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DEVELOPMENT OF VECTORS FOR *Agrobacterium*-MEDIATED GENETIC TRANSFORMATION OF PLANTS CONTAINING THE SYNTHETIC *CRY1Ab* GENE ENCODING RESISTANCE TO LEPIDOPTERAN PESTS

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The research was aimed to develop genetic constructs for *Agrobacterium*-mediated plant transformation, containing the synthetic *cry1Ab* gene, and their testing through the transformation of tobacco, followed by a molecular genetic analysis of the obtained plants to confirm the transformation event. Basic methods of DNA cloning, *Agrobacterium*-mediated transformation of *Nicotiana tabacum* L. by leaf disc method, selection of transformants *in vitro*, analysis of the transgene presence in plant DNA, detection of *cry1Ab* gene expression by PCR with reverse transcription were used. In the course of the study, the vectors pCB182 and pCB241 that contained the synthetic gene *cry1Ab* were constructed. *Agrobacterium*-mediated transformation of tobacco was carried out by created vectors and regenerant plants containing transgenes in their DNA were obtained. Expression of *cry1Ab* transgene in the obtained transformants of tobacco by the RT-PCR method was confirmed. As a result of the *Agrobacterium*-mediated transformation of plants with pCB182 and pCB241 vectors containing the synthetic *cry1Ab* lepidopteran resistance gene it is possible to obtain transgenic plants with expression of the transgene.

Key words: *cry1Ab*, *Nicotiana tabacum* L., *Agrobacterium*-mediated transformation, PCR-analysis, transgenesis.

Human agricultural activity is constantly faced with obstacles that lead to significant crop losses. One of these natural factors, and perhaps the most important of it, is the impact of insect pests on crop yields. In particular, current estimates of worldwide productivity losses of wheat, rice, corn, potatoes and soybeans associated with the major 137 pathogens and pests are 8–41% [1] national and global levels. Quantitative, standardized information on crop losses is difficult to compile and compare across crops, agroecosystems and regions. Here, we report on an expert-based assessment of crop health, and provide numerical estimates of yield losses on an individual pathogen and pest basis for five major crops globally and in food security hotspots. Our results document losses associated with 137 pathogens and pests associated with wheat, rice, maize, potato and soybean worldwide. Our yield loss (range. Pest control) is an ongoing process. The use of synthetic insecticides does considerable harm not only to the target species but also to the environment, including humans. The influence of chemical poisons leads to the destruction of ecosystems, also their ability to accumulate in the soil calls into question the possibility of rapid restoration of natural balance. In addition, the development of mechanisms

of resistance to chemical insecticides is a significant problem in populations of insect pests. Thus, from 1938 to 1976, the number of insects insensitive to agricultural poisons increased from 7 to 364, doubling since then every 6 years [2]. This is why the question arises about the creation of new methods of pest control, which will be characterized by the biological origin of the active substances and their ability to rapidly biodegrade in the environment, a narrow range of target species, a harmlessness to other living organisms.

The sought-after remedy are Bt-toxins. The natural source of the toxin is the anaerobic chemoorganotrophic bacterium *Bacillus thuringiensis*, which resides in the soil. A feature of the bacterium is the ability to form peculiar inclusions in the cytoplasm in the process of sporulation which are crystals of prototoxin with a molecular weight of 130–145 kDa with an insecticidal effect [3]. These crystals are unique to each strain of *B. thuringiensis*. The differences between them are revealed at the primary level of organization of protein molecules, as a direct realization of genetic information. The features of the secondary structure directly affect the rate of transition of prototoxin from crystalline form to soluble, that is, the biological activity of the toxin. Some strains of *B. thuringiensis* are able to produce 5–9 varieties of prototoxin instead of one. The peculiarities of the amino acid composition and structural organization of the active toxins formed from prototoxin molecules cause their receptor affinity for the cells of the midgut of a very limited range of insect species. This targeted action makes Bt-toxin an ideal remedy against crop pests [4]. At the very beginning of the use of *B. thuringiensis* as an alternative to chemical insecticides, direct spraying of the bacterial material (in sporulated form) was applied on the fields damaged by the pest. In the end, due to the growing acreage, this method was considered economically inappropriate for mass use. In addition, spraying over large areas of an extraneous bacterial agent alters the composition and balance of the soil biota, which in the long run is capable of disrupting local ecosystems [5]. An alternative to spraying of bacterial material in the fields is the transfer of bacterial Bt-toxin genes to the plant genome by genetic transformation methods, followed by the production of genetically modified plants capable of synthesizing and accumulating the toxin on their own, thereby protecting against pests [6–7]. The data accumulated over recent years have made it possible to characterize a large number of new *B. thuringiensis* strains and specific toxin action for a wide range of insect species. The complex use of bacterial material and genetically modified plants has put Bt-products at the forefront. As of 2015, the worldwide use of Bt-biopesticides is around 97% [8].

Genetic constructs containing *cry1Ab* gene are the most often used for the formation of plant resistance against *Lepidoptera* insects [8]. The obtained transgenic varieties have been industrially grown since 1996, and their use is increasing annually. In particular, as of 2017, the global area allocated for the cultivation of biotechnological crops in 2017 is estimated at 189.8 million hectares [9].

The purpose of this study was to create genetic constructs containing the synthetic *cry1Ab* gene adapted for expression in the plant genome for *Agrobacterium*-mediated transformation of plants. The efficiency of the vectors was determined by transformation of the tobacco model object (*Nicotiana tabacum*), followed by molecular genetic analysis of the plant material obtained.

Materials and Methods

Genetic constructs. When developing constructs containing *cry1Ab* gene, binary vectors pICBV16 and pICBV19 (Icon Genetics GmbH, Germany) were used for *Agrobacterium*-mediated plant transformation. They contained the *uidA* β-glucuronidase reporter gene under the control of the 35S promoter, also selective neomycin phosphotransferase II (*nptII*) gene in pICBV16 vector, or phosphinothricinacetyl transferase (*bar*) gene in pICBV19 vector.

Cloning reactions were performed using the InsTAclone™ PCR Cloning Kit (Thermo Fisher Scientific). Purification of DNA fragments for ligase reaction was performed using the Silica Bead™ DNA Gel Extraction Kit (Thermo Fisher Scientific).

By preparative restriction of the pICBV16 construct, *uidA* gene was removed using restriction enzymes NcoI and BamHI. The isolated and purified DNA fragment with *cry1Ab* gene was embedded to the vector pTZ57R. *Escherichia coli* XL-1 Blue strain was transformed using the resulting structure. Preparative restriction of the recombinant DNA obtained was performed using NcoI and BamHI restriction enzymes, with the isolation of a region containing *cry1Ab* gene. Ligation of the obtained insert fragments (the coding sequence of *cry1Ab* gene) and the

linearized vector pICBV16 was performed (the final construct was named PCB241). Preparative restriction of the recombinant DNA obtained was performed using EcoRI and BamHI restriction enzymes, with the isolation of a region containing 35S promoter and *cry1Ab* gene. By preparative restriction of the pICBV19 construct using the EcoRI and BamHI restriction enzymes, *uidA* gene and 35S promoter were removed. Ligation of the obtained insert fragments (the coding sequence of *cry1Ab* gene and 35S promoter) and the linearized vector pICBV19 led to creation of pCB182 construct. Schemes of final structures are shown in Fig. 1.

Recombinant DNA was transferred into *Agrobacterium tumefaciens* GV3101 cells, resulting in bacterial colonies containing genetic constructs pCB182 or pCB241.

Plant material. The leaves of aseptic tobacco plants (*Nicotiana tabacum* L.) of Petit Havana variety grown on MS nutrient medium [10] without phytohormones, at +25 °C and 16-hour daylighting have been used in the experiments.

Agrobacterium-mediated transformation. *Agrobacterium*-mediated transformation of *N. tabacum in vitro* plants was carried out by leaf disks method [11]. After transformation, leaf explants were transferred to regenerative selective MS medium containing salts, vitamins and carbohydrates, 1 mg/l 6-benzylaminopurine (BAP), 0.1 mg/l 1-naphthylacetic acid (NAA), 500 mg/l cefotaxime (Cx) to inhibit the growth of bacteria, and 100 mg/l kanamycin, or 5 mg/l phosphinothricin as selective agents, depending on the vector used for transformation. The frequency of regeneration (FR) on selective media was estimated by the percentage of the number of explants formed shoots to the total number of explants transferred. The regenerated plants were grown *in vitro* at phytohormone-free MS medium containing 500 mg/l Cx and a suitable selective agent at the indicated concentration.

Isolation of total plant DNA. Isolation of total DNA from plant leaves was performed by the method [12]. DNA was dissolved in 50 µl of TE buffer pH 8.0. The presence of DNA in the solution was confirmed by 0.8% agarose gel electrophoresis in TBE buffer [13].

The concentration of DNA in the solution was determined by spectrophotometric measurement of the level of adsorption of UV-light with a wavelength 260 nm (A_{260}) [14]. The purity of the DNA was evaluated by the ratio A_{260}/A_{280}. Spectrophotometric measurements were performed using a photometer BioPhotometer v. 1.35 (Eppendorf). The DNA content of the solution was brought to a final concentration of 30 µg/ml by adding TE buffer.

PCR conditions. A Mastercycler® Personal amplifier (Eppendorf) was used to conduct the polymer chain reaction (PCR). The reaction mixture (20 µl) contained 13.9 µl of sterile deionized water, 2 µl of 2 mM dNTP, 2 µl of 10x Green Buffer, 0.5 µl of 10 mM forward primer, 0.5 µl of 10 mM reverse primer, 0.1 µl of DreamTaq™ DNA polymerase (Thermo Fisher Scientific). The primers (Metabion, Germany) used in the study are presented in Table 1.

The PCR reaction for detection of *vir* D1 gene sequence was performed in order to exclude from the list of tested samples those who contaminated with agrobacterial DNA. Confirmation of the fact of agrobacterial contamination allows to reject false positive results of the study. Detection of *vir* D1 gene sequence was performed according to the Lipp João protocol [16].

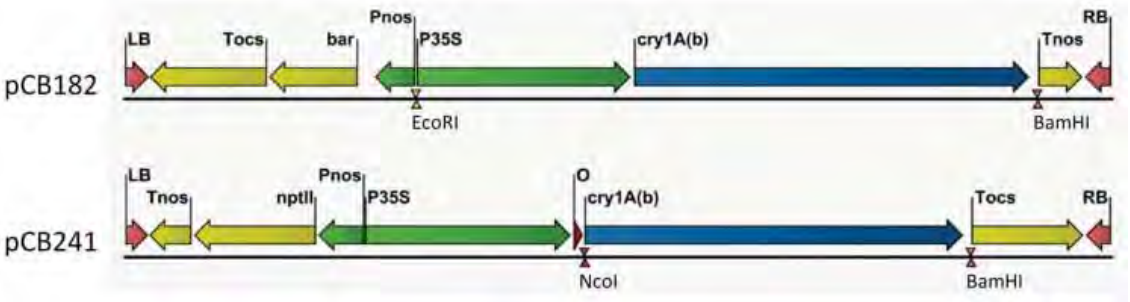

Fig. 1. **Scheme of T-DNA constructs PCB182 and PCB241:**
Tocs — octopinsynthase gene terminator; *Tnos* — nopalinsynthase gene terminator; *bar* – phosphinotricinacetyl transferase gene as selective, the product which confers phosphinothricin resistance; *nptII* — neomycin phosphotransferase II gene as a selective, with product, which confers kanamycin resistance; *Pnos* — nopalinsynthase gene promoter; *P35S* — 35S RNA gene promoter of cauliflower mosaic virus; *OΩ* — omega sequence that enhances the gene expression; *cry1(A)b* — the coding sequence of **cry***1(A)b* gene

Table 1. **The primers used to detect gene sequences**

Primer name	Gene	Sequence	Amplicon length, bp.; reference
Npt1	*nptII*	5′ — GAG GCT ATT CGG CTA TGA CTG — 3′	700; [15]
Npt2		5′ — ATC GGG AGC GGC GAT ACC GTA — 3′	
VirD1-1	*vir D1*	5′ — ATG TCG CAA GGC AGT AAG CCC A — 3′	437; [16]
VirD1-2		5′ — GGA GTC TTT CAG CAT GGA GCA A — 3′	
barpr5	*bar*	5′ — GCG GTC TGC ACC ATC GTC AAC — 3′	551; [17]
rev581		5′ — CAG ATC TCG GTG ACG GGC AGG AC — 3′	
CryO5	*cry1Ab*	5′ — AGG ATT CGC TAC GCT AGC AC — 3′	475
CryO6		5′ — GGA GAT TCC TCT CGT CGC TG — 3′	

Note: bp — base pairs.

The amplification program for the detection of *bar* gene sequence was performed according to Sakhno protocol [17]. Amplification program for detection of *nptII* gene sequence was performed by the TD-PCR method (Touchdown-PCR) [18]. The amplification program for *cry1Ab* gene consisted of: primary denaturation — 4 min at 94 °C; 35 cycles: 30 s at 94 °C, 30 s at 55 °C, 28 s at 72 °C; final elongation — 10 min at 72 °C, with following cooling of reaction mixture to 22 °C.

The amplification products were separated in a 1.2% agarose gel with 0.5 μg/ml ethidium bromide in TBE buffer at an electric field voltage of 6 V/cm during 45 min. The gel image in ultraviolet light was documented on the mLKB Bromma 2011 Macrovue Transilluminator using a Canon EOS 600D digital camera. Electrophoresis images were processed by a GIMP bitmap editor (GNU Image Manipulation Program, www.gimp.org) and a Microsoft PowerPoint text-and-graphic editor.

Reverse transcription. To determine whether transcription of *cry1Ab* gene in the plant genome occurs, total RNA was isolated by the method [19] from the leaf material of rooted regenerants. The cDNA synthesis was initiated by the Oligo dT18 primer (Thermo Fisher Scientific) from mRNA and was performed by Maxima Reverse Transcriptase (Thermo Fisher Scientific) enzyme according to the manufacturer's instructions. The newly synthesized cDNA first strand was analyzed for the presence of *cry1Ab* gene by PCR using the specific CryO5 and CryO6 primers.

Growing transformants in soil. Plants that showed the presence of transgenic sequences in their genome during molecular genetic analysis were transferred to the soil mixture and grown under greenhouse conditions to obtain seed material. The soil mix consisted of peat, sand and turf, in a 2:1:1 ratio. The cultivation was carried out under the conditions of 12-hour light day, at an air temperature of 24 °C.

Results and Discussion

As a result of molecular genetic manipulations, binary vectors pCB241 and pCB182 were constructed. They were containing *cry1Ab* gene under the control of *35S* cauliflower mosaic virus promoter, the selective neomycin phosphotransferase II (*nptII*) gene in case of the vector pCB241, or phosphinothricinacetyl transferase (*bar*) gene for the vector pCB182, under the control of the promoter of bacterial nopalinsynthase gene.

To evaluate the effectiveness of the vectors obtained, the transformation of tobacco was performed using *A. tumefaciens*, which cells contained the binary vector PCB241 or PCB182. As a result of the transformation, the tobacco plants capable to rooting on selective media with 100 mg/l kanamycin or 5 mg/l phosphinothricin were obtained. The beginning of plant regeneration from explants transformed with the pCB241 construct was observed after 5 weeks of cultivation on a selective bacteriostatic medium (Fig. 2), whereas after transformation with the pCB182 vector, plant regeneration occurred after 14 weeks. The percentage of explants that formed plants (regeneration frequency, RF) on a selective medium after transformation with vector pCB182 was significantly lower

Table 2. **Results of Agrobacterium-mediated transformation of *N. tabacum* using vectors pCB182 and pCB241**

Vector	RF,%	Rooting regener-ants, units	Sensitive regener-ants,%	PCR results (+)			
				Analyzed samples, in total	Selective gene, samples	cry1Ab, samples	Selective +cry1Ab, samples
pCB182	22	86	14	54	51	19	19
pCB241	84	123	66	28	25	12	12

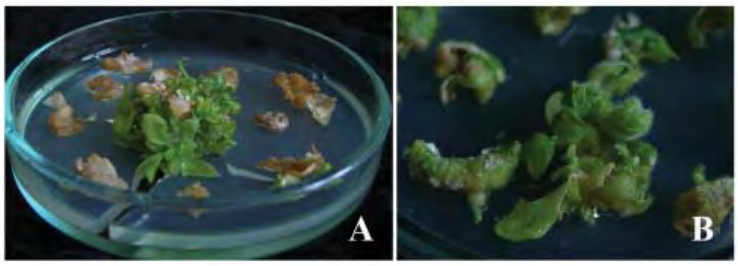

Fig. 2. **Regeneration of tobacco shoots on selective media after *Agrobacterium*-mediated vector t ransformation with pCB182 vector (*A*) or pCB241 (*B*)**

comparing with the transformation using vector pCB241 (Table 2).

After transformation with the pCB241 construct, as well as pCB182, a significant number of regenerants appeared to be sensitive to the selective agent upon further cultivation in banks with selective medium (Table 2). This may be due to a change in transgene expression. Factors that can cause such a phenomenon include: tissue culture, chimerism, transgene integration site (positional effect), copy number (dosing effect), transgenic mutation and epigenetic gene inactivation [20, 21]. Gene silencing, downregulation, or loss of expression can occur at the transcriptional or post-transcriptional level [22]. In the analysis of transgenic lines of rice Chareonpornwattana with co-authors [23] observed that gene silencing correlated more with transgene expression level than with copy number. It has been found that high promoter activity can lead to hypermethylation and, as a consequence, termination of gene transcription in both monocotyledonous and dicotyledonous plant species. The high percentage of plants that showed signs of sensitivity to the selective agent after prolonged cultivation, and the low rate of regeneration on the selective medium observed after transformation with the pCB182 vector, may also be due to the toxic effect of *cry1Ab* gene product on the plant cell, since Cry proteins of GM plants are more solubilized comparing with molecules of the wild type (which are crystalline) and therefore acquire new properties within these plants [24]. In general, up to 23% of plant objects after transformation can show signs of gene silencing [23].

The use of alternative regulatory systems and methods of transformation leads to more stable results. Thus, the adding into the rice genome of *cry1Ab* synthetic gene under the control of corn ubiquitin promoter *Pubi* is characterized by a high (up to 76%) frequency of transformation among the total number of regenerants, stable inheritance [25] and transgene expression [26], a significant level of accumulation of protein product in green parts of plants.

A study by Jabeen [27] demonstrated that the expression levels of *cry1Ab* gene in chloroplasts are significantly higher than those in a nucleus. Thus, the introduction of this transgene into the plastid system is a promising area of work.

DNA of regenerants resistant to selective agents (82 plants in total: 54 plants obtained after transformation with pCB182 construct and 28 plants obtained after transformation with pCB241 vector) was analyzed by PCR. The analysis aimed to identify *bar* gene sequences (for plants transformed with pCB182 construct only), *nptII* (for plants transformed with pCB241 construct only), *cry1Ab*, and *vir* D1 (to control the possibility of agrobacterial contamination).

Among the studied plants, according to the results of PCR analysis to detect the sequence of *vir*D1 gene, only 4 samples showed the presence of the amplicon of the proper size, which may indicate an agrobacterial

contamination of the plant material. The results obtained with these samples were excluded from further research as potentially false positives.

The following PCRs were performed to identify selective genes that are part of the transforming vectors to confirm their transfer to the plant genome: *bar* gene for plants transformed by the pCB182 construct and *nptII* gene for plants transformed by the pCB241 construct.

PCR detection of sequence of *bar* gene in plant DNA obtained after transformation by the pCB182 vector showed that 94.4% of the samples demonstrate the presence of a corresponding 551 bp amplicon. (Table 2, Fig. 3), which indicates the transfer of the gene to the plant genome.

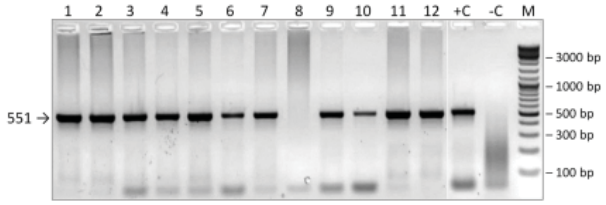

Fig. 3. **Electrophoregram of *bar* gene amplification products from DNA of tobacco regenerants obtained after transformation with pCB182 vector**
Tracks No. 1–7, 9–12 — DNA of *N. tabacum* lines 1-1, 1-2-1, 2-1, 2-2, 1-3-1, 4-1-1, 4-1-2, 2-3, 4-2, 1-4, 1-5; No. 8 — control (DNA of the non-transformed plant *N. tabacum*), +C — positive control (DNA of *N. tabacum* pICBV19); –C — negative control (TE buffer); M – molecular weight marker GeneRuler™ DNA Ladder Mix (Thermo Fisher Scientific). Expected amplicon size is 551 bp

Previous attempts to detect the sequence of *nptII* gene using PCR have resulted in a significant number of nonspecific amplicons on electrophoregrams. Therefore, TD-PCR (Touchdown-PCR) was used to achieve a positive result. TD-PCR allows achieving the accumulation of a specific product with a stable composition of the reaction mixture only by optimizing the temperature cycle. The initial primer annealing temperature increases by 10 °C, but gradually decreases in subsequent cycles. Thus, the specificity of the reaction increases [28].

Touchdown-PCR performed to detect the sequence of *nptII* gene in the DNA of plants obtained after transformation by the pCB241 vector showed the presence of the desired 700 bp amplicon in 89.3% of the samples (Table 2, Fig. 4).

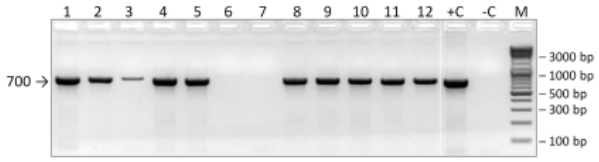

Fig. 4. **Electrophoregram of *nptII* gene amplification products from DNA of tobacco regenerants obtained after transformation with pCB241 vector**
Tracks No. 1–6, 8–12 – DNA of *N. tabacum* lines 24-1, 25-1, 18-1, 12-1, 31-1, 22-1, 38-1, 37-1, 43- 1, 44-1, 46-1; No. 7 – control (DNA of the non-transformed plant *N. tabacum*), +C — positive control (DNA of *N. tabacum* pICBV16); –C — negative control (TE buffer); M — molecular marker GeneRuler ™ DNA Ladder Mix. Expected amplicon size — 700 bp

PCR analysis of the DNA of the analyzed regenerants to detect the sequence of *cry1Ab* gene (Fig. 5) revealed the presence of a corresponding amplicon of 475 bp in length in 35.2% of samples obtained after transformation by vector pCB182 and 42.9% of samples obtained after transformation by vector pCB241. The presence of incomplete T-DNA copies in one or more plant genome sites in transformed plants after *Agrobacterium*-mediated transformation has also been shown by other researchers [29].

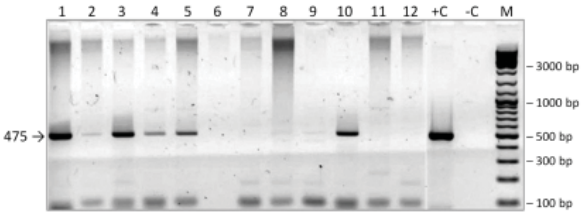

Fig. 5. **Electrophoregram of *cry1Ab* gene amplification products from DNA of tobacco regenerants obtained by transformation with pCB182 construct**
Tracks No. 1–7, 9–12 – DNA of regenerants of *N. tabacum* lines 1-1, 1-2-1, 2-1, 2-2, 1-3-1, 4-1-1, 4-1- 2, 2-3, 4-2, 1-4, 1-5; No. 8 — control (DNA of non-transformed *N. tabacum* plant); +C — positive control; –C — negative control (TE buffer); M — molecular marker GeneRuler ™ DNA Ladder Mix (Thermo Fisher Scientific). Expected amplicon size — 475 bp

For the reverse transcription reaction, total RNA was isolated from the leaf material of 4 rooted plants transformed with the pCB182 construct, and 4 plants transformed with the pCB241 vector. Lines 2-1 (182), 1-3-1 (182), 4-2 (182), 3-2-1 (182), 24-1 (241), 2-4-2 (241), 37-1 (241), 43-1 (241) were used for the study.

PCR analysis of the cDNA synthesized during the reaction showed the presence of *cry1Ab* gene in all samples. A typical electrophoregram is presented in Fig. 6. At the same time, all controls worked in a quality and expected manner.

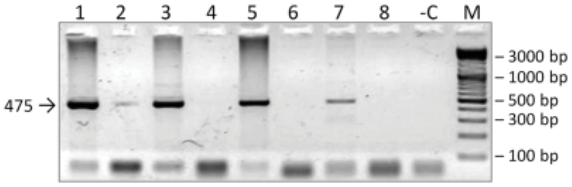

Fig. 6. **Results of the reverse transcription reaction using the transformants obtained by vector pCB182**
Tracks No. 1, 3, 5, 7 — cDNA of transformants of *N. tabacum* plants analyzed; Tracks No. 2, 4, 6, 8 — negative controls (without revertase); –C — negative control (without RNA); M — molecular marker GeneRuler™ DNA Ladder Mix (Thermo Fisher Scientific)

Thus, the expression of *cry1Ab* transgene in the obtained *N. tabacum* transformants was significantly confirmed.

Plants that showed a clear presence of *cry1Ab*, *bar* and *nptII* genes in PCR studies were transferred to the soil for grown and seed production (Fig. 7). Seed generation T1 was obtained after self-pollination, which indicates that there is no influence of transforming vectors on plant fertility.

Conclusions

Thus, in the course of the work, genetic constructs pCB182 and pCB241 were developed. The vectors contained synthetic *cry1Ab* gene, which confers resistance to Lepidopteran pests. Vectors are intended for plant modification by *Agrobacterium*-mediated transformation. The ability of the obtained constructs to transfer *cry1Ab* gene to the plant genome of tobacco, a model object of biotechnology, is demonstrated. Transcriptional activity of *cry1Ab* transgene in the genome of *Nicotiana tabacum* was detected. The expression of a foreign gene did not affect the fertility of the plants.

Fig. 7. **Transformed tobacco plant in soil**
Vector pCB241, line 2-4-2

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СТВОРЕННЯ ВЕКТОРІВ ДЛЯ *AGROBACTERIUM*-ОПОСЕРЕДКОВАНОЇ ГЕНЕТИЧНОЇ ТРАНСФОРМАЦІЇ РОСЛИН ІЗ ЦІЛЬОВИМ СИНТЕТИЧНИМ ГЕНОМ *CRY1Ab*, ЯКИЙ КОДУЄ СТІЙКІСТЬ ДО ШКІДНИКІВ РЯДУ ЛУСКОКРИЛИХ

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Метою дослідження було створення генетичних конструкцій для *Agrobacterium*-опосередкованої трансформації рослин, які містили б синтетичний ген *cry1Ab*, та їх тестування шляхом трансформації тютюну з подальшим проведенням молекулярно-генетичного аналізу отриманих рослин для підтвердження події трансформації.

Застосовували базові методики клонування ДНК, *Agrobacterium*-опосередковану трансформацію *Nicotiana tabacum* L. методом листкових дисків, селекцію трансформантів *in vitro*, аналіз присутності трансгенів у рослинній ДНК, детекцію експресії гена *cry1Ab* методом ПЛР зі зворотною транскрипцією. У ході дослідження було сконструйовано вектори pCB182 і pCB241 із вмістом синтетичного гена *cry1Ab*. Створеними векторами здійснено *Agrobacterium*-опосередковану трансформацію тютюну та отримано рослини-регенеранти, які містили трансгени у своїй ДНК. Підтверджено експресію трансгена *cry1Ab* в одержаних трансформантах тютюну методом ЗТ-ПЛР. У результаті *Agrobacterium*-опосередкованої трансформації рослин векторами pCB182 і pCB241 із вмістом синтетичного гена *cry1Ab* стійкості до лускокрилих комах можна отримувати трансгенні рослини, у яких відбувається експресія трансгена.

Ключові слова: *cry1Ab*, *Nicotiana tabacum* L., *Agrobacterium*-опосередкована трансформація, ПЛР-аналіз, трансгенез.

СОЗДАНИЕ ВЕКТОРОВ ДЛЯ *AGROBACTERIUM*-ОПОСРЕДОВАННОЙ ГЕНЕТИЧЕСКОЙ ТРАНСФОРМАЦИИ РАСТЕНИЙ С ЦЕЛЕВЫМ СИНТЕТИЧЕСКИМ ГЕНОМ *CRY1Ab*, КОДИРУЮЩИМ УСТОЙЧИВОСТЬ К ВРЕДИТЕЛЯМ ОТРЯДА ЧЕШУЕКРЫЛЫХ

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Целью исследования было создание генетических конструкций для *Agrobacterium*-опосредованной трансформации растений, содержащих синтетический ген *cry1Ab*, и их тестирование путем трансформации табака с последующим проведением молекулярно-генетического анализа полученных растений для подтверждения события трансформации.

Использовали базовые методики клонирования ДНК, *Agrobacterium*-опосредованную трансформацию *Nicotiana tabacum* L. методом листовых дисков, селекцию трансформантов *in vitro*, анализ присутствия трансгенов в растительной ДНК, детекцию экспрессии гена *cry1Ab* методом ПЦР с обратной транскрипцией. В ходе исследования были сконструированы векторы pCB182 и pCB241, содержащие синтетический ген *cry1Ab*. Созданными векторами осуществлена *Agrobacterium*-опосредованная трансформация табака и получены растения-регенеранты, содержащие трансгены в своей ДНК. Подтверждена экспрессия трансгена *cry1Ab* в полученных трансформантах табака методом ОТ-ПЦР. В результате *Agrobacterium*-опосредованной трансформации растений векторами pCB182 и pCB241, содержащими синтетический ген *cry1Ab* устойчивости к чешуекрылым насекомым, можно получать трансгенные растения, в которых происходит экспрессия трансгена.

Ключевые слова: *cry1Ab*, *Nicotiana tabacum* L., *Agrobacterium*-опосредованная трансформация, ПЦР-анализ, трансгенез.

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