

## Supplementary Materials

**Supplementary Table S1.** Primer sequences of *PtrMTP* genes used for qRT-PCR analysis

| Gene name         | Forward primer (from 5' to 3') | Reverse primer (from 5' to 3') |
|-------------------|--------------------------------|--------------------------------|
| <i>PtrMTP1.1</i>  | ACGCATGTGAATTGATTGAGAG         | GTTCTTTGGCATTCTTGGAGTT         |
| <i>PtrMTP1.2</i>  | TTCTTGTTGCTGCATTCCGT           | GCATCTTTTGGGTGCTCGT            |
| <i>PtrMTP3.1</i>  | AGGCATAAAAGCCAACAGTCTC         | CATAAGTTCGGCGTGGAGTC           |
| <i>PtrMTP3.2</i>  | TCGTTCTAGGCACAACAATCG          | GGCAACCACCTCATCCATC            |
| <i>PtrMTP4</i>    | ATGATCATACTCACAACCACGA         | TCTTTGCTGGAGTACCTGATAC         |
| <i>PtrMTP5</i>    | GTATTCTACTGCTGAATTGGCG         | CACAGCTAACGAGAATGACATG         |
| <i>PtrMTP6</i>    | GCTTTACTGGGTAACAAAACGA<br>G    | CAAGAATAGCCCCCTCCAACAC         |
| <i>PtrMTP7</i>    | CAAAAAGGTTTGCTTTTTGGGG         | GCTCGTTGACTATGTTGATCAC         |
| <i>PtrMTP8.1</i>  | GAGAGTTGTGGACCGAGTGGA          | GGAAGGATTTTAACGTCGCA           |
| <i>PtrMTP8.2</i>  | TTTCGGAGTATGGATGATTGTAA<br>T   | GCTCTGTAACAGCCTCTGACC          |
| <i>PtrMTP8.3</i>  | GGCATTCTGTCATCTTGATTTT         | GAAACCAACGCCAACTCTGAC          |
| <i>PtrMTP8.4</i>  | GGGAAATGACAAAGACAATGAG<br>G    | AAGAATGCCACCAGCCATAAG          |
| <i>PtrMTP8.5</i>  | CCAAAACAACAGGCTTAACAGA         | CATCATCCTCATTGTCTGTTGTC        |
| <i>PtrMTP8.6</i>  | GAAACAGTATTGCCCCGTGCT          | AGTTCAATGTCAACACGCTCG          |
| <i>PtrMTP9</i>    | TTTTGCCTGGAAGTTTAAGCG          | ACTGCCAATGATCTGCTCTCAGT        |
| <i>PtrMTP10.1</i> | CCATTACTTTGCGGAGGTTG           | TGCAGTGAAAGGTTGGGCT            |
| <i>PtrMTP10.2</i> | AAAAGCCAAACCATTACCACC          | TCCTGCCCCTTATCCATTTC           |
| <i>PtrMTP10.3</i> | CAAATCAGGTTGTGGGAAATAA         | CAGACAAGTGGAAACAGGAAAG         |
| <i>PtrMTP10.4</i> | ATGAAGTGGAATCATTCGTTG          | TTTGGTTTTTTCATAGCGTAAG         |
| <i>PtrMTP11.1</i> | GAGTTTTAGGTCCTGAAGATGAT<br>G   | ATGGCTAATGAACCACTTCTAACA       |
| <i>PtrMTP11.2</i> | AGGCCATTAGACACATTGATAC<br>A    | AAGAGATCATATTCACCTACAACC<br>A  |
| <i>PtrMTP12</i>   | GTATCTTCAGTCATTCCGTTGC         | CAAATCCTTGATTCCGACATCC         |
| <i>PtrEF1a</i>    | GGCAAGGAGAAGGTACACAT           | CAATCACACGCTTGTCAATA           |
| <i>PtrUBQ</i>     | GGCAAGACCATCACTCTCGA           | ACCTCAAGGGTGATGGTCT            |

**Supplementary Table S2.** Primer sequences of *PtrMTP* genes used for yeast expression plasmids construction

| Primer name        | Primer sequence (from 5' to 3')         | Restriction enzyme site |
|--------------------|-----------------------------------------|-------------------------|
| PtrMTP4-pYES2-F    | GGGGT <u>ACCAT</u> GGAACATGACGGAGTCTC   | <i>KpnI</i>             |
| PtrMTP4-pYES2-R    | GCTCTAGATCTGAAAGCTACTACTCAATTTG         | <i>XbaI</i>             |
| PtrMTP6-pYES2-F    | CCCAAGCTTATGGGATTCAAATTCTACAAAA<br>G    | <i>HindIII</i>          |
| PtrMTP6-pYES2-R    | GCTCTAGATCCCAACTACTCATTGACAAAC          | <i>XbaI</i>             |
| PtrMTP8.1-pYES2-F  | GGGGT <u>ACCAT</u> TGGAGGATATGAATTCTGG  | <i>KpnI</i>             |
| PtrMTP8.1-pYES2-R  | GCTCTAGACTAACTGTTGGGTAAACCTGC           | <i>XbaI</i>             |
| PtrMTP8.4-pYES2-F  | GGGGT <u>ACCAT</u> TGGATACGCTGAAATCCTTT | <i>KpnI</i>             |
| PtrMTP8.4-pYES2-R  | GCTCTAGAAAAGTCAAGGATCATTGCCAG           | <i>XbaI</i>             |
| PtrMTP9-pYES2-F    | GGGGTACCATGGCGAGCAGACAGAGC              | <i>KpnI</i>             |
| PtrMTP9-pYES2-R    | GCTCTAGAGGCAGTCTCCATTAAGGATT            | <i>XbaI</i>             |
| PtrMTP10.4-pYES2-F | GGGGT <u>ACCAT</u> TGGTGAGCAAACAGAGC    | <i>KpnI</i>             |
| PtrMTP10.4-pYES2-R | GCTCTAGACAAGGTAGTCTCCATTAAGG            | <i>XbaI</i>             |

Notes: The sequence of restriction enzyme site in the primer was underlined.

**Supplementary Table S3.** The sequence similarity of MTP proteins between *P. trichocarpae* and *A. thaliana*

| Query             | AtMTP1   | AtMTP2   | AtMTP3   | AtMTP4   | AtMTP5   | AtMTP6   | AtMTP7   | AtMTP8   | AtMTP9            | AtMTP10               | AtMTP11  | AtMTP12  |
|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|-------------------|-----------------------|----------|----------|
| <b>sbjct</b>      | Identity | Identity | Identity | Identity | Identity | Identity | Identity | Identity | Identity          | Identity              | Identity | Identity |
| <b>PtrMTP1.1</b>  | 74.38%   | 64.52%   | 67.68%   | 43.21%   | 26.45%   | 37.50%   | ND       | ND       | 28.12%            | 28.12%                | ND       | 26.30%   |
| <b>PtrMTP1.2</b>  | 72.46%   | 62.47%   | 62.91%   | 43.58%   | 28.93%   | 37.50%   | ND       | ND       | ND                | ND                    | ND       | 27.73%   |
| <b>PtrMTP3.1</b>  | 65.31%   | 57.77%   | 65.48%   | 40.53%   | 27.22%   | 30.19%   | 25.00%   | 21.92%   | ND                | ND                    | ND       | 29.94%   |
| <b>PtrMTP3.2</b>  | 63.47%   | 53.92%   | 60.63%   | 37.29%   | 29.17%   | 30.19%   | 20.80%   | 21.28%   | ND                | ND                    | ND       | 27.85%   |
| <b>PtrMTP4</b>    | 45.65%   | 46.20%   | 49.12%   | 51.72%   | 20.80%   | 31.34%   | ND       | ND       | ND                | ND                    | ND       | 23.76%   |
| <b>PtrMTP5</b>    | 19.82%   | 20.49%   | 20.68%   | 20.73%   | 73.77%   | 23.23%   | 35.00%   | 21.64%   | ND                | ND                    | ND       | 33.59%   |
| <b>PtrMTP6</b>    | 25.71%   | 25.44%   | 27.72%   | 23.47%   | 21.43%   | 63.51%   | 29.03%   | 21.40%   | 21.85%            | 22.22%                | 26.27%   | 28.36%   |
| <b>PtrMTP7</b>    | 21.18%   | 21.55%   | 25.37%   | ND       | 35.00%   | 20.13%   | 72.26%   | ND       | ND                | 21.05%                | ND       | 29.81%   |
| <b>PtrMTP8.1</b>  | ND       | ND       | ND       | ND       | ND       | 23.73%   | ND       | 67.00%   | 55.19%            | 49.87%                | 52.24%   | 29.31%   |
| <b>PtrMTP8.2</b>  | ND       | ND       | ND       | ND       | ND       | 20.06%   | ND       | 66.58%   | 54.88%            | 54.57%                | 52.44%   | ND       |
| <b>PtrMTP8.3</b>  | ND       | ND       | ND       | ND       | ND       | 22.04%   | ND       | 66.83%   | 55.36%            | 54.88%                | 54.57%   | 33.33%   |
| <b>PtrMTP8.4</b>  | ND       | ND       | ND       | ND       | ND       | ND       | ND       | 72.29%   | 59.11%            | 58.76%                | 59.30%   | ND       |
| <b>PtrMTP8.5</b>  | ND       | ND       | ND       | ND       | 22.13%   | 20.26%   | ND       | 70.18%   | 58.42%            | 58.42%                | 57.89%   | ND       |
| <b>PtrMTP8.6</b>  | ND       | ND       | ND       | ND       | ND       | 20.67%   | ND       | 66.85%   | 53.49%            | 52.91%                | 52.33%   | 26.51%   |
| <b>PtrMTP9</b>    | ND       | ND       | ND       | ND       | ND       | 20.56%   | 31.91%   | 53.45%   | 64.58%(cover,92%) | 67.92%(cover,86%)     | 58.20%   | 29.41%   |
| <b>PtrMTP10.1</b> | ND       | ND       | ND       | ND       | 24.49%   | 19.38%   | ND       | 55.42%   | 69.64%            | 73.57%(e,0;cover,92%) | 61.04%   | 28.57%   |
| <b>PtrMTP10.2</b> | ND       | ND       | ND       | ND       | 40.00%   | 20.42%   | ND       | 55.12%   | 69.25%            | 72.82%(e,0;cover,92%) | 60.76%   | 27.72%   |
| <b>PtrMTP10.3</b> | ND       | 33.33%   | ND       | ND       | ND       | 21.18%   | 22.22%   | 53.22%   | 66.26%            | 67.73%(e,0;cover,93%) | 60.49%   | ND       |
| <b>PtrMTP10.4</b> | ND       | ND       | ND       | ND       | ND       | 20.14%   | ND       | 53.29%   | 65.62%            | 68.01%(e,0;cover,86%) | 58.31%   | 29.41%   |
| <b>PtrMTP11.1</b> | ND       | ND       | ND       | ND       | 25.00%   | 19.80%   | ND       | 54.26%   | 66.67%            | 60.98%                | 82.03%   | 24.75%   |
| <b>PtrMTP11.2</b> | ND       | ND       | ND       | ND       | 23.08%   | 19.80%   | ND       | 53.78%   | 60.05%            | 61.25%                | 81.52%   | 24.00%   |
| <b>PtrMTP12</b>   | 27.0%    | 30.77%   | 27.97%   | 25.88%   | 29.84%   | 26.67%   | ND       | ND       | 28.57%            | 23.81%                | ND       | 60.02%   |

Note: In the sequence alignment, each MTP protein of *Arabidopsis thaliana* was used as the query sequence, and all MTP proteins of *P. trichocarpae* were used as the subject sequence. ND stands for E-value  $\geq 1$ .

**Supplementary Table S4.** The sequences and the Pfam annotations of conserved motifs in PtrMTP proteins

| Motif ID       | Motif sequence                                         | Length | NSites | E-value  | Pfam                                                           |
|----------------|--------------------------------------------------------|--------|--------|----------|----------------------------------------------------------------|
| <b>MEME-1</b>  | LWLYCRSFRNEIVRAYAQDHYFDVVTNIVGLIAAVLANKFY<br>WWIDPAGAI | 50     | 13     | 3.1E-422 | Cation_efflux (IPR027469), Cation efflux family                |
| <b>MEME-2</b>  | YFVEVDIVLPEEMPLKEAHNIGETLQEKJEKLPEVERAFVHL<br>DFECTHKP | 50     | 13     | 7.8E-390 | ZT_dimer (PF16916); Dimerisation domain of<br>Zinc Transporter |
| <b>MEME-3</b>  | YPIGKLRMQPVGIVIFASVMATLGLQI                            | 27     | 12     | 1.9E-219 | No motif was found in Pfam.                                    |
| <b>MEME-4</b>  | JALYTITNWSGTVIENVVSLVGRTAPPEFLQKLTYLWNHH               | 41     | 13     | 2.6E-310 | No motif was found in Pfam.                                    |
| <b>MEME-5</b>  | ASTLDSLLDLLSGFILWFTHLSMKKPNIY                          | 29     | 12     | 1.1E-202 | No motif was found in Pfam.                                    |
| <b>MEME-6</b>  | ANJVLFAAKVYASIKSGSLAI                                  | 21     | 19     | 1.7E-117 | No motif was found in Pfam.                                    |
| <b>MEME-7</b>  | KKQRNINVQGAYLHVLGDSIQSIGVMIGGAIWYKPEWKIID<br>LICTLIFS  | 50     | 5      | 5.1E-115 | Cation_efflux (PF01545), Cation efflux family                  |
| <b>MEME-8</b>  | JVDKAQPKMSSNQELWLIAMVSATVVKL                           | 29     | 12     | 1.8E-113 | No motif was found in Pfam.                                    |
| <b>MEME-9</b>  | DLYKTLGRQKKISEYYRKQEELLEGFNEVDALTELGIIP                | 39     | 10     | 7.8E-104 | No motif was found in Pfam.                                    |
| <b>MEME-10</b> | IKHIDTVRAYTFGVH                                        | 15     | 12     | 1.4E-083 | No motif was found in Pfam.                                    |
| <b>MEME-11</b> | GWEATPRQSYGYFRIELGALISIQLIWL                           | 29     | 5      | 3.7E-053 | No motif was found in Pfam.                                    |
| <b>MEME-12</b> | HELHIWAITVGKILLACHVMIKPDABADM                          | 29     | 5      | 3.2E-049 | No motif was found in Pfam                                     |

**Supplementary Table S5.** The *cis*-regulatory elements in the promoter regions of *PtrMTP* genes

[illegible]

| Function                 | cis-acting elements  | PtrMTPs |    |    |    |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     |     |     |     |     |  |
|--------------------------|----------------------|---------|----|----|----|---|---|---|---|---|---|---|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|--|
|                          |                      | 1.      | 1. | 3. | 3. |   |   |   |   |   |   |   | 8. | 8. | 8. | 8. | 8. | 8. | 10. | 10. | 10. | 10. | 11. | 11. |  |
|                          |                      | 1       | 2  | 1  | 2  | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4  | 5  | 6  | 9  | 1  | 2  | 3   | 4   | 1   | 2   | 12  |     |  |
| Phytohormone responsive  | ABRE ABA             |         |    |    |    | 5 |   |   |   | 2 |   | 3 | 1  |    | 2  |    | 6  | 5  | 1   |     | 6   | 2   | 1   |     |  |
|                          | P-box gibberellin    |         |    |    |    |   |   |   |   |   |   |   |    |    |    | 1  |    |    | 1   | 1   |     |     |     |     |  |
|                          | GARE-motif           |         |    |    |    |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     |     |     |     |     |  |
|                          | Gibberellin          |         |    |    |    |   |   |   | 1 |   |   |   |    |    |    |    |    |    |     |     |     |     |     |     |  |
|                          | TATC-box gibberellin |         |    |    |    |   |   |   |   |   |   |   |    |    |    |    |    |    | 1   |     |     |     |     |     |  |
|                          | SARE                 |         |    |    |    |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     |     |     | 2   |     |  |
|                          | CGTCA-motif MeJA     |         |    |    |    |   |   |   |   | 1 |   |   |    |    |    |    |    |    | 1   |     | 1   |     | 2   |     |  |
|                          | TGACG-motif MeJA     |         |    |    |    |   |   |   |   | 1 |   |   |    |    |    |    |    |    | 1   |     | 1   |     |     |     |  |
|                          | TGA-element auxin    |         |    |    |    |   |   |   |   |   | 1 |   | 1  |    |    |    |    |    |     |     |     |     |     |     |  |
|                          | AuxRR-core auxin     |         |    |    |    |   | 1 |   |   |   | 1 |   | 1  | 1  | 1  |    |    |    |     |     |     | 1   | 1   |     |  |
|                          | ERE                  |         |    | 1  | 1  |   | 3 | 3 | 1 | 2 |   | 4 |    | 1  |    |    | 1  | 2  |     |     | 3   |     |     |     |  |
| Biotic stress responsive | AT-rich sequence     |         |    |    | 1  |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     |     |     |     |     |  |
| Secondary metabolism     | MBSI                 |         |    |    |    |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     |     |     | 1   |     |  |
|                          | O2-site zein         | 2       |    |    | 1  |   |   | 1 |   |   |   |   |    |    |    |    |    |    |     |     |     |     |     |     |  |
| Circadian control        | Circadian            |         |    |    |    |   |   |   |   |   |   |   | 1  | 2  | 2  |    |    |    |     |     |     |     |     |     |  |
| Site-binding             | Unnamed 1            |         |    |    | 1  | 1 | 1 | 1 |   | 3 | 1 | 3 | 2  |    | 2  |    | 3  | 2  | 1   |     | 3   | 3   |     |     |  |
|                          | Box III              |         | 1  |    |    |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     |     |     |     |     |  |
|                          | CCAAT-box            |         |    |    | 1  |   |   |   |   |   |   |   |    |    |    |    |    |    |     |     | 1   |     |     |     |  |
|                          | AT-rich element      |         |    | 1  | 1  |   |   |   |   | 1 |   |   |    |    |    |    | 1  |    |     |     | 1   |     |     |     |  |