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BIOINFORMATIC ANALYSIS OF GH_SRNA5DPA12 MICRORNA

Abstract: *MicroRNAs (miRNAs) are a widely distributed and newly identified class of small non-coding RNAs, typically consisting of 20–24 nucleotides. To date, many plant miRNAs have been shown to be evolutionarily conserved across species, with some retained from mosses to angiosperms. miRNAs play crucial roles in plant development. These RNAs regulate gene expression at the post-transcriptional level by directing mRNA cleavage or repressing transcription. miRNAs are involved in nearly all stages of plant development, including seed germination, shoot apical meristem development, leaf morphogenesis, floral organ formation, and the determination of flowering time. In this study, we focused on identifying the target gene of the small RNA Gh_sRNA5dpa12, which was isolated from cotton flowers five days after pollination.*

Keywords: *microRNA, cotton, Gh_sRNA5dpa12, Far1-Related Sequence (FRS) gene family, FHY3 and FAR1, FRS (FAR1-RELATED SEQUENCE) genes*

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БИОИНФОРМАЦИОННЫЙ АНАЛИЗ MIPHK GH_SRNA5DPA12

Аннотация: *МикроРНК (миРНК) — это широко распространённый и недавно идентифицированный класс малых некодирующих РНК, как правило, состоящих из 20–24 нуклеотидов. На сегодняшний день установлено, что многие растительные миРНК эволюционно консервативны и сохраняются у различных видов, от мхов до покрытосеменных растений. миРНК играют важную роль в развитии растений. Эти РНК регулируют экспрессию генов на посттранскрипционном уровне путём направленного расщепления мРНК или ингибирования трансляции. миРНК участвуют практически на всех этапах развития растений, включая прорастание семян, развитие апикальной меристемы побега, морфогенез листьев, формирование цветочных органов и определение времени цветения. В данном исследовании*

основное внимание уделено идентификации целевого гена малой РНК Gh_sRNA5dpa12, выделенной из цветков хлопчатника на пятый день после опыления.

Ключевые слова: *микроРНК, хлопчатник, Gh_sRNA5dpa12, семейство генов Far1-Related Sequence (FRS), FHY3 и FAR1, гены FRS (FAR1-RELATED SEQUENCE)*

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GH_SRNA5DPA12 MI RNA SINING BIOINFORMATIK TAXLILI.

Abstrakt. *MikroRNKlar (miRNKlar) kodlanmaydigan 20-24 nukleotidlar ketma-ketligiga ega kichik RNKlarning keng tarqalgan yangi sinfidir. Bugungi kunga kelib, ko'pgina o'simlik miRNKlari evolyutsion tarzda turdan turga, ba'zilari angiospermlardan moxlarga qadar saqlanib qolgan. miRNKlar o'simliklar rivojlanishida alohida rol o'ynaydi. RNKlarning ushbu turi transkripsiyadan keyingi darajada maqsadli gen ekspressiyasini bevosita parchalanish yoki transkripsiya ingibitsiya qilish orqali boshqaradi. miRNKlar o'simliklarning*

deyarli barcha rivojlanish bosqichlarida masalan, nixollik davrida, kurtaklar apikal meristema rivojlanishida, barg morfogenezida, gul organining shakllanishida va gullash vaqtini aniqlashda ishtirok etadi. Ushbu maqolamizda go`za gulinnig changlangandan keying 5 kunida ajratib olingan Gh_sRNA5dpa12 kichik RNK sinning maqsadli genini aniqlash yuzasidan tadqiqotlar olib borilganligi ko`rsatib o`tilgan.

***Kalit so‘zlar**: mikroRNK, paxta, Gh_sRNA5dpa12, Far1-Related Sequence (FRS) genlar oilasi, FHY3 va FAR1, FRS (FAR1-RELATED SEQUENCE) genlari*

Introduction. In the cell, RNA molecules are divided into coding and non-coding types. Coding messenger RNAs (mRNAs) act as intermediaries between protein-coding genes and their corresponding proteins. In contrast, non-coding RNAs (ncRNAs) are functional RNA molecules transcribed from DNA that do not code for proteins but participate in various cellular processes. Non-coding RNAs include ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), as well as gene expression regulators such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) [1].

Based on their origin, structure, associated effector proteins, and biological functions, small regulatory non-coding RNAs—typically 20–24 nucleotides in length—are classified into several groups, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs, found in animals). In addition, other small RNAs that can influence the transcription process—such as promoter-associated small RNAs (PASRs), terminator-associated small RNAs (TASRs), and transcriptional boundary-associated RNAs (TBARs)—are also noteworthy [2].

In plants, siRNAs and miRNAs represent the two main classes of small RNAs (sRNAs), both of which are involved in transcriptional gene silencing and post-transcriptional gene silencing (PTGS).

MicroRNAs (miRNAs) participate in post-transcriptional gene regulation through transcript cleavage, inhibition of transcription, chromatin remodeling, and other epigenetic modifications, including DNA methylation and histone

modification [3]. Since the discovery of the first miRNA, lin-4, in the nematode Caenorhabditis elegans [4], miRNAs have been extensively studied, and numerous miRNAs have been identified in various plant species. The first plant miRNA was discovered in Arabidopsis thaliana [5].

As of today, the miRBase database has documented a total of 7,440 pre-miRNAs from 75 flowering plant species—including 59 dicots and 14 monocots. Of these, 738 mature miRNAs are encoded by 604 pre-miRNAs in Oryza sativa, while Arabidopsis thaliana contains 428 mature miRNAs derived from *326 pre-miRNAs [6]. miRNAs are widespread among plant lineages and are highly conserved across major groups, including mosses, gymnosperms, monocots, and dicots [7].

Plant miRNAs regulate gene expression by inducing mRNA cleavage or transcriptional repression [6]. They play critical roles in both the cytoplasm and nucleus, affecting gene activity through various mechanisms [7]. Primarily, plant miRNAs function via post-transcriptional mRNA cleavage, which is typically based on perfect or near-perfect complementarity to their target sequences. Imperfect base pairing, on the other hand, results in translational repression of the target mRNA [3].

MicroRNAs (miRNAs) or small RNAs are 21–24 nucleotide-long non-coding RNAs that arise from the processing of precursor genes. Similar to other organisms such as humans and animals, the plant genome also contains a large number of small RNA-encoding genes. Functional studies have revealed that small RNAs play key roles in plant growth and development, metabolism, and responses to abiotic and biotic stresses [8,9]. Due to their sequence complementarity with mRNAs, small RNAs can negatively regulate gene expression via mRNA degradation, translational repression, or chromatin modification. These regulatory effects can result in morphological and physiological abnormalities, significantly impacting plant development [10].

The regulation of mRNA transcripts by small RNAs demonstrates a unique gene expression control strategy in plants, which in turn provides opportunities to

enhance agronomic traits in crops [11]. With the advancement of high-throughput sequencing technologies, the genomes of many plant species have been sequenced, enabling the discovery of a wide array of miRNAs and other small RNAs [12]. In particular, extensive studies have been conducted in cotton (Gossypium spp.).

Cotton not only supplies natural fiber for the global textile industry but also serves as a feed source for livestock [13]. Consequently, efforts have focused on improving fiber quality and yield, promoting early maturation, and enhancing resistance to abiotic and biotic stress factors. As a result, numerous miRNAs and their target genes have been identified in cotton [14–17].

Abdurakhmonov (2008) investigated small RNAs involved in cotton fiber development from the day of pollination through the tenth day post-pollination. This research revealed that the expression of small RNAs fluctuates during various stages of fiber development. Among the small RNAs identified from 5-day post-anthesis (5 DPA) ovules, one was designated Gh_sRNA5dpa12, which is involved in the regulation of phytochrome-related photoreceptor genes, known to respond to far-red light [14]. These photoreceptor genes, particularly phytochrome A1, have been shown to play a crucial role in fiber development, fiber quality, and the enhancement of agronomic traits in cotton [18].

Therefore, in this study, we aimed to investigate the origin of the identified small RNA Gh_sRNA5dpa12 and determine its target gene.

Materials and Methods. The small RNA Gh_sRNA5dpa12 was selected from among the miRNAs reported in the study published by I.Yu. Abdurakhmonov (2008) [14]. Bioinformatic analyses, including sequence comparisons, were performed using the BLASTn tool of the NCBI GenBank database and the SEQUENCHER 5.4 software.

Results. The Gh_sRNA5dpa12 small RNA sequence was aligned against the genomes of Gossypium hirsutum, G. barbadense, G. arboreum, G. herbaceum, and G. raimondii using the NCBI GenBank database, which allowed for the

identification of its genomic origin. However, no corresponding target gene for this small RNA was identified within these cotton genomes.

Subsequent alignment with the model plant Arabidopsis thaliana genome revealed that the putative target gene of Gh_sRNA5dpa12 belongs to the Far-Red Light Related Sequence (FRS) gene family, specifically the FRS10 gene. In the next phase, the A. thaliana FRS10 gene was aligned with the G. hirsutum genome to identify the cotton ortholog targeted by Gh_sRNA5dpa12. The analysis showed that Gh_sRNA5dpa12 aligns with the second exon of the Gh_FRS10 gene in G. hirsutum with 91% sequence similarity, differing by five nucleotides. No significant similarity was observed between Gh_sRNA5dpa12 and other members of the FRS gene family.

Structural analysis of the FRS10 gene revealed that, unlike its A. thaliana counterpart—which comprises three exons and two introns—the FRS10 gene in Gossypium species consists of four exons and three introns.

Discussion. The discovery of small RNAs (sRNAs) in plants has revealed that not only genes and transcription factors play essential roles in plant development, but also that small RNAs function as key regulators at the post-transcriptional level. With the advancement of modern technologies, research in plant biotechnology over the past decade has significantly expanded to identify and characterize these regulatory sRNAs. As a result, the expression profiles of many sRNAs have been established. However, despite these efforts, the identification of specific small RNAs and their corresponding target genes in many plant species remains incomplete.

The small RNA Gh_sRNA5dpa12, which we are currently studying, also falls into this category. It was initially isolated from Gossypium hirsutum, but its target gene had not yet been identified. Through bioinformatic analysis, we determined that Gh_sRNA5dpa12 targets a gene belonging to the Far-Red Light-Related Sequence (FRS) gene family, specifically the FRS10 gene.

The FRS10 gene (Far-Red Light-Related Sequence 10) is a member of the larger FRS gene family, which is thought to have originated from transposons, based on its genetic structure and the conserved motifs and domains it contains. This gene family includes FHY3, FAR1, and other FRS (FAR1-RELATED SEQUENCE) genes [19]. In Arabidopsis thaliana, the FRS gene family is well characterized and consists of 12 known members [20]. However, this gene family has not yet been studied in cotton.

Despite the lack of data on FRS genes in cotton, studies in model plants indicate that FRS gene family members are involved in several important biological processes, including plant growth and development, flowering time regulation, and responses to abiotic and biotic stress factors.

Our findings suggest that Gh_sRNA5dpa12, isolated from cotton, targets the FRS10 gene, indicating that the expression of FRS10 may be partially regulated by this small RNA.

Conclusion. This study focused on the bioinformatic identification and target prediction of the small RNA Gh_sRNA5dpa12, previously isolated from *Gossypium hirsutum*. Although its target gene was not initially known, comparative genomic analysis revealed that Gh_sRNA5dpa12 is likely to target the FRS10 gene, a member of the Far1-Related Sequence (FRS) gene family. While the functions of FRS genes have been extensively studied in *Arabidopsis thaliana*, their roles in cotton remain largely unexplored. Our findings suggest that Gh_sRNA5dpa12 may regulate FRS10 gene expression at the post-transcriptional level, implying a potential role in cotton development or stress response. This insight lays the groundwork for further functional studies of FRS genes in cotton and highlights the significance of small RNAs in gene regulation and crop improvement.

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