

Risk Assessment and Monitoring of GMOs in the Czech Republic

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Introduction

Genetically modified (GM) crops represent not only a substantial innovation in plant breeding and agriculture but also a relatively new reality to wide public and environmental specialists because the history of GM crops is still relatively very young. Since the first introduction of GM varieties in 1996 in the United States those became widely used in many countries during the next ten years. Following the official data of the International Service for the Acquisition of Agri-biotech Applications (ISAAA) in 2006, the first year of the second decade of commercialization of biotech crops, the global area of GM crops continued to climb for the tenth consecutive year at a sustained double-digit growth rate of 13%, or 12 million hectares (30 million acres), reaching 102 million hectares (ISAAA, 2006). Among 22 states growing wide scale some of GM crops, there were also six European Union's Member States which decided to use outputs of these modern technologies in their own agricultural practice. Some of them, e.g., France and Portugal returned to the GM maize growing after some period of national bans for GM varieties cultivation, while the others, like Czech Republic (CZ) and Slovakia, started with its wide scale cultivation for the first time in 2005 and 2006, respectively.

The aim of this paper is to give an insight into some fundamental aspects related to the biological safety of GM crops at the time until they were adopted in CZ, especially, how the country was preparing to it in different related areas and which measures have been taken to ensure the safe biotech crops cultivation and handling, in relation to the environment. Using some selected examples of case studies a brief overview of various activities performed in CZ in the field of GM organisms (GMOs) detection, identification and monitoring of possible GM crops impacts will be given.

What does it mean the GMO and how can public be assured about its safety?

Genetically modified organisms, as compared to their close relatives, have introduced some traits which are usually uneasy or impossible to transfer when using classical breeding methods of crossing and recombination. To introduce a new genetic material to some plant species, e.g. enabling them the tolerance to specific herbicide(s) or insect pests, the methods of gene engineering were used, which in principle, are also based on natural mechanisms of genetic modifications and transfer of traits. It is the unconventional method of GMOs origin and some public uncertainty about their safety what lead, especially in Europe, to a specific legislative and handling with **GMOs**. On the other hand, GMOs represent a quite novel technology which should be carefully tested before its general acceptance, like any other technologies. Any GM crop before its release to the environment (either for field experiments or commercialization – market) is, for many years, evaluated step-by-step to comply with numerous safety criteria. Those are especially the safety concerning human and animal health, the environment and biodiversity of organisms. Every transferred trait and a case are assessed individually in so called case studies. Every risk, apart from its probability,

has been taken into account. Potential risks of GMOs are, in fact, very low because only limited number of genes (transgenes) of known effects of the donor organism (eg., bacteria in case of some herbicide tolerant events and Bt-crops, but also plants, or even crop's own genes in some other herbicide tolerant analogues) are transferred into best crop varieties.

Czech Republic has taken the first biosafety measures to GM crops very soon

Already in 1990, based on a joint initiative of Assoc. Prof. Miloš Ondřej of the University of South Bohemia and senior scientist of the Institute of Plant Molecular Biology AS CR in České Budějovice and Professor of the Institute of Biotechnology of Charles University in Prague, Prof. Jaroslav Drobník, the first scientific committee (Czech Committee for Plant Transgenesis) aimed mainly at the registration and voluntary regulations of GMOs handling (all kinds of work with living GMOs, including transport and exchange) was established. Its activities were focused especially on experiments with GM microorganisms performed at laboratories, for which a term "contained use" is generally accepted, and both laboratory- and small scale field trials using the GM plants over the whole country. Although, at that time there was no special national legislation covering the handling with GMOs, the rules developed by the Committee based on former European Directive 90/220/EEC were generally accepted by academic institutions, universities and biotechnological companies. Since the potential risks of GMOs to an environment, especially its living parts were the main interest of the Committee, it started very soon to co-operate tightly with the Ministry of the Environment of the Czech Republic (ME), and a representative of ME became one of its executive board members. The Committee immediately after its setting up started to organize regular inspections of GMO laboratories, and especially, of the field experiments with GM crops. These inspections were aimed mainly

- at the verification of GMO's origin, presence of specific foreign genes (coding for newly introduced traits declared beforehand) using the biotests and molecular biology methods
- monitoring of GM plants at places of previous field trials, and
- possible gene transfer to wild related species

At the end of nineties, about twenty field locations (especially, those where herbicide tolerant winter oilseed rape and sugar beet, maize tolerant to European corn borer – Bt-maize were grown) were supervised annually, some of them repeatedly, and plant samples were collected for analyses. In the centre of Committee's activities were also courses for GMO advisors, plant breeders, PhD. students and an extension service focused on the public (lectures, discussions, presentations in media, e.g., articles in newspapers and journals, broadcast- and TV interviews and films).

One of the greatest challenges to the Committee was its participation in the process of preparation of the first Czech legislative for GMOs, the Act No. 153/2000 Coll. "on the use of genetically modified organisms and products and amendment of some related Acts", which

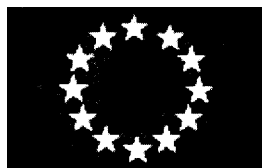
came into power on 1st January 2001, as well as at the preparation of the Decree No. 373/2000 Coll. "Requirements on contained space and protective measures for microbiological laboratories" and the Decree No. 374/2000 Coll. "on specific conditions of handling with genetically modified organisms and products" performed by ME. This legislative covered predominantly the possible risks of GMOs to the environment. The other risks, e.g. potential effects to human and animal health were, in this respect, covered by different acts and decrees prepared by other ministries, Ministry of Agriculture (MA) and Ministry of Health (MH), who also participated at the above mentioned legislative. Based on the Act No. 153/2000 Coll. an official Czech Committee for Genetically Modified Organisms and Products was established as an advisory body of ME for GMOs. It is based on specialists of academic institutions, universities, agricultural institutes, Central Institute for Supervising and Testing in Agriculture (CISTA), the State Phytosanitary Administration of CZ, medical and veterinary organizations, and representatives of ME, MA and non-governmental organizations (NGOs). Therefore its expertise covers a wide spectrum of activities, including also the GMO authorizations, international co-operation, especially those with the European Commission (EC), UNEP, OECD, Cartagena Protocol on Biosafety of GMOs, etc.

Since the year 2005 the new Act 346/2005 Coll. which is harmonized with the Directive 2001/18/EC "on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC", regulates all the GMO handling related to the environmental risks in CZ. One of its important parts represents the monitoring of possible (predictable) impacts on the environment performed during the field experiments (release following letter B of the Directive), and preceding necessarily to the commercial GMO release (following letter C of the Directive), and a post-release monitoring aimed at identification of possible unexpected or delayed GMO effects on the environment when the specific GM crop and trait combination release into the market.

What subjects support GMO biosafety studies in CZ?

There are numerous institutions and agencies supporting environmental studies of possible GMOs' impacts. At the beginning, it was especially the Ministry of the Environment interested in such studies but later also the Ministry of Agriculture started to support annual studies and its grant agency was supporting mainly the long-term studies. Some projects are supported by Grant Agency of the Czech Academy of Sciences and Grant Agency of the Czech Republic too.

Co-operation of CZ in biosafety programmes of EU



It should be noted that the Czech Republic also actively participates in European programmes of the 6th Framework Programme, aimed, e.g., at the safe introduction of GM crops into agricultural systems of European Union countries, known as SIGMEA "Sustainable

Introduction of GM Crops into European Agriculture". CZ cooperated in this programme with other EU countries on biosafety studies of oilseed rape (University of South Bohemia, České Budějovice – USB) and sugarbeet (Czech University of Life Sciences, Prague – CULS). One of the main outputs of this 3,5 year lasting programme was a recommendation of systematic tools for a sustainable co-existence of different agricultural systems with those based on GMOs, officially submitted to EC representatives on an occasion of the Third International Conference on Coexistence between Genetically Modified (GM) and non-GM based Agricultural Supply Chains – GMCC, held in Seville, Spain (20-21st November, 2007). Some other programmes deal with the standardization of methods used for sampling and detection of GMOs. For instance, National Reference Laboratory for Identification of GMOs and DNA Fingerprinting at the Institute of Crop Production, Prague (ICP) and the Institute of Chemical Technology, Prague co-operate with the European Commission Joint Research Centre at Ispra, Italy (JRC) and European Network of Genetically Modified Organisms Laboratories (ENGL).

Risk assessment as a basic part of GMO authorisation in CZ

Release of GMOs into the environment is in CZ regulated, both by national and EU legislatives. Risk Assessment (RA) analyses of intended- and unintended releases of any GMO into the environment represent a fundamental step of the decision making process. It consists of a careful evaluation of various kinds of risks to different components of the environment, including also any possible risks of harms to human being and animals. Such type of work is done by Czech Committee's experts based on documents submitted together with a company's or institute's Dossier and known literature data. If needed, other specialists are also invited to assess specific risks. In case, that an event is for the first time introduced into the country under letter C, specialists can use the data and experience of other countries where the release preceded. But in such case, it is of a great advantage to dispose with the own national data sets of field experiments which reflect possible geographical, climatic, wild flora and fauna differences as well as the diversity of agricultural systems used. Such attitude represents a fundamental part of all the Czech GMO evaluations performed to date. Therefore, e.g., both ministries (ME and MA) supported biosafety-related projects on specific crops and events already many years in advance, before the actual market release could/has take(n) place. In case of any doubts or uncertainty about specific risk(s), the gradual process of release *laboratory --), field experiments --* market release* is stopped until new satisfactory data are available. Based on the national legislative a public participation at any GMO's release to the environment represents an integral part of the process.

Examples of selected monitoring studies

Winter oilseed rape (OSR)

OSR is one of the main crops for which genetically modified cultivars have been developed and released for commercial use in some countries. Biological properties of OSR make this species to be one of the most problematic GM temperate crops to ensure the safe wide scale cultivation. As an invasive species, grown usually on broad acreages and producing huge

amounts of pollen and seeds, OSR can persist in fields for years and slowly spread to surrounding ecosystems. Based on this fact a special attention was paid to OSR in numerous projects supported by ME and MA. Therefore OSR was also the subject of an intensive risk assessment research performed within the European Science Foundation (ESF) project AIGM (Assessment of the Impact of Genetically Modified Plants, AIGM–ESF in which CZ participated in 1998-2001) and the EU SIGMEA (2004-07) project with the aim of identifying possible risks and consequently defining basic rules for transgene confinement.

Introductory studies of the Institute of Plant Molecular Biology AS CR (IPMB AS CR) and Faculty of Biological Sciences, USB in České Budějovice in herbicide tolerant oilseed rape (HT-OSR) were aimed at the development of sampling methodology and of a simple method for herbicide tolerance assessment suitable for laboratory testing of thousands OSR plants (Figs.1-4), evaluation of cross-pollination frequency in various distances from HT-OSR field and comparisons of its viability and persistence to the conventional OSR under specific CZ conditions. In these studies, very important results were obtained, showing that there is no difference in the ability of GM OSR to survive or spread within the field as compared to common OSR cultivars. It was shown also, that the number of hybrids, as an indicator of cross-pollination, decreases substantially in a few meters distance of the pollen source. Both results could serve to decision-making authorities for the improvement of the knowledge-based assessments of GM OSR potential risks and taking adequate measures to minimize them. Nevertheless, the interpretation of outcomes of such studies should reflect the requirements of local authorities, specific geographical and biological conditions and local farming practices. Such studies can serve as the basis for local oversight and monitoring systems and the preparation of Co-existence rules.

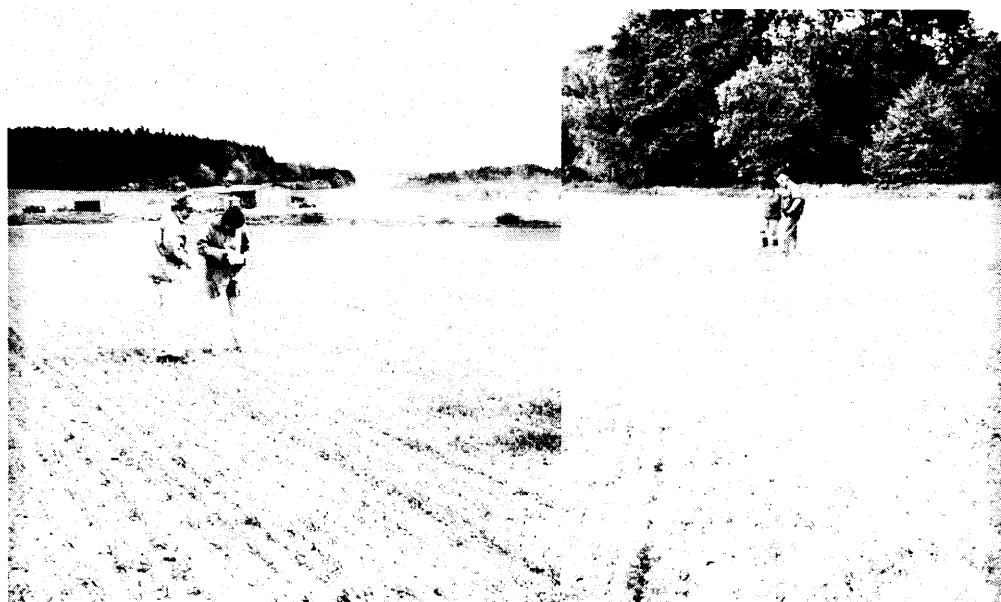


Fig. 1. Monitoring of former experimental field areas for the presence of GM OSR plants. Author: S. Rakouský, USB

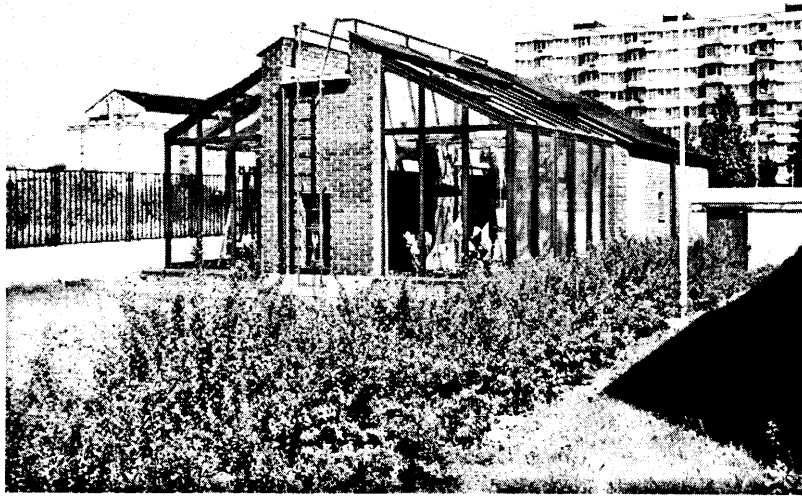


Fig. 2. Glasshouse for testing GM plants.

Author: S. Rakouský, USB

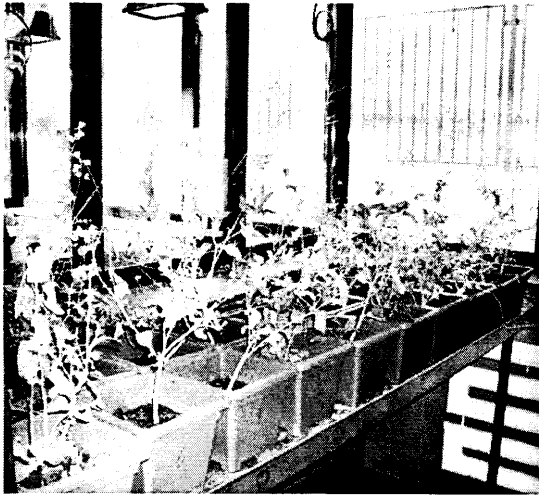


Fig. 3. Contained cultivation of GM OSR

Author: S. Rakouský, USB



Fig. 4. Screening of oilseed rape seedlings to herbicide tolerance. Sensitive plants (at bottom) did not survive after spraying, while tolerant ones are



More detailed studies were performed within the EU SIGMEA project where experimental fields were monitored at regular intervals for the presence of OSR survivors and volunteers. Moreover, data from eight different locations and for five seasons after the HT OSR was grown (2002 – 2006) were obtained (Tab. 1). Results obtained to-date, indicate that GMOSR plants originating from the soil seed bank could be still present in experimental fields five years after GM rape cultivation. Their frequency can be suppressed efficiently using proper agronomy measures to minimize possible risks of contamination of non-GM rape or to reduce to an acceptable level the likelihood of gene transfer to related species. As compared to some other European countries (U.K., France, Germany) risks of the possible gene transfer to some wild species under CZ conditions are very low, due to the nearly absence of close-related species in wild and field flora. Thus, possible cross-contamination of non-GM rape production (conventional or organic), either via transfer of pollen or seeds, would represent a more serious problem.

Sampling date	Location [No.]	OSR volunt. fr. [No./m2]	Samples total [No.]	OSR samples [No.]	Other species samples [No.]
09/05/02-18/06/02	1, 2,3, 4, 7	0-0,33	197	114 (33*)	18 (16*) YC, 65* YF
20/05/03-19/06/03	1, 2, 3, 5, 6	0-0,10	133	91	42* YC, WR
06/05/04-02/06/04	1, 3, 5, 6, 7,8		297	259	34 BM , 4 TR
19/04/05-07/10/05	1, 2, 3, 5, 6,7		261	205	22 YC, 11* YR, 7 SP, 15 FP, 1 TC
12/05/06-13/06/06	1, 3, 5, 6, 7	0-0,20	238	177	4 FP, 41 (15)* SP, 8* TM, 8* WR ,

* - field borders or its near surrounding

BM - Black mustard (*B. nigra* (L.) Koch), TR - Turnip rape (*B. rapa* L.) - **both volunteers**

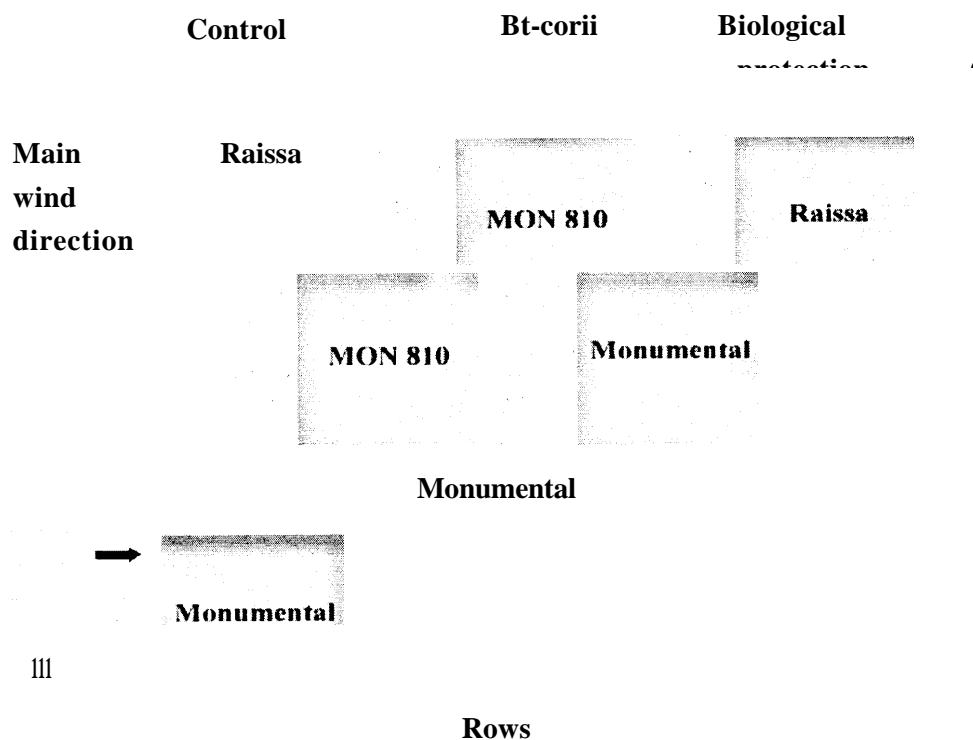
FP - Field penny cress, SP - Shepherd's purse, TC - Thale cress, TM – Treacle mustard, YC - Yellow charlock, YF - Yellow fieldcress, YR - Yellow-rocket, WR - Wild radish - all wild related species

Table 1. An example of monitoring studies outputs performed by USB under the EU SIGMEA project. Author: S. Rakouský, USB

Feral populations of GM OSR which could serve as an additional source of contaminating pollen and seeds, were not found during those limited studies indicating that the relatively strict rules used in CZ for the management of OSR farming practice could be also in favour of the safe GM rape cultivation. The study has helped also to improve the sampling methodology and HT transgene detection in OSR as well as to refine the new oversight systems (and policies) in preparation for the introduction of co-existence rules by the national GMO authorities in the Czech Republic.

Bt-maize

Climatic changes are usually connected also within some shifts in spectra of crop diseases and pests. Especially, during the last decade one of the most serious maize pests, European corn borer (ECB) has spread over a large part of the Czech Republic causing damages of stems and grain. Based on these findings, numerous studies on various strategies of maize fields' protection were initiated to compare not only their efficiency but also to minimize negative effects of treatments to the environment and human health. As to the results of ICP, Prague of two biological methods used to protect maize (first one based on the use of a predatory wasp *Trichogramma*, second one using Bt-maize varieties), the highest efficiency against ECB has shown the Bt-strategy (100%), repeatedly in all three years (Figs. 5, 6). Furthermore, it was



3 protection strate--ics

iiiiiiiiial arca per strate^gy - 0.3 lia total field arca - 1 lia

Fig. 5. Scheme of field trials aimed at a comparison of various methods of maize protection

against European corn borer (ECB) pest. After F. Kocourek, ICP

proved that Bt-maize grain contains up to 60% less of cancerogenic aflatoxins as compared to conventional maize due to the suppression of *Fusarium* fungi outgrowth on undamaged cobs.

Efficiency of Strategies:

Trichogramma

- against ESB 35-69 %

- against *Fusarium*

in maize cobs

30 - 66 %

Bt-corn

- against ESB

100%

- against *Fusarium* in maize cobs 100%



Fig. 6. Comparison of the efficiency of two biological methods of maize protection against ECB pest. After F. Kocourek, ICP

Other field experiments performed by the Institute of Entomology AS CR (IE AS CR), České Budějovice gave further proof of the safety of maize Bt-technology. No significant effects of Bt maize to other non-target insect species (especially predators) were found (Tab. 2).

<u>Date</u>	<u>Non- Bt maize</u>	<u>Bt maize</u>
June 22	1.0 ± 1.0	2.2 3.5
July 11	9.0 ± 3.5	15±6.3
July 30	18.2 ±	20 9.6



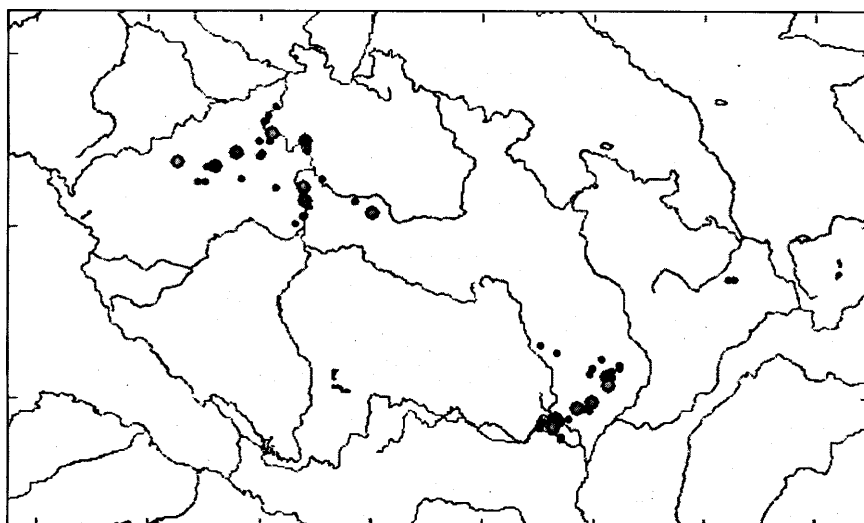
Table 2. Occurrence of an insect predator *Orius* sp. on experimental plots with conventional-and Bt maize After F. Sehnal, IE AS CR

Wheat

Limited number of experiments has been done using the non-GM wheat varieties to evaluate potential risks of their modified derivatives. These experiments and risk assessments were conducted especially in ICP, Prague in co-operation with the Institute of Botany AS CR Průhonice (IB AS CR). Some objections have been risen especially against possible

future cultivation of HT wheat analogues due to the probable HT wheat natural crossings to some perenial weedy *Elytrigia* (former name *Agropyron*) species and possible formation of HT hybrids (Fig. 7).

Distribution of *Agropyron* Intermedium



51N

50N

49N

12E 14E 15E 16E 18E 10E

Fig. 6. Distribution of perenial *Elytrigia* (*Agropyron*) species over CZ territory. Authors: J. Ovesná, ICP and F. Krahulec, IB AS CR

Sugar beet

Studies of CULS on sugar beet have focused mainly on the presence of weed beet species coming from the seed impurities which easily hybridize to sugar beet forming the long-time persisting populations in areas of an intensive sugar beet growing, their dynamics and possibilities to control beet weediness. It was shown that a careful checking of the quality of imported seed lots represents a crucial precondition of successful weedy beets control.

Potato and other crops

Potato, due to its dual use (for food and feed, industrial applications) is for many years in the centre of interest of biotech companies. Two basic types of modified potatoes have been tested in field experiments in CZ so far. With the aim to change the sugar metabolism in potato tubers one of modifications is based on the introduction of a bacterial gene coding for an enzyme phosphofructo-kinase which is able to metabolize free sugars accumulated in a response to a low temperature also at temperatures below 8 °C. Other introduced genes cause modifications of starch composition (e.g. high amylopectin and low amylose content). Monitoring studies confirmed previous assumptions of specialists that only very low levels of potential GM potato risks could be expected under conditions of its growing on a territory of CZ, e.g. most of tubers persisting in the soil due to harvest losses dies during the first winter and no closely-related species are present in wild flora.

Very low potential risks were found also for GM flax during a period of 7-year studies of the research and breeding institute Agritec Plant Research (APR) and Agritec Ltd., Šumperk performed both, in glasshouse and field experiments. Any attempts to cross flax and linseed cultivars to wild relatives occurring in CZ flora were unsuccessful as well as to detect any overwintering plants or volunteers arising of the seed bank following the harvest (Figs. 8, 9). The institute established also an extensive research programme aimed at biosafety studies using a non-GM pea before releasing its modified analogues to field trials.

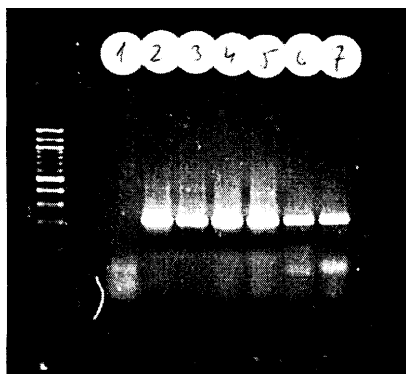


Fig. 8. To detect the presence of specific introduced genes (transgenes), the methods of molecular biology are widely used like polymerase chain reaction (PCR). Its products are separated by gel electrophoresis and visualized under UV light. Presence of bands of specific molecular weight at samples indicates the transgene presence.

Author T. Kocábek IPMB AS CR



Fig. 9. Experiments on artificial crossings of flax to wild-related species present in nature (left) and simulations of waste composting after flax seed purification from hulls (right).

Author E. Tejklová, Agritec Plant Research

Summary

During the past decade the Czech Republic (CZ) has established an extensive research basis

and a network for an efficient co-operation both, at the national- and international levels in the GMO risk assessment and monitoring studies to use the science-based knowledge in a decision making process of GMOs' releases to the environment. This makes a country the qualified and respected partner on international GMO fora. The Czech Republic also very soon has initiated an extension service to the public to explain the fundamentals of genetic modifications, possible risks and benefits to the environment and a community. An intensive work and effort spend in both directions has significantly contributed to the fact that the public acceptance of GMOs in CZ is the highest among all European countries. Czech Republic is in a favour of use of modern biotechnologies, including those based on GMOs, where appropriate and of benefit, both to the public and environment.