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Second International Conference "Genetically modified organisms: The History, Achievements, Social and Environmental Risks"

Saint Petersburg, Russia. December 6–8, 2022

Saint Petersburg State University

AGROTECHNOLOGY FOR THE FUTURE

НАУКА И УНИВЕРСИТЕТЫ
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2022 31 десятилетие науки и технологий

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Second International Conference "Genetically modified organisms. The history, achievements, social and environmental risks"

Second international conference "GMO: history, achievements, social and environmental risks" was held on December 6–8, 2022 in Saint Petersburg State University, Saint Petersburg, Russia in mixed format (full-time and remote participation).

The conference was attended by scientists from Russia (from Saint Petersburg to Vladivostok), China, USA, Germany. They discussed the history of the development and application of genetic engineering methods, promotion of their results in society. Of great interest was the section devoted to the application of genetic engineering methods in fundamental science aimed to study the plant growth and development. A number of reports aimed to study the fundamental problems of biology and medicine using genetically modified microorganisms and animals. Many reports reflected practical results that are of interest for agriculture and medicine. The whole section was devoted to environmental research related to genetic engineering, in particular biodiversity of "agrobacteria" and natural GMOs that arose without human intervention. Discussion of society's attitude towards GMOs concluded the conference, where the importance of training specialists in interdisciplinary areas, as well as closer interaction between biologists and lawyers for improving legislation in the field of GMOs was noted.

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

Prof. Tatiana V. Matveeva

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The fiscal and tax policies on the development of GMOs for agriculture in China: retrospect, status quo and prospect

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The authors study the fiscal and tax policies on GMOs for agriculture in China, mainly focusing on income tax and value added tax, in order to analyze how the polices implement China's GMO strategy in the field of agriculture, reflect the development history of GMOs for agriculture in China and give suggestions on perfection for future policies based on China's GMO strategy.

Firstly, the authors illustrate China's past and present strategy for GMO technology application. As a whole, China permits the technical research on GMOs for agriculture, promotes very cautiously the related industrialization, and attaches great importance to safety supervision.

Secondly, by examining the past and present fiscal and tax policies relating to the abovementioned GMO strategy, the basic features of China's policies are as follows: in terms of fiscal policy, the financial funds have been invested in GMO development and supervision in a bidirectional and balanced way; in terms of tax policy, there are some tax incentives, but the ones solely for GMO development and supervision are few.

Finally, the fiscal and tax policies are important and necessary tool to implement GMO strategy. China's future fiscal and tax policies need to be adapted to the future GMO strategy at issue.

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Chloroplast genomes and GMOs. History, features and perspectives on plastid transgenesis in plant biotechnology

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Chloroplasts provide life on our planet, by creating carbohydrates, amino acids, lipids and O_2 through the process of photosynthesis. Two billion years ago, a eukaryotic ancestor entered symbiosis with photosynthetic cyanobacterium, giving rise to chloroplasts. During evolution, chloroplasts transferred most protein-coding genes of free-living bacteria to the nucleus, but retained a semi-autonomous genetic status — their own genomes and transcription and translation machinery.

The genetics of plastids began in 1909, when Baur and Correns described the non-Mendelian, maternal inheritance of *Pelargonium* leaf color: green, white and variegated. The presence DNA in chloroplasts (chDNA) was prove in the 1960s, and later have been shown, that male chDNA in zygotes is specifically degraded, assuring maternal inheritance in most plants.

The plastid transformation was first described in the unicellular green alga *Chlamydomonas reinhardtii* [1]. This method of plant biotechnology is an alternative to traditional introduction of foreign DNA into the nucleus, and has a number of advantages: (a) the large copy number of chDNA offers high-level transgene expression; (b) site-specific integration of foreign genes reduces the number of transgenic lines to be evaluated; (c) the lack of gene silencing or position effects in chloroplast genomes; (d) the chloroplast employs a prokaryotic gene expression system, and allows the expression of polycistronic genes [2]. Thus, chloroplasts are becoming attractive hosts for the introduction of new agronomic traits, as well as for the biosynthesis of high-value pharmaceuticals, biomaterials and industrial enzymes.

Keywords: chloroplasts; plastid genetics; plastid transformation.

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Natural GMOs: a history of research

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"*Agrobacterium*" — mediated transformation underlies the production of most modern lines of transgenic plants. At the same time, in nature, plants are described that have been transformed by "*Agrobacterium*" without human intervention. They are called natural GMOs.

Such plants were first described by White in 1983 [1] within the genus *Nicotiana* L., and the phenomenon of horizontal gene transfer from "*Agrobacterium*" to plants was considered unique for representatives of this genus for a long time. Only in 2012, another genus of natural GMOs was found. It was *Linaria* Mill. In 2019, the list of species of natural GMOs was increased by an order of magnitude and is constantly updated until now [2].

Several stages can be identified in the history of natural GMO research: 1. Description of individual examples of natural GMOs. 2. Estimation of the frequency of the horizontal gene transfer from "*Agrobacterium*" to plants based on the analysis of NGS data. 3. Description of the diversity of cT-DNA in terms of composition and copy number [3]. 4. Studies of the functions of individual pGMO genes [4]. 5. Phylogenetic studies of natural GMOs [5].

During the analysis of cT-DNA of natural GMOs, it became clear that they differ in the composition and intactness of transgenes, which can be interpreted from the point of view of the lack of a common function in all cT-DNAs. Genes that were not previously found in known "*Agrobacterium*" strains, are identifiedIn cT-DNAs, indicating a greater biodiversity of "*Agrobacterium*" than previously thought [2]. Many cT-DNA genes are intact and expressed. For some it was possible to identify products [4]. All these discoveries lead us to understanding of the role of horizontal gene transfer from "*Agrobacterium*" to plants during their evolution.

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

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The development of approaches to create new symbiotic systems

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Plants interact with a wide range of soil microorganisms, while interactions with symbiotic microorganisms are the most important for them. Symbiosis with nitrogen-fixing nodule bacteria provides a significant advantage in the existence of plants in nitrogen-poor soils. Since only plants from the *Fabales* and *Rosales* (a single representative *Parasponia*) enter into symbiosis with nodule bacteria, an idea about expanding the number of plants entering into such interactions became popular. To solve the problem of constructing new symbiotic systems, it is necessary to provide recognition of the symbiont (by transferring receptor genes into non-legume plants), morphogenesis of the nodule, and its infection.

We have managed to introduce the genes encoding receptors to surface components of rhizobia into the non-legume plants, and it provided increased colonization by nodule bacteria. It may improve the growth and development of non-legume plants and ensure their greater resistance to phytopathogens. Genome analysis of non-legume plant hop *Humulus lupulus* showed the existence of genes involved in symbiosis regulation, but some important regulators such as gene encoding NIN transcription factor were lost. Using genetic engineering approaches, the hop plants carrying the gene encoding NIN transcription factor were created. Analysis of these plants may provide important information about regulation of organogenesis in non-legume plants.

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Systemic control of symbiotic nodulation in legume plants: genetic engineering in functional studies of key regulators

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Legumes are important suppliers of vegetable protein. In symbiotic interactions with soil bacteria rhizobia, legume plants form nitrogen-fixing nodules on their roots, where molecular nitrogen is fixed and incorporated into organic compounds. A host plant controls the number of symbiotic nodules to meet its nitrogen demands. The presence of high amount of nitrate in the soil suppresses the formation of nodules. CLE (CLAVATA3/EMBRYO SURROUNDING REGION) peptides produced in the root in response to rhizobial inoculation and/or nitrate were shown to control the number of symbiotic nodules. Previously, we have identified the *MtCLE35* gene upregulated by the rhizobia and the nitrate treatment in *Medicago truncatula*, which systemically inhibited nodulation when overexpressed. Using genetic engineering approaches we increased its transcriptional activity in transgenic roots, which almost completely prevented the formation of symbiotic nodules. Moreover, we obtained stable transgenic lines overproducing the MtCLE35 peptide, and found that their had lower shoot and root biomass in comparison to the wild-type plants. The study of metabolome of MtCLE35-overproducing plants revealed the increased level of amino acids in the roots, suggesting the stimulating effect of MtCLE35 on amino acid synthetic pathways.

In addition, we obtained several knock-out lines, where the *MtCLE35* gene was edited using the CRISPR/Cas9-mediated system. The detailed analysis of these plants will allow us to understand the mechanisms of MtCLE35 action in the regulation of plant nitrogen status and symbiotic interaction with rhizobia.

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Composite plants of cucumber and buckwheat as a tool to study auxin distribution and transport in the root system

Elena L. Ilina, Alexey S. Kiryushkin, Vera A. Puchkova, Kirill N. Demchenko
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Genetic transformation of most dicotyledonous plants by *Rhizobium rhizogenes* (also known as *Agrobacterium rhizogenes*) results in production of composite plants plants consisting of wild-type shoot and transgenic root system. Composite plants are the suitable model for investigation of hormonal mechanisms related to development of the root system as regulatory links between the root system and the shoot maintains in such plants.

In most plants initiation of lateral root primordia occurs above the elongation zone [1]. However, in cucurbits and some other species, including important cereal crop buckwheat (*Fagopyrum esculentum* Moench), lateral root primordia initiation and development occurs in the apical meristem of the parental root [2, 3].

The phytohormone auxin is a key regulator of lateral root development. Fusions of auxin-responsive promoters and reporter genes can be used to study the role of auxin in the development of root system of non-model plants such as cucumber (*Cucumis sativus* L.) and buckwheat [4].

The "agrobacterium" — mediated transformation technique of cucurbits [5] has been adapted for buckwheat. *R. rhizogenes* strain R1000 was used in all transformations. Set of binary vectors based on pKGW-RR-MGW or pKGW-MGW was developed to study auxin response maxima (*DR5::mNeonGreen*) or auxin transport (fusions of genes encoding auxin efflux proteins *PIN* and *mNeonGreen*).

Pattern of auxin response maxima was similar in both species and included quiescent center and initial cells, columella, xylem cell files and lateral root primordia on all stages of development. Members of CsPIN1 (CsPINb and CsSoPIN1) group contributed unequally in generation of auxin maximum required for lateral root primordium initiation.

The research was supported by the RFBR grant 20-016-00233-a.

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Komagataella phaffii yeast as a model organism in biotechnology and fundamental research

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The choice of a model organism in biology is based on various factors, such as practical significance and ease of manipulation. *Saccharomyces cerevisiae* yeast is one of the most widely used and well-studied eukaryotic models. However, developments in NGS, proteomics, metabolomics and gene editing methods allow other species to become the object of fundamental research. A good example of such emerging model organisms is the yeast *Komagataella phaffii*.

K. phaffii belongs to a unique group of eukaryotic methylotrophs that can use methanol as the sole source of carbon and energy. On the other hand, *K. phaffii* seems to be more characteristic of the common ancient yeast ancestors than the rapidly evolving *S. cerevisiae*. Comparative studies between *S. cerevisiae* and *K. phaffii* will shed light on the mechanisms of evolution of metabolic pathways and regulatory systems. Such studies are accelerated by the practical importance of *K. phaffii* as a common microbial production host in biotechnology.

In our studies, we demonstrate that some amino acids greatly affect gene expression in *K. phaffii* [1]. Transcriptome analysis revealed drastic changes in gene expression when proline was present in the media. About 18.9% of total protein-coding genes were differentially expressed, including genes involved in methanol utilization [2]. Our results show that the unique methanol metabolism pathway is regulated not only by methanol, but also by other carbon sources. Therefore, our findings suggest that the regulation of methanol metabolism pathway is integrated into other cellular regulatory networks. Methanol metabolism, acquired by *K. phaffii* during evolution, is tightly associated with nitrogen an amino acid metabolism.

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Identification of SNPs and InDels probably associated with the development of spontaneous tumors in radish (*Raphanus sativus* L.)

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Radish (*Raphanus sativus* L.) is an agronomically important root crop belonging to the *Brassicaceae* family. A genetic collection of inbred radish lines, which was based on contrasting genetically determined traits including trait "ability to develop tumors", was created at St. Petersburg State University in the middle of the 20th century [1]. Full genetics network controlling this trait still remains unclear [2], and elucidation of its mechanisms can help reveal the key regulators of systemic mechanisms that control cell proliferation and differentiation.

The purpose of this work is to identify single nucleotide polymorphisms (SNPs) and InDels in genes which are candidates for participation in the tumor development process within in the tumor-forming radish line compared to the non-tumor radish line.

We have assembled the genomes of two lines contrasting in the ability to tumorigenesis, annotated them, aligned sequences per assembly, identified candidate genes and differences in the structure of these genes in contrasting radish lines using bioinformatics tools. Bioinformatics data were confirmed by the sequencing by Sanger method.

As a result, in the tumor-forming radish line we identified 151 genes with InDels in their coding regions, which led to various variants of frameshift. Moreover, we detected 39 genes with single nucleotide substitutions (SNPs). According to the gene pathway enrichment analysis, the corresponding genes were classified into the several groups.

Thus, the data obtained will allow us to clarify in more detail the genetic control mechanisms of the tumor development in radish.

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NF-Y genes in the somatic embryo development

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For many important legumes, protocols for their efficient transformation and regeneration have not yet been developed. Somatic embryogenesis (SE) — the embryo development from somatic cells — has an important application in biotechnology: reproduction and obtaining of transgenic plants.

There are transcription factors involved in embryogenesis processes, which can stimulate the development of somatic embryos, such as the *LEC1* gene in *Arabidopsis thaliana*.

The LEC1 belongs to the NF-YB family. NF-YB together with NF-YA and NF-YC subunits form heterotrimeric complex NF-Y. In plants, each subunit is encoded by several genes, and combinations of different subunits are able to regulate various processes in plant.

We hypothesized that the LEC1 orthologues in legumes can stimulate SE in combination with specific NF-YA and C subunits. The aim of our study was to find new SE regulators among *NF-Y* genes in legumes.

In the model legume object *Medicago truncatula* we find *NF-Y* genes highly expressed during SE. Among these genes, potential LEC1 partners were selected with the use of the yeast two-hybrid system. Analysis of calli with overexpression of *LEC1* orthologues didn't demonstrate increased SE capacity, but overexpression of one of the *NF-YA* genes increased callus weight.

We are planning to investigate the effect of co-overexpression of *NF-YA*, *B* and *C* genes on the regeneration processes.

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MtWOX2 gene in somatic embryogenesis of *Medicago truncatula*

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Somatic embryogenesis (SE) is one of the plant regeneration pathways. This process is very similar to zygotic embryogenesis, but embryos develop not from zygote, but from vegetative tissues. This process is widely used in biotechnology for plant transformation and propagation. Somatic embryos can derive directly from the vegetative tissues or through the formation of callus. The search for SE stimulators is very important for plant biotechnology.

Several genes were reported to be the regulators of this process, among them the *WOX (WUSCHEL-RELATED HOMEOBOX)* family members are presented. These genes encode homeodomain-containing transcription factors, participating in different developmental processes. *WOX2* is known for its participation in zygotic embryogenesis. It is expressed in the zygote and later in the apical domain of the embryo. We study the role of this gene in the somatic embryogenesis. Overexpression of *MtWOX2* in some cases leads to the development of embryogenic calli with increased size. We performed transcriptome analysis of *Medicago truncatula* calli with overexpression of this gene compared to the calli overexpressing *GUS*.

It was shown that *MtWOX2* overexpression led to the changes in expression levels of genes, enriched with several GO pathways, including groups related to oxidative stress and ROS formation, response to toxic substance and auxin-activated signaling pathway. Among differentially expressed genes there are members of several TF families, e.g. MADS-box, BHLH, MYB, bZIP and others. These genes may regulate embryogenic callus development. Together, these results can be used for the search of new morphogenic regulators applicable for plant transformation.

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

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Plant somatic cells can be reprogrammed into totipotent embryonic cells that are able to form differentiated embryos in a process called somatic embryogenesis (SE). SE can occur naturally in various plant species and it is widely used for clonal propagation, transformation and regeneration of different crops. This process is regulated by hormone treatment and many proteins, among which WUSCHEL-related homeobox (WOX) transcription factors are believed to play crucial roles. The WOX family is involved in the regulation of a wide range of key developmental programs in different plant organs and tissues. CLE peptides are well-known hormonal regulators of plant development. *WOX* and *CLE* genes can be related to each other through feedback regulatory loops.

Our previous studies have shown that *MtWOX9-1* stimulates SE in *Medicago truncatula* [1] and overexpression of the *MtWOX9-1* gene increases the expression level of the *MtCLE08*, *MtCLE16*, *MtCLE18* genes in SE. In this study, we examine the overexpression effect of *MtCLE08*, *16*, and *18* on the expression level of *MtWOX9-1* gene and a number of other *MtWOX* genes, which were shown to change expression in SE according to the transcriptomic data. No significant impact of *MtCLE* overexpression on any *MtWOX* gene under study was found.

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

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Development of a transgenic tissue visualization system in representatives of *Fabaceae*

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Plant organisms are the objects of green biotechnology, which have been used by humans in various areas of life. The family of legumes (*Fabaceae*) that is being studied in this work is not an exception here. It is a very diverse family widespread throughout the globe.

Barrel medic (*Medicago truncatula*) and common pea (*Pisum sativum*), members of the legume family, were selected in this study for the development of an vital imaging system for transgenic tissues. As a part of the work, we tested an imaging system with post-mortem staining based on the *GUS* reporter, as well as a system for vital detection of transgenic tissue with fluorescent proteins GFP and DsRed. At this stage, vital imaging systems based on betalain and anthocyanin staining of transgenic tissues are designed and currently being adapted to the model objects under study.

The results of this study may be useful for subsequent attempts to solve the problem of the low efficiency of transformation of *P. sativum*. Moreover, such a system should be useful in the study of gene regulatory networks and factors that regulate the process of induction and subsequent development of somatic embryos. In addition, it can be used to track the dynamics of expression of genes of interest on living objects, while studying other fundamental and biotechnological processes.

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Features of the regulation of the transcription factor NIN, which determined its participation in the control of nodule organogenesis in legumes plants

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The transcription factor NIN (NODULE INCEPTION) is a master regulator of forming legume-rhizobial symbioses in plants. This transcriptional factor is the founder of the family of NIN-like proteins, which are widely represented in most part of groups of terrestrial plants, but only in legumes and some representatives of the *Rosales* was recruited to play a key role in the regulation of the development of rhizobial symbiosis. We assume that there are some features in the structure of the protein itself, which are associated with different co-regulators during various processes, which determines the selective involvement of NIN in the regulation of infection and organogenesis, and also there are many regulatory regions in the gene promoter that are associated with different TFs on different developmental stages of symbiosis.

We found that this protein may contain structural features in the form of a specific transcription factor amino acid pattern that was characteristic of legumes with an indeterminate nodule type. In addition, we conducted a search and analysis of promoter elements, which showed activity in the roots of non-legume plants.

In order to study the role of the identified promoter elements associated with cytokinins in the regulation of morphogenesis, we conducted an experiment on the treatment of mutant pea plants with exogenous cytokinins followed by transcriptomic analysis. As a result of this work, we have identified new target genes that can be activated by NIN in the control of later stages of nodule morphogenesis.

The work was supported by the RSF grant 22-26-00279.

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Development of a testing system for regeneration regulators in *Pisum sativum* L.

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Pisum sativum L. (pea) is one of the most important agricultural crops, because its seeds have high protein content, and, due to its ability have symbiotic relationships with nitrogen-fixing bacteria, these plants need less fertilizers. Nevertheless, we are faced with the need to improve old and create new methods for obtaining novel varieties of peas and other agricultural plants. The formation of regenerated pea plants is difficult to achieve in the *in vitro* culture. Accordingly, transformation of this species is a laborious process. In this regard, the search for morphogenic regulators of somatic embryogenesis (SE) in pea is an urgent problem. A number of publications reported on the genes regulating the SE process in a model plant from the legume family, *Medicago truncatula* [1]. In our study, we search for the *in vitro* cultivation system in peas, suitable to test the effect of putative SE regulators in this species. We tested several pea transformation techniques using different explant variants: embryonic axes from mature and immature seeds, as well as shoot apexes. Out of the tested options, the transformation of mature seeds turned out to be optimal. We also designed a set of DNA constructs *in silico*, which are suitable for the search of morphogenic regulators in peas.

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Assembly of genetic constructs for analysis of three promoters in plants

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To study the processes of plant development, it is often necessary to find out in which tissues and cells the gene is expressed. To do this, genetic constructs with promoters and fluorescence reporters are used.

The aim of our work is to create a construct for the simultaneous analysis of the activity of three promoters: auxin-responsive DR5 promoter, cytokinin sensor TCS, and any other promoter necessary for research purposes. We have selected fluorescent proteins with different spectral characteristics that make it easy to distinguish them from each other: red (mCherry), blue (TagBFP), green (mNeonGreen). Hygromycin B resistance gene we use to select transgenic plants. For cloning, we used the MoClo vector system. So far, we have created 5 plasmids containing hygromycin resistance gene, *mCherry*, *mTagBFP*, and *DR5* and *TCS* promoters. The next step is to create a single vector that will contain all components.

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Transgenic plants-immunomodulators for animal husbandry

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In modern pharmacology, there is a tendency to move from low molecular weight drugs to protein drugs. Interferon is among them. Interferon is used in animal husbandry to prevent and treat viral and bacterial diseases, as a single drug and as an adjuvant for vaccines and antibiotics to increase their efficiency and reduce the amount of use. After interferon therapy, meat, milk and eggs can be consumed without restrictions. Currently, interferon for veterinary purposes is obtained using genetically modified organisms (mainly bacteria). The limiting factor is the high cost of the active substance isolating and purifying, which is up to 80–90% of the product cost. Edible plants-producers can solve this problem. Plants have the lowest price per unit of biomass, are not infected by mammalian pathogens, and have an eukaryotic system of protein synthesis.

Transgenic tobacco plants (*Nicotiana tabacum* L.) for the production of bovine gamma-interferon were obtained by agrobacterium mediated transformation at the Department of Genetics and Biotechnology, Saint Petersburg State University. Testing of this model showed that interferon is successfully produced in plants and retains biological activity, including oral administration. Problems were also identified: tobacco toxicity and low levels of protein accumulation. At the next stage of the experiment, it was decided to transform edible carrot (*Daucus carota*) plants, modify the target protein (shorten) to increase its resistance to proteolytic degradation, and use the pSRD1 root-specific promoter. At present, the corresponding transgenic constructs have been obtained, the effectiveness of which will be determined first on the carrot hairy roots, and then on whole carrot plants.

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Heterologous expression of β-alanine betaine biosynthesis gene increases *Nicotiana tabacum* resistance to abiotic stresses

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Plant genetic modification in order to increase their tolerance to various abiotic stresses has been of exceptional importance in recent years. Heterologous expression of glycine betaine (GB) biosynthetic genes leads to increased salt and drought tolerance in various plant species by maintaining the osmotic balance with the environment and stabilizing the quaternary structure of complex proteins. However, GB biosynthesis in transgenic plants is limited by choline availability. Members of the *Plumbaginaceae* family accumulate β-alanine betaine (βAB) instead [1]. The synthesis of βAB is not limited by the availability of choline, as it follows the methylation pathway of the aproteinogenic amino acid β-alanine.

For the first time, we have generated *Nicotiana tabacum* plants expressing the β-alanine N-methyltransferase (*LlBANMT*) gene of *Limonium latifolium*. Transgenic plants were much less affected by such abiotic stresses as increased salinity, excessive illumination, and low temperature. The experimental *Nicotiana tabacum* lines had lower rates of chlorophyll degradation under stress conditions compared to the control plants. *LlBANMT* expression also resulted in less biomass loss under stress conditions, which was associated with higher activities of reactive oxygen species detoxification systems and healthier cell membranes. The presented data demonstrate for the first time the protective properties of *LlBANMT* heterologous expression and shed light on the mechanisms of its action.

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Evolution and epidemiology of global populations of nursery-associated *Agrobacterium*

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Agrobacterium tumefaciens is unique in that it can facilitate the interkingdom transfer of DNA and genetically modify its plant host. While *Agrobacterium* has been coopted for use in the genetic modification of plants, it is also a major pathogen, causing crown gall disease in the nursery, orchard, and vineyard industries. Pathogenicity in *Agrobacterium* is the result of two components. First is the Ti plasmid, which carries virulence genes and the transferred T-DNA region. The second component is the chromosome of *Agrobacterium*, which comprises diverse bacterial lineages and multiple species-level groups. The Ti plasmid can be transferred from strain to strain, diversifying the pathogen and complicating efforts to understand its epidemiology. This system provides an opportunity to study transmission of plasmids and their impact on disease persistence and spread. However, the movement of plasmids, and diversity of chromosomal lineages, means that conventional methods of using whole genome SNPs to track outbreaks are not sufficient, and new techniques must be developed. Additionally, Ti plasmids, like *Agrobacterium*, are genetically diverse and represent multiple plasmid types. Using a framework of >200 sequenced *Agrobacterium* genomes isolated from around the world, and a previously developed model of Ti plasmid types, we modelled their epidemiology. Key to this study was that we first separately analyzed plasmids and strain. Combining results revealed links between nurseries, potential horizontal transfer of the plasmid between strains within nurseries, global spread of plasmids, and long-term persistence of plasmids in the agricultural system. Agricultural practices have the potential to promote the diversification of pathogens and the emergence of new pathogen lineages.

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Overexpression of the *Ib-rolB/C* gene perturbs biosynthesis of caffeoylquinic acid derivatives in transgenic calli of sweet potato

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Ipomoea batatas is a root crop widely cultivated in South America, Africa, and Asia. This is also a source of caffeoylquinic acid derivatives (CQAs) with potential health-promoting benefits. Sweet potato genome carries two separate cellular T-DNA (cT-DNA) regions (*Ib*T-DNA1 and *Ib*T-DNA2). Especially, *Ib*T-DNA2 contains five ORFs homologous to the *Agrobacterium rhizogenes* T-DNA, namely ORF13, ORF14, ORF17n, ORF18/ORF17n, and RolB/RolC proteins [1]. Unfortunately, presently there is insufficient information on *Ib*T-DNA2 genes' function in the physiological processes of sweet potatoes.

In this study, expressional levels of the *Ib*T-DNA2 genes and the effect of *Ib-rolB/C* overexpression were examined using *I. batatas* cell culture. We discovered that *I. batatas* cT-DNA genes were not expressed in callus, and abiotic stresses and chemical elicitors affected their transcriptional levels weakly. Additionally, two *Ib-rolBC*-transgenic cell lines have been established though *Agrobacterium*-mediated transformation of *I. batatas* callus cells. Overexpression of *Ib-rolB/C* gene reduced biomass accumulation of transgenic cell lines by 1.2–1.6 times and increased the CQAs content by 1.5–1.9-fold. To justify the metabolic fluctuations, the study also looked into the expression patterns of the major biosynthetic genes, namely *bPAL*, *IbC4H*, *Ib4CL*, *IbHCT*, and *IbHQT*. The obtained data demonstrated that the overexpression of the *Ib-rolB/C* reduced the *IbPAL* transcript but considerable increase in the transcript levels of the *IbHQT*. We propose that this result was obtained through as-yet-uncharacterized signaling pathways activated by RolB/RolC.

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Natural GMOs in the genus *Nicotiana* L.

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Soil bacteria "*Agrobacterium"* are able to transfer fragments of their plasmids, so-called T-DNA, into plants. T-DNA integrated into plant's genome is called cellular T-DNA (cT-DNA) [1]. Plants transformed in nature are considered natural genetically modified organisms (nGMOs). For the first time, nGMOs were described within the genus *Nicotiana*. To date, more than 50 nGM species are known [2, 3], among which nGMO in the genus *Nicotiana* are the most well studied. Within this genus 3 subgenera are distinguished, those are *Tabacum, Petunioides*, and *Rustica*. CT-DNAs in natural genetically modified representatives of the subgenus *Tabacum* are studied in detail [4, 5]. We know how many cT-DNA those species carry as well as the composition of the cT-DNA, which allows us to propose scenarios for the acquisition of cT-DNA by these species during their evolution. Species *N. noctiflora* and *N. glauca* belong to the subgenus *Petunioides* and they are not so well studied. We sequenced and assembled the genomes of *N. noctiflora* and *N. glauca*, to analyze their cT-DNAs. In the *N. glauca* genome we confirmed the presence of one cT-DNA, gT, discovered in 1983, and showed no other inserts. In the genome of *N. noctiflora* 2 cT-DNAs of different composition were found, NnT-DNA1 and NnT-DNA2. The data suggest a single agrotransformation act in the evolution of the species *N. glauca*, while the species *N. noctiflora* was transformed several times. Further study of cT-DNA in *Nicotiana* representatives belonging to different evolutionary branches of the genus will help to clarify the evolutionary history of the genus *Nicotiana*. In addition, the identification of changes that have occurred in the cT-DNA since its entry into the plant genome will help to elucidate the processes that occur with transgenes in plant genomes over long time intervals.

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PhaseAll: a simple tool for read-based allele phasing

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The currently used genome assembly algorithms do not provide for allele phasing. This can lead to the loss of important information about the genotype of diploid and polyploid individuals. Here we introduce PhaseAll, a simple tool for allele phasing based on short reads obtained by second-generation sequencing. As input data, the tool takes paired reeds in SAM format. PhaseAll iterates sequentially through each alignment position. When a polymorphic position (SNP, insertion or deletion) is first encountered, a unique mutation is written to each allele. For each subsequent polymorphic position, a test is made to verify whether it is located on the same pair of reads (one DNA fragment) as the previous one. If two mutations are located on the same fragment, they are considered to belong to the same allele. If no fragments are found that connected at least one pair of neighboring polymorphic positions, an «X» is written in the allele sequences. This means that the alleles can swap at this position.

PhaseAll is written in python 3. SAM files are processed using the pysam library. PhaseAll is designed to separate only two alleles. To avoid possible sequencing errors, the user can set a read depth threshold below which the polymorphic position will be skipped. Some indels can cause errors in allele phasing, so PhaseAll has an option to skip indels for more accurate SNP reconstruction. The tool was tested on sequences of agrobacterial origin in the *Camellia* L. genome in more than 100 samples. PhaseAll is available for download on the GitHub: https://github.com/pzhurbenko/PhaseAll.

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Genetic engineering approaches to study of the opines of natural GMOs

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A few dozen naturally transgenic plants have been described to date [1]. Many of them contain DNA-sequences homologous to opine biosynthesis genes. Chemically, opines fall into two major structural classes: secondary amine derivatives and sugar-phosphodiesters [2]. The concentration of these molecules in the crude plant extract of nGMOs could be less than 1 pmol, which makes it difficult to study them. However, structure clarification of plant opines reveal their function in plants. The aim of our work is the development of protocol for large scale production, purification and definition of the chemical structure of plant opines.

The approach includes amplification of full-length opine synthase gene by PCR and cloning it using pENTR™/D-TOPO™ Cloning Kit (Thermo Fisher) according to its manual; subcloning in pDest527 using LR Clonase™ II Plus enzyme (Thermo Fisher) according to its manual; transformation NiCo21(DE3) chemically competent *E. coli* cells with obtained recombinant plasmids. After the confirmation of transgenic nature, the *E. coli* cells should be grown on medium with ampicillin 100 mg/l to an optical density at 600 nm 0.5–0.6. Then an equal volume of LB medium with 1 mM isopropylthio-β-galactoside (IPTG) should be added for induction opine synthesis. In case of synthesis of sugar-phosphodiesters, cultural media should also contain 0.02 M arabinose, glucose, and sucrose, respectively. Cells are cultivated during 4 hours for induction of opine synthesis. After that cells are pelleted by centrifugation for 10 minutes at 5000 rpm/min. The original strain and cells grown on the medium without IPTG are used as controls.

The cells are extracted with 80% methanol. The culture liquids are evaporated and redissolved in 80% methanol. The opines are separated by normal-phase chromatography using a CHROMABOND® Flash BT. Thin-layer chromatography is used to analyze the fractions, and to confirm the component opines-like compounds. High-resolution mass spectra are recorded on a Bruker micrOTOF 10223 mass spectrometer (electrospray ionization), eluent 80% MeOH. The ^{31}P NMR spectra are acquired on a Bruker Avance 400 spectrometer (400, 100, and 162 MHz, respectively). The ^{1}H spectra are analyzed in D_2O, with residual solvent signals (7.26 ppm for ^{1}H nuclei) as the internal reference. The ^{31}P NMR is measured relative to H_3PO_4.

The one phosphoric acid residue in the opine structure was confirmed by NMR spectroscopy.

We applied this method to characterize agrocinopine A-like compound in *Nicotiana* and propose it to produce opine-like plant compounds for further biochemical analysis.

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Prospects for the use of natural transgenic cultivated peanut (*Arachis hypogaea* L.) in breeding

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Common peanut (*Arachis hypogaea* L.), or peanut, is an annual herbaceous plant from the legume family (*Fabaceae*). Russia is one of the largest buyers of peanuts, and at the same time, in the south of the country, a number of zones meet the requirements for the cultivation of this crop. However, at present there are no commercial peanut crops in Russia, and selection work is almost not carried out. It is necessary to identify a new source material for breeding and breeding new high-yielding varieties. In the genomes of species of the genus Arachis, homologues of agrobacterial opine synthase genes, cucumopine synthase (cus) and deoxyfructosylglutamine synthase (mas2`), have been identified. The expression of these genes can affect the economically valuable traits of the plant, since the synthesis of various opines affects the composition of the microbiome in the rhizosphere. We have analyzed the expression of the cus gene in various organs of 9 peanut lines from the VIR collection, which have different geographical origin, belong to different cultivar types, and differ in morphological characters. As a result of the analysis, the organ-specific expression was shown; samples were identified that were contrasting in terms of the level of cus gene expression, including those with a high level of expression in the roots (kk-168, 416, 751). For the first time, data were obtained on the work of the cus gene at different stages of plant development on accessions k-168 and k-1157. An increase in the level of expression in the roots during flowering was revealed; during seed germination, the expression is lower. Further analysis and search for a correlation between the expression level of the cus gene and the manifestation of economically valuable traits in peanuts can provide new material for creating promising varieties.

The studies were carried out using the equipment of the resource center of the Science Park of Saint Petersburg State University “Development of molecular and cellular technologies”.

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Molecular genetic and bioinformatic approaches for the allele reconstruction of the *rolB/C*-like gene in representatives of the genus *Vaccinium* L.

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Agrobacterium mediated transformation is one of the most studied examples of horizontal gene transfer between pro- and eukaryotes. During this process a part of the Ti-plasmid — T-DNA — is transferred into the plant cell genome. These sequences could be preserved in the genomes during evolution and inherited in a series of sexual generations. Such plants are described within the genus *Vaccinium* L. [1]. Our research team is currently analyzing natural transgenes in *V. oxycoccos* L., *V. japonicum* Miq., *V. conchophyllum* Rehder, *V. emarginatum* Hayata, *V. myrtilloides* Michx., *V. virgatum* Ait., *V. corymbosum* L., *V. darrowii* Camp, *V. smallii* A. Gray, *V. praestans* Lamb., *V. ovalifolium* Sm., *V. myrtillus* L., *V. uliginosum* L., *V. vitis-idaea* L.

Previously, analyzing the natural transgenes in another genus (*Camellia* L.) [2], we showed the importance of reconstructing the allelic states of transgenes for phylogenetic studies.

In this paper, we present a comprehensive approach for studying the allelic state of the *rolB/C*-like gene in plants of the genus *Vaccinium*. It combines molecular genetic and bioinformatic research methods.

Molecular genetic methods involve Sanger sequencing of a gene sequence in a large number of samples, while for each sample the sequence is presented as a set of polymorphic positions in binary form. Allele resolution occurs based on the description of alleles in homozygotes and a series of "subtractions" of known alleles in heterozygous samples. The second method involves the analysis of SRA (Sequence Read Archive) sequences available in the databases. SRA is a repository of high-throughput sequencing raw data.

Based on our work, we can conclude that both of these approaches make it possible to describe the allelic state of the *rolB/C*-like gene in representatives of the genus *Vaccinium*.

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

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

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Homologues of octopine/vitopine synthase genes in natural GMOs

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The process of horizontal gene transfer causes the appearance of natural genetically modified organisms. At the moment, it is known that over 7% of dicotyledonous plant species are naturally transgenic, i.e. nGMO [1]. These plants contain the genes of agrobacteria, which are integrated in the nuclear genome during infection. In some species of naturally transgenic plants, agrobacterial genes have been preserved for millions of years of evolution. Among these genes, genes encoding octopine/vitopine synthase (*ocs/vis*) can be distinguished [2].

The study of homologues of octopin/vitopin synthase genes in naturally transgenic plants: their structures and diversity, products of encoded enzymes will allow us to establish the functions and evolutionary role of homologues in nGMO. Currently, bioinformatic and genetic engineering methods are used to solve these problems.

*ocs**vis*-like were found in 7 species: *Albizia julibrissin* Durazz., *Cenostigma pyramidale* (Tul.) Gagnon & G.P.Lewis, *Paulownia fortunei* (Seem.) Hemsl., *Pterocarya stenoptera* C.DC., *Rehmannia glutinosa* Steud., *Santalum album* L., *Viscum album* L. In total twenty one *ocs/vis* sequences are known in 17 nGMO species. Twenty sequences are intact. This may indicate the functional significance of these genes for nGMO.

Phylogenetic analysis of currently known *ocs/vis*-like genes of *Agrobacterium*, *Rhizobium* and natural GMOs suggests that diversity of studied genes is wider, than it was estimated based on agrobacterial sequences. On the phylogenetic tree constructed by the neighbor-joining method, 6 clusters for *ocs/vis* can be distinguished. Three clusters contain nGMOs and "agrobacteria", showing the relationship of the T-DNA sequences of nGMO with those of currently known strains of of *Agrobacterium*/*Rhizobium*. Three clusters contain only nGMOs. One of them consists of species that belong to the Cannabaceae family. Other clusters are heterogeneous. No significant ecological similarities were found among the studied species.

The obtained results can be used to study the diversity of ancient and modern strains of agrobacteria, their host specificity and the possible role of their genes in plant evolution.

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

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New cellular T-DNAs in naturally transgenic plants

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Naturally transgenic plants represent the result of *Agrobacterium*-mediated gene transfer. T-DNA of soil bacteria *Agrobacterium* integrated into plant's genome is called cellular T-DNA (cT-DNA) [1]. Today, more than 50 species of naturally transgenic plants, or natural GMO (nGMO) are known [2, 3]. The function of cT-DNA in plants remains unknown. It is assumed that the fixation of transgenes could give plants different selective advantages depending on which genes had been integrated into the plant [4]. In order to clarify this issue, it is necessary to study more naturally transgenic plants. Until recently, the list of nGM plants contained less than 2 dozen species, but a search through genomic and transriptomic sequencing data made it possible to more than double this list [2]. In this work, we used the same approach, looking for cT-DNA genes in whole genome sequencing data that have appeared in the NCBI WGS since 2021. We found 14 new species of naturally transgenic plants, among which the most extended cT-DNAs were found in *Triadica sebifera, Lonicera japonica*, and *Lonicera maackii*. The cT-DNAs in these species are organized as imperfect inverted repeats. In the genomes of the species *Paulownia fortunei, Apocynum venetum, Elaeagnus angustifolia, Erythroxylum havanense, E. densum, E. daphnites*, *E. cataractarum, Ceriops decandra, Camellia oleifera, Silene uniflora*, short cT-DNAs containing only opine synthesis genes were found. We also estimated the approximate age of the cT-DNAs. The first described examples date back to the Late Paleogene, and the process continues to the present. Thus, we can conclude that natural GMOs are a widespread phenomenon, many aspects of which remain unclear, requiring additional research on the topic.

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Optimization of CRISPR/Cas9 method for transgenesis of model microalgae *Chlamydomonas reinhardtii*

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In this work we knocked out the *LTS3* gene of the microalgae *Chlamydomonas reinhardtii* using the TIM technique optimized for the available equipment. We achieved transformation efficiency of 68.8%, knockout of this gene lead to the death of *C. reinhardtii* cells after several division cycles.

The creation and study of genetically modified organisms in fundamental research allows a deeper understanding of the basic processes in the cells with the prospect of further applying this knowledge in practice. Microalgae are an interesting object for genetic engineering because of the great prospects for their application in biotechnology, but in almost every case it is necessary to develop new strategies and transformation methods for the introduction of genetic constructs into the cell. CRISPR/Cas revolutionized the field of genome editing due to its simplicity, efficiency and accuracy compared to previously used methods, which over time simplified the development of protocols [1]. Currently, the most effective method of transformation is TIM (Targeted Insertional Mutagenesis) [2], developed for the microalgae *Chlamydomonas reinhardtii* P.A. Dang. – model object of photosynthesis genetics.

To test and optimize the TIM technique [2] in our lab, we carried out a knockout of the *LTS3* gene, a transcriptional activator of chlorophyll biosynthesis genes in heterotrophic conditions [3].

We used glass beads agitation and electroporation ("Gene Pulser Xcell", Bio-Rad, USA) methods in order to introduce into *C. reinhardtii* cells of the CC-125 (*wt*, *mt+*) strain the ribonucleoprotein complex SpCas9/sgRNA and double-stranded donor DNA with paromomycin resistance gene.

The effectiveness of transformation varied from 10.6% to 68.8%. Probably, the *LTS3* gene product plays a key role in the pathway of chlorophyll biosynthesis, since its knockout led to the death of *C. reinhardtii* cells after several division cycles.

The transformation protocol optimized for the equipment available in our lab can be further refined and used to study the functions of other *C. reinhardtii* genes.

Keywords: genetic engineering; genome editing; microalgae; *Chlamydomonas reinhardtii*; CRISPR/Cas; GATA transcription factors.

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Methylotrophic yeast *Komagataella phaffii* as Neoleukin producer

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Methylotrophic yeast *Komagataella phaffii* (also known as *Pichia pastoris*) is widely applied in biotechnology for recombinant protein production. *K. phaffii* particularly proved to be a successful host system for the synthesis of immunomodulators such as interferons [1].

In this study, we engineered *K. phaffii* strains capable of producing the immunomodulatory protein Neoleukin (Neo-2/15). Neo-2/15 is an interleukin-2 mimetic, designed by *in silico* methods [2]. In preclinical studies on murine cancer models, Neo-2/15 showed superior therapeutic effect to inerleukin-2 with reduced toxicity.

In this work, we show that *K. phaffii* can successfully synthesize and secrete Neo-2/15. We have obtained a number of *K. phaffii* strains, including Mut^S and Mut^+, with different Neo-2/15 expression cassettes integrated into the genome, carrying up to five copies of Neo-2/15 gene. In fact, the higher number of Neo-2/15 gene copies in *K. phaffii* genome allowed a higher protein yield.

In this study, we further developed a split marker approach [3] for yeast transformation using two DNA fragments, comprising of the expression cassette and the overlapping fragments of the marker gene. This allowed us to generate Mut^S strains with two copies using pPICZαB vector, which is not originally intended for Mut^S strain generation.

As a result, we demonstrated that *K. phaffii* is a perspective producer of Neo-2/15, providing wide opportunities to increase the production of this therapeutic protein.

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Design of COMT-Knockout mouse as a preeclampsia mode

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Preeclampsia is a multisystem pregnancy disorder that occurs after 20 weeks of gestation, leading to e.g. preterm labor. It is characterized by hypertension, proteinuria, edema, and multiple organ dysfunction. Up to 8% of pregnancies are complicated by preeclampsia, which is one of the most serious causes of maternal and perinatal mortality [1]. For research of pregnancy disorders and development of therapy for it, a mouse model can be used due of the fact that pregnancy development in mice, especially at early stages, is somewhat similar to human and is well-studied, in particular, in terms of molecular biology [2]. One of the possible options for creating mouse models of preeclampsia is considered to be a mutation in the *COMT* gene encoding catechol-O-methyltransferase [3]. This enzyme plays an important role in the catecholamines conversion and it also catalyzes the O-methylation of hydroxyestradiol producing methoxyestradiol. *COMT* gene knockout results in a phenotype similar to preeclampsia with elevated blood pressure and proteinuria [3]. The previous model was obtained through classic transgenesis methods with Neomycin cassette insertion in the *COMT* locus potentially influencing the results of the experiments. The development of the genome editing systems and its active utilization at Saint Petersburg State University made it possible to obtain a *COMT*-KO mouse line using CRISPR/Cas9 technology which had not been done in Russia before. This model will allow to effectively study the development of preeclampsia and ways to prevent and treat it.

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The sweet protein brazzein as a promising natural sweetener

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In the modern world, due to the overconsumption of sugar-containing products, the problem of obesity is relevant. Among the many sweeteners that minimize sugar intake, a group of sweet-tasting proteins is up-and-coming. Brazzein is the smallest of the sweet proteins (54 aa, 6473 Da), and it is also safe for obese and diabetic people since it does not affect blood sugar and insulin levels. Brazzein has high thermal stability over a wide pH range: from 2 to 8 [1]. To increase the level of sweetness of brazzein, mutant variants of this protein were created through site-directed mutagenesis, the sweetest of which is triple mutant H31R/E36D/E41A, which is 22,500 times sweeter than sucrose [2]. Since the content of brazzein in the fruits of the natural source (*Pentadiplandra brazzeana*) is extremely low (0.2%), various methods have been developed to obtain brazzein using heterologous expression systems, which used as producers: bacteria (*Escherichia coli*, *Lactococcus lactis*), yeast (*Pichia pastoris*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*), plants (*Zea mays*, *Oryza sativa*, *Lactuca sativa*, *Nicotiana tabacum*) and animals (*Mus musculus*) [3–5]. Despite the short peptide sequence, the industrial production of recombinant protein faced several problems, including low protein yield (e.g in mouse milk it was detectable on western blot analysis only) and loss of sweetness. An extremely relevant and promising way to obtain recombinant brazzein is the optimization of extracellular expression in bakers yeasts with the GRAS (Generally recognized as safe) status, since the safety of these microorganisms for human health can potentially significantly reduce the number of brazzein purification steps and thereby reduce its cost to consumers.

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Genetically modified yeasts in studies of human amyloidosis

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Amyloid protein aggregation is a key factor in the development of a variety of serious diseases in humans, commonly named as amyloidoses (Alzheimer's and Parkinson's diseases, type II diabetes, etc.), and a determinant of protein-based inheritance in lower eukaryotes. In yeast, translation termination factor Sup35 is one of the most extensively studied amyloidogenic proteins. Aggregation of Sup35 (induction of [*PSI*$^+$] prion) decreases its functional activity and leads to the suppression of nonsense-mutation as stop-codons become recognized as meaningful more frequently. This phenomenon is the basis of phenotypic detection of Sup35 aggregation in yeast strains possessing nonsense mutation *ade1-14* in *ADE1* gene.

Yeast is convenient model for genetic, biochemical and molecular biology studies. Yeast genome can be easily edited and plasmids can be used for induction of gene expression. Yeast is suitable for analysis of mammalian genes and proteins and thus can be applied for the analysis of amyloidogenic properties of proteins associated with human diseases. Phenotyping detection of [*PSI*$^+$] prion can be modified for the analysis of amyloid aggregation of mammalian proteins in yeast.

We use genetically modified yeasts *Saccharomyces cerevisiae* adopted for amyloid biology research. The mutations leading to auxotrophy toward certain amino acids (leucine, lysine, tryptophane, histidine) and nucleobases (adenine, uracil) were implemented into yeast genome allowing phenotyping detection of [*PSI*$^+$] and the usage of plasmids for the investigation of mammalian protein in yeast.

Application of yeast-based experimental system for studies of different aspects of human amyloidoses is discussed.

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Application of genetically modified microorganisms for potential human amyloids search

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Amyloids are fibrous protein structures often found in patients with severe diseases, such as Alzheimer's, Parkinson's diseases etc. A number of studies have shown that the production of heterologous amyloidogenic proteins in *Saccharomyces cerevisiae* strains results in formation of amyloid aggregates with properties similar to those found in mammals.

Amyloid aggregates formed in yeasts usually do not have their own phenotypic manifestation. To assess amyloidogenic potential of individual proteins a yeast test-system was developed under supervision of Prof. Y.O. Chernoff. The system is based on usage of genetically modified *S. cerevisiae* cells auxotrophic for certain growth factors, allowing effective phenotypic selection to search for amyloidogenic proteins within proteomes of various organisms [1]. Using this test-system, our laboratory evaluated amyloid potential of a spectrum of human proteins, the amyloidogenicity of which was previously predicted by bioinformatics algorithms. The proteins that have shown amyloidogenic potential in yeast-based model are being currently tested *in vitro* and *in vivo*. Some mutant *Escherichia coli* strains can be applied for studying propensity of heterologous proteins to form amyloids *in vitro*. Thus, application of genetically modified microorganisms makes it possible to identify new human amyloidogenic proteins and to improve predictive ability of bioinformatics algorithms.

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Search and analysis of mutations affecting the aggregation of amyloid beta peptides

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Alzheimer's disease (AD) is a fatal neurodegenerative disorder and the most common form of dementia in late life. According to the generally accepted hypothesis, the main cause of AD is the aggregation of the amyloid beta (Aβ) peptide which leads to the formation and accumulation of plaques around a brain cells. Aβ isoform that prevails in plaques consists of 42 amino acid residues and is termed Aβ42. Here, we apply the yeast-based assay for searching mutations that affecting human Aβ42 peptide aggregation. This assay is based on phenotypic detection of amyloid aggregation, nucleated by the attachment of Aβ to prion domain of the yeast protein Sup35 in yeast *S. cerevisiae* (Chandramowlishwaran et al. 2018 J. Biol. Chem. 293:3436). As a result of screening, 70 derivatives with single or multiple mutations, altering amyloid nucleation were identified. Effects of most interesting mutations on biochemical and cytological parameters of Aβ aggregates, as well on the Aβ amyloid structure have been investigated. Results of these experiments shed light on modes and pathways of Aβ aggregation.

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Transgenic plants — a threat to local flora?

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The paper covers major threats associated with wide-range introduction and cultivation of transgenic plants due to germplasm mixing with that of indigenous species of natural plant communities and risks of transgenic plants' adverse impact on the environment. Among them are: influencing non-target species, invasive power, possibility of GMP's escaping into the environment by horizontal gene transfer as well as harmful effect on the soil biota.

Currently, herbicide- and pest-resistant genetically modified plants (GMP) became an integral part of contemporary agrotechnologies in many economies [1]. However, most countries lack national strategy providing science-based substantiated procedure of creating, distribution and safe production of GMP. Rapid development of agricultural biotechnology and GMP production offered many economical benefits but also caused concern due to their potential environmental impact. To date, truly negative effects of GMP production, revealed in the course of growing, are known: harmful effect of entomocide Cry-proteins (Bt endotoxins) on non-target biota, target phytophage resistance to insecticidal plants, phytophage species succession to replace the species eliminated in the agrocoenosis. Vertical transfer of GMP transgenes (repollination between transgenic plants and wild species or isogenic varieties), as well as slow decomposition of transgenic plants' remains — all these factors can have remote environmental consequences [2, 3]. Wind-dispersed pollen of insecticidal GMP contaminates soil and open water reservoirs by toxins, thus posing potential hazards for aquatic organisms and geobionts (including rhizospheric organisms).

Thus, uncontrolled GMP production and introduction, creates a real threat of losing biodiversity and genetic diversity of indigenous plants due to "biological contamination". Therefore, GMP cultivation and monitoring in the fields is of exceptional importance and must be regulated by a science-based national strategy. This strategy would allow to exclude agroecological and environmental genetic risks, to keep the genetic diversity of natural plant communities.

Keywords: transgenic plants; germplasm; plant communities; environmental and genetic risks; GMP monitoring.

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Biorisk assessment of genetic engineering — lessons learned from teaching interdisciplinary courses on responsible conduct in the life sciences

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Genetic engineering is one of the ground-breaking technologies developed in the 20th century with great prospects for improving human, animal, and plant health, providing food security as well as environmental protection in times of climate change. From the early beginning on, scientists were debating about benefits and risks of genetic engineering and actively proposed measures for safe use of this technology. This led to the concept of "biosafety" which aims at protecting humans and the environment from unwanted consequences of the use of genetic engineering. Genetic engineering could be misapplied for even enhancing the threat potential of pathogens or toxins. The concept of "biosecurity" has been introduced to protect biomedicine and the life sciences of being misused for criminal or hostile purposes by malicious actors. The new WHO "Guidance framework for the responsible use of the life sciences" is the most recent example for improving awareness among partitioners in science to strengthening biorisk assessment strategies [1]. But still, there is the need for implementing any of such proposed biorisk assessment frameworks on national and institutional levels. Ideally, comprehensive training of students in the life sciences in biosafety and biosecurity should be mandatory worldwide and embedded in the curricula of their bachelor and master study programmes [2]. But how to engage students and improve their understanding for cross-disciplinary approaches in strengthening biosafety and biosecurity? Which teaching techniques are appropriate? Is there an opportunity for innovation? [3] The work presented here addresses these questions and provides insights in joint teaching activities of the last two years.

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Legal aspects of new genetic technologies

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The development of genomic editing technologies has significantly intesificated the shortcomings of the legal regulation for genetic technologies as in general and as for new technologies of genetic engineering in particular. Because of imperfected legislation for genetic engineering, and in some cases because of its archaism, new genomic technologies, such as genetic editing technologies, which are growth drivers in science, could not be drivers in the economics. The outdated terms and concepts framework and the legal indetermination of using the products based on new genetic technologies (including genome editing) are barriers to achieving the goals to ensure the technological independence of Russia.

The basis of the current legal regulation system of genetic engineering in Russia is a focus to the process and technologies, i.e. methods of the product producing. This means that the changes within genetic information is not important, but only the method of development is. Such way of regulation provides a priori chronic lag in legislation, which could finally lead to technological lag as whole country. The transition to a product-oriented system, when the analysis of base of genetic information changes makes possible to clearly answer to the question of the methods used for this, will allow us to avoid the shortcomings within development of synthetic biology methods indicated earlier, as now as in the future.

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