ODDÍL 2. PEDAGOGIKA, VÝCHOVA, FILOZOFIE, FILOLOGIE

*§2.1 DIRECTIONS OF USE FOR GENETICALLY MODIFIED PLANTS IN AGRICULTURE (***Stoliar S.,** Polissia National University, **Trembitska O.,** Polissia National University, **Klymenko T.,** Polissia National University)

Introduction. Cultivated plants suffer from weeds, rodents, insect pests, nematodes, phytopathogenic fungi, bacteria, viruses, adverse weather and climatic conditions, which significantly reduces the yield of agricultural plants. Along with the protection of plants, there is the task of increasing the productivity of agricultural crops, their food (fodder) values, and the task of creating plant varieties.

It all began in 1972, when Paul Berg (Stanford University, USA) for the first time combined two genes isolated from different organisms in a test tube. And he received a "molecular" hybrid, or recombinant DNA, which could not have formed by itself under natural conditions [1].

Over the past 15 years, 25,000 different transgenic crops have been field tested, of which: 40 % are resistant to viruses, 25 % are resistant to herbicides, and 25 % are resistant to insecticides; crops of transgenic herbicide-resistant plants (corn, soy, cotton) in the whole world amount to more than 28 million hectares [2].

GM plants are plants into which foreign genes are inserted for the purpose of developing resistance to herbicides and pesticides, increasing resistance to pests, and increasing their yield. They are obtained by introducing a gene from another organism into the DNA of a plant. Donors can be microorganisms, viruses, other plants, animals, and even humans.

For example, a frost-resistant tomato was obtained, in the DNA of which the gene of the North American sea flounder is embedded. A scorpion gene was used to create a wheat variety resistant to drought. The first transgenic products were developed by Monsanto (USA). The first plantings of transgenic cereals were made in 1988, and in 1993, the first products with GM components appeared on sale [3].

Weed control: herbicide-resistant crop varieties

Resistance to herbicides is an important trait for agricultural crops, which makes it possible to significantly reduce production costs and increase yields due to more effective weed control.

With the help of traditional selection, it is extremely difficult to breed herbicide-resistant varieties. There are no varieties of agricultural plants resistant to the most widely used herbicides of total action, glyphosate and glifosinate. Genetic engineering solves this problem quite simply – by transferring genes from herbicide-resistant microorganisms into the genetic material of plants. With this in mind, the first genetic engineering research was funded mainly by the largest multinational companies that specialized in the production of the above-mentioned pesticides, as they were primarily interested in creating plant varieties resistant to their products. Due to the relatively simple nature of the genetic control of this trait, a good study of the relevant genes makes it much easier to obtain herbicide-resistant GM plants than, for example, drought- or salinity-resistant ones [4].

As a result of studying the mechanism of action of herbicides, it was found that most often they affect one enzyme important for plant metabolism, binding to it and thus weakening its activity. This leads to disturbances in the growth and development of plants treated with the herbicide, and they die.

Tolerance to herbicides is usually caused by a single gene mutation. Two main mechanisms of stability are known. The first

of them - "target mutation" – is associated with a change in the sequence of amino acids in the part of the enzyme molecule in which it binds to the herbicide. As a result, the herbicide "does not react" to its target, the enzyme retains its activity, and the organism becomes tolerant to the action of the herbicide. This mechanism is typical for resistance to such herbicides as glyphosate (Roundup), sulfonylurea, imidozolinone, etc. The second mechanism is related to the production of enzymes in resistant organisms capable of deactivating the herbicide, for example by attaching any chemical radical to it (acetyl group, nitrate ion, etc.). This mechanism works in organisms tolerant to the herbicide glufosinate ammonium [1].

Soybean resistant to the herbicide glyphosate is the undisputed leader among all transgenic crops. The appearance of GM varieties led to a real revolution in soybean cultivation technology. The fact is that cultured soybeans develop quite slowly in the early stages. The competitiveness of adult plants is also low. This means that it is practically impossible to grow an acceptable crop of this crop without the use of herbicides.

The "target" in the plant is the enzyme 5-enolpyruvylshikimate-Z-phosphate synthetase (EPSPS), which plays an important role in the synthesis of aromatic amino acids (tyrosine, phenylalanine, and tryptophan). Under the action of the herbicide, plants resistant to it show symptoms of nitrogen starvation (due to a lack of the specified amino acids - the "building material" for protein synthesis), and they die within two weeks. It is worth noting that glyphosate belongs to a new generation of herbicides that are relatively safe for human health and the environment, because its "target" is only found in plants, fungi and bacteria and is absent in animals. Glyphosate breaks down relatively quickly (about a week) after getting on plants or soil.

In some bacteria, EPSPS coding genes have been found that carry point mutations. The result of the mutation is the

replacement of one amino acid in the area of the enzyme where it binds to the herbicide glyphosate. In this regard, the herbicide loses its ability to deactivate such a mutant enzyme, and the bacterium becomes resistant to its action.

Several EPSPS genes with a "target mutation" were isolated and cloned: aro A from bacteria of the genus Aerobacter; sml from Salmonella; cp4 from Agrobacterium. The last of these mutant genes, i.e. gene cp4 from the soil bacterium Agrobacterium tumefaciens CP4, is incorporated into transgenic commercial soybean varieties grown around the world. The genetic construct, created by recombinant DNA technology to transfer this gene into plants, also contains the CaMV35S promoter from Cauliflower mosaic virus, the terminal sequence from the nopaline synthetase gene of A. tumefaciens, and a small sequence from Petunia that encodes a chloroplast transit peptide required for delivery mutant EPSPS to chloroplasts – the place of synthesis of aromatic amino acids in the cell. To transfer this design into the genetic material of soybeans, the method of ballistic transfection was used – "bombardment" of cells with the help of a "gene gun" [2].

Transgenic soybeans do not have selective antibiotic resistance genes because the glyphosate resistance gene itself can be used as a selective one. About a thousand different varieties of glyphosate-resistant soybeans, which are grown on different continents, were obtained with the help of traditional selection, in which a single plant with the genetic engineering modification described above was used as the source of the mutant EPSPS gene. Thus, GM soybean varieties differ from conventional ones only in that they produce two types of the same EPSPS enzyme. The first is your own, which can bind to the herbicide, and the second is introduced by the bacterium, which does not bind to the herbicide. It is the presence of the latter that makes these varieties resistant to the action of glyphosate, as it preserves their life after

the crops are treated with the herbicide. The very fact that the bacterial EPSPS is capable of performing the functions of a plant analogue testifies to their significant similarity, including in the sense of safety for human health [1].

The second novel element is a chloroplast transit peptide that delivers transgenic EPSPS to chloroplasts, which is a short chain of amino acids that is rapidly degraded during food digestion.

Control of the number of insect pests of agricultural crops

Another important problem of crop production is increasing the effectiveness of controlling the number of insect pests of agricultural crops. For this purpose, pesticides are most often used - chemical or biological (derived from microorganisms that produce substances toxic to insects). The use of the latter is better from the point of view of safety for human health and the environment. At the same time, the effectiveness of chemical means of plant protection remains much higher than that of biological means. Areas of combined application are also being developed, along the lines of ecological and organic production technologies.

The so-called Bt-toxin, which is obtained by cultivating soil bacteria Bacillus thuringiensis, is widely used as a biopesticide. These bacilli were described at the beginning of the last century, and in the 1930s it was established that they are able to produce products toxic to insects, which have a high selectivity of action. This means that the Bt protein isolated from a certain strain of the bacillus is able to destroy a specific type of insect (eg, beetles) and does not affect others (eg, butterflies, bees). The selectivity is due to the specific mechanism of Bt-protein toxicity. If the drug enters the digestive tract of an insect sensitive to it, it undergoes transformational changes: under the action of a certain proteolytic enzyme in an alkaline environment (pH 7.5–8.0), a small part (approximately a third) is separated from the original protein

molecule, which is active form of this protein. Only it is able to attach to specific receptors in the middle part of the insect's digestive tract and cause cell lysis. The insect stops feeding, the body becomes dehydrated and, eventually, its vital activity ceases [4].

In insects insensitive to certain Bt-protein preparations, the described processes do not occur, and the Bt-protein is simply digested in them. Given that warm-blooded animals and humans have a different digestive tract than insects and have different proteolytic enzymes, Bt protein usually does not pose a threat to these organisms. In addition, the Bt protein is a very unstable protein that easily denatures during heating and is quickly digested by gastric juice. In the almost fifty-year history of using preparations based on Bt-protein, not a single case of allergies or its toxicity to humans, including employees of the enterprises where it is produced, has been noted.

Since the 1960s, biological preparations based on Bt protein (bitoxybacillin, lepidocide, colepterin, dendrolin, baciturin, etc.) have been widely used in agriculture and forestry to combat insect pests. Along with a number of advantages (non-toxicity, non-allergenicity, high selectivity of action, rapid destruction in an acidic environment and under the influence of ultraviolet rays, inability to accumulate in the plant and soil), biological preparations at the same time have a significant drawback that reduces their effectiveness: they are able to protect the plant only for a very short time time. Genetic engineering made it possible to solve this problem. The bacterial gene responsible for the production of the Bt protein was isolated from the DNA of bacteria, cloned, and in some cases significantly modified in its entirety for the artificial synthesis of its individual active fragments, combined with the necessary regulatory elements and embedded in various types of agricultural plants. The most commonly used variants of Bt genes are cryIA (b) from B. thuringiensis v. kurstaki (for corn), cryIA (c) from

B. thuringiensis v. kurstaki (for cotton), cryIIIA from B. thuringiensis v. tenebrionis (for potatoes) [2].

Especially high efficiency of transgenic Bt-protein is noted for corn and cotton. Pests of these crops - the larvae of the European corn borer, cotton bollworm and pink bollworm - live on the surface of the plant for a very short time. Then they penetrate the tissues of the plant and gnaw through the passages there, thus causing great damage to the health of the plants and the harvest. Considering the fact that in transgenic varieties Bt-protein is formed in all green tissues of the plant and is constantly present there, this enables the plant to protect itself from pests throughout the growing season. At the same time, the transgenic Bt protein is highly effective at extremely low concentrations. Bt protein is completely absent in mature grain and silage: it cannot be detected even with the most sensitive analytical methods.

GM varieties resistant to insect pests are more advanced products of genetic engineering compared to the first herbicide-resistant forms. During their creation, more precise mechanisms were used to regulate the activity of transgenes due to the use of non-viral promoters, but plant promoters. Thus, in Bt-corn, the promoter of the phosphoenolpyruvate carboxylase gene of the same corn is used, which ensures the expression of Bt-genes exclusively in the green tissues of the plant (leaves, stems). Thanks to this, there is no Bt protein in mature grain and silage. Another promoter was used to create Bt potatoes - ats 1A of the small ribulose-1,5-bisphosphate carboxylase subunit of Arabidopsis Arabidopsis thaliana (a small weed of the cruciferous family). The Bt gene, regulated by a photosensitive promoter, is expressed 100 times more strongly in the light than in the dark. Therefore, 100 times less Bt protein is produced in tubers than in leaves (0.09–0.053 μg of Bt protein per 1 g raw weight of tubers). Therefore, neither transgenic potatoes nor transgenic corn contain the products of the integrated bacterial gene in their crop.

According to their consumer properties, they are completely identical to the varieties obtained by traditional breeding methods [3].

Protection against viral diseases of agricultural plants

Viral diseases are the cause of quite significant crop losses for a number of crops, primarily those that reproduce vegetatively, as well as pumpkins, tomatoes, and some others. The development of fundamentally new approaches in the fight against viral diseases is of great practical importance on a local and global scale for the preservation of the biodiversity of agricultural crop varieties, which are characterized by unique properties, but have disappeared from production due to the spread of dangerous phytopathogens.

Modern genetic engineering technologies for creating virus-resistant plant varieties are based on the use of the so-called cross protection method. It is based on the phenomenon of increased resistance of plants to aggressive forms of a certain virus, provided that they were previously infected with a less harmful form of the same type of virus. The mechanism of this phenomenon has not been fully elucidated, but it is widely used in Japan to protect tomatoes from damage by tomato and cucumber mosaic viruses, in Brazil to protect citrus fruits, papaya, zucchini, etc.

The following approaches are used for the genetic engineering of virus-resistant forms for the purpose of safety: use CP genes, which are previously modified in such a way that they cannot be transferred from plant to plant; isolate CP genes from natural "non-transmissible" strains; operate with genes from strains unable to infect plants in natural conditions; manipulate shortened CP genes, which code for the formation of defective, non-functioning CP proteins. It became possible to provide protection against viruses even in the case of the insertion of a defective SR gene, when the information RNA formed by its reading is not capable of translation.

Of all the variety of virus-resistant forms obtained, few have been approved for commercial use: papaya, resistant to mottle virus, two forms of zucchini, resistant to several viruses, and potato varieties with complex resistance to the Colorado potato beetle (Bt gene) and to one of the potato viruses: -virus (PVY) or leaf curl virus (PLRV).

The described genetic engineering technology for protecting plants from viruses makes it possible to obtain varieties that are identical in their consumer properties to the varieties of traditional selection. For a long time, people have been safely consuming products of transgenes of CP proteins, because these viral proteins are constantly present in food made from potatoes, zucchini, etc. Moreover, in ordinary varieties, the concentration of these proteins can be tens or even hundreds of times higher than in transgenic forms, because they are not resistant to viruses and therefore accumulate them in their tissues.

International relations in the cultivation of GM plants

The American Agency for International Development (USAID) has been active in the field of biotechnology for more than ten years, considering it as one of the parts of a broader strategy to promote the development of agriculture. USAID-supported science and technology research focuses on both new genetically engineered plant and animal vaccines, as well as less controversial approaches, such as molecular markers, that aid in traditional breeding programs.

In addition, USAID provides technical assistance in the development of the biotechnology regulatory system, which promotes the safe development and application of biotechnology; in solving problems related to the protection of intellectual property rights during the transfer of biotechnology; supports local organizations in Africa to which the public addresses concerns about biosafety.

The USA - the world's largest producer and consumer of GMOs - is the leader both in terms of cultivated areas and the degree of acceptance of transgenic food by society. GM crops are widely used, accounting for 40% of the country's corn, 81% of soybeans, 65% of canola and 73% of cotton, and these numbers continue to grow.

GM products are used in the production of both food for humans and feed for animals without the requirement of special labeling if transgenic sources are present in the products.

The spread of GM soybeans is facilitated by the proximity of Argentina, whose government supports the cultivation of GM crops and where the share of transgenic soybeans is 90%, and corn and cotton – 50 %.

After a five-year hiatus, Europe has come close to allowing the commercial cultivation of GM crops. In 1998, after the EU adopted rules for the use of GM products, France, Italy, Denmark, Greece and Luxembourg banned GM products. The EU has now adopted new rules for the certification and labeling of GMOs and has softened its position significantly, even as individual states insist on introducing additional restrictions on GM crops. In general, since 1998, only Spain has used a limited permit for the cultivation of GM crops, where small amounts of GM corn are grown.

The Philippines became the first Asian country to approve the cultivation of GM crops, starting with insecticide-producing Bt corn in December 2001. This precedent is expected to give impetus to the cultivation of GM rice in this region.

In China, the cultivation of GM plants was in full swing until 2000, when the government suddenly imposed restrictions. It was believed to be a reaction to the company launched by opponents of GMOs in the West. But some American experts believe that the country simply took a time-out and is engaged in breeding its own GM varieties, which would be comparable in

yield to foreign ones. Currently, half of Chinese cotton is transgenic; its main supplier is Monsanto.

Japan has made agrobiotechnology one of the priorities of its research budget, despite the fact that the use of GM products is met with strong public resistance. 38 GM products are approved for commercial use, another 55 have already passed food safety studies at the Ministry of Health, but none have found commercial use due to lack of demand.

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