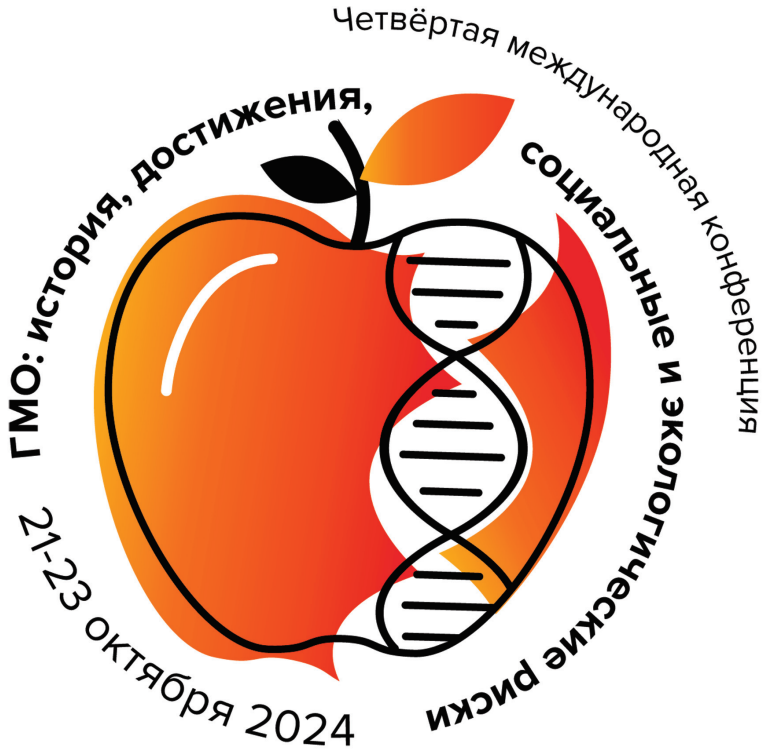
Fourth International Conference

Genetically modified organisms: The History, Achievements, Social and Environmental Risks

Saint Petersburg, Russia, October 21–23, 2024



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Saint Petersburg, Russia, October 21–23, 2024

Saint Petersburg State University

AGROTECHNOLOGY FOR THE FUTURE

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Fourth International Conference "Genetically modified organisms: The History, Achievements, Social and Environmental Risks"; 2024, October 21–23, Saint Petersburg: Eco-Vector; 2024. 44 p. doi: https://doi.org/10.17816/ecogen2024

ISBN 978-5-907886-00-1

Fourth International Conference "Genetically modified organisms: The History, Achievements, Social and Environmental Risks" was held from October 21 to 23, 2024, at Saint Petersburg State University in Saint Petersburg, Russia. The conference was conducted in a mixed format, with both on-site and online participation. It attracted researchers from Russia, China, the USA, Moldova, Tajikistan, Belarus, Israel, and Kyrgyzstan.

Founding source: 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 2022 April 22 on providing a grant in the form of subsidies from the Federal budget of Russian Federation.

Keywords: genetically modified organisms; basic research; genome editing; agriculture; medicine; environment; society.

For citation: Fourth International Conference "Genetically modified organisms: The History, Achievements, Social and Environmental Risks"; 2024, October 21–23, Saint Petersburg: Eco-Vector; 2024. 44 p. doi: https://doi.org/10.17816/ecogen2024

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М34 **Материалы** Четвертой Международной конференции «ГМО: история, достижения, социальные и экологические риски», 21–23 октября 2024 г.; Санкт-Петербург: Эко-Вектор, 2024. 44 с. doi: https://doi.org/10.17816/ecogen2024

ISBN 978-5-907886-00-1

В издании представлены материалы Четвертой Международной конференции «Генетически модифицированные организмы: История, достижения, социальные и экологические риски», состоявшейся 21–23 октября 2024 г. в Санкт-Петербургском государственном университете. Конференция проходила в смешанном формате, с очным и онлайн-участием. В ней приняли участие исследователи из России, Китая, США, Молдовы, Таджикистана, Беларуси, Израиля и Кыргызстана.

Финансирование. Конференция проведена при поддержке Министерства науки и высшего образования Российской Федерации в соответствии с соглашением № 075-15-2022-322 от 22 апреля 2022 г. о предоставлении гранта в форме субсидии из федерального бюджета Российской Федерации.

Ключевые слова: генетически модифицированные организмы; фундаментальные исследования; редактирование генома; сельское хозяйство; медицина; окружающая среда; общество

Для цитирования: Материалы Четвертой Международной конференции «ГМО: история, достижения, социальные и экологические риски», 21–23 октября 2024 г.; Санкт-Петербург: Эко-Вектор, 2024. 44 с. doi: https://doi.org/10.17816/ecogen2024

ISBN 978-5-907886-00-1

Издатель: ООО «Эко-Вектор»
Адрес: 191181, Санкт-Петербург, Аптекарский переулок, д. 3, литера А, помещение 1Н
E-mail: info@eco-vector.com
WEB: https://eco-vector.com

Оригинал-макет изготовлен
ООО «Эко-Вектор»
Мыльников С.В. (научный редактор)
Смирнова И.В. (корректор)
Еленин В.А. (верстка)
Формат 60 × 90/8. Усл.-печ. л. 5,5.
Тираж 70 экз.

ООО «Типография Экспресс В2В».
191180, Санкт-Петербург, наб. реки Фонтанки, д. 104, лит. А, пом. 3Н, оф. 1.
Тел.: +7(812) 646-33-77.
Подписано в печать 10.10.2024
Выход в свет 20.10.2024

Contents

DOI: https://doi.org/10.17816/ecogen2024

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Saint Petersburg State University, Saint Petersburg, Russia

Fourth International Conference "Genetically modified organisms: The History, Achievements, Social and Environmental Risks" was held from October 21 to 23, 2024, at Saint Petersburg State University in Saint Petersburg, Russia. The conference was conducted in a mixed format, with both on-site and online participation. It attracted researchers from Russia, China, the USA, Moldova, Tajikistan, Belarus, Israel, and Kyrgyzstan.

The conference papers were traditionally organized into six sections:

1. GMOs for Basic Research.
2. Genome Editing Technologies.
3. GMOs for Agriculture.
4. GMOs for Medicine.
5. GMOs and the Environment.
6. GMOs and Society.

Additionally, there was a poster session. A distinctive feature of this conference was the large number of presentations in the GMOs and Environment section, as well as a significant representation of talks focused on the genetic engineering of animals. The abstracts are presented in this special issue. 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 2022 April 22 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: GMO; basic research; genome editing; agriculture; medicine; environment; society.

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DOI: https://doi.org/10.17816/ecogen2024

Development of Catechol-O-methyltransferase deficiency mouse for preclinical tests and research

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Catechol-O-methyltransferase (COMT) is an enzyme involved in catecholamine degradation. COMT along with monoamine oxidase are two major enzymes responsible for degradation of dopamine, epinephrine and norepinephrine in mammals. Variations in the COMT gene are associated with schizophrenia, depression and other mental disorders [1], and also there is evidence of association between COMT and preeclampsia [2]. There are two COMT isoforms: a membrane-bound isoform, highly abundant in brain; and a soluble isoform present in cytoplasm, less active that membrane-bound form. The involvement of COMT in nervous system function makes COMT-deficient animals a useful model for studying mental disorders and can provide valuable information for cognitive research.

To better understand the impact of COMT in pathogenesis, we developed a new line of COMT knockout mice using microinjection of sgRNA/Cas9 mRNA construct into zygotes. The resulting knockout mouse line has the deletion of 20 nucleotides in COMT coding sequence, that leads to the absence of both COMT isoforms. We confirmed the absence of COMT activity using HPLC by observing the lack of homovanillic acid — dopamine metabolism product, that can't be produced without COMT.

The line we obtained did not show strong phenotypic manifestations, but it could be used in complex studies or in crosses with other knockout lines. Such multi-knockout models could be interesting objects for studying mental disorders, especially resistant ones, since there is evidence that some variants of the COMT gene in combination with other disorders lead to the development of treatment-resistant depression [3].

Funding source. This work was supported by a St. Petersburg State University grant for the development of scientific research, ID 95445540 (121082000087-7).

Keywords: Catechol-O-methyltransferase; CRISPR/Cas; knockout mice.

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DOI: https://doi.org/10.17816/ecogen2024

Transgenic rye: the past problems and the future perspectives

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Monocots are not natural hosts for *Agrobacterium*, but some protocols for *Agrobacterium*-mediated transformation of cereals and *in vitro* regeneration, which provide stable transgenic plants for corn, wheat, rice, and barley [1], have been developed. Rye, belonging to the group of minor cereals, is now becoming an object of scientific interest due to resistance to unfavourable environmental factors, poor soils and a positive effect on human health. Its relatively recent domestication and open pollinated nature allow rye to keep a variability of genetic traits which are not present in other crops. Despite the fact that rye is recalcitrant species to *in vitro* regeneration, stable transgenic rye plants have been obtained using *Agrobacterium*-mediated transformation of immature embryos [2] and particle bombardment of embryogenic callus [3]. However, despite such complex *in vitro* cultivation procedures the transformation frequency varied from 0.8 to 3.8% for genotypes with high regenerative capacity [4].

Recent research articles show that known systems of transformation *in planta* have been improved and new systems have been developed without the need for regeneration *in vitro*. Transgenic maize [5–7], wheat [8] and rice [9, 10] were produced using the pollen-tube pathway, ovary-drip, infection of pre-imbibed embryos, shoot apical meristem injury under *in vivo* conditions and agro-imbibition. Though these techniques have some limitations and inconsistency, their simplicity and accessibility are very attractable to exploit them on rye.

For rye, the development of protocols for making transgenic plants without an *in vitro* cultivation step could significantly facilitate the process of transferring valuable traits without the need to use complex selection schemes for cross-pollinating rye.

Funding source. This work was supported by the St. Petersburg State University for a research grant (project ID Pure 115624290).

Keywords: *Agrobacterium*; transformation; rye.

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DOI: https://doi.org/10.17816/ecogen2024

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DOI: https://doi.org/10.17816/ecogen2024

Project oriented genetic engineering courses

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The active development in the biotechnology and genetic engineering industries creates a demand for relevant specialists. In training such personnel, both fundamental knowledge and methodological skills in these fields are essential. One approach to addressing this need is to introduce practical tasks into theoretical courses at higher education institutions, integrating them within the scope of a single investigation.

The creation of a plasmid for protein production is one of potential projects. This task allows for the coverage of all key techniques in genetic engineering, including DNA purification, molecular cloning, and PCR. The project can be logically concluded with an assessment of the efficiency of protein production. In addition to teaching laboratory methods, it is also important for students to develop skills in working with nucleic acid sequences *in silico*. This includes constructing plasmid maps and annotating them.

Today, a complete set of open-access tools exists and can address all current tasks in genetic engineering. Moreover, these tools also facilitate adequate planning and verification of future experiments before starting. The task of *in silico* designing the experiment, along with plasmid construction and verification, can be completed in practice within 1–2 weeks of full-time training. If time constraints or lack of access to laboratory facilities arise, the project can be adapted to the classroom setting and executed throughout the semester. Even in this scenario, it is possible to maintain all components related to *in silico* tasks, preparation of solutions, reaction mixtures, and writing protocols.

Funding source. Various iterations of this educational project have been implemented at Sirius University and Alferov University.

Keywords: genetic engineering; education; practical courses.

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DOI: https://doi.org/10.17816/ecogen2024

Improving the digestibility of seed storage proteins in sorghum by RNA silencing of the gamma-kafirin gene: inheritance and expression of the RNAi genetic construct in mutants of cv. Avance and their hybrids

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RNA interference technology is an effective tool for functional genomics, genetic engineering and plant breeding, since it allows creating mutants with altered expression of genes encoding various biological and agronomically valuable plant traits. We have previously obtained a mutant of grain sorghum (cv. Avance) carrying a genetic construct for RNA silencing of the γ-kafirin gene using *Agrobacterium*-mediated genetic transformation [1]. It was found that the construct is stably inherited during self-pollination up to the T_5 generation. qPCR analysis showed that in different plants from T_4 generation, the genetic construct is present in 1 or 2 copies. At the same time, cases of construct elimination were registered, while its fragment carrying the *bar* marker gene was preserved in the genome of T_4 plants. RT-qPCR analysis revealed that the expression level of the γ-kafirin gene in developing kernels of T_4 mutants was reduced by 3.5–84.1 times. Plants carrying the RNA silencing construct had a modified endosperm type (either completely floury or with a thin, often "blurred" vitreous layer), improved *in vitro* grain protein digestibility (up to 85–89%, compared to 52–59% in the original cultivar). Elimination of the construct was accompanied by a reversion of the endosperm type (thick vitreous layer) and decreased protein digestibility. Homozygous lines with different numbers of construct copies were identified, and it was shown that the copy number does not affect the digestibility level. Plants of all transgenic lines containing the RNA silencing construct differed from the original cultivar in reduced height, 1000-grain weight, and grain yield per panicle. It was found that the RNA silencing construct is inherited during hybridization, improving the digestibility of grain proteins in hybrids; recombinants combining high protein content (13.9%) with high digestibility (up to 85–92%) were isolated in F_2 families. The F_1 hybrid of the CMS line A2 KVV-181 with one of the mutant lines gave a higher yield of digestible protein (by 22.7%), compared to the non-transgenic hybrid A2 KVV-181/Avance.

Funding source. The research was supported by the grant of the Russian Science Foundation No. 24-16-00063.

Keywords: RNAi; gamma-kafirin; endosperm; *in vitro* protein digestibility; sorghum.

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DOI: https://doi.org/10.17816/ecogen2024

Obtaining the Brainbow transgenic mouse for *in vivo* cell lineage fate observation

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"Brainbow" is a name for the transgenic Cre-mediated multicolor cell labeling strategy. Cells are transfected with plasmid harbouring different fluorescent proteins flanked by different lox sites. Cre recombinase performs excision of the lox flanked genes randomly changing expression patterns of the transgene-encoded fluorescent proteins [1]. This system was used for labelling cells in mice, fruit flies and zebrafish [2].

In our laboratory we have obtained the Brainbow transgenic mice using the method of the pronuclei microinjection of the Brainbow 3.0 plasmid fragment which contains CAG promotor and coding sequences for 3 fluorescent proteins: mOrange, flanked by *loxP* sites, green EGFP (flanked by *lox2272* sites along with mOrange) and red mKate [3]. When no Cre-recombinase is present mOrange is expressed and cells demonstrate orange fluorescence.

By crossing our transgenic Brainbow mice with a transgenic line expressing NSE-Cre recombinase under the neuron-specific NSE (neuron-specific enolase) promoter, we will produce mice with both transgenes. The NSE-Cre recombinase transgenic mice exhibit tissue-specific Cre-mediated recombination. When Cre recombinase is active, due to stochastic *loxP* or *lox2272* recombination occurrence either EGFP or mKate are left in the transgene in the genome and can be expressed thus providing a wide colour labelling variation for different cells. Moreover, every daughter cell of the same progenitor cell will have the same fluorophore expression pattern. Therefore, these obtained double-transgenic mice provide an opportunity to observe ontogenetic fate of the uniquely labeled cell lines.

Funding source. This work was supported by a St. Petersburg State University grant for the development of scientific research, ID 95445540 (121082000087-7).

Keywords: transgenesis; Brainbow; Cre recombinase; mouse; fluorescent proteins.

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DOI: https://doi.org/10.17816/ecogen2024

Current state of research in porcine genome editing and genetic modification

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Pork is the most widely consumed meat, and Russia is among the top five producers. This industry faces numerous challenges, including infectious and genetic diseases, as well as coping with environmental conditions [1].

First transgenic pigs were created in 1985, however, most of the research is still primarily focused on their use as models for human diseases and for organ transplantation [2]. Pigs have been transformed with genes for growth hormone, insulin-like growth factor 1, phytase, delta 12 fatty acid desaturase and bovine alpha-lactalbumin to improve productivity and meat quality, but none of these transgenic events reached the market [3]. This may be due to public concern towards GMOs.

Genome editing allows to improve livestock without introducing foreign DNA and opens up new prospects in agriculture. Knockout of the myostatin gene in pigs was performed via CRISPR/Cas genome editing in 2015. Virus resistant pigs were produced in 2016, and cold resistant pigs with improved pork production — in 2017 [2]. In 2023, a group of five gene-edited pigs with male sterility was approved for human consumption in US. Researchers at Washington State University undertook the authorization process to demonstrate that genome edited pigs are safe to eat [4]. However, CRISPR pigs are not yet raised on an industrial scale.

Thus, genome editing is a promising tool for increasing the efficiency of pig farming. Since agricultural research is currently less developed than biomedical research, such studies will have great novelty and potential for practical application.

Funding source. This research was funded by the Ministry of Science and Higher Education of Russian Federation, grant number 075-15-2024-666.

Keywords: sus scrofa domesticus; pig; genome editing; transgenic animals.

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DOI: https://doi.org/10.17816/ecogen2024

Hypotheses on the mechanisms of photoperiodic regulation of storage root development based on transcriptomic studies on radish (*Raphanus sativus* L.)

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Light conditions are one of the main factors controlling the plant development and architecture, including the growth of hypocotyl and internodes, formation of lateral shoots and leaves, and transition from vegetative growth to reproductive growth. It is well known that plant development is influenced by both photoperiod and light quality (including light wavelengths, intensity and direction). The 600–700 nm red light (RL) and 700–800 nm far-red light (FRL) are key light-quality factors, which affect different aspects of plant growth and development. The RL and FRL are primarily sensed through the phytochrome family of plant photoreceptors, which are master regulators of all photomorphogenetic responses [1, 2]. Along with other developmental processes, photoperiod and light spectrum regulate the formation of underground plant storage organs, such as modified shoots (tubers and bulbs) and storage roots [3]. At least for the development of potato tubers, the genetic mechanisms of photoperiodic control of tuber formation are well studied. Among them are numerous genes acting in the photoperiodic regulation of flowering, such as genes of *FT* family ("florigens") and *CONSTANS-like1* [4]. However, nothing is known about the mechanisms of photoperiodic regulation of storage root formation. At the same time, the development of main root crops such as sweet potato cassava, radish and carrot demonstrates a seasonality and depends on the day length and light spectrum [3]. In our studies we have demonstrated the dependence of radish storage root formation on the day length and RL/FRL balance. The long day and RL were shown to stimulate storage root formation, which allows us to suppose the role of phytochromes signaling in this process. At the same time, FRL in radish promoted flowering and does not allow formation of storage root. In the transcriptome analysis we have shown that the main players of photoperiodic development such as genes of *CONSTANS, FT, SOC1* families were downreglated in the radish leaves and roots under RL. At the same time, RL leaded to upregulation of root growth inductors, such as *WOX11, WOX5* and *PLETHORA* genes in the radish roots. Our results allow us to identify possible mechanisms regulating the switchover of two opposing developmental programs — flowering and storage root development, depending on RL/FRL balance.

Funding source. This work was supported by the Ministry of Science and Higher Education of the Russian Federation in accordance with the agreement No. 075-15-2022-322 dated 2022 April 22 on providing a grant in the form of subsidies from the federal budget of the 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: photoperiod; light spectrum; red light; storage root; transcriptome; *Raphanus sativus*.

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DOI: https://doi.org/10.17816/ecogen2024

Superweet protein thaumatin II in the selection of garden plants

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Thaumatin II protein from the West African plant *Thaumatococcus daniellii Benth*. has unique properties — it is 3000 times sweeter than sucrose and extremely resistant to heating in an acidic environment. Thaumatin II has been studied in detail and is approved for use as a food additive E957. Thaumatin belongs to the family of 5 pathogenesis-related proteins and participates in the protection of plants from fungal pathogens. The combination of thaumatin properties makes it a promising candidate for use in breeding to enhance the resistance of fruit and berry plants to fungal pathogens, and to improve the taste of their fruits. An urgent task is also to obtain plants that produce recombinant thaumatin II for the needs of the food industry. strawberries are an attractive object for improving the assortment by bioengineering methods due to genetic limitations associated with high heterozygosity and polyploidy, which complicate the implementation of traditional breeding programs. Agrobacterial transformation using the CBE21 strain of *A. tumefaciens* containing the binary vector pBIThau35 has yielded 19 independent transgenic lines of the Fireworks variety and 15 lines of the Selecta variety. Accumulation of thaumatin II was detected by the Western blot method in 17 of the 19 lines and in 14 of the 15 lines, respectively. Field evaluation of the obtained strawberry lines of the Feuworks and Select varieties showed that in 10 of the 17 lines of the Fireworks variety with detectable thaumatin II expression, produced the same yield as the original variety and have the same phenotype, however 7 lines showed some somaclonal variability. The morphological characteristics of 12 lines of the Selecta cultivar out of 14 thaumatin II accumulating lines were similar to those of the original variety, while the two lines had slightly smaller leaves and plant height. Evaluation of disease resistance showed a correlation between thaumatin II expression and increased resistance to *Botrytis cinerea*. Most of the studied transgenic lines of the Fireworks and Selecta varieties, expressing the thaumatin II protein, exhibited a significantly smaller area of necrotic lesions observed on leaf discs in both laboratory and field experiments. Fruits of the lines of both varieties were characterized by a sweeter taste with a light aroma of tropical fruits. The results of field tests indicate pronounced differences in the organoleptic qualities of the fruits and resistance to gray rot (*Botrytis cinerea*) among the transformed strawberry lines. The gene for the super-sweet protein thaumatin II from *Thaumatococcus danielli* was also transferred to the pear to improve the taste of the fruit. Based on the coding sequence cloned by Unilever and the pBI121 vector, a binary vector was created for the expression of the thaumatin gene in plants. Successful transformation of the Burakovka pear variety was obtained using a disarmed supervirulent agrobacterial strain CBE21. The insertion of the thaumatin gene was confirmed by PCR analysis and its expression in various tissues was verified by the Western blot method. In the fruits of the transgenic line, taste modification was observed, determined by the organoleptic test. The expression of TL proteins in plants has at least three applications: 1) increasing resistance to phytopathogens, especially of non-climacteric fruits and berries in the ripening stage; 2) Creation of new forms of garden plants with sweeter fruits and berries; 3) Modification of the native taste and aroma of traditional varieties

Keywords: thaumatin; strawberry; pear; genetic transformation.

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DOI: https://doi.org/10.17816/ecogen2024

The *hmg1* gene affects the reproductive sphere of transgenic tobacco plants

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Transgenic plants serve as convenient models for studying metabolism. Isoprenoids are formed through two metabolic pathways: the acetate mevalonate pathway (MVAP) in the cytoplasm and the 2-methyl-D-erythritol-4-phosphate pathway in the chloroplasts. The MVAP is responsible for synthesizing brassinosteroids, membrane sterols, polyterpenes, and other compounds. A key stage of the MVAP is mevalonate synthesis, which is catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase, encoded by the HMG family of genes.

In this study, transgenic lines of tobacco were analyzed: C-lines expressed the hmg1 gene from *Arabidopsis thaliana* under the 35S CaMV promoter, while A-lines with suppressed hmg1 expression were obtained using antisense RNA strategies [1]. Control plants were also included for comparison. The morphology of the transgenic plants was studied previously [2]. In A-lines, lipid content decreased by 8–10%, and sterols decreased by 34–58% relative to the control. Photosynthetic C14 levels in A-lines decreased by 3.6–7.3%. The C-lines did not differ from the control.

A-lines exhibited lower seed germination rates, decreasing by 8–26% compared to the control. Pollen from C-lines showed the same fertility as the control, whereas pollen from A-lines exhibited a 30% decrease in fertility. In A-lines, pollen fertility assessed by two methods [3] revealed that dehydrogenase activity was lower than that measured by the acetocarmine method, indicating that sterilization occurs late in male gametophyte development.

Both genetic constructs caused abnormalities in ovules, such as abnormal shape, micropyle orientation, incomplete rotation, and underdeveloped integuments compared to the control. In C-lines, these pathologies were less common than in A-lines. Thus, suppression of hmg1 gene expression led to a decrease in terpenoid content and disruptions in the generative sphere of tobacco.

Keywords: tobacco; *hmg1* gene; mevalonic acid; fertility; antisense RNA.

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DOI: https://doi.org/10.17816/ecogen2024

Development of an *Escherichia coli*-based screening system for the search of SARS-CoV-2 main protease inhibitors

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The novel coronavirus, SARS-CoV-2, first identified in Wuhan, China, in December 2019, has led to a global pandemic with severe impacts on public health and economies worldwide. Despite the availability of preventive vaccines, ongoing viral mutations pose a challenge to their effectiveness, highlighting the urgent need for novel antiviral treatments. The RNA genome of SARS-CoV-2 encodes several proteins critical to its replication, including the main protease, known as Mpro or 3CL-pro, which is essential for processing viral proteins. Due to its critical role and absence of human analogs, Mpro is a key target for the development of antiviral drugs. In our research, an *Escherichia coli*-based screening system was developed, to facilitate the search for effective Mpro inhibitors. The system employs the enzymatic activity of β-galactosidase, encoded by the *LacZ* gene in *E. coli*, which hydrolyzes its substrate X-Gal, producing a blue color in the colonies, to indicate the effect of potential inhibitors. In this system, *E. coli* cells are engineered to express both the gene for Mpro, and β-galactosidase. The *Mpro* gene sequence was optimized for expression in *E. coli*, and the recognition site for Mpro was inserted into the *LacZ* gene without disrupting the reading frame. Following transformation of the bacterial cells with plasmids containing the modified *Mpro* gene and the *LacZ* gene with the inserted Mpro recognition site, the cells are grown on a medium containing X-Gal. Potential inhibitors are introduced to assess their effect on Mpro activity. Inhibitors that effectively block Mpro result in β-galactosidase hydrolyzing X-Gal, producing blue colonies. Conversely, if an inhibitor does not inhibit Mpro, Mpro will cleave its recognition site, disrupting *LacZ* expression and leading to white colonies due to the lack of active β-galactosidase. This screening system offers a practical and efficient method for evaluating compounds that may inhibit Mpro and thereby could be utilized in the development of new antiviral drugs.

Funding source. This work was performed in accordance with agreement No. 075-15-2022-322, dated 2022 April 22, 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 for the Future".

Keywords: SARS-CoV-2; Mpro; X-gal; *LacZ*; screening system; inhibitors; *E. coli*.

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DOI: https://doi.org/10.17816/ecogen2024

Administrative and legal features of ensuring the customs authorities' prohibitions and restrictions on cross-border movement of GMOs in traditional and innovative society

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Globalization has led to the emergence of new types of goods that require the development and implementation of specialized control technologies. Genetically modified organisms (GMOs) have become main international trade object, requiring a need for administrative and legal mechanisms to ensure their safe cross-border movement. In this scope customs authorities play a part by controlling the import and export of GM-containing goods [1, 2].

While international trade in GMOs is taking pace, the risks to national food security and environmental sustainability are increasing. The imperative for stringent control and regulation of GMO imports stems from the potential negative impacts on public health and ecosystems. As countries have different security measures at the border, it is useful to explore both traditional approaches and innovative solutions in this area.

India, for example, practices a traditional approach to regulating the movement of GMOs. The National Bureau of Plant Genetic Resources is responsible for issuing permits to import GMOs for scientific purposes. The technology to control the movement of GMOs across borders includes documentary checks, quarantine measures and laboratory tests. Customs authorities work closely with the National Bureau, providing a high level of control and prevention of GMOs smuggling.

Germany, by contrast, demonstrates an innovative approach to control the movement of GMOs. The country is actively using advanced technologies such as molecular analysis and digital systems to monitor and track GMOs in international trade. German customs authorities apply a blockchain technology to ensure transparency and control of supply chains, which can minimize the risk of GMO smuggling or document falsification. The key focus of control in Germany is the use of automated data analysis systems, which accelerate the process of checking documents and detecting discrepancies in imports and exports.

In conclusion, the administrative and legal issues governing prohibitions and restrictions of customs authorities on the cross-border movement of GMOs depend on national priorities and the level of technological development. The experiences of India and Germany highlighted the importance of developing and improving customs technologies in the context of increasing globalization and use of GMOs.

Keywords: GMO; cross-border movement; customs authorities.

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DOI: https://doi.org/10.17816/ecogen2024

Obtaining a targeted deletion in the iucA gene of *Klebsiella pneumoniae* using the CRISPR/Cas9 editing system

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The increasing incidence of antibiotic resistance among various nosocomial pathogens is one of the global problems of the last decades. Recently, the combination of virulence genes (different operons of siderophore synthesis) and antibiotic resistance genes (metallo-β-lactamases) simultaneously in one strain of *Klebsiella pneumonia* was reported, which is a previously unencountered phenomenon [1].

In pathogenic bacteria, siderophores (chelators of trivalent iron) play an important role in virulence and have most often plasmid localization. The objective of this study was to create a deletion of the plasmid-localized NIS synthetase gene *iucA*, which is involved in the synthesis of the siderophore aerobactin, in order to characterize its functional role in the development of hypervirulence in *K. pneumoniae*, which also possesses multiple antibiotic resistance.

The pUC19_CRISPR_ΔiucA system was used to edit the *K. pneumoniae* genome with a selective marker for zeocin resistance. The pUC19_CRISPR vector contains the *Streptococcus pyogenes* Cas9 nuclease gene, genes of the Lambda red bacteriophage λ recombination system, and guide RNA (sgRNA) encoding sequence, combined with a homologous recombination cassette carrying the desired mutation in the *iucA* gene.

We used the plasmid pUC19_CRISPR_ΔiucA to transform the hypervirulent antibiotic-resistant *K. pneumoniae* strains isolated from patients in St. Petersburg hospitals. Among transformants we isolated several strains with the complete loss of pLVPK, the plasmid responsible for hypervirulence.

Thus, the use of the CRISPR/Cas9 system for the genome editing of *K. pneumoniae* is a highly efficient method that can be applied to other *Enterobacteriaceae*.

Funding source. The research was supported by the St. Petersburg State University, grant PURE ID 95445540 (121082000087-7).

Keywords: *Klebsiella pneumoniae*; CRISPR/Cas9; hypervirulence; antibiotic resistance.

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Potato genome editing: different genes — different issues

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Solanum tuberosum L. is an important agricultural crop. Complex tetraploid genome and difficult crossing strongly hinder potato breeding. Modern instruments for genomic manipulations, such as CRISPR/Cas9, enable to speed up this process. However, editing efficiency should be very high in order to affect all alleles of the polyploid genome. It is believed that editing efficiency strongly depends on sgRNA, but our experience shows that target also plays an important role. We successfully applied CRISPR/Cas9 genome editing system to inactivate few genes: *StLFY*, *StDMR6-1*, and *StVINV*.

StLFY in potato is a key regulator of the transition to flowering and tuberization. We achieved high rates of all-allelic knockouts for both used independent sgRNAs. *StLFY* knockout led to bright phenotypic alterations both in inflorescences and in stolons [1].

StVINV encodes a vacuolar invertase — a key ferment in cold-induced sweetening in potato tubers. High-temperature processing of these tubers results in dark-colored products with high amount of acrylamide, a neurotoxin and potential carcinogen [2].

StDMR6-1 gene is considered as a significant susceptibility gene for *Phytophthora infestans* infection because of it's involved in a suppression of plant immune response based on the salicylic acid pathway [3].

These genes were edited simultaneously with different sets of sgRNAs (four sgRNAs for the each gene). We achieved high rates of all-allelic knockouts for *StVINV*. It was shown, that editing efficiency for all sgRNAs targeted on *StDMR6-1* was dramatically lower than for other both genes. Therefore it was possible to obtain only few plants with *StDMR6-1* all-allelic knockouts.

Funding source. This work was partially funded by the Comprehensive Research Program "Development of Potato Breeding and Seed Production". Agreement No. 075-03-2024/4.

Keywords: *Solanum tuberosum*; CRISPR/Cas9; Agrobacteria transformation; *LEAFY*; *DMR6-1*; *VINV.*

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DOI: https://doi.org/10.17816/ecogen2024

Aged GRIN3A-KO mice have no signs of amyloid aggregates in the brain.

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Ionotropic receptors selectively binding N-methyl-D-aspartate (NMDA) are glutamate-controlled ion channels involved in the regulation of numerous neural processes throughout development. These receptors function as heterotetramers, usually formed from two heterodimers. Each heterodimer consists of a glycine-binding subunit (GluN1) paired with one from glutamate-binding subunits (GluN2A, GluN2B, GluN2C, and GluN2D) and occasionally with one of glycine-binding subunits GluN3A or GluN3B. However, the specific role and physiological impact of the GluN3 subunits, which are involved in glycine binding, remain unclear. While their presence does alter receptor function, the physiological roles of these subunits continue to be an area of active research.

The *GRIN3A* gene encoding the GluN3A subunit is expressed actively during early development but its expression diminishes with age. Certain single-nucleotide polymorphisms in the *GRIN3A* gene have been linked to addictions, schizophrenia, and cognitive impairments. Beta-amyloid oligomers, which are associated with Alzheimer's disease, have been shown to bind to NMDA receptors, suggesting a potential link between NMDA receptor dysfunction and neurodegenerative diseases [1]. Excitotoxicity and multiple cognitive impairments have been observed in age-related GRIN3A-KO mice, suggesting that a deficiency in the GluN3A subunit may be a key pathogenic factor in sporadic Alzheimer's disease (AD) [2]. However, another study using this model did not report cognitive impairment or formation of beta-amyloid aggregates in GRIN3A-KO mice [3]. Both these studies used the *GRIN3A* knockout mice, which were generated by integrating a neomycin cassette into the genome. In contrast, our new *GRIN3A* knockout mouse line was created using CRISPR/Cas9 technology, avoiding additional genomic changes. We performed immunohistochemical staining on brain sections from 19-month-old GRIN3A-KO mice using amyloid-specific OC antibodies and did not detect any extracellular amyloid aggregates. Further research is needed to clarify the impact of the GluN3A subunit on receptor function and its overall effects on the organism.

Funding source. This work was supported by a St. Petersburg State University grant for the development of scientific research, ID 95445540 (121082000087-7).

Keywords: NMDA receptors; Alzheimer's disease; GluN3A; CRISPR/Cas; knockout mice.

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DOI: https://doi.org/10.17816/ecogen2024

Identification of probable regulators of spontaneous tumor formation in higher plants in the genomic researches on radish (*Raphanus sativus* L.) inbred lines

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Tumor formation is a pathological process that can occur in virtually all multicellular organisms and results from the uncontrolled proliferation of a group of cells. Higher plants contain functional orthologues of many mammalian tumor suppressors and oncogenes, but mutations in these genes in plants have not led to tumor formation, suggesting a very different principle of organization of the systemic control of cell division in plants [1]. Most examples of plant tumors arise as a result of interactions with a variety of pathogens and phytophages, from bacteria and viruses to nematodes and arthropods [2]. Spontaneous tumors in higher plants which can develop without any pathogen invasion and depend on plant genotype, are more rare. For example, spontaneous tumor formation on the taproots is consistently observed in certain inbred lines of radish (*Raphanus sativus* var. *radicula* Pers.) of the SPbSU genetic collection [3, 4]. We have sequenced the genomes of two closely related radish inbred lines that differ in their ability to spontaneously form tumors. This is the first attempt to sequence the genome of plants with spontaneous tumor formation. We have identified numerous single nucleotide variants (amino acid substitutions, insertions or deletions) that are likely to be associated with the ability to form tumors. More than a hundred SNVs in the CDS of protein-coding genes of the tumor radish line are thought to lead to changes in protein structure ("stop lost" / "stop gained" or a frameshift). Among them, we selected 108 SNVs which are in the homozygous state in the tumor radish line. The presence of the selected SNVs in the radish tumor lines was tested by sequencing the amplicons of the corresponding gene regions in seven tumor and fourteen non-tumor lines of the SPbSU genetic collection [5]. As a result, we found that the *RsERF018* gene contains the insertion close to start codon in most tumor radish lines, which allows us to propose this gene as a candidate regulator of spontaneous tumor formation. The *RsERF018* encodes ethylene-regulated transcription factor which takes part in the regulation of cambium activity [6]. This gene needs to be further investigated as a possible regulator of spontaneous tumor formation.

Funding source. This work was supported by the Ministry of Science and Higher Education of the Russian Federation in accordance with the agreement No. 075-15-2022-322 dated 2022 April 22 on providing a grant in the form of subsidies from the federal budget of the 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: spontaneous tumours; genomic sequence; single nucleotide variants; *Raphanus sativus*.

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DOI: https://doi.org/10.17816/ecogen2024

Genome editing approach for developing herbicide-resistant lines of brassica crops

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Brassicaceae family includes a number of valuable crops used for different proposes — food, oil (including oil for biofuel), forage. One of the most relevant directions of brassica crops selection is endowing a resistance for herbicides [1]. The imidazolinone group of herbicides is promising due to low toxicity for mammals and relatively low concentrations of working solutions. It is known that resistance for some herbicides, including imidazolinones, is associated with acetolactate synthase gene (*ALS*), also known as acetohydroxyacid synthase (AHAS). The gene is involved in the biosynthesis of branched-chain amino acids. ALS-mediated herbicide resistance, which usually based on single amino acid substitution, is common for different plants [2, 3]. For *B. napus* and *A. thaliana* and "resistant" alleles also were revealed from natural populations [4] or obtained due to mutagenesis [5]. Thus, for *ALS* gene of brassica plants, a region near the C-terminus plays the key role. It was confirmed by introducing different amino acid substitutions in this region using base-editing approach in *Arabidopsis ALS* gene [6]. It seems that the mutated region is more important than exact amino acid change.

In the present work we are using different CRISPR/Cas9 in order to introduce different mutations in *ALS* gene of *B. napus*, *B. oleracea* and *Camelina sativa* — important *Brassicaceae* crops. On the one hand, we are applying a pHSE901 vector for base editing, which contains a cytidine deaminase. On the other hand, if exact substitution is not important, "classic" CRISPR/Cas9, which makes double-stranded breaks in target region, can be applied. This approach has much higher editing efficiency, however usually produce indel mutations. During regeneration from calli after agrobacteria-mediated transformation transgenic plants are selected by antibiotics in the *in vitro* conditions. Adding of herbicides in cultivation media will allow to select cells, which possess only necessary mutation — that provide resistance and don't affect viability.

ALS gene has another interesting feature — it is also a popular gene for elaboration of genome editing protocols for different plants [7]. Agrobacteria-mediated transformation of brassica crops, especially *B. oleracea*, strongly depends on genotype. Therefore, we are using *ALS* gene also in order to develop efficient editing protocols for lines, relevant in current breeding.

Funding source. The work was supported by the Russian Ministry of Science and Higher Education, project No. 075-15-2023-582.

Keywords: CRISPR-Cas9; *Brassica*; agrobacterium transformation; herbicide; ALS.

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DOI: https://doi.org/10.17816/ecogen2024

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DOI: https://doi.org/10.17816/ecogen2024

Evolutionary fate of natural transgenes in plants

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Agrobacterium (*Rhizobium*) — mediated transformation is the primary method for obtaining commercially viable lines of genetically modified (GM) plants. This technique utilizes a natural vector system whereby certain species of *Agrobacteria* and *Rhizobium* possess plasmids, a fragment of which (T-DNA) can be transferred to plants and integrated into their chromosomes. As a result of T-DNA gene expression, transgenic cells divide and form transgenic tissues within a normal plant [1]. In rare cases, whole plants can naturally regenerate from transgenic cells, similar to what occurs in GM plant laboratories. These naturally transformed plants are known as naturally transgenic plants or natural GMOs. Current estimates suggest that approximately 7 percent of dicotyledonous plants contain traces of Agrobacterium-mediated transformation in their genomes [2, 3]. These plants can serve as a convenient model for studying the long-term environmental risks associated with cultivating man-made GMOs.

Through the analysis of genomic data from numerous natural GMO species, several scenarios of the evolutionary consequences of transformation can be identified. These scenarios include the loss of transgene function [4], which may result in the complete elimination of the previously introduced T-DNA sequence, the preservation of intact expressed sequences of transgenes that have retained their original functions [5], and the evolution of sequences according to eukaryotic scenarios [2]. The presentation will discuss examples that illustrate each of these scenarios.

Funding source. The work was performed using the equipment from the "Development of Molecular and Cellular Technologies' Resource center of St. Petersburg State University. This work was supported by the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement No. 075-15-2022-322 dated 2022 April 22 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 for the Future".

Keywords: naturally transgenic plants; cT-DNA; transgenes.

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Approaches to the analysis of genetic modified plants transformed by antimicrobial peptides genes

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The search for efficient and environmentally friendly alternatives to chemical pesticides is a subject of interest, particularly the natural immunity mechanisms in plants, such as antimicrobial peptides (AMPs). Previously, several transgenic crop lines have been introduced with alien plant *AMP* genes, demonstrating an increased resistance to economically significant bacterial and fungal phytopathogens. It is believed that such resistance improvements are due to the antimicrobial (antifungal) effects of the synthesized recombinant peptides.

In practice, most studies primarily establish a positive correlation between the enhanced resistance of transgenic plants to phytopathogens and the elevated level of *AMP* genes' transcriptional activity.

The current assessment seems inadequate for understanding the cause of resistance acquired by such transgenic plants. For instance, in some cases where *AMP* genes were introduced, an increased resistance of transgenic plants was achieved without the accumulation of translational products (of the peptide itself) in tissues and organs. This is likely due to the up-regulation of host resistance-associated genes caused by the alien gene.

AMP gene expression products are usually detected via Western blotting, as well as through the targeted extraction of a recombinant peptide from plant tissue, followed by its analytical identification (using methods such as mass spectrometry and N-terminal sequencing). There are a few successful examples describing identification of AMPs in transgenic plants: for instance, a 5-kDa-defensin from chili pepper (*Capsicum annuum*) was confirmed by Western-blot analysis in a protein-peptide extract obtained from transgenic tomato plants [1]. Another rare example of fully isolation and structure validation is thaumatin-like protein from maize (*Zea mays*) which has been expressed in different heterologous systems (*Arabidopsis thaliana*, insect cells and tomato plants). There has been provided a full structure analysis including mass spectrometry and protein sequencing [2]. Not only is the latter method preferable for quantifying peptides, but it is also necessary for gaining insight into the mechanisms of AMPs post-translational modifications in foreign organisms. This is due to the complicated processing of AMP precursor proteins. Genes coding for such precursors are often used in vectors designed for subsequent plant transformation.

To confirm the efficiency of using *AMP* genes in creating plants resistant to pathogens, it is essential to establish a positive correlation between three factors: the level of gene transcriptional activity, the target peptide content in tissues and organs, and the rate of plant resistance to phytopathogens. Only these three factors can decidedly verify the antimicrobial activity of heterologous AMPs as the cause of resistance acquired (or not) by the transgenic plant. These are important in estimating the prospects of its practical application in the future.

Funding source. This study was supported by the Russian Science Foundation (grant No. 19-76-30005-P).

Keywords: transgenic plants; antimicrobial peptides; gene expression; phytopathogen resistance.

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DOI: https://doi.org/10.17816/ecogen2024

Biological activity of cucumopine-like compounds of *Arachis hypogaea* L.

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Arachis hypogaea L., commonly known as cultivated peanut, is a widely cultivated agricultural crop and a natural genetically modified organism (GMO) [1]. Its genome contains genes that are homologous to the agrobacterial cucumopin synthase [2]. Currently, the biological activity of these cucumopine-like compounds remains unexplored, although the related genes are expressed. Our objective is to investigate the effect of products derived from homologous cucumopine synthase genes expressed in *A. hypogaea* on the growth of microorganisms.

The study utilized pure cultures of Ascomycetes: *Candida tropicalis* (Bain.) Berkh., *Candida albicans* (DC.) Harrison, *Scheffersomyces shehatae* (Kurtzman & Robnett) Barbosa, Ge & Sampaio, *Scheffersomyces cryptocercus* (Yamada, Ito, Abe & Tak.) Kurtzman, *Pachysolen tannophilus* (Dell, Krog. & Klop.) Kurtzman & Robnett, *Ogataea methanolica* (van der Walt & Keller) Kurtzman & Boekhout. Additionally, *Saccharomyces cerevisiae* (Bull.) Krieger strains from the Peterhof Genetic Collection of Yeast Strains were included. Cultures commonly used in biopreparations for plant protection were also analyzed: *Trichoderma viride* Pers., *Trichoderma harzianum* Rifai., and *Bacillus subtilis* (Ehrenb.) Cohn, strain B-10 from the collection of the All-Russian Research Institute of Plant Protection.

An extract enriched with cucumopine-like compounds was sourced from a transgenic *Escherichia coli* strain containing *cus*-like gene found in the peanut genome [3], was added to the LB medium. The same nutrient medium without opine served as the control. The growth rate of the strains at 27 °C on both control and experimental media was measured using a FlexA-200 spectrophotometer (ALLSHENG, China) at a wavelength of 630 nm. The experiment was conducted in quadruplicate.

The addition of cucumopine-like compounds to the cultural medium showed the most significant growth-stimulating effect on *Pachysolen tannophilus* and *Bacillus subtilis* strain B-10. These organisms may serve as models for studying the metabolism of opines and their biological roles. The obtained data indicate a specific influence of cucumopine-like compounds on the growth of certain microorganisms. Further research will focus on clarifying the role of opines in the interaction between plants and the microbial community, as well as the development of new biotechnological applications in the fields of agriculture and microbiology.

Keywords: Natural GMO; *Arachis hypogaea*; cucumopine-like compounds; microbial growth stimulation.

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DOI: https://doi.org/10.17816/ecogen2024

Hyperparasitic properties of entomopathogenic fungi towards rust pathogens

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Entomopathogenic fungi (EPF) of the genera *Lecanicillium*, *Akanthomyces* and closely related species can parasitize on rust and powdery mildew pathogens in nature in the absence of a suitable insect host. EPF isolated from phytopathogenic fungi exhibit high activity not only towards arthropods, but also towards phytopathogens [1, 2]. Such EPF are of interest as producers of biopreparations with a complex activity. The efficiency of rust suppression by entomopathogenic isolates was studied with the model phytopathogen *Puccinia recondita*, the causative agent of brown rust. The technique developed for assessing the resistance of wheat varieties [3] with modification for EPF was appplied. Segments of wheat leaves sprouts were sprayed by a suspension of *P. recondita* urediniospores (3–5 thousand spores/ml), and after 1 hour by a suspension of spores of EPF with 5×10^6 spores/ml. On the 7th day after treatment, it was found that all the studied ten isolates were able to suppress the development of rust pustules. On the 10th day the number of normal pustules significantly decreased, and an abundant growth of EPF mycelium was observed above the pustules. The efficiency of the most active isolates was 94–100%. EPF isolates also suppressed rust development in experiments on wheat plants, but their efficiency was lower than on isolated leaves and reached 36–70%. A more detailed study of the process of hyperparasitism of EPF on rust fungi was fulfilled using the Vl 72 and Vl 61 strains of the *Akanthomyces muscarius*, transformed and labeled with a fluorescent protein (GFP) [4]. For this purpose, mature *P. recondita* pustules were infected by EPF. Wheat sprout segments collected every day after infection, were fixed in Carnoy's solution and examined using an AxioImager M1 fluorescence microscope. On the 1st day after *A. muscarius* infection the fungus formed growing tubes and appressoria, on the 2nd day hyphae grew around the pustules and on their surface. On the 3rd day changes of the color of the pustules, thickening of the hyphae of *A. muscarius* and growth at the attachment sites, penetration inside and further growth on the inner walls of the urediniospores were observed. On the 4th day thinning of the urediniospore membranes and their rupture were detected. Subsequently, a mass of spores and growing mycelium around the pustules were observed. EPF (Vl 72 and Vl 61 strains) were tested as a wettable powder, containing the blastospores on the roses grown in the Botanical Garden of Komarov Botanical Institute (St. Petersburg). EPF actively suppressed the development of the rust pathogen *Phragmidium* sp. at a concentration of 1×10^8 spores/ml, the efficiency of *A. muscarius* isolates was 60%.

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

Keywords: hyperparasitism; *Akanthomyces muscarius*; rust fungi; GFP; biocontrol.

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DOI: https://doi.org/10.17816/ecogen2024

Genetic engineering for the study of acaricide / insecticide resistance

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The use of chemical protection agents is currently the primary method for controlling the population of arthropod pests in agricultural crops. However, the intensive use of insecticides is associated with the development of resistance to these agents over prolonged periods. Understanding the mechanisms of resistance development, as well as timely identification of resistant arthropod populations, can form the basis for developing effective pest control strategies in agriculture.

One of the most important mechanisms of insecticide resistance development is target site insensitivity, where single nucleotide polymorphisms (SNPs) can lead to changes in the amino acid sequence of the translated protein, resulting in reduced insecticide efficacy or complete lack of susceptibility.

Currently, there is insufficient data on the contribution of individual genes/alleles to the mechanism of arthropod resistance to chemical agents. Nevertheless, significant progress has been made in recent years due to advancements in bioengineering technologies.

The CRISPR-Cas9 genome editing technology allows for targeted gene modifications in insects to study their functions. Using CRISPR-Cas9, mutations can be created in genes associated with resistance to examine their impact on insecticide susceptibility. For example, this technology was employed to study the involvement of the Nicotinic Acetylcholine Receptors *(nAChRs)* gene in the resistance of the fruit fly *Drosophila melanogaster* to the insecticide Spinosad [1].

Genetic engineering methods are also applied in the creation of diagnostic systems that identify alleles leading to pesticide resistance [2]. An example of such work is diagnostic system we created for identifying *cytochrome b* alleles responsible for bifenazate resistance in spider mites *Tetranychus urticae*. The diagnostic system for detecting the G132A mutation consists of several components: allele-specific oligonucleotides, positive and negative controls. A plasmid containing a fragment of spider mite DNA with the G132A single nucleotide substitution, assembled through site-directed mutagenesis, was used as a positive control.

Keywords: genetic engineering; site-directed mutagenesis; insecticide resistance; target site mutation; resistance management; bifenazate; *Tetranychus urticae.*

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DOI: https://doi.org/10.17816/ecogen2024

In planta genome editing with viral vectors

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Virus-induced genome editing (VIGE) is a new technique of CRISPR/Cas delivery to plant cells [1–3]. Transient expression from a viral vector allows to produce transgene-free plants. However, most of the plant viruses are not transmitted by seeds. This poses a major challenge in editing of crops, as inoculated tissues must be regenerated into plants using *in vitro* culture.

Transformation of *Arabidopsis* usually occurs *in planta*, and the possibility of using viral vectors to perform floral dip transformation is poorly studied. We created a vector to perform knock-in of a *bar* gene in the genome of *A. thaliana*. The target gene with a promoter and terminator was cloned to C3003 module via Gibson assembly and combined with A0508 and B2103 modules in pTRANS_231 transformation backbone, containing BeYDV viral replicon. All vectors were a gift from Daniel Voytas [3]. The resulting vector was introduced to *A. tumefaciens* and used for floral dip transformation of *Arabidopsis*. The seeds were collected, and the seedlings from these seeds were sprayed with Basta herbicide.

Survival rate of the seedlings was 5 times lower than after transformation with a similar knock-out genetic construct without viral replicon. However, we successfully obtained plants, PCR-positive for *bar* gene. Unfortunately, integration of the gene occurred not in the protospacer region, targeted by gRNA, but in a random place of the genome. This was probably due to the absence of homology arms in the construct. Our results indicate that it is possible to produce edited seeds using viral vectors.

Funding source. The research was supported by a grant from the Russian Science Foundation No. 24-76-10065, https://rscf.ru/project/24-76-10065/

Keywords: *Arabidopsis thaliana*; genome editing; knock-in; BeYDV.

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DOI: https://doi.org/10.17816/ecogen2024

Optimization of methods for increasing the productivity of plant cell cultures producing recombinant proteins

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Bioproduction of recombinant pharmaceuticals in plant expression systems is considered a promising alternative to existing platforms based on mammalian or bacterial cells. Cultivation of plant cells in controlled bioreactor conditions ensures the production of high-quality proteins, and rapid cell growth, low cost of nutrient media components and the complete absence of the risk of contamination by pathogens of animal origin are their great advantages. Despite the success of using plant cell cultures for the commercial production of pharmaceutically valuable proteins, there are still many unsolved problems in this area, the most important of which is the insufficiently high yield of recombinant protein. We believe that site-specific integration of target genes into a region of the genome that is characterized by a consistently high level of expression can solve the problem of low levels of expression of recombinant proteins.

To find the most successful regions of the genome, we used several approaches — theoretical and practical. A number of regions of the genome carrying housekeeping genes and characterized by consistently high levels of expression were analyzed. A series of knock-ins carrying the insertion of the target gene into the region of the histone H3.3 gene of *Arabidopsis thaliana* was obtained; the resulting cell lines yielded recombinant protein up to 2% of the ORB. A number of transgenic plants were also obtained and analyzed, carrying an insertion of the target gene in a random region of the genome and not having phenotypic mutations. One of the obtained transgenic lines carried an insertion in the 3' untranslated region of the AT4G39600 gene and gave a stable yield of recombinant protein of 16% of the ORB, both in whole plants and in cell culture.

In the course of our work, we also experimentally selected the optimal composition of the construct for successful site-specific integration of target genes. The key elements of a successful design are DNA regions homologous to the intended insertion site and additional recognition sites for the Cas9 endonuclease. Different methods of delivery of constructs for knockin were also tested. Biolistic delivery showed the highest efficiency, however, difficulties in obtaining monoclonal lines of suspension plant cells may offset this advantage. Moreover, cell cultures induced from *Arabidopsis* leaves are often characterized by high aggregation, which negatively affects cell productivity. We hypothesized that mutations in the GAUT family of genes may result in changes in intercellular adhesion and the formation of smaller aggregates compared to wild-type cultures. Using the CRISPR/Cas9 system, we knocked out the GAUT1 gene encoding the protein homogalacturonan α1,4-galacturonosyltransferase 1, which is the enzyme responsible for the synthesis of pectins. However, the resulting cell culture was characterized by an increased number of large aggregates, compared with the control line, and a reduced yield of the target recombinant protein. Thus, to reduce cell aggregation in culture it is necessary to look for other targets.

Funding source. Work on genomic editing of GAUT1 was carried out with the financial support of the Ministry of Science and Higher Education, grant No. FWNR-2022-0022.

Keywords: *Arabidopsis*; gene editing; knock-in; plant cell culture; recombinant proteins.

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DOI: https://doi.org/10.17816/ecogen2024

The search for novel potential participants of systemic control of nodulation in *Medicago truncatula*

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The symbiosis between *Fabaceae* family plants and rhizobia, a group of nitrogen-fixing bacteria, is an exceptional phenomenon that allows plants to absorb atmospheric nitrogen due to endogenous microorganisms and results in the formation of lateral organs — nodules — from the cells of an irregular meristem. Since this symbiotic interaction requires energy expence, plants have a system of autoregulation of nodule formation (AON) that can limit the number of rhizobia infections and nodule numbers through the systemic signal pathway the main role in which belongs to the regulatory peptides of the CLE and CEP families.

CLE peptides are negative regulators of nodule formation, acting by a negative feedback mechanism. Among them, MtCLE12, MtCLE13 and MtCLE35 have been characterized previously [1]. In *Medicago truncatula* with overexpression of the corresponding genes, nodule formation is suppressed. Along with these genes, the expression of some other *CLE* genes, in particular the *MtCLE29* gene, is also upregulated during the development of symbiotic nodules [2]. According to the amino acid sequence, the MtCLE29 peptide is similar to other known CLE peptides — regulators of the development of symbiotic nodules. However, MtCLE29 function remains unknown. To identify the role of this protein in the systemic regulation of nodule formation, we obtained constructs for overexpression of the *MtCLE29* gene under the constitutive promoter of the 35S Cauliflower mosaic virus (CaMV).

On the contrary, CEP peptides are positive regulators of nodule formation. For example, treatment of *M. truncatula* roots with the MtCEP7 peptide has been shown to increase the number of nodules [3]. However, the mechanism of action of CEP peptides in the development of symbiotic nodules remains unexplored. To study the function of MtCEP7 in more detail, we created a plasmid vector with the insertion of the *MtCEP7* coding sequence under a constitutive promoter (35S:: *MtCEP7*).

Using the obtained genetic constructs, we will study the functions and mechanisms of action of MtCLE29 and MtCEP7 peptides, as well as the possible interaction between peptides of the CLE and CEP families in the control of the development of symbiotic nodules in *M. truncatula*.

Funding source. This work was supported by the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement according to contract No. 075-15-2022-322 date 2022 April 22 on providing a grant in the form of subsidies from the Federal Budget of the Russian Federation. The grant was provided for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future".

Keywords: symbiotic nodule, systemic control, regulatory peptides, CLE.

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DOI: https://doi.org/10.17816/ecogen2024

Prospects of GMO application in modern agriculture

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In modern crop production, the requirements for the timely creation of new approaches to plant protection and soil recultivation are determined by such factors as the rapid spread of pests and phytopathogenic microorganisms, their development of resistance to pesticides and antibiotics, and soil pollution with toxicants. Progress has been made in research aimed at increasing the effectiveness of biopesticides (insecticidal proteins of *Bacillus thuringiensis* have been transferred to other bacteria, the range of controlled pests has been expanded, the stress resistance of the producer strain has been increased, etc.) [1], soil bioremediation (GM microorganisms are used to convert toxic heavy metals — Cd, Hg, Ni, Cu, As, and Fe) [2], as well as reducing the harmfulness of phytopathogens (*Pseudomonas syringae, Xylella fastidiosa*) and displacing pathogenic variants by them [1].

Bacteriophages are of great interest as agents for controlling bacterial pathogens. Modern methods of synthetic biology allow not only to modify the phage genome but also to create completely artificial phages. Successes have been achieved in changing the specificity of phages, overcoming bacterial defense systems, and introducing genes encoding proteins toxic to bacteria. Using engineered phages (including exogenous dispersin B and broad-spectrum endolysins), the efficiency of bacterial biofilm destruction has been increased. Introduction of genes encoding depolymerase into the phage genome helps to destroy the polysaccharide capsule of *Erwinia amylovora* strains and facilitates phage penetration into the cell [3, 4]. Considering the number of sequenced microorganisms and the achievements of genetic engineering, the prospects for using GM microorganisms for plant-growing purposes are constantly expanding.

Keywords: GM microorganisms; plant protection; bacteriophages.

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The *Camelina sativa* agrobacterium-mediated transformation

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Camelina sativa (L.), commonly known as gold-of-pleasure, has experienced renewed interest in recent years as an oilseed crop of the Cruciferous family. Its center of origin is located in southeastern Russia. It adaptable to various climates and soil types, often grown in rotation with other crops.

The oil derived from *C. sativa* is utilized in various industrial applications, including biofuel production, varnishes, paints, and as animal feed. Additionally, gold-of-pleasure has a valuable source of Omega-6 and Omega-9, linolenic fatty acids, and beta-carotene, making it highly applicable in the food industry. In terms of composition and nutritional value *C. sativa* (L.) oil is comparable to cedar and flaxseed oil, however, it is more cost-effective and retains its properties longer. Notably, gold-of-pleasure oil is a leader in vitamin E content.

Currently, there is active development of new *C. sativa* varieties worldwide. Scientists are using the CRISPR-Cas9 genome editing system to improve the gold-of-pleasure. They are focused on enhancing its oil quality, increasing seed yield, and developing herbicide-resistant varieties. Effective plant transformation is essential for delivering the components of the editing system. In this study, we utilized camelina cultivars Omich, Isilkulets, and Crystal (generously provided by L.A. Gorlova, VNIIMK, Krasnodar) to optimize transformation protocol. We compared two distinct approaches: the in planta floral dip transformation and the in vitro callus-mediated transformation.

Keywords: CRISPR-Cas9; *Camelina sativa* (L.); agrobacterium transformation.

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DOI: https://doi.org/10.17816/ecogen2024

Development of in planta model system for evaluation interactions between viral protein VPg and plant eIF4E factors

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Potato virus Y (PVY) is among the most pernicious plant pathogens, which affects a broad spectrum of economically significant crops within the *Solanaceae* family, such as tobacco, potato, tomato, and pepper. PVY exploits a host's translation initiation factor eIF4E via a protein VPg to initiate the viral proteins synthesis. PVY resistance can be achieved if eIF4E is unable to interact with VPg.

It has been shown that even single substitution in the eIF4E structure is sufficient to stop the promotion of a viral infection. Also, some "resistant" eIF4E alleles are known for different plant. However, introduction of single mutations into the plant genome is associated with many difficulties, therefore most experiments on the interaction between viral VPg and mutant eIF4E have been performed in yeast systems. The obtained results have to be verified in plants. In order to examine resilience of artificial eIF4E *in planta* a convenient model is needed.

Nicotiana tabacum L. possesses a multigene family of translation initiation factor genes, comprising eight eIF4E variants. Previously was demonstrated that VPg interacts mainly with two of these factors: eIF4E-1 and eIF(iso)4E. Knockout of these eIF4Es can increase the resistance to PVY.

In our study, we engineered two genetic constructs containing distinct sgRNAs to induce InDel mutations in both genes encoding these translation initiation factors in tobacco plants. These constructs were delivered into the plants via *Agrobacterium*-mediated transformation. Subsequently, we selected tobacco lines with targeted eIF4E knockouts.

Next step is to evaluate PVY resistance of knockouted tobacco lines and use them for overexpression of artificial eIF4E factors.

Funding source. This work is supported by State Task No. 0431-2022-0004.

Keywords: CRISPR-Cas9; *Nicotiana tabacum*; resistant to PVY; VPg.

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DOI: https://doi.org/10.17816/ecogen2024

Investigation of the biological activity of octopine on various species of Ascomycetes

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The appearance of naturally transgenic plants (natural GMOs) is associated with the integration and preservation in the nuclear genome of T-DNA sequences obtained from phytopathogenic agrobacteria, such horizontally transferred sequences are called cellular T-DNA (cT-DNA) [1]. Many of these natural transgenes remain intact and have the potential to be expressed, such as the homologues of the octopine/vitopine synthase genes, for example. However, the evolutionary role of these genes in naturally transgenic plants remains unknown [2]. Currently, there is a widespread hypothesis, which suggests that the products of these genes, octopine and vitopine, may act as attractants for symbiotic or "beneficial" microorganisms in the rhizosphere of nGMOs [3]. Fungi can be one of the groups of such microorganisms, moreover, genes related to the metabolism of opines and octopine in particular have been found in many species of Ascomycetes [2].

Thus, we have conducted several experiments to investigate the biological activity of octopine on various species of fungi that are associated with plants. One of our assumptions was that octopine could be a source of organic nitrogen. However, the data obtained during the cultivation of arginine auxotrophic yeast strains showed that octopine cannot be used by them as a source of arginine. Next, we conducted experiments to study the growth rate of seven yeast-like fungi and five mycelial fungi on solid nutrient media containing octopine. It was found that significant differences in growth rate between experience and control were found only in two species. It has been shown that octopine inhibits the growth of *Scheffersomyces shehatae* (H.R. Buckley & van Uden) Urbina & M. Blackw., and it is also able to accelerate the growth of *Colletotrichum* sp. Corda, in which a sequence similar to octopine synthase has been found. Thus, it can be assumed that naturally transgenic plants synthesizing octopine are able to influence the growth of these species of fungi, thereby changing the species composition of microorganisms of the rhizosphere and phyllosphere.

Further investigation of the biological activity of octopine will allow us to accumulate data that will help verify the hypothesis about the effect of horizontally transferred opine synthase genes in nGMOs on the species composition of microorganisms in the phytomicrobiome.

Funding source. The work was supported by the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement No. 075-15-2022-322 dated 2022 April 04 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". The work was performed using the equipment of the Resource Center of St. Petersburg State University "Development of Molecular and Cellular Technologies".

Keywords: biological activity of octopine; octopine/vitopine synthase; nGMO; horizontal gene transfer.

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DOI: https://doi.org/10.17816/ecogen2024

Creation of a DNA construct for CRISPR/Cas editing of the *trolC* gene of *Nicotiana tabacum* L.

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The species *Nicotiana tabacum* L. belongs to the group of naturally transgenic plants, as homologues of the *plast* genes from agrobacteria — *trolA, B* and *C* — have been found in its genome. The plant homologue of the rolC oncogene is not only present in the genome as part of cT-DNA (cellular T-DNA), but is also expressed in young tissues and probably contributes to improved stress tolerance. It is known that TA is the only one of the three *N. tabacum* cT-DNAs that contains a homologue of *rolC*. It is organised as imperfect inverted repeat, containing two copies of the *trolC* gene. However, the molecular functions of this gene remain largely unknown. To date, technologies such as RNA interference induction and gene knockout using the CRISPR/Cas9 system have been used to study gene functions. Previously, we created transgenic tobacco plants with reduced expression of *trolC* by induction of RNA silencing [1–3]. The aim of this study was to create a genetic construct for knockout of the *trolC* gene using the CRISPR/Cas9 system. For this purpose, *trolC* gene sequences (FN667969.1, X91881.1, AYMY01228844.1, KJ599826.1, AF281249.1) were searched in GenBank and aligned using "MEGA11". The sequence with the most conserved sites was loaded into the CRISPOR RNA guide RNA selection system. The 501 bp long input sequence contained 72 potential guide sequences, each of which was then checked against the RNAfold WebServer site to select the most optimal gRNA structure for interaction with the Cas protein. Four gRNA sequences of gRNAs were selected, which were introduced into the plasmid pMOD_B2103 using the Golden Gate reaction, and cloned in the strain XL1-blue of *Escherichia coli*. To verify the correctness of the construct assembly, DNA was isolated from single colonies, PCR-analysed, and sequenced. DNA containing the target genetic constructs was inserted into the pTRANS_230 plasmid using the pMOD_A0503 and pMOD_C3003 modules. The modular construct was further cloned in *E. coli* strain DH10, followed by single colony sampling, PCR and sequencing. The created target DNA construct was used to transform cells of *Agrobacterium rhizogenes* strain A4 and *Agrobacterium tumefaciens* strain AGL by electroporation to further creation of hairy roots and transgenic tobacco plants with knockout of the *trolC* gene.

Keywords: CRISPR/Cas; *trolC*; *Nicotiana tabacum*; genome editing.

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DOI: https://doi.org/10.17816/ecogen2024

Obtaining and evaluation of protein content in hairy roots and callus culture of *Glycine max* varieties Okskaya and Svetlaya

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Economically, soybean (*Glycine max*) is one of the most important crops, as well as the nutritional and energy standpoint. The tendency to increase the cultivation and consumption of this crop is not accidental, because soybeans contains a large amount of protein, providing consumers with essential amino acids [1]. Due to its high oil content, soya is also an excellent raw material for biofuel production. At the same time, soya has a beneficial effect on the cardiovascular system and lipid metabolism of human [2].

The long period of soybean cultivation in usual way, the influence of biotic and abiotic environmental factors create significant difficulties for study of soybean and its biochemical characteristics. Cell and tissue culture methods, as well as the production of *G. max hairy roots* through transformation by bacterium *Rhizobium rhizogenes* have great potential for the study of processes occurring in root and aboveground parts of the plant, and can be used as a tool for studying protein and its synthesis. In addition, with development of biotechnology, it has become possible to improve the characteristics of soybean, including the reduction of allergenic and toxic proteins.

Hairy roots of *G. max* were obtained using the bacterium *Rh. rhizogenes* strain A-4. It was shown that despite the presence of *rol*-genes in *hairy roots* cultures, their biomass increase was insignificant. It was 2,5 g for soybean variety Okskaya and 0,6 g for soybean variety Svetlaya. Protein content in *hairy roots* of *G. max* of Svetlaya and Okskaya varieties varied from 0,36 mg/g to 1,37 mg/g and from 0,23 mg/g to 1,7 mg/g, respectively. Callus cultures of soybean of two varieties were inducted on medium with different concentrations of hormones. The best medium for induction and cultivation were MS3 (2 mg/l BAP + 0,2 mg/l NAA) for callus of Svetlaya variety and MS4 (1 mg/l BAP + 0,1 mg/l NAA) for callus of Okskaya variety. The maximum biomass growth of callus of soybean varieties Okskaya and Svetlaya was 14,8 g on MS3 medium and 7,11 g on MS4 medium, respectively, which is 6 and 12 times higher than the growth parameters of *hairy roots* (2,5 g and 0,6 g). The maximum protein content in callus of soybean varieties Svetlaya and Okskaya was found to be 8,48 mg/g on MS4 medium and 11,7 mg/g on MS2 (0,1 mg/l kinetin + 3 mg/l 2,4-D) medium, respectively, which is 5–8,5 times higher than in *hairy roots* of these varieties (1,7 mg/g and 1,37 mg/g). Moreover, the protein content in callus is independent of the cultivar *G. max*, but correlates only with cultivation medium.

Keywords: soybean; hairy roots; callus; protein content.

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DOI: https://doi.org/10.17816/ecogen2024

The use of genetically modified yeast strains of *Saccharomyces cerevisiae* for modeling human diseases associated with altered expression of AID/APOBEC cytosine deaminase genes

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Cytosine deaminases of the AID/APOBEC family perform diverse functions in vertebrate cells, including regulation of cholesterol transport, cardiac and skeletal muscle development, retinal and optic nerve regeneration, innate immunity, and defense against retroviruses and retrotransposons. The deaminase AID initiates antibody isotype switching, immunoglobulin gene conversion, and somatic hypermutation. During these processes, AID/APOBEC enzymes catalyze the cytosine deamination in target DNA or RNA, leading to either changes in genetic information or degradation of the nucleic acid. Dysregulation of AID/APOBEC activity harms humans and animals. The untargeted deamination may result in undesirable mutations in tumor suppressor genes or proto-oncogenes, reduced infection resistance, or immunodeficiency. Thus, identifying factors that regulate the activity of AID/APOBEC cytosine deaminases is of significant relevance. To address this issue, we developed and implemented an approach utilizing genetically modified *Saccharomyces cerevisiae* yeast strains expressing human cytosine deaminase genes. Yeast lacks its own cytosine deaminases; however, when vertebrate AID/APOBEC genes are expressed in yeast cells, an active protein is produced that non-specifically deaminates cytosine in the genomic DNA of these microorganisms. This results in an increased frequency of mutagenesis, which can be quantitatively and precisely measured. We compared mutagenesis frequencies in strains expressing deaminase genes under various genetic and environmental conditions. We successfully generated *S. cerevisiae* strains harboring the human *APOBEC3A*, *APOBEC3B*, and *AID* genes, as well as the sea lamprey *Petromyzon marinus PmCDA* gene, recognized for its high activity as a positive control. We studied the effects of individual amino acid residue substitutions within the protein, disruption of repair systems, and exposure to external physical and chemical agents. We identified specific amino acid substitutions that enhance or diminish the enzyme's activity and demonstrated that recombination repair and base excision repair significantly influence the mutagenic activity of cytosine deaminases in our heterologous expression system.

These results validate the effectiveness of the developed model and pave the way for further exploration of factors that may modify AID/APOBEC activity, which is crucial for advancing therapeutic and diagnostic strategies against oncological and infectious diseases.

Acknowledgments. The authors acknowledge the support from the Research Park, St. Petersburg State University.
Funding source. The research was funded by the Russian Science Foundation, grant No. 20-15-00081.

Keywords: AID/APOBEC deaminases; yeast; mutations.

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DOI: https://doi.org/10.17816/ecogen2024

Formation of nodule-like structures in the transformed root culture of *Artemisia annua* L. *in vitro*

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In the literature, the formation of nodules is noted in a number of representatives of the *Fabaceae* and *Rosacae* [1, 2], but is not represented in *Asteraceae*. It was possible to obtain a transformed root culture of *Artemisia annua* (annual wormwood) that forms nodule-like structures, when co-cultivated in vitro with a wild type strain of the phytopathogenic bacterium *Rhizobium rhizogenes*. Stable induction of nodule-like structures on the transformed roots of annual wormwood in vitro may be a convenient model for experimental confirmation of the concept of symbiogenome formation. Transformed root culture, or hairy roots, is obtained from nodular explants of sterile *A. annua* seedlings. The formation of nodule-like structures *in vitro* was induced by a complex of stress factors: high doses of antibiotics and long-term cultivation of the root culture on a depleted nutrient medium in a refrigerator. Cytomorphological study of the culture revealed that the forming nodule-like structures belong to the deterministic type. Young nodules are light brown in color, angular and rounded, due to the development of cells of primordia. Mature nodules are dark brown, covered with a bark-like membrane. Mature nodules lack the meristem and histological structures found in known non-deterministic nodules of other families. On a liquid nutrient medium, nodule-like structures existed within one and a half to two months, localized at the tips of young roots and in the nodes of their branching in the form of dark "necklaces". On a solid medium, the roots grow with the formation of nodule-like structures almost over its entire surface in the form of tubercles. At first, they are light colored, but as they grow and age, they also darken. Histological sections of transformed roots with nodule-like structures revealed the presence of cells with bacteria and infectious filaments. This study could be the first step towards artificial symbiogenesis and clarifying the possible role of *rol*- and especially *vir*-genes of rhizobia in this process.

Keywords: transformation; *Artemisia annua*; *Rhizobium rhizogenes*; nodule-like structures.

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DOI: https://doi.org/10.17816/ecogen2024

Development of transgenic *Arabidopsis* with enhanced expression of class III peroxidase gene by floral dip method

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Class III peroxidases oxidizing a wide range of substrates by H_2O_2 [1]. They are represented by multigene families and perform multiple functions [2]; the role of individual genes is not studied enough. AtPRX39 peroxidase from *Arabidopsis* is highly homologous to TPX1 peroxidase from tomato and has functional sites for binding sinapyl alcohol [3], that allows suggesting its role in cell wall lignification and responses to stress but there are no data on the role of this enzyme in cell wall lignification *in vivo*. The study aimed at the development of transgenic Arabidopsis with enhanced expression of this peroxidase.

The coding fragment of *AtPrx39* gene was cloned in the direct orientation into the pMD19 T-vector (Takara Bio Inc., China) for transformation *Escherichia coli* XL1-Blue strain. Obtained plasmid DNA was isolated and sequenced and cloned into the pBI121 vector under the control of a CaMV35S promoter using BamHI and SacI restriction sites. It was transferred into *E. coli* strain DH5α. Bacterial clones were selected by PCR and restriction of plasmid DNA; the vector was transferred to *A. tumefaciens* strain GV3101. Selection was done on LB medium with the addition of rifampicin and kanamycin.

Arabidopsis Col-0 inflorescences were immersed for 5 min in a suspension of agrobacteria in the media (MS, sucrose, MES, Silwet L-77) with acetosyringone. Inoculated plants were until seeds ripened. The selection of seeds was carried out on 1/2 MS medium with of kanamycin. The transgenic status of Arabidopsis was confirmed using PCR for *nptII* genes and CaMV35S promoter. As a result, ten T3 plant lines were obtained for future studies of the role of *AtPrx39* gene in the lignification of cell walls in transgenic *Arabidopsis*.

Keywords: peroxidases; lignification; agrobacterium-mediated transformation.

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DOI: https://doi.org/10.17816/ecogen2024

A yeast-based assay to identify mutations that alter amyloid aggregation in mouse prion protein

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Amyloids are protein aggregates characterized by a fibrillar structure, with intermolecular cross-β-sheets. The accumulation of amyloid proteins in tissues is associated with several severe and socially impactful diseases including Alzheimer's disease and Parkinson's disease. A particularly concerning category of amyloids is infectious prions, which can be transmitted between organisms and cause fatal, incurable diseases. Examples of prion diseases include scrapie in sheep, kuru and Creutzfeldt-Jakob disease in humans.

The yeast model *Saccharomyces cerevisiae* has been employed for studying amyloids and prions. Research has shown that when producing amyloidogenic mammalian proteins in yeast, they form aggregates that, in most cases, are biophysically and biochemically similar to those found in diseased mammals [1, 2]. Although these heterologous aggregates typically do not exhibit toxicity to yeast cells, they also do not cause significant phenotypic changes.

Here we employed a yeast-based assay [3] to conduct large-scale screening for mutations that affect the amyloid aggregation of the mouse prion protein (PrP). Through this screening, we identified several mutations that effectively prevent PrP aggregation, along with novel mutations which may be associated with the onset of hereditary prion diseases.

Funding source. This work was supported by the St. Petersburg State University (project No. 95444727).

Keywords: PrP; prions; *Saccharomyces cerevisiae*; mutations.

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DOI: https://doi.org/10.17816/ecogen2024

Lichenization-associated genes originating from horizontal gene transfer and gene family expansion contributed to the evolution of algal-fungal symbiosis

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Lichen is the most well-known example of algal-fungal symbiosis, in which photobiont and mycobiont mutually coexist and exchange metabolic products. Recent studies suggest that the lichen-forming ability of fungi and algae is linked to several horizontal gene transfer (HGT) events, both between the symbionts themselves and from other organisms to them. Three candidate genes for HGT (encoding tellurite-resistance/dicarboxylate transporter, nitrilase/cyanide hydratase and oxidoreductase/retinol dehydrogenase) from the fungi into non-obligate lichen photobiont *Trebouxia decolorans* were found only in the genomes of symbiotic green algae [1]. An origin by HGT was identified for the two candidate genes, encoding the glycoside hydrolase 8 and glutathione S-transferase family proteins, which are present in most studied symbiotic *Trebouxiophyceae* species [2].

Lichen formation requires the establishment of a symbiotic interface and metabolic coordination between partner organisms [2, 3]. Gene expression analysis of *Trebouxia* sp. TZW2008 in monoculture and in co-culture with mycobiont *Usnea hakonensis* revealed changes in the expression of more than 3000 genes, including the GATA family transcription factors (GATA-TFs) — conserved proteins with a zinc-finger domain, involved in the regulation of light-induced processes, nitrogen and carbon metabolism [2]. Our results show that the sequences of some algal GATA-TFs share similarities with fungal GATA-TFs, which may be possible evidence of HGT followed by gene family expansion. *In silico*, *in vivo*, and *in vitro* studies of fungal and algal symbiosis-related genes will provide insight into the genetic nature of the processes underlying the formation, synchronization, and adaptation of partner organisms in lichen associations.

Keywords: symbiosis; lichen; algae; horizontal gene transfer; gene family expansion; evolution; GATA factors.

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DOI: https://doi.org/10.17816/ecogen2024

Biosafety of Russian transgenic potato: theory and experimental trials

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The importance of updating the regulatory framework in the field of genetic technologies and biosafety concerns about growing transgenic plants in Russia have necessitated the creation of a textbook titled "Biosafety of Russian transgenic potato: theory and experimental trials". Prepared for publication book summarized the scientific theoretical approach to assessing potential environmental risks and their experimental verification in domestic conditions. Separate chapters of the book examine a model for identifying and characterizing environmental risks of GM plants, intra- and interspecific transgene flow, insect resistance management strategies, negative impacts on non-target organisms, *etc*.

The book's originality consists of data on the experimental biosafety testing of three transgenic potato varieties of Russian selection, conducted in various agroclimatic zones of the Russian Federation under controlled conditions [1]. GM potato lines were obtained by transformation using *Agrobacterium tumefaciens* containing the pBt12 vector, the expression cassette of which included genes encoding resistance to the herbicide glyphosate and the Bt toxin *CryIIIa* (providing resistance to the Colorado potato beetle).

The book presents data from comprehensive 3-year field trials of the obtained Elizaveta, Lugovskoy and Nevsky potatoes transgenic lines, which showed that they largely retained all the characteristics of the original varieties in reproductions, demonstrated high (100%) resistance to the Colorado potato beetle and confirmed that genetic modification using transgenesis did not reveal any immediate or significant risks. We hope that textbook provides recommendations, rules and examples for theoretical and practical applications.

Funding source. The Ministry of Science and Higher Education of the Russian Federation in accordance with agreement No. 075-15-2022-318 date 2022 April 20. The grant was provided for state support for the creation and development of a world-class scientific center, "Agrotechnologies for the Future".

Keywords: biosafety; ecology risks assessment; Russian transgenic potato; field tests.

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DOI: https://doi.org/10.17816/ecogen2024

Genome-editing tools to increase plastome transformation frequency in higher plants

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Currently, genome-editing technologies are widely used in plant bioengineering, including the plastid genome modification [1]. In particular, we propose to use such tools to increase the frequency of plastid genome transformation by increasing the number of double-strand breaks in it. For this purpose, several genetic constructs have been created at the laboratory of plastid genome modification in higher plants at the Institute of Cytology and Genetics SB RAS. The first construct includes the gene encoding *Cas9* with the *Lch* signal of chloroplast localization and the *mCherry* gene as a fluorescent marker, combined in one reading frame. Using this construct, the nuclear transformants of tobacco (*Nicotiana tabacum* L. cv. Petit Havana) were obtained, in which the Lch-Cas9-mCherry fusion protein will be constitutively produced and delivered to plastids. These nuclear tobacco transformants will be used for subsequent transplastomic transformation with the help of two other genetic constructs. One of them contains the *gfp* gene as a target fluorescent marker (transgene), bordered by sequences homologous to the insertion site located between the isoleucine and alanine transport RNA genes in tobacco plastome. Another construct carries a guide RNA homologous to the transgene insertion region. The combined delivery of both genetic constructs into plastids will be carried out through bioballistics, as well as using carbon nanotubes functionalized with a positively charged polymer, such as polyethyleneimine or chitosan. This approach, we believe, will facilitate more efficient incorporation of target constructs into the plastome according to the principle of homologous recombination and a higher yield of transformants.

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

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

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DOI: https://doi.org/10.17816/ecogen2024