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FLC-like factors in wheat (cv. Mv15) and *Conyza* sp.

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ABSTRACT Plants have evolved multiple pathways to regulate flowering time. The repressor pathway maintains the vegetative phase until the promotion pathways initiate reproductive development. The promotion pathways include the long-day, autonomous and vernalization pathways of flowering. A MADS-box protein encoded by FLOWERING LOCUS C (FLC) may be a major repressor of flowering in vernalization pathway since the transcript level of FLC is negatively regulated by vernalization. Special primer pairs were constructed here to identify the FLC genes in a monocotiledonous plant with strong requirement for vernalization and in a weed. This study describes the first example for the presence of FLC-like factors in winter wheat *Triticum aestivum* L. cv. MV15 and in the horseweed *Conyza canadensis* L. (Cronq.).

KEY WORDS

flowering
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FRI

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The transition from vegetative growth to flowering is a major developmental switch in the plant life cycle. Flowering is regulated by interacting genetic pathways (Levy and Dean 1998). The repressor pathway maintains the vegetative phase until the flowering promotion pathways initiate reproductive development. The promotion pathways include the long-day, autonomous and vernalization pathways of flowering (Martinez-Zapater et al. 1994).

In many plant species, flowering is promoted by a period of exposure to low temperature (vernalization). Vernalization often occurs early in a plant's development, and flowering may occur after many weeks of growth in warmer temperatures. This indicates that the vernalization signal is maintained through a number of cell divisions after the cessation of the inductive cold treatment.

Late flowering *Arabidopsis* ecotypes with strong requirement of vernalization contain dominant allele of FRIGIDA (FRI) which appears to increase the level of a MADS-box protein encoded by FLOWERING LOCUS C (FLC) (Michaels and Amasino 1999; Sheldon et al. 1999). FLC may be a major repressor of flowering (Sheldon et al. 2000). The level of FLC transcript and protein is negatively regulated by vernalization. The decrease in FLC transcript is mitotically stable; once transcript levels are decreased, they remain low during the subsequent growth of the plant. In a number of plant species the magnitude of the promotion of flowering by cold treatment is proportional to the duration of the treatment and there is a quantitative relationship between the duration of cold treatment and the extent of down-regulation of FLC activity.

Reduction in the level of genomic methylation largely substitutes for the low-temperature treatment in promoting flowering, implicating a reduction in DNA methylation level as a component in the vernalization response (Finegan et al. 1998).

This study describes the first example for the presence of FLC-like factors in the monocotiledonous plant *Triticum aestivum* L. cv. MV15 winter wheat with strong requirement for vernalization to induce flowering and in the horseweed *Conyza canadensis* L. (Cronq.).

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Materials and Methods

DNA isolation and PCR amplification: DNA was isolated from seedlings of bread wheat *Triticum aestivum* L. cv. MV15 and leaves of *Conyza canadensis* L. (Cronq.) by plant DNAzol Reagent (GIBCO BRL) according to the manufacturer's instructions. PCR amplification was carried out as described by Henrion et al. (1992). Primers were designed by programs accessible free on-line. For database queries needed for primer design BLASTN 2.2.2 (Altschul et al. 1997) and Fasta33_t (Pearson and Lipman 1988) programs were used. The sequences of the oligonucleotide primers used for the PCR amplifications were:
5'-CCGAACTCATGTTGAAGCTTG (FLC5F),
5'- AAACGCTCGCCCTTATCAGC (FLC7R) and
5'-GTATTGACTTAGTTCCGTC (FLC71R).

Sequencing: For direct sequencing PCR products were purified with Prep-A-Gene DNA Purification Kit (Biorad). For cycle sequencing ABI Prism BigDye Kit (Applied Biosystems) was used and the electrophoresis was executed on ABI PRISM 310 Genetic Analyser (Applied Biosystems) according to the manufacturer's instructions. Primers used for the direct sequencing were those used for the amplification (FLC5F and FLC7R).

Results and Discussion

The timing of floral initiation is critical for reproductive success, and plants have evolved multiple pathways to regulate flowering time. The MADS-box protein FLC seems to be the central regulator of the induction of flowering by vernalization pathway since the level of FLC determines the extent of the vernalization response in the promotion of flowering.

Table 1. Position of exons in the *A. thaliana* FLF (FLC) (AF116528) gene sequence.

gene	cDNA	cDNS	lenght(bp)
I	1-294	1-294	294
II	3764-3822	295-353	59
III	3994-4054	354-413	60
IV	4145-4245	414-514	101
V	4924-4963	515-554	40
VI	5158-5198	555-595	41
VII	5593-5928	596-929	334

The aim of the present work was to identify FLC-like sequences in the monocotiledonous bread wheat and in the horseweed *C. canadensis* on the basis of similarities with the previously described *A. thaliana* sequences. The 943 bp long cDNA sequence of FLF (FLC) mRNA (AF116527) and the 6051 bp long complete sequence FLF (FLC) gene (AF116528) were aligned first using the ClustalW program. This alignment revealed the presence of seven exons and their locations in the *A. thaliana* FLC gene.

To construct primers, which may result in 4-900 bp long PCR products from wheat and horseweed, both the lenghts and positions of the exons were taken into consideration. Primer pairs were constructed for Exons V and VII. These primers resulted in a 243 bp product of wheat and a 300 bp long product of horseweed. Products were sequenced (Figure 1.) and found to exhibit some similarities with *A. thaliana* FLC as well as some other proteins *e.g.* gipsy-like retro-transposon or hypothetical polyprotein of *T. monococcum* and a possible protein of rice which has not been identified yet.

CCGAACTCATGTTGAAGCTTGACCACTGCGGTGCT
GGCAGATCTCACAGGAGGAGGGGGAGGGAGACGC
CTCTGATTGAAGCATTTGTTGGCATAGTGACCCTTC
TGTTGGCACTTGTTGCACGTGACCTCCGAGAGCGG
ACGGTGATACGGCCGGCAACTCATGTTGAAGCTTG
AGACGAAGTCTTGTTCTGAAAGCCAGGGTTGTGTG
GGTGGGAAGATCCRCTGCCACCTATGCTCTCTA

AACTTCCACTTTACCAGTAACACTGTCCTCCATAAC
ATATGCATCAGATGACATACATAATCTTGTATCCGAC
AAATMCAATATGTCAGAAGMTCACAAGTAMMGGG
TTGAGATTTTTATATACACATATAGTTTGTTTTTGTTA
CATAGTTGATCTTACTATTTACGCTTACATACATAAGC
TACAAACTCGTTATTAGCACTAAGGTTTGATGGTCA
ATAAAGAGAGTTGTAAGTAAAAAAGGTAGCTATAAT
GGTGGAGATGCAGATAACTAGCGACGGAACTAAGT
TCAATACCAC

Figure 1. Sequences of FLC-like factors of wheat and horseweed, respectively.

This is the first description of FLC-like factors from a monocotiledonous plant and a weed. Study on the changes in the expression of these gene sequences during vernalization needs further experiments and are underway in our laboratory.

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