

O'ZBEKISTON RESPUBLIKASI
OLIIY TA'LIM, FAN VA INNOVATSIYALAR VAZIRLIGI

FARG'ONA DAVLAT UNIVERSITETI

**FarDU.
ILMIY
XABARLAR**

1995-yildan nashr etiladi
Yilda 6 marta chiqadi

6-2025
TABIIY FANLAR

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ВЕСТНИК.
ФерГУ**

Издаётся с 1995 года
Выходит 6 раз в год

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UO'K: 633.511:577.21:577.218

G'O'ZA (*G. HIRSUTUM* L.) TOLA RIVOJLANISHINING MIKRORNK LAR TOMONIDAN BOSHQARILISHI**РЕГУЛЯЦИЯ РАЗВИТИЯ ВОЛОКНА ХЛОПЧАТНИКА (*GOSSYPIUM HIRSUTUM* L.) МИКРОРНК****REGULATION OF FIBER DEVELOPMENT IN COTTON (*GOSSYPIUM HIRSUTUM* L.) BY microRNAS****Usmanov Dilshod Erkinbayevich¹** ¹O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, biologiya bo'yicha PhD**Abdulkarimov Sharofiddin Sayfidinovich²** ²O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, kichik ilmiy xodim**Sobirov Botirjon Masharif o'g'li³** ³O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, kichik ilmiy xodim**Xusanbayeva Shahnoza Rustamjon qizi k.i.x.⁴** ⁴O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, tayanch doktorant**Abdug'afforov Azamat Tojiboy o'g'li⁵** ⁵O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, kichik ilmiy xodim**Juraqulov Durbekjon Saydulla o'g'li⁶** ⁶O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, stajyor-tadqiqotchi**Sharifjonov Abrorbek Abdujabbor o'g'li⁷** ⁷O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, kichik ilmiy xodim**Buriev Zabardast Tojiboevich⁸** ⁸ O'zbekiston Respublikasi Fanlar akademiyasi Genomika va bioinformatika markazi, b.f.d. prof**Annotatsiya**

G'o'za (*Gossypium* spp.) — jahon miqyosida iqtisodiy va ilmiy ahamiyatga ega bo'lgan asosiy texnik ekinlardan biri hisoblanadi. U asosan yuqori sifatli tabiiy tolasi uchun parvarishlanadi. Hozirgi vaqtda *Gossypium* turkumigaga mansub 50 dan ortiq yovvoyi, yarim yovvoyi va madaniy turlar mavjud bo'lib, ulardan to'rttasi *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. va *G. herbaceum* L. madaniy holda yetishtiriladi. Ular orasida *G. hirsutum* L. eng keng tarqalgan va global paxta yetishtirish hajmining 90% dan ortig'ini tashkil etadi. Mazkur tur o'zining yuqori agrobiologik salohiyati, turli iqlim sharoitlariga nisbatan moslashuvchanligi, kasalliklarga chidamliligi hamda tola sifati bilan boshqa turlardan ajralib turadi. Shu sababli, u seleksiya, genetik modifikatsiya va zamonaviy biotexnologik yondashuvlar

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yordamida tola yetishtirishda asosiy obyekt sifatida qaralmoqda. So'nggi yigirma yil ichida molekulyar biotexnologiyada qo'llanilayotgan texnologiyalar ushbu turkum genomni chuqur tadqiq qilish imkonini berdi. Ushbu maqolada g'ozaning *G.hirsutum* L. turi genomida mavjud FAR10 geni RNK texnologiyasi orqali o'rganildi. FRS10 geni ekspressiyasini pasayishi erta va to'liq unuvchanlik fenotipini namoyon qildi. Unuvchanlik nazorat O'simliklarga nisbatan 3 kunga erta va unuvchanlik fozi esa 99% tashkil etdi. Bu kabi natijalar kelgusida gen muhandisligi va seleksiya ishlarida ushbu gendan samarali foydalanish imkoniyatini beradi.

Аннотация

Хлопчатник (*Gossypium* spp.) — одна из основных технических культур, имеющая важное экономическое и научное значение в мировом масштабе. Он выращивается в основном ради получения высококачественного природного волокна. В настоящее время известно более 50 диких, полудиких и культурных видов рода *Gossypium*, среди которых четыре вида (*G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. и *G. herbaceum* L.) культивируются человеком. Среди них *G. hirsutum* L. является наиболее распространённым видом, на долю которого приходится более 90 % мирового производства хлопка. Этот вид отличается высокой агробиологической потенцией, адаптивностью к различным климатическим условиям, устойчивостью к болезням и высоким качеством волокна. Поэтому он рассматривается как основной объект для селекции, генетической модификации и современных биотехнологических подходов в производстве хлопка. За последние два десятилетия технологии молекулярной биотехнологии позволили глубоко исследовать геном этого рода. В данной статье был изучен ген FRS10 в геноме хлопчатника *G. hirsutum* L. с использованием технологии РНКи. Снижение экспрессии гена FRS10 проявило фенотип ранней и полной всхожести. Всхожесть оказалась на 3 дня раньше по сравнению с контрольными растениями, а процент всхожести составил 99 %. Подобные результаты открывают перспективы эффективного использования данного гена в генной инженерии и селекционной практике.

Abstract

Cotton (*Gossypium* spp.) is one of the most important industrial crops worldwide, with both economic and scientific significance. It is primarily cultivated for its high-quality natural fiber. Currently, more than 50 wild, semi-wild, and cultivated species of the genus *Gossypium* are known, among which four species (*G. hirsutum* L., *G. barbadense* L., *G. arboreum* L., and *G. herbaceum* L.) are domesticated. Among them, *G. hirsutum* L. is the most widespread species, accounting for over 90% of global cotton production. This species is distinguished by its high agrobiological potential, adaptability to various climatic conditions, resistance to diseases, and superior fiber quality. Therefore, it is considered the main target for breeding, genetic modification, and modern biotechnological approaches in cotton improvement. Over the past two decades, advances in molecular biotechnology have enabled in-depth studies of this genus's genome. In this study, the FRS10 gene in the genome of *G. hirsutum* L. was investigated using RNA interference (RNAi) technology. Downregulation of FRS10 expression resulted in a phenotype of early and complete germination. Germination occurred 3 days earlier compared to control plants, with a germination rate of 99%. Such results indicate that the FRS10 gene could be effectively utilized in genetic engineering and breeding programs.

Kalit so'zlar: *G. hirsutum* L., Far Red Related Sequencing 10 (FRS10), RNK interferensiya, Coker-312.

Ключевые слова: *G. hirsutum* L., Far Red Related Sequencing 10 (FRS10), РНК-интерференция, Coker-312.

Key words: *G. hirsutum* L., Far Red Related Sequencing 10 (FRS10), RNA interference, Coker-312.

KIRISH

G'oz'a dunyo iqtisodiyotida eng muhim tabiiy va sifatli tola beruvchi texnik ekinlardan biri hisoblanadi. G'oz'a dunyo to'qimachilik sanoatini tabiiy tolaga bo'lgan ehtiyojini qoplash bilan bir qatorda chorva uchun ham ozuqa manbaidir [1,2]. G'oz'a tolasi bu urug'tukchalari bo'lib, ular "trixomalari" deb ataladi. Trixomalarning tola bo'lib pishib yetilgunga qadar o'ziga xos to'rtta boshlash (initiation), cho'zilish (elongation), ikkilamchi qalinlashish (secondary thickening) va pishib yetilish (maturation) kabi bosqichlardan iborat [3]. Bu bosqichlar orasida tola rivojlanishining boshlanish davri tola salmog'ining belgilash bosqichi sifatida qaraladi, chunki bu bosqichda tolalar sonini shakllanishi boshlanadi. Tola rivojlanishining birinchi bosqichi changlanish vaqtida g'oz'a ovullarining epidermal hujayralaridan boshlanadi [4]. G'oz'a tolasi ustida olib borilgan tadqiqotlar natijasida MYB transkriptom faktorlar [5], saxoroza sinteziga javobgar (SUS) geni [6], steroid reduktaza (*GhDET2*) geni [7], arabina galaktan oqsili (*GhAGP4*) [8], *GhTUB1* [9], *MIXTA* (*GhMML4_D12*) [10], *PhyA1* [11] genlari tolaning erta rivojlanishibi boshqarilishida katta ahamiyatga ega ekanligi aniqlangan. Paxta tolasining rivojlanishining boshlanish, cho'zilishi, ikkilamchi qalinlashish va pishish bosqichlariga changlanishdan so'ng 60 kun sarflanadi. Bu vaqt ichida tolaning rivojlanishiga aloqador genlarning holati turli darajada ekspressiyalanadi [12,13,14,15,16,17]. G'oz'a tolasining transkriptom ma'lumotlarni tahlil qilish natijasida tola rivojlanishida nafaqat genlar balki kichik hamda micro RNK (siRNK va miRNK) larni ham ahamiyati kattaligi ma'lum bo'ldi [18].

Kichik RNKlar o'zining nishon genlarini transkripsiya yoki posttranskripsiya darajasida giston modifikatsiyalari va mRNKni to'g'ridan-to'g'ri parchalash hamda translyatsiyani repressiyaga uchratish orqali boshqara oladigan 18-32 nukleotid uzunligidan iborat bo'lgan endogen oligonuklotidlar hisoblanadi [19,20]. Bu kabi RNK lar 1993-yilda *C.elegans* model organizmida kashf qilinganidan buyon bu boradagi bilimlar juda keng taraqqiy etib kelmoqda [21,22,23]. Kichik RNK larning organizmlardagi ahamiyati shu darajada keng tarqalganki hujayrada kechadigan barcha morfobiokimyoviy jarayonlarning boshqarilishidagi roli o'ziga xos o'rin egallaydi [24,25,26,27].

O'simlik miRNKlari o'simlik organlari rivojlanishining turli bosqichlarida, stressga chidamlilikni oshishida, fitogormon signalida, o'sish fazasining o'zgarishida, kasalliklarga chidamliligining shakllanishida hamda erta gullash va pishib yetilishda muhim tartibga soluvchi omil sifatida ko'riladi [28,29,30,31]. Paxta tolasining rivojlanishida ham microRNK larning roli katta. Abdurakhmonov va jamoasi tomonidan tola rivojlanishining g'o'za guli changlanishdan keying 0 kundan 10 kungacha gul tugunchasini rivojlanishida microRNK larning o'rganilish, tolaning rivojlanishining har bosqichida qaysi microRNK larning ekspressiyasi kuchayishi va susayishini ochib berdi. Bu microRNK larni tahlil qilish jarayonida uzoq qizil nurga javobgar genlarga nishon qilgan microRNK lar ham aniqlandi [17]. Ushbu miRNKni bioinformatik usullar yordamida chuqur tahlil qilinishi uning nishon geni fotoretseptor FHY3/FAR1 genlar oilasining vakili FRS10 gen ekanligi ma'lum bo'ladi. Fotoretseptor genlar, xususan fitoxromA1 geni g'o'za tolasining rivojlanishi va sifatining yaxshilanishida muhim o'rin tutishi olib borilgan tadqiqotlarda aniqlangan [11]. Fotoretseptor genlar oilasi 1993-yili Whitelam tomonidan *Arabidopsis thaliana* mutant o'simliklarini tadqiq qilinishi natijasida uzoq qizil nurga sezuvchanligi past bo'lgan namunalarda aniqlandi. Bu o'simliklar uzoq qizil nur ostida boshqa nurlardan farqli ravishda gipokotel uzunligini namoyon qilgan. Ularni *far-red elongated hypocotyls1 (fhy1)*, *fhy2*, *fhy3* deb nomlangan [32]. FHY1 geni va uning gamologi FHL (FHY1-LIKE) fotomorfogenezni boshqarishda ishtrok etadi [33]. FHY2 esa fitoxromA genini kodlaydi va uzoq qizil nurga javob beradi [32]. FHY3 va uning gomologi FAR-RED IMPAIRED RESPONSE1 (FAR1) transpozonlardan kelib chiqqan transkripsiya faktorlarini kodlaydi [34]. FHY3 va uning homologi FAR1 o'simliklarning o'sishi va rivojlanishida hal qiluvchi ahamiyatga ega. Bundan tashqari, FHY3 va FAR1 FRS (FAR1-RELATED SEQUENCE) va FRF (FRS-RELATED FACTOR) oilalarining asoschilaridir [35]. *A.thaliana*da FRS genlar oilasining 12 ta, FRF ning 4 ta vakillari aniqlangan [36]. FHY3 va FAR1dan tashqari, ko'pgina FRS va FRF oilaviy oqsillarining fiziologik va molekulyar mexanizmlari va ularning o'simlik morfologik tuzilishidagi funksiyalari noma'lum bo'lib qolmoqda [35]. FRS oilasi vakillari (FRS6, FRS8, FRS9) genlarining *A.thaliana*da funksiyasi aniqlangan [35]. Bu oilaning boshqa vakillarining o'simlikdagi funksiyasi haqida ma'lumotlar deyarli yo'q. Ushbu oila vakillarining g'o'za turilarida mavjudligi bioinformatik tahlillarga asoslanib o'rganilgan bo'lsada, ularning funksiyalari o'rganilmagan. Ushbu malolqada g'o'za guli changlanishdan keyingi tola rivojlanishining 5 kunlik tugunchasidan ajratib olingan GH5DPA12 miRNKning [17] nishon geni bo'lgan FHY3/FAR1 genlar oilasining vakili FRS10 genini g'o'za tolasini morfologiyasida tutgan o'rni haqidagi dastlabki ma'lumotlar keltirilgan.

MATERIAL VA METODLAR

Tadqiqotimizda Gh5DPA12 miRNK [17] asosida olingan RNKi genetik konstruksiyasiga ega g'o'za o'simligining *G.hirsutum* L. turiga mansub Coker-312 tizmasida foydalanildi. RNKi konstruksiyasini tutgan o'simliklarni tuproq sharoitida parvarishlab, tajriba olib borildi. Tajriba Genomika va bioinformatika markazning Maxsus Urug'chilik tajriba xo'jaligida 90x20x1 sxemasi asosida uch takrorda, xar bir takror 35 ta o'simlikda ekib o'rganildi. Tajribada RNKi genetik konstruksiyasiga ega bo'lmagan T1 avlodidan ajralib chiqqan nol segregant va Coker-312 tizmalaridan nazorat o'simlik sifatida foydalanildi. O'simliklarni molekulyar tahlillardan o'tkazishda nihollik davrida amalga oshirildi. O'simliklar barigidan genom DNKsi Delloport usulida ajratildi [37]. Ajratilgan gDNK lar NanoDrop 1000 Spektrofotometr yordamida sifat va miqdor tekshirildi va 50 ng/ul keltirildi hamda RNKi gen-konstruksiya uchun maxsus praymerlar yordamida PZR tahlillari o'tkazildi. Amplifikatsiya jarayonida olingan fragmentlari 1,5 % agarozda gelida tekshirildi. Morfologik belgilarni hisoblashda Excel va ImageJ bundled with 64-bit Java 1.8.0_112(70MB) dasturlaridan foydalanildi.

NATIJA VA MUHOKAMA

Tajriba maydonchasiga ekilgan chigitlar 7 kun o'tgach RNKi tizmalarda unib chiqish boshlandi va ushbu jarayon o'simliklarda unib chiqishlar soni 50 foizga yetganda hisobga olinib boshlandi.

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Ushbu fazada RNKi chigitlari nazorat o'simliklardan 3 kun erta unib chiqqanligi aniqlandi. Chigitlarning unib chiqish foizida ham farqlar kuzatildi. RNKi tizmalarda unib chiqish foizi o'rtacha 97,3% ni, nazorat o'simliklarda: Coker-312 da 94% va nol segregantda 94,6% ni tashkil etdi. Erta unib chiqqan o'simliklarni RNKi genetik konstruksiyaga ega ekanligini PZR yordamida konstruksiya uchun maxsus praymerlar yordamida identifikatsiya qilindi. Identifikatsiya natijalariga ko'ra 99% o'simliklarda RNKi genetik vektor konstruksiyasi mavjud ekanligi aniqlandi. O'simliklarda unuvchanlik ko'rsatkichidan tashqari ularning o'sishi va rivojlanishida ham sezilarli farqlar kuzatildi. RNKi tizmalar fenotipik jihatdan nazorat o'simliklardan erta gullash, gipokotel qismining uzayganligi, ildiz tizimida ikkilamchi yoni ildizlar hamda hosil shoxlarining soni ortganligi bilan farq qilishi kuzatildi.

Chigit unuvchanligi ko'p hollarda fizologik hodisa bo'lib, unuvchanlik qobiliyati ularning saqlash va urug'larning yarovizatsiya davrini o'tashiga bog'liq hisoblanadi. Tinim davrini o'tagan chigitlarda unuvchanlik foiz katta bo'lib, deyarli to'liq unib chiqadi [38]. Biz olib borgan tadqiqotda fotoretseptor genlar ham unuvchanlik jarayonini boshqarilishda ishtirok etishi ma'lum bo'ldi. G'o'za o'simligida FRS10 geni ekspressiyasini susayishi urug'larda erta unuvchanlik fenotipini keltirib chiqardi. Shu kabi xolat *A.thaliana* mutant o'simliklarida kuzatilgan. *A.thaliana*da FRS6 geni ekspressiyasini susayishi ular erta unuvchanlik fenotipini ko'rsatgan [36]. Bundan tashqari ushbu genlar oilasi vakillarining bog'lanish geni hisoblangan fitoxrom A geni ekspressiyasini g'o'za o'simligida susayishi erta unuvchanlik, gipokotel uzayish, erta gullash va pishish kabi bir qancha fenotipik belgilarni namoyon qilgan [11]. Bizning tadqiqotimizda g'o'za GhFRS10 geni ekspressiyasini susayishi RNKi o'simliklarda erta unuvchanlik, gipokotel uzayish va ildiz tizimini kuchli rivojlanishi belgilarini namoyon bo'lish, FRS genlar va fitoxrom A geni o'rtasida kuchli bog'liqlik borligini yana bir bor isbotlaydi. O'z navbatida bu genning o'simlikdagi funksiyasining kamayishi o'simliklarga ijobiy ta'sir ko'rsatishi ma'lum bo'ldi.

XULOSA

Natijalardan shu narsa ma'lum bo'ldiki o'simliklarda miRNA lar ko'pgina morfobiologik jarayonlarning borishiga katta ta'sir o'tkazadi. Xususan g'o'zaning 5 kunlik shona tugunchasidan ajratilgan miRNA sintezining ko'payishi FRS10 genini faoliyatini to'xtatadi. Natijada g'o'zada eng muhim ko'rsatkichlardan hisoblangan unuvchanlik ko'rsatkichi ertaligi aniqlandi. Demak bu genning *G.hirsutum* L. turida faoliyatining kamayishi chigit unuvchanligini erta boshlanishiga va unuvchanlik foizini katta bo'lishiga olib keladi. Bu kabi ijobiy natijalar FRS genlar oilasi vakillarini g'o'za o'simligini morfologik shakllanishda katta ahamiyatga ega ekanligini ko'rsatadi.

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