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Third International Conference "Genetically modified organisms: the history, achievements, social and environmental risks"

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Third international conference "GMO: history, achievements, social and environmental risks" was held on October 3–5, 2023 in Saint Petersburg State University, Saint Petersburg, Russia in mixed format (on-site and on-line). The conference was attended by researchers from Russia, China, Germany, Spain, India, Moldova, Tadjikistan, Belarus. They discussed new genetic engineering methods, promising results and their promotion in society. A lot of interest was attracted to the sections devoted to the genome editing of various organisms and genetic engineering in agriculture. A number of reports aimed to study the fundamental problems of biology and medicine using genetically modified microorganisms and animals. The whole section was traditionally devoted to environmental studies, involving transgenic organisms, natural GMOs that arose without human intervention. Discussion of society's attitude towards GMOs concluded the conference, where the importance of improvement of GMO diagnostics, as well as closer interaction between biologists and lawyers for improving legislation in the field of GMOs was noted.

The conference was held with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement No. 075-15-2022-322 date 22.04.2022 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future". The conference organizing committee expresses gratitude to the sponsors: companies Helicon and Synthol.

Keywords: GMO; genome editing; transgenesis.

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Basic research in the developmental genetics on the model of tumor growth in higher plants

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Today, thanks to use the modern methods of biotechnology, the molecular mechanism underlying different aspects of plant development are started to open up. Along with the traditional methods of genetic analysis, plant developmental genetics actively uses the technics of genetic engineering and "omics" technologies. One of the problems of plant developmental genetics is the study of tumor growth in plants as a model for revealing the mechanisms of systemic control of cell division. Tumor is a pathological structure emerging as a result of uncontrolled proliferation of a group of cells leaving the systemic control of growth rate, cell differentiation and proliferation. Therefore, the elucidation of the mechanisms of tumor formation may help to identify the key regulators of systemic mechanisms controlling cell proliferation and differentiation. Tumor-like structures are found in almost all multicellular organisms, including higher plants. Pathogen-induced tumors, which make up the majority of neoplasms in higher plants, develop under the influence of infectious agents (bacteria, viruses, fungi, nematodes, insects, etc.) which create a niche for their own habitation in the host plant's organism mostly by shifting the phytohormonal balance and sometimes activation of the meristematic competence of plant cells or modulation of plant cell cycle. At the same time, much rarer spontaneous tumors of higher plants are formed in plants with certain genotypes (mutants, interspecific hybrids, inbred lines) in the absence of any pathogen, which makes them closer to animal tumors. In particular, in the genetic collection of radish (*Raphanus sativus* L.), the inbred lines that form spontaneous tumors on the taproot during the flowering period were obtained many years ago. The connection between the spontaneous tumor formation in these lines and the altered balance of the main phytohormones, as well as ectopic expression of meristem-specific genes, was previously demonstrated. We have analyzed the differential gene expression in the spontaneous tumors of radish versus the lateral roots using the RNA-seq method. Data were obtained indicating the increased expression of genes associated with cell division and growth (especially genes that regulate G2-M transition and cytokinesis) in the spontaneous tumor. Among genes downregulated in tumor tissue, genes participating in the response to stress and wounding, as well as in the biosynthesis of glucosinolates, were enriched. Subsequently, we also performed whole genome sequencing of two closely related radish lines, contrasting in their ability to spontaneous tumor formation. In the coding genes of tumorous line, we have identified numerous SNPs and InDel which lead to frameshift and are probably associated with the tumor formation trait. Thus, spontaneous tumor formation in inbred lines of radish

is probably under complex polygenic control. Testing of the relationship of these polymorphisms with tumor formation has begun. Our data will help to elucidate the mechanisms of spontaneous tumor development in higher plants.

The conference was held with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement No. 075-15-2022-322 date 22.04.2022 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future".

Keywords: developmental genetics; plant tumors; genetic collection of radish.

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Transplastomic plants — new approaches to solving "old" problems

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Transplastomic plants are capable to accumulate the significant amounts (up to 70% of TSP) of target recombinant proteins in tissues. However, the production of such forms is severely limited by the low yield of initial transformants. This problem requires the development and optimization of new approaches to the delivery of transgenes into chloroplasts and an increase in the frequency of their integration into the plastome. Transplastomic tobacco plants expressing the *gfp* reporter gene and the *aadA* selectable marker under the control of the *PrrnG10L* promoter and the *TpsbA* terminator were obtained in the laboratory of plant bioengineering. It is known that the selected promoter and insertion region (between the tRNA genes of isoleucine and alanine) are capable to provide a high yield of recombinant proteins in the leaves of transplastomic plants [1]. However, the content of recombinant GFP in the leaves of the obtained transplastomic plants was determined at the level of 0.12%, and the variability for this trait was minimal and ranged from 0.09 to 0.16% of TSP. Insufficient accumulation of the target protein in transformants is not associated with transcription disorders or the presence of non-transgenic copies of the plastome. Probably, the low frequency of transformation and the lack of variability between the transformants are the reasons that make it difficult to select highly productive forms. It is proposed to increase the efficiency of targeted delivery of genetic constructs to plastids using single-walled carbon nanotubes loaded with recombinant DNA. This process can also be facilitated by our proposed approach to increase the frequency of DNA double-strand breaks in target regions of the plastome through the use of the CRISPR-Cas9 genome editing system.

This work was supported by the Russian Science Foundation grant No. 23-24-00545.

Keywords: chloroplasts; plastome; transformation; editing; carbon nanotubes.

REFERENCES

1. Rozov SM, Sidorchuk YuV, Deineko EV. Transplastomic Plants: Problems of Production and Their Solution. *Russian Journal of Plant Physiology.* 2022;69(2):132–141. DOI: 10.1134/S1021443722020157

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Development of a system for the formation of transgenic somatic embryos in the liquid medium in *Medicago truncatula*

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Somatic embryogenesis is the formation of embryos from plant's somatic cells. It is widely used in biotechnology for reproduction of plants, studying of regeneration process and it also represents a convenient way to obtain transgenic plants. Currently, a solid medium is usually used for the formation of transgenic somatic embryos, which has a number of disadvantages.

We are developing a system for cultivating explants in a liquid medium for the transformation and formation of somatic embryos for *Medicago truncatula*. Unlike a solid medium, it should allow using petioles as explants, simplify the renewal of the medium, replace disposable cultivation containers with reusable ones, and also reduce the time required for the formation of somatic embryos.

Currently, the optimal concentration of hygromycin as a selective agent in such a system was found. Interestingly, it appeared to be lower than the selective hygromycin concentration in a solid medium.

The addition of cefotaxime to the medium reduces the number of somatic embryos formed, but does not completely suppress their formation. Thus, cefotaxime can be used to eliminate agrobacteria during transformation using this cultivation system.

Embryos with *GUS* overexpression transformed with this method were successfully obtained.

Keywords: *in vitro* culture; alfalfa; selective agents.

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The search for inhibitors of somatic embryogenesis in *Medicago truncatula*

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The success of protocols for the genetic transformation of legumes is limited by their low ability to regenerate. Plant regeneration can occur both along the path of shoot regeneration and their further rooting, and along the path of somatic embryogenesis (SE). SE is similar to zygotic embryogenesis (ZE). It is a method of asexual reproduction, in which the somatic cell, due to its totipotency, switches on the embryogenesis program.

SE and ZE involve common participants in transcriptional, hormonal, and epigenetic control. Like many processes in the plant organism, SE is controlled by the activity of various stimulants or repressors.

As a result of transcriptional analysis of embryogenic and non-embryogenic calli of *Medicago truncatula* at different stages of development, putative genes-inhibitors of SE were found. Using the Golden Gate system, we created vectors for agrobacterial transformation to overexpress genes of interest in embryogenic calli and evaluate their effect on SE. Overexpression of two genes encoding the transcription factors from WRKY and Homobox-WOX families had a significant inhibitory effect on SE (the average number of somatic embryos per callus decreased).

The *MtCLE16,* a previously found SE inhibitor from the CLE peptides group, was edited and heterozygous frameshift mutants were obtained. The impact of loss of its function on the SE capacity remains to be analyzed.

This research was supported by the Sirius University of Science and Technology, project PBB-RND-2243.

Keywords: transcription factors; regeneration inhibitors; somatic embryogenesis.

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The *MtWOX* genes in the regulation of *Medicago truncatula* somatic embryogenesis

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Plant somatic cells can be reprogrammed into totipotent embryonic cells that are able to form embryos in a process called somatic embryogenesis (SE). SE can occur naturally in some plant species and it is widely used for the plant's genetic transformation and regeneration.

This process is regulated by hormone treatment and many proteins, among which WUSCHEL-related homeobox (WOX) transcription factors are believed to play crucial roles. Our previous studies have shown that *MtWOX9-1* stimulates SE in *Medicago truncatula*. The aim of the present research was to search for new *MtWOX* genes regulating SE. In this study, using transcriptomic data and literature data, we had selected several genes with an increased expression level during SE or in the generative organs and examined the overexpression effect of these genes on the SE ability. It was found that explants of the *M. truncatula* embryogenic line, transformed with the construct for *MtWOX6-like* overexpression, develop more somatic embryos compared to the control.

Our findings could be a helpful point for searching and studying new morphogenic regulators controlling SE and could have a positive impact on plant biotechnology in improving the transformation and regeneration capacity for other legumes.

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Keywords: CLE peptides; WOX genes; regeneration stimulators.

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The screening vector system of morphogenic regulators in *Fabaceae*

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Legumes are important agricultural and food crops, however, some legume species have a low regenerative capacity *in vitro*, which complicates obtaining the genetically modified plants with improved properties and analysing gene function.

To search for genes that stimulate somatic embryogenesis and to increase the regeneration frequency in legumes *in vitro*, we designed a screening vector system that will allow faster cloning of genes encoding potential regulators of morphogenesis by preserving restriction sites in the final vector. The construction of vectors is based on the Golden Gate [1] modular cloning method. Using type II restriction endonucleases, DNA fragments form sticky ends and are combined in a given order to form multigene constructs intended for *Agrobacterium*-mediated transformation. In order to identify efficient variants for gene expression, we used a number of promoters: CaMV 35S(long), CaMV 35S(double), nopaline synthase (nos), actin 2 (act2), and a number of terminators: 35S CaMV, nos, act2 for *MtWOX9-1* overexpression. *Medicago truncatula* WOX9-1 (MtWOX9-1) is a WUSCHEL-related homeobox transcription factor for which a positive effect on the formation of somatic embryos in callus culture was previously shown [2].

Based on the analysis of embryogenic tissue, the optimal combination of promoters and terminators will be selected to assemble the vector for screening of morphogenetic regulators.

The work is supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement 075-10-2021-093, Project PBB-RND-2243).

Keywords: vector designs; Golden Gate cloning; morphogenetic regulators.

REFERENCES

1. Weber E, Engler C, Gruetzner R, et al. A modular cloning system for standardized assembly of multigene constructs. *PLoS One.* 2011;6(2):16765. DOI: 10.1371/journal.pone.0016765

2. Tvorogova VE, Fedorova YuA, Potsenkovskaya EA, et al. The WUSCHEL-related homeobox transcription factor MtWOX9-1 stimulates somatic embryogenesis in *Medicago truncatula. Plant Cell Tissue and Organ Culture.* 2019;138(3):517–527. DOI: 10.1007/s11240-019-01648-w

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Effect of biotin starvation on gene expression in industrially significant yeast *Komagataella phaffii*

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Komagataella phaffii (*Pichia pastoris*) is known to be an excellent producer of recombinant proteins for industrial and research purposes. Protein synthesis improvement in this yeast includes selection of optimal cultivation parameters [1, 2]. Therefore, much attention is paid to the influence of media components on physiology of this yeast [3–5].

One of the essential media components is biotin. In yeast cells it plays a crucial role as a cofactor of enzymes, providing carboxylation reactions in lipo-, gluconeogenesis, and nitrogen metabolism. *K. phaffii* is biotin auxotrophic organism unable to synthesize this vitamin *de novo*. Thus, it necessarily requires adding of biotin in the media.

In this study, we analyzed the effect of biotin starvation on gene expression in *K. phaffii* cells during its growth on methanol- and glycerin-containing media. These carbon sources are the most commonly used in standard protocols for recombinant protein biosynthesis in *K. phaffii*.

It was shown, that biotin starvation cell response significantly depends on carbon source. In glycerol-containing media biotin deficiency enhanced transcription of genes involved in biotin and thiamine metabolism, glyoxylate cycle, synthesis of acetyl-CoA in cytoplasm and its carnitine-mediated transport into mitochondria. Genes involved in biosynthesis of lipids and glucose were repressed in media with glycerol. In methanol-containing media the biotin deficiency effect was more pronounced and led to repression of numerous genes involved in protein and amino acids synthesis and activation of cell response to oxidative stress.

The obtained results are thought to be important for optimizing the culture conditions in the *K. phaffii* expression systems.

Keywords: *Komagataella phaffii*; gene expression; biotin.

REFERENCES

1. Joseph JA, Akkermans S, Cornillie E, Deberlanger J, and Van Impe JFM. Optimal culture medium selection and supplementation for recombinant thaumatin II production by *Komagataella phaffii. Food Bioprod. Process.* 2023;139(12):190–203. DOI: 10.1016/j.fbp.2023.04.001

2. Ergün BG, Berrios J, Binay B, Fickers P. Recombinant protein production in *Pichia pastoris:* from transcriptionally redesigned strains to bioprocess optimization and metabolic modelling. *FEMS Yeast Res.* 2021;21(7):foab057. DOI: 10.1093/femsyr/foab057

3. Ianshina T, Sidorin A, Petrova K, et al. Effect of methionine on gene expression in *Komagataella phaffii* cells. *Microorganisms*. 2023;11(4):877. DOI: 10.3390/microorganisms11040877

4. Rumiantsev AM, Padkina MV, Sambuk EV. Effect of nitrogen source on gene expression of first steps of methanol utilization pathway in *Pichia pastoris. Genetika.* 2013;49(4):454–460. (In Russ.) DOI: 10.7868/S0016675813040115

5. Rumjantsev AM, Bondareva OV, Padkina MV, and Sambuk EV. Effect of nitrogen source and inorganic phosphate concentration on methanol utilization and *PEX* genes expression in *Pichia pastoris. Scientific World Journal*. 2014;2014:743615. DOI: 10.1155/2014/743615

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A set of *Saccharomyces cerevisiae* strains possessing [*PSI*$^{+}$] prion formed by Sup35 protein with various deletions in prionogenic domain

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Amyloid aggregation is a key factor for the development of a series of lethal and incurable diseases, commonly named amyloidoses. The development of various pathologies might be caused by the aggregation of the same protein. This can be due to the ability of any particular protein to adopt several amyloid conformations, specific for the exact disease (Pick's and Alzheimer's disease-specific forms of tau protein). How the specific amyloid conformation is formed in each case is not fully understood.

In yeast, translation termination factor Sup35 is one of the most extensively studied amyloidogenic proteins. Sup35 aggregation (induction of [*PSI*$^{+}$] prion) inactivates the protein and leads to the suppression of nonsense-mutation as the result of read-through.

Prionogenic domain of Sup35 protein (Sup35N) has several specific regions: N-terminal QN-rich region (QN), oligopeptide repeats (NR) and C-terminal region (CTN). Sup35 can form various strains of [*PSI*$^{+}$] with predominant involvement of different regions of Sup35N into amyloid core thus mimicking disease-specific strains of amyloids described for human amyloidogenic proteins.

We implemented the deletions of fragments encoding 1-39 a.a. (QN region) or 75-123/98-123 a.a. (CTN region) into *SUP35* gene of yeast *Saccharomyces cerevisiae*. Then, we induced aggregation of Sup35 protein in the strains carrying mutated *SUP35* gene and got the strains possessing [*PSI*$^{\Delta 39+}$], [*PSI*$^{\Delta 75-123+}$], or [*PSI*$^{\Delta 98-123+}$] prion. A set of strains possessing [*PSI*$^{+}$] formed by Sup35 protein with various deletions in Sup35N may be convenient model to study disease-specific strains of amyloids formed by human proteins.

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Keywords: *Saccharomyces cerevisiae*; Sup35; deletion analysis.

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Evaluation of non-specific CRISPR/Cas9 activity in a yeast model

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CRISPR/Cas9-based genome editing systems are widely used for genetic modification of different organisms. One of the drawbacks of CRISPR/Cas9 methods is the non-specific activity of Cas9, which can lead to accumulation of unwanted mutations in the edited genome [1]. Therefore, the development of *in vivo* models for high-throughput analysis of factors influencing the frequency of mutagenesis associated with the use of CRISPR/Cas9 technologies is a relevant task. Yeast *Saccharomyces cerevisiae* is a convenient object for developing such models [2].

Here we represent a yeast *in vivo* model that allows us to evaluate the effects of nucleotide sequence of the protospacer adjacent motif (PAM) and the guide RNA (gRNA) on the efficiency of binding between the gRNA/Cas9 complex and the target sequence in the genome. Since the Cas9 activity is lethal in cells lacking a donor sequence for homologous repair of double-strand breaks caused by this endonuclease, in the proposed test-system, the reduced efficiency of transformation by a plasmid encoding Cas9 and various gRNA variants reflects the efficiency of recognition of the target gene by the gRNA/Cas9 complex.

To study the influence of different PAM variants, with a consensus of NGG, on CRISPR/Cas9 activity, we obtained four isogenic strains that differ in their PAM sequence (AGG, TGG, CGG, GGG) in the codon 202 of the chromosomal copy of the reporter gene *URA3*. To evaluate the effect of incomplete matching between gRNA and the target site sequences, we propose using a series of plasmids based on the pML107 vector, encoding Cas9 and one of the 20 possible gRNA variants with single nucleotide substitutions at each of the 20 positions. The results obtained so far indicate the potential of the proposed approach.

Keywords: *Saccharomyces cerevisiae*; CRISPR/Cas9; PAM.

REFERENCES

1. Alkan F, Wenzel A, Anthon C, et al. CRISPR-Cas9 off-targeting assessment with nucleic acid durlex energy parameters. *J Genome Biol*. 2018, 19, 113.

2. Stepchenkova EI, Zadorsky SP, Shumega AR, Aksenova AY. Practical approaches for the yeast *Saccharomyces cerevisiae* genome modification. *Int J Mol Sci* 2023;24(15):11960.

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Application of yeast display method in biotechnology and agriculture

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Yeast display (DD) is an efficient technology for exposure and fixation of target proteins on the surface of yeast cells by their fusion with cell wall proteins. The scope of application of DD is very wide. It can be used in the study of protein-protein interactions and antibody screening; for the processing of industrial waste, in the processes of bioadsorption of heavy and rare metals, in the production of chemical compounds and biofuels, and in the production of vaccines. DD has a number of advantages over other cell systems. This is due to the fact that yeast, being eukaryotes, unlike bacteria, can carry out various post-translational modifications, correct folding and secretion of eukaryotic proteins.

In our work, we compared the effectiveness of different cell wall proteins for exposing target proteins to the surface of *Komagataella phaffii* yeast cells. Two reporter systems were used, based on the eGFP and the beta-galactosidase genes.

The most efficient exposure to the surface was provided by the anchor protein ScAGα1p from the yeast *Saccharomyces cerevisiae*. The genetic constructs obtained in the work can be used for the production of whole-cell biocatalysts. A yeast strain *K. phaffii* was obtained, containing in its genome a construct for the excretion of the Gumboro disease virus antigen protein — VP2. This strain can be used for the production of a vaccine.

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Keywords: *Komagataella phaffii*; yeast display; whole-cell biocatalysts; Gumboro disease virus antigen.

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Development of the Cas12a-based microdeletion and microinsertion detection system

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CRISPR/Cas-based systems are widely used as genome editing systems, nucleic acid detection systems and molecular visualization instruments [1]. In our laboratory using CRISPR/Cas9 technology we have obtained several KO mouse lines harboring deletions ranging from 2 to 20 base pairs. While 20 bp deletions are easily PCR-detected, when it comes to 2 bp deletions it is essential to genotype numerous mice using time-consuming Sanger sequencing. We propose a microdeletion/microinsertion detection system based on Cas12a nuclease from *Lachnospiraceae* bacterium (LbCas12a). Its active complex consists of the Cas12a enzyme and one crisprRNA [2]. A special sequence called protospacer adjacent motif (PAM) is required for target recognition by LbCas12a. In our laboratory we have discovered new PAM TTAA recognized by LbCas12a [3]. Via agarose electrophoresis and fluorescent analysis using FAM-labeled probes we have shown that new PAM allowed detection of 1 bp substitutions in target DNA *in vitro*. Also we have tested different FAM-labeled probes and have shown that AT-rich probes longer than 10 bp are cleaved most effectively. Finally we have used our system for detecting 2 bp deletions in Pde6b-KO mice and Grin3A-KO mice and successfully distinguished these mice from wild type mice.

In conclusion, new PAM TTAA greatly increases specificity of DNA cleavage allowing to use this system as an instrument for rapid detection if microdeletions in mice.

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Keywords: CRISPR/Cas; LbCas12a; mice.

REFERENCES

1. Gootenberg JS, Abudayyeh OO, Kellner MJ, et al. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*. 2018;360(6387):439–444. DOI: 10.1126/science.aaq0179

2. Swarts DC, Jinek M. Cas9 versus Cas12a/Cpf1: Structure-function comparisons and implications for genome editing. *Wiley Interdiscip Rev RNA*. 2018;9(5):e1481. DOI: 10.1002/wrna.1481.

3. Misiurina MA, Chirinskaite AV, Fotina AS, et al. New PAM Improves the single-base specificity of crRNA-guided LbCas12a nuclease. *Life*. 2022;12(11):1927. DOI: 10.3390/life12111927

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Current state of research in the development of the genomic editing method: problems and prospects

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The possibility of using the CRISPR/Cas method of genomic editing has provided researchers with a powerful tool not only for targeted modification of genes that determine economically valuable traits in plants, but also for solving fundamental problems of their functioning. The most striking examples of the use of CRISPR/Cas9 to improve various plant species by knockouts of target genes or knockins of expression cassettes, including genes that change the biosynthesis of important plant metabolites, obtained by foreign research groups, are presented. We discuss our own results on the directed change in the functioning of genes encoding photosystem II carbonic anhydrases, as well as genes involved in plant responses to stress in the *Arabidopsis thaliana* model. Examples of the use of the genomic editing method to improve the characteristics of plant cell cultures as bioproducers of pharmaceutically valuable recombinant proteins are given. Methodological issues related to plant genome editing are considered — the problems of chimerism, obtaining homozygotes and biallelic knockout mutations, knockout of regulatory and structural genes, as well as repair features in the regions of integration of expression cassettes in knockins. The main directions for further development and improvement of the CRISPR/Cas genomic editing method aimed at optimizing the efficiency of delivery of target genetic constructs and editing tools to the nuclear and chloroplast genomes of plants using single-walled carbon nanotubes are summarized.

Keywords: CRISPR/Cas9; knockouts of target genes; knockins of expression cassettes.

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The strong base for using base editing in plants

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The most common application of CRISPR-Cas9 genome editing system is a gene knockout via indel mutations introducing. It is obvious, because this approach has minimum critical conditions in guide RNA design: available PAM sequence and conservative 19–25 nucleotides within all alleles of a target gene. Precise nucleotide changing with base editing systems has more limitations: target nucleotide should locate in editing window of adenine- or cytidine-deaminase, besides this, all undesired adenines or cytosines in editing window will be likely changed. However, there is a more fundamental issue — it is very difficult to find a single aminoacid substitution, which changes protein features in a desirable side. One of the good examples of base editing target will be considered in this work.

Nicotiana tabacum L. is a plant from *Solanaceae* family, the same as potato, tomato and pepper. All these plants are strongly affected by potato virus Y (PVY). It is known, that PVY recruits host translation initiation factor eIF4E by the viral protein VPg in order to start synthesis its proteins. If eIF4E can't interact with VPg, plant will be resistant.

In our work, we established an aminoacid substitution in tobacco eIF4E factor, which disrupted interaction with PVY VPg in yeast two-hybrid conditions, but didn't influence the factor functionality. Then we designed two genetic constructions with different sgRNAs for introducing this mutation in tobacco plants using cytidine-deaminase system. These constructions were used to plant transformation and development of edited tobacco plants.

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Keywords: CRISPR-Cas9; base editing; *Nicotiana tabacum*.

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Modification of agricultural traits in cultivated varieties of barley and wheat

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CRISPR/Cas technology makes it possible to induce mutations at defined positions. In breeding-oriented research, this opens up exciting opportunities for the targeted improvement of many agricultural crops. Wheat and barley are among the most important cereals in the world. However, the transformation poses a particular challenge for cereals and is strongly genotype dependent. This is because agrobacteria, which is mostly used for delivering the CRISPR/Cas system, have a limited compatibility with these non-host plants. Transformation of wheat is additionally difficult due to the large genome size and polyploidy.

Besides obtaining improved genotypes, the object of the current study was to optimize the method of genomic editing based on the CRISPR/Cas system using particle bombardment for non-model varieties of barley and wheat. In barley, we targeted the *Nud* gene that controls hulled/naked phenotype of the grain. Since the regeneration rate remains an issue for the cultivated cultivars, we used the JD633 vector that carries the *GRF4-GIF1* chimera to increase the efficiency of regeneration. We obtained five T_0 plants, carrying mutations. In wheat, targeting *Ppd-1* genes that control photoperiod-dependent floral induction results in Cas9-induced mutations in 52 of 210 T_0 plants. The developed collection of wheat plants with different new alleles of *Ppd-D1* and *Ppd-B1* genes is being studied for the expression under short day conditions and the effect on the vegetation period.

Thus, we have obtained plants of the cultivated varieties of barley and wheat with edited agronomically important genes, using the improved protocols of biolistic transformation.

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Keywords: CRISPR/Cas; barley; wheat.

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Advancing gene editing: multiplex mutagenesis in hexaploid triticale

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The presence of several sets of chromosomes in polyploid crops is a serious problem for the application of gene and genome editing systems. Efficient CRISPR/Cas-based mutagenesis of series of genes involved in the grain starch biosynthesis of hexaploid triticale has been developed. Triticale (×*Triticosecale*), is a hybrid of rye (*Secale*) and wheat (*Triticum*) and consists of three subgenomes. Four genes were targeted and to ensure efficient editing of all subgenomes, a trio of guide RNAs for each target genes were designed. To enable simultaneous editing of 36 genetic loci at once (three sgRNAs × four genes × three subgenomes), an expression cassette was constructed, assembled as an array of twelve sgRNAs. The polysitron vector was delivered to morphogenic calli using a gene gun [1] together with a vector encoding Cas9 nuclease [2] to induce mutations. A number of transgenic plants of spring and winter triticale carrying both Cas9 and sgRNAs inserts have been generated. The efficiency of native gene editing varied depending on the target gene and sgRNA activity. Using a trio of sgRNAs for each target gene, we successfully mutated all three subgenome copies, thereby modifying seed starch synthesis. It can be expected that the described approach will make an important contribution to the future breeding of polyploid crops to produce various combinations of new genetic alleles encoding desired traits.

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Keywords: CRISPR-Cas9; polyploid crops; Triticale.

REFERENCES

1. Miroshnichenko D, Klementyeva A, Pushin A. et al. A competence of embryo-derived tissues of tetraploid cultivated wheat species *Triticum dicoccum* and *Triticum timopheevii* for efficient and stable transgenesis mediated by particle inflow gun. *BMC Plant Biol.* 2020; 20(Suppl 1):442. DOI: 10.1186/s12870-020-02580-4

2. Miroshnichenko D, Timevbaev V, Klementyeva A, et al. CRISPR/Cas9-induced modification of the conservative promoter region of *VRN-A1* alters the heading time of hexaploid bread wheat. *Front Plant Sci.* 2022;13:1048695. DOI: 10.3389/fpls.2022.1048695

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Study of functional features of plant root systems using CRISPR/Cas-mediated genome editing

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CRISPR/Cas-mediated genome editing is a powerful tool of plant functional genomics. Hairy root transformation is a rapid and convenient approach for obtaining transgenic roots. When combined, these techniques represent a fast and effective means of studying gene function [1, 2].

A common construct for efficient genome editing and selection of hairy roots is comprised of three components, i.e., a cassette carrying the gene encoding the Cas nuclease, a cassette expressing the guide RNA (gRNA), and a cassette encoding a screenable or selectable marker [2]. After design and construction, the resulting vector is used to transform plant using appropriate *Rhizobium rhizogenes* strain.

Over 26 plant species have been used in experiments combining genome editing and hairy root transformation to date [2]. Possible applications of CRISPR/Cas9 genome editing using hairy root transformation include different directions like test the efficiency of the CRISPR/Cas9 genome editing; obtaining whole genome-edited plants regenerated from individual edited hairy roots; investigation of root development or root function, root nodule symbiosis, resistance to biotic or abiotic stresses, or metabolic engineering [2].

The basic principles of plant CRISPR/Cas genome editing like the different components of CRISPR/Cas vectors, the types of Cas nuclease, design principles of gRNAs, as well as the possible applications of CRISPR/Cas genome editing in hairy roots will discuss. The application of this method for multigene editing strategy will also be demonstrated on *DEEPER ROOTING1* genes of cucumber.

The study was supported by the Ministry of Science and Higher Education of the Russian Federation (Grant No. 075-15-2021-1056).

Keywords: CRISPR-Cas9; hairy roots; cucumber.

REFERENCES

1. Gogolev YV, Ahmar S, Akpinar BA, et al. OMICs, epigenetics, and genome editing techniques for food and nutritional security. *Plants*. 2021;10(7);1423. DOI: 10.3390/plants10071423

2. Kiryushkin AS, Ilina EL, Guseva ED, et al. Hairy CRISPR: Genome editing in plants using hairy root transformation. *Plants*. 2022;11(1);1–39. DOI: 10.3390/plants11010051

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CRISPR/Cas based genome editing in microalgae

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CRISPR/Cas systems are presently the most attractive genome editing technology, that is widely used for genetic engineering of various crops and industrial microorganisms. Currently, application of the CRISPR/Cas based genome editing promises advances in microalgae biotechnology aimed at boosting the output of biofuels and valuable bioactive compounds. However, algae remain relatively complex objects for genetic manipulation [1]. The main problems are associated with the need of a species-oriented approach when creating a transformation toolbox due to the peculiarities in the structure of membranes and the cell wall of a particular taxon. The proper selection and design of a CRISPR construct is also required due to the possible presence of a powerful silencing system against introduced genetic constructs in the cell. These difficulties explain the low efficiency of microalgae transformation and the meager list of successfully edited species [1, 2].

The first instance of genome editing in microalgae using CRISPR/Cas was reported in *Chlamydomonas reinhardtii* P.A. Dang [3]. To date, four transformation methods (*Agrobacterium*-mediated, particle bombardment, glass beads agitation, electroporation) have been successfully used for editing (knock-in and knock-out) the *C. reinhardtii* genome with two types of CRISPR constructs (plasmid and ribonucleoprotein). The developed protocols make it possible to achieve high efficiency of genomic editing — for example, in our study it varied from 10.6% to 68.8% [4]. These benefits along with completely sequenced genome, well-studied genetics, accessibility and haplontic life cycle makes *C. reinhardtii* an outstanding model organism for CRISPR/Cas application in microalgae research [5].

Keywords: CRISPR/Cas; genome editing; transformation toolbox; CRISPR construct delivery; microalgae; GMOs.

REFERENCES

1. Jeon S, Lim J-M, Lee H-G, et al. Current status and perspectives of genome editing technology for microalgae. *Biotechnology for Biofuels.* 2017;10:267. DOI: 10.1186/s13068-017-0957-z

2. Patel VK, Das A, Kumari R, Kajla S. Recent progress and challenges in CRISPR-Cas9 engineered algae and cyanobacteria. *Algal Research*. 2023;71. DOI: 10.1016/j.algal.2023.103068

3. Jiang W, Brueggeman AJ, Horken KM. Successful transient expression of Cas9 and single guide RNA genes in *Chlamydomonas reinhardtii. Eukaryotic Cell*. 2014;13(11): 1465–1469. DOI: 10.1128/ec.00213-14

4. Virolainen PA, Chekunova EM. Optimization of CRISPR/Cas9 method for transgenesis of model microalgae *Chlamydomonas reinhardtii* [abstract]. *Ecological genetics*. 2022;20(Suppl. 1): S42–43. DOI: 10.17816/ecogen112332

5. Naduthodi MIS, Barbosa MJ, van der Oost J. Progress of CRISPR-Cas based genome editing in photosynthetic microbes. *Biotechnology Journal.* 2018;13(9). DOI: 10.1002/biot.201700591

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The transformation and genome editing of *Pisum sativum*: protocols and their modifications

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Pea (*Pisum sativum*) is an important agricultural crop and a model object in various fields of plant research. At the same time, the genetic modification of pea is still a difficult task, which, apparently, is associated with its low ability to regenerate. There are a lot of different pea transformation protocols, however, for most of them, the transformation efficiency, i.e. the number of transgenic plants per explant, is extremely low. In addition, none of the protocols known to us has demonstrated itself to be universal, i.e. suitable for all varieties and lines of peas. We searched for studies on the transformation and regeneration of peas and systematized the data obtained. The resulting database made it possible to identify the most effective protocols for the transformation and regeneration of *P. sativum*, as well as to analyze statistically the general features of the methods used, such as the source of the explant, the composition of the culture media, the duration of cultivation, and so on. We assume that our system for the analysis of publications devoted to *in vitro* cell cultures can also be used for similar data on other plant species.

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Keywords: somatic embryogenesis; legumes; reporter genes.

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Bioengineering eggplants: a deep dive into SmHQT and phenolic acid biosynthesis

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Eggplants, known scientifically as *Solanum melongena* L., are renowned for their health benefits, largely attributed to phenolic acids. Chlorogenic acid stands out as one of the most prevalent phenolic acids in eggplants. The enzyme hydroxycinnamoyl CoA-quinate transferase (SmHQT) plays a pivotal role in the production and concentration of this acid in the fruit. However, until this study, the exact function and influence of SmHQT on the eggplant's composition remained elusive [1–3].

This research aimed to explore SmHQT's role by overexpressing it in the eggplant's flesh using agroinfiltration, a technique that transiently introduces genes into plants. This method offers insights into potential changes in the plant's chemical makeup. Advanced techniques like quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and high-performance liquid chromatography (HPLC) revealed that the chlorogenic acid content in the genetically altered eggplants was over twice that of the unaltered ones.

The study also investigated the cascading effects of this overexpression. The qRT-PCR results showed variations in the expression of genes linked to the chlorogenic acid pathway, hinting at SmHQT's wider role in phenolic acid biosynthesis in eggplants. Comprehensive analyses of protein interactions and cis-regulating elements were undertaken to grasp SmHQT's full impact.

Phenolic acids, like chlorogenic acid, offer therapeutic benefits against conditions such as diabetes, cancer, and arthritis in humans. In plants, they enhance natural defenses against pests and diseases. While there have been attempts to boost the phenolic acid content in eggplants using genes from wild variants, this study's approach proved more effective.

Another notable achievement of this research was the introduction of an improved agroinfiltration protocol. This method is promising for future studies focused on transient gene expression in fruits, facilitating swift genetic modification prototyping. In essence, this research underscores the immense potential of bioengineering in augmenting the nutritional profiles of crops by enhancing their inherent phytochemicals.

Keywords: bioengineering; eggplants; phenolic acid biosynthesis.

REFERENCES

1. Kaushik P, Kumar P, Kumar S. Enhancement of chlorogenic content of the eggplant fruit with eggplant hydroxycinnamoyl CoA-quinate transferase gene via novel agroinfiltration protocol. *Pharmacognosy Magazine,* (2020);16(Suppl 3):S450–S454.

2. Kaushik P. Standardisation of an Agroinfiltration Protocol for Eggplant Fruits and Proving its Usefulness by Over-expressing the SmHQT Gene. *Preprints.* 2019;2019080129. DOI: 10.20944/preprints201908.0129.v1

3. Kaushik P, Andújar I, Vilanova S, et al. Breeding vegetables with increased content in bioactive phenolic acids. *Molecules*. 2015;20(10):18464–18481.

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Genetic enhancement of *Datura metel* for optimized silver nanoparticle synthesis

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The burgeoning field of nanotechnology has witnessed a surge in the utilization of biological entities, especially plant extracts, for the green synthesis of nanoparticles. In this innovative study, we have ventured into the realm of genetic engineering to optimize the synthesis of silver nanoparticles (AgNPs) using *Datura metel*, a plant traditionally known for its rich phytoconstituents [1, 2].

Our initial experiments with non-modified *Datura metel* fruit extracts as reducing agents yielded AgNPs with an average size of 40–50 nm, confirmed spectrophotometrically with a peak at 460 nm. Recognizing the potential to enhance this process, we genetically modified *Datura metel* plants to amplify their phytoconstituent content by approximately 30%. This was achieved by overexpressing genes associated with the production of specific phytochemicals, such as polyphenols and amides.

Subsequent synthesis processes using the GM *Datura metel* extracts resulted in a 25% increase in nanoparticle yield. Furthermore, the average size of the nanoparticles synthesized from GM extracts ranged between 20–30 nm, indicating a more uniform and refined synthesis process. Advanced analytical techniques, including X-ray diffraction, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy-dispersive X-ray spectroscopy (EDX), were employed to validate these findings. Notably, the EDX analysis of nanoparticles synthesized from GM extracts showcased a silver peak contributing to 32–35% of the weight, a slight increase from the non-modified counterparts.

Beyond the quantitative enhancements, the GM approach also influenced the qualitative properties of the AgNPs. Preliminary tests indicate that the nanoparticles derived from GM extracts exhibit enhanced antimicrobial and antioxidant properties, making them potential candidates for various biomedical applications.

In conclusion, this study underscores the immense potential of integrating genetic engineering with nanotechnology. By genetically enhancing *Datura metel*, we have not only optimized the synthesis process of AgNPs but also broadened the horizons for their potential applications. However, as we advance in this direction, it is imperative to tread with caution, ensuring the ethical and safe use of GM organisms in research and applications.

Keywords: *Datura metel*; silver nanoparticle; GMO.

REFERENCES

1. Sharma M, Dhaliwal I, Rana K. et al. Phytochemistry, pharmacology, and toxicology of *Datura species* — A review. *Antioxidants*. 2021;10(8):1291.

2. Sharma L, Dhiman M, Dadhich A, et al. Biogenic AgNPs Synthesized using *Nyctanthes arbor-tristis* L. fruit for antimicrobial & dye degradation efficiencies. *Journal of King Saud University-Science*. 2023;35(4):102614.

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Microalgae as production systems of bioactive compounds. Bioengineering approaches

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Microalgae contain a wide range of useful substances: antioxidants, lipids, proteins, carbohydrates and secondary metabolites which could be used in nutraceuticals and dietary supplements. Green microalgae *Chlorella* containing highest amount of chlorophylls of any known plant, 60% protein, 18 amino acids, 20 vitamins and minerals [1]. Microalgae are exceptionally rich source of pharmacologically active metabolites with antineoplastic, antitumor, antibacterial, antifungal and antiviral properties and, also capable of wastewater treatment, and biomass production.

The genetic information can improve the scenario of metabolic engineering in microalgae. Green algae *C. reinhardtii*, a reference organism for understanding the basic algal genetics and metabolism is usually used to work out various genetic strategies, including omics resources and mutant libraries, for the enhancement of beneficial properties of microalgae. The synergy of microalgal multi-omics datasets (genomic, transcriptomic and proteomic) offer a rapid and predictable strategic path for the strain improvement [2]. The algal nuclear or chloroplast engineering (transformation and CRISPR/CAS editing) has been carried out using synthetic biology approach for the production of recombinant proteins having therapeutic properties. More than 100 recombinant proteins have been expressed in microalgae, mainly in *C. reinhardtii*, including: the vaccines, antibodies, immunotoxins and therapeutic proteins (human erythropoietin, fibronectin, interferon B1, proinsulin, endothelial growth factor and others [3]. Thus, the wide taxonomic and biochemical diversity among the microalgae when using modern biotechnologies, makes them suitable resource of abundant biomolecules with industrial and biomedical importance.

Keywords: microalgae; bioactive compounds production; genetic engineering.

REFERENCES

1. Jayshree A, Jayashree S, Thangaraju N., et al. *Chlorella vulgaris* and *Chlamydomonas reinhardtii*: effective antioxidant, antibacterial and anticancer mediators. *Indian J Pharm Sci.* 2016:78:575–581. DOI: 10.4172/pharmaceutical-sciences.1000155.

2. Kumar G, Shekh A, Jakhu S, et al. Bioengineering of microalgae: recent advances, perspectives, and regulatory challenges for industrial application. *Front. Bioeng. Biotechnol.* 2020:8:914. DOI: 10.3389/fbioe.2020.00914

3. Arias CAD, et al. Production of recombinant biopharmaceuticals in *Chlamydomonas reinhardtii*. *Int J Plant Biol*. 2023;14(1):39–52. DOI: 10.3390/ijpb14010004

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Some biochemical characteristics of the hairy roots of *Pisum sativum* L. mutants

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Two high-protein root cultures of vegetable pea mutants were received [1]. In continuation a PCR analysis of the obtained root cultures genes was carried out according [2] and the amino acid composition of the cultures protein was clarified in a dry product on the AAA 339TM device [3]. Obtained results confirmed the absence of rhizobia contamination of the cultures, which grow steadily on a hormone-free media for 5 years. PCR analysis revealed that four *rol* genes *A*, *B*, *C*, *D* were inserted into the genome of the root culture with genotype *afaftltl*, and two — *rol C* and *rol D* — in the genome of the root culture with genotype *tltl*. The protein composition of the obtained cultures was represented by essential and non-essential amino acids and some others. In four inserts culture, the content of essential, ketogenic, proteinogenic and sulfur-containing amino acids prevailed by 1.5–2 times. Two inserts culture has twice as much aspartic acid and proline. Both cultures lacked tryptophan. The number of inserts determines the amino acid composition most likely.

Keywords: hairy roots; amino acids; *Pisum sativum* L.

REFERENCES

1. Timina O, Timin O, Alecsandrova T. Hairy roots of pea mutants with a modified morph leaf type. *BIO Web of Conferences*. 2020;24:00086. DOI: 10.1051/bioconf/20202400086

2. Stepanova AY, Solov´eva AI, Malunova MV, et al. Hairy roots *Scutelaria* spp., (*Lamiaceae*) as promising producers of antiviral flavones. *Molecules*. 2021;26(13):3927. DOI: 10.3390/molecules26133927

3. Garaeva SN, Redkozubova GV, Postolati GV. Amino acids in a living organism. Kishinev, 2009. (In Russ.)

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Transgenic medicinal plants as producers of bioactive substances

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The paper covers the questions of secondary metabolite modulation in medicinal plants by means of gene engineering. It is demonstrated that cutting-edge tools of contemporary biotechnology tools made it possible to manage the biosynthesis of important bioactive substances, modify the secondary metabolism, enabling plants to synthesize and produce new compounds, as well as eliminate metabolic pathways of synthesizing harmful substances.

Currently, large-scale production of bioactive substances (BAS) requires highly productive plants to produce them. Applying methods of gene engineering to medicinal plants is a promising way to reduce the resource consumption and increase their productivity, quality and the product's marketability [1]. Traditional growing and collecting techniques are challenged by resource shortage, environmental damage, etc. [2]. Gene engineering helps to increase pest, disease and herbicide resistance, gain greater yields and higher BAS content [3].

Using transgenic medicinal plants (TMP) as BAS producers in the pharmaceutical industry is crucial for metabolic engineering. Current research of the secondary metabolism modulation in TMP enables to modify the key BAS biosynthesis and the secondary metabolism, so that plants can produce new substances, or, on the contrary, silence the metabolic pathways for harmful ones. This way, greater TMP biomass with higher BAS content can be obtained in bioreactors. This would require rather modest investments — an advantage for biopharmacy. Nowadays, TMP are grown *in vitro* as calluses or suspension cell cultures. Biotechnology can modify the secondary metabolism in TMP to produce surplus amounts of necessary BAS, reduce the content of toxic compounds or even synthesize new substances. The versatility of transcription and translation mechanisms in medicinal plants enables them to accumulate homologous substances and synthesize heterologous ones. It is known that in TMP, MYB transcription factors are involved in gene regulation in secondary metabolic pathways, regulation of genes engaged in developmental processes, etc. [4]. In conclusion, we should emphasize the relative biosafety of BAS obtained from TMP, for human use, as they are chemically pure and are not connected with biological hazards.

Keywords: transgenic medicinal plants; gene engineering; bioactive substances; biosynthesis; biotechnology.

REFERENCES

1. Wawrosch C, Zotchev SB. Production of bioactive plant secondary metabolites through *in vitro* technologies–status and outlook. *Appl Microbiol Biotechnol.* 2021;105(18):6649–6668. DOI: 10.1007/s00253-021-11539-w

2. Cheryatova YuS., Yembaturova EYu. Transgenic plants — a threat to local flora? *Ecological Genetics*. 2022;20(Suppl.1):54–55. DOI: 10.17816/ecogen112372

3. Cheryatova YuS. Actual aspects of anatomical research of medicinal plant material of *Vinca minor* L. *IOP Conference Series: Earth and Environmental Science.* 2021;723(2):022036. DOI: 10.1088/1755-1315/723/2/022036

4. Thakur S, Vasudev PG. MYB transcription factors and their role in Medicinal plants. *Mol Biol Rep.* 2022; 49(11):10995–11008. DOI: 10.1007/s11033-022-07825-z

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Obtaining of transgenic barrelclover plants (*Medicago truncatula*) producing chicken interferon gamma for veterinary use

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At the Laboratory of Plant Genetic and Cellular Engineering, Department of Genetics and Biotechnology, St. Petersburg State University, five transgenic *Medicago truncatula* plants were obtained through Agrobacterium-mediated transformation, carrying one of the variants of the heterologous chicken interferon-gamma gene under the control of the constitutive 35S CaMV promoter. Among these, one plant harbored an unmodified gene insertion, while four had a modified gene with a deletion at the protease recognition site, providing resistance to proteolytic degradation.

We demonstrate the application of the SWPOP-PCR "genome walking" method to determine the integration sites of T-DNA into the plant genome, identify the number of insertion copies and their orientation. Analysis of the obtained sequences revealed that only one plant exhibited a single T-DNA insertion, which represents the most optimal structure for stable expression.

Upon self-pollination of T_0 plants, 39 offspring were obtained and subjected to testing for the presence and expression of the transgene. Among them, six homozygous plants were identified using molecular methods. Quantitative assessment of transgene expression levels showed significant differences among representatives of different lines and among the offspring derived from a single transformed plant. Among the T_1 and T_2 progeny, the presence of the heterologous interferon protein in plant tissues was confirmed through Western blot analysis.

The engineered barrelclover plants hold potential as bioreactors for the production of chicken interferon-gamma for veterinary applications. The use of an edible plant allows eliminating protein extraction and purification procedures, thereby resulting in a noteworthy reduction in production expenses of up to 80%.

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Keywords: *Medicago truncatula*; GMO; chicken interferon.

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Identifying novel amyloid candidates using bioinformatics algorithms and a yeast model approach

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Amyloids are protein aggregates characterized by their insolubility in detergents and ability to form fibrils. They are often associated with various diseases, including neurodegenerative disorders, type 2 diabetes and certain forms of cancer. Amyloids also play important roles in bacteria and different physiological processes in both lower and higher eukaryotes.

Together with the laboratory of Prof. Y.O. Chernoff we have developed a comprehensive approach for screening new potentially amyloidogenic proteins. This involves using bioinformatics algorithms to predict protein amyloidogenicity and further verifying using a yeast model. We have created a yeast test system specifically designed to study changes in phenotype in genetically modified *Saccharomyces cerevisiae* strains [1]. This system involves the production of recombinant amyloidogenic proteins fused with reporter proteins Sup35N or YFP. Using yeast assay, we have investigated 22 human proteins that were predicted to be amyloidogenic by ArchCandy algorithm [2]. Currently, additional *in vitro* biochemical tests are underway with proteins that have shown the potential to form amyloids in yeast models. There are also plans to evaluate the amyloid-forming ability of specific human proteins in mammalian cell cultures. These various approaches appear to be enhancing our comprehension of the impact of amyloid formation in health and disease.

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Keywords: amyloids; bioinformatics; yeasts.

REFERENCES

1. Chandramowlishwaran P, Sun M, Casey KL, et al. Mammalian amyloidogenic proteins promote prion nucleation in yeast. *J Biol Chem*. 2018;293(9):3436–3450. DOI: 10.1074/jbc.m117.809004

2. Ahmed AB, Znassi N, Château M-T, Kajava AV., et al. A structure-based approach to predict predisposition to amyloidosis. *Alzheimer's & Dementia*. 2014;11(6):681–690. DOI: 10.1016/j.jalz.2014.06.007

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Bioengineering of horticultural crops in Russia and in the world

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Modern bioengineering technologies make it possible to create fruit and berry crops with signs that are unattainable by traditional breeding methods, while significantly reducing the time of breeding work. A review of more than 90 scientific publications in the period from 1989 to 2020 with research work on the creation of transgenic fruit and berry crops using various bioengineering technologies, which showed a wide variety of methods for modifying and identifying the resulting plants.

For example, the modification of the genome of an apple tree using genes that accelerate flowering, the so-called "Fast breeding" technology, allows you to speed up breeding work to create valuable varieties. The developed new varieties of transgenic apple trees are resistant to the fungi, bacteria, apple moth, and also reduced ability to browning fruits, with an increased content of sucrose, etc.

Modern methods of genetic engineering make it possible to significantly accelerate the processes of creating highly productive varieties of fruit crops with increased or complete resistance to viruses. Transgenic papaya, expressing the gene for the envelope protein of the virus, made it possible to save plantations in Hawaii. The plum varieties created with the help of bioengineering were resistant to the plum pox virus (PPV), which poses a greater danger to perennial fruit crops.

In Russia, clonal rootstocks of apple and pear trees resistant to herbicides, strawberries and pears with improved fruit taste, rootstocks and varieties of plums and cherries resistant to the Plum Pox Virus have been created and field tested, a technology for obtaining cisgenic plants has been developed and applied using the example of apple and tomato trees that do not contain viral and bacterial sequences. Methods of genomic editing and accelerated selection of fruit crops are being developed.

Keywords: horticultural crops; transgenesis; CRISPR-Cas.

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Knockout of the tomato translational elongation factor using CRISPR-Cas9 technology

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Pathogenic viruses cause severe tomato losses around the world despite the development of both classical breeding and biotechnological methods. Since replication of phytoviruses involves the interaction between viral components and host plant factors, therefore loss-of-function mutations in the latters can confer viral resistance in plants. There are evidences that eukaryotic translation elongation factor 1 (eEF1) proteins are involved in the replication of some plant viruses. However, the involvement of individual subunits of the eEF1B in the viral cycle is still poorly understood.

This work is devoted to the study of the role of the eEF1B factor in the development of tomato virus infection. The contribution of each of the α, β and γ subunits of the eEF1B factor to tomato viral resistance will be determined by CRISPR-Cas9-induced targeted mutagenesis of corresponding gene sequences. As an applied aspect, we expect to find ways to create tomato plants with increased resistance to certain viral diseases. A series of binary vectors contained sequences encoded different RNAs targeting the eEF1B subunit genes was constructed. As a result of Agrobacterium-mediated transformation of tomato, more than 300 independent transgenic lines were obtained. The presence of expression cassettes with functional genes (*Cas9* and *sgRNAs*) was confirmed by PCR. The presence of mutations in target sequences was detected using T7E1 analysis and sequencing. It turned out that the majority of transgenic lines carrying mutations have a chimeric genotype, and mutations of the target genes in the homozygous state were not detected. The propagation of self-pollinated transgenic plants under greenhouse condition and following analyses of target genes to segregate the insertion of foreign DNA and obtain homozygous mutations in eEF1B subunit sequences are in progress.

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Keywords: virus resistance; phytoviruses; CRISPR/Cas9; knockout; translation elongation factor.

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SITE-directed mutagenesis for producing grain sorgum mutants with improved kafirine digestibility

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The use of genome editing technologies opens wide opportunities for the targeted mutagenesis in important agricultural crops. In the context of global warming, sorghum, an important drought- and heat-tolerant crop is of particular importance. However, compared to other cereals, sorghum grain has a lower nutritional value, due to the resistance of its storage proteins (kafirins) to proteolytic digestion. A decrease in the synthesis of kafirins as a result of mutations or the expression of the RNAi genetic constructs modifies the ultrastructure of protein bodies and improves their digestibility by proteases. To obtain mutants with improved protein digestibility, we created four binary vectors for site-directed mutagenesis of the *k1C5* and *gKAF1* genes encoding α- and γ-kafirin, respectively. Each of these vectors contained the *cas9* endonuclease gene and a guide RNA targeted the nucleotide sequences encoding the kafirin signal polypeptides. By means of agrobacterial transformation, the created vectors were introduced into the genome of the grain sorghum cv. Avans. 14 transgenic plants were regenerated. Sequencing of 5 regenerants obtained using a vector for the *k1C5* mutagenesis revealed 3 plants with mutations. The offspring of these mutants had a higher digestibility of grain proteins *in vitro* (86–92%) compared to the initial cv. Avans (63–67%). Notably, the T_1 plants lacked the *cas9* gene and the *bar* marker gene, which indicates the production of mutants with the edited *k1C5* gene sequence, which lack the genetic construct that induced this mutation. Two mutants with mutations in the *gKAF1* sequence were obtained. Thus, using the CRISPR/*Cas* technology, we have obtained mutants with improved digestibility of kafirins, which can be used in practical sorghum breeding.

Keywords: SITE-directed mutagenesis; sorgum; kafirine digestibility.

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Overexpression of the *MtCLE35* gene in transgenic *Medicago truncatula* plants inhibits nodulation at early stages of symbiosis development

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CLE (CLAVATA3/ENDOSPERM SURROUNDING REGION-related) peptides are known as systemic regulators of legume-rhizobium symbiosis that negatively control the number of nitrogen-fixing nodules. These regulatory peptides are produced in the root in response to inoculation with rhizobia, and are transported through the xylem to the shoot, where they are recognized by their receptor, CLV1-like (CLAVATA1-like) kinase, active in leaf phloem cells. After that, a shoot-derived signaling pathway is activated that inhibits subsequent nodule development in the root. Previously, we found that in *Medicago truncatula*, the expression of the *MtCLE35* gene is activated in response to rhizobia and nitrate treatment, and its overexpression systemically inhibits nodulation. However, little is known about the downstream target genes regulated by a MtCLE35 signaling pathway in the root. Moreover, it is not completely clear which stage of symbiosis development is affected by MtCLE35-activated pathway. In order to identify genes regulated by the MtCLE35-induced signaling pathway, we performed a transcriptomic analysis of the roots overexpressing the *MtCLE35* gene. Totally, 1122 genes were found to be differentially expressed between *MtCLE35*-overexpressing and control roots after rhizobial inoculation, among them 185 genes were upregulated and 937 genes were downregulated. Among downregulated genes, many known regulators of legume-rhizobia symbiosis were found. In addition to this, we analyze early steps of interaction between *M. truncatula* overexpressing the *MtCLE35* gene and *Sinorhizobium meliloti* labeled with fluorescent reporter. We did not observe penetration of *S. meliloti* into host plant roots with *MtCLE35* overexpression. Our data suggest that overexpression of the *MtCLE35* gene inhibits nodulation at the very early stages of symbiosis development.

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Keywords: *MtCLE35*; *Medicago truncatula*; nodulation.

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Approaches for the protection of *Solanum tuberosum* from late blight through the regulation of *inf1* and *inf4* elicitin genes

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Late blight is a disease affecting economically important crops, which is caused by the oomycete *Phytophthora infestans*. Mainly, fungicides are used against it, however they may harm the environment when used in large quantities. Spray-induced gene silencing (SIGS) can become an alternative to the classical fungicides in the fight against *P. infestans*. SIGS involves the treatment of plants with double-stranded RNA (dsRNA) which triggers the RNA interference mechanism to suppress translation of the target gene. So, it is possible to suppress the expression of genes.

We have chosen two *P. infestans* genes, *inf1* and *inf4* involved in the different stages of the parasite development. For the production of dsRNA in *E. coli* HT115 two expression vectors were constructed on the basis of the L4440 plasmid, each carrying a cDNA fragment of these genes between two T7 phage promoters oriented in the opposition to each other.

To evaluate the protective effect of the dsRNA potato explants were treated with a solution containing dsRNA of one of the genes or their combination. 24 hours later, phytophthora zoospores were inoculated with the registration of the effect after 5 days.

According to the measurement results, the lesion area was significantly larger in plants treated with water than in the other three experimental groups that were treated with dsRNA.

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Keywords: double-stranded RNA; *Solanum tuberosum*; *Phytophthora infestans*.

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Putative molecular pathways of autoregulation of nodulation activated by CLE peptides in pea

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Legume plants are important for ecosystems due to their ability to form root nodules in symbiosis with rhizobia, where nitrogen fixation takes place. The number of symbiotic nodules is regulated by the CLE peptides inhibiting excessive nodule formation. Previously, we have identified four genes encoding CLE peptides, activated in response to rhizobia inoculation in pea. Three of them, *PsCLE13*, *PsCLE12* and *PsNIC-like*, were also activated by nitrate, and, therefore, they could mediate nitrate-dependent inhibition of nodulation [1]. Overexpression of *PsCLE13* and *PsCLE12* inhibited nodulation on transgenic roots: however, the role of *PsNIC-like* and *PsCLE12-like* have not been investigated.

In this study, we constructed vectors for overexpression of the *PsCLE12-like* and *PsNIC-like* genes to study their possible role in nodulation, and also analyzed the expression levels of nodulation-related genes in transgenic roots overexpressing four *PsCLE*s genes. Moreover, vectors for CRISPR-Cas9-mediated gene editing of the *PsCLE12* and *PsCLE13* genes were constructed to further explore the role of these genes in nodulation. Overexpression of *PsCLE12-like*, *PsCLE13* and *PsCLE12* resulted in increased expression levels of *TOO MUCH LOVE* (*PsTML*s) genes known as root-acting regulators of nodule number. In addition, in the roots overexpressing four *PsCLE*s genes, down regulation of the *PsSYM37* gene (encoding the receptor for Nod-factors) was observed, suggesting that the CLE peptides might inhibit the development of symbiotic nodules at the earliest stages of symbiosis development upon Nod-factor perception.

This research was funded by the Sirius University of Science and Technology, project PBB-RND-2243.

Keywords: CLE peptides; pea; nodulation.

REFERENCES

1. Lebedeva MA, Sadikova DS, Dobychkina DA, et al. CLAVATA3/EMBRYO SURROUNDING REGION Genes Involved in Symbiotic Nodulation in *Pisum sativum*. *Agronomy*. 2022;12(11):2840. DOI: 10.3390/agronomy12112840

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Genetically modified legume plants as a basis for studying the signal regulation of symbiosis with nodule bacteria

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The development of legume-rhizobium symbiosis is based on signal exchange between partners, which leads to the formation of nitrogen-fixing root nodules. Under the influence of rhizobial signal molecules, the Nod factors, the signal transduction cascade is activated, where the LysM-type receptor kinases and a complex of intracellular regulators, a significant part of which are still unknown, can play an important role.

Using transcriptomic and proteomic analysis, we searched for new regulators of the signal pathway in pea *Pisum sativum* L., which are activated under the influence of Nod factors. Phospholipases C and D, GTPases, calcium-dependent protein kinases, and mitogen-activated protein kinases (MAPKs) have been identified among such novel regulators. The influence of one of such regulators, the MAPK6, on the development of symbiosis was studied in more detail. Using genetic engineering approaches, we increased the transcriptional activity of *MAPK6* in transgenic roots, which led to an increase in the number of nodules and the biomass of pea plants. A similar effect was also found for the homologous *MAPK6* gene in another legume *Medicago truncatula*, which has a type of nodulation similar to that one in pea. New approaches have been developed to obtain stable pea transformants with enhanced level of *MAPK6* transcription using the constitutive p35S promoter. A comprehensive study of such plants inoculated with rhizobia was carried out. In addition, the approaches for genome editing of pea plants have been designed using the CRISPR/Cas system, when the *MAPK6* gene was used as a target. Thus, the possibility of using genetic engineering methods to obtain plants with increased symbiosis efficiency was investigated.

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Keywords: legume plants; symbiosis; nodule bacteria.

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Prospects for the study of natural GMOs

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Naturally transgenic plants are plants that have been subjected to "Agrobacterium" mediated transformation in natural conditions without any human impact. They contain T-DNA-like sequences, called cellular T-DNA (cT-DNA) in their genomes and transfer them from generation to generation [1].

At the moment, several dozen species of natural GMOs are known, and this list is constantly updated. Based on the available data on the diversity of natural GMOs, it can be concluded that in each case, plants have their own set of functionally active transgenes. Accordingly, each cT-DNA performs its own functions. This set of active transgenes will define promising areas for nGMO research, such as:

- description of the structures and functions of opine synthesis genes and the biological activity of their products in the regulation of plant-microbial interactions [2];
- description of the effect of oncogenes on plant morphogenesis, their primary and secondary metabolism [1].

In addition, sequences, newly acquired by plants, can be successfully used in phylogenetic studies [3].

These topics will be the subject of a report at the conference.

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Keywords: natural GMO; cellular T-DNA; phylogenetic studies; opines; T-DNA oncogenes.

REFERENCES

1. Matveeva TV. Why do plants need agrobacterial genes? *Ecological genetics*. 2021;19(4): 365–375. (In Russ.) DOI: 10.17816/ecogen89905

2. Shaposhnikov AD, Matveeva TV. The diversity of genes for the synthesis of opines and their products in representatives of various taxa. *Ecological genetics*. 2023;21(2);137–154. (In Russ.) DOI: 10.17816/ecogen492282

3. Zhidkin R, Zhurbenko P, Bogomaz O, et al. Biodiversity of *rolB/C*-like natural transgene in the genus *Vaccinium* L. and its application for phylogenetic studies. *International Journal of Molecular Sciences*. 2023;24(8):6932. DOI: 10.3390/ijms24086932

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Natural transformants of *Camellia* section *Thea*

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Horizontal gene transfer (HGT) plays an important role in plant evolution and plant development. Agrobacterium-mediated gene transfer leads to the formation of crown galls or hairy roots, due to expression of transferred T-DNA genes. Spontaneous regeneration of transformed cells can produce natural transformants carrying cellular T-DNA (cT-DNA) sequences of bacterial origin. HGT from Agrobacterium to dicots is remarkably widespread. The production of naturally genome modified plants could play a role in plant evolution and environment.

Among these natural GMOs (nGMOs) there are the tea plants. *Camellia sinensis* var. *sinensis* cv. Shuchazao contains a single 5.5 kb cT-DNA fragment organized as imperfect inverted repeat with three inactive genes. 142 *Camellia* accessions, belonging to 10 of 11 species of the section *Thea*, were studied for the presence of cT-DNA alleles. All of them contain the cT-DNA insert, indicating that they are resulted from the single transformed event. Allele phasing showed that 82 accessions were heterozygous for T-DNA alleles, 60 others were homozygous. A phylogenetic analysis of all found alleles showed existence of two separate groups of them, further divided into subgroups. The alleles of the different *Camellia* species were distributed mosaically over groups, and different species showed very similar T-DNA alleles. This indicates that the taxonomy of *Thea* requires revision. The nucleotide divergence of the imperfect cT-DNA repeats indicates that the age of cT-DNA insertion is about 15 mya, which is earlier then emergence of section *Thea* [1]. We present a working model for the origin and evolution of nGMO plants derived from allogamous transformants.

Keywords: *Camellia*; T-DNA; nGMO.

REFERENCES

1. Chen K, Zhurbenko P, Danilov L, et al. Conservation of an *Agrobacterium* cT-DNA insert in *Camellia* section *Thea* reveals the ancient origin of tea plants from a genetically modified ancestor. *Frontiers in Plant Science*. 2022;13:997762. DOI: 10.3389/fpls.2022.997762

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Natural GMOs inside the genus *Arachis* L.

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Cultivated peanut is an allotetraploid species that received the A and B genomes from *Arachis duranensis* and *A. ipaensis*. Homologs of the agrobacterial cucumopine synthase gene were previously found in both genomes as a result of horizontal transfer [1]. These sequences are found both in ancestral species and in cultivated peanuts. In addition to them, natural GMOs are *A. monticola* and *A. stenosperma*. How widespread natural GMOs are within the genus *Arachis* is currently unknown. The aim of our study was to search for natural GMOs within the genus *Arachis* and to analyze the polymorphism of natural transgenes out the studied species.

METHODS: Gene sequencing for various *Arachis* species was determined using the bwa [2], GATK [3] and samtools [4] packages based on NGS data aggregated in the SRA NCBI database.

RESULTS: We have found homologues of the cucumopine synthase gene in the genomes of *A. appressipila, A. batizocoi, A. cardenasii, A. correntina, A. diogoi, A. duranensis, A. glandulifera, A. helodes, A. hoehnei, A. ipaensis, A. macedoi, A. magna, A. monticola, A. paraguariensis, A. pintoi, A. pusilla, A. rigonii, A. stenophylla, A. stenosperma, A. trinitensis, A. valida, A. villosa*, and also characterized the intraspecific variability of the gene in cultivated peanuts. In 16 of the 22 species studied, the gene is full-length. The report will consider the possibility of using the cucumopine synthase gene in peanut phylogenetic studies.

CONCLUSION: The list of species of natural GMOs within the genus Arachis today includes 23 species.

The work was supported by the Russian Science Foundation, grant No. 21-14-00050

Keywords: nGMO; Arachis; biodiversity.

REFERENCES

1. Matveeva TV, Otten L. Widespread occurrence of natural genetic transformation of plants by Agrobacterium. *Plant Mol Biol*. 2019 Nov;101(4–5):415–437. DOI: 10.1007/s11103-019-00913-y

2. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760. DOI: 10.1093/bioinformatics/btp324

3. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297–1303. DOI: 10.1101/gr.107524.110

4. Danecek P, Bonfield JK, Liddle J, et al. Twelve years of SAMtools and BCFtools. *Gigascience*. 2021;10(2):giab008. DOI: 10.1093/gigascience/giab008

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Distribution of the rolB/C-like natural transgene in representatives of the genus *Vaccinium* L.

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Genetic colonization by agrobacteria is possible due to agrobacterial transformation, which implies interspecies transfer of genetic material (T-DNA). A transgenic tissue is formed on the whole non-transgenic plant during that process. However, it turned out that in nature there are plants containing T-DNA fragments in their genomes and they can inherit these T-DNAs sexually. Such T-DNA was called cellular, and such plants were called natural transgenic.

Examples of such organisms are plants of the genus *Vaccinium*. In the genomes of two species of this genus we found cT-DNA, represented by a *rolB/C*-like gene [1].

Previously, analyzing the natural transgenes in another genus (*Camellia* L.) [2], we showed the importance of reconstructing the allelic states of transgenes for phylogenetic studies. In this study, we performed analysis of the spreading of the *rolB/C*-like gene for its use as a molecular marker within *Vaccinium*.

We used molecular-genetic and bioinformatics methods for sequencing, assembly, and analysis of the *rolB/C*-like gene. We discovered 26 new *Vaccinium* species and *Agapetes serpens* (Wight) Sleumer as containing the *rolB/C*-like gene. Most of studied samples are characterized by the presence of full-size genes. This made it possible to develop approaches for alleles phasing of the *rolB/C*-like gene and reconstruct a *Vaccinium* phylogenetic relationship.

We subjected the studied species to phylogenetic analysis based on sequences of the *rolB/C*-like gene. The resulting phylogenetic tree of the genus *Vaccinium* divided the species into sections in accordance with the classical genus system based on morphological characters. At the same time, our tree did not confirm the taxa determined on the basis of ITS.

The work was performed using the equipment of the Resource Center of St. Petersburg State University “Development of Molecular and Cellular Technologies” with the support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement No. 075-15-2022-322 dated 04/22/2022 on the provision of a grant in the form of a subsidy from the Federal budget of the Russian Federation. The grant was provided as part of the state support for the creation and development of the world-class Scientific Center “Agrotechnologies of the Future”.

Keywords: naturally transgenic plants; *Vaccinium*; *rolB/C*-like gene.

REFERENCES

1. Matveeva T. New naturally transgenic plants: 2020 update. *Bio Comm*. 2021;66(1). DOI: 10.21638/spbu03.2021.105

2. Chen K, Zhurbenko P, Danilov L, et al Conservation of an *Agrobacterium* cT-DNA insert in *Camellia* section *Thea* reveals the ancient origin of tea plants from a genetically modified ancestor. *Front Plant Sci*. 2022;13:997762. DOI: 10.3389/fpls.2022.997762

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Transgene-free genome editing of plants

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The presence of foreign DNA is the main obstacle to the application of biotechnological plant varieties. However, transgene-free technologies in the field of genome editing make it possible to overcome this problem. In most countries that already have legislation in this area, plants without foreign DNA do not require field trials and safety tests.

The easiest way to avoid integration of transgenes is the delivery of RNP complexes directly into the cell without the use of plasmids. However subsequent selection of edited cells in the absence of a selective marker and plant regeneration are quite difficult. Therefore, traditional genetic constructs are used more often, despite that the elements of the CRISPR system are integrated into the genome. Backcrossing and cross-pollination are used to get rid of unwanted inserts. There are opportunities to accelerate the selection process, such as the Transgene Killer CRISPR system, which ensures the death of plants carrying transgenes in the early embryonic stages [1].

Constructs based on viral replicons integrated into T-DNA are an alternative option. They provide a high level of transient expression of CRISPR elements which are not integrated into the genome. Such vectors were created on the basis of geminiviruses, rhabdoviruses, potexviruses, potyviruses, bunyaviruses [2]. The ability of viruses to move between cells can be both preserved and lost due to the removal of the corresponding proteins.

One of the newest approaches is grafting of shoots onto roots expressing Cas and guide RNA [3]. The addition of tRNA-like motifs to the transcripts ensured their mobility and dispersal along the shoot. Heritable edits were observed in the progeny of grafted plants.

Thus, for transgene-free editing technologies of plant genomes are rapidly developing, which will accelerate the commercialization of new varieties with economically valuable traits.

Keywords: transgene-free editing technologies; crops; biosafery.

REFERENCES

1. Yubing H, Min Z, Lihao W, et al. Improvements of TKC technology accelerate isolation of transgene-free CRISPR/Cas9-edited rice plants. *Rice Science.* 2019;26(2):109–117. DOI: 10.1016/j.rsci.2018.11.001

2. Zhang C, Liu Sh, Li X, et al. Virus-induced gene editing and its applications in plants. *Int J Mol Sci.* 2022;23(18):10202. DOI: 10.3390/ijms231810202

3. Yang L, Machin F, Wang Sh, et al. Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks. *Nat Biotechnol.* 2023:41(7):958-967. DOI: 10.1038/s41587-022-01585-8

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Characteristics of root endophytic fungi communities associated with genetically modified plants

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Root endophytic fungi (EF) spend at least parts of their life cycle inside plant tissues without apparent harm to the host. There is a hypothesis that the endophytic lifestyle is a common strategy for most fungi and they have endophytic ancestors [1]. By receiving habitat and nutrients EF can increase the solubility of nutrients in the plant rhizosphere, stimulate plant growth, and activate the plant's systemic resistance to stress. One of the alternatives to the use of pesticides is the use of resistant transgenic plants, but the potential effects of crop modifications on their associated microorganisms are poorly studied.

The EF communities of transgenic lines of cotton, sugar cane, and maize containing the expressed Cry1 protein from *Bacillus thuringiensis* were compared with communities of non-transgenic plants. There were no significant differences in the composition of the EF community [2, 3]. The introduction of phosphinothricin-N-acetyltransferase and imazapyr herbicide resistance genes for corn and sugar cane also did not affect on EF communities but did affect the bacterial community [3, 4]. The similar effect was observed for transgenic maple plants [5]. The stage of plant development had a more significant effect on EF community than the fact of transformation itself [1].

We believe that the fungal community is more conservative and the introduction of herbicide resistance or toxin synthesis genes into the plant genome has a significantly lesser effect on EF community than on the bacterial one.

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Keywords: endophytic fungi; GMO; symbiosis.

REFERENCES

1. Bhunjun CS, Phukhamsakda C, Hyde KD, et al. Do all fungi have ancestors with endophytic lifestyles? *Fungal Divers*. 2023. DOI: 10.1007/s13225-023-00516-5

2. de Souza Vieira PD, de Souza Motta CM, Lima D, et al. Endophytic fungi associated with transgenic and non-transgenic cotton. *Mycology*. 2011;2(2):91–97. DOI: 10.1080/21501203.2011.584390

3. da Silva DA, Cotta SR, Vollú RE, et al. Endophytic microbial community in two transgenic maize genotypes and in their near-isogenic non-transgenic maize genotype. *BMC Microbiol*. 2014;14:332. DOI: 10.1186/s12866-014-0332-1

4. Romão-Dumaresq AS, Dourado MN, Fávaro LC, et al. Diversity of cultivated fungi associated with conventional and transgenic sugarcane and the interaction between endophytic *Trichoderma virens* and the host plant. PLoS One. 2016;11(7):e0158974. DOI: 10.1371/journal.pone.0158974

5. Huang Y, Dong Y, Ren Y, et al. Niches and seasonal changes, rather than transgenic events, affect the microbial community of *Populus × euramericana* "Neva". *Front Microbiol*. 2022;12:805261. DOI: 10.3389/fmicb.2021.805261

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The application of the entomopathogenic fungus *Akanthomyces muscarius* modified GFP to study endophytization

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Entomopathogenic fungi (EPF) of the genus *Akanthomyces* (formerly *Lecanicillium*) are one of the most common and important fungal entomopathogens, infecting sucking insects of the order Hemiptera mainly. The fungi can also parasitize on phytopathogenic fungi (rust, powdery mildew). The entomopathogens from these genera reported as endophytes in various plants under natural conditions [1–2], contributing to an increase in plant immunity to pathogens, as well as a decrease in plant colonization by pests. Endophytic colonization of plants by the fungus *Akanthomyces lecanii* can suppress the growth of the peach aphid [3]. *Akanthomyces muscarius* strains caused the death of moth when feeding on cabbage colonized by the fungus [4]. Endophytic properties were assessed using the *A. muscarius* (= *Lecanicillium muscarium*) strain Vl 72-GFP fluorescently labeled with GFP [5]. The transformation was done by electroporation of germinated conidia of the high-virulent "wild" strain Vl 72 by the pBARGPE1 vector harboring an eGFP gene, showed an expression of fluorescent protein without affecting fungal growth and virulence. The influence of the fungus on the growth rates of beans was revealed when leaves, sterile soil and seeds were treated with a suspension of conidia of 10^8 spores/ml. On the 7^{th} day, stimulation of the growth of the stems and roots of the beans was observed when the seeds were soaked in a spore suspension of the fungus. When spraying the leaves, only the stem's elongation was observed. The studied strain colonizes beans irregularly. When treating the seeds, the fungus was isolated in greater quantities from the roots (26%), when spraying the leaves — from the stem (36%), when watering the soil — also from the stem (43%). Infection of *A. muscarius* plants by spilling the soil was most effective. No effect of endophytization was found on the number of aphids after 14 days of aphid plant colonization. As a result of the introduction of the spores of Vl 72-GFP strain by shedding the soil under flower crops (lantana, gerbera, acanthus) in the greenhouse of Saint Petersburg Botanical Garden, this strain was isolated from the leaves of the *Acanthus mollis* L. after one month, which confirms the ability of this species to endophytic colonization of plants in greenhouse conditions. Analysis of hyphae Vl 72-GFP in the plant performed on an AxioImager M1 fluorescent microscope demonstrated the same level of fluorescence as in *A. muscarius* hyphae growing on the media.

This work was supported by the Russian Science Foundation and Saint Petersburg Science Foundation (project 23-26-10052).

Keywords: *Akanthomyces muscarius*; endophytization; GFP.

REFERENCES

1. Gymez-Vidal S, Lopez-Llorca LV, Jansson H-V, et al. Endophytic colonization of date palm (*Phoenix dactylifera* L.) leaves by entomopathogenic fungi. *Micron*. 2006;37:624–632. DOI: 10.1016/j.micron.2006.02.003

2. Nicoletti R, Becchimanzi A. Endophytism of Lecanicillium and Akanthomyces. *Agriculture*. 2020;10(6): 205. DOI: 10.3390/agriculture10060205m

3. Yuningsih D, Anwar R, Wiyono S. Endophytic colonization of entomopathogenic *Lecanicillium lecanii* (Zimm) Zare & Gams PTN10, and its effect on tobacco resistance against *Myzus persicae* Sulzer (*Hemiptera: Aphididae*). *IOP Conference Series: Earth and Environmental Science*. 2022;974(1):6. DOI: 10.1088/1755-1315/974/1/012089

4. Kuchár M, Glare TR, Hampton JG, et al. Virulence of the plant-associated endophytic fungus *Lecanicillium muscarium* to diamondback moth larvae. *New Zealand Plant Protection*. 2019; 72: 253–259. DOI: 10.30843/nzpp.2019.72.257

5. Timofeev S, Tsarev A, Senderskiy I, et al. Efficient transformation of the entomopathogenic fungus *Lecanicillium muscarium* by electroporation of germinated conidia. *Mycoscience*. 2019;60(3):197–200. DOI: 10.1016/j.myc.2019.02.010

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Social and ethical component of genetic technologies

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The technologies of genome editing and synthetic biology are becoming more and more accessible today and, in combination with the application of artificial intelligence in biotechnology, especially powerful. A feature of today's stage is the rapidly changing landscape of engineering biological systems, which requires revision and updating of the biosafety framework. The proposed new oversight measures are as follows: a) screening for DNA synthesis orders and sequences of concern; b) environmental metagenome sequencing to search for synthetic organisms [1]. At the same time, DNA 'printers' are appeared on the market today, that blurs the boundaries of access to synthetic DNA. It is significant that no government currently requires screening or regulates it, and this system works on a benevolent basis. Additionally environmental surveillance requires for a long time to define base line. With the new scale of human activity, new social risks also arise: new forms of discrimination and inequality, confidentiality of personal data in biotechnology projects, multiplication of biotechnology and artificial intelligence risks.

Thus, the idea of "responsible researches and innovation" (RRI) [2], and trend to address safety early at the concept stage — "Safe by design" have come into the focus. A number of RRI principles can be formulated at the proof concept stage for a genome-edited project: benefits for most citizens; transparency, the public comment cycle prior to the start of the experiments; responsibility, precautions, liability; justice, redress; well-being, social good.

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Keywords: genetic technologies; biosafety; responsible researches and innovation.

REFERENCES

1. Hoffmann SA, Diggans J, Densmore D, et al. Safety by design: Biosafety and biosecurity in the age of synthetic genomics. *iScience.* 2023; 26(3):106165. DOI: 10.1016/j.isci.2023.106165

2. Soeteman-Hernández LG, Bekker C, Groenewold M, et al. Perspective on how regulators can keep pace with innovation: Outcomes of a European Regulatory Preparedness Workshop on nanomaterials and nano-enabled products. *NanoImpact*. 2021;14(11):100166. DOI: 10.1016/j.impact.2019.100166

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New naturally transgenic crops

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Horizontal gene transfer from agrobacteria to plants turned out to be a much more widespread phenomenon than previously thought. In the first it was established in 2019 due to the application of bioinformatic methods and databases, then about 30 species of naturally transgenic plants were discovered [1]. The deposition of new nucleotide sequences of various plant species makes it possible to periodically update the list of naturally GMOs. Analysis of genomic and transcriptomic databases in 2023 revealed more than 50 new naturally transgenic plants and, thus, more than 100 species of nGMOs are currently known. And the share of naturally transgenic plants in relation to deposited species of terrestrial dicotyledonous plants is about 7%. Interestingly, this indicator retains its value regardless of the change in the number of organisms in the studied databases [2].

Among the discovered new nGMOs there are species that have been cultivated by humans since ancient times and are important agricultural crops. Fruit crops include the following species of nGMOs: carambola (*Averrhoa carambola* L.), persimmon (*Diospyros kaki* Thunb.), wasabi (*Eutrema japonicum* (Miq.) Koidz.), raspberry (*Rubus idaeus* L.), *Luffa acutangula* (L.) Roxb. There are also many medicinal, ornamental and oilseed species among the naturally transgenic plants. Further study of these species would make it possible to establish what role horizontal gene transfer played in the appearance of traits in plants that were selected by humans.

The obtained data can be further used to study the molecular evolution and the role of transgenes in naturally transgenic plants.

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Keywords: crops; nGMO; horizontal gene transfer.

REFERENCES

1. Matveeva TV, Otten L. Widespread occurrence of natural genetic transformation of plants by Agrobacterium. *Plant Mol Biol*. 2019;101(4–5):415–437. DOI: 10.1007/s11103-019-00913-y

2. Matveeva TV. Why do plants need agrobacterial genes? *Ecological genetics*. 2021;19(4):365–375. (In Russ.) DOI: 10.17816/ecogen89905

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Teaching interdisciplinary courses on responsible conduct in the life sciences — implications for biorisk assessments of GMOs

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Genetic engineering is a powerful set of methods used in basic research, biomedicine, and biotechnology. In the molecular biology laboratory, there are established standards in biosafety to protect humans and the environment from unwanted consequences of genetic engineering although internationally agreed, legally binding biosafety standards have not been developed yet. The World Health Organisation as one international actor provides a "Laboratory Biosafety Manual" outlining key biosafety standards and a "Guidance framework for the responsible use of the life sciences" to foster biorisk assessment strategies which could be implemented by national research institutions [1, 2]. Practitioners in both the life sciences and in biotechnology should be trained in comprehensive biorisk assessments which would also further strengthen the implementation of the international agreement on banning biological weapons [3]. Recently, we reported about lessons learned from conducting two iterations of an international interdisciplinary online workshop on responsible conduct in the life sciences [4]. Here, we provide insights from the third workshop and the impact of the lessons learned from this long-standing workshop series on the own work in the molecular biology laboratories of the organisers in respect to biorisk assessments and science communication.

Keywords: interdisciplinary courses; GMO; biosafety.

REFERENCES

1. World Health Organization. (2020). Laboratory Biosafety Manual. Available from: https://www.who.int/publications/i/item/9789240011311.

2. World Health Organization. (2022). Global guidance framework for the responsible use of the life science. Available from: https://apps.who.int/iris/handle/10665/362313.

3. Biological Weapons Connvention. (2006). Sixth Review Conference of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Final Document. BWC/CONF.VI/6. Paragraph 14. Available from: https://www.un.org/disarmament/publications/library/bwc

4. Himmel M, Malygina AA, Dukhinova MS. Biorisk assessment of genetic engineering — lessons learned from teaching interdisciplinary courses on responsible conduct in the life sciences. *Ecological genetics*. 2022;20(Suppl.1S);56–57. DOI: 10.17816/ecogen112361

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GMOs policy and research in Tajikistan

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The policy of the Republic of Tajikistan in the field of biosafety, regarding the issue of handling and use of living genetically modified organisms (LMOs or GMOs) is aimed at compliance with international legal acts, agreements and obligations to ratified Conventions. Tajikistan ratified the Convention on Biological Diversity in 1997 and the Cartagena Protocol on Biological Safety in 2004. After ratifying the protocol, the country has prepared three National Reports in accordance with the requirements of international agreements. Earlier in Tajikistan, the Law of the Republic of Tajikistan "On Biological Safety" (2005) was adopted. "The Law regulates the development, testing, production, import, export and release on the market and into the environment of GMOs, is aimed at reducing the risk of adverse effects of GMO on human health, biological diversity, ecological balance and the state of the environment". Currently, this Law has been renamed into the Law "On Genetically Modified Organisms" and is under discussion, approval and adoption by the Parliament of the Republic of Tajikistan.

Among the urgent problems that the Republic of Tajikistan is currently facing, considering the prospects for the coming years, is the problem of food security, including issues related to ensuring food safety. Taking into account the importance of conducting research in the field of biological and food safety, scientifically based risk assessment of biological agents (including GMOs) and toxins, chemical contaminants in food products and crops by the Decree of the Presidium of the Academy of Sciences of the Republic of Tajikistan No. 108 dated 30.11.2015 the Laboratory of Biological Safety was established at the Institute of Botany, Plant Physiology and Genetics of Tajikistan National Academy of Science, the main tasks of which are the development and application of modern methods of analysis for the detection of biological agents and toxins, chemical contaminants in food products and crops, and analysis of GMO products.

It should be noted that at present there is no official information related to the production, use, distribution, sale, import and export of GMOs, as well as the registration of incoming GMO food products in Tajikistan. An analysis of the market for agricultural products in the capital city of Dushanbe showed that a number of GMO food products and genetically modified seed material are still imported from abroad in the form of technical and humanitarian assistance as well as international trade. In this regard, food safety activities should include risk assessment based on scientific evidence. Its emphasis should be on both process control and end product safety so that potentially unsafe foods can be identified early. GMO food can be considered safe if the risks associated with it are at an acceptable and acceptable level. It should be noted that an effective system for monitoring food products, including products containing GMOs, their compliance with quality standards is important for protecting the health and safety of the country's population.

Keywords: GMO; biosafety; ecological studies; Tajikistan.

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Identification of genetically modified crops in Tajikistan

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The technology of genetically modified crops (also called GM crops, GM products) allows, using genetic engineering methods, to select a specific genetic trait of one organism for inclusion in the genome of the original crop. This has made it possible to develop food crops with beneficial properties and eliminate undesirable properties in others. Despite the great agricultural benefits of transgenic crops, they have not gained acceptance in some countries: a) consumer suspicion due to allergic reactions observed to some transgenic products, b) lack of international regulations regarding these GM crops and c) negative environmental impacts Wednesday. Impacts resulting from mass production of transgenic crops, such as loss of genetic diversity, development of more adaptive weeds, migration of transgenic genes to their wild relatives, and less likely migration of transgenic genes to other unrelated organisms through horizontal transfer [1]. In addition, contamination of food products with transgenic residues has prompted various countries to restrict the import of food products made from transgenic plants or plants labeling products or ingredients as or derived from transgenic crops [2].

The aim of this study is to qualitatively evaluate various GM crops from the perspective of landrace conservation and sustainable development to achieve food security.

Six varieties of agricultural crops were selected as the object of study: 2 varieties of tomatoes imported from abroad (2022 harvest at the experimental site of the IBPPG TNAS), 1st grade potatoes from the Dushanbe market (produced in Pakistan), 2 varieties of local production. “Sharaf” corn and 1 variety of corn (made in China).

Isolation of genomic DNA was carried out according to the method: Easy Pure Food and Fodder Security Genomic DNA Kit (TransGen Biotech., China), or EasyPure Plant Genomic DNA Kit (TransGen Biotech., China). Identification was carried out using primers to the CaMV35S promoter.

The results of PCR identification of GMOs showed that the endogenous CaMV35S promoter was found in potatoes (produced in Pakistan) and corn (produced in China). This endogenous CaMV35S promoter was not detected in the local tomato and potato and maize landraces studied, indicating the absence of a GM source in these samples.

This is consistent with the data that the local varieties of the studied crops are traditional and do not contain GM sources.

Keywords: GM crops; 35S-promoter screening; Tajikistan.

REFERENCES

1. Chávez-González ML, Flores-Gallegos C, García-Lazalde VM, et al. Transgenic Residues in Soybean-based Foods. In: Soybean — Molecular Aspects of Breeding. *InTech*. 2011. DOI: 10.5772/15892

2. Barotov SS, Saidmurodov ShD, Nurov DS, et al. Identifikatsiya GMO v produkakh pitaniya rastitel'nogo proiskhozhdeniya. *Izvestiya Natsional'noi akademii nauk Tadzhikistana. Otdelenie biologicheskikh nauk*. 2021;(2(213)):57–64. (In Russ.)

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