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ДОКЛАДЫ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

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ASSESSMENT OF THE INTEGRATED CHITINASE GENE STABILITY IN WHEAT LINES AFTER BIOBALLISTIC TRANSFORMATION

Abstract. The synthesis of PR-proteins (pathogenesis related proteins), the most studied of which are chitinases and β-1,3-glucanases, occurs in response to infection with pathogens in plants. Information about the exact role of individual PR proteins within plant immunity makes it possible to use certain specific antifungal proteins for the development of transgenic plants with increased resistance to fungal diseases. At the same time, it is important not only to obtain a plant with the desired trait, but also to fix in it the stable expression of the transferred gene and the inheritance of the acquired trait in generations.

Herein we have studied the stability of the chitinase gene insertion in T1, T2 and T3 generations of transformed wheat lines obtained by the method of cis-gene transfer. Primary transformed regenerant plants were obtained as a result of bioballistic transformation of the chitinase gene into immature wheat germ of Saratovskaya 29 and Kazakhstanskaya 19 varieties. Following screening of regenerant plants by PCR for the presence of the target gene made it possible to select 6 lines presumably carrying the insert based on variety Saratovskaya 29 and 2 lines based on variety Kazakhstanskaya 19.

The seed material of the selected regenerant plants was cultivated in soil conditions and the seeds of the T1 generation were obtained. DNA amplification of 8 selected lines St-29№25, St -29№43, St-29№44, St-29№33, St-29№26, St-29№35, Kz-19№1, Kz-19№2 with specific primers revealed insert-carrying lines, partially cleaved lines and lines with a high degree of insert instability. According to the results of the T2 and T3 generations PCR analysis, a complete absence of insertion in the St-29№35 and St-29№33 lines was revealed, a partial cleavage of the trait in the St-29№43 and Kz-19№2 lines was revealed, and the stable inheritance of the chitinase gene in four lines St-29№25, St-29№44, St-29№26 and Kz-19№1.was confirmed. These lines were selected as promising for in-depth study of their resistance to fungal diseases and further replication.

Key words: transformed plants, DNA, wheat chitinase gene, insert stability.

Introduction. The modern level of biological technologies development, and in particular, genetic engineering, allows to perform targeted modifications in the genome of living organisms. This approach has found wide application in research aimed at adding the desired properties to any agricultural plant without changing its nutritional value.

One of the most important and critically difficult moments in obtaining plants with new features was the selection of an effective method for introducing foreign genes.

The discovery of a natural transformation system using soil Ti-plasmids - an ideal natural vector - was a breakthrough in the transformation of plant cells. However, recently, the method of bioballistic

transformation is increasingly used to transfer the "necessary" information and obtain plants with improved intrinsic properties, which allows to introduce exclusively target plant genes into the genome and does not require the participation of bacterial DNA. Nowadays bioballistics is one of the most effective methods for transforming not only monocotyledonous, but at the same time it is also successfully used on dicotyledonous plants. Callus and suspension cultures, cultivated immature embryos, and embryogenic pollen are usually used as the starting material for transformation. Monocotyledonous plants as corn, rice, barley, and dicotyledonous plants such as tobacco, potatoes, beets, soybeans, rapeseed, alfalfa, tomatoes, carrots, cabbage, grapes were transformed with a bioballistic gun. [1, 2].

Since the early 2000s, it has been proposed to divide genetically modified organisms into groups depending on the source of transgenes. [3-6]. Organisms the genome of which have been introduced with genes of the same species or species with their own regulatory elements with which they can interbreed are called cisgenic or intragenic. With this type of transformation, the gene is introduced with the regulatory regions of foreign genes. In this regard, it is believed that cisgenic plants are not genetically modified, although they are obtained by genetic engineering methods. Plants obtained by introducing constructs that alter the expression of plant genes by triggering the process of RNA interference are also referred to as cisgenic plants. To date, there are known cisgenic soybean lines (Treus ™, Plenish ™) with a modified composition of fatty acids in the seeds and resistance to herbicides based on sulfonylurea, tomatoes (Huafan No. 1) with delayed maturation, glyphosate-resistant corn lines (GA1 and its derivatives) [2]. Currently, transgenic technologies are increasingly used to increase the resistance of varieties and hybrids to environmental stressors, for example, to the action of various pathogens - viruses, bacteria, fungi and insects [7-8].

Wheat is the world's primary food crop. However, it is one of the last crops to be genetically transformed, and despite numerous field trials, genetically modified wheat has not yet been grown commercially.

Numerous protocols for the transformation of wheat have been developed, but today its efficiency is significantly lower than that of other cereals [9] due to the large (17000 Mb) and complex (hexaploid) genome and genotype-dependent reactions of wheat tissue cultures can lead to silencing of transgenes [10]. Nonetheless, particle bombardment is a reliable and well-used wheat transformation protocol [11], although it suffers from the lack of complex transgenic integration schemes such as DNA positioning / copying or fragmentation during bombardment. [12]. Moreover, identification and maintenance of regenerated callus without contamination for a certain period of time is complicated as well [13]. When developing and producing new plants by the bioballistic transformation method, some parameters such as the selection of plasmid, wheat genotypes and the composition of the medium for cultivating explants and regenerant plants are considered [14].

As a result of bioballistics, a successful transformation of *Triticum monococcum* L. has already been carried out and plants resistant to the herbicide phosphinothricin have been obtained.. Analysis of the expression of marker genes and treatment of regenerant plants with herbicides showed that the *gfp* and *bar* genes are stably integrated into the genome of *Triticum monococcum* and the acquired trait is inherited over several generations. [15].

An attempt was made to overcome the susceptibility of soft wheat *Fusarium graminearum* by introducing the rice chitinase gene (Cht-2) by bombardment of immature wheat embryos

The incorporation of Cht-2 into the genome of the transformants was confirmed by dot blot analysis and the evaluation of the transformants by PCR assay using specific primers. The transformation efficiency (number of transgenic plants / number of embryos) composed 6.01%. The biochemical characteristics of the transformants were studied and it was determined that the content of total protein, phenolic compounds and the activity of the antioxidant enzymes peroxidase and catalase significantly decreased in new plants, as well as under stress conditions caused by infection with F. graminearum, the activity of phenylalanine ammonia lyase and chitinase increased significantly. compared to non-transgenic plants [16].

In response to infection with phytopathogens - viruses, bacteria, fungi and insects, PR-proteins (pathogenesis related proteins) are synthesized [17-19]. The most studied of these are chitinases and P-1,3-glucanases, which inhibit the growth of certain types of bacteria and fungi. These enzymes hydrolyze the main components of the cell wall - chitin and P-1,3-glucan. In addition, chitinases and P-1,3-glucanases are encoded by single genes, which prompts interest in them. [17, 18, 20, 21]. The

determined role of PR proteins in plants immunity enables to use anti-fungal proteins for the development of transgenic plants with increased resistance to fungal diseases. Such plants have already been obtained and have been observed to be resistant to fungal pathogens. [16, 22-26].

The aim of this work is to study the inheritance stability of the chitinase gene insertion in transformed wheat lines of T1, T2, and T3 generations obtained by the method of cisgenic bioballistics.

Materials and methods. The objects of the study were transformed lines of soft wheat *Triticum aestivum* based on Saratovskaya 29 and Kazakhstanskaya 19 varieties of T1, T2, and T3 generations obtained by the method of bioballistic transformation, and DNA samples isolated from these plants. In all experiments, non-transformed wheat plants of Saratovskaya 29 and Kazakhstanskaya 19 varieties was used as the control.

DNA isolation from transformed and non-transformed (control) plants was carried out from 100 mg of leaf plates of 2 - 3 week old seedlings using a GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Fisher Scientific).

The presence of the target chitinase gene insert of was determined by PCR in a reaction volume of 20 µl, with the addition of 2 µl DNA, 2 µl HotTaq x10 buffer (Sileks, Russia), 2 mM MgCl2, 0.2 mM dNTP, direct (ACC CTG TTG TTT GGT GTT ACT TCT GC) and reverse (GCA GTA GCC CCA GGA GTA GG) primers - 10 pmol each, and 1 unit. HotTaq DNA polymerase (Sileks, Russia) on Mastercycler ep gradient S (Eppendorf, Germany) in the following mode: initial denaturation at 95 ° C 15 min; (95 °C - 60 sec; 58°C – 60 sec; 72°C – 60 sec) – 35 cycles; 72°C – 5min.

Amplification products were separated by electrophoresis in 1% agarose gel in 0.5 x TBE buffer at a voltage of 15 V/cm for 30 min. After electrophoresis, the gel was stained with a solution of ethidium bromide (1 µg / ml), followed by visualization in ultraviolet light on a GelDoc device (Bio-Rad, USA) in transmitted ultraviolet light at a wavelength of 260 to 360 nm.

Results. Wheat regenerant plants of the Saratovskaya 29 and Kazakhstanskaya 19 varieties presumably carrying an insert of the chitinase gene were obtained as a result of several series of bioballistic cisgenic transformation of immature embryos. Transformation was performed with genetic constructs with the wheat chitinase gene (Chit) applied to gold particles using a ballistic device Biolistic Particle Delivery System PDS-1000/He (Bio-Rad) according to the manufacturer's protocol and according to the established method [27].

The transformed regenerant plants were grown in soil under the conditions of a culture light room to the state of a ripe ear. Further studies and determination of the inheritance stability of the insert were carried out on lines that had no visible defects during cultivation, had a sufficient amount of seed material for planting, and, the most important criterion, had a confirmed insertion of the target chitinase gene according to the results of preliminary PCR analyzes. The collected seed material of six lines № 25, 26, 33, 35, 43 and 44 of regenerant plants based on the Saratovskaya 29 variety and two lines №1 and №2 of the Kazakhstanskaya 19 variety was planted in the soil and plants of the T1 generation were obtained. In all further studies, plants of the original varieties were planted in parallel as a control.

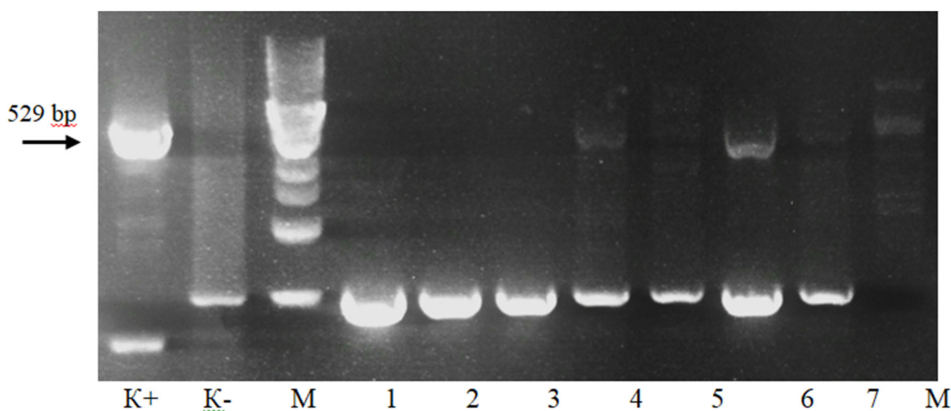
The plants was evaluated according to such parameters as plant height, lodging, flowering and heading periods. In general, according to the results of all visual observations, no visible differences from the control, untransformed seedlings of varieties Saratovskaya 29 were revealed. Significant differences in the growth rate, flowering and heading times were also not found when comparing the transformed lines with each other for all the indicated parameters.

In total, during the growing season of 375 wheat plants of the T1 generation, 468 samples of leaf bsamples were collected from 6 transformed lines based on the Saratovskaya 29 variety, 2 lines based on the Kazakhstanskaya 19 variety and 2 control variants. DNA was isolated to conduct the polymerase chain reaction, and, in order to exclude false negative results, the samples were analyzed three times. A total of 468 DNA samples from transformed plants were examined.

PCR for the presence of the chitinase gene insert was conducted with primers designed in such a way that it was possible to anneal simultaneously a part of the construct promoter and the subsequent chitinase gene sequence after the promoter (ACCCTGTTGTTTGGTGTTACTTCTGC; GCAGTAGCCCCAGGAGTAGG; amplicon size 529 bp). This design was developed to exclude false positive results by amplifying the native wheat chitinase gene. Therefore, the detected amplification product of 529 bp in size indicated the presence of the target chitinase gene in the sample.

Figure shows the results of DNA amplification isolated from transformed wheat plants. Plasmid (K +) with the chitinase gene and DNA (K-) from non-transformed wheat plants of Saratovskaya 29 variety served as controls.

The generalized screening data of transformed T1 generation wheat lines and control plants of varieties Saratovskaya 29 and Kazakhstanskaya 19 for the presence of target inserts of the chitinase gene are shown in the table below (Table).



Screening results for the presence of the target chitinase gene. K+ - positive control (plasmid containing the chitinase gene), K- - negative control, M – DNA ladder (100 bp), 1-3, 5 – plants without the target chitinase gene, 4, 6, 7– plants carrying the target chitinase gene

These tables show that not all lines selected for preliminary screening had the insertion of the target gene. Based on the revealed percentage, the plants of the T1 generation were divided into three groups.

The first group included plants T1 St-29№35 и St-29№33, characterized by a high degree of the insert instability. Regarding line St-29№35, it can be assumed that it was chimeric at the time of the initial analysis, or its definition as carrying the insert was erroneous, or the localization of the insert caused its complete cleavage in the T1 generation.

The second group consisted of T1 plants of the St-29№43 иKzз-19№2 lines, in which partial cleavage occurred and the stability of the insert was 79.55% and 60%, respectively. These two lines can be further studied to clarify the point of the insertion.

The third group of plants, which combined plants of the lines St-29№25, St-29№44, St-29№26 and Kz-19№1, was distinguished by the inheritance stability of the target chitinase gene in the T1 generation.

To assess the stability of the detected insert in T2 wheat transformant plants, seeds of 8 lines of transformed wheat were sown in an experimental field and 357 T1 plants were cultivated. Seeds of the following wheat lines were sown: St-29№25, St-29№44, St-29№26, Kz-19№1, which we identified as carriers of a stable insert and line St-29№43 и Kz-19№2 with partial cleavage in plants by the presence of an insert, lines St-29№35 and St-29№33without an insert.

Total 363 leaf samples were collected during the wheat plants vegetation season in the field, followed by DNA isolation from leaf samples analysis for the presence of an insert under the same conditions as in the previous year. The reaction was carried out three times to exclude false negative results.

The screening results of T2 generation experimental plants of 8 transformed wheat lines and control plants of Kazakhstanskaya 19 and Saratovskaya 29 varieties are given in Table.

The data in Table demonstrate that further partial cleavage occurs in the lines St-29№43 и Kz-19№2 and the stability of the insert is only 76.92% and 76.6%, respectively, which suggests that for obtaining a new wheat line with target traits of resistance to fungal diseases, these lines cannot be used. In the line St-29№35 and St-29№33, which we indicated as not showing the insertion of the target chitinase gene in the T1 generation, the insertion was not detected in the T2 generation either.

In contrast to the mentioned earlier lines, the plants of the lines St-29№25, St-29№44, St-29№26 и Kz-19№1 confirmed the stability of the chitinase gene inheritance in the T2 generation. In this regard, these four lines were used in further replication in the T3 generation. The line St-29№43, which showed a partial splitting of the trait, was also taken for reproduction and analysis of the next generation.

Under the conditions of the experimental field, the 5 lines of transformed wheat of T3 generation was obtained. In total, 232 plants of the T2 generation lines St-29№25, St-29№44, St-29№26, Kz-19№1, which were identified as carriers of the stable insert and the St-29№43 line with partial cleavage in plants by the presence of the insert, were cultivated. In addition, control plants of varieties Saratovskaya 29 and Kazakhstanskaya 19 were planted as well.

The presence of an insert was determined by amplification of DNA isolated from 456 leaf samples collected from experimental plants during the growing season. The results of screening experimental plants of the T3 generation of five transformed wheat lines and control plants are given in Table.

Again, chitinase gene insertions were found in lines St-29№25, St-29№2, St-29№44 и Kz-19№2 and the stability of inheritance ranged from 80 to 96%. Further partial cleavage occured and the stability of the insert composed only 71.4% for the St-29No.43 line.

Results of screening of new wheat lines transformant plants for the presence of a target chitinase gene insert

Name of line	T1			T2			T3		
	Number of plants, each.	The presence of the chitinase gene	Total positive plants, %	Number of plants, each.	The presence of the chitinase gene	Total positive plants, %	Number of plants, each.	The presence of the chitinase gene	Total positive plants, %
Saratovskaya 29 (control)	45	-	-	46	-	-	46	-	-
St-29 №25	49	48	97,96	48	48	100	48	46	95,8
St-29 №26	47	45	95,8	46	42	91,3	47	43	91,6
St-29 №33	45	14	31,11	42	0	0			
St-29 №35	48	0	0	43	0	0			
St-29 №43	44	35	79,55	39	30	76,92	42	30	71,4
St-29 №44	50	45	90,0	45	43	95,55	47	38	80,9
Kazakhstanskaya 19 (control	48	-	-	45	-	-	48	-	-
Kz-19 №1	47	47	100,0	47	47	100	48	41	85,4
Kz-19 №2	45	27	60,0	47	36	76,6			

Conclusions. The development of strategies for the host plant resistance against crop diseases is the foundation of future agricultural production. The development of technologies for rapid gene isolation, molecular labeling methods, the results of whole genome sequencing, as well as the development of knowledge of the field pathosystem are the potential for the implementation of effective and long-term programs for the creation of new varieties resistant to environmental stressors while reducing dependence on pesticides [28].

When transforming plants, an important factor is not only to obtain a transgenic plant with the desired trait, but it is also necessary that the transferred gene is expressed in the new plant and the acquired trait is inherited in generations [27].

Primary transformed regenerant plants were obtained as a result of cis-gene transfer of the chitinase gene into immature wheat germ of Saratovskaya 29 and Kazakhstanskaya 19varieties. And primary screening of them by PCR for the presence of the target gene enabled to select 6 lines based on variety Saratovskaya 29 and 2 lines based on variety Kazakhstanskaya 19, presumably bearing an insert.

The seeds of the T1 generation were obtained from the selected regenerant plants by cultivation in the soil, which served as the material for the subsequent generations of T2 and T3.

In the field, leaf samples (plates) of the studied plants were collected to identify the lines carrying the wheat chitinase genes insertion. The presence of an insert was determined by PCR using designed primers capable of identifying the target gene. DNA amplification of 8 selected lines линий St-29№25, St-29№43, St-29№44, St-29№33, St-29№26, St-29№35, Kz-19№1, Kz-19№2 revealed lines carrying the insert, lines with partial cleavage and lines characterized by a high degree of instability of the insert.

The 4 lines St-29№25, St-29№2, St-29№44 and Kz-19№2 with the stability of chitinase inheritance ranged from 80 to 96%were established as a result of PCR analysis of wheat generations T1, T2 and T3. Partial cleavage occurred and the stability of the insert was less than 80% for St-29№43 and Kz-19№2 lines. Insertions of the desired gene were not found in lines St-29№35 and St-29№33.

Thus, the lines St-29№25, St-29№44, St-29№26 and Kz-19№1were selected as promising for in-depth study of their resistance to fungal diseases and further replication.

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БИОБАЛЛИСТИКАЛЫҚ ТРАНСФОРМАЦИЯДАН КЕЙІН БИДАЙ ЛИНИЯЛАРЫНА ЕНГІЗІЛГЕН ХИТИНАЗА ГЕНІНІҢ ТҰРАҚТЫЛЫҒЫН БАҒАЛАУ

Аннотация. Биобаллистика қазіргі кезде біржарнақты өсімдіктерді трансформациялауда тиімді әдістердің бірі болып саналады, бірақ бұл әдіс қосжарнақтыларға да сәтті қолданылуда. Баллистика арқылы күріш, жүгері, темекі, картоп, қызылша, соя, жүзім және басқа да дақылдар өзгеріске ұшырады.

2000 жылдан бастап жеке реттелетін элементтері бар гендердің бір түрінің немесе түраралық гені енгізілген организмдерді цисгендік немесе интргендік деп атауға ұсынылды. Цисгенді өсімдіктер гендік инженерия негізінде алынғанымен, олар гендік модификацияланған деп саналмайды. Тұқымдағы май қышқылының құрамы өзгерген және сульфонилмочевина негізіндегі гербицидке төзімді соянық цисгенді линиялары, қызанақтың жетілуін бәсеңдететін, сонымен қатар глифосфатқа төзімді жүгері линиялары белгілі. Қазіргі уақытта трансгенді технологияларды қоршаған ортаның стрестік факторларынан, мысалы, түрлі қоздырғыштан – вирус, бактерия, саңырауқұлақ және жәндіктер әсерінен құтылу әрі сорттар мен будандардың тұрақтылығын арттыру үшін күнен-күнге қолданысы артуда. Цисгендік биобаллистикалық тасымалдау элементтері ретінде ген жиынтығы емес, керісінше бір генмен кодталатын өсімдік қоздырғышының зақымдануын инактивациялауға қатысатын ақуыздар қолданылады.

Өсімдіктер табиғатында патоген індетіне қарсы PR ақуызының (pathogenesis related proteins) синтезі жүреді, солардың ішінде көп зерттелгені – хитиназалар мен β-1,3-глюканазалар. Өсімдік иммунитетіндегі жеке PR ақуызының нақты рөлін білу арқылы саңырауқұлақ ауруына төзімділігі жоғары трансгенді өсімдіктерді өсіруде белгілі бір антифунгецитті ақуыз қолдануға мүмкіндік береді. Сонымен қатар, қажетті белгі-нышаны бар өсімдік алумен қатар, берілетін геннің тұрақты экспрессиясы мен өсімдік тұқымында сол қасиетті бекіту маңызды.

Жұмыста цисгеннің ауысу әдісімен алынған бидайдың Т1, Т2 және Т3 тұқымына енгізілген хитиназа генінің тұрақтылығы зерттелді.

Хитиназа генін Саратовская 29 және Казахстанская 19 сорттарының жетілмеген бидай тұқымына биобалистік трансформация нәтижесінде бастапқы трансформацияланған регенерант өсімдіктері алынды. ПТР скринингі арқылы мақсатты гені бар болжамды 6 линия, соның ішінде Саратовская 29 сорты негізінде 6 линия мен Қазақстанская 19 сорты негізінде 2 линияны сұрыптап алуға мүмкіндік берді. Таңдалған қалпына келтіретін өсімдіктердің тұқымдық материалы топырақта өсіріліп, Т1 буынының тұқымы алынды. Сұрыпталған 8 линияның – Ст-29№25, Ст-29№43, Ст-29№44, Ст-29№33, Ст-29№26, Ст-29№35, Кз-19№1, Кз-19№2 ДНҚ-сын арнайы спецификалық праймермен амплификациялау нәтижесінде ген енгізілген 3 линия анықталды: енгізілген гені бар линиялардың жартылай ыдырауы арқылы және жоғары дәрежедегі тұрақсыздығы негізгі сипаты болып саналатын линиялар. Тұрақсыз тұқым қуалайтын дәрежедегі линиялар зерттеуден алынып тасталды және әрі қарайғы зерттеулерде қолданылмады. Жарым-жартылай ыдырайтын линиялар Т2 буынын алу мақсатында жұмыстың келесі кезеңіне жіберілді. ПТР анализінің нәтижелері бойынша Т1 буынында мақсатты хитиназа генінің енгізілгенін көрсетпеген Ст-29№35 және Ст-29№33 линиясы Т2 буынында да анықталмаған. Ст-29№43 және Кз-19№2 линиясында одан әрі жарым-жартылай ыдырау үдерісі тіркеліп, тұрақтылығы 76,92% және 76,6% сәйкесінше құрады, сондықтан бұл линияларды кейінгі тәжірибеде қолдануға мүмкіндік болмады. Ст-29№25, Ст-29№44, Ст-29№26 және Кз-19№1 линиясы хитиназа генінің Т2 буынында тұқым қуалайтын тұрақтылығы дәлелдеді. Осыған байланысты Т3 буынын көбейту мақсатында аталған төрт линия арықарай да қолданылды. Белгінің жарым-жартылай ыдырайтын қасиеті сипатталған Ст-29№43 линиясы келесі тұқымды көбейту мен талдау үшін таңдалды. Тәжірибелік өріс жағдайында трансформацияланған бидайдың Т3 5 линиясы алынды. Тұрақты енгізбенің тасымалдаушысы

ретінде Ст-29№25, Ст-29№44, Ст-29№26, Кз-19№1 линиясының Т2 буынының 232 өсімдігі және Ст-29№43 линиясының жарым-жартылай ыдырайтын енгізбесі бар өсімдіктер өсірілді.

Хитиназа генінің тұрақты тұқым қуалайтын қасиеті Ст-29№25, Ст-29№44, Ст-29№26 және Кз-19№1 төрт линияда анықталды. Келесі зерттеулерде Ст 29№25, Ст-29№44, Ст-29№26 және Кз-19№1 линиясының зертханалық және далалық жағдайда саңырауқұлақ ауруына төзімділігін және кейін көбею үдерісін қарастырылады.

Саңырауқұлақ қоздырғышына сезімталдықтың төмен және орташа дәрежесін көрсеткен сұрыпталған линиялар, жаңа сорт жасау және қоздырғыш пен өсімдіктің өзара әсерін зерттеу үшін қолданылуы мүмкін.

Түйін сөздер: трансформацияланған өсімдіктер, ДНҚ, бидай хитиназа гені, енгізбе тұрақтылығы.

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ОЦЕНКА СТАБИЛЬНОСТИ ВСТРОЕННОГО ГЕНА ХИТИНАЗЫ В ЛИНИЯХ ПШЕНИЦЫ ПОСЛЕ БИОБАЛЛИСТИЧЕСКОЙ ТРАНСФОРМАЦИИ

Аннотация. Биобаллистика на сегодняшний день является одним из самых эффективных методов трансформации однодольных, но при этом также с успехом применяется на двудольных растениях. С использованием баллистики были трансформированы рис, кукуруза, табак, картофель, свекла, соя, виноград и др.

С начала 2000-х годов были предложены организмы, в геном которых были введены гены одного с ними вида или видов с собственными регуляторными элементами, с которыми они могут скрещиваться, называемые цисгенными либо интрагенными. Считается, что цисгенные растения не являются генномодифицированными, хотя они и получены методами генной инженерии. Известны цисгенные линии сои с измененным составом жирных кислот в семенах и устойчивостью к гербицидам на основе сульфонилмочевины, с замедленным созреванием томатов, устойчивые к глифосату линии кукурузы. В настоящее время трансгенные технологии все чаще используются для повышения устойчивости сортов и гибридов к стрессовым факторам окружающей среды, например, к действию различных патогенов – вирусов, бактерий, грибов и насекомых. В качестве элементов для цисгенного биобаллистического переноса используют белки, участвующие в процессах инактивации поражения патогенами растений и кодируемые одним геном, а не семейством генов.

В растениях в ответ на инфицирование патогенами происходит синтез PR-белков (pathogenesis related proteins), наиболее изученными из которых являются хитиназы и β-1,3-глюканазы. Знание точной роли отдельных PR-белков в иммунитете растений дает возможность использовать определенные специфические антигрибные белки при создании трансгенных растений с повышенной устойчивостью к грибным болезням. При этом, важно не только получить растение с нужным признаком, но и закрепить в нем стабильную экспрессию перенесенного гена и наследование приобретенного признака в поколениях.

Нами проведено исследование стабильности вставки гена хитиназы в поколениях Т1, Т2 и Т3 трансформированных линий пшеницы, полученных методом цисгенного переноса.

В результате биобаллистической трансформации гена хитиназы в незрелые зародыши пшеницы сортов Саратовская 29 и Казахстанская 19 получены первичные трансформированные растения-регенеранты, скрининг которых методом ПЦР на наличие целевого гена позволил отобрать предположительно несущие вставку 6 линий на основе сорта Саратовская 29 и 2 линии на основе сорта Казахстанская 19. Семенной материал отобранных растений-регенерантов был культивирован в почвенных условиях и получены семена поколения Т1. Амплификация ДНК 8 отобранных линий Ст-29№25, Ст-29№43, Ст-29№44, Ст-29№33, Ст-29№26, Ст-29№35, Кз-19№1, Кз-19№2 со специфическими праймерами выявила линии, несущие вставку, линии с частичным расщеплением и линии, характеризующиеся большой степенью нестабильности вставки. Линии с нестабильно степенью наследования были выбракованы и не использованы в дальнейших исследованиях. Линии с частичным расщеплением были включены в следующий этап работы с целью получения Т2 поколения. По результатам ПЦР анализа в линиях Ст-29№35 и Ст-29№33, не показавших в поколении Т1 вставку целевого гена хитиназы, в поколении Т2 так же вставки не выявили. В линиях Ст-29№43 и Кз-19№2 происходит дальнейшее частичное расщепление и стабильность вставки составила 76,92% и 76,6%, соответственно, поэтому данные линии не могут быть использованы в последующих экспериментах. Линии Ст-29№25, Ст-29№44, Ст-29№26 и Кз-19№1 подтвердили стабильность наследования

гена хитиназы в поколении Т2. В связи с этим, эти четыре линии были использованы в дальнейшем тиражировании в Т3 поколении. Линия Ст-29№43, показавшая частичное расщепление признака, также была нами взята для размножения и анализа следующего поколения. В условиях экспериментального поля было получено поколение Т3 5 линий трансформированной пшеницы. Всего осуществлено культивирование 232 растений Т2 поколения линий Ст-29№25, Ст-29№44, Ст-29№26, Кз-19№1, определенных нами как носителей стабильной вставки и линии Ст-29№43 с частичным расщеплением в растениях по наличию вставки.

Стабильное наследование гена хитиназы подтверждено в четырех линиях Ст-29№25, Ст-29№44, Ст-29№26 и Кз-19№1Дальнейшие исследования предусматривают изучение устойчивости линий Ст-29№25, Ст-29№44, Ст-29№26 и Кз-19№1 к грибковым заболеваниям в лабораторных и полевых условиях с последующим тиражированием.

Отобранные линии, показавшие низкую и среднюю степень поражаемости к грибным патогенам, могут быть использованы для создания новых сортов и при изучении взаимодействия патоген-растение.

Ключевые слова: трансформированные растения, ДНК, ген хитиназы пшеницы, стабильность вставки.

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