Discussion following the ECJ decision on mutagenesis – response of the Czech Republic

January 2019

Position of the Czech Republic regarding possible amendment of Directive 2001/18/EC

The EU legislative framework on the use of genetically modified organisms (GMO), namely Directive 2001/18/EC, was adopted nearly 20 years ago. The definition of GMO and the listed techniques of genetic modification that together set the scope of the Directive are even older, being based on the situation in nineteen eighties. Progress of biotechnology has been enormous since that time, especially during the last few years. Therefore the Czech Republic authorities agree with the opinion of many experts that the Directive should be reviewed to reflect the current developments.

It should be noted that problems with the implementation are not limited to new plant breeding techniques only – the Directive is not suitable for the use of modern biotechnology in medicine as well (clinical studies with GM medical products of gene therapy and vaccines).

Following the ECJ ruling on mutagenesis, some amendments to the Directive should be adopted, to differentiate between organisms obtained by transgenesis (insertion of genes from other species) and organisms with only small, precise changes in the inherent DNA of the species, obtained by gene editing.

In the longer term, Czech experts recommend to change the legislative framework entirely in such a way that the regulatory procedure is not triggered by the production technique but by the characteristics and potential risks of the final organism or product.

We are aware of the fact that biotechnology – above all GM crops - is politically very sensitive issue. Therefore any changes in regulations have to be preceded by a broad discussion involving scientists, industry, farmers, other stakeholders and the public. In the Czech Republic the debate already started in the second half of 2018; this year it should result in concrete proposals of amendments of Directive 2001/18/EC.

Detection - Statement of the Czech National Reference Laboratory for GMO

New breeding techniques, namely gene/genome editing, represent a useful tool for genetic improvement of agricultural organisms. Some examples have been shown and introduced on the market; products are under development either by companies or at the universities and research centres as experimental materials.

There are important aspects that should be taken into consideration and various scenarios assessed:

- Companies would place a product on the market along with information about the edited site and submit a method for detection and quantification. In principle an event specific method can be designed, validated and verified by reference laboratories. Financial resources will be needed to perform the validation and to purchase adequate chemicals and standards provided that PCR platform will be used (personal costs - 3 weeks of 2 persons, reference material, if available, PCR/qPCR reagents, overheads 20% covering power, water supply, support of technical/economy department) and costs for an accreditation of the method by an official accreditation body,

  Increasing number of such organisms and derived products could be expected. No general screening protocols can be developed for such cases, multiplex and high throughput approach will

be required. Laboratories will need probably more advanced equipment, incl. NGS machine and specialised operators.

- Numerous unauthorized products or products from countries where regulations are not applied could be present in imported goods or on the market. Assumed that NRL should identify such cases, not only equipment is needed but highly qualified bioinformation for data comparisons is necessary. It is questionable whether even wide knowledge of contemporary allelic variants in individual varieties and genetic resources could help to differentiate between natural and "induced" change. Cost - benefit analysis should be performed.

Information on gene editing projects in the Czech Republic

In the Czech Republic, new mutagenesis techniques (gene editing) are applied in contained use only, mostly for basic research. GM crop breeding projects are not supported by the government agencies, due to the negative perception of GMOs in EU. According to relevant experts, the ECJ ruling makes it practically impossible to bring any gene edited plants to commercialisation.

Examples of specific projects with gene editing of plants

Project financed by the European structural funds, call Excellent Research, CZ.02.1.01/0.0/0.0/ 16_019/0000827, Plants as a tool for sustainable global development (2018-2022), Palacký University Olomouc
The objective of the project is to acquire new knowledge about growth regulation, phenotype formation and stress adaptation of plants. Novel multidisciplinary approaches will be applied which use the newest technologies and take advantage of international network of cooperating top-class research institutions. The basic plant research will enable further development of targeted projects.

Project financed by the European structural funds, Priority axis 1: Strengthening capacities for high-quality research, CZ.02.1.01/0.0/0.0/16_019/0000738, Centre for Experimental Plant Biology (7/2018-6/2023)
The aim of the project is to extend the research capacity of the Institute of Experimental Botany of the Czech Academy of Sciences, and two partners from the South Moravian region (Mendel and Masaryk University - CEITEC) in Experimental Plant Biology at these locations: the city Prague, in the region of Liberec, Olomouc and South Moravia.

Projects financed by the Czech Grant Agency:
- CRISPR-Cas barley genome editing: prospective tool for modern breeding (2017-2019),
- Gibberellin biosynthesis and signal transduction – identification of novel targets for plant growth regulation (2018-2020),
- Distinct transcription factor families controlling meristem activity and organogenesis in *Arabidopsis* (2017-2019)
- Genetic and cell biology approaches to study regulation of YODA (MAP3K4) signaling by HSP90 proteins in *Arabidopsis* (2017-2019)

The projects exploit new genome editing techniques TALEN and CRISPR to get new knowledge that can be applied for breeding of economically important crops with higher yields with resistance to biotic abiotic stresses, improvement of technological parameters of production processes, and increase of minerals contents. Through this new technologies plants hormonal regulations of important synthetic pathways are studied.

Institute of Experimental Botany - plants

- Impact of humanised glycosylation pathway on protein accumulation and traficking in plant seeds. The CRISPR/Cas 9 technique is used for blocking the plant glycosylation pathway. The aim of the research is to produce human proteins (antibodies, vaccines).
- Impact of the climate change on resistance of plants to pathogens. The climate change brings frequent periods of dry and hot weather. The resulting stress changes the resistance of plants to pathogens, new pathogens may occur in our region as well. In this project, interactions of plant immune system with pathogens will be studied. The CRISPR/Cas 9 technique will be used to identify genes that could improve the resistance to pathogens. In the long term, gene editing could enable breeding of resistant crops.
- Basic research on flowering of *Chenopodium* plants. The CRISPR/Cas 9 technique is used for knock-out of various genes.

Negative experience of the Institute of Experimental Botany: One project proposal of the Institute has been dismissed by the authorities in spite of the fact that the CRISPR/Cas 9 technique was planned to be used only in a preparatory phase for identification of target genes. For breeding, EMS (chemically induced) mutants were to be used.

Examples of projects with organisms other than plants:

Czech Centre for Phenogenomics (CCP), Institute of Molecular Biology (IMG) – laboratory mice
Through its membership in INFRAFRONTIER and International Mouse Phenotype Consortium (IMPC, http://www.mousephenotype.org/), CCP is a partner in a collective global network that aims to analyse the effect of loss of function gene mutations in mice. The goal of this consortium is to discover functional insight for every gene by generating and systematically phenotyping 20,000 knockout mouse strains – this should result in an 'Encyclopaedia of mammalian gene function', representing a very ambitious goal with huge impact in future biomedicine. To achieve the goal, IMPC relies on mouse models, the most important tools at our scientific disposal in understanding mammalian gene function. The fundamental genetic similarity between mice and humans allows researchers to infer a human gene's function based on studies with laboratory mice. One powerful technique is to turn off, or "knockout", the activity of a mouse gene to assess which biological systems are impacted. This gives insights to how a similar gene in humans may contribute to disease when its activity is altered.
The CCP generates knockout mouse strains using embryonic stem cell resources or for now by novel methods using the CRISPR/Cas9 or TALEN technology, which is very cost-effective and opens also other possibilities, e.g. to simultaneously produce point mutants relevant for human diseases. The production of mouse strains is tracked within the "international Micro-injection tracking system" (iMits, https://www.i-dcc.org/imits) and the strains are made available to the research community via public repositories.