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Position

Genetically modified organisms (GMO) in agricultural production and their monitoring

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Genetically modified organisms (GMO) in agricultural production and their control

Introduction

Nature has a number of natural mechanisms for the exchange of genetic material. Oriented at nature, molecular biological methods were developed, which allow a direct and targeted recombination of nucleic acids. Today, during transfer of genetic material onto selected organisms, usually genetic sequences are used, which everywhere appear naturally. Due to numerous advantages, these products isolated from GMU are used increasingly in the production of food and feeds. Live GMO are also commercially used for food, growth media and pharmaceuticals (nutraceuticals) production and to produce renewable resources.

During the approved marketing in the sense of the Act on Genetic Engineering and during regionally and temporary restricted outdoor tests, GMO can come into contact with animated nature. The distribution and manifestation of newly introduced gene constructs can take place vertically (sexual, within species or closely related species) or horizontally as gene transfer (parasexual or asexual, crossing species borders). Horizontal gene transfer has, at the moment, been sufficiently proven for bacteria, but not for plants, humans and animals. In Germany, the behaviour of transgenes in the respective organisms and possible environmentally or health relevant results can be deducted from a relatively large number of outdoor test using genetically engineered agricultural crops and from large scale production over several years in the USA. These, however, are no exact trials for risk assessment and a widely based safety research has started. Furthermore, it is possible to deduct implications for humans and the environment in the direct comparison with conventionally or mutagenically bred plants. Possible, publically discussed risks could be spontaneous hybridisation (outcrossing of transgenes in closely related species), allergens and resistances the increase or decrease of the biodiversity or the displacement of traditional agricultural plants and animals. Regionally and temporary restricted outdoor tests, which are started to answer questions regarding the biological safety, partly deliver results, which are still not always fully in concordance with the observations from agricultural practise. In the future, farming accompanying monitoring programmes are to be started in accordance with the common position of the EU Council of Ministers and the EU Commission (Dir. 90/220/EEC) on the deliberate release into the environment. An important criterion for the risk assessment for the legislative authorities on EU and national levels, who regulate the release and the marketing of the GMO, is the OECD demands for sequential equivalence (composition, nutritive value, planned use, etc.) with comparable food and feeds, where the risks are known from experience.

The monitoring of the legal regulations is in the responsibility of the ministries in charge of the Länder. A number of national and international laws and regulations, such as the Genetic Engineering and Animal Welfare Act, the Food Law or the Novel Food and Novel Feed Regulation (present as draft), the European Council Guidelines 98/95 and 90/220, together regulate comprehensively the handling and marketing of GMO. Together, they have the common aim to control the reproduction and promotion of GMO, to be able to act regulating on a legal basis on discovery of a potential danger. In Europe, there is an obligation to label transgenic plants, animals and microorganisms. Therefore, in agriculture and the food industry, a documentary evidence of live, inactivated or dead GMO and isolated and processed ingredients from GMO must be kept. The new version of the Guideline 90/220/EEC will be expected to bring regulations regarding the general and case specific monitoring and surveillance opportunities of the obligation to label GMO's in environment and foods. The necessary monitoring and verification programmes are to be promoted on Länder-level by the Agricultural Analysis and Research institutions (LUFA), which in Germany have to monitor the sustainability of production sites, the quality of plant and animal raw materials for food, industry raw material and feed production and seeds and feeds themselves. For monitoring of transgenic plants, animals and bacteria, several LUFA (among others Hameln, Jena, Kassel, Leipzig, Rostock, Speyer) have begun or already built monitoring laboratories, in order to fulfil the legal mandate. The Association of German Agricultural Analytic and Research Institutes (VDLUFA) has accompanied the setting up of the analysis laboratories with workshops for "Food an feed biotechnology" and "Horizontal gene transfer" at the congresses 1995 in Garmisch-Partenkirchen and 1998 in Giessen and will continue to do so. The findings to date are summarised in the book "Lebensmittel, Nahrungsketten und Gentechnik" (VDLUFA-Schriftenreihe, H. 48, 197 p., 1998).

This VDLUFA position, which represents the opinion of the majority of the VDLUFA members, is mainly based on the above publication and is meant to be used for objectification of the discussion.

Using genetic engineering in agricultural production

The production of transgenic plants and microorganisms can be carried out using microinjection (DNA-injection with extremely thin glass capillaries into the nucleus of individual cells, DNA= desoxyribonucleic acids), electroporation of protoplasts (following the production of cells without cell membrane, increase of plasma membrane permeability for DNA through use of short electro pulses), lipofection (transfer of DNA-loaded fat particles through the lipidous cell membranes), the ballistic transfer (bombarding cells with DNA-loaded high speed particles) or the use of viral or bacterial transfer mechanisms (e.g. the agrobacterium system). Worldwide, GMO is used per year for more than 10,000 release tests. Within the EU, the first place of the release applications is take y maize with 29 %, followed by rape (23 %), sugar beet (16 %) and potatoes (10 %).

In Germany, the sugar beet takes first place in front of rape, potatoes and maize (Biologische Bundesanstalt (BBA)-Genetic engineering databank). Worldwide already more than 45, in Europe
approx. 14 transgenic arable crops are approved for cultivation. The aims of genetic changes of agricultural crops for the gain of agriculture,
consumers and environment could be:

(1) Building and improving of resistances to
- diseases and damage by viruses, bacteria, fungi, nematodes and / or insects
- herbicides
- noxious substances (heavy metals, environmental chemicals)
(2) Tolerance improvement towards
- environmental influences (temperature, dryness, salt)
(3) Resistance and competitiveness improvement towards
- wild herbs (e.g. improved capability for nutrient acquisition)
(4) Improvement of the composition and quality by changing
- the primary ingredients (starch, oils, fats, proteins),
the secondary ingredients (aroma, roughage, vitamins, toxins) or the introduction of
- new ingredients (technical enzymes, hormones, neutraceuticals etc.)
(5) Improvement of the yield and marketability by improved
- nutrient uptake, material translocation and photosynthesis performance,
- synergism between humans, animals, plants and associative or symbiotic microorganisms
- feed utilisation
- storability of the harvested goods (stock piling)
- production of industrial raw materials according to requirements.

Spreading of transgenes in the environment

a) Microorganisms (prokaryots)

In nature, the gene transfer in procaryots (bacteria) is possible and proven over very distant relations.
Resultantly, the respective genes are able
to establish themselves and spread in an ecosystem. This is also the case for genetically engineered (transgenic)
microorganisms. Bacteria, as ubiquitous organisms, are often
subjected to changing environmental conditions and / or a multitude of organic and inorganic foreign matter. Quick adaptation to the pressure for selection pressure and / or the gaining or the modification of enzyme coding genes are bacterial survival strategies.
The natural adaptability is increased through gaining resistance and other genes
and in microorganisms takes place through

∑ conjugation (exchange of circular, in the cytoplasm free floating DNA (= plasmids with transposable elements) after direct cell contact).

Transformation (uptake of free DNA into a competent = available for reception receiver cell) and in reduced scope though

∑ transduction (targeted gene transfer with viruses as vectors).

As, however, especially the microbial biosystem is by no means researched and understood, preventative measures must also be taken against speculative risks.

b) Humans and transgenic food

Per meal, humans take up more than 300 mg of foreign genetic material (DNA, RNA = ribonucleic acid) though meat, fruit, salad etc. Nucleases and organism specific defence systems (intestinal flora, somatic cell barriers, immune system, nuclear membrane) prevent very effectively that foreign genetic material that was taken up is integrated into the genome. Quite like proteins, carbohydrates and fats, the foreign genetic material is quickly broken up into its basic building blocks (deoxyribonucleoside and ribonucleoside monophosphates. As nucleotides are chemically identically built in all living beings, all organisms use the nucleotides taken up to newly synthesise body's own DNA and RNA. In addition to these cell internal protective mechanisms against foreign DNA, to carry out successful transformation and expression in eucaryot cells, prerequisites are that

(a) the released DNA stays intact in the environment for a certain length of time,
(b) the potential receiver cell is competent (ready to transform),
(c) the taken-up DNA can resist the cells own restriction system,
(d) the recipient (receiver cell) integrates the foreign DNA into the own genome and also propagates it
and
(e) the signals for transcription (RNA synthesis through DNA dependant RNA polymerase) and translation (translation of the information saved in the RNA into Proteins) are recognised by the recipient and expressed, that means turned into proteins.

c) Probability of a gene transfer in ecosystems

Nature has foreseen a fast disassembly of the DNA into nucleotides. This also applies to foreign DNA. Therefore, the half-life of highly molecular DNA in the digestive tract and in waste water usually is only a few minutes. However, small amounts of DNA in chain length down to intact genes can remain. These could, however, with relatively low probabilities, be transferred to microorganisms in the soil, the water or in the digestive tract. More current research seems it possible that nucleotide sequences could be transported up into the blood stream of mammals. An integration of intact foreign genes into the mammal genome, however, has not been scientifically proven. When looked at from the point of the evolution,

the DNA recombination is relatively conservative. This is also the case for bacteria. For eucaryonts
(fungi, plants, animals), which mainly or only reproduce sexually,

No specific mechanisms of a horizontal gene transfer have been observed; apart from transformation, which has only been observed in a few fungi.

The possibility for horizontal gene transfer diminishes in the ranks

bacteria > fungi > soil animals > plants > mammals

greatly. In laboratory experiments, it was demonstrated that sorption to clay minerals or humus colloids makes the DNA more resilient to the break down through nucleases. When compared to the digestive tract, in fresh and sea water biotopes and in soils, the DNA half life could increase from a few minutes to 3 to 80 hours.
Therefore, in nature a successful transformation should be restricted to certain micro ecosystems (e.g. the rhizosphere = few µm to mm deep root soil contact zone).
The bacterial growth and reproduction is particularly stimulated in the rhizospere due to the photosynthetic supply with energy rich compounds.
Intensive
bacterial growth could promote horizontal gene transfer. The ability for natural transformation and production of extracellular DNA was observed in bacteria.
Interfaces between eukaryonts- (animals, plants) and microorganisms, such as the rhizosphere, therefore need special consideration for the risk assessment. To make the species spanning transmission of transgenes less probable within the world of bacteria, it would be sensible and desirable, to incorporate new properties not into the plasmides, which can be exchanged easily through conjugation, but instead integrate them into the bacterial chromosome.
For plants, it would be sensible and desirable, to incorporate new properties not into the pollen, but into the chloroplasts of the female gametes.

Possibilities for monitoring

All GMO produced foreign proteins are a transgenic product, which was added to the species-own
genes in germinable or capable of division cells of animals, plants or bacteria.
The genes which are to be transferred are
equipped on the DNA with specific signal structures of the transcription (promotor and terminator DNA sequences), which determine the initiation
point and the initiation frequency of the messenger RNA (m-RNA) synthesis and stop the RNA synthesis. With this, the expression is made possible in the respective receiver organism
and in the individual organs. Additionally, so called marker genes (phenotypically easily distinguishable markers) can be present (e.g. genes for resistance to antibiotics).

The GMO detection can be carried out in various detection levels:
effect levels / phenotypically (changed characteristic = suspicion of altered gene, e.g. the delayed softening of the Flavr Savr®tomatoes)
protein level (e.g. highly specific antibody reaction) and
nucleic acid levels (e.g. polymerase chain reaction for multiplication and for the detection of a known foreign gene).

Approved but not declared GMO, which can also be present in food as ingredients, can be very difficult to identify, especially when present as mixtures.
This genetically engineered alteration can only be proven by linking many specific individual tests.
To date, for unknown genetically engineered alterations no practical procedure exists.
Possibilities to detect unknown genetically engineered alterations can be
(a) the use of specific primer mixes for common marker genes and promotor sequences (primer = starter DNA sections, necessary for the specific reproduction of the transgene using polymerase chain reaction),
(b) the still expensive and may be in 5-10 years time realisable DNA chip technology and
(c) DNA fractioning using a particular restriction enzyme, followed by two dimensional electrophoretic sorting of the fragment mix by size and adenine- thymine content.

Using that to find the "needle in the haystack" is very elaborate and expensive, as checking a potential GMO and its follow-on products cannot be restricted to one universal detection method and even the combination of several methods does not in every case lead to the clear detection. In the future, the approval procedure for marketing of GMO (Guideline 90/220/ EEC) should contain the respective

gene sequences of the newly introduced gene construct.
The planned changes of the Guideline 90/220/ EEC also contain a gene register, in which the gene characteristics, the nucleotide sequences and the genetic engineering procedures of the GMO are registered. This data is then available for detection processes and monitoring.

Global GMO application requires new organisational structures

The Novel Food Regulation, published 14. February 1997, the novel Feed Regulation, present as draft, of the European Parliament and the Council, the EEC Council Guideline 98/95, which contains far reaching regulations regarding genetically engineered seeds and varieties, and the planned changes to the Guideline 90/220 EEC can, combined with national and international food, genetic engineering and animal protection laws and accompanied by practical implementing provisions, warrant the safe and controlled marketing of GMO.

The procedure for release of products, where sufficient information about their safety Is present, should be made easier and obligatory monitoring should be introduced for products with a limited marketing approval. As ecological Impacts usually are only apparent after longer periods of time, besides the certification, production and marketing control, the monitoring after approval plays a very important role in risk management.

Approval processes, which foresee a thorough analysis of the various safety aspects for environment and end user, only are as valuable as their adherence can be monitored safely, practically, standardised and cost efficient. In addition to surveillance of the declaration of already approved GMO, it is also important to be able to check GMO that have been marketed without approval. Here, the inspection authorities in their entirety must collaborate. As the regulations of the Novel Food Regulation are not in place for feeds, these until now do not have to be labelled. After implementation of the Novel Feed Regulation, as seen with the Novel Food Regulation, the monitoring orders should increase.

The Agricultural Analysis and Research Institutions monitor for the last approx. 150 years agricultural food production and the environment. They have available a variety of physical, chemical, microbiological, seed technical, feed chemical and local methods, which are regularly validated in interlaboratory testing and are continuously adapted to the legal requirements. They are in close contact with Universities and further public research institutes (e.g. Biologische Bundesanstalt, Federal Research Centre for Nutrition (BfE), Bundessortenamt, Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV), Federal food test agencies) and industrial research facilities. Within the framework of the international method development, the specialist group seeds of the LUFA as a member of the International Seed Testing Association is at the moment taking part in an interlaboratory comparison for the determination of GMO in seeds (Maize). With their long-term tested environmental chemistry, microbiological methods and their knowledge about local conditions, and equipped with modern molecular biology laboratories, LUFA can build up, in close collaboration in particular with the Robert-Koch-Institute as approval body, an effective GMO monitoring with site specific risk assessment and warrant an environmentally relevant consumer protection.