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Cloning and characterization of a flowering time gene from *Thellungiella halophila*

Qiaoyun Fang[1,2], Jun Liu[2], Zhengkai Xu[2,3], and Rentao Song[2,3]*

[1] The Key Laboratory of Gene Resource Utilization for Severe Diseases, Ministry of Education, Anhui Medical University, Hefei 230032, China
[2] School of Life Sciences, Shanghai University, Shanghai 200444, China
[3] Shanghai Key Laboratory of Bio-energy Crop, Shanghai University, Shanghai 200444, China

***Thellungiella halophila* (*T. halophila*) (salt cress) is a close relative of *Arabidopsis* and a model plant for salt tolerance research. However, the nature of its later flowering causes some difficulties in genetic analysis. The *FRIGIDA* (*FRI*) gene plays a key role in the *Arabidopsis* vernalization flowering pathway, whose homolog in *T. halophila* may also be a key factor in controlling flowering time. In order to study the molecular mechanism of vernalization responses in *T. halophila*, a full length cDNA named *ThFRI* (*Thellungiella halophila FRIGIDA*) was isolated from the young seedlings of *T. halophila* by RT-PCR and RACE. The *ThFRI* cDNA was 2017 bp in length and contained an open reading frame encoding a putative protein of 605 amino acids. The *ThFRI* showed significant homology to *AtFRI* (74.5% at the nucleotide level and 63.9% at the amino acid level). To study its function, *ThFRI* cDNA was transformed into *Arabidopsis thaliana*, driven by CaMV 35S promoter. Transgenic plants expressing *ThFRI* exhibited late-flowering phenotype, which suggests that *ThFRI* is the funtional *FRI* homolog in *T. halophila*. The cloning and funtional characterization of the *FRI* homolog of *T. halophila* will faciliate further study of flowering time control in *T. halophila*.**

Keywords *Thellungiella halophila*; vernalization; *FRIGIDA*; *ThFRI*; *Arabidopsis thaliana*

In plant development, the transition from vegetative to reproductive phase is critical for seasonal changes. The timing of reproductive transition is determined by developmental status and environmental conditions. This combination promotes flowering at the appropriate time by coupling the accumulation of sufficient nutrients to favorable environmental conditions [1–4]. Genetic research has revealed that multiple pathways have evolved to regulate flowering time in many plant species. These pathways monitor both a plant's developmental state and environmental cues, such as photoperiod and temperature.

Previous studies on genes controlling flowering time have been conducted predominantly in *Arabidopsis*. The *FRI*, *FLC*, *CO*, *FT* and *FCA* have been isolated by generating mutants with altered flowering times [1,5,6]. *FRI* and *FLC* were found to be the two key loci determining flowering time in *Arabidopsis*; they act synergistically to cause late flowering [7–11]. The *FRI* gene encodes a novel protein with two predicted coiled-coil domains [12,13]. Functional *FRI* alleles accelerate *FLC* messenger RNA accumulation, which in turn inhibits flowering [9], unless down-regulated by vernalization. The *FRI* alleles are thought to promote early flowering in the absence of vernalization [8]. Thus, *Arabidopsis* mutants with non-functional or weak *FRI* alleles have been widely used as research materials because of their early flowering [14]. For example, Columbia (Col) carries a dominant *FLC* but a recessive *FRI* allele, and *Landsberg erecta* possesses a weak *FLC* and a recessive *FRI*.

T. halphila (salt cress), a typical halophyte, is an extremophile that is native to harsh environments [15]. It can grow in a medium containing 500 mM NaCl and can survive at −15 ºC. *T. halophila* has many features that make it a useful model system, such as its relatively small genome (twice the size of *Arabidopsis*), small size, copious seed production, self-pollination and genetic transformation by the floral dip procedure. Therefore, *T. halophila* has also been used as a research model to study plant salinity

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*corresponding author: Tel, 86-21-66133225; Fax, 86-21-66135163; E-mail, rentaosong@staff.shu.edu.cn

tolerance [16,17]. However, as a late flowering plant, *T. halophila* has a major drawback as a genetic model because of the prolonged period of vernalization treatment to induce early flowering. Given that *T. halophila* is closely related to *Arabidopsis* and that most of their genes are similar (>90% similarity in cDNA sequences) [16,17], it is possible to clone and characterize genes in the vernalization pathway of *T. halophila* based on genetic information from *Arabidopsis* [18].

In this study, the cDNA of the *FRI* homolog from *T. halophila* was cloned and functionally characterized in *Arabidopsis*. We showed that *ThFRI* is highly homologous to *FRI* and that heterologous expression of *ThFRI* in *Arabidopsis* (Col) could restore the late-flowering phenotype. These data suggest that *ThFRI* is the functional homolog of *FRI* in *T. halophila*. The cloning of *ThFRI* will facilitate future studies of flowering time control in *T. halophila* and the genetic engineering involved in early flowering *T. halophila* for plant salt tolerance.

Materials and Methods

Plant material and treatment

Arabidopsis thaliana (Col) and *T. halophila* (stock number: CS22504) were cultivated in a growth chamber at 23 ºC under a 16 h light and 8 h dark cycle. For aseptic growth, seeds were surface-sterilized and plated on a growth medium of 1/2 Murashige Skoog (MS), 0.3% sucrose, 0.9% agar with pH 5.8, and stratified for 3 d at 4 ºC in the dark before germination in the growth chamber. Five independent transgenic lines were grown under the same conditions and used for flowering time measurement.

Total RNA extraction and cDNA synthesis

Total RNA extraction and cDNA synthesis were performed [18]. Total RNA was extracted from the seedlings of plants using the Trizol reagent (Tiangen, Shanghai, China) according to *Arabidopsis* laboratory manual. Genomic DNA contamination was removed by RNase-free DNase I (Invitrogen, Carlsbad, USA) treatment at 37 ºC for 30 min. First-strand cDNAs were synthesized from 4.0 μg of total RNA with the 3′-RACE kit (Invitrogen) according to the manufacturer's instruction.

Cloning full-length cDNA of *ThFRI*

Based on the high sequence similarity between *Arabidopsis* and *T. halophila* cDNA [16,17], *ThFRI*/P1 and *ThFRI*/P2 primers were designed according to *Arabidopsis FRI* (**Table 1**). The cDNA fragment from *T. halophila* was amplified by RT-PCR, and cloned into pSK-T vector (GENE-Tech, Shanghai, China) for sequencing analysis.

To obtain the 5′ missing portion of the cDNA, two specific primers, *ThFRI*/P4 and *ThFRI*/P5, were designed based on sequence information acquired from the partial cDNA fragment. The 5′-RACE was performed using the FirstChoice™ RLM-RACE kit (Ambion Inc., Austin, USA) according to the manufacturer's instructions.

Table 1 Oligonucleotide primers used in the study

Primer name	Primer sequence (5′ to 3′)
ThFRI/P1	GTGTAGCAAAGGTCTGCGTAA
ThFRI/P2	CTGAGACCATAGGGACAAGCC
3′ adaptor primer	CTGATCTAGAGGTACCGGATCC-dT$_{(20)}$
ThFRI/P3	GATTGCTGGTGCTCTGAAACGGTCA
5′-RACE adapter	GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGC UUUGAUGAAA
Outer primer	GCTGATGGCGATGAATGAACACTG
Inner primer	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG
ThFRI/P4	CTCGCAGGGATCATATGCGAATCAT
ThFRI/P5	CACGAACTTCGCTGGCTCCTTGGCTA
ThFRI/P8	CATG*CCATGG*ATGATCCCTGCCCGTGGCTA
ThFRI/P9	CTAG*TCTAGA*TTACATTAATCCTATGTGGT
ThFRI/P11	CCGGAGATGATAACGTCGAATG
ThFRI/P12	CAGAGCACCAGCAATCTCAGAA
UBQ10/P1	GGTGTCAGAACTCTCCACCTCAAGAG
UBQ10/P2	TCAATTCTCTCTACCGTGATCAAGATC

Random-primed RT and nested PCR reactions were performed to amplify the 5′-end of the *ThFRI* cDNA (**Fig. 1**). The 3′-RACE was performed by the anchored primer and internal gene specific primer to obtain the 3′ missing portion of *ThFRI* cDNA (**Table 1**). PCR products were purified from a 1% agarose gel and cloned into pSK-T vector for further sequence identification. Full length *ThFRI* cDNA was then amplified by gene specific primers (**Table 1**) with nested PCR.

Three independent clones were sequenced to minimize errors introduced during cloning. The full-length *ThFRI* sequence was submitted to GenBank (accession No. DQ089808).

Sequence analyses of *ThFRI*

A homology search was performed with the BLAST program (http://www.ncbi.nlm.nih.gov/blast.cgi). Alignment analysis was performed with VECTOR NTI 8.0 software (http://www.informaxinc.com). The deduced protein sequences were analyzed using the ExPASy Proteomics Server (http://us.expasy.org).

Construction of heterologous expression vector for heterologous expression in *Arabidopsis*, the ORF of *ThFRI* cDNA was amplified by *ThFRI*/P8 and *ThFRI*/P9 (**Table 1**). *Nco*I and *Xba*I sites were introduced at their respective 5′-ends. PCR product was digested with *Nco*I and *Xba*I, and cloned into corresponding sites of the vector pAVA321 [19]. The vector contained dual 35S promoter from CaMV, a translational enhancer sequence of tobacco etch virus and a 35S transcriptional terminator from CaMV. The expression cassette from the resulting construct was released by *Bam*HI and *Kpn*I and sub-cloned into corresponding sites of pPZP211 [20], making the heterologous expression construct pPZP211-*ThFRI* (**Fig. 2**).

Plant transformation

Expression constructs pPZP211-*ThFRI* and pPZP211, the latter as a negative control, were transformed into *Agrobacterium tumefaciens* GV3101 via electroporation. *Agrobacterium* containing these constructs were used to transform *Arabidopsis thaliana* by floral dip method [21]. *Arabidopsis* transformants were selected on agar plates containing 1/2 MS medium and 50 μg/ml kanamycin.

Semi-quantitative RT-PCR analyses

Total RNA was extracted from *Arabidopsis* plants and treated with DNase I (RNase-free) to remove genomic DNA contamination [18]. First-strand cDNA was synthesized by Superscript II (Invitrogen). RT-PCR was performed on first-strand cDNA using gene-specific primer sets (**Table 1**). To normalize the RT mixtures, *Arabidopsis UBQ10* was used as internal control. The following conditions were used for PCR: 40 s at 94 ºC, 40 s at 56 ºC, and 1 min at 72 ºC for 30 cycles. For each primer set,

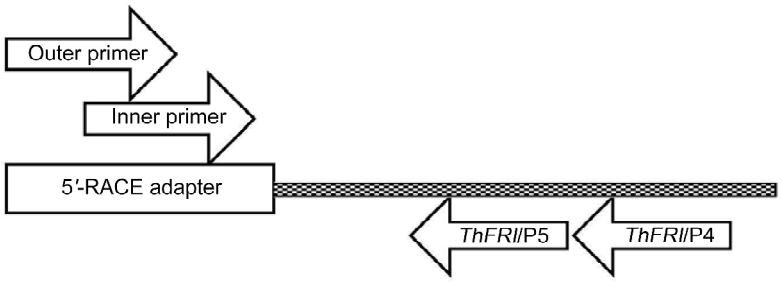

Fig. 1 Schematic display of two gene specific primers, *Thellungiella halophila FRIGIDA* (*ThFRI*)/P4 and *ThFRI*/P5, and two corresponding universal primers for 5′-RACE.

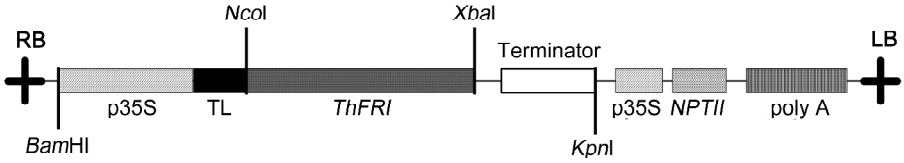

Fig. 2 Schematic diagram of transformation vector pPZP211-*ThFRI* The *ThFRI* complementary DNA was inserted in sense orientation between *Nco*I and *Xba*I sites. p35S, cauliflower mosaic virus 35S promoter; LB, left border; *NPTII*, neophosphotransferase gene; RB, right border; terminator, the 35S transcriptional terminator from CaMV; TL, the translational enhancer sequence of tobacco etch virus.

three independent biological repeats were performed.

Results

Isolation of full-length *FRI* cDNA from *T. halophila*

It has been shown that the flowering behavior of *T. halophila* mimics the winter annual *Arabidopsis* in which *AtFRI* acts as the major flowering inhibitor [22]. This suggests a *FRI* homolog could exist in *T. halophila*. Because most genes in *T. halophila* have an approximately 90% sequence similarity to *Arabidopsis* counterparts at the cDNA sequence level [16,17], specific primers were designed based on *Arabidopsis AtFRI* cDNA (GenBank accession No. AF228499) to obtain a partial cDNA fragment from *T. halophila* by RT-PCR. Sequence analysis of this partial fragment revealed a sequence with 80% similarity to *AtFRI*, suggesting it could be the *FRI* homolog in *T. halophila*. The 5′-RACE and 3′-RACE were carried out to obtain the 5′ and 3′ missing portions of the cDNA, respectively. Subsequently, full-length cDNA was obtained and designated *ThFRI* (GenBank accession No. DQ089808).

Sequence analysis of *ThFRI* cDNA

The full-length sequence of *ThFRI* cDNA is 2017 bp and contains a 1818 bp ORF that encodes a protein of 605 amino acids. The full-length nucleotide sequence and the deduced amino acid sequence are shown in **Fig. 3(A)**. The cDNA contains a 46 nucleotides 5′ untranslated region (UTR) and a longer 3′ UTR of 153 nucleotides, including the polyA-tail. The deduced protein has a molecular weight of 67.86 kDa and a theoretical isoelectric point of 7.42.

ThFRI's 605 amino acids were found to be significantly homologous only to *AtFRI* in the GenBank database, with 74.5% identity at nucleotide level and 63.9% identity at amino acid level **[Fig. 3(B)]**. However, a comparison between *ThFRI* and *AtFRI* protein sequences revealed some differences; for example, the region between amino acids 108 and 157 showed much lower identity (24.1%), and 14 amino acids were deleted in *ThFRI* at amino acid 548 [**Fig. 3(B)**]. In addition, extra amino acids were found in *ThFRI* at the N-terminal and C-terminal (seven and three amino acids respectively). Overall, significant sequence homology was found across the entire gene, suggesting that the cloned *ThFRI* was an ortholog of *AtFRI* in *T. halophila*.

Heterologous expression of *ThFRI* in *Arabidopsis* restored late-flowering phenotype

Arabidopsis thaliana (ecotype Col) flowers early due to a recessive *FRI*-Col allele. If a functional *FRI* allele were introduced into *Arabidopsis* (Col), *FRI* protein would accelerate the *FLC* messenger RNA accumulation, which, in turn, would inhibit flowering and cause late-flowering phenotype [9]. To assess its biological function and determine whether *ThFRI* is functional, a complementary test was carried out in the *Arabidopsis* (Col) with recessive *FRI*-Col. We constructed pPZP211-*ThFRI*, the heterologous expression construct of *ThFRI* (see "Materials and Methods") (**Fig. 2**).

Expression constructs were transformed into *FRI*-Col *Arabidopsis* by the floral dip method [21]. Transgenic lines were selected by kanamycin resistance and detected by PCR with specific primers according to recombinant plasmid (data not shown). Five independent transgenic lines (designated OV1-OV5) were selected for further analysis (**Fig. 4**). The expression of *ThFRI* in transgenic *Arabidopsis* was verified by RT-PCR using gene-specific primers *ThFRI*/P11 and *ThFRI*/P12 (**Table 1**). All positive transgenic lines had *ThFRI* transcripts except the OV4 line, which was proved to be a false-positive transgenic line; no such transcript was detected in negative control plants that transformed with empty pPZP211 [**Fig. 4(E)**]. Late-flowering phenotype analysis was carried out with those confirmed transgenic lines. They all displayed well-characterized late-flowering phenotype: significantly more rosette leaves were found before flowering [**Fig. 4(A,B)**]. The first 10 rosette leaves formed on transgenic lines, as represented by OV1, were apparently larger than those of control plants [**Fig. 4(C)**]. Within the same transgenic line, there was no significant difference in *ThFRI* expression level or flowering time among individual plants [**Fig. 4(D, E)**]. These results indicate that heterologously expressed *ThFRI* could functionally complement the *FRI*-Col in *Arabidopsis* and cause late-flowering phenotype in transgenic *Arabidopsis*. Therefore, we demonstrated that *ThFRI* is a functional *FRI* homolog in *T. halophila*.

Discussion

AtFRI has been shown to be a key regulator in the flowering time pathway of *Arabidopsis*. In this study, *ThFRI* was isolated from *T. halophila*, and characterized in *Arabidopsis* (Col) with recessive *FRI*-Col allele. By RT-PCR with primers designed on *AtFRI* and the RACE method (**Fig. 1**), the full-length cDNA *ThFRI* was cloned from *T. halophila* (**Fig. 3**). Sequence analysis indicated that *ThFRI* and *AtFRI* shared significantly high similarity. Heterologous expression of *ThFRI* in *Arabidopsis* with *FRI*-Col resulted with late-flowering phenotype (**Fig. 4**), which

(A)

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aaatgatggcgatgaatgaacactgcgtttgctggatgattcgcatatgatccctgcccgtggctactcccatcattatccatccacgacggaggagaaaccgtcgtcgccggcgacgatt  121
                                                  M  I  P  A  R  G  Y  S  H  H  Y  P  S  T  T  E  E  K  P  S  S  P  A  T  I   25
ccgcggctacaccagcgagatcaatctgaacgacggggagacttcccggcgattaacagaaccgaacctaccaataaggaaatcacctccggggactctaaacatccgcaattcatgaaa  241
 P  R  L  H  Q  R  D  Q  S  E  R  R  G  D  F  P  A  I  N  R  T  E  P  T  N  K  E  I  T  S  G  D  S  K  H  P  Q  F  M  K   65
tcaatcgacgatttagctaagttctcagctgcatttgacgcctttaaacgccactacgacgacttgcagaagcacatggatgacatcgagaacgccattgaatccaagttcaagagcaac  361
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 G  V  D  D  S  S  S  H  S  P  E  H  D  A  S  R  E  I  A  T  A  I  V  C  P  P  P  P  E  E  A  E  T  A  P  E  M  I  T  S  145
aatgataaggcggaggggcaacgattgtgcgagtcgatgtgcagcaaaggcctgcgcaaatacatatacgcgaatatctccgagcgagctaagttaatggaggagattcctgcaggtttg  601
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 K  L  A  K  E  P  A  K  F  V  L  E  C  I  G  K  F  Y  L  Q  G  R  K  A  F  S  H  D  S  H  M  I  P  A  R  Q  V  S  L  L  225
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 I  L  E  C  F  L  L  M  I  E  P  G  E  E  K  V  K  S  M  I  E  S  S  V  K  E  E  A  E  A  A  A  F  A  W  K  R  R  I  M  265
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 S  G  T  S  E  I  A  G  A  L  K  R  S  P  F  L  V  P  I  V  S  G  I  V  D  S  C  L  K  R  G  T  N  I  E  A  L  E  I  V  345
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tactctccggttcatggacagagactgcctcgagagtactctcctccagttcatgggcaacaacagataccatatggtctacaaagggtttacaggcattcaccatctgtagaaagatta 1801
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ttgactttgcctcatcacaggtctcctcgtaactcatcacaagaccacataggaggaatgtaaatatgtaacacaaaggttttgtttttagagcagtcaaaaaattatttaaagatcaa 1921
 L  T  L  P  H  H  R  S  P  R  N  S  S  Q  D  H  I  G  G  M  *                                                            605
ggcttaataagcaaaaaaaattctactctttgtaatttgacatttcattcagatgctaatgttattgcttctcaggttaaaaaaaaaaaaaaaaaa                          2017
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(B)

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ThFRI protein  MIPARGYSHHYPSTTEEKPSSPATIPRLHQRDQSE-RRGDFPAINRTEPTNKEITSGDSK   59
AtFRI protein          MSNYPPTVAAQPTT--TANPLLQRFHQSFQRRRELPKIVETESTSMDTTTGQSK   51

ThFRI protein  HPQFMKSIDELAKFSAAFDAFKRHYDDLQKHMDDIENAIESKFKSNGVDDSSS-----HSP  115
AtFRI protein  QPQFLKSIDELAAFSVAVETFKRQFDDLQKHLESIENAIESKLESNGVVLAARNNNFHQP  111

ThFRI protein  EIIDASR----EIATATVCPPPPEEADTTAPEMITSNDKAEGQRLCESMCSKGLRKYIYANIS  172
AtFRI protein  MLSPPRNNVSVETTVTVSQPSQ  EIVPETSN  KPEGGRMCELMCSKGLRKYIYANIS  166

ThFRI protein  ERAKLMEEIPAGLKLAKEPAKFVLECIGKFYLQGRKAFSHDSEMLPARQVSLLILESFLL  232
AtFRI protein  DQAKLMEEIPSALKLAKEPAKFVLDCIGKFYLQGRKAFTKESPMSSARQVSLLILESFLL  226

ThFRI protein  MLEPGEEKVKSMIESSVKLEAEAAAEAWKRRQMNEGKLATAEAADARGLLLLVACFGVPS  292
AtFRI protein  MPDRG--KGKVKTESWTKDEAEAAAVAWKRRQMTEGGLAAAEKVDARGLLLLVACFGVPS  284

ThFRI protein  SFRSMDLLDLIRQSGTSFTAGALKRSPFLVPVVSGIVDSCIKRGTNIEALEIVFTFGMED  352
AtFRI protein  NFRSTDLLDLIRMSGSNFTAGALKRSQFLVPVVSGIVESSIKRGMHIEALEIVYTFGMED  344

ThFRI protein  KTSPSSLLTPFLRKSKESFELAKRKAHSPTAFKEAIEKQLAALLSVTKCLEAHKLDPAKE  412
AtFRI protein  KTSAALVLTSFLKMSKESFERAKRKAQSPLAFKEAMTKQLAVLSSVMQCMETHKLDPAKE  404

ThFRI protein  LPGWPIKEQIVKLEKDTLQIDKQMEEQARSISLMEEAVLIKRLYNQQMKRPRLSEMEMPP  472
AtFRI protein  LPGWQIKEQIVSLEKDTLQIDKEMEEKARSISLMEEAVLAKRVYNQQMKRPRLSPMEMPP  464

ThFRI protein  AASSSYSPIYRDRNFSSHIDGDRDEISALVSSYLGPSSSFPHRSSLRRSPEYMVPP--GG  530
AtFRI protein  VTSSSYSPIYRDRSFPSQRDDQDEISALVSSYLGPSTSFPHRS--RRSPEYMVPLPHGG  522

ThFRI protein  LGRSVSAYEIILLPSSYSP              VIGQRLPRDYSPPVIIGQQQLPYGLQRVY  576
AtFRI protein  LGRSVTAYEIILAPTSYSPGHIGHIRLIHRQYSPSLVIGQRIIPLQYSPPTIIGQQQLPYGIQRVY  582

ThFRI protein  RHSPSVERLILPHHRSPR-NSSQDHIGGM  605
AtFRI protein  RHSPSEERYLGLSNQRSPRSNSSLDPK  609
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Fig. 3 The full length cDNA of *Thellungiella halophila FRIGIDA* (*ThFRI*) and its deduced amino acid sequence (A) cDNA sequence and deduced amino acid sequence of *ThFRI*. Nucleotide positions are given on the right side of the sequence in the 5′ to 3′ orientation. The start codon, ATG, and the stop codon, TAA, are underlined and italicized. The deduced amino acid sequence is shown beneath the nucleotide sequence. The cDNA clone has been deposited in GenBank (accession No. DQ089808). (B) Alignment of *ThFRI* and *AtFRI* protein sequences. Identical amino acids are highlighted in black, and positive amino acids are highlighted in gray.

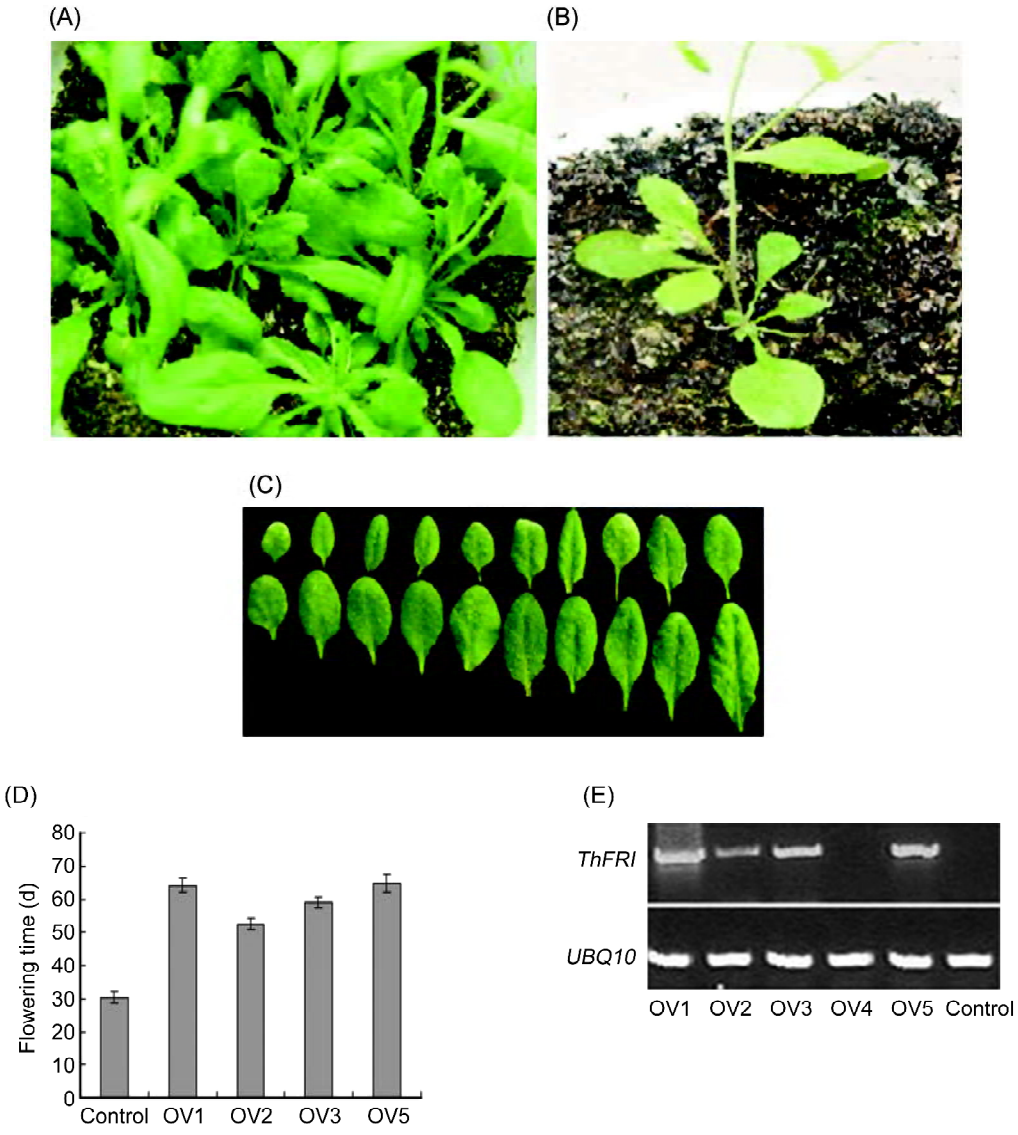

Fig. 4 Analysis of transgenic *Arabidopsis* plants with 35S::*Thellungiella halophila FRIGIDA* (*ThFRI*) (A) Late-flowering phenotype of transgenic *Arabidopsis* with 35S::*ThFRI*. (B) Early-flowering phenotype of transgenic *Arabidopsis* with empty expression vector. (C) Display of first 10 rosette leaves formed on transgenic *Arabidopsis* plants with 35S::*ThFRI*; the sample in the lower row is from OV1 and in the upper from the control plants. (D) Flowering time of four transgenic *Arabidopsis*. Control is a negative transgenic line transformed with empty vector; OV1, OV2, OV3 and OV5 are independent transgenic lines. Plants were grown under LD conditions. The y-axis indicates the days prior to flowering. (E) The expression of *ThFRI* in transgenic *Arabidopsis*. The expression of *ThFRI* in transgenic *Arabidopsis* plants was detected by RT-PCR using gene-specific primers from *ThFRI*. The upper panel shows the RT-PCR result of samples from five independent *ThFRI* transformation events and a negative transgenic plant transformed with empty vector as control. The RT-PCR products of *UBQ10* were used as an internal control, as shown in the lower panel.

indicated that *ThFRI* had functions similarly to *AtFRI*. These results suggested that *ThFRI* and *AtFRI* are functionally conserved during evolution, and that the heterologous expression of *ThFRI* could result in flowering delay in closely related species, such as *Arabidopsis*. As a result, the vegetative growth was extended, which could be valuable in some crop and vegetative plants [24]. By controlling the expression of *FRI*, vegetative growth could be rationally manipulated.

Our data showed that the heterologous expression of *ThFRI* in *Arabidopsis* could give rise to the late-flowering phenotype. However, the heterologous expression of

ThFRI could only extend the vegetative growth period, but not fully complement *AtFRI*-deficiency in *Arabidopsis* (Col). *Arabidopsis* ecotype with wild-type *AtFRI* had about twice the vegetative growth period before flowering (about 4 months) as compared with *ThFRI* heterologous expression lines from this study [25]. Such differences suggested that there might already be some functional divergence between *AtFRI* and *ThFRI*, although differences due to expression pattern changes could not be ruled out. The results showed that the heterogeneous expression of *ThFRI* could partially restore the late-flowering phenotype in *Arabidopsis*. Due to its excellent salinity tolerance, *T. halophila* is considered as a model system for salinity tolerance studies. However, its long life cycle presents a major limitation for lab research. Genetic manipulation at *FRI* locus would create early flowering *T. halophila*. *FRI* takes effect synergistically during the formation of late flowering behavior in *Arabidopsis*; loss of its function would promote early flowering. With ethane methyl sulfonate mutagenesis or RNA interference technique, loss-of-function *ThFRI* mutants could be obtained, resulting in early flowering *T. halophila*. A previous study of another key gene involved in the vernalization pathway in *T. halophila*, *ThFLC*, had demonstrated the feasibility of genetic engineering of short life cycle *T. halophila* through the vernalization pathway [18]. The cloning of the functional *FRI* homolog in *T. halophila* was a critical step towards better understanding of both flowering time regulation in *T. halophila* and the engineering of early flowering *T. halophila* as a model plant for salt tolerance study.

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