An organ-specific role for ethylene in rose petal expansion during dehydration and rehydration. Daofeng Liu, Xiaojing Liu, Yonglu Meng, Cuihui Sun, Hongshu Tang, Yudong Jiang, Ali Khan Muhammad, Jingqi Xue, Nan Ma, and Junping Gao.
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SUPPLEMENTARY DATA

Measurement of fresh weight and water potential

Fresh weight and water potential were used to monitor the water status of cut flowers during dehydration and rehydration.

For measurement of fresh weight, initial weight of cut flowers was measured individually after their stem ends were snipped off before pretreatment. Weight of each flower was taken again in each time point of dehydration and rehydration, respectively. The change in fresh weight was presented as the percentage of initial weight.

Water potential of cut flowers was measured using the pressure chamber method according to previous reports (Scholander *et al*., 1965) during dehydration and rehydration.

Silencing of *RhACSs* and *RhETRs* in rose flowers by VIGS

A) Cloning of *ACS* and *ETR* genes in rose

In terms of cloning of *ACS* and *ETR* genes from rose flowers, among the five *RhACS* genes, *RhACS1*.*RhACS2* and *RhACS3* were previously isolated and reported by Ma et al. (2005). The cDNA fragments of *RhACS4* and *RhACS5* were obtained from NCBI database, and their 3’ end were amplified by 3’ RACE PCR the present work.

RhETR1-5 were previously isolated and reported by Xue et al. (2008).
The primers of *RhACS1-5* genes and *RhETR1-5* genes were designed based on their specific domains of each gene.

B) Conformation of gene silencing

To determine the expression levels of *RhACS1* and *RhACS2* in sepals and gynoecia along with the expression levels of *RhETR1*, *RhETR3* and *RhETR5* in petals of silenced flowers, primers were designed outside the 3' end region used for VIGS to avoid amplification of virus RNA from the silencing vectors (Supplementary Table S3). This ensured that only the endogenous *RhACS1, RhACS2, RhETR1, RhETR3* and *RhETR5* transcripts were examined in VIGS-silencing flowers. The rose *Ubi1* gene was used as the internal standard in sepals, petals and gynoecia.

C) Ratio of height to diameter of flowers

Maximum height and diameter of each flower were measured using a vernier caliper on the 2nd vase day after dehydration treatment.

Transformation of the *RhACS1* and *RhACS2* Promoters in Arabidopsis

A) Construction of the *RhACS1* and *RhACS2* Promoter-GUS Fusion

The 2164 bp fragment of 5'-upstream region of *RhACS1* (accession no.HF562220) and the 1584 bp fragment of 5'-upstream region of *RhACS2* (accession no. HF564634) were isolated from rose genomic DNA according to the instructions of the LA PCR in vitro cloning kit (TaKaRa, Dalian, China). The amplified sequences were digested by *SphI* and *XbaI* and then inserted into the binary vector pBI121 in order to replace the cauliflower mosaic virus 35S promoter which lies upstream of the ß-glucuronidase gene.

B) Gene transformation in Arabidopsis

Arabidopsis thaliana Columbia (Col) plants were transformed according to the floral dip method (Clough and Bent, 1998) using the *Agrobacterium tumefaciens* GV3101 strain carrying the constructs of pBI121-Pro$_{RhACS1}$::GUS or pBI121-Pro$_{RhACS2}$::GUS. Arabidopsis seeds were surface-sterilized using 5% NaOCl

for 10 min, washed off and stratified at 4°C for 3 d, and then sown on MS medium to allow for germination. Eight-day-old seedlings were transplanted to 7-cm pots filled with 1:1 mixture of peat and vermiculite in a controlled room under 23 ± 1℃, 16/8 h (day/night), and 80-100 μm^{-2} s^{-1} illumination. The plants were watered every 4 days. The T0 seeds collected from transformed plants were germinated on the MS medium containing 50 mg L^{-1} kanamycin (Km) to screen for positive transformants.

C) Treatments of transgenic Arabidopsis

For dehydration treatments, inflorescence of the tranformants mentioned above were cut down completely and dehydrated for 2 h in plastic Petri dishes with the lid removed at 22℃ under light (80-100 μm^{-2} s^{-1} illumination) conditions.

D) GUS assay

For the histochemical GUS analysis, inflorescence after dehydration treatments were incubated into a GUS staining solution (75.5 mM sodium phosphate (pH 7.0), 0.1% Triton X-100, 0.05 mM K_3/K_4FeCN, 10 mM EDTA, 20% methanol (v/v) and 50 μg mL^{-1} 5-bromo-4-chloro-3-indolyl glucuronic acid) at 37℃ overnight. Inflorescence were then cleared by ethanol and imaged shortly thereafter.

Supplementary Figures

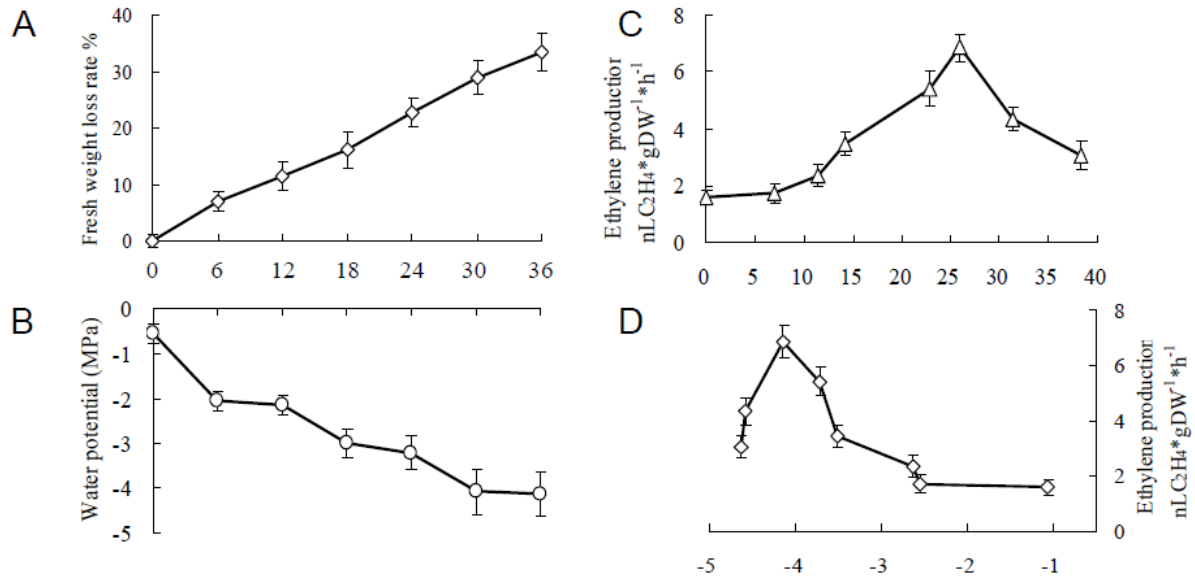


Figure S1. Changes in fresh weight loss rate during dehydration. (A) water potential (B) as well as ethylene production of different fresh weight loss (C) and water potential (D) in rose flowers. Flowers at stage 2 were used for 36 h dehydration. Each value represents means of 30 replicates, Bar=S.E.

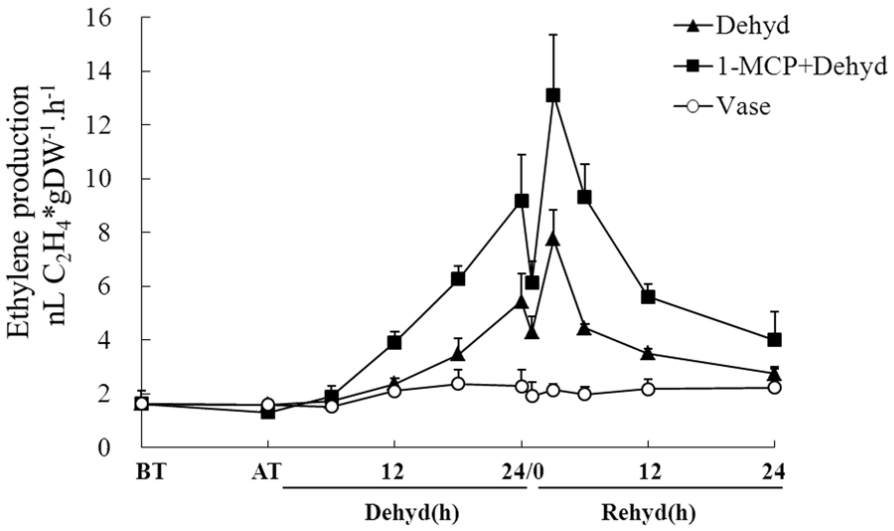


Figure S2. Ethylene production in whole flowers. Dehyd, dehydration, flowers placed in DW were under air condition for 12 h, and then were subjected to dehydration for 24 h. 1-MCP + Dehyd, flowers placed in DW were pretreated by 2 ppm 1-MCP for 12 h, then were subjected to dehydration for 24 h. BT, before treatment; AT, after treatment.

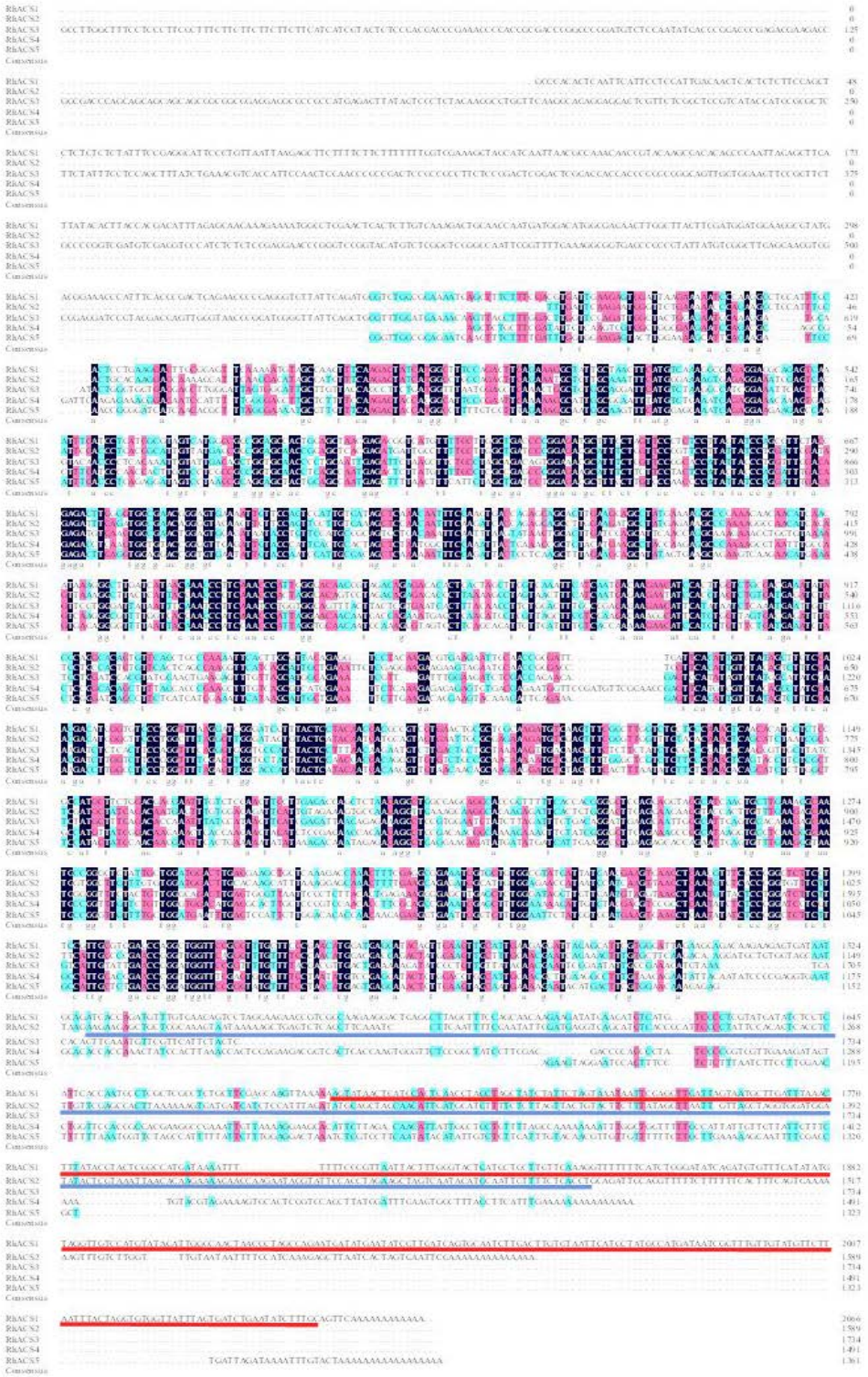


Figure S3. Alignment of cDNA sequence of *RhACS1-5* genes and the specific fragments used in VIGS. Underlined in red indicates 3’ specific fragment of *RhACS1* gene used in VIGS. And underlined in blue indicates 3’ specific fragment of *RhACS2* gene used in VIGS.

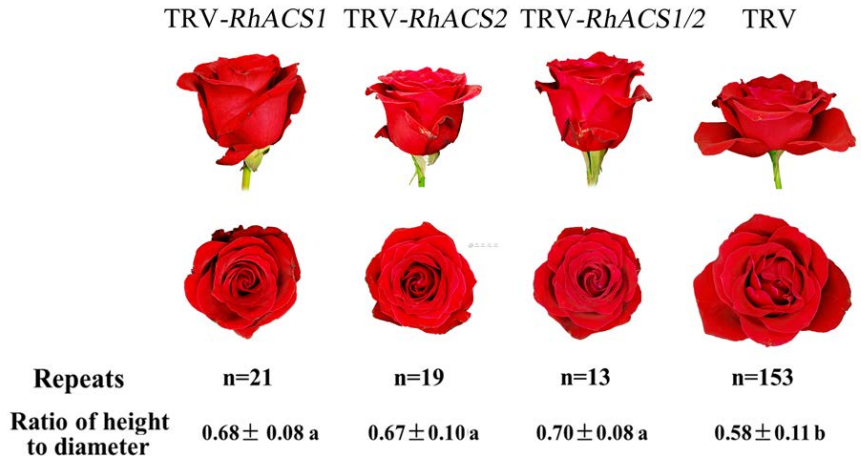
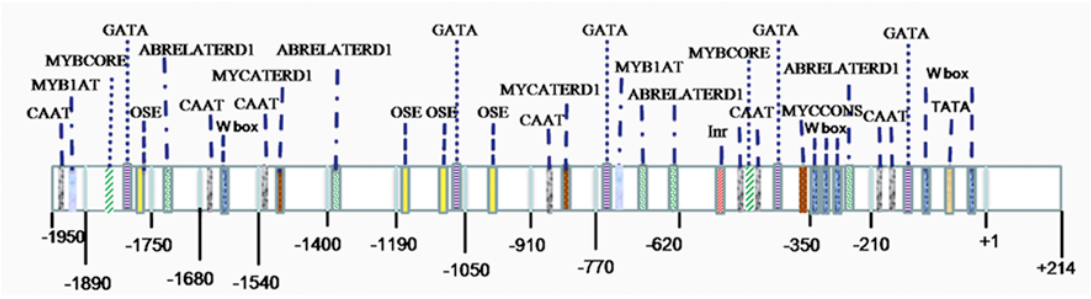


Figure S4. The phenotype of *RhACS1*-, *RhACS2*- or *RhACS1/2*-silenced flowers (upper) and ratio of height to diameter (lower). The photographs were taken on the second day after 24 h dehydration. n, replicates of rose flowers.

A

-1950 TAGAAATAGTCAATCTCATGCTAAATAGTAAAAAGTACGTACGTAATAATTATAACCATG
-1890 ATCAAAATGTGAAAATGGGCCTTATGAAGTCAAGGAAAAAGGGAGTGAAATGATTTCTGTTAAAAGTCAA
-1820 AAAGGACAAATCACCACCTTATAGTAGGCGATACAAAATTAAAATTAAAACAAACTCTTCCCTTTTAATT
-1750 AGTAGGTAACAGTAGTTCCTCATACTAATTTTGTACTCTACGTGCTTATTGTTCCCATCCAAAATTTGAA
-1680 ATTTGTACTGACCCCAATAAAGTATACAAAAGCGACAAGTACTTACCCTAATTATAACCCCCACAGACCA
-1610 AAGAGAGCCACAGACCACGTTTTCGTGGCCTTTCAGCACCACCGCGTAAATGAAACAAAAAAACGTTTTTG
-1540 GATGCCCGCAATATTTTGTGAAACCGATCATGTGGAGCCTATACTTCTTAGATTGCCCAACTAGTTGGGA
-1470 ATGTATGATGGGAATGATTTGTTGGCCATTCTGAGAAAAGAGAGCAAAGGGTGTGATGCGAAATGTATAA
-1400 ACGCATTCAACGTGGAAGCCAAAATGGGCAGGGTTTTGTGGGACTTGGGTACTCGAAAGGAACCTAAGGA
-1330 AAATAGAACTGCCCTTAATTACTCAGTCGTCCTCGTGTGCGAGTCTTGTGACATTTAACTATTTAGGTGC
-1260 ACAAGTCCAAAGAGCCGCCCAAGAAGTAATATTAGTATACTTTATTCTAGTTAACATTAAAATCTAACTT
-1190 TCTCTCTTTTAAATTTCATCCATAAAATGATCCACGGCTCATTGAAAATCTATTCTATATATTCGATCAT
-1120 TCTAGTCAACATTAAAATCTAACTTTCTCTCTTTTAAATTTCATCGATAAAATGATCCACGGCTCATTGA
-1050 AAATCTATTCTATATATTCGATCATTCTAGTCAACATCAAAATCTAACTTTCTCTCTTTTAAATTTCATC
-980 CATAAAATGATCTACGGCTCACTGAAAATCTATTCTATATATCTAATATGCAACAACAGTACATTGTTTC
-910 GTGATCATCACAACAGTAATTGTTTCGTGATCATCACAACAATACATTGTTTCACTCAAATATTGTCATC
-840 ATGTGTCATAGGGTAAAACAAGTAACACCAAGAATTCTTATAGTAGAGTTCTTACTTCAAATTACCCTTG
-770 ATTAGGATAGGCAACTTGCTAGTCTGAATTCCTTATCAAGAAACTTTGACATAACCCGAACATTGTATTC
-700 GAAAACCACTGGAGAATTACGTGGTACTTTCGTGATCACCCAAAAGTTTCTCATTTTATAAAATAACAAA
-630 TAATTACGTGATACTTTCATAATCACCCAAAAGTTTCTCATTTTATAAAATAACAAATAATTCTTTTGAA
-560 TCGGACTGCAAATTAGAATGAACTCAAAGAAATTGTCTCAAACTTCATCACAAAAATCCAATAACACTGT
-490 TGTCAATTCCAACTAGCATACGATGGACGGAGAGAGTGGGGAATGTCATCCTAGAACTTAAATACAAATT
-420 TAGTTTATGTTACTAGTAGATATCGCATATCTTATGTTGGCTAACCCATTCCTATTCTCACTTGTGAGTA
-350 ATGACTTTGCAACCGGCCACATAACCTCGATCAACACCATAGAGGAGAATTTCAAAAAAGAAATACGAAC
-280 AAGTACACTTCCATACGTGACTTGCTATGATCAAGCTCAACACGCAAGCCCTTAATTACGACTCGTTGAC
-210 CAATAACCCAAGCACAATTTTGTTCTGTTTTTTCCATTCCTCGGTGGGGGCGGGTTTTTCCACATTTGAA
-140 TCTTGCAGAGTTGGATATGGACTTCTGACCCGAAAGAAAAAGAAAAATTTGAAATGGAATTTTCGCACTA
-70 AATTTTTAGAGCGAGCTTTGCCAAACTGTCTTCCACTGCCTATAAATTCCCACACATACCTGACCCTTCC
1 CCCCACACTCAATTCATTCCTCCATTGACAACTCAGTCTCTTCCGGCTCTCTCTCTCTCTCTCTGTTTCC
71 GAGGCATTCCCTTCTCTTTTTTTCGAGGCATTCCCTTATTCTTTTTTGGTCGAAAGGTAGCATCAATTAA
141 CAAGGCTCAAAGCCAAACAACAGTACAAGGGACACAACCCAATTTAGATCTTGATTATACACTTACCACG
211 ACATG



B

-1433 TCTGAGTGATCTACGGAAAGCGAGACCATTGGA
-1400 GGTCGTATGGTTTCTTCTACAAGAGTCAACTGTAATTGATCGTGTAGCACAACACCTTAAATATGACATG
-1330 GGTGTTTTCTCTGTGTATTCTATGGTTACTTCCACAGCAGCCAGCTTTCAAGCAAGACTGATTTTAGCAT
-1260 TTCTCATTGTCATAGATGCTGGTTTTAAAATATAAGACGCAGATGAGACCATGCTGTAAGTACCAAGTAT
-1190 TTTCATAATTCTACAAGCCGGTCATCGTCATGCCACCATAAATTCTATCGGAGTGATAAATTTGTCATTA
-1120 TAAAGATGCTTATGAAATGGATTATAGAGAAGTGTGAATTAGTAATTTCTAACCCATAAAATGGTTCGTT
-1050 GGGGCATTTCATTGACCCCCTTTTGGCTCCACGGCCTCCACCACAGATACAAAGTCCTACTGGTTTGGTG
-980 AGACTAATCACCCAATATAGTGATCGTTCACATTTTATATTGAGATATGAAGTAATAGTTGAAATCAAGT
-910 TTAATAGAACTGTGTTTTTAGTTTGAAAATCCTCATGTCCGTCCTTTTCAGTATAAACATACAATTTTGA
-840 AAGTATAGTAGTCTATTCTAAGCTTAATATGCATTGTTGATTCAAAATCGTAACTTTAACATGTCAGTAA
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-630 ATATGAGATCTTTGGTTCAAATCCATTGAACACTCATGTTTCGCATTTTTCATCCCCCATGCCATTAGAG
-560 AATTCTCCGTGTAATGTGTGTTACCGTGTAGGCATGTACATAACTCGTTTTGGTACAAACTTCACAGGGA
-490 TGGTTTGACTGAATAGAGACCTATTCAGTGAACAGCGAACACTGGACTATATATTGATGATGGCTGACAC
-420 TCTGACAGTTAAGGTACCTTCAATAATGCCTTGTCCTTACATAAAGGAAGTGACAATGAAACAGCTTTCC
-350 CCAACTGGCTTAGATTTTGAGCCCATCTATTTAGTTTAACATTGATTGAAATTTTTTTTTAAATTTTTGT
-280 TATTATTATTATTTTTTTATATAATGAATGGTGACTCGAATTTAAGATCTGGATCCTAGGTAGTGGGTGT
-210 TCTAACCACTGACACCATATCGTCAACATTGATTGAAACTCAAACACAAATCAACTTCTACCTAGATTTT
-140 CAAAATTAAAGAAGCATCAGTTGCAGCTTCATGGAAACTCCCAAGAAATACAAGAACAAAAAGTCCGACG
-70 CTTATTTTTTTTGCATAGCCATCTGAGACCAACCTCATCCATAACGTCTATAAATACCATTCAGGAAGTT
1 GTATTCTTCATTCAACTGCAAAACAATTTCGTGCATTTTACGAGTTCCCAAAGGACCTGACCTTTTAGCT
71 AGCTAGCTCTCTTTTCTCCTTTTTGGTAGATTTCTCATTGCACTGAAGGTATCCATCGATCTTTAAGGCA
141 TTACAAGAAAATG

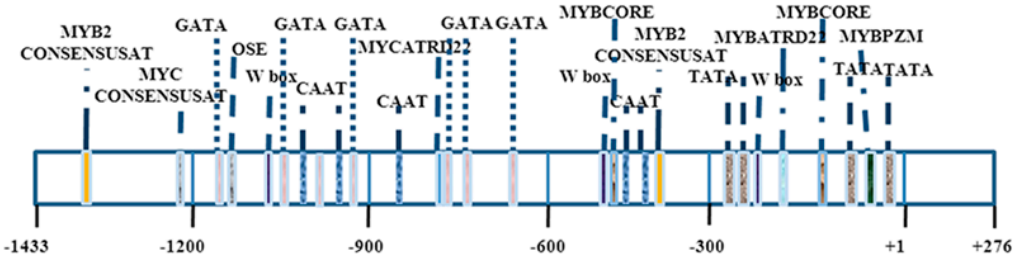

Figure S5. Promoter of *RhACS1* and *RhACS2*. (A) The *RhACS1* promoter. Upper, The sequence and putative *cis*-elements detected in the region. Lower, A pictorial representation of the promoter region of *RhACS1* with potential *cis*-elements binding sites. +1, transcription start site. (B) The *RhACS2* promoter. Upper, The sequence and putative *cis*-elements detected in the region. Lower, A pictorial representation of the promoter region of *RhACS2* with potential *cis*-elements binding sites. +1, transcription start site.

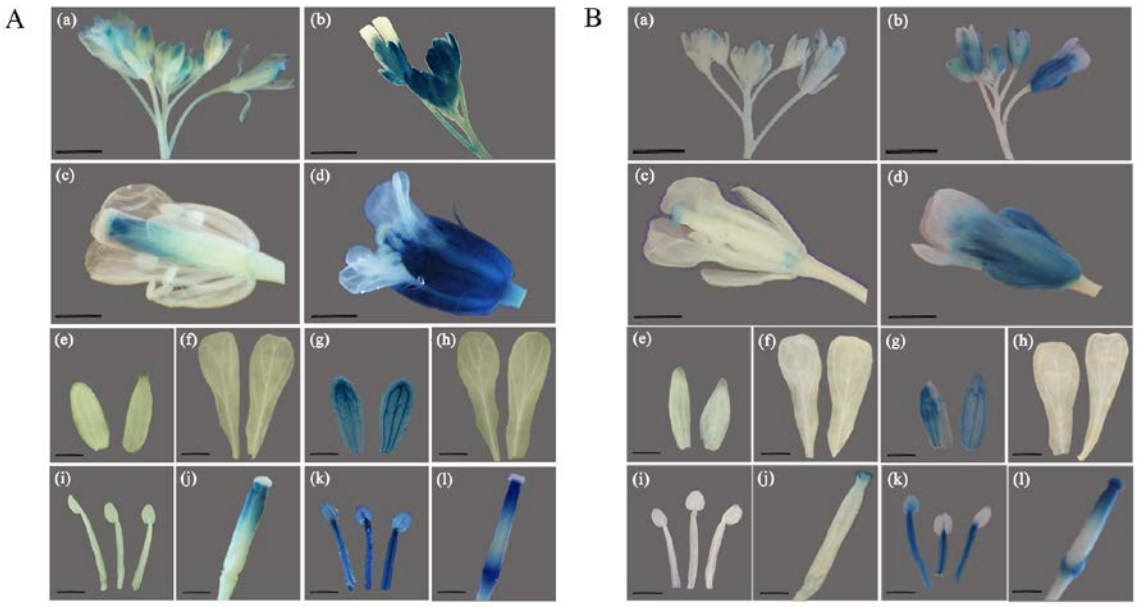

Figure S6. Induction of the *RhACS1* promoter activity by dehydration in flowers of transgenic Arabidopsis. A and B represent two independent lines. Expression of the *uidA* reporter gene, which encodes ß-glucuronidase (GUS), was driven under the control of the *RhACS1* promoter sequences. Arabidopsis transgenic plants harbouring this fusion were stained for GUS activity so that localized activity appears as a blue precipitate. All plants were grown under long day length (16 h light: 8 h dark) conditions, and cut down for a 2 h dehydration phase during inflorescence development stage. Expression in non-dehydration treatment: (A) inflorescence, (C) flower, (E) sepals, (F) petals. (I) stamens, (J) pistil; after 2 h dehydration treatment: (B) inflorescence, (D) flower, (G) sepals, (H) petals. (K) stamens, (L) pistil. Bars, (A,B) 3 mm; (C–D) 1 mm; (E-L) 500 µm.

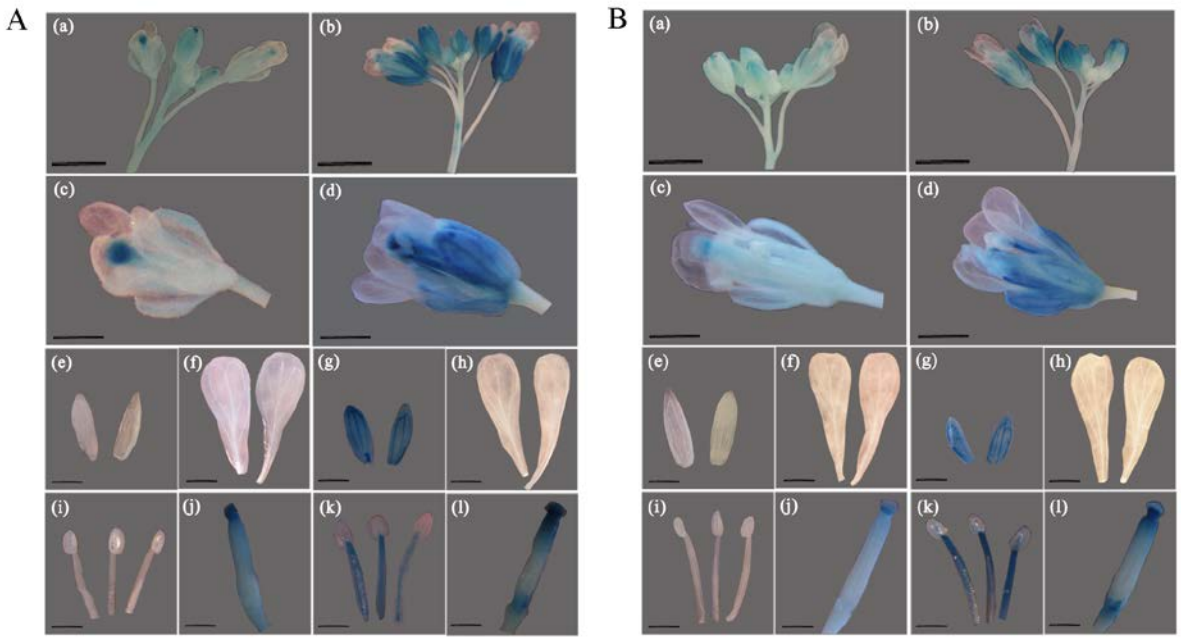

Figure S7. Induction of the *RhACS2* promoter activity by dehydration in flowers of transgenic Arabidopsis. A and B represent two independent lines. Expression of the *uidA* reporter gene, which encodes ß-glucuronidase (GUS), was driven under the control of the *RhACS1* promoter sequences. Arabidopsis transgenic plants harbouring this fusion were stained for GUS activity so that localized activity appears as a blue precipitate. All plants were grown under long day length (16 h light: 8 h dark) conditions, and cut down for a 2 h dehydration phase during inflorescence development stage. Expression in non-dehydration treatment: (A) inflorescence, (C) flower, (E) sepals, (F) petals. (I) stamens, (J) pistil; after 2 h dehydration treatment: (B) inflorescence, (D) flower, (G) sepals, (H) petals. (K) stamens, (L) pistil. Bars, (A,B) 3 mm; (C–D) 1 mm; (E-L) 500 μm.

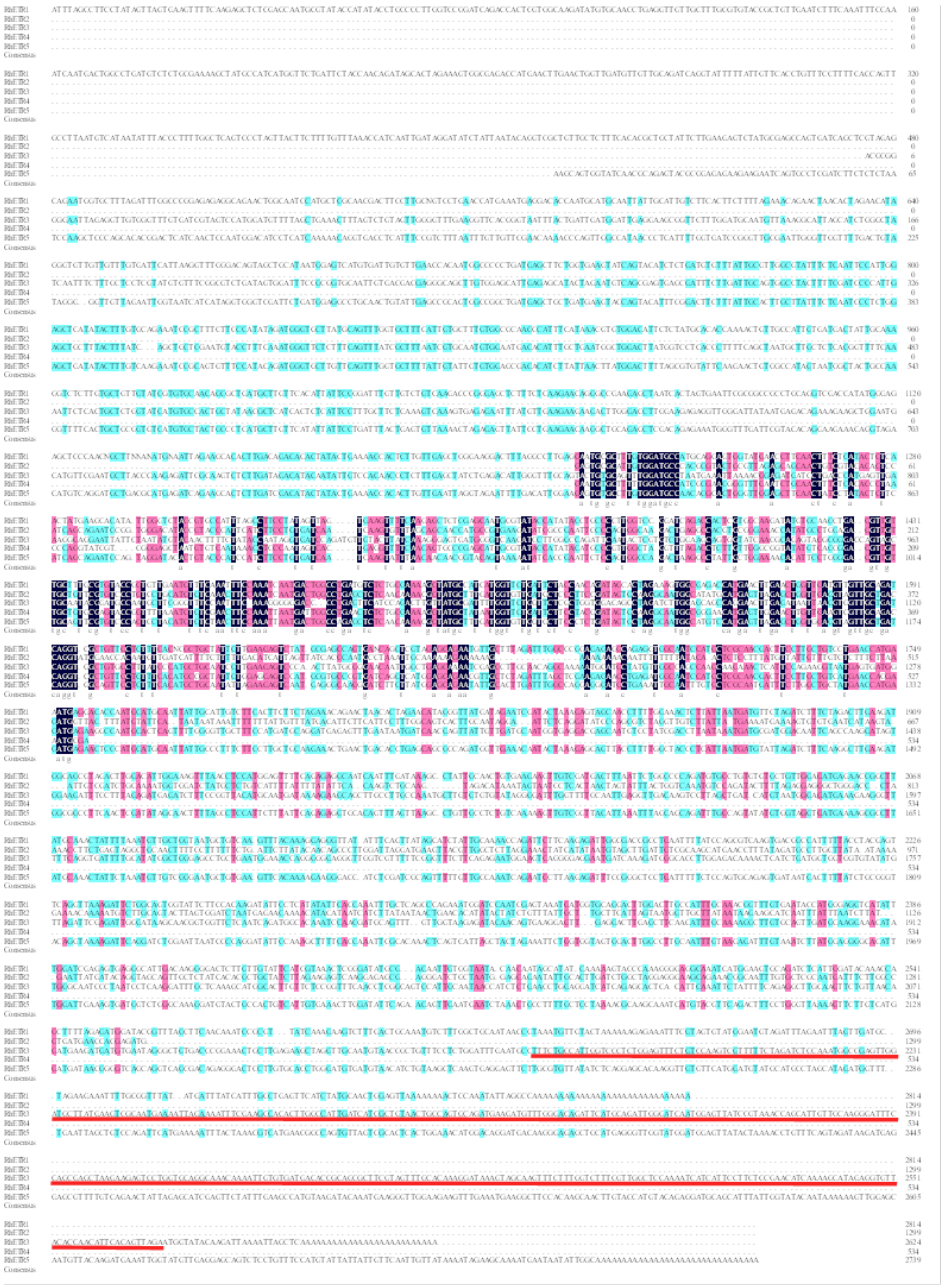

Figure S8. Alignment of cDNA sequence with *RhETR1-5* genes and the specific fragments used in VIGS. Underlined in red indicates 3’ specific fragment of *RhETR3* gene used in VIGS.

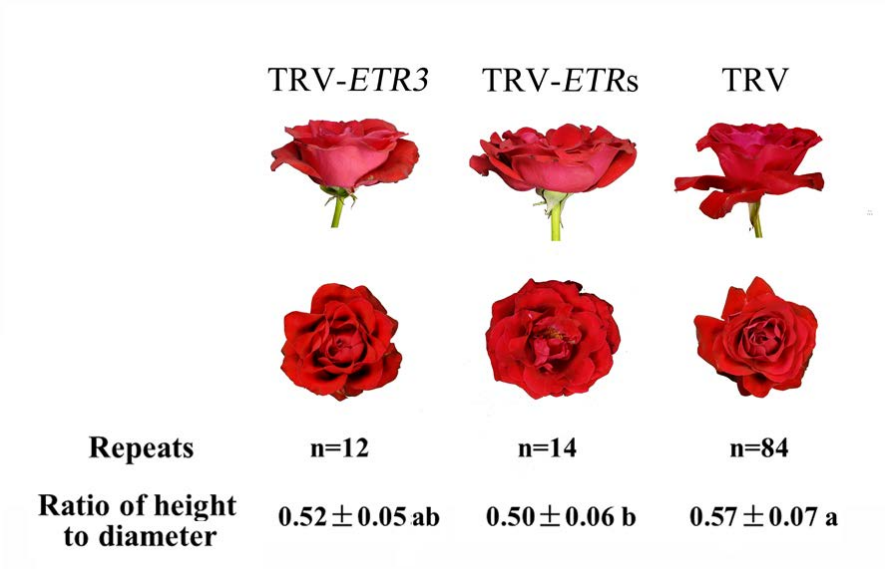

Figure S9. The phenotype of *RhETR3*- or *RhETRs*-silencing flowers (upper) and the ratio of height to flower diameter (lower). The photographs were taken on the second day after 24 h dehydration. n, replicates of rose flowers.

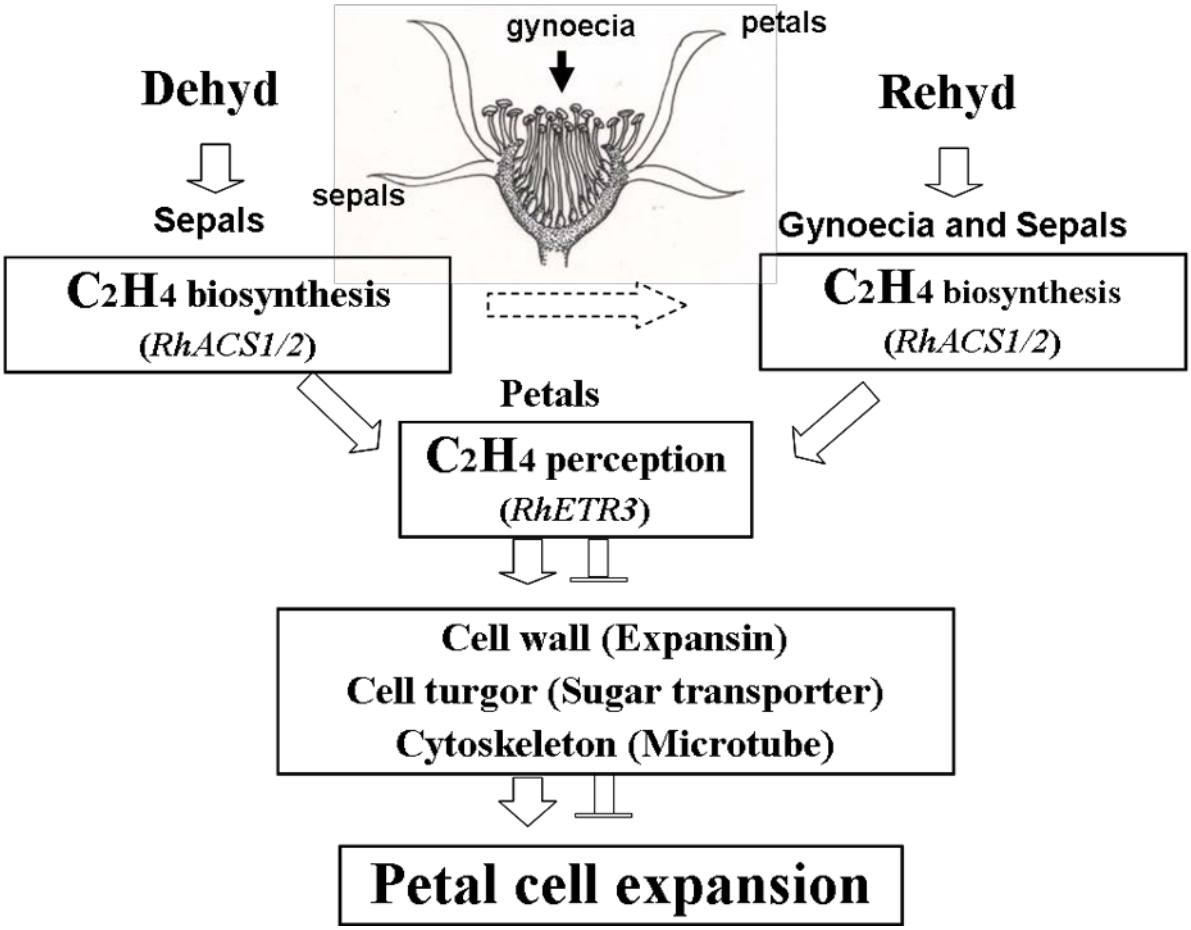

Figure S10. Proposed model of dehydration-affected flower opening mediated by ethylene in rose flowers. Solid arrows indicate positive regulation. Tee arrows indicate negative regulation. Dashed arrows indicate uncertain processes.

Supplementary Tables

Table S1. Primer sequences of various genes in RT-PCR analysis

Gene	Forward primer	Reverse primer
RhACS1	5’ - GCGTTACAGAGGTCCTACAAG-3’	5’ -ACAAACCCGGAACCAGCCTGG-3’
RhACS2	5’- CCTGCCAGAGTTCAGAAATGCTGC-3’	5’-GCAATGCTGATGAACCTTGGCTGAG-3’
RhACS3	5’ - GCCTTGGCTTTCCTCCCTTC - 3’	5’-ACCCAACTCGTCGTACGGAT-3’
RhACS4	5’-TAATATGTCCGAGGATACTC- 3’	5’-AATGTTGTCTAAGAATGTGC-3’
RhACS5	5’- GAGTCCATTCTTGGACACACCAAC-3’	5’- CTAATCAAGCGGTCGAAAATTGC-3’
RhETR1	5’-TGTGCCATTTAGCCTTCCTGTA-3’	5’-CCTGATCTGCAACAACATCAAC-3’
RhETR2	5’ - CGCTATGCTTTGATGGTGTT-3’	5’ -GCAGCCTACTCAGAAGGTTTT-3’
RhETR3	5’ - GCTCATCACTCTCATTCCTTTGC - 3’	5’- GCATTGGCATCCGTATTGCAGC- 3’
RhETR4	5’ - ACACCCTAACCCAGGTATCGTCG-3’	5’ - ATGGATTGCCATCTCAGCTTCTC- 3’
RhETR5	*5*’ - ATGGCTACTGCCAAGGTTTTCA-3’	5’ - CAGGAATGTGTTTTCCAGCAAT-3’
RhUbi1	5’ - CACCCTCGCCGACTACAA-3’	5’ - AGTGATGGTCTGCCGGTTAAG-3’

Table S2. Primer sequences of various genes for construction of VIGS vectors.

Gene	Forward primer	Reverse primer
RhACS1 (VIGS)	5’-GCTCTAGAAGTATAACTCATCCACTCAAC-3’	5’-GCGAGCTCGCAAAGATATTCAGATCACTA-3’
RhACS2 (VIGS)	5’-GCTCTAGAAAGAAGAGCTGCTGGCAAAG-3’	5’-GCGAGCTCCAGGTGAGAAACAATTGCATG-3’
RhETR3 (VIGS)	5’-GCTCTAGAAGTATAACTCATCCACTCAAC-3’	5’-GCGAGCTCGCAAAGATATTCAGATCACTA-3’
RhETRs (VIGS)	5’-CGTCTAGACAGAAGCACTCTTGATCGACAT-3’	5’-TACCCGGGTTTGACAGAGGCAACAGGC-3’

Table S3. Primer sequences of *RhACSs* and *RhETRs* genes in RT-PCR analysis for gene silencing

Gene	Forward primer	Reverse primer
RhACS1 (cmjc)	5'-CGACTTGTGACACGGACGCATA-3'	5'-CAGAAGGAAGGCAACGCACCT- 3'
RhACS2 (cmjc)	5'- AAACTCAGCATCTAATCGCATC-3'	5' - GCAACTTCCATAGTCTGGTCGT-3'
RhETR1 (cmjc)	5' -AAGAGTCTATGCGAGCCAGTG-3'	5' - TCCTGCACCATCATTTACTCG- 3'
RhETR3 (cmjc)	5' -TGCATATGGTTGGGAGCCTG-3'	5' - AGCCCTATTCACATCATCTTCGTC-3'
RhETR5 (cmjc)	5' -GATGGTCTGGGCAAAGGAT-3'	5' - AGACACAACTCGGGTGAGATAG-3'

Table S4. The proportion of each floral tissue contributed for increased ethylene production

	Sepals	Petals	Androecia	Gynoecia	Receptacles	Whole flower
Dehyd 18 h	44.3%	43.7%	9.4%	0.0%	2.6%	100.0%
Dehyd 24 h	65.4%	26.6%	7.2%	0.0%	0.7%	100.0%
Rehyd 1 h	9.2%	5.5%	3.5%	79.5%	2.3%	100.0%
Rehyd 3 h	36.4%	34.4%	5.6%	19.4%	4.2%	100.0%

Percentage of ethylene production in each floral parts. Dehyd, dehydration; Rehyd, rehydration. Each value represents means of 30 replicates.