

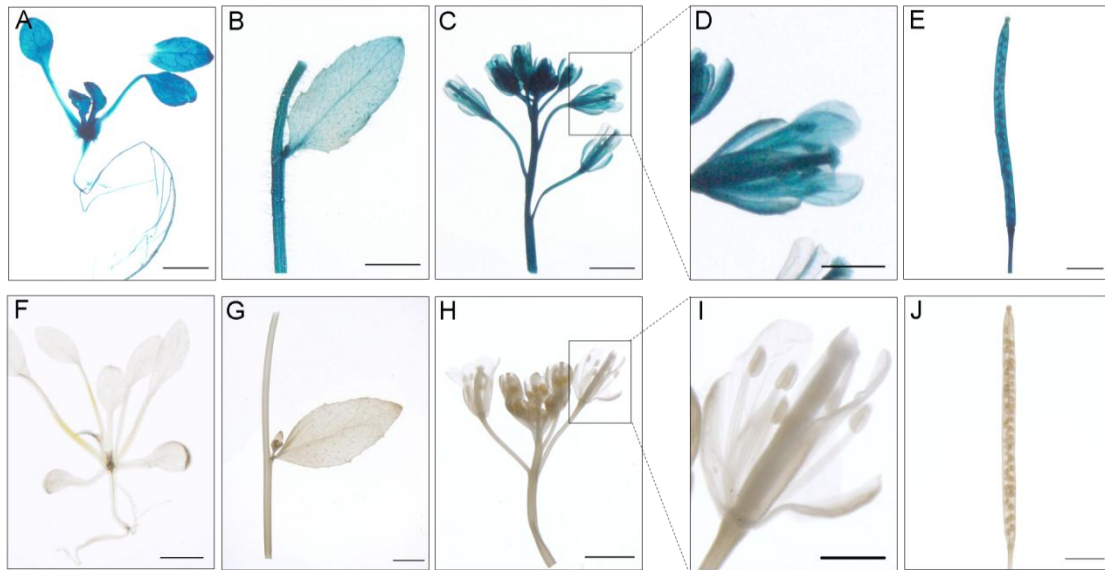
Supplementary data



Supplementary Fig. S1. Opening flower morphology of the parent plants 181A and H5 under relatively low temperature. A, the whole flowers of sterile parent 181A; B, the sterile flower with petals and sepals peeled off; C, the whole flowers of fertile parent H5; D, the fertile flower with petals and sepals peeled off. The petals of sterile parent are shrunk (A) and the stamens of sterile parent are shorter than pistil (B).

1 atgttggtgctcggagattgggtgcttctgctctcgtatccccctgagtcctggtgactcttgcaactccttctctcttttcatcagatcc
M L C R R L V L V S R I P L S P V G T L A T P L L S F I R S
91 tcatgcaaacgaggctactctggtctcggcagcgatagaaatctctcatcttacaagagagactgagaagtggctggtcgatatacaag
S C E R G Y S G L G S D R N L S S Y K E R L R S G L V D I K
181 aagaaggatgctgtagctctggttccagtccatgattagggtctcctcttccacggtcattggatttcaataaactggttagtgagta
K K D A V A L F Q S M I R S R P L P T V M D F N K L F S A V
271 gccagaacgaaacagatgatctcgtggtggtctctgcaagcaaatggaactgcaagggtgacacatagcatttacacgctgagatt
A R T K Q Y D L V L D L C K Q M E L Q G I A H S I Y T L S I
361 atgatcaattgcttctgcccctctgagggaactcggtttctggttttctggtgagggtaagatggtgaggcttgggtgatgagcctgacaca
M I N C F C R L R E L G F A F S V M G K M L R L G Y E P D T
451 atcacattotcaacttggatcaacggttttagtctggtggtgtagagtttccgaaagctggtgagtttagttgatcgtatggtggaaatggag
I T F S T L I N G L C L V G R V S E A V E L V D R M V E M E
541 gttatacacaatctcatcagctcaacactattgtcaatggacttctctccaagtggaagtgtctgaggcaatggctttgatcagatcga
V I P N L I T L N T I V N G L C L Q G E V S E A M A L I D R
631 atgattggataatggatgccaaaccaatgaaactgatctatggtccggtttgaacagaatggtcaagtcaggtaaacactgcctggccttg
M M D N G C Q P N E R T Y G P V L N R M C K S G N T A L A L
721 gatctgctcagaagatggaacacagaagatcaagctcagatgacacatacaatctcatcattgacagctctttgcaaatgaggggagc
D L L R K M E H R K I K L D A V T Y N F I I D S L C K D G S
811 ctgcaagatgactcagccttttcaatgaaatggaaaccaaaggatcaaaaacaaatgtctttacctacaactctctcattagaggcttc
L E D A L S L F N E M E T K G I K P N V F T Y N S L I R G F
901 ttagtgctcagaagatgggatgattggtgcacagctgctgagggatatgatcacaaggggaatcacccccacccgtcatcactttcaattct
C S A G R W D D G A P L L R D M I T R G I T P T V I T F N S
991 ttgattgatgttttggaaagtggaaagcttactgaggctcaagatttgtacaacgagatgatcacaagggcacaatctctgatatc
L I D S F V K V G K L T E A Q D L Y N E M I T R G T Y P D I
1081 attacataaactctatgataaatggctgtgcaatgaaaaacgcttagatgagccaaccagatgctggatctgatggttagcaaggaa
I T Y N S M I N G L C N E K R L D E A N Q M L D L M V S K E
1171 tgcgctcctgatctcgtgacttataatacccttataaatggatactgtaaggctaaacgagttgatgaaggatgagacatttccgcaaa
C D P N I V T Y N T L I N G Y C K A K R V D E G M R H F R K
1261 atgtctgtcaaggagtggtgccaatacagctcattataacactctccaaggggtttgtcaatcaggaaaacttaattggtgcacaa
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1351 gaactcttcaggagatggtctcctcaagggttcatcctgatattataacctacaaaatttctggtgagattgtgtgacaatggaaga
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1441 gtagaaggaggttgggaataacttgatcaaatgcacaagagtaacatggaacttgatattggtttatataatcatcattcacgggatg
V E E A L G I L D Q M H K S N M E L D I G L Y N I I I H G M
1531 tgcaatgcaataaggtcagatgcttggagttgttctgtagcctacggtcgaaggagtgaaaccagacgctcaagacatatactaca
C N A N K V D D A W S L F C S L R S K G V K P D V K T Y T T
1621 atgattggaggttgtgtaagaaggatcgctgtctggaagcggcagatttataagaagatggaagaggtgggttggcacaatgat
M I G G L C K K G S L S E A G M L C K K M E E D G I A P N D
1711 tgtacatacaaacactcttatcaggggacatctccgagatggtgacttaacaaaatcagcaaaacttcaagaagaatgaagaggtgtggg
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1801 ttctctgcagatgcttcaaccataaagattggtatggtatcggatggtagaatgaagaaaagctttctggatgctttcttag
F S A D A S T I K I V M D M L S D G R M K K S F L D M L S *

Supplementary Fig. S3. Nucleotide sequence of recessive *rfn* allele from 181A and the deduced amino acids. The mutations are indicated by red.



Supplementary Fig. S5. GUS assay from different tissues of *Pro_{Rfm}*: GUS introduced *Arabidopsis* plants. A, seedling after two weeks screened by hygromycin; B-F, staining of the stem and leaf (B), inflorescence (C), flower (D) and silique (E) from reproductive stage. F-J, staining of the corresponding tissues from wild-type plants. Scale bars: A-C, E-H, J, 2 mm; D, I, 500 μ m.

Supplementary Table S1. The primers used in this study. Underlines indicate the restriction sites.

Prime name	Primer sequence (5'-3')	Purpose
BnSR02-F	CTCTTCTACGGTCAGTGGCA	Fine mapping
BnSR02-R	GAGATTGAGGATTGGGGTTG	
BnSR28-F	TTTGAAAGAGCGGTTATGA	Fine mapping
BnSR28-R	ACATGCTCGATCGTGCTTAT	
BnSR33-F	GCTTGGTATCGTTCTCCGTC	Fine mapping
BnSR33-R	TCTGGCTTGTTACTCAAGCG	
BrSC64-F	CTGCTGCGAGTTACTTGAAGC	Fine mapping and BAC
BrSC64-R	AGGATTCTGCTTTTGTGGTTTC	screening
BrSC65-F	ATAGCAGAAGCAGCATTGAAAG	Fine mapping and BAC
BrSC65-R	GCCTGGAGTGAVAAGAAGACG	screening
BrIP77-F	TGACGTGAGGGCGATTCTTG	Fine mapping
BrIP77-R	TAATGGACCAGGCGGTGATG	
BrIP81-F	TCGCTCCACCAACAATAAAC	Fine mapping
BrIP81-R	TCAGTCCATGAGATCAAGACG	
ORF2-F	CG <u>gaattc</u> GTCCAACCACAATGGCTTTAAC	Binary construct of <i>Rfn</i>
ORF2-R	CG <u>ggatcc</u> ATTGCTAAGGCGAATCCGGTTG	
Rfn-F	GTTCTTGCTGTAAAGCGTTGT	Transgenic plants
Rfn-R	GTTGCGTTTTTCATTGCTTGTC	detection
RfnRT-F	CTTGCAGAGATCCAACACGAGAT	qRT-PCR of <i>Rfn</i> or <i>rfn</i>
RfnRT-R	CAAAACCGAGTTTCTGGAGGC	
Pro-F	CC <u>taattaa</u> GTCCAACCACAATGGCTTTAAC	GUS-fusion
Pro-R	<u>Aggcgccc</u> TGTTTGTTTTTTTTTTCTTCAAATCTAC	
Rfn-Xba-F	GC <u>tctaga</u> ATGTTGTGTCGGAGATTGGT	GFP-fusion
Rfn-Xba-R	GC <u>tctaga</u> ATTTCTATCGCTGCCGAGAC	
insitu-F	ATGTAAGAAAGGCTCGCTGC	RNA <i>in situ</i>
insitu-R	TAAGTCACCACCTCTGAGATGAG	hybridization

orf222-F	TGGTGGAAAAGATCGTACAAGT	RNA gel blot
orf222-R	AAGACCCGGAAGTGGTGATC	
orf139-F	CGATCCACAAACTGACGCAT	RNA gel blot
orf139-R	TTGTGCCGAGTCAAATCTGC	
BnActin-F	CCCTGGAATTGCTGACCGTA	qRT-PCR (control)
BnActin-R	TGGAAAGTGCTGAGGGATGC	

Supplementary Table S2. Fertility of T₁ progeny derived from five independent T₀ transgenic plants by selfing and test-cross.

No.	Source	Total plants	Fertile plants	Sterile plants	Expected ratio	χ^2 value ^a
T ₁ -04	selfing	61	47	14	3:1	0.05
T ₁ -20	selfing	96	70	26	3:1	0.13
T ₁ -21	selfing	132	125	7	15:1	0.07
T ₁ -29	selfing	122	111	11	15:1	1.16
T ₁ -46	selfing	99	91	8	15:1	0.30
T ₁ -04	test-cross	65	32	33	1:1	0
T ₁ -20	test-cross	88	51	37	1:1	1.92
T ₁ -21	test-cross	82	55	27	3:1	2.34
T ₁ -29	test-cross	78	60	18	3:1	0.07
T ₁ -46	test-cross	84	62	22	3:1	0.02

^a χ^2 (0.05, 1) = 3.84