**Isomiddinova Orastabonu Lutfiddinovna**

Tayanch doktorant

**Kamburova Venera Seitumerovna**

Ilmiy ishlar bo’yicha direktor o’rinbosari b.f.d.

**Raxmatova Nodira Rahimovna**

O’simliklar proteomika va metabolomika laboratoriyasi mudiri, PhD.

**Imamxojaeva Azoda Sobirovna**

Bo'limi mudiri, PhD.

**Mamatkulova Shahnoza Xakberdiyevna**

Tayanch doktorant.

O’ZRFA Genomika va bioinformatika markazi

e-mail: isomiddinovaorastabonu@gmail.com

# GEN-NOKAUT QILINGAN G’O’ZA NAVLARINING NISHON BO‘LMAGAN ORGANIZMLARGA TA’SIR QILISH EHTIMOLINI BAHOLASH

***Annotatsiya.*** *Ushbu maqolada pHellsgate-8::PHYA1 gen muhandisligi konstruksiyasi asosida yaratilgan gen-nokaut navlarining nishon bo‘lmagan organizmlarning genomlariga ta’sir qilish ehtimoli baholangan. Tadqiqot ob’ektlari sifatida quyidagi organizmlar tanlangan: odam (Homo sapiens), asalari (Apis mellifera), qizilqanot qo‘ng‘iz (C. septempunctata), g’o’za kuzgi g‘ujasi (Helicoverpa armigera) va g’o’za bitasi (Aphis gossypii). Bu organizmlarning genomlari GenBank ma’lumotlar bazasi orqali olingan. Gen-nokaut g’o’za navlarining nishon bo‘lmagan ta’sirlarini baholash ESFA tomonidan GMO (genetik modifikatsiyalangan organizmlar) xavfsizligini aniqlash uchun tavsiya etilgan bioinformatik tahlil qoidalariga muvofiq amalga oshirildi. Tadqiqot davomida ketma-ketliklar o‘xshashligini baholash uchun E-value testi qo‘llanildi.*

*Natijalarga ko‘ra, siRNA molekulalarining yo‘naltiruvchi va yo‘lovchi iplariga nisbatan nishon bo‘lmagan organizmlarning genomlarida sezilarli gomologiya aniqlanmadi. Tadqiqot natijalari shuni ko‘rsatadiki, pHellsgate-8::PHYA1 konstruksiyasi nishon bo‘lmagan organizmlar uchun zararli ta’sir ko‘rsatmaydi. Bu holat transgen g’o’zadan genlarning vertikal yo‘l bilan uzatilish ehtimoli past ekanligini bildiruvchi adabiyotlar ma’lumotlariga ham mos keladi.*

***Kalit so‘zlar**: genetik jihatdan modifikatsiyalangan organizmlar, gen-nokaut navlari, g’o’za, ekologik xavf, nishon bo‘lmagan ta’sir.*

**Исомиддинова Орастабону Лутфиддиновна**
Аспирантка
**Камбурова Венера Сеитумеровна**
Заместитель директора по научной работе, к.б.н.
**Рахматова Нодира Рахимовна**
Заведующая отделом, PhD
**Имамходжаева Азода Собировна**
Заведующая отделом, PhD
**Маматкулова Шахноза Хакбердиевна**
Аспирантка
Центр геномики и биоинформатики АН РУз
e-mail: isomiddinovaorastabonu@gmail.com

## ОЦЕНКА ВЕРОЯТНОСТИ НЕЦЕЛЕВОГО ВОЗДЕЙСТВИЯ ГЕН-НОКАУТНЫХ СОРТОВ ХЛОПЧАТНИКА

***Аннотация.** В статье проведена оценка вероятности нецелевого воздействия ген-нокаутных сортов хлопчатника, созданных с помощью генно-инженерной конструкции pHellsgate-8::PHYA1 к гену фитохрома A1 хлопчатника Gossypium hirsutum L., на геномы нецелевых организмов: человека (Homo sapiens), медоносной пчелы (Apis mellifera), божьей коровки (C. septempunctata), хлопковой совки (Helicoverpa armigera) и хлопковой тли*

*(Aphis gossypii). Доступ к геномам нецелевых организмов был получен через GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).*

*Оценка риска вероятности нецелевого воздействия ген-нокаутных сортов хлопчатника проводилась в соответствии с рекомендациями ESFA о порядке выполнения биоинформатического анализа для идентификации потенциальных нецелевых генов в рамках оценки безопасности ГМ-растений на основе РНК-интерференции. Для оценки достоверности гомологии исследуемых последовательностей был выбран E-value тест.*

*В результате исследования выявлено, что геном ни одного из нецелевых организмов не дает достаточной гомологии ни с направляющей, ни с пассажирской цепью siRNA.*

*Полученные данные позволяют утверждать, что исследуемая генетическая конструкция pHellsgate-8::PHYA1 к гену фитохрома A1 хлопчатника Gossypium hirsutum L. не обладает нежелательными эффектами на нецелевые организмы. Результаты работы совпадают с имеющимися литературными данными о низкой вероятности вертикального переноса генов от трансгенного хлопчатника.*

***Ключевые слова:*** *генетически модифицироанные организмы, ген-нокаутные сорта, хлопчатник, экологические риски, нецелевое воздействие.*

**Isomiddinova Orastabonu Lutfiddinovna**
PhD Candidate
**Kamburova Venera Seitumerovna**
Deputy Director for Scientific Work, PhD
**Rakhmatova Nodira Rahimovna**
Head of Department, PhD
**Imamkhojaeva Azoda Sobirovna**
Head of Department, PhD
**Mamatkulova Shakhnoza Khakberdiyevna**
PhD Candidate
Center of Genomics and Bioinformatics, ASU Academy of Sciences of the Republic of Uzbekistan

e-mail: isomiddinovaorastabonu@gmail.com

***Abstract:*** *The article assesses the likelihood of non-target effects of gene-knockout cotton varieties created using the genetic engineering construct pHellsgate-8::PHYA1 on the genomes of non-target organisms: humans (Homo sapiens), honey bees (Apis mellifera), ladybug (C. septempunctata), bollworm (Helicoverpa armigera) and cotton aphid (Aphis gossypii). Genomes of non-target organisms were accessed through GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The risk assessment of the potential for off-target effects of gene-knockout cotton varieties was carried out in accordance with the ESFA guidelines on the procedure for performing bioinformatics analysis to identify potential off-target genes in the framework of RNA interference-based safety assessment of GM plants. To assess the reliability of the homology of the studied sequences, the E-value test was chosen.*

*As a result of the study, it was revealed that the genome of none of the non-target organisms provides sufficient homology with either the guide or the passenger strand of siRNA.*

*The data obtained allow us to state that the studied genetic construct pHellsgate-8::PHYA1 does not have undesirable effects on non-target organisms. The results of the work coincide with the available literature data on the low probability of vertical gene transfer from transgenic cotton.*

***Key words:*** *genetically modified organisms, gene knockout varieties, cotton, environmental risks, non-target effects.*

**Kirish**. Transgen mahsulotning maqsadsiz biologik ta’sirlariga doir xavflarni aniqlash va baholash ilmiy nuqtai nazardan eng murakkab masalalardan biridir. Bir tomondan, toksik moddalarning ta’sir doirasiga tushishi mumkin bo‘lgan oziq-ovqat zanjiri ishtirokchilarini, shu jumladan yirtqichlik va parazitizmga oid barcha potentsial ekologik aloqalarni to‘liq aniqlash va hisobga olish murakkab [1,2]. Boshqa tomondan, ko‘plab biotik va abiotik omillar mavjudki, ularni laboratoriya yoki cheklangan dala sharoitida to‘liq takrorlash deyarli imkonsiz. Bunday omillar toksik ta’sir ehtimoli, intensivligi va shakliga sezilarli ta’sir ko‘rsatadi. RNA

aralashuvi (RNAi) yoki gen tahrirlash texnologiyalariga asoslangan genetik konstruktsiyalar tomonidan yuzaga keladigan kechikkan ta’sirlar – ya’ni toksinning birlamchi ta’siri bilan maqsadsiz nishon o‘rtasida bir necha vositachilar bo‘lgan hollarda – yuzaga chiqishi uchun bir necha yil va organizmlarning bir necha avlodi o‘tishi kerak bo‘lishi mumkin. Shu sababli, transgen mahsulotlarning maqsadsiz ta’siri va uning oqibatlari bo‘yicha ishonchli xulosa faqat uzoq muddatli monitoring asosida, turli ekologik sharoitlarda, bir yoki bir nechta bog‘liq biologik ob’ektlar ustida o‘tkazilgan keng miqyosli tajribalar orqali berilishi mumkin [3,4]. GMOlarning maqsadsiz ta’sirlarini baholash uchun zarur bo‘lgan eng muhim ma’lumotlar quyidagilardan iborat: transgen belgini olgan organizmning tabiiy tarqalish hududlari, uning yashash muhitidagi boshqa organizmlar (ayniqsa hayvonlar) bilan potentsial ekologik o‘zaro aloqalari (ya’ni retsipient yoki parental organizmning biologik xususiyatlari). Shuningdek, transgenning toksik potensialini va ta’sir xarakterini aniqlashda, genetik modifikatsiya qanday donor organizmga tegishli ekanligi, transgen faoliyatiga ta’sir etuvchi regulyator va boshqa funksional elementlar haqida ma’lumotlar muhim hisoblanadi. GMOning biologik xususiyatlarini tavsiflashda quyidagilar hisobga olinadi: transgen tomonidan kodlangan oqsilning faolligi va xossalari, transgen ekspressiyasining darajasi hamda uning qaysi o‘simlik qismlarida ifodalanishi (masalan, faqat yashil qismlarda yoki changda ham) [4]. GMOning atrof-muhit bilan o‘zaro ta’sirini baholashda esa, GMO ta’siriga tushishi mumkin bo‘lgan nishon va maqsadsiz organizmlarni aniqlash va tavsiflash, shuningdek, ularning o‘zaro ta’sir mexanizmi va kutilgan natijalari to‘g‘risidagi ma’lumotlar muhim ahamiyat kasb etadi. Agar GMO bioreaktor sifatida ishlatiladigan bo‘lsa, u holda undan foydalanish sharoitlarini baholashda ekin maydonini yovvoyi hayvonlarning kirib kelishidan himoya qilish choralariga alohida e’tibor qaratilishi kerak [4]. RNAi konstruktsiyalarining maqsadsiz biologik ta’sirlarini prognoz qilish uchun bioinformatik yondashuvlardan foydalanish tavsiya etiladi [4]. Bu metodlar orqali ifodalanadigan RNKning organizm genomidagi ichki genlar bilan o‘xshashlik darajasi (gomologiyasi) aniqlanadi [5,6]. Shu sababli, bunday vositalardan

foydalanish maqsadsiz ta’sirlarni minimallashtirish nuqtai nazaridan maqsadga muvofiq sanaladi [5]. Shu munosabat bilan, mazkur tadqiqotning maqsadi – pHellsgate-8::PHYA1 gen konstruksiyasining quyidagi maqsadsiz organizmlar genomiga ehtimoliy ta’sirini baholashdan iborat bo‘ldi: odam (*Homo sapiens*), asalari (*Apis mellifera*), qizilqanot qo‘ng‘iz (*Coccinella septempunctata*), g’o’za kuzgi g‘ujasi (*Helicoverpa armigera*) va g’o’za bitasi (*Aphis gossypii*).

**Tadqiqot materiallari va metodikasi**. Tadqiqot materiali sifatida pHellsgate-8::PHYA1 gen-muhandislik konstruksiyasi, shuningdek, quyidagi organizmlarning annotatsiyalangan genetik ma’lumotlari tanlab olindi: odam (*Homo sapiens*), asalari (*Apis mellifera*), qizilqanot qo‘ng‘iz (*Coccinella septempunctata*), g’o’za kuzgi g‘ujasi (*Helicoverpa armigera*) va g’o’za bitasi (*Aphis gossypii*). Ularning genomlariga GenBank ma’lumotlar bazasi orqali kirish ta’minlandi (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Gen-nokaut qilingan g’o’za navlarining maqsadsiz biologik ta’sir ehtimolini baholash Yevropa oziq-ovqat xavfsizligi agentligi (ESFA) tomonidan RNA aralashuvi asosidagi genetik modifikatsiyalangan o‘simliklar xavfsizligini aniqlash uchun tavsiya etilgan bioinformatik tahlil metodikasi asosida amalga oshirildi [7]. Kichik interferens RNK (siRNA) molekulalarining taxminiy baholanishi va tuzilishi siDirect 2.0 onlayn vositasi (http://siDirect2.RNAi.jp/) yordamida amalga oshirildi [8]. Baholash jarayonida Ui-Tei, Amarzguioui va Reynolds qoidalaridan foydalanildi [8]. Har qanday maqsadsiz ta’sir ehtimolini minimallashtirish maqsadida, boshlang‘ich siRNA-dupleksning barqarorligini nazorat qilish uchun maksimal erish harorati (Tm) 21,5°C darajasida belgilandi.

Taxminiy baholangan siRNA molekulalari keyinchalik NCBI (National Center for Biotechnology Information) bazasidagi odam (Homo sapiens) genom va transkriptom ma’lumotlari bilan solishtirilib, ketma-ketliklar o‘xshashligi (gomologiyasi) mavjudligini aniqlash uchun skriningdan o‘tkazildi. Bundan tashqari, ushbu siRNA molekulalari asalari (Apis mellifera), qizilqanot qo‘ng‘iz (C. maculata), g’o’za kuzgi g‘ujasi (Helicoverpa armigera), g’o’za bitasi (Aphis gossypii) hamda g’o’za tripsi (Thrips gossypii Jakh.) genomlari bilan ham

taqqoslandi. Ma’lumotlar GenBank orqali olinib, BLAST (blastn) algoritmi – ya’ni o‘xshash bo‘lgan nukleotid ketma-ketliklarini aniqlovchi tizim yordamida tahlil qilindi [9].

BLAST parametrlari qisqa so‘rov ketma-ketliklari bilan aniq moslikka erishish uchun quyidagicha sozlandi: segment uzunligi — 16 nukleotid, kutilyotgan (E-value) chegarasi — 1000 qilib belgilandi, bu esa maxsuslikni yuqori darajada nazorat qilish imkonini berdi. Qamrovi 85% va undan yuqori bo‘lgan siRNAlar tahlildan chiqarib tashlandi. Gapocha ochish, kengaytirish va mos kelmaslik uchun belgilangan jarima qiymatlari mos ravishda 2, 2 va -3 etib belgilandi.

**Tadqiqot natijalari**. ESFA tomonidan ishlab chiqilgan ko‘rsatmalarga asoslanib, pHellsgate-8::PHYA1 konstruksiyasi orqali RNA aralashuvi (RNAi) jarayonida yuzaga kelishi mumkin bo‘lgan siRNA molekulalarining maqsadsiz ta’sir ehtimoli baholandi. Buning uchun eng samarali deb topilgan taxminiy baholangan siRNA molekulalarining yo‘naltiruvchi va yo‘lovchi (passajir) zanjirlari quyidagi organizmlarning annotatsiyalangan genomlari bilan solishtirildi: odam (*Homo sapiens*), asalari (*Apis mellifera*), qizilqanot qo‘ng‘iz (*Coccinella septempunctata*), g’o’za kuyasi (*Helicoverpa armigera*) va g’o’za bitasi (*Aphis gossypii*). Genetik ma’lumotlar GenBank ma’lumotlar bazasi orqali olindi (http://blast.ncbi.nlm.nih.gov/Blast.cgi) va ketma-ketliklar o‘xshashligini aniqlash uchun BLAST algoritmiga (blastn — bir nechta o‘xshash ketma-ketliklar uchun) murojaat qilindi [9].

Tadqiqotda siRNA ketma-ketliklari bilan organizm genomlari o‘rtasidagi gomologik o‘xshashlik darajasining ishonchliligini baholash uchun E-value testi tanlandi. Tahlil natijalari 1-jadvalda keltirilgan. Jadval ma’lumotlariga ko‘ra, o‘rganilgan organizmlardan hech birining genomi siRNAning yo‘naltiruvchi yoki yo‘lovchi zanjirlari bilan etarlilik darajasida gomologik moslik namoyon qilmagan.

1-jadval – siRNA yo‘naltiruvchi va yo‘lovchi zanjirlarining maqsadsiz ta’siriga oid BLASTN tahlil natijalari

| Tavsif | Max Score | Total Score | Query Cover | E value | Per. Ident |
|---|---|---|---|---|---|
| ***Homo sapiens*** | | | | | |
| **Yo'naltiruvchi zanjir** | | | | | |
| Homo sapiens chromosome 1, GRCh38.p14 Primary Assembly | 36.2 | 72.4 | 95% | 0.21 | 100% |
| Homo sapiens chromosome 6, GRCh38.p14 Primary Assembly | 36.2 | 70.4 | 85% | 0.21 | 100% |
| Homo sapiens chromosome 5, GRCh38.p14 Primary Assembly | 34.2 | 34.2 | 80% | 0.82 | 100% |
| Homo sapiens chromosome 8, GRCh38.p14 Primary Assembly | 34.2 | 34.2 | 80% | 0.82 | 100% |
| Homo sapiens chromosome 18, GRCh38.p14 Primary Assembly | 34.2 | 34.2 | 80% | 0.82 | 100% |
| **Yo'lovchi zanjir** | | | | | |
| Homo sapiens chromosome 1, GRCh38.p14 Primary Assembly | 36.2 | 36.2 | 85% | 0.21 | 100% |
| Homo sapiens chromosome 6, GRCh38.p14 Primary Assembly | 36.2 | 36.2 | 85% | 0.21 | 100% |
| Homo sapiens chromosome 7, GRCh38.p14 Primary Assembly | 34.2 | 34.2 | 80% | 0.83 | 100% |
| Homo sapiens chromosome 8, GRCh38.p14 Primary Assembly | 34.2 | 34.2 | 80% | 0.83 | 100% |
| Homo sapiens chromosome 12, GRCh38.p14 Primary Assembly | 34.2 | 68.4 | 80% | 0.83 | 100% |
| Homo sapiens chromosome 18, GRCh38.p14 Primary Assembly | 34.2 | 34.2 | 80% | 0.83 | 100% |
| ***C. septempunctata*** | | | | | |
| **Yo'naltiruvchi zanjir** | | | | | |
| Coccinella septempunctata genome assembly, chromosome: 8 | 32.2 | 86.7 | 90% | 0.46 | 100% |
| PREDICTED: Coccinella septempunctata WD repeat-containing protein 26 (LOC123309530), transcript variant X2, mRNA | 30.2 | 30.2 | 71% | 1.8 | 100% |
| PREDICTED: Coccinella septempunctata WD repeat-containing protein 26 (LOC123309530), transcript variant X1, mRNA | 30.2 | 30.2 | 71% | 1.8 | 100% |
| Coccinella septempunctata genome assembly, chromosome: 2 | 30.2 | 58.5 | 95% | 1.8 | 100% |
| Coccinella septempunctata genome assembly, chromosome: 3 | 30.2 | 30.2 | 71% | 1.8 | 100% |
| PREDICTED: Coccinella septempunctata carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase 1 (LOC123310748), mRNA | 22.3 | 22.3 | 52% | 445 | 100% |
| PREDICTED: Coccinella | 22.3 | 22.3 | 52% | 445 | 100% |

| Tavsif | Max Score | Total Score | Query Cover | E value | Per. Ident |
|---|---|---|---|---|---|
| septempunctata actin-related protein 2/3 complex subunit 5-C (LOC123310855), mRNA | | | | | |
| **Yo‘lovchi zanjir** | | | | | |
| PREDICTED: Coccinella septempunctata WD repeat-containing protein 26 (LOC123309530), transcript variant X2, mRNA | 32.2 | 32.2 | 76% | 0.46 | 100% |
| PREDICTED: Coccinella septempunctata WD repeat-containing protein 26 (LOC123309530), transcript variant X1, mRNA | 32.2 | 32.2 | 76% | 0.46 | 100% |
| Coccinella septempunctata genome assembly, chromosome: 8 | 32.2 | 32.2 | 76% | 0.46 | 100% |
| Coccinella septempunctata genome assembly, chromosome: 3 | 32.2 | 32.2 | 76% | 0.46 | 100% |
| Coccinella septempunctata genome assembly, chromosome: 5 | 30.2 | 30.2 | 71% | 1.8 | 100% |
| Coccinella septempunctata genome assembly, chromosome: 2 | 30.2 | 60.5 | 95% | 1.8 | 94.74% |
| PREDICTED: Coccinella septempunctata homeobox protein PKNOX2-like (LOC123320252), transcript variant X1, mRNA | 22.3 | 22.3 | 52% | 445 | 100% |
| ***Apis melifera*** | | | | | |
| **Yo’naltiruvchi zanjir** | | | | | |
| PREDICTED: Apis mellifera succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (LOC550667), transcript variant X2, mRNA | 32.2 | 32.2 | 76% | 0.13 | 100.00% |
| PREDICTED: Apis mellifera succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (LOC550667), transcript variant X1, mRNA | 32.2 | 32.2 | 76% | 0.13 | 100.00% |
| PREDICTED: Apis mellifera uncharacterized LOC113219042 (LOC113219042), transcript variant X2, mRNA | 30.2 | 30.2 | 71% | 0.51 | 100.00% |
| PREDICTED: Apis mellifera uncharacterized LOC113219042 (LOC113219042), transcript variant X1, mRNA | 30.2 | 30.2 | 71% | 0.51 | 100.00% |
| PREDICTED: Apis mellifera titin (LOC551259), transcript variant X1, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Apis mellifera | 28.2 | 28.2 | 85% | 2.0 | 94.44% |

| Tavsif | Max Score | Total Score | Query Cover | E value | Per. Ident |
|---|---|---|---|---|---|
| putative pre-mRNA-splicing factor ATP-dependent RNA helicase PRP1 (LOC551824), transcript variant X2, mRNA | | | | | |
| PREDICTED: Apis mellifera putative pre-mRNA-splicing factor ATP-dependent RNA helicase PRP1 (LOC551824), transcript variant X1, mRNA | 28.2 | 28.2 | 85% | 2.0 | 94.44% |
| **Yo‘lovchi zanjir** | | | | | |
| PREDICTED: Apis mellifera succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (LOC550667), transcript variant X2, mRNA | 32.2 | 32.2 | 76% | 0.13 | 100.00% |
| PREDICTED: Apis mellifera succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (LOC550667), transcript variant X1, mRNA | 32.2 | 32.2 | 76% | 0.13 | 100.00% |
| PREDICTED: Apis mellifera E3 ubiquitin-protein ligase RNF123 (LOC410136), transcript variant X2, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Apis mellifera putative pre-mRNA-splicing factor ATP-dependent RNA helicase PRP1 (LOC551824), transcript variant X2, mRNA | 28.2 | 28.2 | 85% | 2.0 | 94.44% |
| PREDICTED: Apis mellifera putative pre-mRNA-splicing factor ATP-dependent RNA helicase PRP1 (LOC551824), transcript variant X1, mRNA | 28.2 | 28.2 | 85% | 2.0 | 94.44% |
| PREDICTED: Apis mellifera uncharacterized LOC113219042 (LOC113219042), transcript variant X2, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Apis mellifera uncharacterized LOC113219042 (LOC113219042), transcript variant X1, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Apis mellifera putative deoxyribonuclease TATDN1 (LOC411837), transcript variant X2, mRNA | 26.3 | 26.3 | 61% | 7.9 | 100.00% |
| PREDICTED: Apis mellifera putative deoxyribonuclease TATDN1 (LOC411837), transcript variant X1, mRNA | 26.3 | 26.3 | 61% | 7.9 | 100.00% |

| Tavsif | Max Score | Total Score | Query Cover | E value | Per. Ident |
|---|---|---|---|---|---|
| ***Helicoverpa armigera*** | | | | | |
| **Yo'naltiruvchi zanjir** | | | | | |
| PREDICTED: Helicoverpa armigera serine/threonine-protein kinase ATR (LOC110375320), mRNA | 30.2 | 30.2 | 71% | 0.52 | 100.00% |
| PREDICTED: Helicoverpa armigera myelin transcription factor 1 (LOC110371529), transcript variant X3, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera myelin transcription factor 1 (LOC110371529), transcript variant X2, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera myelin transcription factor 1 (LOC110371529), transcript variant X1, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera hemocytin (LOC110369772), transcript variant X3, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera hemocytin (LOC110369772), transcript variant X2, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera hemocytin (LOC110369772), transcript variant X1, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera prominin-like protein (LOC110378473), transcript variant X10, mRNA | 24.3 | 24.3 | 57% | 32 | 100.00% |
| PREDICTED: Helicoverpa armigera SPRY domain-containing protein 7 (LOC110381373), mRNA | 24.3 | 24.3 | 57% | 32 | 100.00% |
| PREDICTED: Helicoverpa armigera tyrosine-protein kinase transmembrane receptor Ror (LOC110384348), mRNA | 24.3 | 24.3 | 57% | 32 | 100.00% |
| **Yo'lovchi zanjir** | | | | | |
| PREDICTED: Helicoverpa armigera serine/threonine-protein kinase ATR (LOC110375320), mRNA | 30.2 | 30.2 | 71% | 0.52 | 100.00% |
| PREDICTED: Helicoverpa armigera uncharacterized LOC110377987 | 28.2 | 28.2 | 85% | 2.0 | 94.44% |

| Tavsif | Max Score | Total Score | Query Cover | E value | Per. Ident |
|---|---|---|---|---|---|
| (LOC110377987), mRNA | | | | | |
| PREDICTED: Helicoverpa armigera hemocytin (LOC110369772), transcript variant X2, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera hemocytin (LOC110369772), transcript variant X1, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera serine/threonine-protein kinase SMG1 (LOC110375567), transcript variant X2, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera serine/threonine-protein kinase SMG1 (LOC110375567), transcript variant X1, mRNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| 35D18_HaBAC_fin, Helicoverpa armigera BAC, pupae DNA | 28.2 | 28.2 | 66% | 2.0 | 100.00% |
| PREDICTED: Helicoverpa armigera ras-related protein Rab-32 (LOC110383771), transcript variant X2, mRNA | 26.3 | 26.3 | 61% | 8.1 | 100.00% |
| PREDICTED: Helicoverpa armigera alsin (LOC110371117), transcript variant X1, mRNA | 24.3 | 24.3 | 57% | 32 | 100.00% |
| 33M07_HaBAC_fin, Helicoverpa armigera BAC, pupae DNA | 22.3 | 22.3 | 52% | 126 | 100.00% |
| ***Aphis gossypii*** | | | | | |
| **Yo'naltiruvchi zanjir** | | | | | |
| Aphis gossypii genome assembly, chromosome: 2 | 32.2 | 60.5 | 85% | 0.38 | 100.00% |
| Aphis gossypii genome assembly, chromosome: 1 | 30.2 | 147 | 95% | 1.5 | 100.00% |
| **Yo'lovchi zanjir** | | | | | |
| Aphis gossypii genome assembly, chromosome: 2 | 32.2 | 62.4 | 90% | 0.38 | 100.00% |
| Aphis gossypii genome assembly, chromosome: 3 | 30.2 | 30.2 | 71% | 1.5 | 100.00% |
| Aphis gossypii genome assembly, chromosome: 1 | 30.2 | 116 | 85% | 1.5 | 100.00% |

Shunday qilib, *Homo sapiens* transkriptlari uchun E-testi qiymati yo'naltiruvchi zanjirda 0,21–0,82, yo'lovchi zanjirda esa 0,21–0,83 diapazonida o'zgarib turdi. *C. septempunctata* genomi uchun ushbu qiymat yo'naltiruvchi va yo'lovchi zanjirda 0,46–445 oralig'ida edi. *Apis mellifera* uchun E-testi qiymati

yo‘naltiruvchi zanjirda 0,13–2,0, yo‘lovchi zanjirda esa 0,13–7,9 ni tashkil etdi. *Helicoverpa armigera* genomida bu ko‘rsatkich yo‘naltiruvchi zanjirda 0,52–32, yo‘lovchi zanjirda esa 0,52–126 oralig‘ida kuzatildi. *Aphis gossypii* uchun esa E-testi qiymati yo‘naltiruvchi hamda yo‘lovchi zanjirlar uchun 0,38–1,5 darajasida bo‘ldi. Ushbu qiymatlar nolga nisbatan sezilarli darajada farq qiladi va shunday qilib, siRNA ning yo‘naltiruvchi hamda yo‘lovchi zanjirlarining past homologiysini ko‘rsatadi.

Genetik konstruktsiya pHellsgate-8::PHYA1 dan hosil bo‘lgan siRNA zanjirlari (yo‘naltiruvchi va yo‘lovchi) inson **(***Homo sapiens*), asalari (*Apis mellifera*), xonqizi qo’ng’izi (*C. septempunctata*), g’o’za tunlami (*Helicoverpa armigera*) va g’o’za shirasi (*Aphis gossypii*) genomlariga nojo‘ya ta’sir ko‘rsatmaydi. Ushbu natijalar transgen g’o’za o‘simligidan genlarning vertikal ko‘chishi ehtimolining pastligi haqidagi adabiyotlardagi ma’lumotlar bilan mos keladi [10–12].

Olingan natijalar asosida ta’kidlash mumkinki, tadqiq etilgan pHellsgate-8::PHYA1 genetik konstruktsiyasi nishon bo‘lmagan, ya’ni maqsad qilinmagan organizmlarga hech qanday nojo‘ya yoki zararli ta’sir ko‘rsatmaydi. Bu esa ushbu konstruktsiyaning xavfsizligini va biologik xilma-xillikka salbiy ta’sir ko‘rsatmasligini tasdiqlaydi. Shu bilan birga, olingan ma’lumotlar transgen g’o’za o‘simligi orqali genlarning vertikal ko‘chishi ehtimolining pastligini ko‘rsatgan oldingi adabiyot natijalari bilan to‘liq mos keladi. Demak, ushbu genetik konstruktsiya atrof-muhit uchun xavfsiz hisoblanadi va uning qo‘llanilishi ekologik xavfsizlik talablariga javob beradi.

***Adabiyotlar.***

1. Roderick H., Urwin P.E., Atkinson H.J. Rational design of biosafe crop resistance to a range of nematodes using RNA interference // Plant Biotechnol. J. 2018. Vol. 16, № 2. P. 520–529.

2. Yin Y. et al. Advances in the engineering of the gene editing enzymes and the genomes: Understanding and handling the off-target effects of CRISPR/Cas9 // J. Biomed. Nanotechnol. 2018. Vol. 14, № 3. P. 456–476.

3. De Schrijver A. et al. Quality of laboratory studies assessing effects of Bt-proteins on non-target organisms: minimal criteria for acceptability // Transgenic Res. 2016. Vol. 25, № 4. P. 395–411.
4. ESFA. Guidance on the environmental risk assessment of genetically modified plants // EFSA J. Wiley, 2010. Vol. 8, № 11. P. 1879.
5. Chen L. et al. Trends in the development of miRNA bioinformatics tools // Brief. Bioinform. 2019. Vol. 20, № 5. P. 1836–1852.
6. Kleter G.A. Food safety assessment of crops engineered with RNA interference and other methods to modulate expression of endogenous and plant pest genes // Pest Manag. Sci. 2020. Vol. 76, № 10. P. 3333–3339.
7. ESFA. Minutes of the 118th Plenary Meeting of the Scientific Panel on GMO. Parma, Italy: Parma European Food Safety Authority, 2017. 1–11 p.
8. Naito Y. et al. SiDirect 2.0: Updated software for designing functional siRNA with reduced seed-dependent off-target effect // BMC Bioinformatics. BioMed Central, 2009. Vol. 10, № 1. P. 1–8.
9. Mogren C.L., Lundgren J.G. In silico identification of off-target pesticidal dsRNA binding in honey bees (Apis mellifera) // PeerJ. 2017. Vol. 2017, № 12. P. 1–12.
10. Madhi S.A. et al. Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant // N. Engl. J. Med. Massachusetts Medical Society, 2021. Vol. 384, № 20. P. 1885–1898.
11. Yan S. et al. Pollen-mediated gene flow from transgenic cotton is constrained by physical isolation measures // Sci. Rep. 2018. Vol. 8, № 1. P. 2862.
12. Yan S. et al. Intercrops can mitigate pollen-mediated gene flow from transgenic cotton while simultaneously reducing pest densities // Sci. Total Environ. 2020. Vol. 711. P. 134855.