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Agriculture of a New Generation

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The Nobel Prize in Chemistry for 2020 was awarded to Emmanuelle Charpentier and Jennifer A. Doudna for "the development of a method for genome editing". Over the past 10 years this method has been successfully entering various fields of science and is gradually conquering the world as an opportunity to solve intractable problems.

The genetic revolution – the genome editing method CRISPR/Cas9 is a precise intervention at DNA level that is capable of changing the code of life in just a few weeks. The genetic scissors, as this technology is called, will in future have an enormous impact on the life sciences, completely transforming methods of treatment in medicine for dangerous and hereditary diseases.

But not only in medicine; in agriculture as well this method reveals new research horizons that will completely change our attitude towards animals and agricultural crops.

It is no longer a utopia, a world in which plants intended for feeding the population will be able to withstand extreme climate change and will be resistant to increasingly aggressive pests.

Will agricultural science succeed in creating a more responsible and secure era for consumers, with a healthy and affordable food chain that will operate with fewer resources and will spare the environment?

A new-generation agriculture

According to data from the Food and Agriculture Organization (FAO), nearly 40% of the plant crops serving as staple food for the population are destroyed annually by various plant diseases and pests. This is particularly true in poor regions where agriculture is the main livelihood of the people; the lack of high-quality and affordable food leads to severe economic and social consequences. An additional factor that contributes to the compromising of global food reserves is the globalization of trade, which leads to the uncontrolled spread of invasive species.

The deteriorated plant health environment, caused by climate change, human activities and the improper use of pesticides, is also a key factor not only for the increase in dangerous diseases and pests affecting cultivated plants, but also for the reduction of biodiversity. Therefore, in recent years the public and private sectors have been investing more and more in initiatives for integrated pest management, scientific research and innovative technologies that focus above all not on the consequences, but on the causes and the possibilities for their prevention.

Plant genetics is providing an ever-richer range of options for prevention in the field of agriculture. The aim of genetic modification is to obtain lines of agricultural plants with advantages over classical ones: improved nutritional qualities (for example, enriching rice with carotene – the precursor of vitamin A); resistance to pests and diseases; tolerance to herbicides; increased tolerance to drought or saline soils.

Prevention through genome manipulation – CRISPR/Cas9

Unexpectedly, the answer to all these problems in modern agricultural science lies in the seemingly tiny DNA molecules, whose potential turns out to be unlimited. As early as 1953, the foundations of modern biotechnology were laid with the application in laboratory conditions of *restriction enzymes*, which cut genetic material. Since then, numerous different methods for genome manipulation have been introduced. The revolutionary step in genetics is the introduction of a tool that allows rapid and precise editing of the genome. CRISPR/Cas ("CRISPR") is borrowed from processes that occur in bacterial cells. It is a mechanism that enables bacteria to protect themselves from viral attacks, consisting of two parts – a unique imprint of the virus (encoded in CRISPR) and an enzyme (Cas) that has the ability to cut both strands of DNA. When attacked by a known virus, bacteria use this imprint to direct Cas to its genetic material. Once cut, it is inactivated and the viral attack is prevented. The resulting change can delete or replace specific DNA segments, thereby enhancing or deactivating certain traits.

Along with the advantages of this method in fields such as pharmacy, gene therapy and the treatment of diseases such as HIV, malaria, cancer, diabetes, etc., CRISPR technology is increasingly successfully entering agriculture as well.

Precise genome editing is of enormous interest to the agricultural sector, because everyone knows how much time and effort it takes to create new resistant varieties. There are already numerous crops with improved agronomic performance – rice, wheat, oranges, tomatoes and others that are resistant to pathogens; maize that withstands drought; tomatoes with increased yield. In addition to benefits for farmers, there are benefits for the environment, as fewer resources are used to obtain production, and pesticide use is reduced. The end consumer also benefits, since active work is underway to improve the nutritional value and quality of products. For example, it is entirely possible to control the percentage of gluten in wheat and achieve results of – 85% lower gluten content. And in Asia, research is increasingly being conducted to create rice with increased amylase content, which breaks down complex carbohydrates and converts them into monosaccharides such as glucose. Amylase is an enzyme present in human saliva and plays an active role in the proper glucose metabolism of the body.

Apples resistant to fire blight

One of the latest studies on the CRISPR/Cas method using *Agrobacterium tumefaciens* was published in the Journal of Plant Biotechnology in 2019. The bacterium *Erwinia amylovora*, which causes the disease fire blight in apple, induces infection in the fruit through the DspA/E effector, which interacts with the apple susceptibility protein MdDIPM4. Scientists use CRISPR/Cas9 to create a defective MdDIPM4 protein, which is introduced into apple (cv. Gala and Golden Delicious) via *Agrobacterium tumefaciens.* In this case, the interaction between classical breeding using the bacterium *A. tumefaciens* and revolutionary methods in the creation of new varieties is of particular interest. The bacterium *Agrobacterium tumefaciens* has the ability to transfer DNA into plant cells. Its function in the overall process is to infect a large number of plant species and to induce the formation of plant

tumors in which it develops. The tumors are actually caused by a plasmid of the bacterium called Ti (from *tumor-inducing* in English). Once the plant is infected, the Ti plasmid is transferred from the bacterial cell into a plant cell, integrates into its genome and causes its malignant transformation. The Ti plasmid is not carcinogenic

for animals and humans, and based on it, vectors are created for cloning and expression of foreign genes in plant cells. Through a combination of the two breeding methods in apple, a total of

57 transgenic lines with 75% editing efficiency were obtained. Seven edited lines with loss of function of the MdDIPM4 protein were exposed to fire blight, and the results showed a significant reduction in susceptibility to the disease compared to the control. The results of the study demonstrate the development and application of CRISPR-Cas9 for the creation of gene-edited apples with a minimal footprint of exogenous DNA.

Wheat – the queen of genetic modification

At the other end of the world, drought is not a problem for agricultural crops, and for years varieties resistant to long periods without a drop of rain have been developed. However, prolonged rainfall in Japan often completely destroys farmers’ harvests.

Researchers there are working on a new wheat variety suitable for regions with higher precipitation. With the help of the CRISPR-Cas9 system, they are developing wheat that at a later stage leads to the production of higher-quality flour. For their experiment, the Japanese researchers use a variety from arid zones that is sensitive to moisture. In the case of heavy and prolonged rainfall before harvest, the seeds often germinate in the ears, which subsequently leads to low-quality flour for the food industry. By applying CRISPR-Cas9 via *Agrobacterium*, the team creates wheat lines with a non-functional Qsd1 gene, which regulates seed dormancy or germination. After eight transformations, one of the attempts turned out to be successful. The new variety was crossed with a wild-type wheat to obtain a mutant without transgenes. The resulting plants were irrigated for a week and only 20-30 percent germinated, whereas almost all ordinary wheat seeds exposed to the same conditions germinated. In this case, genome editing and the creation of a new wheat variety resistant to rain took scientists only about one year. By comparison, with conventional breeding techniques a similar development would take approximately 10 years. In classical genetics, scientists use the particle bombardment method (gene gun), in which microscopic particles, e.g. gold, are coated with DNA. Then, under high pressure, the DNA-coated particles must be introduced into the recipient plant. The desired results are awaited for years and are not always as precise and predictable as with the combination of CRISPR-Cas9 and *Agrobacterium.* Of course, not every wheat variety responds to infection with *Agrobacterium* bacteria.

This problem has been corrected by a team of specialists from the Shandong Academy of Agricultural Sciences, China, who successfully targeted wheat genes, opting for CRISPR-Cas9 delivered through *Agrobacterium* – genetic transformation. Thus, they managed to improve the quality characteristics of wheat by using bacteria for more precise insertions of the CRISPR-Cas9 genome editing complex.

Tomatoes – a true genetic miracle

An international team of scientists from Brazil, the USA and Germany created about a year ago a tomato using CRISPR-Cas9 genome editing. The new tomato variety, which has an increased lycopene content, was developed from a wild plant and only within a single generation.

The researchers used as a parental species *Solanum pimpinellifolium* – a wild tomato from South America and ancestor of the modern cultivated tomato, whose fruits are the size of peas and the yield is minimal, but they are highly aromatic and their lycopene content is impressive.

The international team of experts modified the basic wild tomato by applying CRISPR-Cas9 genome editing, with the resulting plants carrying small genetic modifications in six genes that are key to tomato domestication.

The modified tomato has fruits three times larger than the wild type. This corresponds to the size of cherry tomatoes. It has 10 times more fruits, and their shape is oval, unlike the round wild fruits (an important trait, because in case of rainfall round fruits crack faster than oval ones). The plants also have a more compact growth habit. The new tomato variety has a very high content of the carotenoid pigment lycopene, which is a powerful antioxidant and protects the body from oxidative stress. Thus, the selected plant has twice the content of this beneficial pigment compared to its wild parent and five times more than its modern counterparts – cherry tomatoes.

According to an article published in January 2019 in *Trends in Plant Science*, with the new genome editing techniques it is possible to create a tomato that competes with some of the hottest peppers. The results of whole-genome sequencing in tomatoes show that this vegetable crop has the genes for pungency, but does not possess the mechanism that would allow these genes to become active. Thus, through CRISPR-Cas9, tomatoes synthesizing capsaicinoids can be created, claim the researchers who are currently working on this project. The aim is not to satisfy the growing culinary niche, but to increase the production of capsaicinoids for commercial purposes. The active substance in hot peppers (capsaicin) is known for its antibiotic and analgesic properties and for protection against pests.

The future of CRISPR

Despite the significant achievements and opportunities, there are some technical, legal and ethical obstacles to the widespread use of CRISPR that still need to be addressed. One of the major technical problems in genome editing is that the *Cas* enzyme does not always recognize the target precisely and may cut DNA material at other, undesired sites. However, over the past two years researchers have been developing the technology and increasing the accuracy and success rate of edits. Substitutes for Cas are already being tested that can cut only a single base of the DNA molecule or bind to it without cutting, thereby regulating the activity of target genes.

For now, a serious obstacle to the application of innovative breeding in agriculture in Europe is the legal framework that science in the EU must comply with. Under the existing legislation in the Union, genetically modified organisms are banned or access to them is severely restricted and strictly regulated. In 2018, the Advocate General of the European Court of Justice published an opinion in which the main topic was the mutagenesis techniques currently used (mostly treatment with chemical mutagens or radiation). They are not regulated in the same way as genetic modification tools, and the new technologies allow a much more precise intervention and can be regarded as targeted mutagenesis. Moreover, gene editing does not add a new DNA sequence, as is the case with most genetically modified crops, but edits already existing genes. In the USA, where CRISPR-Cas9 is gaining more and more supporters, the Department of Agriculture has announced that plants produced through gene editing will not be subject to regulation by the department. According to the Americans, edited plants are indistinguishable from those obtained through classical breeding. Procedures are yet to be established for how such plants will be described on food labels.

From a moral point of view, gene editing offers more opportunities and applications in agriculture than the ethical dilemmas observed in medicine, for example. The frightening scenarios such as human cloning, designer babies, etc., do not really concern the development of agricultural science. In the context of agriculture, only a regrouping of the major agro-corporations can be expected, which in future will have to shift their focus from the chemical industry and the production of plant protection products to the creation of new resistant varieties of agricultural crops.

The Green Revolution

Genome editing expands traditional tools for plant breeding by introducing new plant traits more quickly and precisely, which in turn saves years or even decades in obtaining new varieties. These innovations in plant genetics will provide crop protection against drought, diseases and pests and at the same time will increase the nutritional and quality value of the final products. Through the CRISPR method, allergens can be controlled and eliminated, which is currently an important step in satisfying the market with healthy and high-quality food. By using science, agricultural producers will be able to meet consumer expectations for a healthy and affordable food chain, ensured with fewer resources and with less impact on the environment.

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