAGC |
| TaABCC4 | F | CATTGCTAGCAATTACTGGATGGC |
|  | R | GATGCGCCCACTTGGAGTGGAATC |
| TaABCC9 | F | AGGTCTGGTGAAGGAATTCGATGC |
|  | R | GAGGTTGGACGAGCGGTC |
| TaABCC11 | F | CATTCTGTGTCGGGGCATGATAGA |
|  | R | TGCATTTTGAGCTAGTTCTCTCCATC |
| TaABCC12 | F | ATTGTCCATCTTCATCACAGTTGAAATGCGG |
|  | R | CTTGATGAGTGCACAGCCAATGTTGACAATCA |
| TaABCC14 | F | ACCACTCTCCTGAGCGCG |
|  | R | AGAGGATCGACGTTGCTCCTTAC |
| Ta18SrRNA | F | GTGACGGGTGACGGAGAATT |
|  | R | GACACTAATGCGCCCGGTAT |
| ChOP-PCR |  |  |
| TaHMA2 | F | TTGGGCTAACTAACCTTCTTCTC |
|  | R | CATTAACTGGCAAACCCACCTA |
| TaABCC2 | F | GTACATCCATGGGATTTGGTTGC |
|  | R | TTGCTCACCATCACCAATACTGT |
| TaABCC3 | F | CTCTGCTTCCGAGGAGCCTC |
|  | R | CAAGGTCAGGGACGTCGTCG |
| TaABCC4 | F | GAGCCTGCTCTCTTGCATTCTT |
|  | R | CTTGCCGAACAGTATGTTCTCCTG |
| TaABCC9 | F | CCGGGCACCCGGTGATCA |
|  | R | ACGAACCTGTCCACGAGCGA |
| TaABCC12 | F | TTAATTTTCCTCTGCATCGCACG |
|  | R | TCAACCATGTATCCACTATAAGTGTCG |

Table 2. Pedigree information of wheat varieties used in this study.
$\left.\begin{array}{clll}\begin{array}{c}\text { S. } \\ \text { No }\end{array} & \text { Varieties } & \text { Parenatge / Pedigree } \\ \hline 1 & \text { Pak 81 } & \begin{array}{l}\text { KVZ//BUHO//KAL/BB } \\ \text { CM33027-F-15M-500Y-0M-76B-OY-OPAK }\end{array} \\ \hline 2 & & \text { Fakhr-e-Sarhad } & \text { PFAU"S"/SERI/BOW "S" } \\ & & \text { CM85295-010-TOPY-2M-0Y-0M-3Y-0M }\end{array}\right]$

