

Genetically modified plants – from the laboratory to practical application in European agriculture. Part I

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Introduction

In March 2012 the agrochemical giant BASF announced that its research on genetically modified potatoes adapted to the growth conditions in Europe, would be cancelled. The company is closing its European branches responsible for biotechnology, downsizing personnel and moving its activities to America and Asia. This is a clear indication of the impact of EU's restrictive policy on GMOs which, combined with the unfavourable political and economic climate, results in a major drop in innovativeness of research and applications for European agriculture [1].

Biotechnology is a unique tool, allowing for enhancing and implementing new properties into domesticated plants. According to OECD definition, biotechnology is the “application of science and technology (...) for the production of knowledge, goods and services.” The development of biotechnology is observed mainly in three fields: 1) agriculture and agri-food processing, 2) industry, 3) pharmacy with medicine and veterinary medicine. Biotechnology is capable of not only assisting conventional agricultural methods, but also enhancing the plants without the barrier of crossing, which is the fundamental technique of inducing genetic variation in conventional breeding. The climate fluctuations trigger changes in biotic communities, for instance the emergence of new pests, as well as weather anomalies that result in prolonged draughts or floods. The manufacturers are interested in technologies that would ensure higher crop volumes by reducing the damage caused by plant diseases and pest, and will improve plant resistance to adverse external factors. Furthermore, biotechnology provides plant breeding with a range of other techniques, such as: doubled haploids, somaclonal variation, molecular marker assisted selection, micropropagation, or *in vitro* regeneration. Genetically modified domesticated plants, resistant to biotic and abiotic stress factors, may be helpful in addressing crucial issues arising from environmental changes. It is generally believed that it will be the plant varieties resistant to abiotic stress factors (improved tolerance to draught, floods, low temperatures, improved utilisation of minerals) that will be of most importance to increased food production. On the other hand, European manufacturers remain sceptical about solutions offered by biotechnology due to lack of acceptance by the market and consumers, and unfavourable legal situation [2].

What is a genetically modified plant organism?

Plants, similarly to other higher-order organisms, have in their cells the deoxyribonucleic acid (DNA) that the genes are made from. A transgenic (genetically modified) plant contains gene or genes that have been modified by humans using genetic engineering. Those modifications can be related to genes already existing in the plant, e.g. increasing or reducing their expression, or can be triggered by inserting a brand new gene. Compared to conventional breeding, the difference

lies within the method of implementing modifications into plant genes. In fact, all domesticated plants have undergone long-term genetic modification processes from growing in the wild to domestication. Human use of plants is related to the process of domestication, guided selection and finally, breeding. Those processes have induced changes in the genetic pool of the population, resulting in the plant acquiring new properties and features. Modifications implemented by way of conventional breeding, often assisted with biotechnology, such as *in vitro* cultures or molecular marker-based selection, have allowed for major progress in the creation of new varieties. Genetic engineering was first used in 1973 to create a recombinant organism – bacteria [3]. In 1979 Bedbrook and his colleagues from Cambridge University proved that plant DNA can also be cloned and replicated in the bacteria, similarly to any other DNA [4]. The age of cloning plant genes began with successful cloning of ribosomal DNA and telomeric repeat sequences in wheat and it revolutionised modern plant breeding. Cloning enabled the cross-species transfer of any gene, including between species that cannot cross. Deoxyribonucleic acid is a universal component of all living organisms, but certain properties are specific only for some species. Therefore, to protect corn from damage by European Corn Borer, instead of spraying the crops with insecticides containing the *Bacillus thuringiensis* bacteria, the gene from that bacteria can be inserted into corn genome using genetic engineering. The modified variety will produce the Cry1Ab protein by itself, protecting the plant from the pest.

The organism, whose genetic material was modified in manner not occurring in natural conditions (crossing or natural recombination), but using genetic engineering techniques, is called a genetically modified organism – GMO (Act of 22 June 2001 on Genetically Modified Organisms (Journal of Laws of 2001, No. 76, item 811) [5].

What plant properties are modified by genetic engineering and how?

Creating new varieties by conventional breeding is a lengthy and arduous process of combining the best genes. Depending on the planned use of the new variety, the inserted genes may be responsible for the plant's resistance to diseases or pests, increasing crop volume, tolerance to low temperatures, draught or other adverse environmental conditions. Before the development of techniques for plant transformation and cell regeneration in *in vitro* cultures the transfer of a gene responsible e.g. for resistance to insects to corn was not possible from the bacterium. Biotechnological techniques enable the breeder to combine a number of favourable properties of a range of organisms. With genetic engineering, theoretically any property can be implemented into plants. The gene can originate from any organism. One advantage of this technology is the fact that instead of the whole gene set, only one or more specific genes responsible for the production of the desired protein are transferred into the organism.

The first transgenic plants – tobacco and petunia resistant to kanamycin – were grown in laboratories in 1983 [6, 7]. Another 10 years had to pass until the first GM plant – tomato with blocked polygalacturonase gene – was marketed in the United States, and another two until the first product from a GM plant – tomato paste – entered the European market. In 1996 the Roundup Ready soybean, resistant to glyphosate (the active substance of i.a. the Roundup herbicide) received approval for use in food and feed in Europe. Since then every year witnesses the registration of new genetic modifications throughout the world. The genetically modified plants registered thus far are usually characterised by one or more of the following properties: herbicide tolerance, resistance to pest, modified amino acid or fat composition, male sterility, changed colour, delayed ripening, insertion of a selection marker or resistance to viral infections.

Transgenic plants are created in the process of genetic transformation, where exogenous genetic material is inserted into the plant cell and inserted into its genome. As a result, a new hereditary property emerges [8]. The foundation of plant transformation methods is the universal nature of deoxyribonucleic acid (DNA) which contains the genetic information of the majority of living organisms. The genetic information encoded in the system of 4 bases is universal and can be successfully read both in bacteria and plants. Genes are specific DNA fragments that encode information on proteins. Even evolutionarily distant organisms feature similar mechanisms of converting genetic information into information of protein amino acids that serve structural and functional purposes in the cell. This is why the DNA fragment from a bacterium is correctly read and interpreted when inserted into a plant cell.

However, before the gene from the bacterium is transferred into the plant cell, it must undergo a range of processes and adaptations. Firstly, the gene must be identified and isolated from the bacterial cell. Then, the gene is cloned, i.e. inserted and replicated in the cloning vector, which is usually bacterial. In order to enable the gene expression in the plant cell, it is necessary to build a gene structure and equip it with regulatory sequences prior to insertion to the plant. A promoter sequence is added to the gene, controlling the time and place of gene expression. The GM plants created thus far usually contain the constructive promoter of cauliflower mosaic virus (CaMV35S). Its main advantage is ensuring the constant gene expression, independent from the type of tissue, external conditions or the age of the plant. Currently, plant transformations are increasingly performed using more specific promoters that activate the genes in specific plant organs, such as the root, at the specific moment of the plant's life or under the influence of a specific stimulus, e.g. light.

Sometimes the inserted gene is modified so that its expression in the plant increases. One example of this is the Bt gene from *Bacillus thuringiensis* in which the A-T base pairs content was reduced, adjusting proportions to more typical for plant genes which are rich in G-C pairs, at the same time retaining the essential amino acid composition of the new protein. The created genetic structure, aside from the promoter, should also include the terminator sequence which signals the end of the encoding sequence. Also, marker sequences are often added to facilitate the identification of plant cells that have successfully undergone the transformation process. This is a practical solution, given the low effectiveness of the transformation process, reaching the maximum rate of a few per cent. The marker genes are often sequences confer the resistance to selection factors, such as antibiotics or active substances of herbicides; sometimes markers also codes for a coloured product, such as green fluorescent proteins (gfp), luciferase or β -galactosidase. In each case those products enable the differentiation of cells that have successfully undergone transformation from other cells. A completed DNA sequence in the form of a construct for transformation is inserted into the plant cell.

The currently bred and used GM plants have been created mainly with the use of one of the following methods of inserting the transgene into the plant cell:

- Using the mechanism adapted from the natural environment, where the *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* bacteria infect the plant cells, transferring their DNA [9]. Those bacteria transmit to the plant a fragment of circular T-DNA– Ti (tumour-inducing) or Ri (root-inducing) plasmid which is then integrated with the plant DNA. In the nature the genes on the Ti/Ri plasmid induce the development of tumor-like growths or roots on the plant by forcing the plant cell to produce substances required for bacteria development. By altering the genetic composition on the plasmid to one favourable for humans the *Agrobacterium* can be used as the vector transferring genes important from the viewpoint of the biotechnologist. For a long time this method was only viable for dicotyledones which are natural hosts for *Agrobacterium*, but since 1994 it has been successfully applied to transformations of monocotyledones, mainly cereals [10]. The transformation process employing *Agrobacterium* comprises the following stages:
 - isolation of the gene of interest from the donor's organism
 - inserting the gene to Ti/Ri plasmid
 - inserting the T-DNA-containing plasmid into *Agrobacterium*
 - combining the plant cell with the bacterial cell
 - T-DNA excision and integration with the plant genome [11].
- Another method of transferring DNA to a plant cell is called microinjection, the biolistic method or the gene gun method. On the gold or tungsten microcarriers with 0.6–4 μm in diameter the previously prepared DNA is coated and injected under pressure into the plant explants. This method of transformation is successfully applied to monocotyledones. One of the products of this method is the popular corn modification MON810 [12].
- The third method of transformation involves the delivery of DNA to the protoplasts. Plant cells stripped from cell walls and subjected to substances such as polyethylene glycol absorb DNA more easily and integrate it with its chromosomal DNA [13]. DNA can also be inserted with the use of electric impulses which create micropores in the membranes of the plant cell that can be then penetrated by DNA. With cell selection and further regeneration of the cells from the protoplast the entire plant can grow and develop.

Genetic modifications of plants in practice – the European Union and the world

Plants are capable of producing almost any protein when provided with appropriate genetic information. The greatest challenges faced by modern agriculture are limited resources of agricultural lands, the increasing food demand and climate changes [14]. With the achievements of biotechnology a more sustainable management of resources is possible through more effective plant protection, reduced fertiliser volumes and irrigation. New plant varieties with improved tolerance to draught and other adverse conditions, such as salinity, low or high temperature, may expand the production areas and respond to climate changes with their improved tolerance, health and more efficient mineral balance better.

One of the key objectives of the modern genetic modifications of plants remains the protection against pests, tolerance to herbicides and resistance to diseases. The varieties capable of more efficient use of nutrients, reducing CO₂ emissions, with modified starch, lipid and amino acid composition, tolerance to salination and other environmental stress factors will become increasingly important. Those plants will be used as biofactories producing substances used in medicine, biodegradable plastics and biofuels and removing contamination from the environment by phytoremediation.

Genetic modifications of plants found on the market and in the environment

Dr Clive James, Chair of the Board of Directors of International Service for the Acquisition of Agri-Biotech Application (ISAAA) is the author of the annual report on the global status of GM crops. In the 2011 report Dr James emphasised the 16th anniversary of introduction of commercial GM crops to cultivation [15]. According to ISAAA's data, until 2011 a total of 1,045 permits for the use of 196 genetic modifications of plants as food and fodders, in the processing industry and in the environment have been granted in 60 countries. The majority of permits concerned the modifications of corn (65), cotton (39), rapeseed (15), potato (14) and soybean (14). The most widely used GM plant is the GTS-40-3-2 soybean, resistant to Roundup herbicide – 25 permits (European Union counted as 1), then MON810 corn resistant to pests – 23 permits, Roundup herbicide tolerant NK603 corn – 22 permits and MON531 cotton resistant to pests – 14 permits globally. According to the EU Register of Authorised GMOs, there are many more genetic modifications of plants present on the EU market in the form of food, feed or processing material than can be released into the environment: UE – 26 modifications of corn, 8 modifications of cotton, 6 modifications of soybean, 3 modifications of rapeseed, 1 modification of potato and 1 of sugar beet (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm) [16].

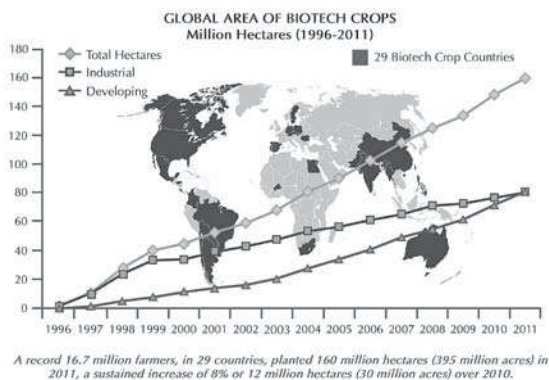


Fig. 1. Global cultivation area of GM plants in 1996-2011 (million hectares)
Source: Clive James 2012 [15]

The currently cultivated GM plants are characterised by tolerance to herbicides (soybean, cotton, rapeseed, corn, sugar beet and alfalfa), resistance to specific pests (corn, cotton) and improved utility properties (potato). Since 1999 a steady growth of the genetically modified crops hectareage has been observed, reaching 160 million hectares in 2011 (Fig. 1) [15]. This marks a 94-fold increase compared to 1999. In the United States the GM varieties grew on 96 million has.; Brazil – 30.3 million has.; Argentina – 23.7 million has.; India – 10.6 million has.; Canada – 10.4 million has.; China – 3.9 million has.; Paraguay – 2.8 million has.; Republic of South Africa – 2.3 million has. The GM crops areas expanded also in 19 other countries. It should be noted that nearly 50% of the GM varieties were grown in developing countries, with 14.5 million hectares cultivated by 15 million smallholders. The five leading countries, growing GM crops on the total area of 71.4 million hectares, included: China and India in Asia; Brazil and Argentina in Latin America; and RPA in Africa.

In the 6 countries of the European Union: Spain, Portugal, Czech Republic, Poland, Slovakia and Romania the pest-resistant GM corn was grown on 11,449,000 hectares in 2011 (year-to-year growth by 26%). 3 countries grew the GM potato “Amflora” for the purposes of industry and feed production. In Poland the MON810 corn varieties (resistant to European Corn Borer and approved for growing in the EU) were grown on similar area over the past 2 years, covering approx. 3,000 hectares.

In Spain, where two pests: European Corn Borer (*Pyrausta nubilalis* [Hbn]) and another species boring stalks and attacking ears – Mediterranean corn stalk borer (*Sesamia nonagrioides* [Lef]) caused substantial economic damage in some regions, the corn with the expression of CryIAb protein was grown on over 50,000 has. [17]. Before 2010 only varieties with one modified property (MON810) were approved for growing in the EU. In March 2010 the list of GM varieties approved for growing was expanded to include the varieties of the “Amflora” potato containing the genetic structure that increases the amylopectin content, to be used in the industry and to produce fodders.

Currently the application for the approval for growing the “Fortuna” potato is being reviewed. This variety is resistant to potato blight, the most dangerous potato disease causing losses of up to \$1.5 billion annually in the EU and up to \$7.5 billion globally.

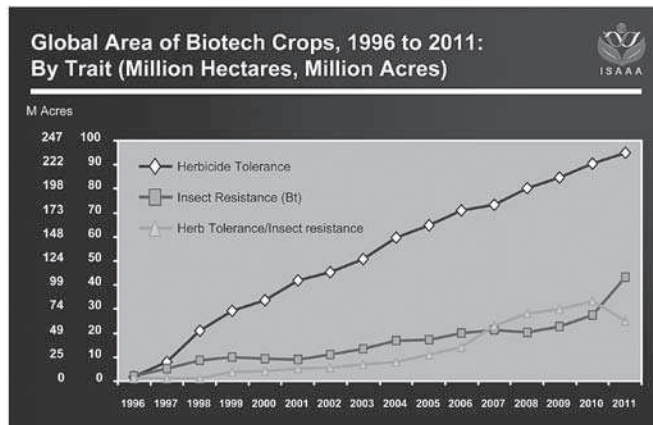


Fig. 2. GM crops area in 1996-2011 (million hectares) according to the modified properties
Source: Clive James 2012 [15]

Globally, the varieties of corn, soybean and herbicide tolerant rapeseed dominate in the total area of GM crops, taking 59% or 93.9 million has. in 2009. A significant increase from 28.7 million has. in 2009 to 42.2 million has. in 2011 should be noted, amounting to 22% of the total global GM crops with two or three modified properties (stacked events), while GM varieties with only one modified property of resistance to insects were grown on 23.9 million has. (15% of the total GM crops area) (Fig. 2) [15]. The percentage increase of varieties with stacked events between 2010 and 2011 was 31%. The increase of herbicide tolerant varieties was only 5%. A 10% drop in the crops with a single modified property of pest resistance was observed. The increase in GM crops was particularly significant in 12 countries (of which 8 are classified as developing). The relatively highest increase in the crops of corn with 3 properties of resistance to 2 pest species (European Corn Borer and Western Corn Rootworm) and tolerance to herbicides as compared to other GM varieties took place in the United States. The corn varieties with 2 properties: resistance to pests and tolerance to herbicides were grown in the Philippines on 411,000 hectares in 2010, which amounted to a 22% growth when compared to 2009.

Genetic plant modifications ready for registration and anticipated in the future

It is difficult to estimate how many new GM varieties will be commercialised, particularly on the European Community market. An increasing number of GM plants are created and registered in the Asian countries for use on domestic markets. Bill & Melinda Gates foundation sponsors the VIRCA project (*Virus Resistant Cassava for Africa*). Also, research is conducted on GM plants from the *Fabaceae* family resistant to pests and fungi, draught tolerant and enhanced with nutrients, such as provitamin A, to be grown in African and some

Asian countries. According to estimates by Stein and Rodriguez-Cerezo [18] in 2015 approx. 120 various genetic modification will have been registered throughout the world. These will be mainly pest resistant and herbicide tolerant varieties, including new varieties resistant to dicamba, with modified quality parameters, resistant to viral infections and more tolerant to abiotic stress. It is believed that in the future the stacked events will dominate in new GM varieties, combining pest resistance, tolerance to herbicides and draught, but also quality parameters such as high omega-3 fatty acid content or increased provitamin A content. Significant importance is also attributed to new varieties that would be resistant to pests with piercing-sucking mouthparts, such as *Meridae*. An important task will be to provide farmers with varieties resistant to pathogens such as: *Fusarium*, *Verticillium*, *Rhizoctonia*, *Pythium* or viruses.

Varieties with better tolerance to biotic stress factors, mainly draught, are becoming more and more important. Since 2010 in Canada and since 2011 in the United States crops include corn varieties with improved tolerance to draught. First commercial application of those varieties in the subtropical regions in Africa will probably take place in 2017. In Australia wheat varieties resistant to draught have been grown, yielding 22% higher crops than the current varieties. This will be a major success, given the decreasing global water resources, wasteful surface irrigation systems in most parts of the world related to intense evaporation and soil salinity (except for Israel which uses mainly drop irrigation in field environment) and, what is often overlooked in Europe, the rise in malaria and schistosomiasis caused by *Schistosoma haematobium*.

In the longer perspective, varieties with improved productivity are anticipated, created by accumulation of a number of traits, including the improved efficiency of basic metabolic pathways, e.g. photosynthesis.

European and Polish legislation on the release of GMO into the environment and the use of genetically modified plants

The basis for numerous specific legal acts related to GMO and issued by the European Union and Poland are the key international legal acts – the Convention of Biological Diversity of Rio de Janeiro and the Cartagena Protocol on Biosafety [19].

The objectives of the Rio de Janeiro Convention of 5 June 1992 are:

“(…) the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.”

Each Contracting Party shall implement appropriate instruments that will limit the damage to the environment of other States or areas beyond the limits of national jurisdiction.

Each Contracting Party shall cooperate with other Contracting Parties in the preparation of plans and strategies for environmental protection and, as far as possible and appropriate, include the conservation and sustainable use of biological diversity in relevant plans and policies. Each Contracting Party shall also identify and monitor elements of biological diversity, and protect them both in natural habitats and outside them. The Convention also imposes the obligation to conduct research on biological diversity in order to protect it, as well as educate and increase social awareness, identify and limit the adverse impacts on the environment. Furthermore, the provisions of the Convention stipulate respecting the genetic resources on the territories of the Contracting Parties, exchange of information on the protection and sustainable use of biological diversity, as well as scientific and technical cooperation, also in the field of biotechnology, and fair share in the benefits arising from the use of genetic resources.

The objectives of the Cartagena Protocol, transposed into the Polish legal order in 2004, are to “to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.” Regulations of the Protocol refer mainly to “development, handling, transport, use, transfer and release of any living modified organisms (...) that prevents or reduces the risks to biological diversity, taking also into account risks to human health.”

The application of GM technology has been strictly regulated by the European Union since the beginning of the 1990s. The law has been drawn up with the objective of protecting the health of humans and animals, and the environment. A genetically modified organism, food product or fodder may be marketed or released into the environment only upon authorisation, i.e. approval for use in a specific manner. Another fundamental principle of the European law is the free movement of goods, including healthy and safe GM products. Those products must be authorised only once to enter the markets of the entire EU. The majority of European law on GMO has been formulated in the years 2000-2003 when the key directives and regulations on GMO were drawn up. Those key documents include:

Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms [20]. This document addresses the issue of deliberate release of genetically modified organisms into the environment for experimental purposes, such as field research on GMO – part B of the Directive, and for placing the products on the market, e.g. GMO seeds for commercial crops use on the EU territory – regulated by part C of the Directive. The Directive provides a definition of the GMO and lists techniques of obtaining GMOs, as well as those techniques that do not result in a transgenic organism within the meaning of the law. The Directive also contains information and methodology of the environmental risk assessment that should be carried out prior to the release of GMOs into the environment. Also, the Directive stipulates the requirement of traceability and labelling of the GMO product at all stages of placing it on the market. The Directive includes provisions on the compulsory monitoring of GMOs after placing it on the market, including the observation of cumulative long-term effect of GMOs on the environment. The Directive also imposes the obligation of maintaining public registers of information on the release of GMOs into the environment.

Regulation (EC) no. 1829/2003 on genetically modified food and feed that addresses the issue of placing on the market foods and feed composed of, containing or produced with the use of GMOs [21]. This legal act provides the principles of a unified, harmonised and EU-regulated procedure of European market authorisation of genetically modified food and feed, including the traceability and labelling requirements for such products. According to the Regulation, the requirement of labelling refers to food, foodstuffs, food additives, feed, feed additives and flavourings used in food production. The labelling requirement threshold in the EU has been established at the 0,9% proportion of the genetically modified ingredient in the product. The labelling requirement does not apply to products in which the proportion of each genetically modified ingredient (soybean, corn) is no higher than 0,9%, provided that this presence is adventitious or technically unavoidable.

Regulation (EC) no. 1830/2003 of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms [22]. The European law stipulates the requirement of traceability of GMO products. In Poland, as in other EU Member States, the basic documents regulating all matters related to GMOs are EU directives and regulations. Additionally, the issue of plant GMOs is

addressed by the Act of 22 June 2001 on genetically modified organisms, Act on fodders and Seeds Act. Furthermore, in 2006 the government of the Republic of Poland published Poland's Framework Position on GMOs which defined the state policy on this matter.

Act of 22 June 2001 on genetically modified organisms is the key document in the Polish law regulating matters related to GMOs [5]. The Act addresses issues such as: enclosed use of GMOs, deliberate release of GMOs into the environment for purposes other than placing on the market, placing GMOs on the market, transit and export of GMOs. The Act also specifies the role of state authorities in GMO-related matters.

According to the provisions of the Act, the decisions on GMOs, the laboratory research on GMOs and the release of GMOs into the environment in Poland are taken by the Minister of Environment after consulting the GMO Committee's opinion. Aside from the Minister of Environment, responsibility for controlling the application of the provisions of the Act lies with customs authorities and a range of inspectorates, such as: General Veterinary Inspectorate, Chief Sanitary Inspectorate or Main Inspectorate of Plant Health and Seed Inspection.

In March 2006 the government of the Republic of Poland took a position on GMOs in which it supported the enclosed use of GMOs according to the requirements set forth by the law and opposed the notion of deliberate release of GMOs into the environment in Poland for experimental purposes, marketing of GM products authorised in the EU in accordance with Directive 2001/18 [20], marketing of GM fodders, and growing genetically modified corn, soybean, potatoes, rapeseed and sugar beet. Polish government accepts only the import of GM food from outside of the European Union and import thereof from EU Member States under the condition of explicit labelling and without the possibility of further processing of such food in Poland.

Implementing the Polish government's Framework Position on GMOs, the Parliament of the Republic of Poland passed an **amendment to the Seeds Act and the Act on plant protection of 27 April 2006**, by which a provision was added that GM varieties would not be entered into the national register and that the seeds of GM varieties may not be marketed on the territory of Poland [23]. The European Commission objected to this Act, citing its incompliance with the law of the European Union. The President of Poland, after vetoing the Seeds Act passed in July 2011, is currently working on his own draft. At the beginning of March this year the government gave positive opinion on the presidential draft, with the exception of provisions prohibiting i.a. the registration of genetically modified plants.

Act of 22 July 2006 on fodders introduced a prohibition of production, marketing and use of genetically modified fodders and genetically modified organisms for fodder purposes [24]. In 2008 a moratorium was passed which expires in December 2012. The Act of fodders passed by the Sejm (Polish Parliament) in mid-July this year extends the period of use of GMOs to December 2016.

Coexistence of GM crops and other types of production

Coexistence is the simultaneous existence of various types of crops or products on the same area. Coexistence is not possible unless we accept the possibility of trade additions of one material in another, such as the threshold of biological additions in seeds. Implementing the principles of coexistence is aimed at providing the farmers with the choice between conventional, organic and genetically modified crops. The consumers in turn have a choice based on the labelling compliant with the legal requirements set forth in the Regulation 1830/2003(CE) concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms. Coexistence does not apply to the safety aspects of GMOs, because those issues have been addressed in the risk assessment prior to placing of the genetically modified

varieties on the market, as required in Directive 2001/18/EC. The recommendations on coexistence refer to potential economic losses, arising from the GMO material penetrating the non-GMO products.

The Commission Recommendation 2003/556/EC [25] and the current Recommendation 2010/C 200/1 [26] on guidelines for the development of national co-existence measures to avoid the unintended presence of GMOs in conventional and organic crops clearly states that no type of cultivation, be it conventional, organic or GMO-based, cannot be excluded from use in the European Union. The Member States cannot prohibit, limit or prevent placing approved GMOs on the market; furthermore, the States should draw up legislation that will avoid unintended GMO presence in other products (Directive 2001/18/EC, Article 26a). The provisions on coexistence should take into consideration the environmental and economic conditions which vary across the Member States. All activities should be consistent with those carried out thus far, not discriminate any users, be based on the analysis of potential profits and losses of undertaking or resigning from the activities, based on economic analysis and reviewed against new scientific data. The system should provide the consumers with as many choice options as possible and ensure practical choice for the producers. In accordance with the principle of proportionality, which is one of the fundamental principles of EU legislation, the measures undertaken for the purpose of coexistence of crops should not exceed the necessary minimum, in order to ensure that accidental presence of trace amounts of GMOs does not exceed thresholds specified in Regulation (EC) 1829/03 and Directive 2001/18/EC, which will prevent unnecessary burdens for the parties interested.

In the implementation of the principles of coexistence consideration must be given not only to biological factors related to the potential transmission of genes between crops, but also conditions arising from the increase in production of the crop, the size of fields and their location. Also, the differences between species and types of production must be taken into account. It is advisable to employ the known and widely used methods of segregation. The sources of the most common problems are seeds impurities, outcrossing, volunteers harvesting and storage methods. The provisions must be adequate to the requirements of the matter (should not encumber the farmers too much). The activities should ensure coordination not only between neighbouring farms, but also between the regional arrangements, therefore should be implemented on an appropriate scale.

The principles of coexistence have been established in the majority of EU Member States [27]. Thus far, Poland has not established its principles of coexistence. The work on developing such principles is under way; the principles of coexistence will be set forth in the new Act on GMO prepared by the Ministry of Environment.

Summary

One of the key practical achievements of biotechnology are genetically modified organisms (GMOs). Cultivated for nearly 20 years, the GMOs are becoming increasingly important in the global food production. Genetic modifications are implemented into plants that play an important role in agriculture, such as corn, soybean or rapeseed, but also model plants (e.g. *Arabidopsis* (thale-cress)) used in genetic research. Among several methods of plant genetic transformation two have found wide practical applications – microinjection of DNA into plant cells or use of *Agrobacterium* to introduce changes into plant cells. Those methods allowed for obtaining a number of new plant varieties characterised by novel properties that could not have been attained with traditional breeding methods. The most popular plant modifications include traits such as pest resistance, herbicide tolerance and modified composition. The area of GMO crops increases every year, reaching 160 million ha. in 2011.

The legal regulations on placing GMOs on the market or releasing them into the environment vary in terms of scope and requirements across different countries. The EU has one of the most restrictive legal systems on GMOs. EU legislation focuses on the protection of human life and animal health, and environmental protection. The regulations also stipulate detailed analysis of each GMO at every stage of the approval process for the use of such organisms. Also, GMOs must be monitored after having been placed on the market. The precautionary principle, underlying the Community regulations, clearly states that all activities related to GMOs must be directed at preventing any negative impacts of the technology, i.a. in agriculture, and that such activities should not be undertaken only after such negative impacts have been observed. The EU law serve as the basis for the legal regulations on GMOs applicable in Poland.

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