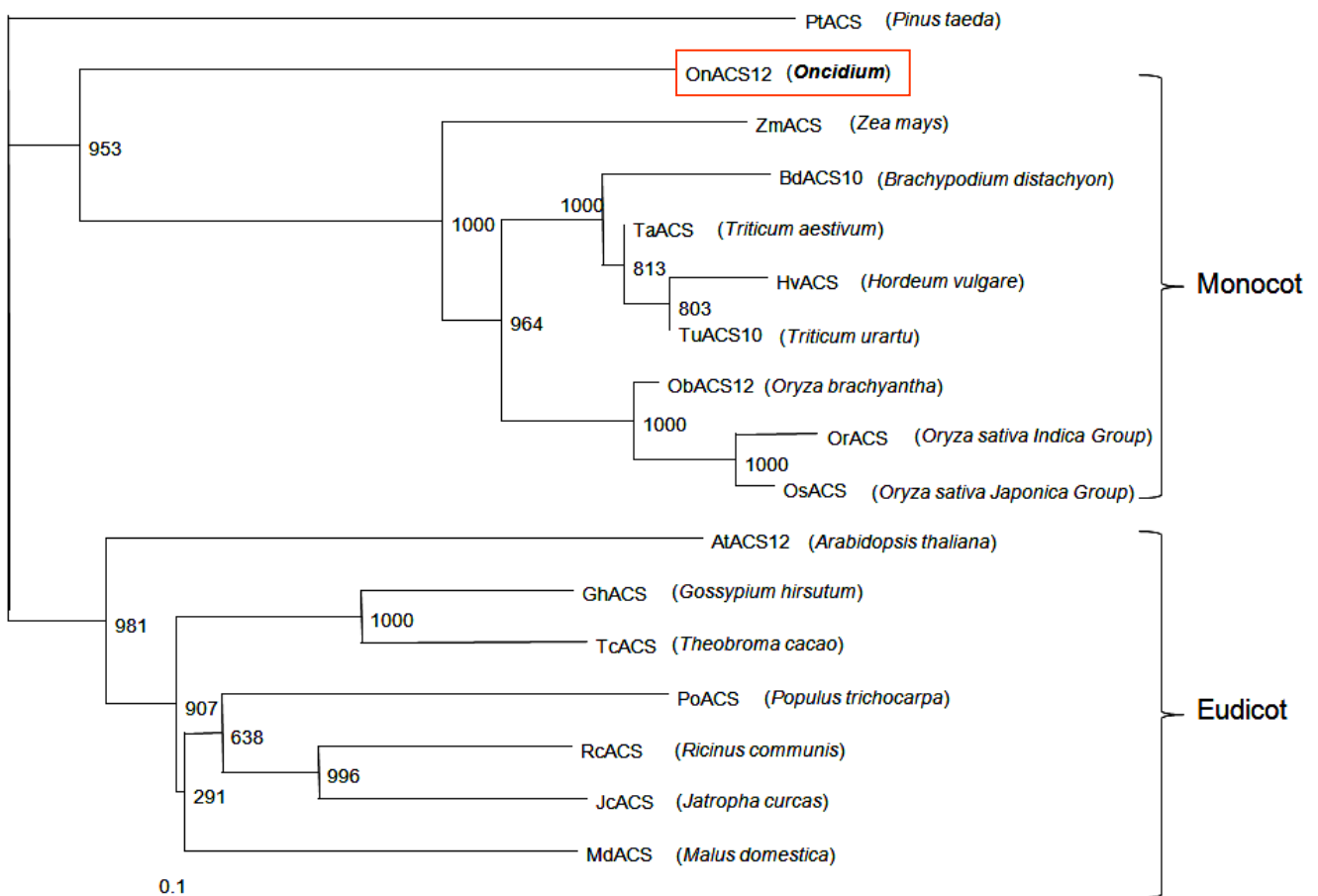


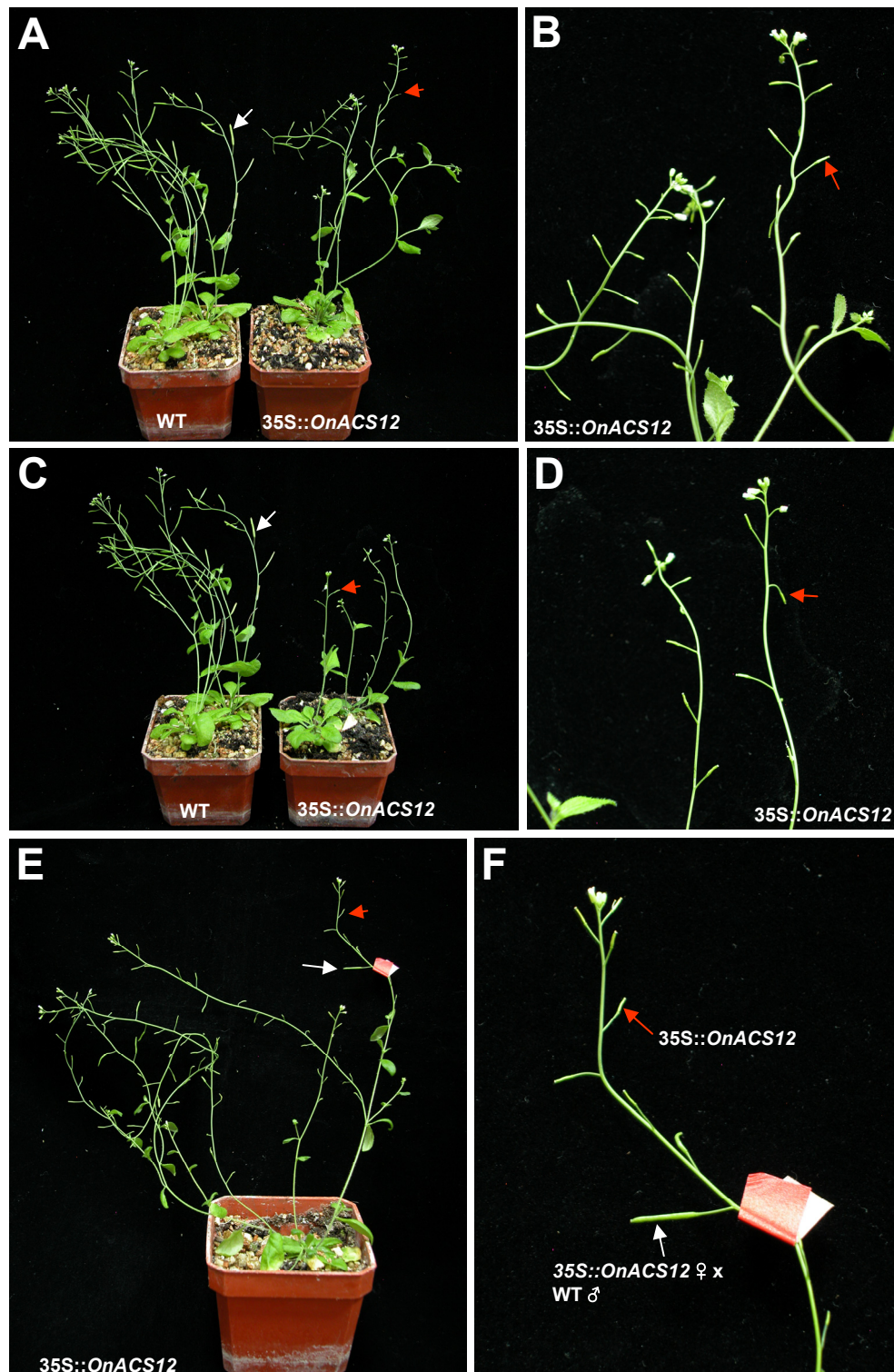
**Supplementary Figure 1** Amino acid sequences alignment of OnACS12 and related ACS proteins.

Alignment of the amino acid sequences of OnACS12 and related ACS proteins GmACS7 (*Glycine max*), LaACS4 (*Lupinus albus*), TcACS7 (*Theobroma cacao*), MdACS (*Malus domestica*), LsACS2 (*Lactuca sativa*), ZmACS (*Zea mays*) and AtACS12 (*Arabidopsis thaliana*). The conserved glutamate residue (E) marked with a triangle in box 1 is involved in adomet specificity (Mccarthy et al. 2001). The tyrosine (Y) residue marked with a full circle in box 2 is involved in the PLP-binding site (Capitani et al. 1999). The lysine (K) residue marked with an asterisk in box 5 is involved in the PLP cofactor active site. The seven boxes region (in red) represented high conserved amino acid domain among ACS proteins (Yamagami et al. 2003). The dark highlights indicate identical residues and gray highlights indicate similar residues. Dashes were introduced to improve alignment. The amino acid sequences were aligned by the BioEdit program using ClustalW Multiple Alignment.



### Supplementary Figure 2 Phylogenetic analysis of ACS proteins.

Amino acid sequences of ACS proteins were obtained via the National Center for Biotechnology Information server (<http://www.ncbi.nlm.nih.gov/>). Names of the plant species of each ACS gene are listed behind the protein names. *Oncidium* OnACS12 was red boxed. The multiple sequence alignment was performed by using ClustalW of DNA Data Bank of Japan (<http://clustalw.ddbj.nig.ac.jp/top-e.html>). The phylogenetic graph was generated by using the TREEVIEW program. Numbers on major branches indicate bootstrap percentages for 1,000 replicate analyses.



**Supplementary Figure 3** Analysis of the indehiscent anther phenotype in 35S::*OnACSI2* *Arabidopsis*.

**(A)** A 52-day-old 35S::*OnACSI2* plant produced short siliques (red arrow on the right), whereas the wild-type plant produced normal elongated siliques (white arrow on the left).

**(B)** Close-up view of the 35S::*OnACSI2* inflorescence from **(A)** showing short siliques (red arrow).

**(C)** Two other 52-day-old 35S::*OnACSI2* plants produced short siliques (red arrow on the right), whereas the wild-type plant produced normal elongated siliques (white arrow on the left).

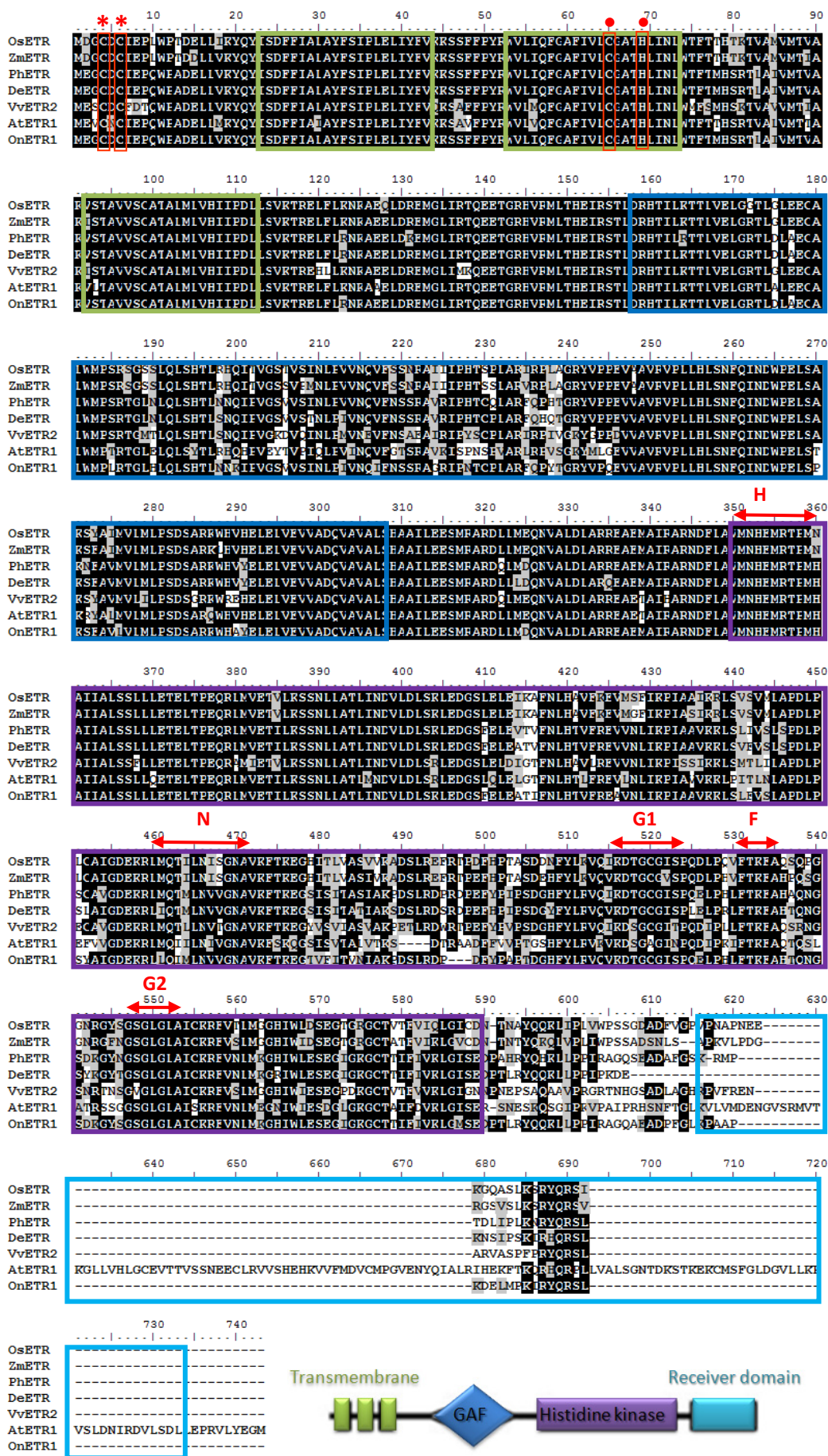
**(D)** Close-up view of the 35S::*OnACSI2* inflorescence from **(C)** showing short siliques (red arrow).

**(E)** The normal elongated silique of a 35S::*OnACSI2* plant after cross-pollination with wild-type pollen (white arrow by the red tape) along with the short 35S::*OnACSI2* siliques without pollination (red arrow).

**(F)** Close-up view of the 35S::*OnACSI2* ♀ x WT ♂ elongated silique (white arrow by the red tape) and the short 35S::*OnACSI2* siliques (red arrow) from **(E)**.

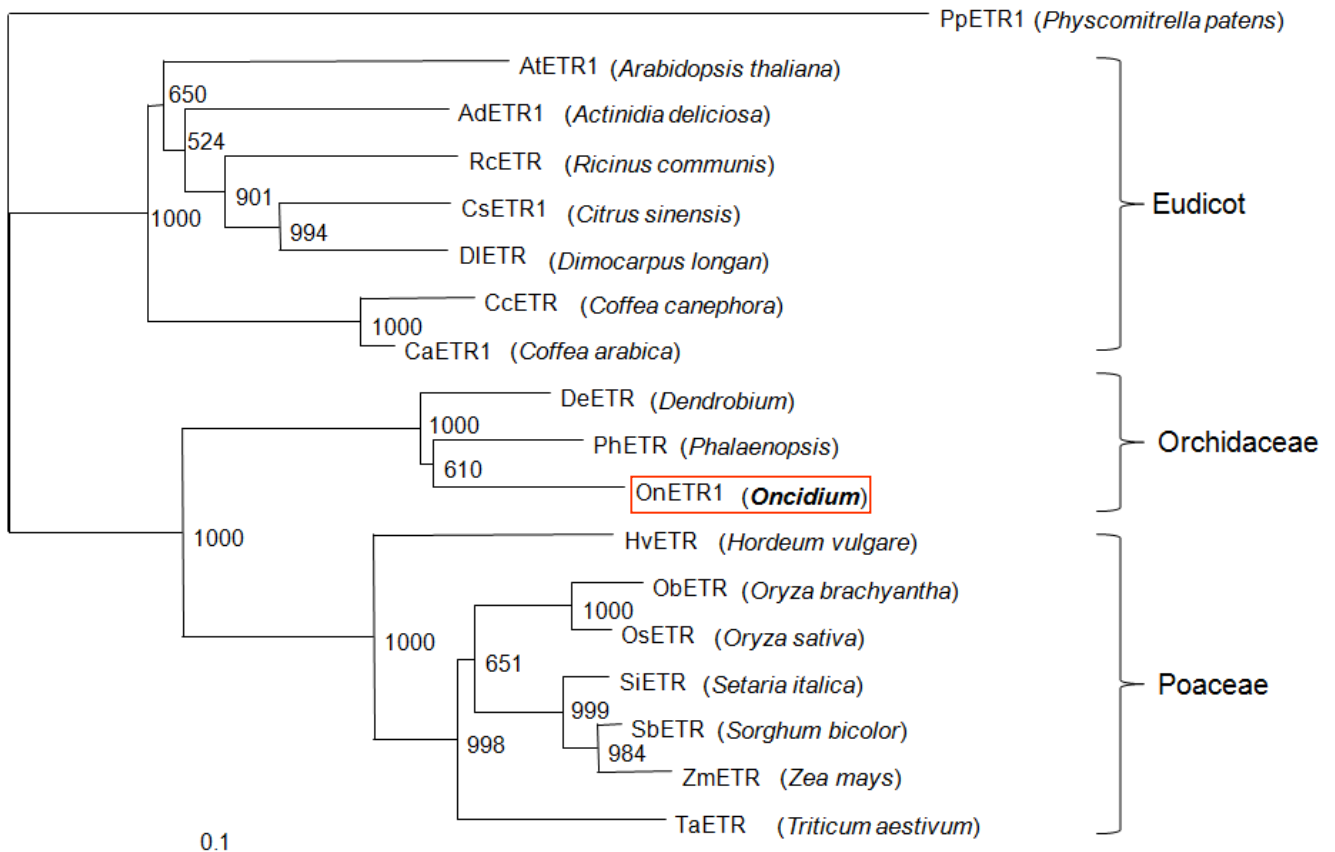


# Huang\_S Fig4



**Supplementary Figure 4** Amino acid sequences alignment of OnETR1 and related ETR proteins.

Alignment of the deduced amino acid sequences of OnETR1 and related ETR proteins OsETR (*Oryza sativa*), ZmETR (*Zea mays*), PhETR (*Phalaenopsis*), DeETR (*Dendrobium*), VvETR2 (*Vitis vinifera*) and AtETR1 (*Arabidopsis thaliana*). The ethylene binding site composed of three transmembrane domains indicated were in green boxes. The blue box region showed GAF domain. The histidine kinase domain (in the purple box) contained five conserved motifs (under the red arrows) including H, N, G1, F and G2 motifs. The cyan box in C terminal showed putative receiver domain (Parkinson and Kofoed 1992). The two cysteine residues Cys-4 and Cys-6 marked with asterisk are involved in forming disulfide-linked dimer required for receptor function. The two conserved residues Cys-65 and His-69 marked with a full circle in the second transmembrane domain are considered to mediate ethylene binding to ethylene receptor and to be candidate ligands for the Cu(I) cofactor (Schaller and Bleecker 1995). The dark highlights indicate identical residues and gray highlights indicate similar residues. Dashes were introduced to improve alignment. The amino acid sequences were aligned by the BioEdit program using ClustalW Multiple Alignment.



**Supplementary Figure 5** Phylogenetic analysis of ETR1 proteins.

On the basis of amino acid sequence of the full-length protein, OnETR1 was closely related to orchid DeETR (*Dendrobium*) and PhETR (*Phalaenopsis*). Names of the plant species for each *ETR* gene are listed behind the protein names. Amino acid sequences of ETR proteins were obtained via the National Center for Biotechnology Information server (<http://www.ncbi.nlm.nih.gov/>). The multiple sequence alignment was performed by using ClustalW of DNA Data Bank of Japan (<http://clustalw.ddbj.nig.ac.jp/top-e.html>). The phylogenetic graph was generated by using the TREEVIEW program. Numbers on major branches indicate bootstrap percentages for 1,000 replicate analyses.



**Supplementary Table 1. Oligo primer sequences used for gene cloning**

| Gene           | Primer ID      | Sequence                            | Restriction site | Used for       |
|----------------|----------------|-------------------------------------|------------------|----------------|
| <i>OnACS12</i> | 35Xba1-F       | 5'-CTCTAGAGTTTTCTCCAAAAGGCAC-3'     | XbaI             | cDNA           |
|                | 35Kpn1-R       | 5'-GCGGTACCGCAGATTCTCATTTCACTGAC-3' | KpnI             |                |
| <i>OnETR1</i>  | OnETR1-F-BamHI | 5'-GGATCCATGGAAGGCTGTGATTGCATT-3'   | BamHI            | cDNA           |
|                | OnETR1-R-KpnI  | 5'-GGTACCCTACAACTTCTCTGATACCG-3'    | KpnI             | cDNA           |
|                | OnETR1(C65Y)-F | 5'-TCCTTTACGGAGCAACCCA-3'           |                  | point mutation |
|                | OnETR1(C65Y)-R | 5'-GCTCCGTAAAGGACAATGAAG-3'         |                  | point mutation |

**Supplementary Table 2. Primer used for real-time quantitative PCR**

| Gene           | Primer ID    | Sequence                            |
|----------------|--------------|-------------------------------------|
| <i>OnETR1</i>  | RT-OnETR1-F  | 5'-TGTCATTGTTTGTGTCTCTTGCTC-3'      |
|                | RT-OnETR1-R  | 5'-CTTAAATAGAAATGCCCGTCAGTAGG-3'    |
| <i>OnACS12</i> | RT-ACS12-F   | 5'-TCTTAATATAGCCAAGGTCAATATCACAC-3' |
|                | RT-ACS12-R   | 5'-CTAAGTCGCTCCATAACCACAGG-3'       |
| <i>CPS</i>     | RT-CPS-F     | 5'-CTATATGGAGATGAAGGAGAAGGAGAG-3'   |
|                | RT-CPS-R     | 5'-AGTGTGGGTGAAGAAGTTAGTTAGG-3'     |
| <i>KS</i>      | RT-KS-F      | 5'-GATAGGTGGATTGTGGAGAATAGATTG-3'   |
|                | RT-KS-R      | 5'-ATACGAGCATCAGATAGTTCAGGAG-3'     |
| <i>GA3ox1</i>  | RT-GA3ox1-F  | 5'-GCGTGCCTTTGGGACTTCTC-3'          |
|                | RT-GA3ox1-R  | 5'-CTTGCGTTGGACAGGTAGCC-3'          |
| <i>GA2ox1</i>  | RT-GA2ox1-F  | 5'-CAAGAGCGTGAGGCATAGGG-3'          |
|                | RT-GA2ox1-R  | 5'-AGTCAATGAAGGTCCAGCGAAG-3'        |
| <i>MYC2</i>    | RT-MYC2-F    | 5'-GAAGAAGATAAAGCAAACCCGAGAC-3'     |
|                | RT-MYC2-R    | 5'-CTCCTCATCAACAGCGTCATCC-3'        |
| <i>WRKY33</i>  | RT-WRKY33-F  | 5'-GCTGCTATGCTGGTCACTCC-3'          |
|                | RT-WRKY33-R  | 5'-GAAGGTTGCTGTGTTGTTATTGTTG-3'     |
| <i>GAI</i>     | RT-GAI-F     | 5'-GATGAATGAAGAAGACGACGGTAAC-3'     |
|                | RT-GAI-R     | 5'-TCGGTGAGCATAGAATCAAGCC-3'        |
| <i>RGL1</i>    | RT-RGL1-F    | 5'-GTTGGTTGGAAACTTGGTCAGC-3'        |
|                | RT-RGL1-R    | 5'-CGGTGCGATTGATTGATTGTCG-3'        |
| <i>RGA</i>     | RT-RGA-F     | 5'-TTTAGGTTACAAAGTTAGGTCATCGG-3'    |
|                | RT-RGA-R     | 5'-CGGAAGAGGAGGAGGATTAAGC-3'        |
| <i>RGL2</i>    | RT-RGL2-F    | 5'-CAATGGCGGATAAGAAGAAGGC-3'        |
|                | RT-RGL2-R    | 5'-TAATGAACAGAGTCGTTTAAGACAGTAG-3'  |
| <i>UBQ10</i>   | RT-UBQ10-F   | 5'-CTCAGGCTCCGTGGTGGTATG-3'         |
|                | RT-UBQ       | 5'-GTGATAGTTTTCCAGTCAACGTC-3'       |
| <i>FT</i>      | RT-AiFT-1    | 5'-ATCCGTTTAATAGATCAATCACTC-3'      |
|                | RT-AiFT-2    | 5'-TCACCAACCAATGGAGATATTC-3'        |
| <i>SOC1</i>    | RT-SOC1-F    | 5'-CTTCTAAACGTAAACTCTTGGGAGAAG-3'   |
|                | RT-SOC1-R    | 5'-CCTCGATTGAGCATGTTCTATG-3'        |
| <i>CO</i>      | RT-CO-F      | 5'-GGCTCCTCAGGGACTCAC-3'            |
|                | RT-CO-R      | 5'-TGGGCGTTCTTGGGTGTG-3'            |
| <i>LFY</i>     | LEAFY-qPCR-F | 5'-CACGCTCTTGATGCTCTCTCC-3'         |
|                | LEAFY-qPCR-R | 5'-TGTCCCTCTGTCTCTCTGTC-3'          |
| <i>MYB26</i>   | RT-MYB26-F   | 5'-ACAGATGGGCTCAAATTGCTAAAC-3'      |
|                | RT-MYB26-R   | 5'-TGATGATGATGACCGGATGATGG-3'       |
| <i>NST1</i>    | RT-NST1-F    | 5'-GCTTAACGGACCCACATCATATTC-3'      |
|                | RT-NST1-R    | 5'-TACGGAGATCGGACGGAAGG-3'          |
| <i>NST2</i>    | RT-NST2-F    | 5'-CGCCGTCGTTCAATGAGGAG-3'          |
|                | RT-NST2-R    | 5'-TCGTGATGGTGGTGTGTTATGG-3'        |
| <i>MYB85</i>   | RT-MYB85-F   | 5'-GAGTGATAGTAACTTGGTGTGGTG-3'      |
|                | RT-MYB85-R   | 5'-ACATCTTCTCGTCATTGATTCTTCC-3'     |
| <i>EDF1</i>    | EDF1-RT-1    | 5'-TCACAAACACAACAAATATGGAATACAG-3'  |
|                | EDF1-RT-2    | 5'-GCTTTGGAGTAGTAGAGATGGAGAG-3'     |
| <i>EDF2</i>    | EDF2-RT-1    | 5'-CTAAACAACACGCCGAGAAACAC-3'       |
|                | EDF2-RT-2    | 5'-GCCGAACCACCACCTGAACC-3'          |
| <i>EDF3</i>    | EDF3-RT-1    | 5'-ACACATATACATTACATTACTAATCTC-3'   |
|                | EDF3-RT-2    | 5'-AATGGAATCTGTAGTTGTAGAGC-3'       |
| <i>EDF4</i>    | EDF4-RT-1    | 5'-CCGAAACATCACGCAGAGAAAC-3'        |
|                | EDF4-RT-2    | 5'-AACCCGACCCGCATCTAAATC-3'         |
| <i>ERF1</i>    | ERF1-RT-1    | 5'-GAGCAGTCCACGCAACAAAC-3'          |
|                | ERF1-RT-2    | 5'-TCTCCGAAAGCGACTCTTGAAC-3'        |
| <i>OnUBQ</i>   | OnUBQ-RT-1   | 5'-CCACGAAGGCGGAGAACAAAG-3'         |
|                | OnUBQ-RT-2   | 5'-AAGGCGAAGATTCAGGATAAGGAG-3'      |

**Supplementary Table 3. Flowering time and leaf numbers of wild-type and 35S::*OnACS12* plants that were grown under long-day (LD, 16 h light/ 8 h dark) conditions.**

| <b>Genotype</b>            | <b>No. of plants</b> | <b>Days of flowering<sup>a</sup></b> | <b>Rosette leaves<sup>b</sup></b> |
|----------------------------|----------------------|--------------------------------------|-----------------------------------|
| <b>WT</b>                  | <b>13</b>            | <b>31.3 ± 1.1</b>                    | <b>8.9 ± 1.3</b>                  |
| <b>35S::<i>OnACS12</i></b> | <b>26</b>            | <b>39.3 ± 2.5</b>                    | <b>9.3 ± 1.4</b>                  |

<sup>a</sup> The average number of days for plants to develop an elongated inflorescence in two centimeters.

<sup>b</sup> The average number of rosette leaves for plants at the time of inflorescence in two centimeters.