J Genet Resour2024; 10(2):23-31 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI:10.22080/jgr.2024.27624.1398*

Gene Expression Analysis of Deduced Protein Sequence from the *FLOWERING LOCUS C* (*FLC*) Homolog during Vegetative and Reproductive Phases in *Lepidium sativum* L.

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ARTICLE INFO

Article history:
Received 29 March 2024
Accepted 14 May 2024
Available 27 June 2024

Keywords:
Flowering repressor
Garden cress
Gene expression
LsFLC gene sequencing
Phylogenetic tree

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p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

The transition of the shoot apical meristem from vegetative to reproductive phases is a critical stage in the plant life cycle. In contrast, the success of reproduction depends on the flowering initiation timing. One of the key repressors of flowering is the gene *FLOWERING LOCUS C* (*FLC*), which is important for determining flowering time. The present study, therefore, aimed to identify and investigate the expression patterns of *Lepidium sativum* genes, considering FLC. Specific primers were designed for the RT-PCR assays, amplifying a 361-nucleotide fragment of the *LsFLC* gene, recorded in the NCBI database under the accession number GenBank: KT582105.1. The sequenced fragment encoded for a deduced protein sequence of 120 amino acids. The expression of *LsFLC* in different plant organs was analyzed at various phenological stages, namely two days (early stage of vegetative development), 13 days (early vegetative stage), 28 days (vegetative maturity or transition stage), and during reproductive development stages, which fall on 33 days (early reproductive stage) and 34 days (flowering stage). Results showed that *LsFLC* expression levels were very high in the root and relatively high in leaves and stems, whereas no expression was allowed in flowers. Indeed, the mRNA levels were much higher during the vegetative phase compared to the transition to flowering, therefore agreeing with the known role of *FLC* as a promoter of vegetative growth and an inhibitor of flowering. Phylogenetic analysis showed that the *LsFLC* gene has a close relationship with Brassicaceae members, in particular with the highest homology with *Arabidopsis lyrata*. The current study has contributed to the knowledge of the functions of LsFLC proteins during the phenology of *L. sativum* and could contribute to strategies developed for improving this species in desirable traits.

Please cite this paper as: Jonoubi, P., Rezanejad, F., & Mahyapoor, F. (2024). Gene expression analysis of deduced protein sequence from the *FLOWERING LOCUS C* (*FLC*) homolog during vegetative and reproductive phases in *Lepidium sativum* L. *Journal of Genetic Resources*, 10(2), 23-31. doi: *10.22080/jgr.2024.27624.1398*

Introduction

The flowering time of a plant represents one of the basic plant attributes that contribute to its adaptation and productivity. The transition to the flowering phase is considered one of the most critical phases in the life cycle of plants (Kim, 2020). It is representative of the transition of the shoot apical meristem from vegetative to reproductive development (Doke and Guha, 2014). Knowing this, during this sensitive stage, the SAM undergoes a modification, which causes the generation of flowers instead of leaves. The molecular mechanisms facilitate this transition from the vegetative to the reproductive phase in plants (Lyu *et al*., 2020). From the developmental biology molecular genetics perspective, flowering induction entails several pathways (Lei *et al*., 2024). Photoperiod, gibberellins, temperature condition, age,

vernalization, and autonomous pathway are yet some of the molecular regulatory pathways of plant flowering (Kinoshita and Richter, 2020). These were *FLOWER LOCUS C* (*FLC*), a member of the MADS-box family, which can act as an effective repressor of flowering (Fan *et al*., 2023). Further studies have also explained the regulatory role of *FLC* over temperature for germination and its involvement in suppressing flowering pathway genes *APETALA1* (*AP1*), *FT*, and *SOC1* (Deng *et al*., 2011). High levels of *FLC* gene expression suppress the levels of SOC1 and FT mRNAs that accumulate in seedlings. On the other hand, a high level of *FLC* expression is induced by vernalization, while a strong interaction attained between *FLC* and vernalization brings about homologous down-regulation of FLC mRNA (Mouradov *et al*., 2002). *FRI* alleles are considered dominant alleles required during vernalization (Zhu *et al*., 2023). At the same time, *FIR* is a key factor responsible for *FLC* expression (Takada *et al*., 2019). Many reports of molecular studies have demonstrated that the downregulation of *FLC* expression advances the vernalization in response to cold exposure (Sharma *et al*., 2020). The induction of flowering for setting seeds in many plant species requires vernalization or a period of prolonged exposure to cold temperatures (Shea *et al*., 2017). *FLC* overexpression represses flowering through both rule-based autonomous and vernalization-dependent pathways (Li *et al*., 2022). Intraspecific variation in the requirement of vernalization is an evolutionary adaptation to the varied climatic regimen in temperate climates. In addition, vernalization affects agricultural production in that early-day flowering may limit the yield potential of vegetable crops (Schiessl *et al*., 2019).

Medicinal plants have long been appreciated for their therapeutic potential in managing various health disorders and are highly valued due to their pharmacological properties (Chaudhary *et al*., 2010). Among these, *Lepidium sativum* presents certain biological and pharmacological characteristics based on which the choice of the plant was considered for the present work. *L. sativum* is an herbaceous annual characterized by being glabrous, erect, and fast-growing. It goes by other names, including "common cress," "garden cress," or "garden pepper-weed" (Shah *et al*., 2021). Seeds from *L. sativum* are highly valued, containing approximately 21% protein, 28% carbohydrates, 7% fiber, and a high amount of fat at approximately 32% (El-Gendy *et al*., 2023). Although these findings provided much information, no published research explored the genetics, expression, and associated mechanisms of FLC gene involvement in flowering for *L. sativum*. Recently, the genus *Lepidium* has been introduced as a model organism using its brief vegetative phase and characteristics similar to those of *Arabidopsis*. Cloning and sequencing of the *FLC* gene in *Lepidium* enable us to investigate its function and role in flowering and other developmental stages. This would not only help in understanding the genetics of plants better but may also unravel some new aspects of the regulatory networks operating during flowering and plant development. Moreover, the study has ascertained this plant's phylogenetic relationship with the other Brassicaceae members by determining the *LsFLC* gene sequence.

Materials and Methods

Preparation of plant samples

Garden cress seeds were obtained from Pakan Bazr Corporation, Esfahan, Iran. The seeds were sterilized with 1% hydrogen peroxide for 20 minutes. They were then washed with distilled water and were transferred to perlite-filled 20×30 cm pots. The plants were fed with Hoagland's solution (Hoagland and Arnon 1950), and the nutrient solution was replenished every three days.

Germination was carried out in a greenhouse under controlled conditions with a 16-hour light/8-hour dark photoperiod. The light period was supplemented at a light intensity of 450 μmol m^{-2} S^{-1} supported by fluorescent and incandescent lamps. Temperature was maintained at 25°C during the day and night, while relative humidity was maintained at 70%.

In the molecular studies, young vegetative leaves were used as the main tissue to identify and sequence the *FLC* homologous gene in the studied plant. To investigate any possible expression of the *FLC* gene in all other plant organs, fresh tissues of leaves, stems, and roots

were collected at different stages of development. Young leaves near the shoot apex and from the young parts of the stem and root, i.e., near the apical meristems, were taken. Sampling was done for five plants, and experiments were conducted in three replicates.
These stages included young plants at the vegetative phase (13 days old and with six leaves), mature plants in the vegetative phase (26 days old and with 33 leaves), and mature plants in the reproductive phase (33days old post-flowering characterized by the appearance and opening of the first flowers).

Bioinformatics analysis

Since the target gene's sequence is unknown, primers were designed based on gene sequences available for other similar plants within the same genus and family. To this end, the same primers used in the study of *FLC* gene expression were employed. The sequence of the homologous *FLC* gene from the following plants was analyzed using the NCBI database: *Arabidopsis thaliana* (Accenssion no: JF318804.1); *Arabidopsis halleri* (KC505459.1); *Raphanus sativus* (AB611009.1); *Brassica oleracea* (AY273161.1); *Sinapis alba* (EF542803.1); *Brassica napus* (JQ255388.1); *Brassica nigra* (KJ733745.1); *Brassica juncea* (KJ489426.1).
The obtained sequences were aligned using MUSCLE software (https://www.ebi.ac.uk/Tools/-msa/muscle).
Based on the conserved regions, referring to the start and end of the Coding DNA Sequence (CDS) region, with consideration to the 5' untranslated region (5`UTR) and 3' UTR, a pair of primers was designed using the Gene Runner software. To assess the suitability of the designed primers, various BLAST software tools were employed based on information provided during the search results.
First, the alignments of amino acid sequences encoded by these obtained results from sequence determination were conducted by using BLAST and then using the MEGA 6.06 software with some *FLC* homologs that exist in the NCBI gene bank, the similarity of which was investigated. Using the Conserved Domain Database by http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml, conserved protein domains in *Lepidium* homologous to FLC were investigated.

In this respect, the data recorded from ImageJ software concerning the intensity of bands that were amplified in the PCR reaction were compared in SPSS software. The statistical analysis was done using Tukey's test at a 95% confidence interval. To illustrate the results in graphical format, Excel 2010 software was utilized. This approach reproduces the methodology used to analyze and interpret the experimental results thoroughly and provides a deeper understanding of such results.

Primer design and RNA extraction

The primer was synthesized by the Pishgaman Company. According to the company's protocols, sterile deionized water was added to the lyophilized primers to prepare the storage solution. Later on, the primers were 31 times diluted with the storage solution to prepare the working solution according to the company's instructions (Table 1).

Table 1. Sequence of primers used.

Primer name	Sequence (5'→3')
Fr1FLC	5`AGAAGCCATGAGAAGAAA
Fr2-FLC	5`CCGACAAGTTACCTTCTC
Rv1FLC	5`CTAATTAAGCAGTGGGAG
Rv2-FLC	5`TTCTGTCTTCCTGGCTCT
Fr-GAPDH	5`CAAGGACTGGAGAGGTGG
Rv-GAPDH	5`TTCACTCGTTGTCGTACC

The RNA from young leaves was extracted according to the instructions of each manufacturer, with slight modifications developed for the GeneAll® RiboExTM kit. RNase-free water was used to elute the RNA sample, which was then stored at 4 °C.
The quality and quantities of the RNA samples were measured using a Nanodrop spectrophotometer. The readings were obtained by measuring absorbance at 260_{nm}. Quality for RT-PCR was checked by running on 1% agarose gel through horizontal electrophoresis. Contamination of proteins and other impurities of RNA samples was checked from the $OD260_{nm}$/ $OD280_{nm}$ and OD 260_{nm}/ OD 230_{nm}, respectively. Subsequently, the RNA samples were kept at -80 °C until further analysis.

cDNA synthesis and RT-PCR analysis

According to the manufacturer's recommendations, total RNA was isolated from

leaves, roots, apical meristems, and flowers of garden cress using the RNeasy Plant Kit from GeneAll, RiboEx, Total RNA isolation solution, Korea. DNase treatment was performed to obtain DNA-free RNA, and the resulting RNA was used for synthesizing first-strand cDNA with Superscript® II Reverse Transcriptase (Invitrogen) and oligo(dT) primers. Expression of cDNA by RT-PCR was performed using degenerate primers against conserved regions of *FLC* gene sequences that were aligned from *Arabidopsis thaliana* cDNA (Accession no. NM_001085094) and *Brassica napus* (AY036888). Cycling conditions for PCR were initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 30 s, and elongation at 72 °C for 1 min with a final extension at 72 °C for 5 min. Then, the PCR products were analyzed by 1% agarose gel electrophoresis and staining with the RedSafe™ (Intron Co., Korea). Gene sequencing was performed on the CDS of *LsFLC* gene.

Phylogenetic analysis

A comparative analysis was performed for the deduced LsFLC protein sequence with other FLC homologous proteins to find the conserved regions and the level of similarity. Several FLC homolog protein sequences were retrieved from the NCBI database and then aligned using the ClustalW2 program to find the shared regions with the deduced LsFLC protein sequence. A phylogenetic tree of these proteins was also constructed using the MEGA software.

Results

Sequence analyses of *LsFLC* gene

Total RNA was extracted from the leaf samples in this study. At 260 nm, the wavelength at which RNA is normally measured, the absorbance of RNA was about OD 0.5 to OD 0.6. The absorption ratio of $OD260_{nm}$/ $OD280_{nm}$ was about 1.9 to 2.1. These showed that the concentration and quality of the extracted RNA were appropriate. Further, three sharp and intense bands corresponding to ribosomal RNAs are manifested in 1% agarose gel, which is indicative of the optimal quality of RNA extracted. This, in turn, dictates its suitability for conducting RT-PCR experiments (Fig. 1 A).

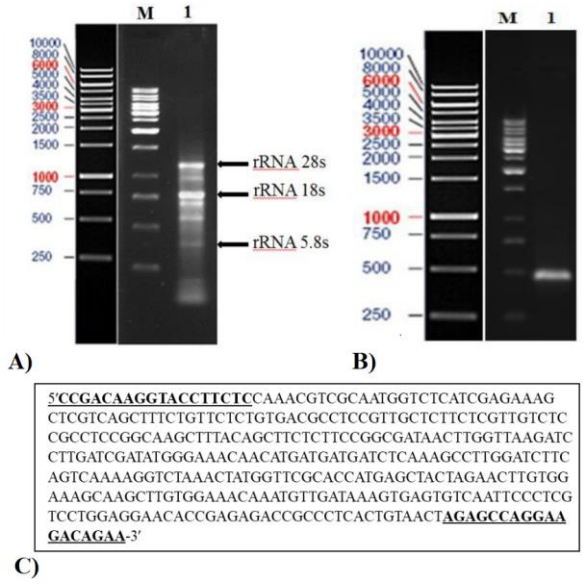

Fig. 1. The electrophoretic profile and registered sequence of *Lepidium sativum* from NCBI data bank: A) The electrophoretic profile of the total RNA extracted from young leaves of *Lepidium sativum*; B) The electrophoretic profile of the RT-PCR product indicates the amplification of a full-length duplication from the *LsFLC* homologous gene in *Lepidium sativum* (Well 1: The band corresponding to a 361-bp fragment was synthesized using the Fr2FLC forward and Rv2FLC reverse primer, M: The molecular weight marker used was a 1kb DNA ladder from Fermentase); C) The NCBI registered sequence of *LsFLC* CDS region with accession number KT582105 which arrows show that were used primer sequences.

Based on the outcome of such analysis, total RNA extracted from a young vegetative leaf was used in RT-PCR experiments. Multiple PCR reactions were made with the obtained cDNA using primer pairs F1-R1, F1-R2, F2-R1, and F2-R2. Contrary to expectations, and as shown in Fig. 1B, no amplification was obtained for the F1 and R1 primers, which had been designed from regions assumed to be conserved at the beginning and end of the gene. Then, under the optimized reaction conditions, the specific single-band amplification of a 361-nucleotide fragment in the middle of the coding sequence of the gene using the F2-R2 primer pair was accomplished. These results suggest that primer selection and optimization during RT-PCR experiments are very crucial in successfully amplifying the target fragment (Fig. 1).

With the results presented in Fig. 1B, complete sequencing of the RT-PCR product was done using primers Fr2FLC and Rv2FLC. Therefore, information on both the start and stop codons within the gene encoding region was attainable, and at the same time, confirmation of sequence integrity of the designed primers could be performed. From the BLAST results, it was clear that this gene demonstrated a high similarity with other homologues from the Brassicaceae family, including a maximum similarity of 92% with *Camelina sativa*. The identified 361-nucleotide fragment was deposited in the NCBI under the accession number KT582105 and was named *LsFLC* (Fig. 1C).

LsFLC deduced protein sequence

The sequence of the region of the *LsFLC* gene, including most of exon 1, full lengths of exons 2 and 3, and additional segments of exons 4 and 5 has been nearly completely determined. Consequently, the LsFLC protein sequence is given in Fig. 2.

N- Terminal

RQGTFSKRRNGLIEKARQLSVLCDASVALLVVSASGKLYSFSSGDNLVKILDRYGKQHD

DDLKALDLQSKGLNYGSHHELLELVESKLVETNVDKVSVNSLVLEEHRETALTVTRAR

KTE C-Terminal

Fig. 2. The deduced protein sequence of LsFLC.

The deduced protein LsFLC of this species was compared with homologues in other plants, and it was found that this protein is 36-90% similar to other homologues. *Cardamine flexuosa* showed the highest similarities (90%), and *Posidonia oceanica* had the lowest similarities (36%), respectively.

Phylogenetic tree analysis

The study of a phylogenetic tree concerning this species and its homologues in other plants confirmed this; the other species belonging to this family are close to the current studied species, *Lepidium sativum* (Fig. 3).

LsFLC gene expression pattern

Studies of *LsFLC* gene expression were done in various vegetative and reproductive organs during different phenological stages in the vegetative development, including two days (early vegetative stage), 13 days (early vegetative stage), and 28 days (vegetative maturity and transition stage), as well as reproductive development stages, including 33 days (early reproductive stage) and 34 days (flowering stage) (Fig. 4A).

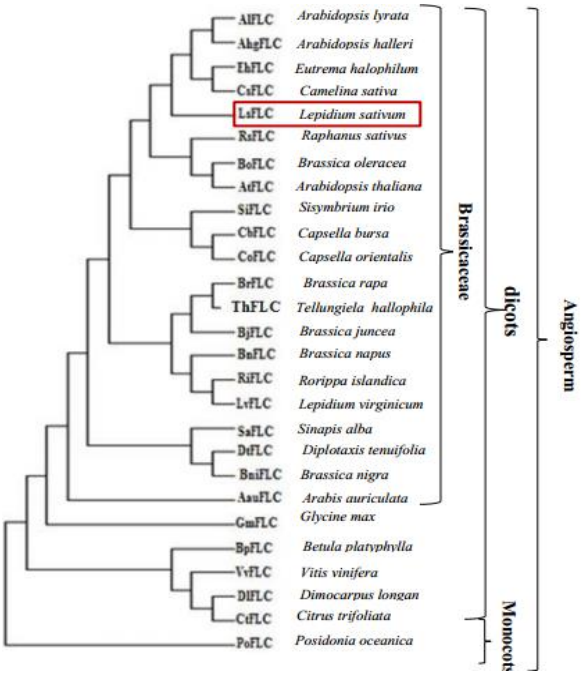

Fig. 3. Phylogenetic tree of FLC homogenous proteins in different species. LsFLC is located inside the box.

By RT-PCR, results indicated a 361-nucleotide fragment for the *LsFLC* gene in some organs, but for the reference gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase), there were 511-nucleotide fragments in almost all the organs. *LsFLC* gene expression was very high in the roots, about 90%, with relatively low expression in leaves, about 65%, and about 30% in the stem. Expression was almost absent during the opening of flowers and only reached about 2% (Fig. 4B).

Comparative analysis showed that the *LsFLC* gene had a higher expression in different organs at the vegetative stage compared to the reproductive phase. The gene expression data were treated with ImageJ and SPSS, where the mean values were compared using Tukey's test. A significance level of $P < 0.05$ was considered and applied to find significant differences (Fig. 4C).

Discussion

Flowering time is a critical determinant of crop yield and, thus, one of the important targets for plant breeding. Changes in flowering time enable plants to adapt to different climatic regions and ecological niches. Unless the genetic and molecular mechanisms underlying the vernalization of the need are fully understood, crops can be bred to adapt to particular kinds of environmental conditions. Further research in the direction of regulatory networks controlling flowering time would contribute to more precision in plant breeding strategies against this important agronomic trait.

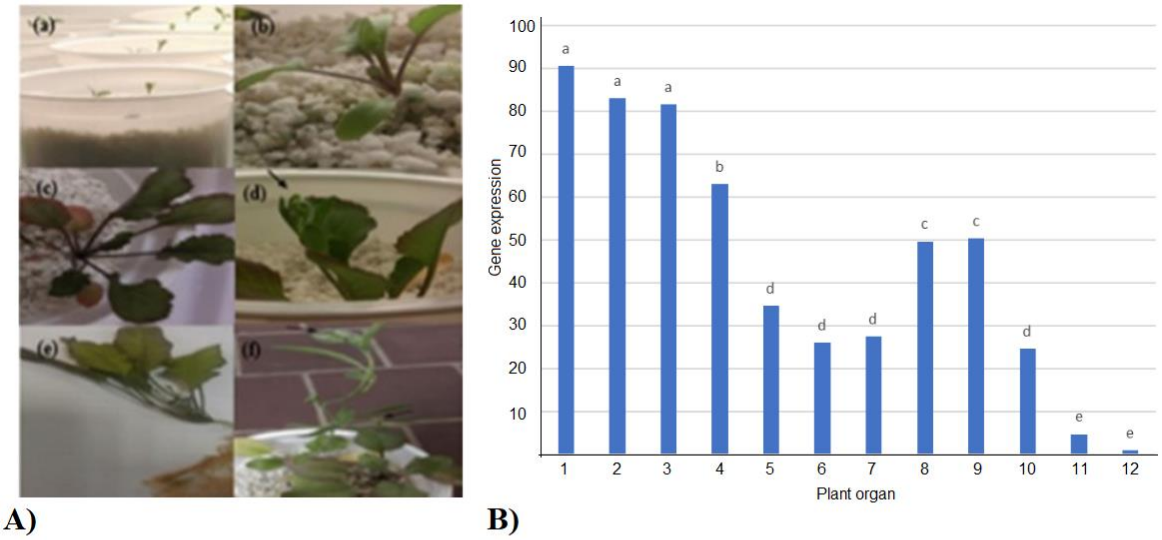

Fig. 4. The phenological stages of vegetative and reproductive development, expression and gel electrophoresis of *LsFLC* gene in *Lepidium sativum:* A) Phenological stages of vegetative and reproductive development in garden cress which a-d showed 4, 13, 28, 31, and 33-day-old plants, respectively; B) Expression of *LsFLC* gene in different plant organs in vegetative and reproductive stages (statistical analysis was conducted using the Duncan test ($P< 0.05$). Different letters indicate statistically significant differences); C) Gel electrophoresis of real-time PCR amplification fragments (1: rootlet, 2: roots in the early vegetative stage, 3: roots in the mature vegetative stage, 4: roots in the reproductive stage, 5: stems in the early vegetative stage, 6: stems in the mature vegetative stage, 7: stems in the reproductive stage, 8: cotyledon leaves in seedling, 9: leaves in the early vegetative stage, 10: leaves in mature vegetative stage,11: leaves in reproductive stage, 12: flowers. In all reactions, the expression of the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) reference gene was considered as a positive control.

Before vernalization, the FLC represses flowering in the shoot apical meristem, and exposure to low temperature reduces the levels of this gene, promoting flowering (Wu *et al*., 2020). The components of the autonomous pathway complex consist of *ii* and *APRF1*. Mutations that

repress these components cause increased expression of FLC, hence late-flowering phenotype independent of photoperiod, which can be complemented by vernalization. This indicates that the *FLC* gene is one of the critical factors regulating flowering time (Qi *et al*., 2022). The dehydration-responsive element-binding protein (DREB1A) transcriptionally activates the expression of the *FLC* gene, and *FLC*, in turn, represses the expression of the key floral activator *SOC1*, resulting in late flowering. On the other hand, *SOC1* is known to act as a negative regulator for the expression of the *DREB1A* gene and forms a feedback loop that includes *FLC*, *SOC1*, and *DREB1A*. This mechanism dampens the level of expression of the regulon *ICE1-DREB1A* and controls flowering time. Over-expression of *DREB1A* probably interferes with this feedback mechanism, thereby leading to increased levels of *FLC*, hence promoting delayed flowering (Kohan-Baghkheirati *et al*., 2022). *FLOWERING LOCUS K* (*FLK*) is an RNA-binding protein with K-homology KH motifs that has been implicated in the regulation of floral transition by repressing the levels of the key floral repressor *FLC* in *Arabidopsis* (Amara *et al*., 2023). In this work, the *FLC* gene showed very high expression in roots, while it was relatively lower in flowers; almost no expression was detected in opening flowers. Moreover, *FLC* expression was lowered from the seedling stage towards vegetative maturity. Zou *et al*. (2012) demonstrated that *Brassica FLC* and *Arabidopsis FLC* have similar functional activity during gametogenesis. In both these species, *FLC* activity is reset in the male reproductive tissue and suppressed as the pollen matures within the flower buds undergoing vernalization. According to Zou *et al*. (2012), the transcriptional activity of *BnFLC.A2* and *BnFLC.A10* exhibited a slight increase during flowering, while *BnFLC.A3b* showed a significant reduction compared to stem elongation levels. The present study showed that transcription of the *LsFLC* gene was low during the reproductive phase of flowering compared with vegetative growth. Recently, Gramzow *et al*. (2023) detected members of *FLC/SQUA*-like clade in the following tissues of gymnosperm: roots, branches, stem, and female cones. This finding showed that these *FLC*-related genes are present in each tissue type, from which their diverse functional role can be established in different plant species. Kumar *et al*. (2016) presented that the application of bioinformatics and experimental approaches demonstrated the importance of several members of the MADS-box transcription factors in various developmental aspects along with different phenological events of apples. Thus, transitions between successive developmental stages of apple phenology are correlated with a change in either *MdMADS* gene expression. In angiosperms, the *MADS*-box gene family is comprised of many distinct clades, among which those dealing with floral organ identity are of primary importance. *FLC* is a transcription factor from the *MADS*-box family (Fan *et al*., 2023). Kumar *et al*. (2016) identified six *DAM* genes and four *FLC*-like genes showing similarities with other dicot species through the comparative phylogenetic analysis of the MADS-box family proteins in apples. Gramzow *et al*. (2023) used modern genomic data to investigate the unusual evolutionary dynamics of *FLC*-like genes, namely their tendency to form clusters of tandemly duplicated genes within a phylogenetic framework. The genomic analysis identified that the duplicated fragment from the coding region of cress *LsFLC* gene was 361 nucleotides in length and shared high similarity with the previously reported ones from the Brassicaceae family (Deng *et al*., 2011). The comparative studies indicated that the coding sequence of the *LsFLC* gene showed a high similarity to *Camelina sativa* at 92%, while the deduced protein, with a sequence of 120 amino acids, was similar at 90% to *C. flexuosa*. Phylogenetic analysis described *L. sativum* as being closer to other members of this family and further confirmed the accuracy of the *LsFLC* gene in this plant species.

Conclusion

In a nutshell, our molecular studies identified a homogenous *FLOWERING LOCUS C* (*FLC*) gene in *Lepidium sativum* L. Herein; this work represents the results of RT-PCR analysis, indicating a 611-nucleotide band corresponding to a segment of the genome of the plant. Sequencing a fragment of its coding region identified a 361-nucleotide fragment from the

FLC gene. Based on this positive BLAST result, the gene has been named *LsFLC*.

Comparative analysis of the deduced protein sequence of LsFLC with other FLC alignments showed amino acid identity ranging from 45 to 91%. In addition, this phylogenetic analysis confirmed the proper sequencing of LsFLC and further established a relationship between LsFLC and other members of Brassicaceae. The expression study of the *LsFLC* gene in different plant organs in stages of development showed high expression in the roots, while less expression was visible in leaves and stems. Notably, expression was absent within open flowers. In addition, the expression level was higher during the vegetative stage compared with the reproductive phase. Such an expression profile supports the role of *LsFLC* in regulating the flowering process. It extends the vegetative period by repressing the onset of the reproductive phase during vegetative growth. Although they are expressed during the transition to flowering, other flowering-promoting genes suppress the expression of *LsFLC*, allowing successful flowering, seed production, and survival across generations. It was assumed that *LsFLC* might play an important role in delaying flower budding and that the results would underpin further theoretical studies on the function of *LsFLC* in flowering time regulation, benefiting further molecular breeding studies.

Acknowledgments

The authors appreciate Kharazmi University and Shahid Bahonar University of Kerman for supporting this research. Perplexity AI and Gtpinf were used to edit and paraphrase the text.

Conflict of Interests

The authors declare no conflict of interest.

References

Amara, U., Hu, J., Cai, J., & Kang, H. (2023). FLK is an mRNA m6A reader that regulates floral transition by modulating the stability and splicing of FLC in *Arabidopsis*. *Molecular Plant*, 16(5), 919-929. https://doi.org/10.1016/j.molp.2023.04.005

Chaudhary, G., Goyal, S., & Poonia, P. (2010). *Lawsonia inermis* Linnaeus: A Phytopharmacological Review. *International Journal of Pharmaceutical Sciences and Drug Research*, 2(2), 91-98. https://ijpsdronline.com/index.php/journal

Deng, W., Ying, H., Helliwell, C. A., Taylor, J. M., Peacock, W. J., & Dennis, E. S. (2011). FLOWERING LOCUS C (FLC) regulates development pathways throughout the life cycle of *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America, 108, 6680-6685. https://doi.org/10.1073/pnas.1103175108

Doke, S., & Guha, M. (2014). Garden cress (*Lepidium sativum* L.) seed - An important medicinal source: a review. *Journal of Natural Products and Plant Resources*, 4, 69-80. http://scholarsresearchlibrary.com/archive.html

El-Gendy, M. S., El-Gezawy, E. S., Saleh, A. A., Alhotan, R. A., Al-Badwi, M. A., Hussein, E. O. S., & Omar, S. M. (2023). Investigating the chemical composition of *Lepidium sativum* seeds and their ability to safeguard against Monosodium Glutamate-induced hepatic dysfunction. *Foods*, 12(22), 4129. https://doi.org/10.3390/foods12224129

Fan, M., Chen, L., Xue, X., Guo, Q., Guo, D., Guo, L., & Hou, X. (2023). Identification and characterization of flowering time regulatory gene *FLC* of *Paeonia ostii* 'Fengdan'. *Scientia Horticulturae*, 310, 111748. https://doi.org/10.1016/j.scienta.2022.111748

Gramzow, L., Sharma, R., & Theißen, G. (2023). Evolutionary dynamics of FLC-like MADS-Box genes in Brassicaceae. *Plants*, 12, 3281. https://doi.org/10.3390/plants12183281

Hoagland, D.R., & Arnon, D.I. (1950). Preparing the nutrient solution. Water-culture method grows. *Plants Without Soil*, 347, 29-31. https://doi.org/citeulike-article-id:9455435

Kohan-Baghkheirati, E., Bagherieh-Najjar, M., Abdolzadeh, A., & Geisler-Lee, J. (2022). A precise expression level of dreb1a gene is required for proper seed germination, vegetative and reproductive development, and seed yield in Arabidopsis thaliana. *Journal of Genetic Resources*, 8(1), 99-110. https://doi: 10.22080/jgr.2022.22267.1279

Kim, D.H. (2020). Current understanding of flowering pathways in plants: focusing on the vernalization pathway in *Arabidopsis* and several vegetable crop plants. *Horticulture, Environment, and Biotechnology*, 61, 209-227 https://doi.org/10.1007/s13580-019-00218-5

Kinoshita, A. & Richter, R. (2020). Genetic and molecular basis of floral induction in, *Arabidopsis thaliana*. *Journal of Experimental Botany*, 71(9): 2490-2504, https://doi.org/10.1093/jxb/eraa057

Kumar,G., Arya, P., Gupta, K., Randhawa,V., Acharya, V. & Singh, A.K. (2016). Comparative phylogenetic analysis and transcriptional profiling of MADS-box gene family identified *DAM* and *FLC*-like genes in apple (*Malus*x *domestica*). *Scientific Reports*, 6: 20695. https://doi.org/10.1038/srep20695

Lei, Y., Gao, J., Li, Y., Song, C., Guo, Q., Guo, L., & Hou, X. (2024). Functional characterization of *PoEP*1 in regulating the flowering stage of tree peony. *Plants*, 13, 1642. https://doi.org/10.3390/plants13121642

Li, Y., Wang, C., Guo, Q., Song, C., Wang, X., Guo, L., & Hou, X. (2022). Characteristics of *PoVIN3*, a key gene of vernalization pathway, affects flowering time. *International Journal of Molecular Sciences*, 23, 14003. https://doi.org/10.3390/ijms232214003

Lyu, J., Cai, Z., Li, Y., Suo, H., Yi, R., Zhang, S., & Nian, H. (2020). The floral repressor gmflc-like is involved in regulating flowering time mediated by low temperature in soybean. *International Journal of Molecular Sciences*, 21. https://doi.org/10.3390/ijms21041322

Mouradov, A., Cremer, F., & Coupland, G. (2002). Control of flowering time: interacting pathways as a basis for diversity. *The Plant Cell*, *14* (suppl-1): S111–S130, https://doi.org/10.1105/tpc.001362

Qi, P. L., Zhou, H. R., Zhao, Q. Q., Feng, C., Ning, Y. Q., Su, Y. N., & He, X. J. (2022). Characterization of an autonomous pathway complex that promotes flowering in *Arabidopsis*. *Nucleic Acids Research*, 50(13), 7380-7395. https://doi:10.1093/nar/gkac551.

Schiessl, S. V., Quezada-Martinez, D., Tebartz, E., Snowdon, R. J., & Qian, L. (2019). The vernalization regulator *FLOWERING LOCUS C* is differentially expressed in biennial and annual *Brassica napus*. *Scientific Reports*, 9,14911, https://doi.org/10.1038/s41598-019-51212-x1

Shah, M. B., Dudhat, V. A., & Gadhvi, K. V. (2021). *Lepidium sativum*: a potential functional food. *Journal of Ayurvedic and Herbal Medicine*, 7(2), 140-149.https://doi.org/10.31254/jahm.2021.7213

Sharma, N., Geuten, K., Giri, B. S., & Varma, A. (2020). The molecular mechanism of vernalization in Arabidopsis and cereals: role of *Flowering Locus C* and its homologs, *Physiologia Plantarum*, 170(3), 373-383, https://doi.org/10.1111/ppl.13163

Shea, D. J., Itabashi, E., Takada, S., Fukai, E., Kakizaki, T., Fujimoto, R., & Okazaki, K (2017). The role of *FLOWERING LOCUS C* in vernalization of *Brassica*: the importance of vernalization research in the face of climate change. *Crop Pasture Sciences.* 69, 30-39, https://doi.org/10.1071/CP16468

Takada, S., Akter, A., Itabashi, E., Nishida, N., Shea, D. J., Miyaji, N., & Fujimoto, R. (2019). The role of *FRIGIDA* and *FLOWERING LOCUS C* genes in flowering time of *Brassica rapa* leafy vegetables. *Scientific Reports*, *9*, 13843 https://doi.org/10.1038/s41598-019-50122-2

Wu, Z., Fang, X., Zhu, D., & Dean, C. (2020). Autonomous pathway: *FLOWERING LOCUS C* repression through an antisense-mediated chromatin-silencing mechanism. *Plant Physiology*, 182, 27-37. https://doi.org/10.1104/pp.19.01009

Zhu, P., Schon, M., Questa, J., Nodine, M., & Dean, C. (2023). Causal role of a promoter polymorphism in natural variation of the *Arabidopsis* floral repressor gene *FLC*. *Current Biology*, 33, 4381-4391. https://doi.org/10.1016/j.cub.2023.08.079

Zou, X., Suppanz, I., Raman, H., Hou, J., Wang, J., Long, Y., ... & Meng, J. (2012). Comparative analysis of *FLC* homologues in Brassicaceae provides insight into their role in the evolution of oilseed rape. *PLoS One*, 7(9), e45751. https://doi.org/10.1371/journal.pone.004