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CISGENIC BIOLISTIC TRANSFORMATION FOR OBTAINING NEW FORMS OF POTATOES WITH IMPROVED RESISTANCE TO LATE BLIGHT

Abstract. This article presents the results of application of cisgenic biolistic transformation for the accelerated production of new forms of potato with increased resistance to late blight. The reason for late blight development is the parasitic organism *Phytophthora infestans*, belonging to oomycetes (pseudo-fungi), which infects valuable agricultural plants. In this study, with the aim of combating *P. infestans*, a number of experiments on the biolistic transformation of the most common potato varieties Aksor and Nevskiy were carried out in Kazakhstan. Two potato genes – *Rpi-vnt1.1* and *StREM1.3* – were selected as targets for introduction. Expression of the first gene should be activated, and the expression of the REMORIN1.3 gene should be suppressed. *Rpi-vnt1.1* was under the control of *Solanum tuberosum* polyubiquitin gene promotor (*Pat*) and *Arabidopsis thaliana* polyubiquitin 5 gene terminator (*ubq5*). Knock-down double stranded RNA-hairpin gene construction for *StREM1.3* silencing was under the control of *Solanum tuberosum* phytochrome B gene promotor (*phyB*) and *Arabidopsis thaliana* hot-shock protein 18.2 terminator (HSP18.2). Three series of biolistic transformation were carried out, as a result of which 636 regenerated plants of potato varieties Aksor and Nevskiy were obtained. DNA was extracted from the plant material of potato transformant plants in the quality and quantity suitable for PCR analysis for the presence of an insert. PCR analysis was carried out, revealing 52 plants carrying the VNT insert. *StREM1.3* silencing gene construction was detected in plant lines by qPCR, based on comparative analysis of of gene expression level and revealed 6 lines with reliably lower *StREM1.3* expression level in comparison with wild-type plants.
Key words: biolistic transformation, cisgenes, *Phytophthora infestans*, RNA-silencing, resistance genes.

Abstract. Late blight caused by the oomycete *Phytophthora infestans* is known to be one of the most harmful diseases of cultivated solanaceous plants such as potato, tomato, eggplant, and pepper. Due to late blight, annual losses of potatoes in the world reach 10-15% and, for example, in the USA alone, they cause damage of more than $ 6 billion [1, 2]. In case of severe damage to potatoes by late blight, in some years, the yield decrease can reach 70% or more.

Considering the fact that *P. infestans* is one of the most complex and variable parasitic organisms affecting agricultural plants, modern methods of genetic engineering and biotechnology are currently used to fight late blight. One of these approaches is the use of direct biolistic transformation of plants by their own resistance genes. It is known that about 74% of the *P. infestans* genome consists of repeating regions, with a large number of genes encoding effector proteins that are necessary for successful colonization of plant cells. RXLR proteins, which are the main class of transported effectors, are encoded by 550 phytophthora genes [3, 4]. These effector proteins that are recognized by plant immune receptors known as R-proteins (Resistance protein).

The use of potato's own genes encoding known R-proteins for biolistic transformation of potato plants may become one of the most successful approaches, which will allow obtaining new lines with increased resistance to most existing phytophthora strains.

The complexity of this approach is that despite the large number of crops that have been successfully modified by the method of biolistic transformation over the past 20 years [5], direct genetic transformation of potatoes is still rarely used to obtain new forms of this crop. Since the publication of the first work on potato bombardment in 2001 [6], the number of such works remains insignificant and mainly devoted to the analysis of the size of the inserted inserts [7], comparison with the method of PEG-mediated transformation of protoplasts [8], and studies on bombardment potato agrobacterial cells carrying three genes of interest [9].

Thus, the experimental work on the transformation of potato plants with genes of resistance to late blight carried out in this study will provide experimental data on the effectiveness of biolistic transformation of potatoes and the possibility of using this method to create new lines with resistance to late blight.

The goal of the research was to study the possibility of obtaining new forms of potatoes with increased resistance to late blight by cisgenic biolistic transformation.

Materials and methods. The object of the study: the varieties taken for the biolistic transformation were 2 varieties of domestic and Russian selection with different resistance to late blight. The domestic variety Aksor is relatively resistant, while the Russian variety Nevskiy is moderately resistant to disease.

Two potato genes - Rpi-vnt1.1 and StREM1.3 – were used as target genes. The Rpi-vnt1.1 gene is supposed to be expressed as an effector in transgenic plants, while the expression of the StREM1.3 gene, on the contrary, should be suppressed.

Genetic engineering constructs.

The sequence of the *Rpi-vnt1.1* gene from *Solanum venturii* was taken from the UniProt database (ID FJ423044) and is a genomic sequence from chromosome 9. It is 4310 bp long and carries one intron [10, 11]. For construction, the CDS sequence (710 ... 3385) was used. The *Rpi-vnt1.1* gene sequence is flanked by the potato *Pat* polyubiquitin gene transcription promoter from *Solanum tuberosum*, taken from the UniProt database (HM439286). At the 3' end, the *Rpi-vnt1.1* gene sequence is flanked by the *Arabidopsis thaliana* ubiquitin 5 (*ubq5*) transcription terminator. The sequence was taken from the GenBank database (At3g62250), the terminator length is 250 bp. [12]. The genetic construct was synthesized and assembled in the pUC57 plasmid by GenScript Limited.

The construction of the knockdown gene with a double-stranded RNA hairpin for StREM1.3 silencing is under the control of the *Solanum tuberosum* phytochrome B gene promoter (*phyB*) and the *Arabidopsis thaliana* heat shock protein 18.2 terminator (HSP18.2). The sequence of the *StRem1.3* gene from *Solanum venturii* was taken from the GenBank database (LOC102577743) and is a 597 bp mRNA sequence that encodes a 155 aa peptide [13]. Based on on-line sequence analysis in the Sfold program [14] with confirmation of the selected target sequences for RNA interference in commercial search engines BLOCK-iT RNAi Designer (ThermoFisher) and siRNA Target Finder (GenScript), two adjacent sites were selected -targets for small interfering RNA (siRNA): +348 - +369 bp and +411 - +432 bp. In the VectorNTI Suite 11.3 program, a sequence was created containing these regions (90 bp) in forward and reverse orientations, separated by an intron from catalase-2 of castor bean (*Ricinus communis*). At the 5'-end, the sequence of the knockdown sequence of the *StRem1.3* gene is flanked by the potato *phyB* (*Solanum tuberosum* phytochrome B) promoter. The sequence is from GenBank (Y14572.1). At the 3' end, the sequence is flanked by the transcriptional terminator of the *Arabidopsis thaliana* heat shock protein 18.2 (HSP18.2) gene. The sequence was taken from the GenBank database (NM_125364.3), the length of the terminator is 249 bp. [15]. The construct was synthesized and assembled on the pUC57 plasmid by GenScript Limited.

Genetically engineered constructs were cloned in chemically competent cells of *E. coli* strain DH-5α. Colony screening was carried out on LB medium containing 100 μg/ml ampicillin.

Isolation of plasmid DNA

Isolation of plasmid DNA was carried out using mini-columns and a DNA Extraction Kit (Thermo). *E. coli* cells carrying the plasmid were cultured overnight in 4 ml of Luria-Bertani (LB) medium on a shaker at 37 °C; further isolation was performed according to the manufacturer's instructions.

Preparation of genetically engineered cassettes for biolistic transformation

Plasmids pUC-patVNThsp and pUC-phyREMhsp were treated with *Eco*RI / *Sal*I restriction endonucleases and eluted from a 1% agarose gel with a QIAEX II Gel Extraction Kit (Qiagen).

Biolistic transformation of plant material

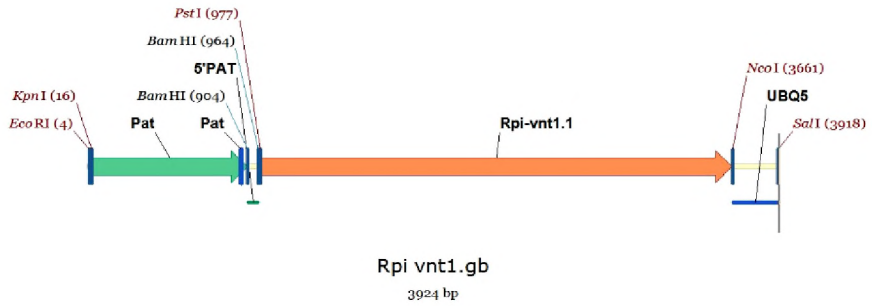
Biolistic transformation of potato internodes was carried out on a Biolistic PDS-1000/He Particle Delivery System (Bio-Rad) according to the manufacturer's instructions. For each experiment, 100 ng of elution-purified MEU, the minimal expression unit, was used. We used microparticles (1 μm) of gold (Bio-Rad) coated with DNA according to the binding procedure [16]. For each design, four to seven shots were fired using both 900 psi and 1100 psi discs.

General molecular biological methods

Determination of the amount of nucleic acids was carried out by measuring the ultraviolet absorption on a spectrophotometer Ultraspec 2000 (Pharmacia) at a wavelength of 260 nm. It was assumed that one absorption unit with a 1 cm wide cuvette corresponds to 35 μg of single-stranded DNA oligonucleotide or 50 μg of double-stranded DNA in 1 ml of solution.

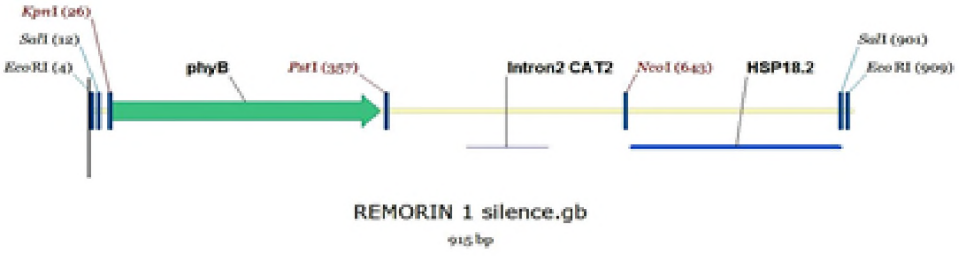
Nucleic acid electrophoresis was performed in a standard way [17] in agarose (1-2%) gel in TAE buffer.

Research results and discussion. To carry out the research, two genetically engineered constructs, Rpi_vnt1.1 and StREM1.3, carrying the sequences of two genes, two promoters and two terminators were constructed (figure 1, 2). All regulatory elements and target genes are cis-genic for the potato genus.



Rpi-vnt1.1 - sequence of the gene for resistance to late blight from *Solanum venturii*; Pat — promoter of transcription of the potato polyubiquitin gene from *Solanum tuberosum*; 5'PAT - 5'-NTP of the potato polyubiquitin gene from *Solanum tuberosum*; UBQ5 - terminator of transcription of the ubiquitin 5 gene from *Arabidopsis thaliana*

Figure 1 - Map of genetic engineering construct Rpi vnt1.1 (VectorNTI Suite 11.3)



phyB - promoter *phyB* (*Solanum tuberosum* phytochrome B gene); Intron2 CAT2 - sequence of the second intron of castor bean catalase-2 (CAT-2); HSP18.2 - transcription terminator of the gene for heat shock protein 18.2 (HSP18.2) from *Arabidopsis thaliana*

Figure 2 - Map of the genetically engineered construction StREM1.3 (VectorNTI Suite 11.3)

The resulting constructs were transformed into chemically competent cells of *E. coli* strain DH-5α for the production of constructs in preparative quantities required for research. Colonies containing the insert were screened in LB medium with 100 μg/ml ampicillin. Isolation of plasmid DNA was performed using mini-columns and a DNA Extraction Kit (Thermo). *E. coli* cells with a confirmed insert were cultured overnight in 4 ml of LB medium on a shaker at 37 °C, after that the plasmid was isolated according to the manufacturer's instructions.

A significant amount of plant explants obtained from internodes of test-tube potato plants were used to carry out biolistic transformation. More than 1000 test-tube plants of each variety Aksor and Nevskiy were produced by micropropagation method.

Obtaining DNA fragments for biolistic transformation of plant material

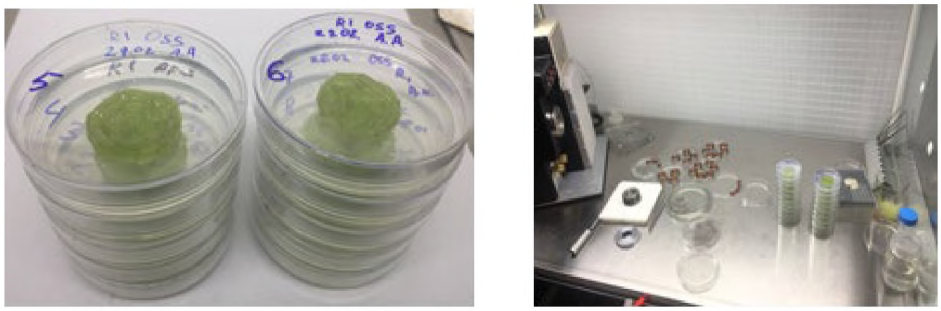
At the next stage of work, after obtaining and checking by restriction analysis of both constructs, we obtained DNA fragments suitable for biolistic transformation of plants. All genetically engineered constructs were treated with *Eco*RI / *Sal*I restriction endonucleases. Since the amount of DNA required for plant transformation was preparative, restriction was carried out in large volumes, after which the restriction mixtures were precipitated with isopropanol, and fragments of the required size were eluted from 1% agarose gel. The purified preparations of DNA fragments were checked for integrity by subsequent electrophoresis, the amount was measured and the purity of the preparation was assessed ($OD_{260/280}$). After that, the DNA concentrations in the obtained preparations were equilibrated and used for plant transformation.

Biolistic transformation of potato internodes

Potato internodes were prepared in advance (50-60 pieces per cup) and subjected to genetic bombardment at the rate of 100 ng of DNA fragment per experiment. The procedure was carried out in a standard manner according to the manufacturer's instructions for the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad), taking into account our optimized parameters.

For the best binding of genetic constructs to gold particles in the experiments, we used PM buffer containing polyethylene glycol and magnesium [16]; the helium pressure during the shot was 1100 psi.

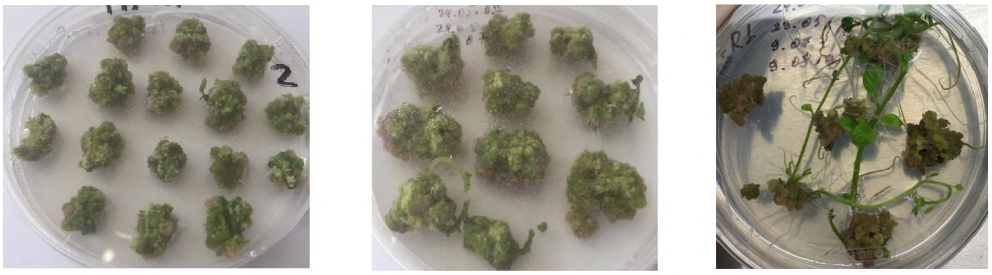
In total, 3 series of biolistic transformations were carried out on 3483 explants of internodes of test tube plants of potato varieties: Aksor (1779 pcs.) And Nevskiy (1704 pcs.) (figure 3).



A - internodes of Aksor potatoes on osmotic medium (OSS), ready for biolistics; B - biolistic transformation

Figure 3 - A series of biolistic experiments on potato internodes

After cultivation of the transformed internodes on the M6 callus-formation medium (MS medium with vitamins 4.43 g/L, 30 g/L sucrose, 2.4 D 2 mg/L and zeatin 0.5 mg/L), 625 calli of the Aksor variety and 556 calli of the Nevskiy variety were obtained, some of which were capable of embryogenesis. Embryogenic calli were passaged on R4 regeneration medium, which is based on MS medium with a standard vitamin content of 4.43 g/L, sucrose 30 g/L and 2 mg/ L gibberellic acid (figure 4).



A - embryogenic calli of the Aksor variety; B - embryogenic calli of the Nevskiy variety;
B - regenerant plants of the Aksor variety

Figure 4 - Regeneration of potato plants on callus cultures after biolistic transformation

According to the results of biolistic transformation, 636 transformed regenerant plants of potato varieties Nevskiy and Aksor were obtained. DNA samples were isolated from all transformants and their quality was checked for suitability for further analysis for the presence of inserted genes. All DNA samples were verified by amplification with primers to the actin reference gene, which is present in all plants. This stage was necessary to exclude false negative results in further experiments when determining the insertion of the target gene. PCR studies with the reference actin gene showed that all DNA samples met the requirements for PCR to detect the insert. Further, quantitative PCR analysis of the DNA of the transformant plants was carried out, which revealed 52 plants of the Aksor cultivar carrying the *VNT* insert.

The presence in the transformant plants of the construct with the *StREM1*.3 gene was also determined by the method of quantitative PCR through analysis of the level of gene expression in comparison with that in control wild-type plants. In total, this method revealed 6 lines of potato plants of the Aksor variety with a significantly lower level of expression of *StREM1*.3, which indicates the presence of this gene in them.

Thus, in general, the results obtained in the course of the conducted studies showed a certain effectiveness of the method of biolistic transformation in relation to potato plants. Two selected genes, *Rpi_vnt1.1* and *REMORIN1.3*, potentially suitable for creating new lines of *P. infestans* resistant potatoes, were transformed into plants by this method. As a result of 3 series of ballistic transformation of potato plants, 636 regenerant plants were obtained, of which 52 plants with the *VNT* insert and 6 lines with *StREM1.3* were found only in plants of the Aksor variety. The absence of gene insert in plants of the Nevskiy variety indicates that the use of the method of cisgenic biolistic transformation requires careful selection of conditions for each variety separately. Potato plants with the identified insert will be tested for resistance to late blight and with a high probability can be promising for creating new lines with improved resistance to the disease.

In general, based on the data obtained in the study, we can suggest that it is possible to create new lines of potatoes with improved qualitative characteristics in a short time based on the method of cisgenic biolistic transformation.

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ЦИСГЕНДІ БИОБАЛЛИСТИКАЛЫҚ ТРАНСФОРМАЦИЯ ӘДІСІН ҚОЛДАНУ АРҚЫЛЫ КАРТОПТЫҢ ФИТОФТОРОЗҒА ТӨЗІМДІ ЖАҚСАРТЫЛҒАН БЕЛГІЛЕРІ БАР КАРТОП ФОРМАЛАРЫН АЛУ

Аннотация. Мақалада цисгенді биобаллистика трансформациясы әдісін қолдану арқылы фитофторозға төзімділігі жоғары картоптың жаңа түрлерін шығару жөнінде зерттеулердің нәтижелері келтірілген. Бағалы ауылшаруашылық өсімдіктерін зақымдайтын, оомицеттерге жататын паразиттік организм *Phytophthora infestans* әсерінен фитофтороз пайда болады. *P. infestans* – күрделі және өзгермелі паразиттік организмдердің бірі болғандықтан, қазіргі кезде онымен күресу үшін гендік инженерия мен биотехнологияның заманауи әдістері қолданылады. Сол тәсілдердің бірі – фитофтора штамның көбісіне төзімді болып келетін жаңа линиялар құру мақсатында төзімділік гені бар өсімдіктердің тікелей биоболистикалық трансформациясын қолдану. Дақылдың жаңа түрлерін алу барысында картоптың тікелей генетикалық трансформация әдісі сирек қолданылатындықтан, аталған тәсілді қолдану қиынға соғады.

Бұл зерттеулерде *P. infestans*-ты бақылау мақсатында Қазақстанда ең көп таралған Аксор және Невский картобының цисгендік биоболистикалық трансформациясы бойынша бірқатар тәжірибелер жүргізілді. NCBI дерекқорында берілген ақпараттар негізінде ген-нысан ретінде картоптың екі гені – Rpi-vnt1.1 және

StREM1.3 таңдалды. Картоптың конститутивті промоторларының бақылауымен таңдалған ген арқылы генетикалық конструкциялар синтезделіп және дизайны құрастырылды.

Биобаллистикамен трансформацияланған өсімдіктерде Rpi-vnt1.1 генінің экспрессиясы белсенділік танытып, REMORIN1.3 генінің экспрессиясы басып-жаншылуы керек. Rpi-vnt1.1 – *Solanum tuberosum* полиубиквитин генінің промоторы (Pat) және *Arabidopsis thaliana* полиубиквитин 5 гендік терминаторы (ubq5) бақылауында болса, ал StREM1.3-ті сайленсингке арналған екітізбекті РНҚ-шпилькасы бар «нокдаун» генінің конструкциясы *Solanum tuberosum* фитохром В генінің промоторы (phyB) және 18.2 *Arabidopsis thaliana* белок терминаторының (HSP18.2) бақылауында жүреді.

Алынған конструкциялар DH-5α штамының E. coli хемикомпетентті клеткаларына зерттеуге қажетті мөлшерде трансформацияланды. Плазмидті ДНҚ бөліп алу үшін «Thermo» фирмасының миниколонналар мен DNA Extraction Kit реагенттер жинағы арқылы жүзеге асырылды.

Биоболистикалық трансформацияны жүзеге асыру үшін картоптың екі сорты пробиркада микроклоналды көбейтілді. Микроклоналды көбейту әдісі арқылы картоптың Аксор және Невский әр сорты үшін 1000-нан астам пробиркалық өсімдіктері көбейтілді. Тәжірибе барысында пробиркалық өсімдігінің түйінаралық бөлігінен алынған эксплантар қолданылды.

Биоболистикалық трансформацияның үш сериясы жүргізу нәтижесінде картоптың Аксор және Невский сортының 636 регенерант өсімдіктері алынды. Барлық трансформанттардан ДНҚ бөлініп және құрамына ген енгенін тексеру үшін сапалылығы тексерілді. ДНҚ-ның барлық үлгілері бүкіл өсімдікте кездесетін актин генінің праймерлеріне ампфликация жүргізу арқылы тексерілді. Бұл кезең мақсатты геннің енгізілуін анықтаған кезде жалған теріс нәтижелерді болдыртпайды. Актиннің референтті гені арқылы жүргізілген ПТР зерттеулерінің нәтижесінде барлық ДНҚ үлгілері вставканы анықтау үшін ПТР жүргізу талаптарына сәйкес келетіндігін көрсетті.

Әрі қарай трансформант өсімдіктердің ДНҚ-сына сандық ПТР анализі жасалып, нәтижесінде *VNT* вставкасы бар Аксор сортының 52 өсімдігі анықталды. Сандық ПТР арқылы гендік экспрессия деңгейінің салыстырмалы талдауы негізінде StREM1.3 генінің құрылымы өсімдік линияларында анықталды. Жабайы типтегі өсімдіктермен салыстырғанда картоптың Аксор сортының 6 линиясында StREM1.3 экспрессиясы едәуір төмен болды, яғни бұл олардың құрамында аталған геннің бар екендігін көрсетеді.

Жүргізілген зерттеулер барысында алынған нәтижелер картоп өсімдігіне қатысты биоболистикалық трансформация әдісінің белгілі бір тиімділігін көрсетті. Бұл әдіс арқылы картоптың *P. infestans*-қа төзімді жаңа линияларын құру үшін таңдалған екі ген Rpi_vnt1.1 және REMORIN1.3 өсімдіктерге трансформацияланды. Анықталған вставкасы бар картоп өсімдіктері фитофтораға төзімділігі үшін әрі қарай тексерілетін болады және ықтималдығы жоғары, ауруға төзімді жақсарған жаңа линияларды алуға болады. Жалпы зерттеу барысында алынған мәліметтерге сүйене отырып, цисгендік биобаллистикалық трансформация әдісі арқылы қысқа мерзімде сапалы жаңа картоп линияларын жасауға болады.

Түйін сөздер: биобаллистикалық трансформация, цисгендер, картоп фитофтора инфестенттері, РНҚ-ны басу, қарсыласу гендері.

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ПРИМЕНЕНИЕ МЕТОДА ЦИСГЕННОЙ БИОБАЛЛИСТИЧЕСКОЙ ТРАНСФОРМАЦИИ ДЛЯ ПОЛУЧЕНИЯ НОВЫХ ФОРМ КАРТОФЕЛЯ С УЛУЧШЕННЫМИ ПРИЗНАКАМИ УСТОЙЧИВОСТИ К ФИТОФТОРОЗУ

Аннотация. В данной статье представлены результаты исследований по использованию метода цисгенной биобаллистической трансформации для ускоренного получения новых форм картофеля с повышенной устойчивостью к фитофторозу. Причиной развития фитофтороза является паразитарный организм *Phytophthora infestans,* относящийся к оомицетам, поражающим ценные сельскохозяйственные растения. *P. infestans* является одним из наиболее сложных и изменчивых паразитических организмов, для борьбы с которым в настоящее время используют современные методы генной инженерии и биотехнологии. Одним из таких подходов служит использование прямой биобаллистической трансформации растений собственными генами устойчивости для создания новых линий с повышенной устойчивостью к большинству существующих штаммов фитофторы. Сложность такого подхода заключается в том, что прямая генетическая трансформация картофеля все еще редко используется для получения новых форм этой культуры.

В данном исследовании с целью борьбы с P. infestans был проведен ряд экспериментов по цисгенной биобаллистической трансформации наиболее распространенных в Казахстане сортов картофеля Аксор и Невский. На основе информации, представленной в базе данных NCBI, в качестве генов-мишеней выбраны два гена картофеля картофеля – Rpi-vnt1.1 и StREM1.3. Осуществлен дизайн и синтезированы генетические конструкции с отобранными генами под контролем конститутивных промоторов картофеля.

Экспрессия Rpi-vnt1.1 гена в трансформированных биобаллистикой растениях должна быть активирована, а экспрессия гена REMORIN1.3 должна быть подавлена. Rpi-vnt1.1 находится под контролем промотора гена полиубиквитина Solanum tuberosum (Pat) и терминатора гена полиубиквитина 5 *Arabidopsis thaliana* (ubq5). Конструкция гена «нокдаун» с двухцепочечной РНК-шпилькой для сайленсинга StREM1.3 находится под контролем промотора гена фитохрома В *Solanum tuberosum* (phyB) и терминатора белка горячего шока 18.2 *Arabidopsis thaliana* (HSP18.2).

Полученные конструкции были трансформированы в хемикомпетентные клетки *E. coli* штамма DH-5α для наработки конструкций в препаративных количествах, необходимых для исследований. Выделение плазмидной ДНК осуществляли с использованием мини-колонок и набора реагентов DNA Extraction Kit фирмы “Thermo”.

Для проведения биобаллистической трансформации предварительно проводили микроклональное размножение пробирочных растений картофеля обоих сортов. Методом микроклонального размножения было произведено свыше 1000 пробирочных растений каждого сорта Аксор и Невский. В экспериментах использовали растительные экспланты, полученные из междоузлий пробирочных растений.

Было проведено три серии биобаллистической трансформации, в результате которых получено 636 растения-регенеранта картофеля сортов Аксор и Невский. Из всех трансформантов выделены образцы ДНК и проверено их качество на пригодность к дальнейшему анализу на наличие встроенных генов. Все образцы ДНК были проверены амплификацией с праймерами к референтному гену актина, который имеется у всех растений. Данный этап позволил исключить в дальнейших экспериментах появление ложноотрицательных результатов при определении вставки целевого гена. ПЦР исследования с референтным геном актина показали, что все образцы ДНК соответствуют требованиям для проведения ПЦР на выявление вставки.

Далее был проведен количественный ПЦР анализ ДНК растений-трансформантов, который выявил 52 растение сорта Аксор, несущее вставку *VNT*. Конструкция с геном StREM1.3 также была обнаружена в линиях растений с помощью количественного ПЦР, на основе сравнительного анализа уровня экспрессии генов. Всего было выявлено 6 линий картофеля сорта Аксор с достоверно более низким уровнем экспрессии StREM1.3 по сравнению с растениями дикого типа, что говорит о присутствии в них данного гена.

Результаты, полученные в ходе проведенных исследований, показали определенную эффективность метода биобаллистической трансформации в отношении растений картофеля. Два выбранных гена *Rpi_vnt1.1* и *REMORIN1.3*, потенциально пригодных для создания новых линий картофеля, устойчивого к *P. Infestans,* были трансформированы в растения данным способом. Растения картофеля с выявленной вставкой в дальнейшем будут исследованы на устойчивость к фитофторозу и с высокой вероятностью могут быть перспективными для создания новых линий с улучшенной резистентностью к заболеванию. В целом, основываясь на полученных в исследовании данных, можно утверждать, что на основе метода цисгенной биобаллистической трансформации за короткий срок можно создать новые линии картофеля с улучшенными качественными признаками.

Ключевые слова: биобаллистическая трансформация, цисгены, картофель Phytophthora infestans, подавление РНК, гены устойчивости.

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