G‘O‘ZANING (*GOSSYPIUM HIRSUTUM* L) HASHAROTLARGA CHIDAMLILIK XUSUSIYATINI OSHIRISHDA AHAMIYATLI GENLARNI TAVSIFLASH

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Annotatsiya. Ushbu maqolada g‘o‘za (*G. hirsutum* L) o‘simligining iqtisodiy ahamiyati, hasharotlar bilan zararlanishi natijasida hosildorlikning kamayishi va bunga yechim sifatida gen muxandisligi yordamida *Cry*, *Cyp*, *Vip* genlari yordamida transgen g‘o‘za liniyalarini olish hamda hasharotlarga qarshi chidamlilikni oshirishda *Cry1Ia12*, *Cry1Ab*, *Cry1Ac*, *Cry3Bb1*, *Cry3*, *Cry2a*, *Cry2AX1*, *Vip3A*, *Vip3Aa14* genlari va *Cyp6* genlari oilasining o‘ziga xos xususiyatlari va ahamiyati bo‘yicha ma’lumotlar keltirilgan. Hozirgi paytda ushbu genlar chuqur tahlil qilinib, o‘zbek paxta navlarining hasharotlarga chidamli navlarini olish ustida tadqiqotlar boshlandi. Yaqin kunlarda hasharotlarga chidamli yangi biotexnologik g‘o‘za navlari yurtimiz dalalarida keng maydonlarga joriy qilinishi rejalashtirilmoqda.

Kalit so‘zlar: *G. hirsutum* L, *Helicoverpa armigera, Bacillus thuringiensis, Cry1, Cry3, Cry2a, Vip3A* va *Cyp6.*

ХАРАКТЕРИСТИКА ГЕНОВ, ВАЖНЫХ ДЛЯ ПОВЫШЕНИЯ ХАРАКТЕРИСТИК УСТОЙЧИВОСТИ ХЛОПКА (*GOSSYPIUM HIRSUTUM L*)

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Аннотация. В данной статье рассматривается экономическое значение хлопчатника (*G. hirsutum* L), снижение продуктивности из-за поражения насекомыми и решение этой проблемы – создание трансгенных линий хлопчатника с использованием генов *Cry*, *Cyp*, *Vip* и средств борьбы с насекомыми. специфические характеристики и важность семейств генов *Cry1Ia12, Cry1Ab, Cry1Ac, Cry3Bb1, Cry3, Cry2a, Cry2AX1, Vip3A, Vip3Aa14* и *Cyp6* в повышении устойчивости. В настоящее время эти гены тщательно проанализированы и начаты исследования по получению устойчивых к насекомым сортов узбекского хлопка. В ближайшее время планируется внедрить на больших площадях полей нашей страны новые биотехнологические сорта хлопчатника, устойчивые к насекомым.

Ключевые слова: *G. hirsutum* L, *Helicoverpa armigera, Bacillus thuringiensis, Cry1, Cry3,Cry2a, Vip3A* и *Cyp6.*

CHARACTERIZATION OF GENES IMPORTANT IN ENHANCEMENT OF INSECT RESISTANCE CHARACTERISTICS OF COTTON (*GOSSYPIUM HIRSUTUM* L)

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Annotation: This article discusses the economic importance of cotton (*G. hirsutum* L), the reduction in productivity due to insect damage, and the solution to this problem, which is the production of transgenic cotton lines using *Cry, Cyp, Vip* genes and anti-insects information on specific characteristics and the importance of *Cry1Ia12, Cry1Ab, Cry1Ac, Cry3Bb1, Cry3, Cry2a, Cry2AX1, Vip3A, Vip3Aa14* genes, and the Cyp6 gene family in increasing resistance. These genes are currently being thoroughly examined, and research is underway to develop insect-resistant Uzbek cotton cultivars. Shortly, new biotechnological

cotton cultivars that are pest-resistant will be introduced over huge portions of our farms.

Key words: *G. hirsutum* L, *Helicoverpa armigera, Bacillus thuringiensis, Cry1, Cry3*, *Cry2a*, *Vip3A* and *Cyp6.*

Kirish. Dunyo bo‘yicha Xitoy, Hindiston, AQSH, Pokiston, Braziliya, Avstraliya va O‘zbekiston davlatlari paxta xomashyosini ishlab chiqarishda yetakchilik qiladi va paxta xomashyosini ishlab chiqarish maydonining 90% i *G.hirsutum* L. bo’lsa, 8 % qismini *G.barbadense*, qolgan maydonlarini esa asosan diploid turlarga mansub (*G. herbacium va G. arboretum* L) g‘o‘za navlari tashkil etadi [1]. Keng miqyosda rasmiy ravishda Amerika paxtasi sifatida tanilgan (*G. hirsutum L.*) dan, to‘qimachilik sanoatida taxminan 40-60%, agrosanoatning turli sohalarida, xususan, paxta chigitidan yog‘ olishda 15-25 %, qog‘oz va sellyuloza sanoatida 20-40% gacha foydalaniladi. Asosiy qishloq xo‘jaligi ekini hisoblangan g‘o‘za hosilining yillik iqtisodiy ulushi butun dunyo bo‘ylab taxminan 600 milliard dollarni tashkil qilishi bo‘yicha ma’lumotlar mavjud [2, 3, 4, 5]. Iqtisodiy jihatdan ahamiyatli bo’lgan bu ekin turini yetishtirishda turli omillar, jumladan, zararkunandalar, turli patogen mikroorganizmlar biotik stressga sabab bo’lsa, qurg‘oqchilik, sho‘rlanish va haroratning o’zgarishi kabi abiotik stressni vujudga keltiruvchi omillar paxta hosilining miqdoriga hamda uning tola sifatiga sezilarli darajada zararli ta’sir ko’rsatadi [6]. Turli tadqiqotlar natijalari shuni ko‘rsatdiki, zararkunandalar va kasalliklar tufayli hosilning yo’qotilish darajasi taxminan 15-30%, ba’zi hollarda esa 50% gacha yetishi mumkin [7]. Shu sababli ham hozirgi kunda hasharotlarga chidamli g‘o‘za liniyalarini olish dolzarb muammo hisoblanadi.

Paxta hosilini hasharotlar bilan zararlanishi.

O‘zbekiston sharoitida g‘o‘za ekiladigan maydonlarda, g‘o‘za o‘sishi va rivojlanishi davrida 214 turdan ortiq zararli hasharotlar rivojlanadi va paxta hosiliga zarar keltiradi [8]. Ko‘rsatilayotgan zararning katta miqdori lepidoptera turkumiga kiruvchi *Helicoverpa armigera*, *Heliothis armigera, Pectinophora* *gossypiella*, *Earius insulana/vitella*, *spodoptera litura* kabi hasharotlarning zarari katta ekanligi hisobga olinsa, ko‘sak qurti 30-80% gacha hosilning jiddiy yo‘qotilishiga sabab bo’ladi [9,10]. Masalan Eronda, mamlakatning shimoliy qismida, 181 ta o’simlikda zararkunandalik qiladigan *H.armigera*, an'anaviy ravishda 10-12 marta petsidsidlar yordamida kurashilishiga qaramasdan, umumiy mahsulotning 30% ini yo’qotilinishiga olib keldi [10]. Pokiston misolida o’simlikning hasharotlar bilan zararlanishiga nazar soladigan bo’lsak, umumiy qishloq xo‘jaligi zararkunandalar tufayli taxminan hosilni 14% i zararlansa, shundan 84 % i paxta hosiliga to‘g‘ri keladi [11]. O‘zbekiston misolida, respublikamiz hududining taxminan 1,4408 millon gektardan ortiqroq yer maydonlarida aynan *G.hirsutum* turiga mansub navlar yetishtiriladi va har yili hosildorlik miqdori 3,5 mln tonnani tashkil etadi. Ammo abiotik va biotik omillar ta’siri natijasida, ayniqsa, hasharotlarning zarari tufayli hosildorlik darajasi pasayib ketmoqda [12, 13].

Hasharotlarga qarshi kurashishda yechim sifatida *B. thuringiensis* va genetik modifikatsiyalangan g’o’za navlari.

An’anaviy tarzda, kimyoviy dori vositalari yordamida hasharotlarga qarshi kurashish ham foydali, ham zararli ta’sir ko‘rsatadi. Shu bilan birga pestidsidlardan haddan tashqari foydalanish, iqtisodiy jihatdan qimmatga tushib, hasharotlarning chidamliligini oshirib, ularning yanada ko‘payishiga hamda atrof-muhitda uzoq muddatli zaharli qoldiqlarning tarqalishi va to‘planishiga sabab bo‘ladi [14, 15]. Yuqoridagi muammolarga yechim topish bilan birgalikda, hasharotlarning ko‘rsatayotgan zararini boshqarish uchun, ilg‘or biotexnologiya yutuqlaridan foydalangan holda, *B.thuringiensis* insektitsid oqsillarini ekspressiyalovchi ekologik toza, genetik modifikatsiyalangan ekinlarni olish eng muqobil strategiya bo‘la oladi. *B. thuringiensis* gramm musbat, spora hosil qiluvchi entomopatogen bakteriya bo’lib, hasharotlarga chidamli genlarning eng muhim manbai hisoblanadi. Bu bakteriya genlari turli hasharotlarning, xususan, lepidopteranlar, koleopteranlar dipteranlarning lichinkalariga qarshi toksik bo’lgan kristall oqsillarni hosil qiladi. Ma’lumot o‘rnida shuni qo‘shimcha qilish lozimki, birinchi marta 1981-yilda *Bt* insektitsid kirstall oqsil geni aniqlandi. Bu esa mavjud GM paxta liniyalarini olinishiga asosiy turtkilardan biri bo’ldi [16, 17, 18]. Hozirda zararkunandalar populyatsiyasini nazorat qilishda, paxta hosildorligidagi yo‘qotishlarni kamaytirish maqsadida butun dunyoda GM paxta liniyalari ishlab chiqilgan va genetik modifikatsiyalangan g‘o‘zalar ekiladigan maydon 13,7 % ga yetdi [19]. *Bt* asosida yaratilgan transgen g‘o‘zalar, Xitoy, Hindiston va boshqa rivojlangan mamlakatlarda yetishtirilmoqda.

Gen muxandisligida ishlatiladigan Bt toksin kodlovchi genlarning ahamiyati.

Ushbu genlar asosan *Cry* genlar oilasiga mansub bo’lib, genetik modifikatsiyalangan g‘o‘zalarning ko‘p qismi *Cry* genlari yordamida olingan. *Cry* genlarining 750 tasi tavsiflandi va ular 73 ta sinfga bo‘lindi. *Cry1* toksin eng ko‘p o‘rganilgan toksinlar sinfi bo’lib, 260 tadan ortiq genlar aniqlangan [20].

Bundan tashqari, *Cry1Ia12 geni* kiritilgan transgen o‘simliklar g‘o‘za zararkunandalari hisoblangan *A. grandis* va *S. frugiperda* kabi hasharotlarga qarshi chidamlilik xususiyatini o‘zida namoyon qiladi va u *B. thuringiensis* S811 shtammida *Cry1Ia12* proteini aniqlangan va *Escherichia coli* da ekspressiya qilingan. Praymerlar yordamida o‘tkazilgan tajribalar shuni ko‘rsatdiki, bu gen 420 bp ligi aniqlandi. Ma’lumotlar bazasida to‘plangan ma’lumotlarga ko‘ra, *Cry1Ia* tipidagi oqsil ketma-ketligi boshqa *Cry1Ia* toksinlari va *Cry1Ia12* 99% o‘ziga xoslik va o‘xshashlikni ko‘rsatdi [21].

B. thuringiensis ning *Cry1Ab va Cry1Ac* genlari ham borki, bu ikki genlar kombinatsiyasi ham hasharotlarga qarshi chidamlilikni oshirishda, birgalikda o‘simlikka transformatsiya qilingan va olingan transgen g‘o‘za Lepidoptera turkumiga kiruvchi zararkunandalarga qarshi kuchli chidamlilikka ega bo‘ldi. Bu transgen g‘o‘za 1996-yilda Avstraliya, Meksika va AQSHda birinchi marta o‘stirilgan [22]. *Cry1Ab* va *Cry2A* genlari 565 bp va 600 bp ekanligi aniqlangan [23].

Muhammad Mubashar Zafar va boshqalarning o‘tkazgan sekvens tahlillariga ko’ra, *Cry3Bb1* 459 bp and *Cry3* 562 bp ekanligi qayd qilindi. Boshqa adabiyotlarda esa bu genlar *Pectinophora gossypiella va Spodoptera lithura* kabi zararkunandalarga qarshi sezilarli darajada kuchli ta’sir etishi va boshqa bir qator genlar bilan birgalikda g‘o‘zadan tashqari, makkajo‘xori va baqlajonga ham transformatsiya qilinganligi haqida ma’lumotlar keltirilgan [24, 25].

Allah Bakhsh, Abdul Qayyum Rao va boshqalarning tadqiqotlariga ko‘ra, *Cry2a* genida ham hasharotlarga qarshi toksiklik xususiyati borligi va 600 bp ekanligi aniqlangan [26, 27]. 1990-yillarda parasporal kristall d-endotoksinlarni (*Cry1Ac* va *Cry2Ab*) ifodalovchi g‘o‘za navlari AQSh, Avstraliya va boshqa turli mamlakatlarda ishlab chiqarila boshlandi [15].

Cry2AX1 geni hasharotlarga *Cry2Aa* va *Cry2Ac* genlaridan ham ko‘ra kuchliroq zararli ta‘sir ko’rsatishi aniqlangan. Guruch va pomidorda bu genning ekspressiyasi natijasida turli hasharotlarga halokatli ta’sir ko’rsatdi. Olingan transgen paxta 88% ko‘sak qurtiga qarshi chidamlilik xususiyatini o‘zida namoyon etdi [28, 29, 30].

B. thuringiensis da vegetativ insektitsid oqsilini ishlab chiqarishga javobgar genlar oilasi *Vip* genlar oilasi hisoblanadi. Hozirda *Vip* genining 100 dan ortiq turlari ma’lum [31, 32]. Keltirilgan ma’lumotlarga ko‘ra, *Vip1* oqsillaridan 15 ta, *Vip2* oqsillaridan 20, *Vip3* oqsillaridan 111 ta va *Vip4* oqsillaridan 5 tasi aniqlanib, nom berilgan [33]. *Vip1* va *Vip2* gen oilalari koleoptera hamda homoptera zararkunanadalariga zaharlilik darajasi yuqori bo’lsa, eng katta *Vip3* genlar oilasi Lepidopteran turkumiga kiruvchi zararkunanadalarga kuchli zaharli ta’sir ko’rsatadi [34, 35]. *Vip3A* genlar oilasiga kiruvchi *Vip3Aa19* geni *Vip3Ac* va *Vip3Aa* oqsili, *Vip3AcAa* genlari bilan g’o’za o’simligi hujayrasiga muvaffaqiyatli transformatsiya qilinganligi bo‘yicha ma’lumotlar mavjud [36, 37].

Vip oilasiga kiruvchi genlar ichida eng keng o‘rganilgan vakili *Vip3A* hisoblanadi. 1996-yilda birinchi marta *B. thuringiensis* ning *AB88* va *AB424* shtammlaridan *vip3A(a)* va *vip3A(b)* genlari topilib, klonlanganligi haqida ma’lumotlar mavjud. *Vip3A* toksinining hatto past dozasi ham turli hasharotlarning rivojlanishiga salbiy ta’sir ko‘rsatadi [32]. Ayniqsa, *S.exigua, H.armigera, S.frugiperda, Heliothis virescens, H. Zea va A.ipsilon* kabi hasharotlarga qarshi zaharli ta’sir ko’rsatish darajasi yuqori hisoblanadi. [38].

Vip3Aa14 genining uzunligi Ritu Bhalla, Monika Dalal va boshqa tadqiqotchilar tomonidan aniqlangan va klonlanib olingandan so‘ng, bir qancha lepidoptera turkumiga mansub *H.armigera, Spodoptera litura, Earias vitella, Pieris brassicae, Plutella xylostella* kabi zararkunandalarda gen ekspressiyasi natijasida ishlab chiqarilgan oqsilning zaharlilik darajasi o’rganilgan. Sekvens tahlillariga ko‘ra, Bt ning 8 xil shtammidagi *Vip3Aa1* geni bilan solishtirilib chiqilganida, 31 ta nukleotidga farq qilganligi aniqlangan [32, 39, 40].

Hasharotlarga chidamlillikni ta‘minlovchi *H. armigera* da aniqlangan genlar.

Cyp6 genlari polifag zararkunandalariga qarshi ta’sir etadigan bir qancha genlarni o‘z ichga oladi [41]. 2018 yilga kelib, 16 000 turdagi o‘simliklarda *Cyp* genlari aniqlangan [42]. *CYP6AB14* va *CYP6AB60* genlari *Spodoptera litura* tarkibida zararli o‘simliklarni zararsizlantirishda ishtirok etsa, *CYPAE14 geni H. armigera* da gossipol katabolizmida qatnashishi aniqlangan [43, 44]. P450 CYP6 oilasi vakillariga juda o‘xshash ketma-ketliklarga ega bo’lib, o‘xshashlik darajasi *H. armigeradan ajratilgan CYP6AE12* 67% ni, *Depressaria Pastinacella* da mavjud *CYP6AE1* 58% ni tashkil etadi. Birinchi marta gossipol induksiyali P450 *CYP6AE14* zararlovchi hasharot qurtlaridan ajratib olingan va o‘ziga xos xususiyatlari aniqlangan [45]. Bundan tashqari bir qator g‘o‘za tunlami genomida mavjud *CYP332A1, CYP4L11, CYP4L5, CYP4M6, CYP4M7, CYP6B7, CYP9A12, CYP9A14* genlar asosida olingan genetik konstruksiyalar va *CRISPR-Cas9* texnologiyasi asosida (*CYP6AE)* ishlab chiqilgan konstruksiya ham ko‘sak qurtiga qarshi kurashda samarali ta’sir etishi to‘g‘risidagi ma’lumotlar adabiyotlarda keltirilib o‘tilgan. [46, 47].

Xulosa. Respublikamiz millliy daromadining katta qismi paxta xomashyosidan kelishini hisobga oladigan bo‘lsak, hasharotlarning zararlilik ta’sir doirasi kundan-kunga kengayib borayotgani katta muammolarga sabab bo’lmoqda. Bugungi kunda gen muxandisligi yordamida hasharotlarga chidamli g‘o‘za liniyalari va navlarini olish olimlar zimmasidagi eng muhim vazifalardan biri ekanligini ta’kidlab o’tishimiz kerak. Bu muammolarga yechim sifatida an’anaviy kurash usullari foyda bermayotgani tufayli, hozirda yangicha muqobil yo‘l sifatida *Cry, Vip, Cyp* gen oilalari vakillaridan foydalanish, hasharotlarga qarshi chidamlilikni oshirishda eng yaxshi yechim hisoblanadi. Shu maqsadda, ushbu genlarni chuqur o’rganib, kelgusida O’zbek paxta navlariga tadbiq etishni rejalashtirdik. Ayni paytda tadqiqot ishlari davom etmoqda.

FOYDALANILGAN ADABIYOTLAR.

1. Dohlman E, Johnson J, MacDonald S, et al. The world and united states cotton outlook: US Dep Agric, Agric Outlook Forum; 2019.
2. Saeed Q, F Ahmad, N Iqbal, SM Zaka (2019). Chemical control of polyphagous pests on their auxiliary hosts can minimize insecticide resistance: A case study of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) in cotton agroecosystem. *Ecotoxicol Environ Saf* 171:721–727.
3. Ali N, I Ullah (2018). *Cotton-Wheat cropping system: Constraints and Management.* University of Agriculture, Faisalabad. Available at /2018/08/15/cotton-wheat-cropping-constraints-management.
4. Siddiqui HA, M Asif, S Asad, RZ Naqvi, S Ajaz, N Umer, M Arshad (2019). Development and evaluation of double gene transgenic cotton lines expressing Cry toxins for protection against chewing insect pests. *Sci Rep* 9; Article 11774
5. Shuli F, AH Jarwar, X Wang, L Wang, Q Ma (2018). Overview of the cotton in Pakistan and its future prospects. *Pak J Agric Res* 31:396–408 .
6. Lobell DB, GP Asner (2003). Climate and management contributions to recent trends in US agricultural yields. *Science* 299:1032–1032.

7. McCook, S. & Vandermeer, J. J. P. The big rust and the red queen: Long-term perspectives on coffee rust research. *Phytopathology* **105**(9), 1164–1173 (2015).
8. Almuxammedov S. N va boshq “G‘o‘zani biologik usulda himoya qilish ”. Toshkent-1998.
9. Hussain, S., 2002. Genetic Transformation of Cotton with Galanthus Nivalis Agglutinin (GNA) Gene. PhD Thesis, The University of Punjab, Lahore, Pakistan
10. Kranthi KR, Naidu S, Dhawad CS, Tatwawadi A, Mate K,Patil E, Bharose AA, Behere GT, Wadaskar RM, Kranthi S. Temporal and intra-plant variability of Cry1Ac expression in *Bt*-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hubner)(Noctuidae: Lepidoptera). Curr. Sci. 2005 Jul 25;89(2):291.
11. Behdad E, Cotton pests, in elementary entomology and important plant pests of Iran (Yadbood Press, Esfahan Iran) 2002, 324-347.
12. Wendel JF, Brubaker CL, Edward PA. Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. American Journal of Batony. 1992; 79:1291-1310.10.1002/j. 1537-2197.1992. Tb 13734.x-DOI.
13. Iqbol MJ, Reddy OUK, El-Zik KM, Pepper AE. A genetic bottleneck in the evolution under domestication of upland cotton *Gossypium hirsutum* L.examined using DNA ingerprinting. Theoretical and Applied Genetics. 2001; 103:547-554.
14. Shahid, M. R., Farooq, J., Mahmood, A., Ilahi, F., Riaz, M., Shakeel, A., Petrescu-Mag, I. V. and Farooq, A. 2012. Seasonal Occurrence of Sucking Insect Pest in Cotton Ecosystem of Punjab, Pakistan. *Adv. Agr. Bio.,* 4. 26-30.
15. Sanahuja G, Banakar R, Twyman RM, et al. Bacillus thuringiensis: a century of research, development and commercial applications. Plant Biotechnol J. 2011;9(3):283–300.
16. Eede GVD, H Aarts, HJ Buhk, G Corthier, HJ Flint, W Hammes, AV Wright (2004). The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. *Food Chem Toxicol* 42:1127–1156.
17. Allah Bakhsh, Abdul Qayyum Rao, Ahmad Ali Shahid, Tayyab Husnain and S. Riazuddin.Insect Resistance and Risk Assessment Studies in Advance Lines of Bt

Cotton Harboring *Cry1Ac* and *Cry2A* Genes (2009). American-Eurasian J. Agric. & Environ. Sci., 6 (1): 01-11.

18. Adamczyk Jr JJ, J Gore (2004). Laboratory and field performance of cotton containing *Cry1ac*, *Cry1f* and both *Cry1ac* and *Cry1f* against beet armyworm and fall armyworm larvae (Lepidoptera: Noctuidae). *Flor Entomol* 87:427–433
19. Cohen, B.M., F. Gould. and J.C. Bentur, 2000. *Bt* rice: practical steps to sustainable use. Intl. Rice Research 25(2): 4-10.
20. James,C.(2014).*Brief49:Global Status of Commercialized Biotech/GMCrops: 2014. ISAAABrief*. Ithaca,NY:International Service for the Acquisition of Agri-biotech Applications,290.
21. Crickmore,N.,Baum,J.,Bravo,A.,Lereclus,D.,Narva,K.,Sampson,K.,etal. (2014). *BacillusthuringiensisToxinNomenclature.*
22. Grossi-de-Sa,M.F.,deMagalhaes,M.Q,Silva,M.S,Silva,S.M.B,Dias,S.C, Nakasu, E.Y.T.,etal.(2007).Susceptibilityof *Anthonomusgrandis* (cotton boll weevil)and *Spodopterafrugiperda* (fallarmyworm)toaCry1Ia-typetoxinfrom a Brazilian *Bacillusthuringiensis* strain. *J. Biochem.Mol.Biol.* 40, 773–782.doi: 10.5483/BMBRep.2007.40.5.77.
23. Fiuza, L.M., C. Nielsen-Leroux, E. Goze, R. Frutos and J.F. Charles, 1996. Binding of *Bacillus thuringiensis* Cry1 toxins to the midgut brush border membrane vesicles of *Chilo suppressalis* (Lepidoptera: Pyralidae): evidence of shared binding sites. Appl. Environ. Microbiol., 62(5): 1544-1549.
24. Muhammad Mubashar Zafar, Ghulam Mustafa , Fiza Shoukat , Atif Idrees , Arfan Ali, Faiza Sharif , Amir Shakeel. 2022. Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. Scientifc Reports. 1-2p.
25. Shrestha, R., Jakka, S. & Gassmann, A. J. J. O. A. E. Response of Cry3Bb1-resistant western corn rootworm (Coleoptera: Chrysomelidae)to Bt maize and soil insecticide. *J. Appl. Entomol.* 142(10), 937–946 (2018).

26. Iannacone, R., Grieco, P. D. & Cellini, F. J. P. M. B. Specific sequence modifications of a *cry3B* endotoxin gene result in high levels of expression and insect resistance. *Plant Mol. Biol.* 34(3), 485–496 (1997).
27. Allah Bakhsh, Abdul Qayyum Rao, Ahmad Ali Shahid, Tayyab Husnain and S. Riazuddin. Insect Resistance and Risk Assessment Studies in Advance Lines of Bt Cotton Harboring Cry1Ac and Cry2A Genes. Am-Euras. J. Agric. & Environ. Sci., 6 (1): 01-11, 2009.
28. Naqvi RZ, Asif M, Saeed M, Asad S, Khatoon A, Amin I, Mukhtar Z, Bashir A, Mansoor S. Development of a triple gene *Cry1Ac-Cry2Ab-EPSPS* construct and its expression in *Nicotiana benthamiana* for insect resistance and herbicide tolerance in plants. Frontiers in plant science. 2017 Jan 24;8:55.
29. Manikandan R, Sathish S, Balakrishnan N, Balasubramani V, Sudhakar D, Udayasuriyan V. *Bacillus thuringiensis* mediated transformation of *indica* rice with synthetic *cry2AX1* gene for resistance against rice leaf folder. J Pure Appl Microbiol. 2014 Aug;8(4):3135-42.
30. Bamishaiye EI, Varanavasiappan S, Balakrishnan N, Udayasuriyan V, Sudhakar D. Genetic Manipulation ofTomato (*Solanum lycopersicum*) cv. PKM-1 using *cry2AX1* gene for insect resistance. Int. J. Curr. Microbiol. App.Sci. 2017;6(12):4309-19.
31. Sakthi AR, Naveenkumar A, Deepikha PS, Balakrishnan N, Kumar KK, Devi EK, Balasubramani V, Arul L, SinghPK, Sudhakar D, Udayasuriyan V. Expression and inheritance of chimeric *cry2AX1* gene in transgenic cotton plants generated through somatic embryogenesis. In Vitro Cellular & Developmental Biology-Plant. 2015 Aug 1;51(4):379-89.
32. Estruch JJ, GW Warren, MA Mullins, GJ Nye, JA Craig, MG Koziel (1996). *Vip3A*, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc Natl Acad Sci* 93:5389–5394.

33. Khan MH, G Jander, Z Mukhtar, M Arshad, M Sarwar, S Asad (2019). Comparison of *in vitro* and *in planta* toxicity of *vip3A* for epidopteran herbivores. *BioRxiv* 2019:1–33.
34. Crickmore, N.; Berry, C.; Panneerselvam, S.; Mishra, R.; Connor, T.R.; Bonning, B.C. Bacterial Pesticidal Protein Resource Center. 2020. Available online: (accessed on 8 August 2020).
35. Sampurna, S.; Maiti, M.K. Molecular characterization of a novel vegetative insecticidal protein from Bacillus thuringiensis e_ective against sap-sucking insect pest. J. Microbiol. Biotechnol. **2011**, 21, 937–946. **2**. Jucovic, M.; Walters, F.S.; Warren, G.W.; Palekar, N.V.; Chen, J.S. From enzyme to zymogen: Engineering Vip2, an ADP-ribosyltransferase from Bacillus cereus, for conditional toxicity. Protein Eng. Des. Sel. 2008, 21, 631–638.
36. Khan MH, G Jander, Z Mukhtar, M Arshad, M Sarwar, S Asad (2019). Comparison of *in vitro* and *in planta* toxicity of *vip3A* for epidopteran herbivores. *BioRxiv* 2019:1–33**.**
37. Peterson JA, Ode PJ, Oliveira-Hofman C, Harwood JD (2016) Integration of plant defense traits with biological control of arthropod pests: challenges and opportunities. Front Plant Sci 7:1794
38. Palma, L.; Hernández-Rodríguez, C.S.; Maeztu, M.; Hernández-Martínez, P.; de Escudero, I.R.; Escriche, B.;Muñoz, D.; Van Rie, J.; Ferré, J.; Caballero, P. Vip3C, a novel class of vegetative insecticidal proteins from Bacillus thuringiensis. Appl. Environ. Microbiol. 2012, 78, 7163–7165.
39. Selvapandiyan, A., Arora, N., Rajagopal, R., Jalali, S.K.,Venkatesan, T., Singh, S.P. and Bhatnagar, R.K. (2001) Toxicity analysis of N- and C-terminus-deleted vegetative insecticidalprotein from Bacillus thuringiensis. Appl. Environ. Microbiol. 67.5855–5858.
40. Doss, V.A., Kumar, K.A., Jayakumar, R. and Sekar, V. (2002) Cloning and expression of the vegetative insecticidal protein (vip3V) gene of Bacillus thuringiensis in Escherichia coli. Protein Express. Purif. 26, 82–88.

41. Nelson, D. R. (1998). Metazoan cytochrome P450 evolution. Comp. Biochem.Physiol. Part C. Pharmacol. Toxicol. Endocrinol. 121, 15–22. doi:10.1016/s0742-8413(98)10027-0.
42. Pottier, M. A., Bozzolan, F., Chertemps, T., Jacquin, Joly. E., Lalouette, L., Siaussat, D.,et al. (2012). Cytochrome P450s and cytochrome P450 reductase in the olfactory organ of the cotton leafworm Spodoptera littoralis. Insect Mol. Biol. 21, 568–580. doi:10.1111/j. 1365-2583.2012.01160.x.
43. Ying-Bo Mao, Wen-Juan Cai, Jia-Wei Wang, Gao-Jie Hong, Xiao-Yuan Tao, Ling-Jian Wang,Yong-Ping Huang & Xiao-Ya Chen**.** Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol.Nature Biotechnology.4November2007; 1-p
44. Over-expression of multiple cytochrome P450 genes in fenvalerate-resistant field strains of Helicoverpa armigera from north of China Li Xu 1, Dongzhi Li 1, Jianying Qin 1, Weisong Zhao 1, Lihong Qiu.Affiliations expand PMID: 27521913 DOI: 10.1016/j.pestbp.2016.01.003.
45. Gonzalez FJ, Gelboin HV (November 1992). "Human cytochromes P450: evolution and cDNA-directed expression". Environmental Health Perspectives. 98: 81–85. doi:10.1289/ehp.929881. PMC 1519618. PMID 1486867.
46. Hung, C. F., Harrison, T. L., Berenbaum, M. R. & Schuler, M. A. *CYP6B3*: a secondfuranocoumarin-inducible cytochrome P450 expressed in Papilio polyxenes. Insect Mol. Biol. 4,149–160 (1995).
47. Wen, Z. M., Pan, L. P., Berenbaum, M. R. & Schuler, M. A. Metabolism of linear and angular furanocoumarins by Papilio polyxenes *CYP6B1* co-expressed with NADPH cytochrome P450 reductase. Insect Biochem. Mol. Biol. 33, 937–947 (2003).