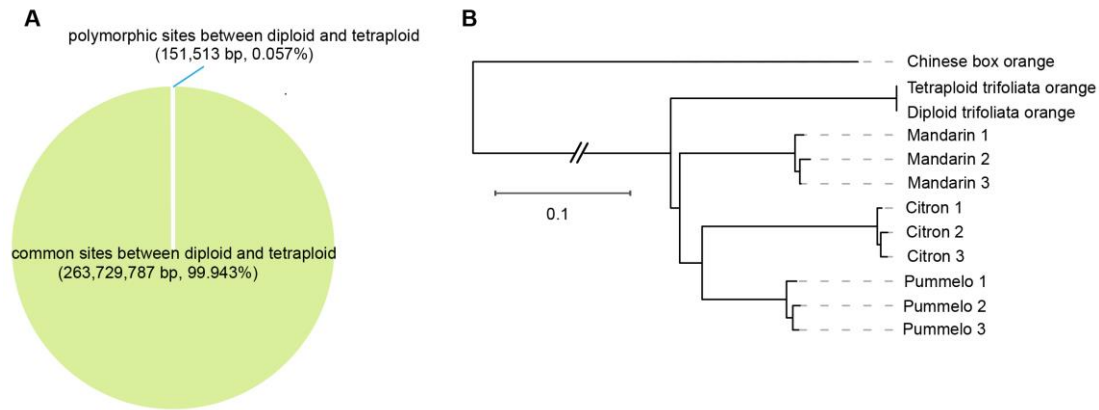


**CHH methylation of genes associated with fatty acid and JA biosynthesis contributes to cold tolerance in autotetraploids of *Poncirus trifoliata***

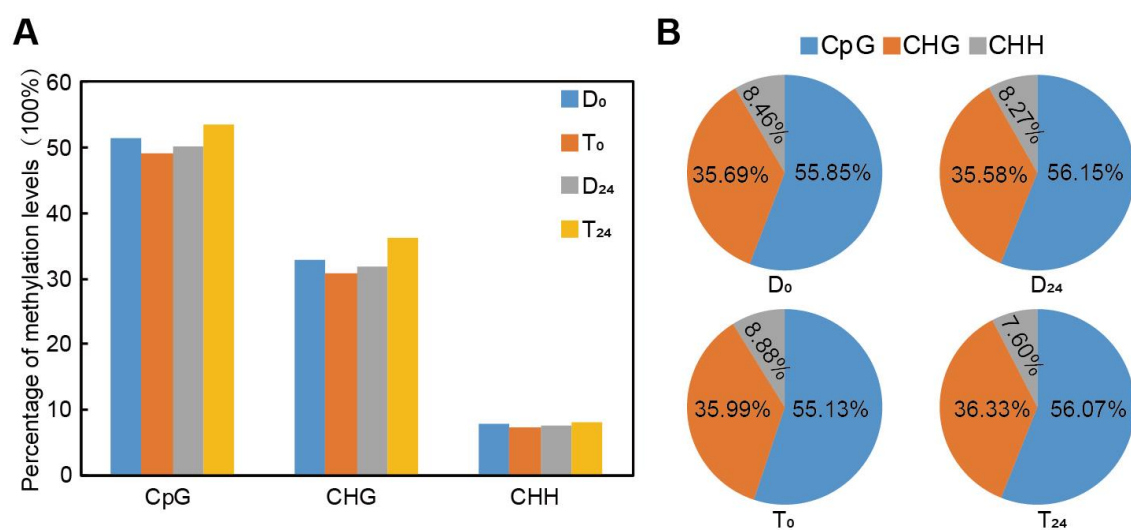
Yue Wang<sup>1</sup>, Lanlan Zuo<sup>1</sup>, Tonglu Wei<sup>1</sup>, Yu Zhang<sup>1</sup>, Yang Zhang<sup>1</sup>, Ruhong Ming<sup>1</sup>, Daharo Bachar<sup>1</sup>, Wei Xiao<sup>1</sup>, Khan Madiha<sup>1</sup>, Chuanwu Chen<sup>2</sup>, Qijun Fan<sup>2</sup>, Chunlong Li<sup>1</sup>, Ji-Hong Liu<sup>1</sup> \*

**SUPPORTING INFORMATION**



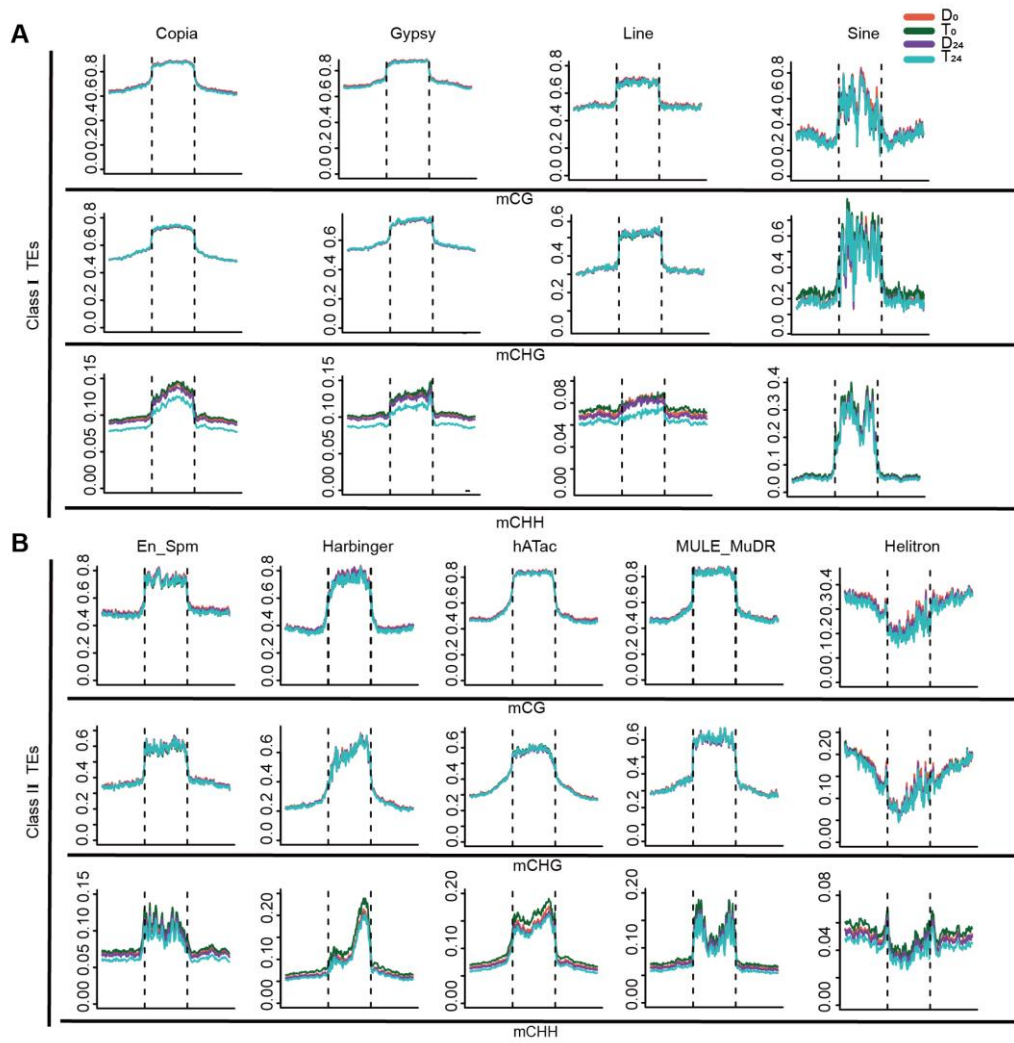
**Figure S1.** Analysis of genomic divergency between tetraploid and diploids

(A) Venn diagram for distribution of common and polymorphic sites, in terms of numbers and proportions, between diploid and tetraploid. The site numbers were obtained by aligning the re-sequencing data of diploid and tetraploid samples to the reference genome. (B) A phylogenetic tree constructed based on the obtained SNPs of tetraploid and diploid, along with nine genotypes of mandarin, pummelo and citron (three for each), using *Atalantia buxifolia* as an outgroup. Bootstrap values = 100.

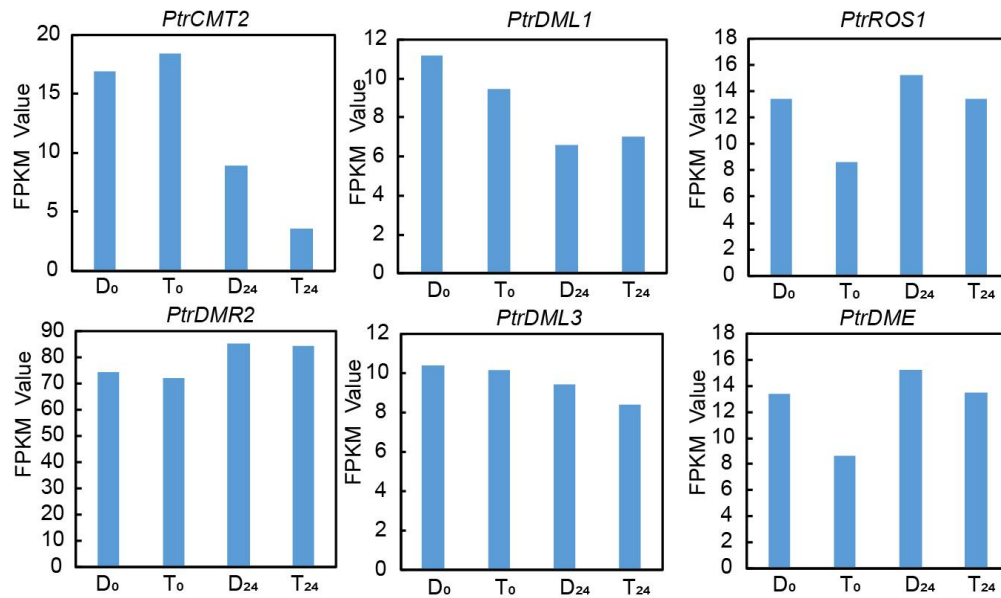


**Figure S2.** Global DNA methylation in diploid and tetraploid

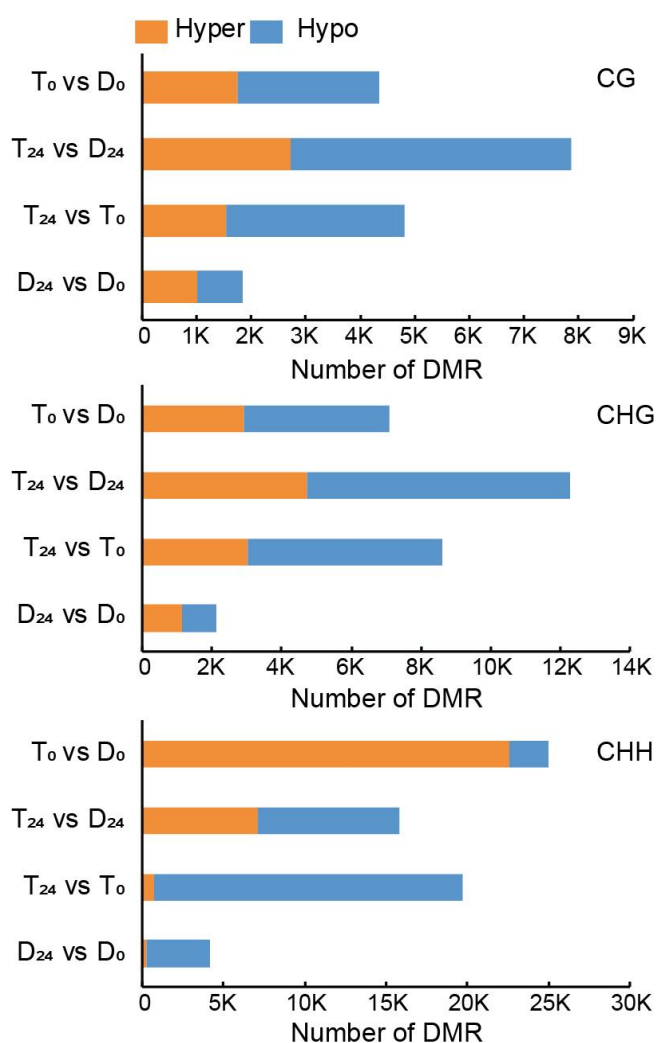
(A) Genome-wide average methylation levels for three methylation contexts (CG, CHG and CHH) in the tetraploids and diploids before and after cold stress. (B) Relative percentage of methylated cytosines for each cytosine context (CG, CHG, and CHH).



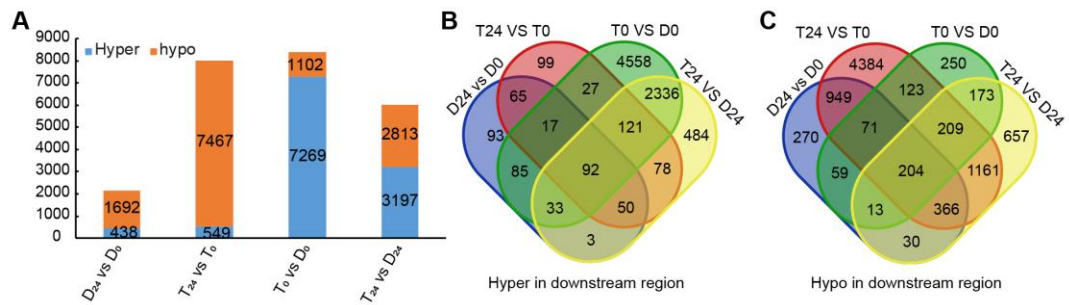
**Figure S3.** Average level of methylation distribution in TEs among Class I (**A**) and Class II (**B**) TEs, including TE bodies and their 2-kb flanking regions



**Figure S4.** Expression patterns of DNA methylase genes and demethylase genes in the tetraploid and diploid

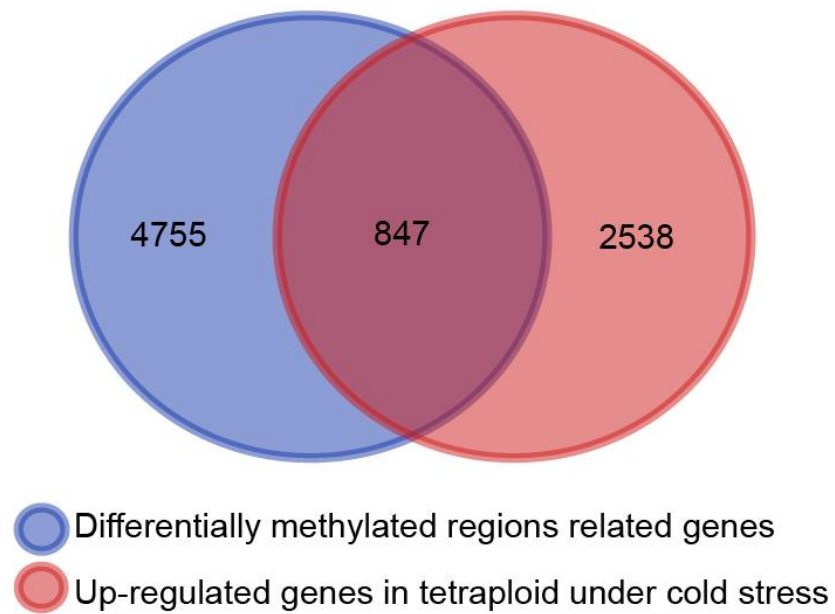


**Figure S5.** Numbers of differentially methylated regions in tetraploid and diploid under control and cold stress conditions in three methylated contexts, CG, CHG and CHH. The window length was set to 200 bp with a 200 bp step size.

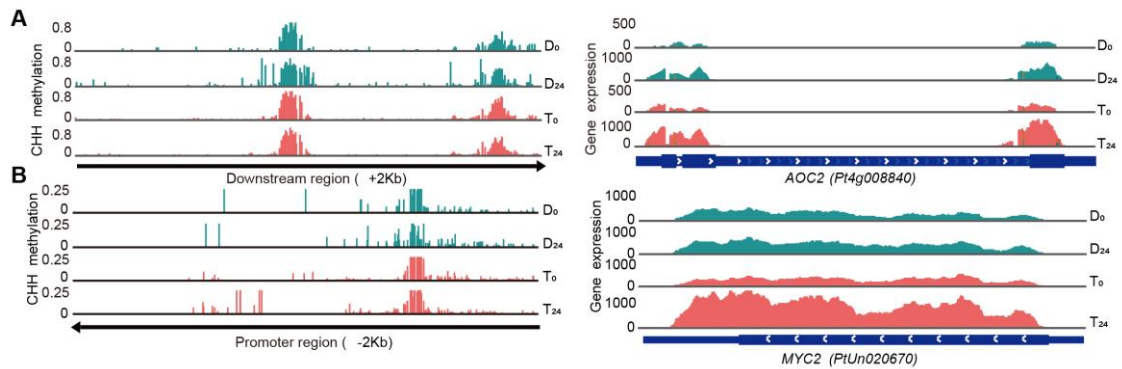


**Figure S6.** Distribution of differentially methylated regions (DMR)-related genes (DMGs) in gene downstream region

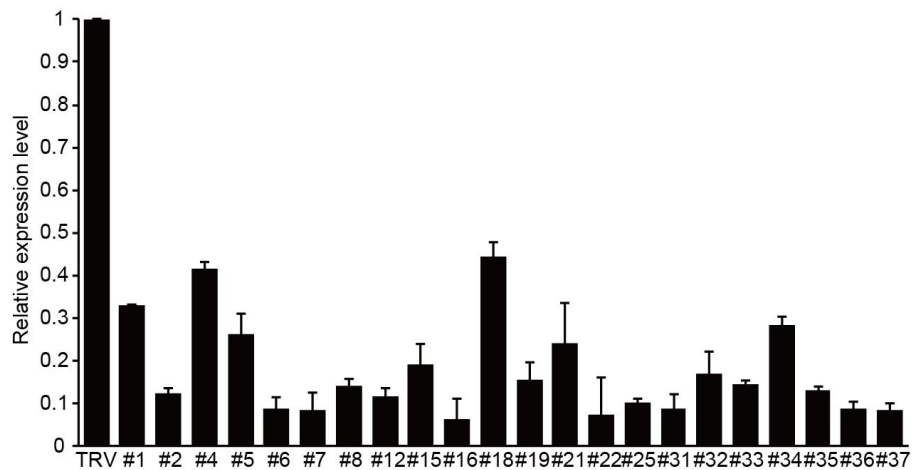
(A) Numbers of DMGs, including hypomethylated and hypermethylated genes, DMGs in the gene downstream regions. (B-C) Comparison of hypermethylated (B) and hypomethylated (C) genes in the gene downstream regions.



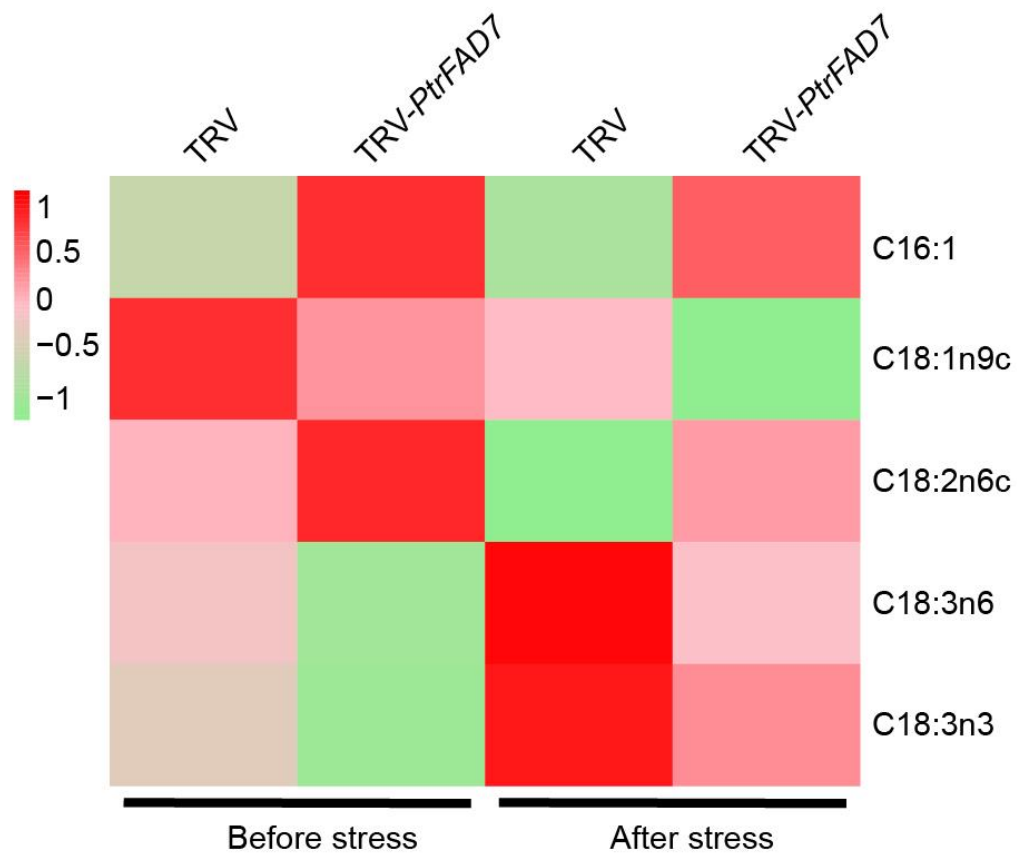
**Figure S7.** Analysis of overlapping between differentially methylated genes and upregulated genes in the tetraploid compared to diploid under cold stress



**Figure S8.** Expression and methylation patterns of JA synthetic and signaling genes. CHH methylation and expression levels of AOC2 (A) and MYC2 (B) were revealed by Genome browser snapshot



**Figure S9.** Expression levels of *PtrFAD7* in positive VIGS plants, using *Actin* as an internal reference gene. Error bars indicate  $\pm$  SE (n = 3)



**Figure S10.** Unsaturated fatty acids (UFAs) levels in the VIGS (TRV-PtrFAD7) and TRV control plants before and after cold stress, as shown by heatmap

**Table S1.** Summary of RNA sequencing and alignment data for each sample

| <b>Samples</b> | <b>Clean Reads</b> | <b>Clean Base (Gb)</b> | <b>Clean Q20 Bases Rate(%)</b> | <b>Clean Q30 Bases Rate(%)</b> | <b>GC Content(%)</b> | <b>Total Mapped Reads (%)</b> |
|----------------|--------------------|------------------------|--------------------------------|--------------------------------|----------------------|-------------------------------|
| D0-1           | 24,870,362         | 7.46                   | 98.02                          | 94.2                           | 43.13                | 94.48                         |
| D0-2           | 24,534,397         | 7.36                   | 98.16                          | 94.52                          | 43.31                | 96.07                         |
| D0-3           | 21,366,138         | 6.41                   | 97.92                          | 94.01                          | 43.23                | 95.71                         |
| T0-1           | 23,883,221         | 7.16                   | 97.97                          | 94.17                          | 43.27                | 95.73                         |
| T0-2           | 23,283,516         | 6.99                   | 98.06                          | 94.33                          | 43.42                | 95.93                         |
| T0-3           | 22,656,740         | 6.8                    | 98.09                          | 94.43                          | 43.44                | 96.03                         |
| D24-1          | 20,110,726         | 6.03                   | 97.73                          | 93.58                          | 42.94                | 95.32                         |
| D24-2          | 19,278,768         | 5.78                   | 97.9                           | 93.98                          | 43.27                | 95.79                         |
| D24-3          | 24,055,260         | 7.22                   | 97.85                          | 93.85                          | 43.02                | 95.33                         |
| T24-1          | 24,937,501         | 7.47                   | 97.94                          | 94.08                          | 43                   | 95.6                          |
| T24-2          | 22,655,394         | 6.79                   | 97.91                          | 94.04                          | 42.97                | 95.1                          |
| T24-3          | 20,168,619         | 6.04                   | 97.91                          | 94.08                          | 42.98                | 94.85                         |
| Average        | 22,650,054         | 6.7925                 | 97.96                          | 94.11                          | 43.17                | 95.5                          |

(D0: diploid before stress; T0: tetraploid before stress; D24: diploid after cold stress; T24: tetraploid after cold stress. "1", "2" and "3" represent three duplicates.)

**Table S2.** Summary of bisulfite sequencing and alignment data for each sample

| <b>Samples</b> | <b>Clean Reads</b> | <b>Clean Bases Number</b> | <b>Clean Q30 Bases Rate(%)</b> | <b>Unique Mapping rate (%)</b> |
|----------------|--------------------|---------------------------|--------------------------------|--------------------------------|
| D0-1           | 95,676,038         | 14,351,405,700            | 93.97                          | 73.7                           |
| D0-2           | 106,245,180        | 15,936,777,000            | 94.08                          | 73.6                           |
| T0-1           | 225,381,314        | 33,807,197,100            | 93.95                          | 74.1                           |
| T0-2           | 205,956,972        | 27,335,627,700            | 94.28                          | 73.9                           |
| D24-1          | 92,611,660         | 13,891,749,000            | 93.27                          | 72.1                           |
| D24-2          | 115,793,234        | 17,368,985,100            | 94.28                          | 73.1                           |
| T24-1          | 182,237,518        | 27,335,627,700            | 94.57                          | 73.8                           |
| T24-2          | 206,651,192        | 30,997,678,800            | 94.12                          | 72.9                           |
| Average        | 153,819,139        | 22,628,131,013            | 94.07                          | 73.4                           |

(D0: diploid before stress; T0: tetraploid before stress; D24: diploid after cold stress; T24: tetraploid after cold stress. "1" and "2" represent two duplicates.)

**Table S3.** Fatty acid contents in diploid and tetraploid during the cold stress process

|    | C8:0 | C10:0 | C11:0 | C12:0 | C13:0 | C14:0 | C14:1 | C15:0 | C15:1 |
|----|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 2B | 0.59 | 1.61  | 0.09  | 0.32  | 0.78  | 1.92  | 0.38  | 0.20  | 0.25  |
| 4B | 0.55 | 1.50  | 0.00  | 0.23  | 0.94  | 1.84  | 0.43  | 0.26  | 0.24  |
| 2A | 0.51 | 1.23  | 0.13  | 0.23  | 0.67  | 1.61  | 0.37  | 0.10  | 0.22  |
| 4A | 0.61 | 0.72  | 0.09  | 0.30  | 0.70  | 1.74  | 0.34  | 0.08  | 0.20  |

| C16:0 | C16:1 | C17:1 | C18:0 | C18:1 | C18:2(n-6) | C18:3(n-6) | C18:3(n-3) | C20:0 |
|-------|-------|-------|-------|-------|------------|------------|------------|-------|
| 17.91 | 0.57  | 0.43  | 5.15  | 8.62  | 17.36      | 0.64       | 40.51      | 0.33  |
| 16.77 | 0.63  | 0.45  | 4.98  | 9.22  | 19.60      | 0.54       | 39.16      | 0.38  |
| 15.27 | 0.59  | 0.39  | 4.47  | 7.66  | 17.67      | 0.74       | 48.93      | 0.29  |
| 14.97 | 0.52  | 0.41  | 4.47  | 7.51  | 20.43      | 0.70       | 51.69      | 0.32  |

| C20:1 | C20:2 | C20:3(n-6) | C20:4(n-6) | C22:0 | C22:1 | C22:2 | C23:0 | C24:0 |
|-------|-------|------------|------------|-------|-------|-------|-------|-------|
| 0.00  | 0.00  | 0.00       | 0.00       | 0.28  | 0.11  | 0.89  | 0.58  | 0.48  |
| 0.04  | 0.08  | 0.00       | 0.00       | 0.25  | 0.17  | 0.78  | 0.43  | 0.53  |
| 0.00  | 0.00  | 0.00       | 0.00       | 0.20  | 0.00  | 0.65  | 0.27  | 0.49  |
| 0.00  | 0.06  | 0.08       | 0.02       | 0.22  | 0.00  | 0.59  | 0.31  | 0.59  |

2B: diploid before stress; 4B: tetraploid before stress; 2A: diploid after cold stress; 4A: tetraploid after cold stress

**Table S4.** The primer sequences used in this study

| Gene            | Primer  | Sequence               |
|-----------------|---------|------------------------|
| <i>PtrCBF1</i>  | CBF1-F  | CGGCCAAGTTTCTGATCCTGG  |
|                 | CBF1-R  | GGGTTACGTAGCTCACATAACC |
| <i>PtrCBF2</i>  | CBF2-F  | GCTGGTCATCGCCAGAGTC    |
|                 | CBF2-R  | CCCATTGCCCCGAATCC      |
| <i>PtrCB4</i>   | CBF4-F  | GTGTGAGCTGCGAGAGCCG    |
|                 | CBF4-R  | CACCTGCAGCCTCAGGG      |
| <i>PtrCIPK3</i> | CIPK3-F | GCCATAGCAGGGGTGTCTACC  |
|                 | CIPK3-R | CCACAAGACCACAAGTCAGCG  |
| <i>PtrCIPK7</i> | CIPK7-F | CTGCTGCTCGACAGAGACGG   |
|                 | CIPK7-R | AGACCACGCATCGGCCAC     |
| <i>PtrLTI</i>   | LTI-F   | CAGTCGTGGATGAGGCTCC    |
|                 | LTI-R   | CCTGTGCCAGTGGAATCGG    |
| <i>PtrMPK3</i>  | MPK3-F  | GGTCTTGCTCGTCCAACCTCG  |
|                 | MPK3-R  | CGGACAAACCCCAGATCAGC   |
| <i>PtrPP2C</i>  | PP2C-F  | GCCGCTGGTGAATTGCACTAC  |
|                 | PP2C-R  | GCTCACACCTACAGGTGGC    |
| <i>PtrFAD7</i>  | FAD7-F  | GTTAGTGCTCCACTGAGGGTG  |
|                 | FAD7-R  | CAGCAGCAGCAGCCAAG      |
| <i>Actin</i>    | Actin-F | CCGACCGTATGAGCAAGGAAA  |
|                 | Actin-R | TTCCTGTGGACAATGGATGGA  |