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MAS TEXNOLOGIYASI ASOSIDA O‘RGIMCHAKKANAGA BARDOSHLI F2 DURAGAYLARNI TANLASH, QIMMATLI XO‘JALIK BELGILARINI BAHOLASH VA ULAR ASOSIDA BOSHLANG‘ICH ASHYOLAR OLISH

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Annotatsiya. *Ushbu maqolada MAS (markerlarga asoslangan seleksiya) usulidan foydalanib o`rgimchakkanaga chidamlir navlar yaratish uchun olib borilgan tadqiqot natijalari yoritilgan. Dastlab, o`rgimchakkanaga chidamli va sezgir, hamda genotipik jihatdan polimorf namunalar so`ruvchi zararkunandalarga chidamlilik geniga spesifik praymerlari yordami ajratib olingan. So`ngra ular o`zaro chatishtirilib duragaylar kombinatsiyalar olingan. Olingan duragay kombinatsiyalarning $F_2$ avlodlaridan spesifik praymerlar, hamda fenotipik tahlillar yordamida o`rgimchakkanaga bordoshli bo`lgan genotiplar tanlab olingan va o`rgimchakkanaga chidamli navlar yaratish uchun olib boriladigan tadqiqotlarda boshlang`ich ashyo sifatida foydalanish uchun tavsiya qilingan.*

***Kalit so`zlar****: MAS, paxta, $F_2$ duragaylar, PZR, chidamlilik, o`rgimchakkana, tola chiqimi, polimorfizm.*

SELECTION OF SPIDER MITE-RESISTANT F2 HYBRIDS BASED ON MAS TECHNOLOGY, EVALUATION OF VALUABLE ECONOMIC TRAITS AND OBTAINING INITIAL MATERIALS BASED ON THEM

Abstract. *In this article, the results of the research conducted to create varieties resistant to spider mite using the MAS (marker-based selection) method are highlighted.*

Initially, resistant and susceptible to spider mite, as well as genotypically polymorphic samples were isolated with the help of specific primers for the resistance gene to sucking pests.

Then they were crossbred and hybrid combinations were obtained. Genotypes resistant to spider mite were selected from the F2 generations of hybrid combinations using specific primers and phenotypic analysis and recommended to be used as starting material in researches to create spider mite resistant varieties.

Key words: *MAS, cotton, F2 hybrids, PCR, resistance, spider mite, fiber yield, polymorphism.*

СЕЛЕКЦИЯ ГИБРИДОВ F2, УСТОЙЧИВЫХ К ПАУТИННОМУ КЛЕЩУ, НА ОСНОВЕ ТЕХНОЛОГИИ МАС, ОЦЕНКА ЦЕННЫХ ХОЗЯЙСТВЕННЫХ ПРИЗНАКОВ И ПОЛУЧЕНИЕ НА ИХ ОСНОВЕ ИСХОДНОГО МАТЕРИАЛА.

Аннотация. *В статье описаны результаты исследований, проведенных по созданию сортов, устойчивых к паутинному клещу, методом МАС (маркерной селекции).*

Первоначально с помощью специфических праймеров к гену устойчивости к сосущим вредителям были выделены устойчивые и восприимчивые к паутинному клещу, а также генотипически полиморфные образцы. Затем их скрестили и получили гибридные комбинации. Из гибридных комбинаций поколений F2 с использованием специфических праймеров и фенотипического анализа выделены генотипы, устойчивые к паутинному клещу, и рекомендованы к использованию в качестве исходного материала в исследованиях по созданию сортов, устойчивых к паутинному клещу.

Ключевые слова: *MAC, хлопок, гибриды F2, ПЦР, устойчивость, паутинный клещ, выход волокна, полиморфизм.*

Kirish

(MAS) ekinlarning eng yaxshi navlarini yaratishning samarali usullaridan biridir [1; 145-156-b.]. MAS usulidan seleksiyada foydalanish orqali urug‘chlik samaradorligini ham keskin oshirish, nav tozaligini nazorat qilish mumkin [2; 55-57-b.]. Ko‘p o‘lchovli Quantitative Trait Locus (QTL) polimorfizmi tufayli markerlar yordamida tanlash samaradorligi keskin ko‘tarilgan [3; 106313-b., 4; 01083-b.]. Paxtaning qimmatli xo‘jalik belgilari bilan bog‘liq allellarning kashf etilishi bu allellarni to‘g‘ridan-to‘g‘ri MAS da qo‘llash imkonini bergan [4; 01083-b.]. F_2 o‘simliklari 3 usul bilan, fenotip (1), kombinatsiyalangan marker-genotip va fenotip (MAS) (2), genotip (3) asosida tanlanadi [5; 1092–1101-b.]. MAS va bekkros chatishtirish bir nechta eng yaxshi ota-onalar qatoridan foydali QTL allellarining o‘ziga xos kombinatsiyalarini tanlash orqali nisbatan kam sonli genlarga ega bo‘lgan liniyalarning urug‘chilik qiymatini va nav tozaligini oshirishning samarali usuli bo‘la oladi [6; 1845-1853-b., 7; 55-67-b.].

So‘nggi yigirma yil davomida seleksiya va urug‘chilikda MAS usulidan foydalanish tez sur’atlar bilan o‘sdi. Bu usulni qo‘llash orqali bir qancha olimlar ko‘plab tadqiqotlar olib borishgan [8; 153–163-b., 9; 593–602-b., 10; 375–389-b., 11; 262–268-b., 12; 2492–2498-b.], Maheswari va boshqalar [13; 17-33-b.]. MAS usuli takroriy tanlov qilish va har qanday turdagi ekinlarda urug‘chiligini yaxshilash uchun muhim vosita ekanligini ta’kidlagan [14; 55-67-b.].

Nayakning tadqiqotlariga asoslanib, navlarni ko‘paytirishda eng samarali bo‘lgan marker yordamida seleksiya usuli morfologik xususiyatlarning nisbiy ahamiyatini hisobga olgan holda yoki hisobga olmagan holda, molekulyar markerlardan foydalangan holda urug‘chilik yo‘nalishidagi jozibador individlarni tanlash usulidir deyish mumkin [15; 183-197-b.]. Bu usul, ayniqsa, chidamli turlarni yaratish uchun yaxshi samara beruvchi usuldir 16; 1-9-b.]. G‘o‘zaning hali nihollik davrlaridayoq spesifik markerlarlar yordamida chidamli genotiplarni ajratib olish mumkin [17; 1-19-b.]. Chidamli navlarni ekish, paxta hosilining oshishi va tannarxning keskin tushushiga paxtadan olinadigan foydaning oshishiga olib keladi [18; 563–577-b.].

Material va metodlar

Tadqiqotni bajarish davomida ananaviy seleksiya va genetika, markerlarga asoslangan seleksiya va statistika usullarida foydalanilgan.

Genomik DNA ajratib olish va vizualizatsiya qilish:

3-4 chinbarg chiqish fazasida har bir genotip yosh barglardan steril qaychi yordamida 0.5 gr namuna olinadi. Namunalar sterillangan distirlangan suv va etanol spirti yordamida yuviladi va eppendorf probirkalariga solinadi. Probirkalarni laboratoriyaga tashish davomida quruq muzdan foydalaniladi, DNA izolatsiyasiga qadar namunalar -20 ºC saqlanadi. DNK izolyatsiyasi Sentiltrimeyhtlaminiumbromid (CTAB) protokoli asosida amalga oshirildi [19].

SSR amplifikatsiyasi.

SSR amplifikatsiyasi PCR amplifikatorida amalga oshirilib, gel elektroforez yordamida tekshirildi. PCR protokoliga ko`ra denaturatsiya +95 ºC haroratda 3 daqiqa, keyin 34 sikl +95 ºC da 30soniya, +55 ºC da 30 soniya, +72 ºC da 1 daqiqa, davom etadi. Har bir PCR jarayoni uchun reaksiya hajmi 15 µL ni tashkil qildi. PCR reaksiyasi master Mix 0.75 µL dNTP (Conc.10 mM),

1.5 µL 10X PCR buffer, 1 µL F(forward) primer, 1 µL R (reverse) primer, 0.5 µL Taq DNA polymeraza (Conc.5 µL), 2 µL template DNA (Conc. 40ng/µL), 8.25 µL ddH2O (distillangan,sterillangan suv) lardan tashkil topgan [19].

Olingan ma‘lumotlarning statistik tahlillari Origin Pro dasturida ANOVA usulida [20], dala fenologik kuzatuvlari «Dala tajribalarini o‘tkazish uslublari» (2007) bo‘yicha olib borildi [21], G‘o‘zaning o‘rgimchakkana bilan zararlanish darajasi Xodjayev Sh.T. (2004) usulida [22] va tola sifati «Agrosanoat majmuida xizmat ko‘rsatish markazi» ning sinov laboratoriyasida Uster HVI Spectrum tola klassifilatsiyasi tizimida tahlil qilindi.

Tajriba natijalari

MAS usulidan foyyadalanib o`rgimchakkanaga bardoshli boshlang`ich manbaalar tanlash maqsadida olingan F2 duragay o`simliklaridan ajratilgan genom DNK si namunalarining tekshiruv natijalariga ko`ra namunalardagi DNK kontsentratsiyasi turli miqdorda bo`lishidan qatiy nazar namunalarning tozalik darajasi PZR reaksiyasi uchun maqbul holda ekanligini ko`rsatgan.

Polemeraza zanjir reaksiyasi (PZR) uchun 1- jadvalda keltirilgan tarkibli “master mix” ishchi aralashmadan foydalanilgan.

1- jadval.

PZR uchun ishchi aralashma (master mix) tarkibi.

Komponentlar	Hajmi
ddH_2O	6 µL
5 x Master Mix	1 µL
Praymer -F	1 µL
Praymer -R	1 µL
DNK	1 µL
Bitta namuna hajmi	10 µL

DNK markerlarining genom bo`ylab ampilifikatsiyasi 35 sikldan iborat standart PZR dasturida amalga oshirilgan (2- jadvalga qarang).

2-jadval

PZR reaksiyasi uchun foydalanilgan amplifikatsiya dasturi bosqichlari.

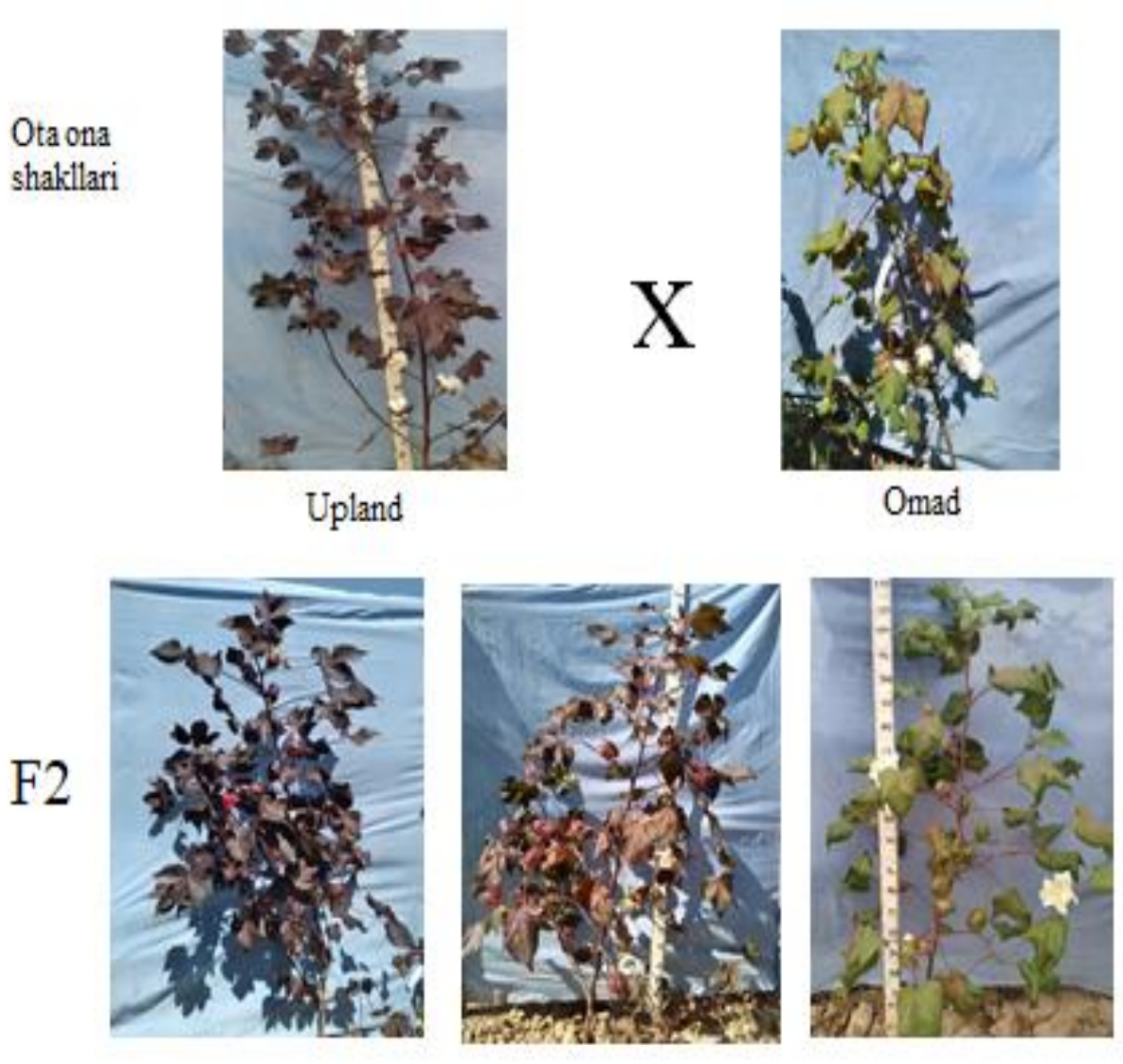
	Boshlang`ich denaturatsiya	Denaturatsiya	Praymerni genomga joylashuvi	Elongatsiya	Yakunlovchi elongatsiya	PZR mahsulotini amplifikatorda saqlanishi
Harorat	95 °C	95 °C	55 °C	72 °C	72 °C	4°C
Vaqt	2 min	30 sek	30 sek	1 min	2 min	∞
Sikl	1x	35x			1x	

Tadqiqot namunalari gel-elektroforez tahlili yordamida PZR amplikonlarning molekulyar og`irligi bo`yicha, “AmpliSize Molecular Ruler, 50bp” molekulyar og`irlik marker yordamida aniqlangan. Namunalarni genotiplash “Microsoft Excel 2021” va iMEC kompyuter dasturi orqali

amalga oshirilgan.

Upland × Omad duragay kombinatsiyasida tanlovlar so`ruvchi zararkunandalarga chidamlilik geni bilan birikkan BNL 1705 SSR praymeri [62;42-b.] yordamida amalga oshirildi.

Chidamsiz shaklida 160-175 juft asosli allellar mavjud bo`lsa, o`rgimchakkanaga chidamli namunada 160- 200 juft asosli allellar mavjudligini kuzatilgan . Upland x Omad duragay kombinatsiyalarida fenotipik jihatdan ham ajralishlar kuzatilgan, Ya`ni chidanli chidamlilik geni bo`yicha chidamlilik geni bo`yicha donor na`munalar poyasi antatsion rangga ega bo`lgan. Chidamsiz namuna poyasi esa yashil rangga ega bo`lgan. Ulardan olingan duragay kombinatsiyalarda antatsion, yashil va oraliq forma (yarim antatsion) rangli poyali osimliklar uchragan (1- rasmga qarang).



1-rasm. Upland, Omad navlari va ularni chatishtirish yo`li bilan olingan ikkinchi avlod durgay kombinatsiyalarda ro`y bergan poyasini rangi bo`yicha fenotipik ajralish.

Upland × Omad duragay kombinatsiyasining ikkinchi avlod o`simliklaridan ajratilgan DNK namunalari BNL1705 DNK marker yordamida PZR skrining qilinib, genotipik

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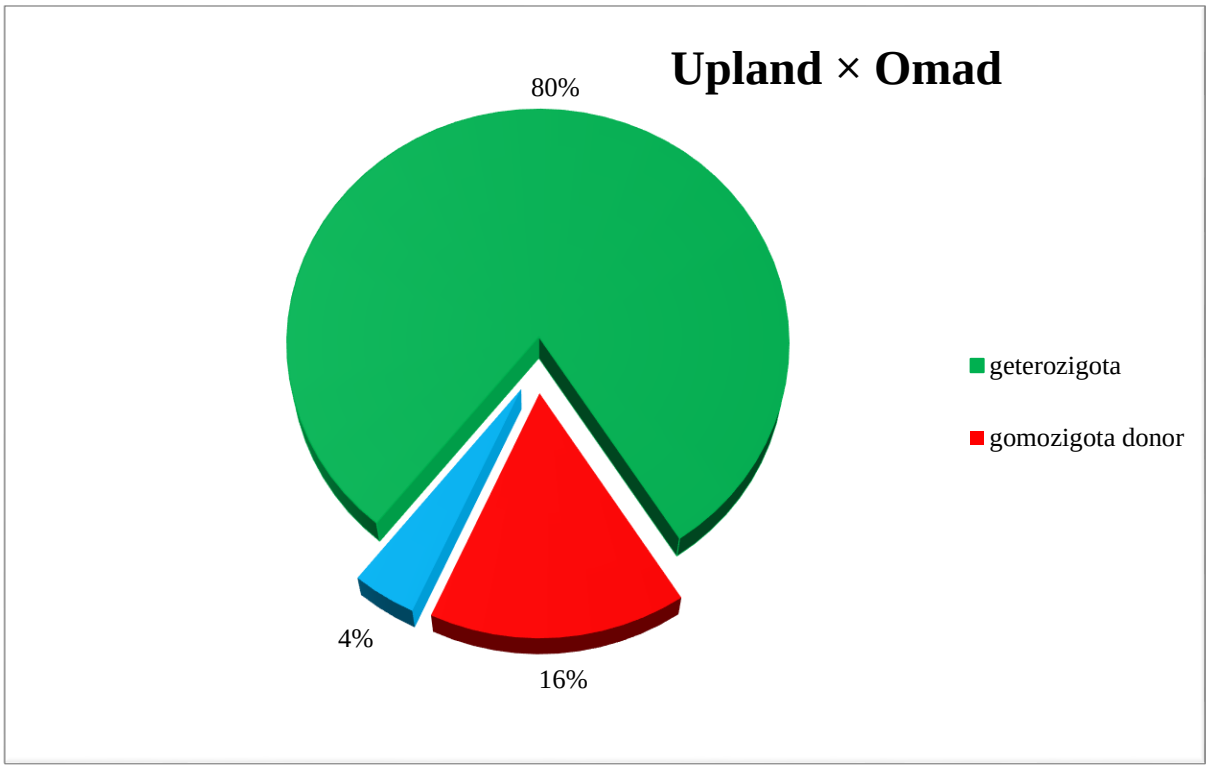
baholanganda, ushbu duragay kombinatsiyasining F_2 avlodida genotipik ajralishlar sodir bo`lganligini ko`rish mumkin. Genotipik tahlil natijasiga ko`ra chidamlilik belgisi bo`yicha geterozigota o`simliklar 80 % ni, chidamlilik geni bo`yicha chidamlilik geni bo`yicha donor allellariga ega gomozigota o`simliklar 16% ni, retsipient allellariga ega gomozigota o`simliklar 4% ni tashkil qilgan (2-rasmga qarang).



2-rasm. Upland × Omad duragay kombinatsiyasining F_2 avlodi o`simliklarida kuzatilgan genotipik ajralish holati.

New Impr × Namangan 77 duragay kombinatsiyasining F_2 avlodidan tanlovlar BNL1705 SSR markerida monomorflik kuzatilganligi bois so`ruvchi zararkunandalarga chidamlilik geni bilan birikkan yana bir SSR marker NAU922 [18, pp. 1–7] bilan amalga oshirildi. Namangan 77 namunasida 190 juft asosda 1ta allell mavjud bo`lsa, New Impr namunasi 110, 190 juft asoslarda ikkita allell mavjud bo`lgan. New Impr × Namangan 77 duragay kombinatsiyalarida 5, 14, 15, 20, 23 raqamli namunalarda ajralishlar kuzatilib chidamsiz namuna allellari bilan bir xil bent bergan (3- rasmga qarang). Bu kombinatsiyada ham chidamsiz namuna allellari bilan bir xil allellarga ega o`simliklarda o`rgimchakkana bilan zararlanish holatlari kuzatilgan.

New Impr × Namangan-77 duragay kombinatsiyasining ikkinchi avlod o`simliklaridan ajratilgan DNK namunalari NAU922 DNK marker yordamida PZR skrining qilingan, genotipik baholanganda, ushbu duragay kombinatsiyasining $F_2$ avlodida genotipik ajralishlar sodir bo`lgan.

Genotipik tahlillar natijasiga ko`ra o`rgimchakkana bilan zararlanishga chidamlilik belgisi bo`yicha geterozigota o`simliklar 60 % ni, chidamlilik geni bo`yicha chidamlilik geni bo`yicha donor allellariga ega gomozigota o`simliklar 20% ni, retsipient allellariga ega gomozigota o`simliklar 20 % ni tashkil qilgan (3-rasmga qarang).

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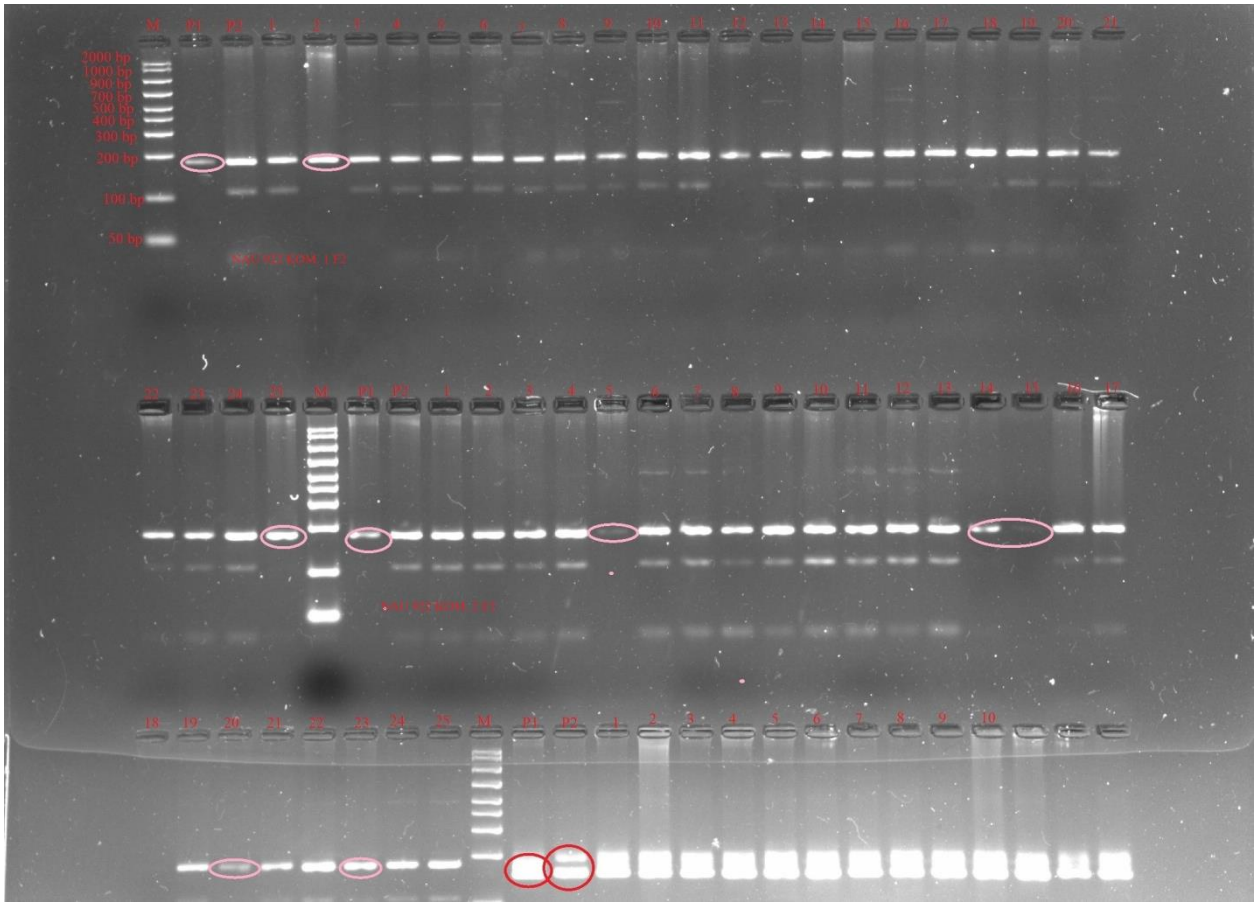

3- rasm. Upland x Omad, New Impr x Namangan 77 duragay kombinatsiyalarining F_2 duragaylari orasida polimorfizm. NAU 922 BNL, 1705 SSR markerlari gelelektroforezidagi rasmi. M-molekulyar og`irlikni bildiruvchi marker, P1, P2 – ota ona shakllari, 1-qator 1-25 Upland x Omad duragay kombinatsiyasi $F_2$ avlod o`simliklari. 2-qator 1-25 New Impr x Namangan 77 duragay kombinatsiyasi F_2 avlod o`simliklari. 3-qator 1-13 BNL SSR marker Upland x Omad duragay kombinatsiyasi o`simliklari.

Tanlash foydalanilgan spesifik markerlarning geterezigotalik va pik qiymatlari genotiplash natijalariga asosan iMEC dasturida hisoblab topilgan (3- jadvalga qarang).

3- jadval.

MAS usulida tanlashda foydalanilgan DNK markerlarining PIC qiymatlari.

DNK markeri	**Praymer sekvensi** (Forward/Reverse)	**He**	**Pic**
BNL1705	F: GCCAATTTAGTATAGGAAGCAAGT	0,1579	0,1454
	R: CATGTATTATTTTCACCCCTCTCT		
NAU922	F: GGAGTTTGGGAAACCCTATC	0,1975	0,1780
	R: CCATGACTTGAAGCAGATGA		

New Impr × Namangan 77 duragay kombinatsiyasida ham polimorfizm kuzatilgan 5, 14,15, 20, hamda 23 raqamli chidamlilik allellari kuzatilib, ushbu o`simliklarda 2023 yil mavsum davomida o`rgimchakkana bilan zararlanish holatlari kuzatilmagan. 1, 12, 21 raqamli o`simliklar retsipient allellariga ega o`simliklar bo`lib, bu o`simliklarda mavsum davomida mos ravishda 30.4

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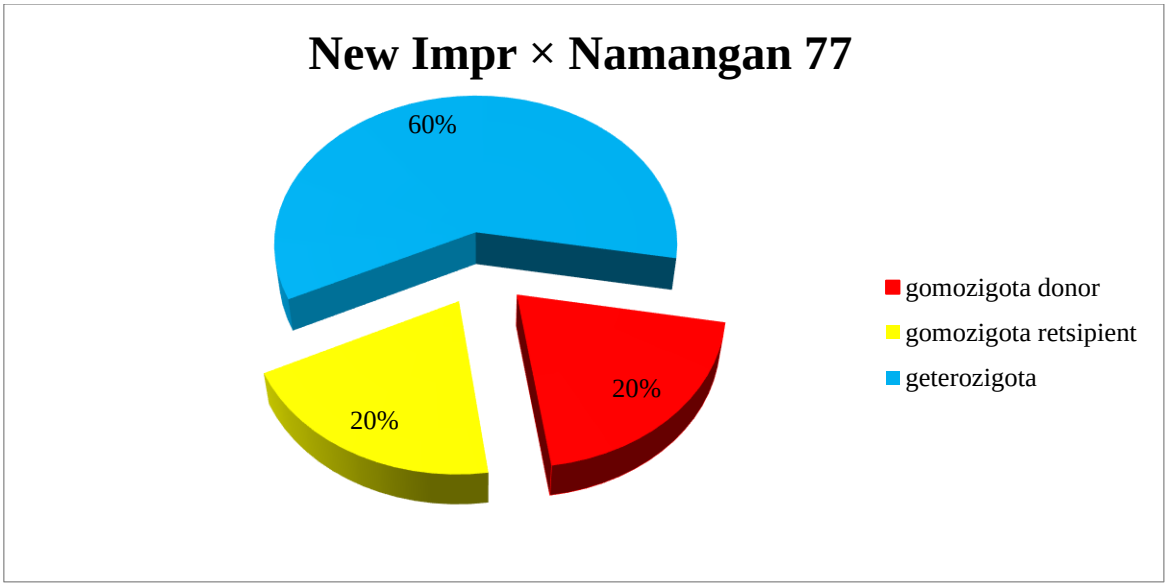
%, 36 %, 24.1 % gacha zararlanish holati kuzatilgan. Geterozigotalik holati nomoyon bo`lgan qolgan namunalarda ham o`rgimchakkana bilan zararlanmaslik, yoki zararlangan taqdirda chidamlilik geni bo`yicha chidamlilik geni bo`yicha donor o`simlik zararlanish darajasidan ortib ketmagan zararlanish darajasi kuzatilgan. Ikkinchi New Impr x Namangan 77 duragay kombinatsiyasidagi 10, 13, 16, 17, 19, 22, 24 raqamli o`simliklar bunga yaqqol misol bo`la oladi. Bundan ko`rinib turibdiki chidamlilik allelliga ega bo`lgan namunalar ham o`rgimchakkana bilan zararlanishi mumkin ammo, hosildorlik ko`rsatgichlariga katta zarar yetkaza olmagan.

New Impr × Namangan 77 duragay kombinatsiyasi PZR tahlillariga asosan F_2 avlodlarda genotipik ajralishlar sodir bo`lgan. Unga ko`ra o`rganilgan New Impr × Namangan 77 duragay kombinatsiyasi $F_2$ avlodlarda 60% geterozigota o`simliklar, 20 % o`simliklar chidamlilik geni bo`yicha chidamlilik geni bo`yicha donor allellariga ega gomozigota o`simliklar, 20 % retsipient allellariga ega gomozigota o`simliklar ekanligi aniqlangan (4-rasmga qarang).



4-rasm. New Impr × Namangan 77 F_2 duragay kombinatsiyasida genotipik ajralish.

PZR tahlillari asosida genotiplangan New Impr × Namangan 77 F_2 avlodi namunalarning genotipik ajralishi bo`yicha zararlanish darajasi o`rganilganda a genotipda Namangan-77 navidan o`tgan allellar nomoyon bo`lgan va o`rgimchakkana bilan zararlanishga chidamlilik xususiyatiga ko`ra Namangan-77 naviga o`xshash F2 avlod o`simliklari hisoblanadi. Tajribalar natijalariga ko`ra a genotip o`simliklarida o`rgimchakkana bilan o`rtacha 30,0 % zararlanish kuzatilgan bo`lib, retsipientdan 2,5% kam zararlangan, o`rgimchakkana bilan zararlanishga chidamlilik geni bo`yicha donor o`simlikdan 22, 1 % ko`p zararlangan. Genotipik ajralish bo`yicha b genotipga mansub o`simliklar o`rgimchakkana bilan zararlanishga chidamlilik xususiyati bo`yicha donor namuna New Impr ga o`xshash osimliklar hisoblanib, bunday o`simliklarda o`rgimchakkana bilan zararlanish kuzatilmagan. Genotipik ajralish bo`yicha keying guruh o`simliklari h genotipga mansub bo`lib, bunday o`simliklarda o`rgimchakkana bilan zararlanishga chidamlilik belgisi bo`yicha donor va retsipient o`simliklari har ikkalasining allellari mavjud bo`ladi. Ya`ni bunday o`simliklar geterozigota holatidagi o`simliklar hisoblanadi. Tajriba natijalariga ko`ra h genotip o`simliklari o`rgimchakkana bilan o`rtacha 6,4 % zararlanishi holati kuzatilgan (5-rasmga qarang).

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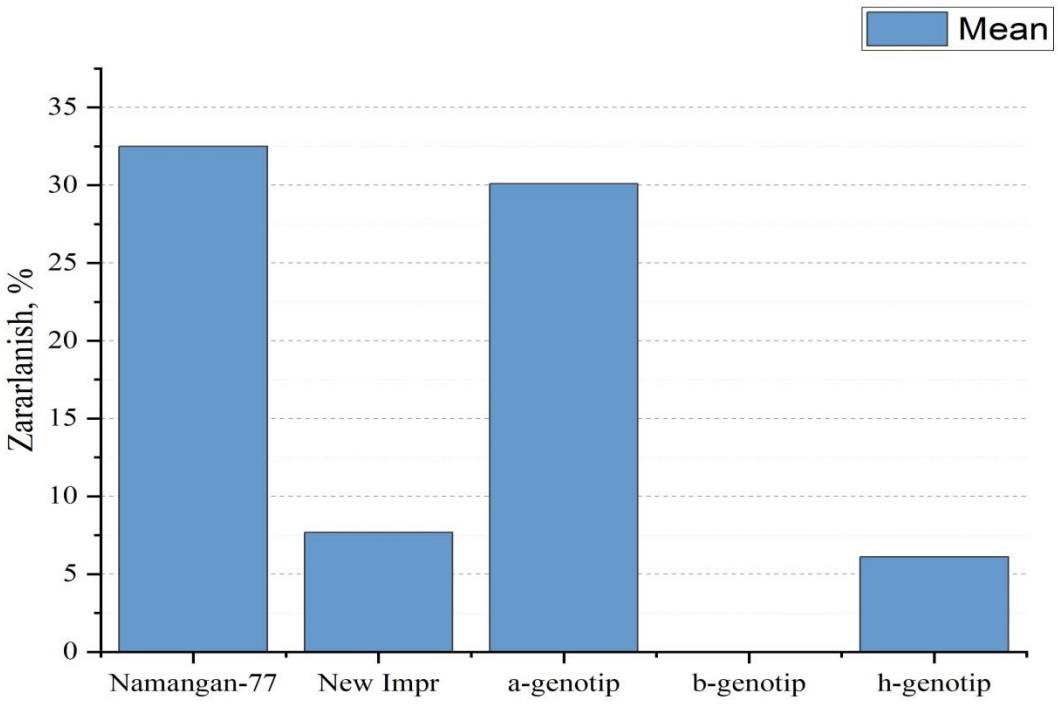
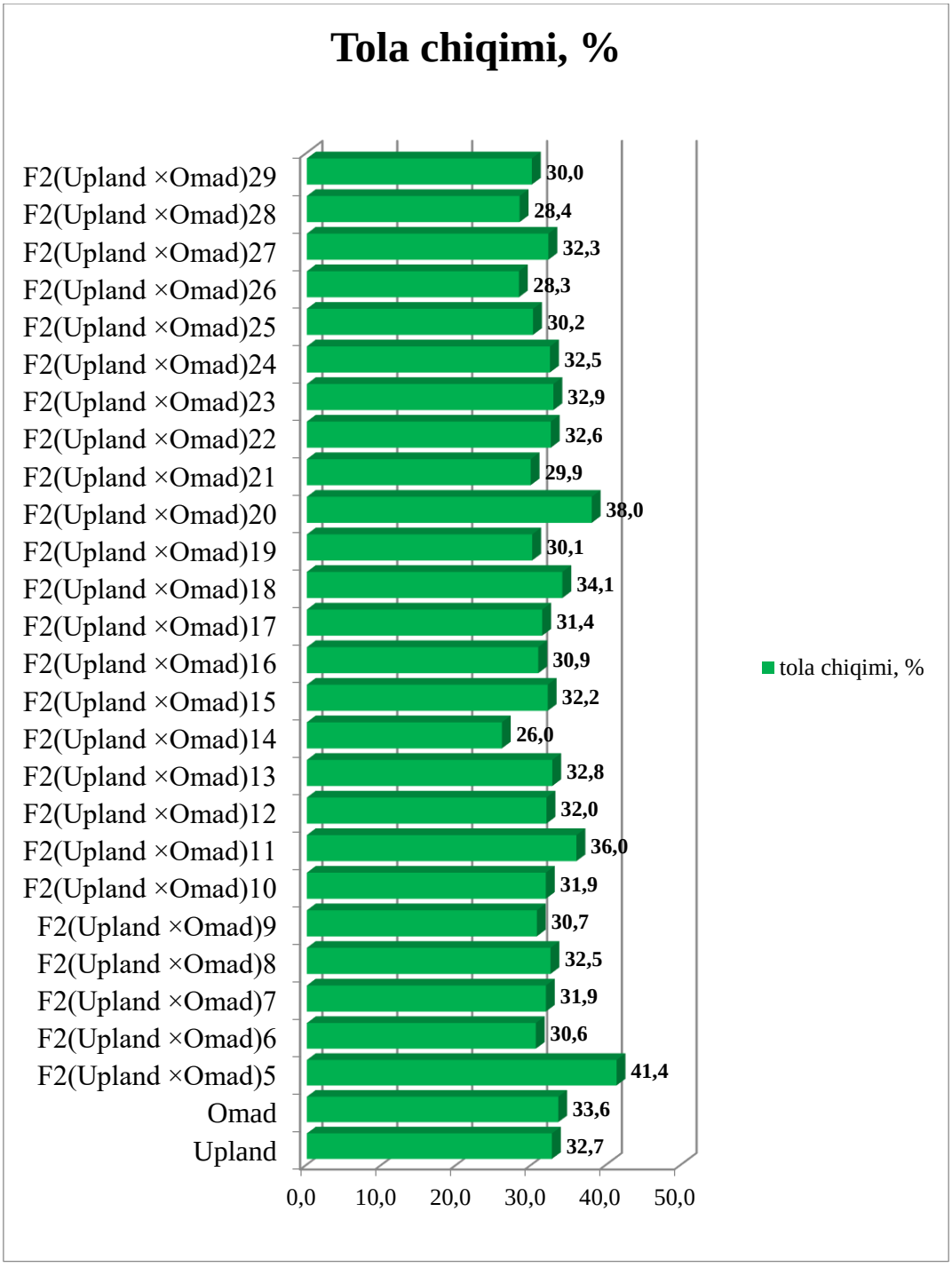


5-rasm. New Impr × Namangan 77 duragay kombinatsiyasida genotipik ajralish bo`yicha zararlanish darajasi.

Genotipik tahlillar uchun o`rganilayotgan o`simliklarning Upland × Omad, hamda New Impr × Namangan 77 duragay kombinatsiyalarining F_2 avlodi o`simliklaridan 25 tadan o`simliklar DNK markerlari asosida yakka tanlovlar qilingan. Ushbu o`simliklarning xo`jalikka qimmatli belgilardan biri tola chiqimi belgisi bo`yicha o`rganilganda (4.28-rasmga qarang), Upland × Omad duragay kombinatsiyasining F_2 avlodi o`simliklaridan Upland × Omad 5/G, Upland × Omad 11/G, Upland × Omad 18/G, Upland × Omad 20/G, genotiplarining tola chiqimi ota ona shakllarining tola chiqimidan yuqori bo`lgan. Hamda ishlab chiqarish talablariga mos bo`lgan (6-rasmga qarang).



6-rasm. Upland × Omad duragay kombinatsiyasi genotiplarining tola chiqimi.

New Impr × Namangan-77 duragay kombinatsiyasi o`simliklarida tola chiqimi belgisi o`rganilganda, New Impr × Namangan-77 32/G, New Impr × Namangan-77 34/G, New Impr × Namangan-77 37/G genotiplari faqatgina onalik shakli New Impr namunasida yuqori tola chiqimiga ega bo`lgan(7-rasmga qarang).

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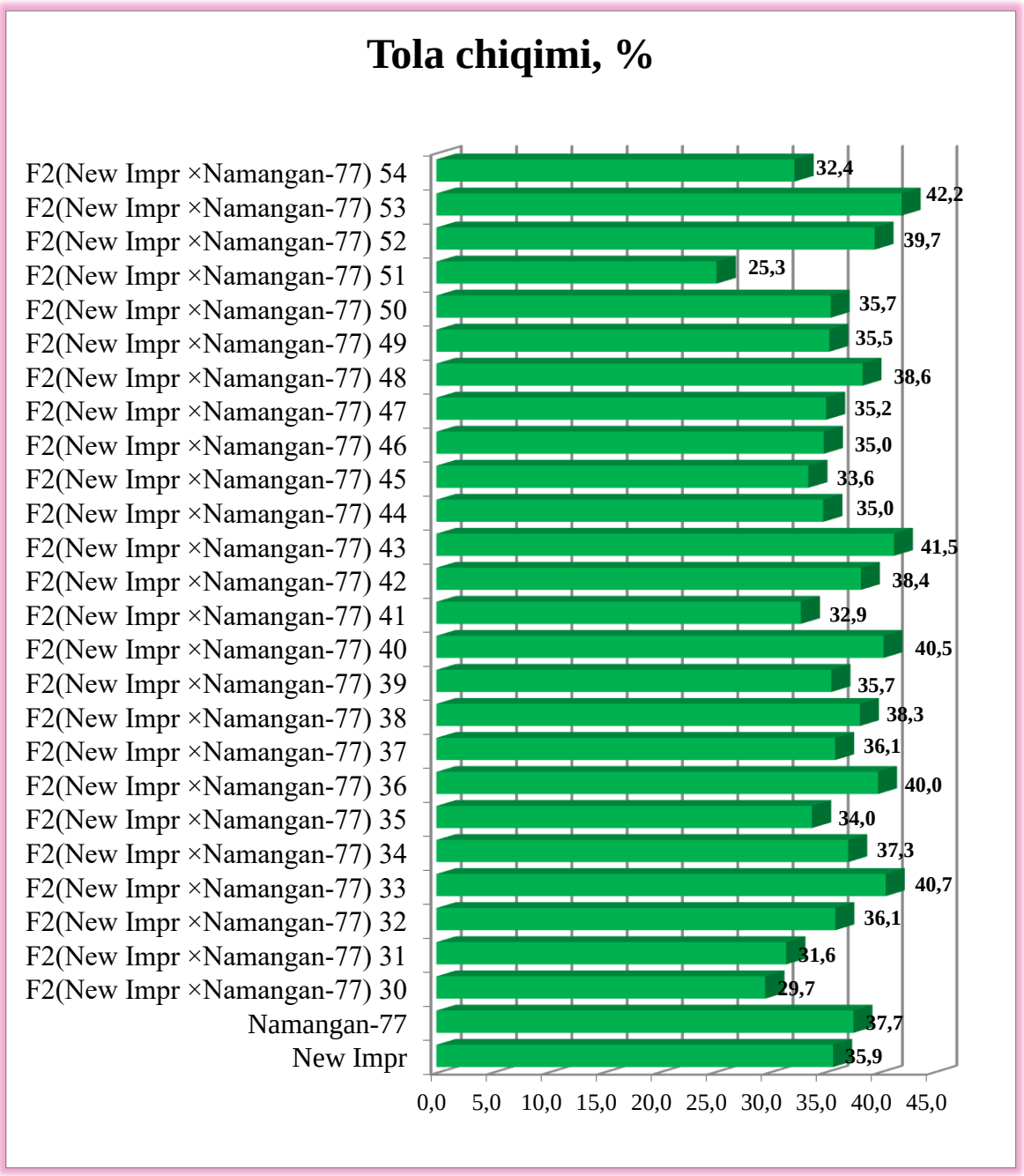

7-rasm. New Impr × Namangan-77 duragay kombinatsiyasi genotiplarining tola chiqimi

New Impr × Namangan-77 33/G, New Impr × Namangan-77 36/38, New Impr × Namangan-77 40/G, New Impr × Namangan-77 42/G, New Impr × Namangan-77 43/G, New Impr × Namangan-77 48/G, New Impr × Namangan-77 52/G, New Impr × Namangan-77 53/G genotiplari ham New Impr ham Namangan-77 namunasi tola chiqimidan yuqori, hamda ishlab chiqarish talablariga mos tola chiqimiga ega ekanligi aniqlangan.

Xulosalar

Ota ona shakllari sifatida tanlangan namunalar chidamlilik belgisiga birikkan DNK markerlari yordamida PZR skrining qilinganda orgimchakkana bilan zararlanishga bardoshli va sezgir navlar orasida o`zaro polimorfizm mavjudligi aniqlangan.

So`ruvchi zararkunandalarga chidamlilikka birikkan BNL1705, NAU922 SSR markerlari o`rgimchakkana bilan zararlanishga chidamlilik geniga ham bog`langanligi aniqlangan.

O`rgimchakkana bilan zararlanishga chidamli va sezgir namunalar o`zaro chatishtirilib olingan duragay kombinatsiyalarda chidamlilik belgisi dominantlik qildi.

Duragay kombinatsiyalarning ikkinchi avlodida fenotipik va genotipik ajralishlar sodir bo`lgan. DNK markerlari yordamida chidamlilik allellariga ega $F_2$ avlod o`simliklari orasidan New Impr × Namangan 77-5/G, New Impr × Namangan 77-14/G, New Impr × Namangan 77-15/G, New Impr × Namangan 77-20/G, New Impr × Namangan 77-23/G, Upland × Omad-19/G, Upland × Omad-33/G, Upland × Omad-35/G genotiplar tanlab olingan. O`rgimchakkana bilan zararlanishga bardoshli bo`lgan boshlang`ich ashyolar yaratilgan.

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