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TECHNOLOGIES OF GENETIC MATERIAL USE RESTRICTION: TYPES, MOLECULAR-GENETIC BASE AND ETHICAL ANALYSIS OF THEIR APPLICATION

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Background. In order to save money, some farms use the seed obtained in the process of cultivation not only for sale, but also for sowing, which has not found supporters among companies engaged in the production of genetically modified seed. To protect their rights, the latter have created technologies to limit the use of genetic material, which are intended to be used for protection the intellectual rights to reproduce plants with a changed genotype. However, these technologies contain also a commercial component and violate a number of moral principles and international acts.

Aim. To describe the types of terminator technologies, their genetic and molecular basis and purpose. To assess a correspondence of their compliance with the international documents and norms.

Methods. Terminator technologies types, genetic bases and application and their analysis from the standpoint of international norms were studied. To achieve the goal, the methods of fact analysis, comparison and generalization were used.

Results. There are two types of terminator technologies (variety- and trait-specific), which are based on the interaction of three genes, which leads to the implementation of certain phenotypic manifestations. These technologies are used to implement nine goals in practice. It was found that the technologies for limiting the use of genetic material are both contradictory and consistent with a number of international legal acts, which did not make it possible to determine clearly the appropriateness of their use in agriculture.

Conclusions. Terminator technologies application is still a controversial fact since they are based on the duality principle: to carry simultaneously a positive and a negative manifestation for people.

Key words: terminator technologies, transgenes, intellectual rights, bioethics.

Given the realities of modern life, producers of plant products in pursuit of consumers are forced to reduce the selling price, reducing the cost of purchasing high-yielding plant varieties. A more efficient way is to use a part of the seed obtained after harvest for next year’s sowing, then the cost of its purchase is reduced to zero.

Genetic material use restriction technology (GMURT) or Genetic use restriction technology (GURT) is a development of US biotechnology companies whose main goal is to reduce the uncontrolled use of genetic material by farmers and ordinary citizens. It involves the first-generation seed sterility, i.e. the inability to obtain the second-generation offspring or specific traits manifestation. This protects the intellectual rights of breeding scientists, whose efforts have resulted in plants with altered genotype obtaining [1]. It is believed that the development of these technologies was influenced exclusively by the commercial component, because in this case the seed manufacturing companies will receive extra profits due to the annual seed or specific chemicals-inductors sale [2, 3]. The article is devoted to the review of existing terminator technologies, their production methods and the difference between their types analysis. Also, the prospects for these technologies use in different countries and ethical aspects related to their use are analyzed. The perspectives for the technology use in agriculture and the advantages and disadvantages that arise are estimated.

1. Technology types to limit the genetic material use

There are two groups of terminator technologies. The first one is V-GURT (variety-specific GURT), there are technologies to limit the genetic material use at the level of varieties, i.e. variety-specific technologies (or terminator technologies, or sterile seed technologies, or suicidal seed technologies) [3, 4]; the second group is T-GURT (trait-based GURT), there are technologies to limit the genetic material use at the level of traits, i.e. trait-specific technologies [5].

Historically, the V-GURT technology was the first, so both types are customary to call terminator technologies. The first patent for this technology was issued in 1998. According to the patent data [3, 6], the production of plants with sterile seed was due to the lethal gene activation during embryogenesis. The main goal of this patent was to ensure the intellectual property (seed) rights [7].

Trait-specific technologies for genetic material limiting are those that control gene expression that is associated with the encoding of a certain trait that remains latent until an external factor acts on the plant. The process is started only in a specific inductor presence, which is sold by the same companies that produce seed [8]. Examples of traits are: drought tolerance and tolerance to high salt content in the soil [3, 9], cryoresistance, tolerance and resistance to pests, tolerance to herbicides, stress [5], production of BAS with industrial application, flowering, germination, color, taste properties, etc. [7]. A feature of this technology type is the ability to "on"/"off" the trait, depending on the husbandry needs and environmental factors [5].

2. Genetic-molecular base of terminator technologies

The patented technology is based on the trigenic structure functioning (Fig. 1).

Molecular mechanisms are common to two types of technology. The gene-1 promoter, which carries information about the protein, which due to the mechanism of positive repression inhibits the functioning of the gene-2 promoter, is always active, so at rest the first gene is expressed. The second gene encodes a specific enzyme — recombinase. Recombinase acts on LOX sites. Because these sites are located on either side of the repressor sequence linked to the gene-3 through a substance encoded by the gene-3 itself, a gene repressing sequence is excised with it. Gene-3 is actually a gene-terminator that encodes the toxin RIP — ribosome inhibiting protein or another specific trait. Seed germination is suppressed by the gene-3 expression in plant embryos in the later development stages only, ensuring the germination of the parental seed grains [3, 4. 8, 10, 11].

2.1 Terminator technologies of the V-GURT type classification by molecular mechanisms of action 2.1.1. The first variation of V-GURT Gene-3 expression is limited by the blocking sequence. To activate the last gene, it is necessary to use an inducer (1.2) (Fig. 2*a*), such as tetracycline. The inducer prevents the gene-1 product binding to the gene-2, resulting in the activation of the gene-2 product, recombinase (Fig. 2*b*). After that, the RIP of the gene-3 is expressed (Fig. 2*c*), which leads to the sterile seed emergence in the second generation after the interaction of P-3 (LEA) with the gene-3 [3, 12].

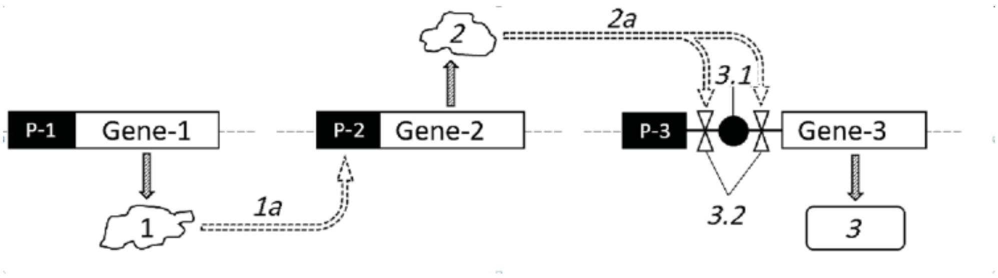

Fig. 1. **The general view on the terminator technologies (schematic representation) [2]:**
1 — protein-repressor of the gene-2 promoter; *2* — recombinase; *3* — a specific trait carried by the gene-3; *P-1, P-2, P-3* — gene-1, gene-2 and gene-3 promoters respectively, *1a* — the effect of the gene-1 product on *P-2*, *2a* — the effect of the gene-2 product on 3.2; *3.1* – the sequence of the gene-3 repressor, *3.2* — LOX sites

2.1.2 The second variation of V-GURT

In this case, gene-1 is inactive, i.e. the seed is immediately unviable (Fig. 3, *a, b*). Germination requires the action of an inducer, the function of which is completely opposite to the previous case (Fig. 3, *c*): tetracycline leads to the gene-2 repression, resulting in no cleavage of the gene-3 blocking sequence. In the next generation, sterile seed are obtained again [7].

2.1.3 The third variation of V-GURT. The least common technology used only for plants with vegetative reproduction (with root crops, tubers, plant organs: leaves, cotyledons, stems). The patent for this technology is owned by Syngenta company. Their patented mechanism ensures the presence of a constantly active gene that blocks the vegetative growth of the plant and seed formation. The action of an inducer is required to repress the gene (similar to the previous variation). The main purpose of this V-GURT variation is to increase the rest period of the plant to extend the shelf life [2].

2.2. Terminator technologies such as T-GURT classification by molecular mechanisms of action

2.2.1 The first variation of T-GURT Gene-3 is under the repressive protein influence (similar to the first variation of V-GURT), as a result of which the useful trait encoded in the gene is not manifested. Action of recombinase which in turn is limited by another protein action, is needed to remove the impact. Unlocking recombinase requires the chemical agent action that will inhibit the repressive action of the protein on gene-2 and allow it to be expressed, resulting in the manifestation of a gene-3 sign. In subsequent generations, gene-3 is inherited in an inactive state, so to express the sign, the treatment with an inductor must be performed annually [12].

2.2.2 The second variation of T-GURT The expression of phenotypic traits by the gene-3 is not limited to the action of a repressor (similar to the second V-GURT variation) associated with this gene, as it is removed by expressed recombinase because gene-2 is not inhibited by the gene-1 product. Thus, the sign remains "on" in a number of generations. Its inactivation requires a stimulus from the inductor, which is sold by the company-supplier of seed [2].

2.2.3 The third variation of T-GURT

This variation of terminator technologies is one of the most perspective and promising. Site-specific DNA recombination systems (gene deletion system) require recombinase and multiple repeats of nucleotide sequences. The term "recombination" means the DNA excision and subsequent association, which may result in integration, deletion or inversion of a gene fragment. The gene deletion system functions due to the combination of the bacteriophage recombination system P1 Cre/lox and *Saccharomyces cerevisiae* — FLP / FRT, which act on short sequences specific to each of the

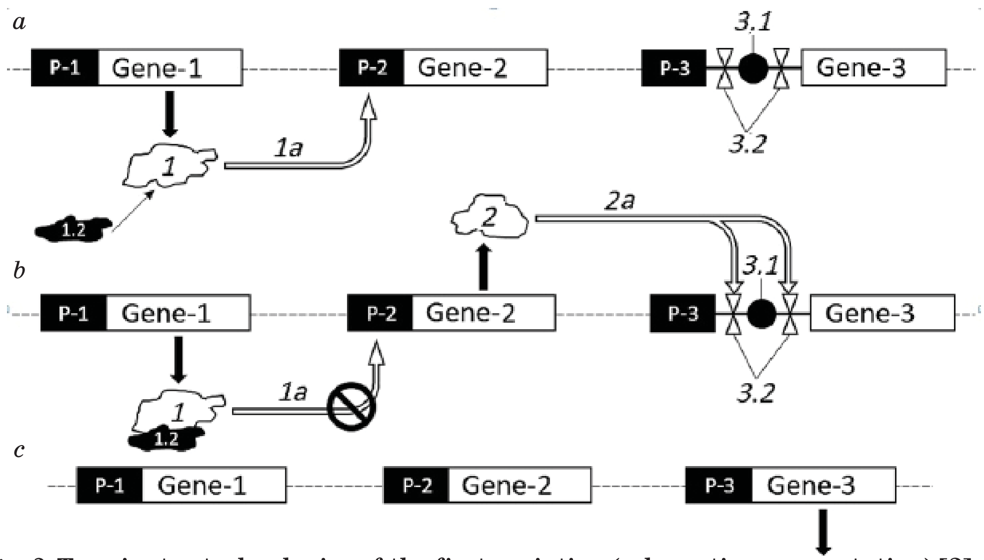

Fig. 2. **Terminator technologies of the first variation (schematic representation) [2]:**
1 — protein-repressor of the gene-2 promoter; *2* — recombinase; *3* — a specific trait carried by the gene-3; *P-1, P-2, P-3* — gene-1, gene-2 and gene-3 promoters respectively, *1a* — the effect of the gene-1 product on *P-2*; *2a* — the effect of gene-2 product on 3.2, *1.2* — *c*hemical inducer, *3.1* — the sequence of the gene-3 repressor, 3.2 — LOX sites

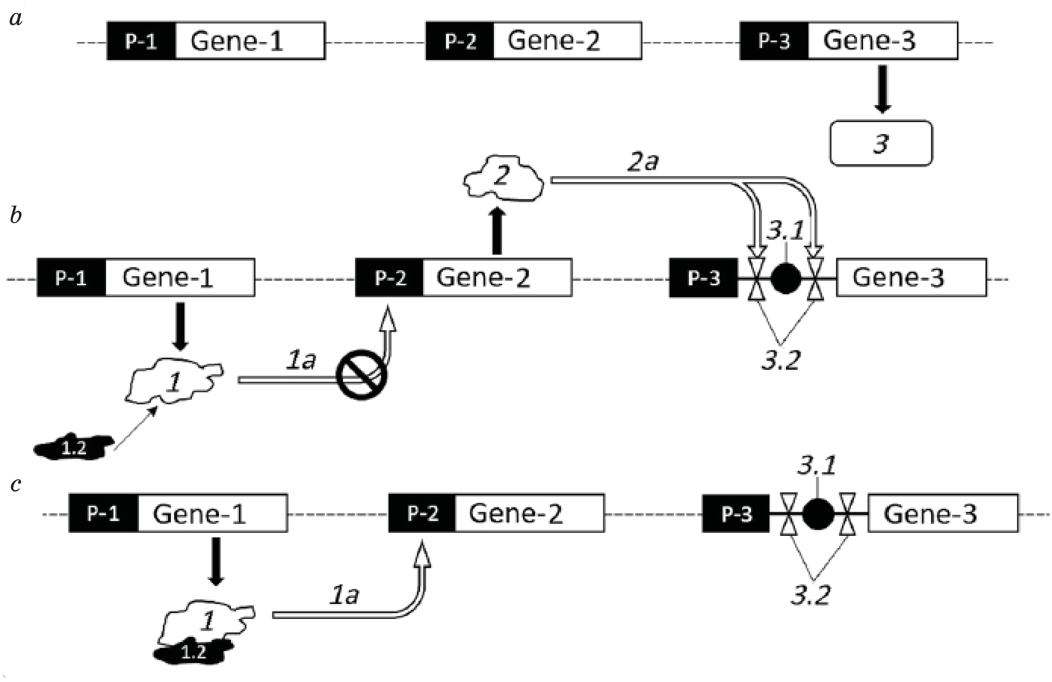

Fig. 3. **Terminator technologies of the second variation (schematic representation) [2] :**
1 — protein-repressor of the gene-2 promoter; *2* — recombinase; *3* — a specific trait carried by the gene-3; *P-1, P-2, P-3* — *g*ene-1, gene-2 and gene-3 promoters respectively, *1a — t*he effect of the gene-1 product on *P-2; 2a* — the effect of the gene-2 product on 3.2; *1.2 — chemical inducer; 3.1* — the sequence of the gene-3 repressor; *3.2* — LOX sites

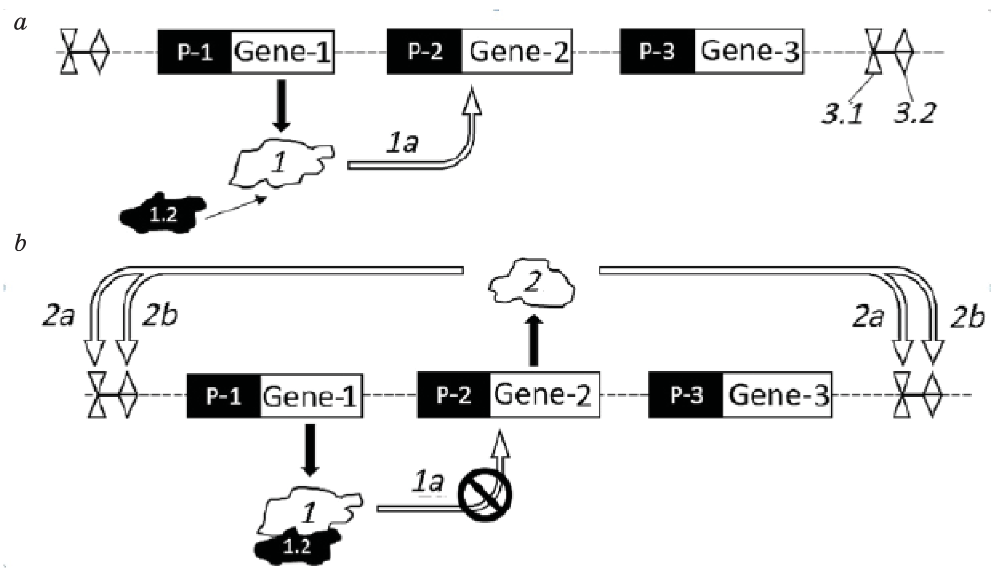

Fig. 4. **T-GURT terminator technologies of the third variation (schematic representation) [7, 17]:**
gene-1 — gene encoding the repressor protein of the gene-2 operator; *1.2* — chemical inducer of the gene-1 product; *gene-2* — restrictase Cre or FLP gene; *gene-3* — target trait gene; *P-1, P-3* — gene-1 and gene-3 promoters, respectively, *P-2* — specific promoter PAB5, *1a* — gene-2 repression, *2a* — LOX Cre sequence excision; *2b* — FLP sequence FRT cutting

systems separately with a length of 30-40 bases [3,we evaluated the system for foreign gene removal in a hybrid aspen clone, INRA 353-53 (*Populus tremula* × *P. tremuloides* 13, 14]. As a result, targeted insertion into identified and well-characterized parts of the gene and its removal is achieved [15]. The system is also used to extract gene markers in order to create markerless transgenic organisms [16].

Cre or FLP recombinase genes, which are under the control of tissue- or stage-specific promoters, and transgenes are administered between two direct repeats of loxP-FRT sequences, as this increases the efficiency of the recombinase gene. The expression product of the latter removes the functional transgenes contained between loxP-FRT, including the gene encoding it. In the case of seed or pollen-specific promoters application, such as PAB5 *Arabidopsis*, FLP protein should be produced only in the seed or pollen. The promoter is under the control of a repressor protein (Fig. 4*a*), as a result of which recombinase is inactive. Only the use of an external inducer (e.g. ethanol) relieves repression, and recombinase removes the sequence in certain places (Fig. 4*b*) [7]. Transgenes must be automatically removed from seed or pollen and accumulated exclusively in the vegetative organs and remain genetically stable. Excised DNA sequences (loxP-FRT sites, trait genes, repressor genes, and Cre and FLP gene) are destroyed by nonspecific cell nucleases and should be easily excised by recombinase [17]. Pollen and seed of transgenic plants must be produced as non-transgenic starting from the next generation and contain the loxP-FRT fusion sequence. That is, the transgene is expressed in the generative organs only in this generation, and without the inducer application in the following the desired trait will not be expressed [7, 17].

Terminator technologies application

Creating pure lines

It is used for those cultures that are propagated only by seed. Using biotechnological tools, genetically engineered seed of the parental forms PP (pure / inbred lines) are developed. One of the transgenic parents contains a LEA promoter, a cutting sequence, a blocking sequence and a lethal gene; the other contains a specific germination promoter and a recombinase gene. When crossing, hybrid offspring F_1 are obtained, which are sold to farmers [10].

Coercion of transgene flow

The leakage of transformed genes occurs due to the spread of viable pollen and seed over long distances by three factors: anthropogenic (transportation, sowing or harvesting), biotic (birds, fish, animals) or abiotic (wind, water). Due to the presence in the nature of weeds that are reproductively compatible with the cultures grown, the genes flow can lead to the emergence of herbicide-resistant offspring of weeds, the so-called "super-weeds" due to cross-pollination. Therefore, the use of terminator technologies can prevent this problem, due to the seed sterility in generation F_2 [11].

Increasing yields

Prolonged use of hybrid offspring for reasons of economy leads to heterozygous loci segregation and an increase in the homozygotes proportion, resulting in declining plant yields [18]. Therefore, the new seed application every year will lead to the maximal production level, within the genetically determined properties [19]. The increase in biomass and yield is due to morphological changes, increasing the duration of flowering time, etc. [20].

Seed market control

Because seed companies create genetically modified plant varieties based on terminator technologies, farms are forced to buy seed or chemicals annually to block or unlock a particular trait or lethal gene [21].

Other ways for use

– Intellectual rights defense of seed companies' specialists (mentioned above).

– Increasing the storage duration of plants with vegetative reproduction (mentioned above).

– Lines tracing, removal of selection cassettes, chromosomal engineering and translocation, specific cell depletion [22]. Thus, due to the mechanisms of genetic manipulation, glyphosate and bentazone-resistant rice varieties were constructed [23].

– Increasing the genetic diversity of many commercially important cultures [24]. Examples of plants that are improved by V-GRUT technologies are: soybeans, rice, cotton, corn [12], tobacco [2], flax, peanuts, sesame, coconut [25].

– Competition of giant manufacturing companies leads to increased investment in agriculture, which ultimately benefits farmers [19].

Ethical problems of terminator technologies

Moral analysis of technologies to limit the use of genetic material

The commercialization of transgenic plants seed is an incentive to increase the impact of intellectual property rights on the possibility to store and use seed. It has also led to the consolidation of the seed and agrobiotechnology industries, which has contributed to the globalization of intellectual property modes [25].

GMURT is a tool for monopolizing the seed market by giant corporations, which increases the dependence of farmers on GM seed and chemical inducers [26]. Agrochemical and seed firms strengthen their market position and influence on farms by concluding technology exchange agreements, thereby restricting freedom of choice when buying seed and reducing the availability of natural seed [27]. But in its principles, Monsanto did not take into account the public interest (especially the rights of farmers) and the state sovereignty [28]. The technology is funded by the US Department of Agriculture to increase its own seed production [4]. It is believed that such approaches can destroy traditional farming methods [29]. Lack of competition and the right to protect intellectual property has led to higher prices for GM seed, including cotton. As a result, Indian farmers began to sell their assets and go bankrupt. Someone takes more decisive steps — suicide. However, the authorities are actively trying to hide the relationship between mortality and rising prices [30].

In 2006, a movement against the use of terminator technology began. This has led to a moratorium on field trials, due to the lack of appropriate biosafety protocols and the commercialization of these technologies, but they continue to exist as a tool to protect against gene leakage [31]. Given the complexity of the genetic and molecular principles of GMURT, there is a possibility that an attempt at biological containment will only exacerbate the problem and the technology will not work. The technology itself creates risks of environmental contamination by transformed sterility genes or others, which can lead to unpredictable consequences [32].

In addition, plants genetic modifications lead to a decrease in the biodiversity of economically valuable varieties, especially in developing countries [29].

The Ukrainian branch of the international seed company Syngenta distributes seed and plant protection products in Ukraine, thus providing Ukrainian farmers with high-quality seed of corn, rape, sugar beet, sunflower, potato, soybean, cereals (wheat, barley, oats, etc.), vegetable crops (tomato, cucumber, zucchini, cabbage, etc.), berry crops, drupaceous fruits (apricot, peaches, plums, cherries, etc.) and others [33]. Syngenta introduces drought- and pest-resistant seed, high-yielding plant varieties, investing heavily in their creation. Therefore, they are obliged to protect their intellectual and property rights through the technology commercialization, which is specified in the Code of Conduct of the company, as well as guided by the principles of rational use of plant biodiversity [34].

Analysis of technologies for restricting the use of genetic material from the standpoint of international legal acts

The UN organization Convention on Biological Diversity [35] generally condemns the use of terminator technologies in the national economy. Producers and defenders of GMURT comply with the provisions set out in Articles 3, 8 (e), 10 (a, b), 16.1, 16.3, 16.5: do not cause significant damage to the environment, contribute to the preservation of protected areas and take measures to prevent destabilization of the ecosystem — V-GURT creation, provide access to the created technologies to the developing countries, at the expense of their sale, create patents in the field of intellectual property; at the same time violate the provisions of Articles 8 (h, j), 10 (c), 11, 15.2, 16.2, 19.2 and 19.3, as they introduce genetically modified plant species that can harm the ecosystem, prevent the use of biological diversity — T-GURT application indirectly influences on the reducing the use of traditional farming methods, which contributes to the biodiversity reduction, restricts access to genetic resources, especially for farmers in developing countries — due to the need to buy seed or inductors.

The use of terminator technology is contrary to Articles 1 and 12 of the International Covenant on Economic, Social and Cultural Rights [36] in the following provisions: the ability to freely dispose of natural resources, violates the right to mental health, but nevertheless complies with Articles 11 and 15 about the need to use the methods of science to improve methods of combating hunger and the ability to use the results of scientific progress.

The International Convention for the Protection of New Varieties of Plants [37] approves the GMURT application as they prevent unauthorized use of new plant varieties by third parties. The sale of such seed is regarded as the need to return the financial investment invested in the creation.

Terminator technologies as ways to protect intellectual property can be considered the subject of protection of the World Intellectual Property Organization, as the Convention on its establishment refers to the assistance of member states in developing methods of intellectual property rights protection [38].

The International Treaty on Plant Genetic Resources for Food and Agriculture contains a list of farmers' rights, namely the preserved seed use, exchange and sale, which terminator technologies ignore [39].

From the point of view of people freedom in economic activity, GMURT appear in a negative sense, which is reflected in the need for annual purchase of special substances or the seed themselves. That is, Article 22 of the Universal Declaration of Human Rights is violated [40].

Given the impact of technology on farmers' bankruptcies, such a scenario is likely to recur in other countries. Thus, there is an inconsistency with Article 1 of the European Social Charter [41].

Conclusions

There are two types of technologies for limiting the use of genetic material (variety-specific and trait-specific), which have different goals: the first contains a gene that leads to the sterility of second-generation seed; gene of the second type encodes information about certain traits. Each of the varieties contains 3 variations of technologies, which differ at the genetic level and by application, as a result of which the phenotypic manifestation and interaction with genes are also different.

Technologies for limiting the use of genetic material have been used in the development of new genetic engineering methods and old errors correction, increasing the harvest amount and extending the shelf life of ornamental and economically valuable plants etc.

Terminal technologies are based on the principle of dualism: they are both a way to protect intellectual rights and a method of controlling the seed market by companies. Technologies do not fully comply with the provisions of the International Treaty on Plant Genetic Resources for Food and Agriculture, the Universal Declaration of Human Rights, the European Social Charter; are protected by the International Convention for the Protection of New Varieties of Plants and the World Intellectual Property Organization; at the same time, it coincides with and contradicts the provisions of the UN organization Convention on Biological Diversity and the International Covenant on Economic, Social and Cultural Rights.

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ТЕХНОЛОГІЇ ОБМЕЖЕННЯ ВИКОРИСТАННЯ ГЕНЕТИЧНОГО МАТЕРІАЛУ: ТИПИ, МОЛЕКУЛЯРНО-ГЕНЕТИЧНА БАЗА ТА ЕТИЧНИЙ АНАЛІЗ ЇХ ЗАСТОСУВАННЯ

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Вступ. З метою економії фермерські господарства використовують отримане в процесі культивування насіння не лише для реалізації, але й для посіву, що не здобуло прихильників серед компаній з виробництва генетично-модифікованого насіння. Ними було створено технології обмеження використання генетичного матеріалу для захисту інтелектуальних прав на відтворення рослин зі зміненим генотипом. Проте ці технології містять ще й комерційну складову та порушують низку етичних принципів і міжнародних актів.

Метою роботи було ознайомити із видами термінаторних технологій, їхньою генетично-молекулярною базою та призначенням, а також встановити їх відповідність міжнародним документам і нормам.

Методи. Досліджували види, генетичні основи і застосування термінаторних технологій та здійснювали їх аналіз з позицій міжнародних норм. Для досягнення мети використовували методи аналізу фактів, порівняння та узагальнення.

Результати. Існує 2 види термінаторних технологій (сорто- та ознако-специфічні), в основу яких покладено взаємодію трьох генів, що сприяє реалізації тих чи інших фенотипічних виявів. Встановлено, що технології, які обмежують використання генетичного матеріалу, водночас і суперечать, і відповідають низці міжнародних правових актів, що не дає змоги чітко визначити доцільність їх використання в сільському господарстві.

Висновки. Використання термінаторних технологій досі залишається суперечливим фактом, оскільки в їх основу покладено принцип дуалізму: позитивний та негативний вплив на людей.

Ключові слова: термінаторні технології, трансгени, інтелектуальні права, біоетика.

ТЕХНОЛОГИИ ОГРАНИЧЕНИЯ ИСПОЛЬЗОВАНИЯ ГЕНЕТИЧЕСКОГО МАТЕРИАЛА: ТИПЫ, МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ БАЗЫ И ЭТИЧЕСКИЙ АНАЛИЗ ИХ ПРИМЕНЕНИЯ

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Вступление. С целью экономии фермерские хозяйства используют полученные в процессе культивирования семена не только для реализации, но и для посева, что не нашло сторонников среди компаний, занимающихся производством генетически модифицированных семян. Ими были созданы технологии ограничения использования генетического материала для защиты интеллектуальных прав на воспроизведение растений с измененным генотипом. Однако эти технологии содержат еще и коммерческую составляющую и нарушают ряд этических принципов и международных актов.

Цель. Ознакомление с видами терминаторных технологий, их генетически-молекулярной базой и назначением, а также установление их соответствия международным документам и нормам.

Методы. Исследовали виды, генетические основы и применение терминаторных технологий и осуществляли их анализ с позиций международных норм. Для достижения цели использованы методы анализа фактов, сравнения и обобщения.

Результаты. Существует 2 вида терминаторных технологий (сорто- и признак-специфические), в основу которых положено взаимодействие трех генов, приводящее к реализации тех или иных фенотипических проявлений. Установлено, что технологии, которые ограничивают использование генетического материала, одновременно и противоречат, и соответствуют ряду международных правовых актов, что не дает возможности четко определить целесообразность их использования в сельском хозяйстве.

Выводы. Использование терминаторных технологий до сих пор остается спорным фактом, поскольку в их основу положен принцип дуализма: положительное и отрицательное влияние для людей.

Ключевые слова: терминаторные технологии, трансгены, интеллектуальные права, биоэтика.