(cc) BY

Oripova Barno Bakhtier kizi
research scientist
Institute of Genetics Plant Experimental Biology Academy
of Sciences of the Republic of Uzbekistan
Tashkent, Uzbekistan

FLD – FLOWERING GENE SEQUENCE PROCEDURE

Abstract*: flowering is an important reproductive activity in plant life, which occurs under the coordinated integrated action of several flowering-related genes. Studying the flowering process at the molecular level will become the basis for creating the possibility of artificially controlling the flowering period of cotton species in the future.*

Keywords*: Cotton, Gossypium L, diploid, tetraploid, gene, in silico PCR, primer.*

Орипова Барно Бахтиёр кизи
соискатель, научный сотрудник
Институт генетики и экспериментальной биологии растений
Академии наук Республики Узбекистан
г. Ташкент, Узбекистан

ПРОЦЕДУРА ОПРЕДЕЛЕНИЯ ПОСЛЕДОВАТЕЛЬНОСТИ ГЕНА ЦВЕТЕНИЯ

Аннотация*: цветение – важная репродуктивная деятельность в жизни растений, которая происходит при согласованном комплексном действии нескольких генов, связанных с цветением. Изучение процесса цветения на молекулярном уровне станет основой для создания возможности искусственного управления периодом цветения видов хлопчатника в будущем.*

Ключевые слова*: Хлопок, Хлопчатник, диплоид, тетраплоид, ген, виртуальная ПЦР, праймер.*

The *FLD*, *TFL1*, *SOC1* and *PIF4* genes were selected based on research by world scientists aimed at the molecular genetic study of the flowering process of plants. The functions of these genes were studied on the model plant *Arabidopsis thaliana* [1, 2, 3, 4,

Content is licensed under the Creative Commons Attribution 4.0 license (CC-BY 4.0)

5], among which the *TFL1*, *PIF4* and *SOC1* genes are amplified in the cotton genome. The *FLD* gene has not been studied in wild and cultivated diploid and tetraploid cotton species. Considering this, *Gossypium L.* cotton genomes and Arabidopsis genome *FLD*, *TFL1*, *SOC1* and *PIF4* genes were downloaded from the National Center for Biotechnology Information (NCBI) database. Primer pairs were designed for these genes using the Primer-BLAST web interface [6]. The presence of this primer in the cotton genome was confirmed using in silico PCR and molecular analysis (Fig. 1–2).

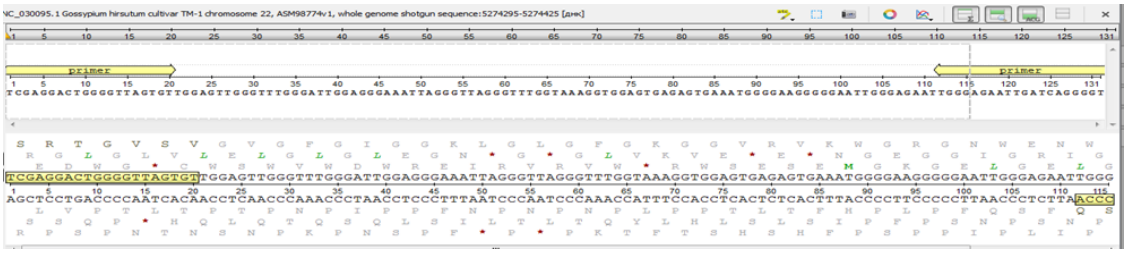

Figure 1. Hybridization of the FLD gene primer with the G. hirsutum genome

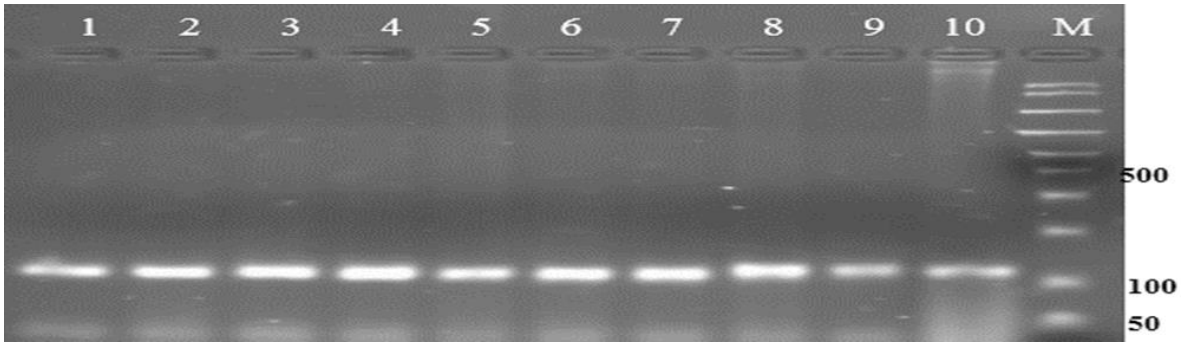

Figure 2. Electrophorogram of the PCR result using the FLD gene marker

M – Marker DNA Ladder (50 нг/µл конц); 1-*G.herbaceum* subsp. *pseudoarboreum*, 2-*G.herbaceum* subsp. *frutescens*, 3-*G.herbaceum* subsp. *africanum*, *G.euherbaceum* A-833., 5-*G.arboreum* subsp *nanking* (white fiber), 6-*G.arboreum* subsp *nanking* (brown fiber), 7-*G.euarboreum A*-352, 8--*G.mustelinum* Watt, 9-*G.hirsutum* subsp *mexicanum var..nervosum* (Yucatan), 10-*G.hirsutum* subsp *mexicanum var.nervosum* (Victoria).

The result of PCR analysis based on the primer of the *Flowering LOCUS D* (*FLD*) gene, which controls early flowering, can be interpreted as follows: samples 2, 3, 4, 5, 6,

Содержимое доступно по лицензии Creative Commons Attribution 4.0 license (CC-BY 4.0)

7 and 10 with 125 nucleotide pairs. alleles based on PCR electrophogram: 1, 8 with 130 pairs of nucleotide alleles and 9 samples turned out to be similar to each other (Fig. 2).

The NCBI database currently contains the genome composition and sequences of diploid and tetraploid, wild and cultivated species of 30 species of the genus *Gossypium* L. However, genes directly or indirectly involved in flowering and flowering-related processes, as well as their activities, have not been fully identified in all types of cotton. In particular, despite the fact that the *FLD* gene is the main gene-phenomenon [7] in the development of flowering time and generative organs of angiosperms, including cotton, the general description, molecular mechanisms of functioning and expression of this gene are reflected in studies conducted mainly on Arabidopsis plant.

Among cotton species, this gene is described only in *G. hirsutum* L.; there is no data confirming the existence of the nucleotide sequence of this gene in other varieties of the genus *Gossypium* L. In this context, we aimed to identify the *FLD* gene in the genome of the cotton species used in our study and include the sequences in the database.

To do this, fasta ortholog files containing 3473 nucleotide sequences of the *FLD* gene of the species *G.hirsutum* L. were first downloaded from the NCBI database. Based on these sequences, 6 pairs of primers (forward and reverse (F/ R)), which synthesize a PCR product 600–700 nucleotides long (Fig. 3).

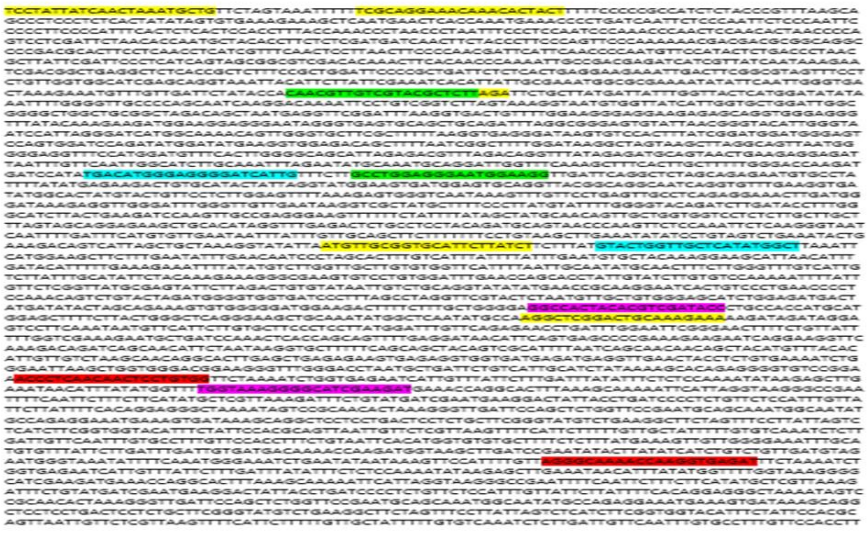

Figure 3. Primer pairs mapped to the FLD gene ortholog present in the G. hirsutum L genome.

Content is licensed under the Creative Commons Attribution 4.0 license (CC-BY 4.0)

Color pairs* represent primer pairs (F/R): 1 yellow *FLD*_1, 2 green *FLD*_2, 3 blue *FLD*_3, 4 yellow *FLD*_4, 5 pink *FLD*_5, 6 red *FLD*_6.

To determine the presence and hybridization of these primers in the genome of the studied samples, PCR screening analyzes were carried out in a volume of 50 µl.

Ensuring the purity (free from other alleles) of PCR product amplicons obtained by amplifying primers based on the *FLD* gene to the genome of research samples ensures the successful completion of the sequencing process. Accordingly, the alleles shown in the electrophorogram were excised with a scalpel under ultraviolet light using a Tanon model 3500 (China) electrophorogram (electrophoresis gel imaging device).

The PCR product from the excised agarose gel extract containing the desired amplicon was purified using the PureLink Quick Gel Extraction kit (Invitrogen) and the results were confirmed by repeated gel electrophoresis analysis. (Figure 4(b)).

Thus, the results obtained on the basis of the molecular and bioinformatic analysis carried out during the study serve as an important step in determining the nucleotide sequences of the *FLD* gene in wild diploid and tetraploid cotton varieties.

References

1. El-Din El-Assal, S., Alonso-Blanco, C., Peeters, A.J.M., Raz, V., and Koornneef, M. A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2 // Nat. Genet. – 2001. – No. 29. – Pp. 435–440. DOI: 10.1038/ng767

2. Méndez-Vigo, B., Savic, M., Ausín, I., Ramiro, M., Martín, B., Picó, F.X. Environmental and genetic interactions reveal Flowering Locus C as a modulator of the natural variation for the plasticity of flowering in Arabidopsis // Plant Cell Environ. – 2016. – No. 39. – Pp. 282–294. DOI: 10.1111/pce.12608

3. Li, P., Filiault, D., Box, M.S., Kerdaffrec, E., van Oosterhout, C., Wilczek, A.M. Multiple FLC haplotypes defined by independent cis-regulatory variation underpin life history diversity in Arabidopsis thaliana // Genes Dev. – 2014. – No. 28. – Pp. 1635–1640. DOI: 10.1101/gad.245993.114

4. Capovilla, G., Schmid, M., and Pose, D. (2015). Control of flowering by ambient temperature // J. Exp. Bot. – 2015. – No. 66. – Pp. 59–69. DOI: 10.1093/jxb/eru416. EDN: UTVRRF

Содержимое доступно по лицензии Creative Commons Attribution 4.0 license (CC-BY 4.0)

5. Michaels, S.D., He, Y., Scortecci, K.C., and Amasino, R.M. Attenuation of Flowering Locus C activity as a mechanism for the evolution of summerannual flowering behavior in Arabidopsis // Proc. Natl. Acad. Sci. – 2003. – No. 100. – Pp. 10102–10107. DOI: 10.1073/pnas.1531467100

6. National Library of Medicine. A tool for finding specific primers [Electronic resource]. – Access mode: https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome

7. The Arabidopsis Information Resource (TAIR). Locus: AT3G10390 [Electronic resource]. – Access mode: https://www.arabidopsis.org/servlets/TairObject?id=35869&type=locus

Content is licensed under the Creative Commons Attribution 4.0 license (CC-BY 4.0)