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**BRIEF OVERVIEW
ON STUDY OF GENETICALLY MODIFIED ORGANISMS
AND ASSESSMENT OF POTENTIAL RISKS OF THEIR USAGE
FOR NATURAL SPECIES**

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Brief information is summarized on the genetically modified organisms (GMOs), methods of their construction, spheres of usage, potential risks of GMOs propagation, necessity, and ranges of control of their usage, *inter alia* their application to aquatic organisms. The data available allow concluding that the expert community currently has no exact answer concerning the scale of GMOs usage in the country, as well as about the degree of genetic safety of their usage in several fields of industry, especially in semi-closed systems for growing plants and rearing of animals and other living beings in the Russian Federation and other countries. Application of molecular genetic markers and new law regulations will help in monitoring GMOs usage in agriculture and other industries in Russia and responding to requests from the Russian Government and social institutions, as well as many challenges on genetic safety.

Keywords: genetically modified organism, GMO, construction, usage, potential risks, aquaculture, agriculture

A *genetically modified organism* is an organism whose genotype has been artificially altered using genetic engineering techniques ([Geneticheski modifitsirovannyi organizm, 2021](#)). Hereinafter, “GMO” and “GM-organism” are used in the meaning defined above. Similarly, the abbreviation “GMO” is used as “genetically modified object” ([Ganzha et al., 2011](#)).

This brief overview provides general understanding of GMOs and some of the latest developments in this area. Detailed information can be found in the references cited. To date, GMOs are found in almost all food products offered in stores ([Zakharova et al., 2015](#)). The methods for GMOs producing are diverse; they are based on the procedures of transfection and transduction, which add to the normal genome of organisms a set of DNA fragments of other organisms modifying its genotype and phenotype (properties) in the desired way and constructing a new transgenic organism. GMO receives new genetic components through special molecular constructions: vectors. Transfection uses a bacterial plasmid as a vector to transfer new genes (gene), that are specially adapted for this transferring. During transduction, modified retroviruses or their special fragments, capable of enzymatic integration into the DNA of the selected organism, usually are the vector. Both processes have the disadvantage of random insertion into alien DNA. This results in the loss of functionality of the inserted genes themselves or in the activation/deactivation of neighboring genes of the cells in the host organism, loading them with harmful

effects, *inter alia* their malignant transformation. In recent years, the technique of genetic material transfer has received significant improvement, which allows more accurate inserting of vectors through the CRISPR/Cas gene editing (Mojica et al., 2005).

GM-organisms can be more efficient and secure in cultivation, breeding, and rearing in closed systems, as well as applicable for the usage in food and medical industries with proper control. As a rule, genetic changes are carried out in accordance with the techniques mentioned above for commercial purposes, but they may be carried out for purely scientific purposes as well. In this overview, only a range of issues related to GMOs usage in human commercial activity is considered. In agriculture and food industry, GMOs mean only organisms modified by the introduction of one or more transgenes into their genome (Kuznetsov, 2005a). The same applies to other living beings, for example, to hydrobionts bred in closed and semi-closed systems; therefore, this is applied to aquaculture farms with full or partial control of reproduction by humans.

The main concerns of GMOs usage relate to their legal, environmental, agrotechnical, and food safety (Kuznetsov, 2005b ; Ganzha & Bannikova, 2010 ; Ganzha et al., 2011 ; Chuyko, 2011), as well as guarantees of the human genome protection under molecular therapy with the CRISPR/Cas gene editing (see <https://en.wikipedia.org/wiki/CRISPR>), which is already included in the medical practice in several countries.

The Food and Agriculture Organization of the United Nations (FAO) considers the usage of genetic engineering techniques to create transgenic plants or other organisms an integral part of agricultural biotechnology. The transfer of genes responsible for useful traits is a natural consequence of the development of a practice on breeding animals and plants. New techniques expanded the abilities of breeders in terms of controllability and efficacy of the process of creating new strains of broods, in particular being able to transmit useful traits between non-breeding naturally species (FAO, 2004 ; Zhuravleva, 2016). Actual agricultural practice has many examples of the successful usage of transgenic plants, that significantly increase crops. In the early 1980s, the first GMO strains were produced in the United States for commercial use. These strains were extensively tested by government agencies: National Institutes of Health (NIH) and Food and Drug Administration (FDA). After evaluating the evidence for the safety of their usage, these strains of organisms were granted market approval.

In a number of countries, including Russia, construction, production, and usage of GMO products is a subject to government regulation; more than 150 regulatory and legislative acts were passed (Verzhkova et al., 2008). In the Russian Federation, several types of transgenic objects have been investigated and approved for usage. Out of them, the most known are: soybean – strains 40-3-2, A 2704-12, and A 5547-127; potato – sorts Russet Burbank Newleaf, Superior Newleaf, Elizabeth 2904/1 kgs, and Lugovskoy 1210 amk; corn – strains GA 21, T-25, NK-603, MON 863, MON 88017, MIR 604, and Bt 11; rice – strain LL 62; and sugar beet – strain H7-1 (Genetically Engineered Mice, 2012 ; Lidder & Sonnino, 2011).

According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA) data, 14 countries were producing crops with biotechnological changes by 2004, and 25 countries – by 2009 (James, 2009). The USA, Brazil, Argentina, India, Canada, and China are leading in terms of GMO producing in crop production. Meanwhile, many countries – Austria, France, Luxembourg, Greece, Switzerland, and New Zealand – have banned the cultivation of GM-plants. Currently, GMOs usage is restricted in a small number of countries (Fig. 1). The distribution of GMOs of plant origin for 25 countries with the specification of breeding objects is presented in a review (Ganzha et al., 2011). Importantly, the introduction of GMOs in growing plants is highly likely to mean their inclusion

in the human diet: either directly or through the food chains when used in the form of food and feed additives in animal husbandry. At the same time, there is no control of their effect on the consumer, or this control is insignificant.

Since 01 January, 2011, fish and other products, which are produced from harvested biological resources, including those obtained from the cultivation and rearing of fish and other aquaculture and mariculture objects, are equated to agricultural ones in Russia. By the Order of the Government of Russia dated 30 November, 2010, No. 953 the corresponding amendments were made to the existing norm “On the classification of types of products as agricultural products and primary processing products made from agricultural raw materials of own production”.

In the fishery, GMOs are tested both on model organisms and on objects of commercial cultivation (Isaeva & Morozov-Leonov, 2005 ; Mikodina, 2008). Specifically, GMOs are described for Atlantic salmon *Salmo salar*, coho salmon *Oncorhynchus kisutch*, Chinook salmon *O. tshawytscha*, coastal cutthroat trout *O. clarkii clarkii*, Nile tilapia *Oreochromis niloticus*, Mozambique tilapia *O. mossambicus*, Japanese rice fish *Oryzias latipes*, common carp *Cyprinus carpio*, channel catfish *Ictalurus punctatus*, African sharptooth catfish *Clarias gariepinus*, fossil cat *Heteropneustes fossilis*, nigorobuna *Carassius auratus grandoculis*, crucian carp *C. carassius*, yellow pike *Sander vitreus*, northern pike *Esox lucius*, Amur catfish *Parasilurus asotus*, weatherfish *Misgurnus fossilis*, pond loach *M. anguillicaudatus*, gilt-head bream *Sparus aurata*, red seabream *Pagrus major*, Wuchang bream *Megalobrama amblycephala*, and zebrafish *Brachydanio rerio* (Ganzha et al., 2011). In the Russian Federation, research on GMOs in aquaculture is carried out on representatives of the most farmed species: rainbow trout, tilapia, carp, etc. (Ganzha et al., 2011).

However, there are very contradictory positions regarding the applicability of genomic technologies, *inter alia* widespread GMOs usage, which deserve serious attention of the expert community, specialized departments, and government agencies. Supporters of the applying GMO-technologies argue that only they, with the current world population, can save the planet from the threat of hunger. Opponents of this approach believe that with the actual level of agricultural technology and mechanization of agricultural production, existing plant varieties and animal breeds obtained in the classical way are capable of providing the world population with high-quality food (Obzor pervogo dnya konferentsii, 2019 ; Obzor dokladov za 23 iyunya, 2019) (materials of the Congress of the All-Union Society of Geneticists and Breeders, 2019).

At the International Genetic Congress in 2019 (The VII Congress of the All-Union Society of Geneticists and Breeders), a complex of ethical, social, legal, and scientific norms was considered in the usage of genetic technologies, *inter alia* construction of GMOs and control of their applying (Obzor dokladov za 20 iyunya, 2019). Despite the multitude of documents, both mentioned and new (Ganzha & Bannikova, 2010 ; Chuyko, 2011 ; Agapov & Ganyuhina, 2016), a single set of legislative acts on this issue has not been taken in the Russian Federation. In this regard, the request of the Government of Russia dated 17 March, 2020, No. MN-7/435-AM is extremely timely; its topic is as follows: “Improving the mechanism for controlling the release of genetically engineered organisms and products obtained with the usage of such organisms or obtained from such organisms, as well as monitoring their effect on humans and the environment using modern technologies”. The literature sources on these issues are partially annotated; those include 42 articles under the section “Methods for determining GMOs and quality control of organic products” alone (Geneticheski modifitsirovannye organizmy, 2017). In the next section, the author offers his vision of the issues – for their consideration by the expert community, as well as in government departments and other government agencies.

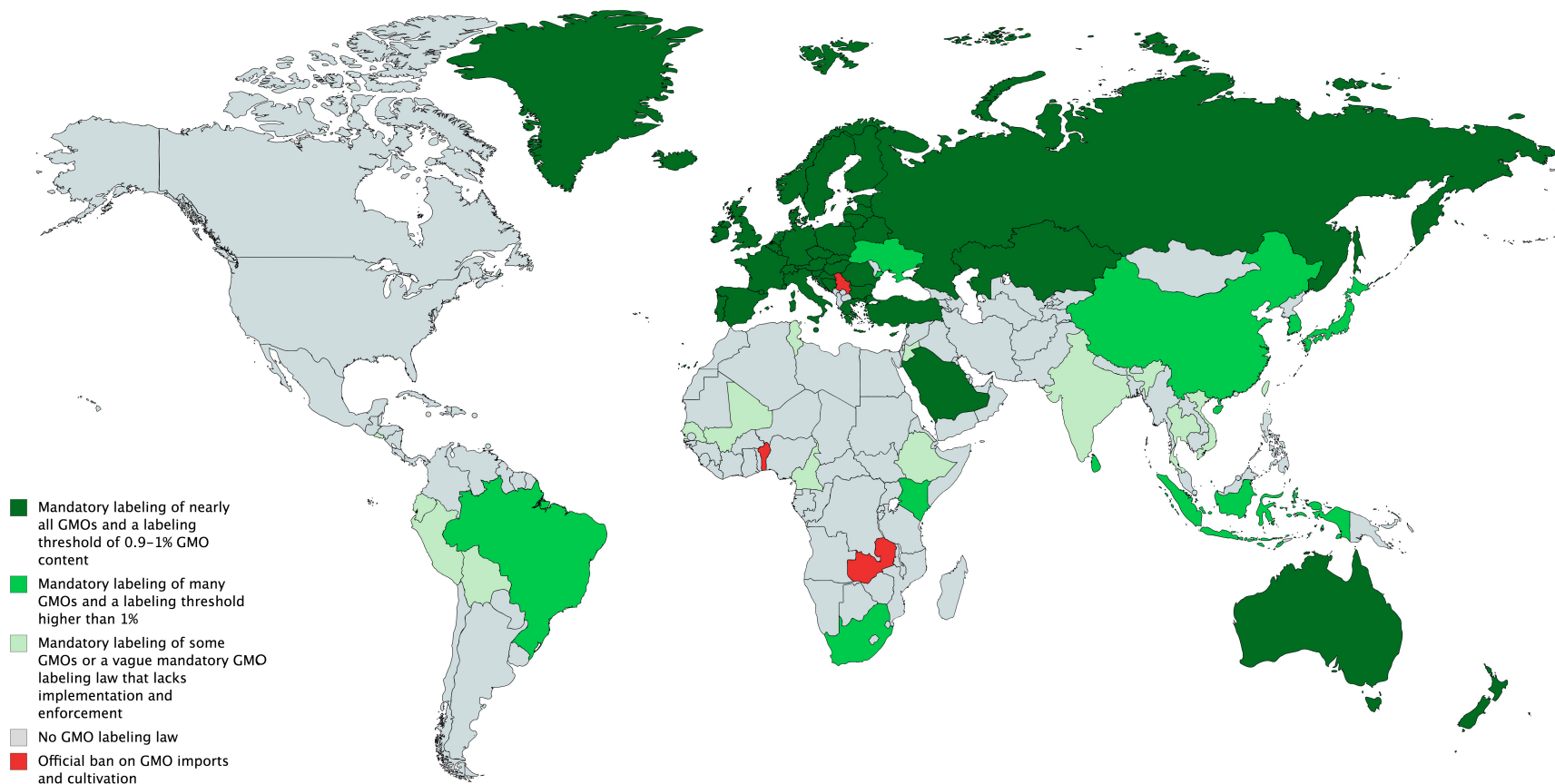


Fig. 1. Prevalence of GMOs in the world. The colors indicate areas of continents with distribution restrictions; the shades of gray indicate areas where GMOs usage is not regulated by law (source: <https://www.techpedia.pl>)

Identification of GMOs, biodiversity monitoring, and molecular markers. Genetic safety problems. The identification of GMOs is an essential component of controlling the prevalence of their practical application. However, among the most important tasks of biology, there is not only monitoring of GMOs and the safety of the usage of genetic technologies, but also the study of ecological aspects in their contact with natural communities, as it is possible in semi-closed reproduction systems. In this regard, the closest attention should be paid not to full-cycle agricultural and other farms where the reproduction of organisms is fully controlled by humans, but to semi-closed breeding systems, *e. g.* ranching, which is carried out at most salmon marine hatcheries, with partial control of the life cycle by the personnel. During rearing, GMO vectors are capable of entering the natural environment due to horizontal transfer; there, they can convey to species undesirable and even harmful in nature properties of transgenic organisms: instability of localization in the genome and provoking alterations of cellular functions during translocations with unpredictable consequences. The key task is to monitor all the biological diversity and take measures aimed at its preserving in order to maintain a comfortable living environment for current and future generations of Russian citizens. The problems of the society accelerated technologization and food trade globalization entail the need for control the quality of food products, sources for drugs, and their composition as well. All this is impossible without a well-functioning system for identifying organisms based on biological molecular markers.

The origins of the application of the variability of biological macromolecules, *e. g.* in biology, have long roots. In general, biological molecular markers have found application in numerous fields depending on the needs of modern society. Let us define what a biological molecular marker is. A *biological molecular marker* (hereinafter MM) is any macromolecule of a living organism, that can be an identifier of a certain function, results of a biochemical, population, or evolutionary process (Kartavtsev & Redin, 2019). The study and usage of MMs have already become a new branch of biomedical science, as evidenced by the presence of special journals: Biomarkers, Current Biomarker Findings, Biomarker Insights, Barcode Bulletin, *etc.* MMs are used in many fields of biology and medicine.

There are three most significant areas:

1. **DNA barcoding.** Molecular markers are applied in the global program for redescribing biological diversity on a modern molecular and bioinformatics basis: iBOL (see <https://ibol.org/>). As a standard marker, or DNA barcode, for most invertebrates and vertebrates, the nucleotide sequence of *Co-1* gene encoding cytochrome *c* oxidase subunit I of mitochondrial DNA (mtDNA) is used. For ease of usage, the first half of the gene is used as a barcode, about 650 base pairs (bp). For plants, other MMs, or barcodes, are more suitable, such as *matK* (maturase), *rbcL* (large subunit of the enzyme ribulose-bisphosphate carboxylase), and fragments of internal transcribed spacers of rRNA, ITS1, ITS2, *etc.* (Zhokhova et al., 2018 ; Shneyer & Rodionov, 2018). At this level, the basis for successful identification is low intraspecific variability (weak nucleotide sequence differences between individuals of the same species), but an order of magnitude greater interspecific divergence of samples (between individuals of different species): on average, about 0.5–1.0 % and 10 % divergence for animals, respectively (Kartavtsev, 2011).

One of the applications of MMs is the identification of hybrids and invasive species. Due to globalization and the intensification of international food trade, identification of samples in export-import operations is of great importance. Falsification of brands, *e. g.* trade marks, under which fish fillets, caviar, and other products are sold, can be accurately identified by MMs, and this helps state-owned and private enterprises to avoid significant economic and reputational losses (Nedunoori et al., 2017).

2. **Molecular markers for the identification of stocks, strains, and breeds of animals.** For this level, *Co-1* and other mtDNA molecular markers are not quite suitable, since they are relatively little variable within the species (although there are exceptions). Usually more conservative in animals, nuclear DNA (nDNA) molecular markers are even less applicable at this level. However, microsatellite nDNA loci and single nucleotide polymorphisms are the most effective in identifying differences between animal populations, breeds, and stocks, as well as in their certification in higher organisms.

3. **Molecular markers in the field of medicine** have acquired the greatest importance, especially in the diagnosis of diseases (specifically, breast, prostate, and colon cancer, *etc.*). They are applied in a forensic science as well: aimed at excluding certain individuals from suspects. The scope of MMs also includes monitoring of genetic safety to assess the risks of usage of recombinant DNA and genetically modified products/objects in food and medical industries. There is a special review on the biomedical problems of DNA barcoding (Zhokhova *et al.*, 2018).

In addition to the obvious applied value in medicine and in the biodiversity description, the areas listed above are useful for the paradigms of general biology and evolutionary genetics, as well as for the scientific component of the iBOL program, being of a decisive importance in identifying species by DNA barcoding. As of 07 August, 2020, the iBOL database accumulates the research results as follows: samples of living organisms – 11,429,832; samples with barcodes – 8,466,913; and species identified by barcodes – 314,777 (The Barcode of Life Data System, 2021, see Taxonomy Browser). All this data is accompanied by documentation unified by the iBOL standards and is available to any user *via* the Internet. The contribution of the Russian Federation and RUS-BOL (Shtrikhkodirovanie zhivykh organizmov na osnove DNK, 2021) to research on DNA barcoding is reflected in the Barcode of Life Data System (BOLD) database: these are 42,174 records published, which form 7,972 barcode clusters represented by 263 organizations (laboratories and creative groups). The records made in BOLD refer to 27,320 species names, representing a total of 6,099 species. In terms of activity, Russia is in the middle of the list of program participants, at the level of Brazil and France.

Thus, on the merits of the above-mentioned request of the Government of Russia, it can be stated as follows: **up to date, the expert community does not have an exact answer to the question of the scale of GMOs usage in the country and the degree of genetic safety of their usage in several fields of industry, especially in semi-closed systems for growing plants and rearing of animals and other living beings in the Russian Federation**, as well as in other countries. The usage of MMs and a new regulatory framework will allow carrying out a more accurate monitoring of GMOs applying in agriculture and other industries in the Russian Federation and answering the questions raised by the government, as well as other important public requests regarding genetic safety.

List of genetic safety measures

- Genetic safety of food products. Carrying out large-scale research aimed at development of guidelines for the control of export-import food flows. Development of specialized MMs for monitoring genetic and environmental safety of GMOs usage. Development of recommendations for monitoring the usage of GMO products as food items, food additives, and drugs.
- Monitoring of invasions and hybridization. Ecological and genetic monitoring.
- Monitoring of gene pools of major agricultural crops and commercially important animals and fish. Monitoring of varietal (breed) diversity based on MMs.

- Monitoring of gene pools of human populations based on MMs and omics technologies, *inter alia* the usage of the latest instrumental base for whole genome sequencing, genome editing, transcriptome, proteomic, and other types of analysis to maintain the health of the nation.
- Funding in the amount of 150 million rubles *per year* for 5 years of a targeted federal program for the study and maintenance of biodiversity on the topic “DNA barcoding as the basis of the program for molecular, genetic, and bioinformatic description of biological diversity of living organisms in Russia. Genetic certification of especially valuable populations and species based on new methods for describing biological diversity: biobanking, environmental DNA, transcriptome analysis, and other approaches”.
- Prolongation of funding of the relevant topics of the Russian Science Foundation on the assessment of the biodiversity of living organisms and the elaboration of specific research and development projects.
- Organization of a system of monitoring by the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing – of GMO products as food items, food additives, and drugs, as well as food manufacturers and chain stores of the Russian Federation – based on molecular markers.

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**КРАТКИЙ ОБЗОР
ИЗУЧЕНИЯ ГЕНЕТИЧЕСКИ МОДИФИЦИРОВАННЫХ ОРГАНИЗМОВ
И ОЦЕНКА ПОТЕНЦИАЛЬНЫХ РИСКОВ ИХ ИСПОЛЬЗОВАНИЯ
ДЛЯ ПРИРОДНЫХ ВИДОВ**

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Представлены краткие сведения о генетически модифицированных организмах (ГМО), методах их создания, областях использования, потенциальных рисках применения, а также необходимости и сферах контроля их использования, в том числе применительно к водным организмам. Приведённые материалы позволяют заключить, что экспертное сообщество в настоящее время не имеет точного ответа на вопрос о масштабах использования ГМО в стране, а также о степени генетической безопасности их применения в некоторых сферах производства, особенно в полузамкнутых системах воспроизводства растений, животных и других объектов в Российской Федерации и за рубежом. Использование молекулярных маркеров генов и новая нормативная база позволят осуществить более точный мониторинг применения ГМО в сельском хозяйстве и других отраслях промышленности в РФ, ответить на запросы Правительства России и общества, а также на ряд других важных вызовов относительно генетической безопасности.

Ключевые слова: генетически модифицированный организм, ГМО, создание, применение, потенциальные риски, аквакультура, сельское хозяйство