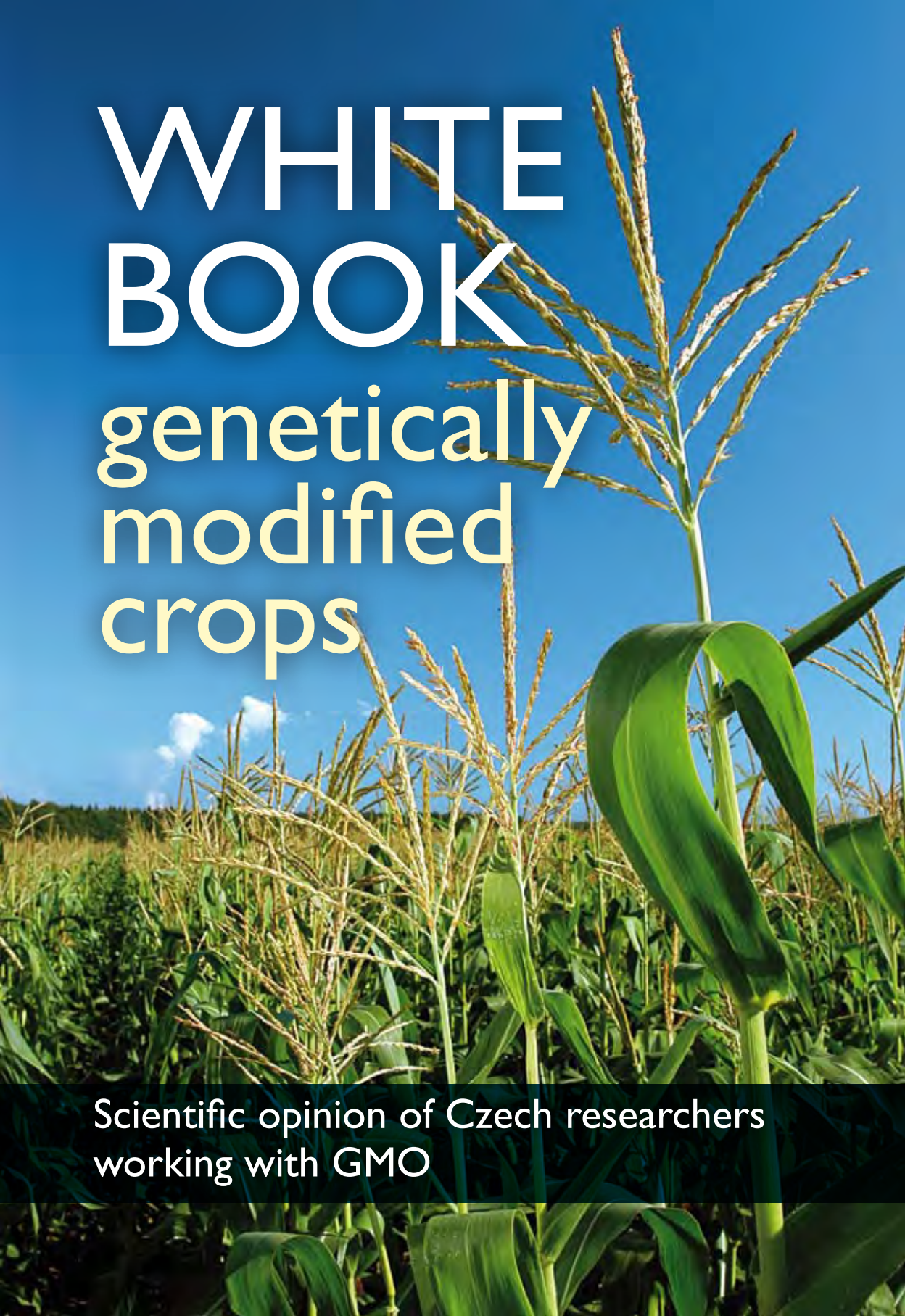

WHITE BOOK

genetically modified crops

Scientific opinion of Czech researchers working with GMO

WHITE BOOK

GENETICALLY MODIFIED CROPS

EU REGULATIONS AND RESEARCH EXPERIENCE FROM THE CZECH REPUBLIC

THE HISTORY
OF MAJOR HUMAN
DISCOVERIES SHOWS THAT
FUNDAMENTALISTIC IDEOLOGY,
IGNORANCE, AND GREED
OFTEN SUPPRESS THE TRUTH,
BUT ONLY FOR A CERTAIN
PERIOD OF TIME.
THIS BOOK WAS PREPARED
WITH THE DESIRE
TO SHORTEN THE PERIOD
OF FALSE APPREHENSION
OF GM CROPS
IN EUROPE.

WHITE BOOK genetically modified crops

EU REGULATIONS AND RESEARCH EXPERIENCE FROM THE CZECH REPUBLIC

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This Book was prepared under the auspices of the 7FP REGPOT-2008-1 project "Building up Modern Biotechnologies for Agriculture" (acronym MOBITAG, GA 229518). Most Czech scientists working with genetically modified organisms (GMOs) relevant for agriculture and related activities were invited to express their opinions and provide a short annotation on their research. Their names and addresses are provided at the end of the book, following a conjoint appeal calling for a revision of the current EU legislation on genetically modified crops. This book, including a call from Czech scientists, is neither an advertisement nor an advocacy for the deployment of GM crops – it is a call for the use of critical intelligence and knowledge in the decision making process on this technology. The book and its contents can be distributed freely.

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1. PURPOSE OF THIS BOOK

Current efforts to combat starvation on a world scale are undermined by a continuous increase in the human population accompanied by a decrease in the acreage of arable land. The threat of food shortage is exacerbated by increasing demands on food safety and quality. High production of high quality food depends largely on efficient cultivars that have hitherto been obtained, primarily, by random mutations. The selection of "spontaneous" mutations and the hybridization of selected plants had been sufficient for millennia, and the highly productive cultivars we grow in the fields today were largely obtained with the aid of mutations induced either by radiation or chemicals. Whilst these techniques remain useful tools of plant breeding, it is feared that many thousands of mutagenic interventions that have already been carried out have practically exhausted the endogenous genetic resources of most crops.[1] Fortunately, the innate resources can now be enriched by procedures that are known as genetic modifications (GM) since this technology provides access to a significantly increased gene pool.

Genetic modification applied to crops today should be called transgenesis because they include the transfer of one or more useful foreign genes into the target plant, thereby conferring a new trait, such as resistance to an insect pest if the transgene encodes an insecticidal protein. As in any other technology (soil tilling, herbicide application, biological control of insect pests, etc.), the production of GM crops constitutes human interference with nature and must be deployed with care. The risks and benefits of GM crops must be compared with other techniques serving the same purpose, for example insecticide application in insect pest control, before their practical deployment is considered. In the case of commercialized GM crops, scientific evidence as well as practical experience[2] has demonstrated that they bring considerable economic benefits to farmers and are more environment-friendly than comparable technologies. However, in spite of their successful worldwide cultivation, the use of GM crops in the European Union has become a controversial subject and the technology is completely rejected by some member states. Since such a condemnation of a modern technology may endanger EU competitiveness, it should be thoroughly analyzed using unbiased scientific methods. This need has been recognized by the Council of the Ministers of Environment that convened on December 4, 2008, and stated[3]:

> (Council...) *INVITES the Member States to ensure* ***full participation of their competent scientific bodies*** *in the consultation the EFSA will undertake during the revision process, by offering their contribution on the project within the required time frame;*

1 Gressel, J. (2008) Genetic Glass Ceilings. Transgenics for Crop Biodiversity. The John Hopkins University Press, Baltimore, Maryland.

2 James, C. A. (2008) Global Status of Commercialized Biotech/GM Crops: 2008. ISAAA Briefs. Brief 38.

3 Council Conclusions on Genetically Modified Organisms (GMOs). 2912th ENVIRONMENT Council meeting, Brussels, December 4, 2008.

and

(Council...) *INVITES the EFSA and the Member States to pursue the formation of an extensive* ***network of European scientific organisations*** *representing all disciplines including those related to ecological issues with the assessment of risks associated with the cultivation* ***or use of GMPs in food and feedingstuffs.***

stressing

(Council...) *EMPHASISES that Member States and the Commission should ensure that systematic and independent research on the potential risks involved in the deliberate release or the placing on the market of GMOs is conducted; NOTES that* ***the necessary resources should be secured for such research by the Community and Member States*** *in accordance with their budgetary procedures, and that independent researchers should be given access to all relevant material.*

Czech scientists working with GM crops responded to this invitation by compiling this White Book that summarizes the results of their analysis of relevant EU legislation and provides examples of conducted research. The Czech scientific community has a long tradition in the investigation of GM crops: it has contributed to the development of genetic modifications, participated in formulating national regulations on genetically modified organisms (GMOs), and has substantially contributed to debates addressing rational concerns, and eventual acceptance, of GM crops by the majority of the public. Czech Republic is among the few EU countries where farmers have gained practical experience with the cultivation of GM crops and this provides positive feedback on current research activities.

Many European scientists are disturbed by the fact that political factors and ideology prevent unbiased assessment of GM technology in some EU countries, with a negative effect on the whole Community. Being aware of the responsibility their country bears during the EU Presidency, Czech scientists decided to formulate their position in support of a scientific approach to GM issues. As with any other technology, the deployment of GM crops will bring benefits with minimal negative effects when used in a rational, scientifically designed way. We hope that this White Book will encourage the politicians as well as the general public to accept this objective viewpoint.

2. EU APPROACH TO AGRICULTURAL BIOTECHNOLOGIES

2.1 CONCISE HISTORY OF EU REGULATIONS

US President Barack Obama recently signed a memorandum on the importance of scientific integrity in government decision making.[4] The Memorandum states that public policy should be guided by the most accurate and objective scientific advice available and that

> *The public must be able to trust that advice, as well, and to be confident that public officials will not conceal or distort the scientific findings that are relevant to policy choice.*

Unfortunately, recent European legislation concerning GM crops does not consider scientific findings but appears to be based on an unjustified belief that transgenesis is the only selection method generating risk[5]. Other biotechnology methods, such as distant hybridisation and mutagenesis induced by radiation or hazardous chemicals, are claimed inherently safe without a need for any control. This "confidence trick" is deliberately applied to an unsuspecting public.

Policy makers neglecting scientific evidence and adhering to unjustified beliefs betray their electorate that expects management of public affairs in the interest of the EU population. However, the *à priori* condemnation of the use of genetic modifications in plant breeding reduces the competitiveness of EU agriculture and is thereby against the interests of EU citizens. Furthermore, the public is misinformed about the principles of genetic manipulation and about the safety of this procedure compared to other plant breeding methods such as radiation or chemical mutagenesis. The current EU rules regulating the development, testing, and deployment of GM crops are very similar to the legislation regulating the use of poisons, narcotics, explosives and chemical weapons (state licence for handling, labelling, protocols setting and saving, personnel specific training, plans of accident handling, regular reports to the state authorities, specific licence for export and import, etc.). Such regulation inevitably arises suspicion in the minds of the public and is easily interpreted as proof that GM crops are dangerous. The misinformed public then rejects GM technology and eventually demands political representatives to ban everything related to the GM crops.

The EU approach to GM crops was initially more objective, but has changed over the years. At the end of 1983, Etienne Dvington, then the Vice-President of the Commission, and the commissioners for agriculture and internal markets proposed the formation

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4 Available at: <http://www.whitehouse.gov/the_press_office/Fact-Sheet-on-Presidential-Memorandum-on-Scientific-Integrity/>

5 Morris, S. H (2007) Parallel biopolitical universes. Nat Biotechnol 25, 33–34; available at: <http://www.nature.com/nbt>.

of a Biotechnology Steering Committee (BSC) to be chaired by the Director-General of DG XII (Science, Research and Development). The proposal was adopted early in 1984, with the Directorate for Science, Research and Development being responsible for drafting most of the documents on biotechnology. However, since July 1985, DG XI (Environment) has been included in the BSC and a new body, the Biotechnology Regulation Inter-service Committee (BRIC), was formed, with the chair being divided between DG III (Internal Market) and DG XI.

The aims of BRIC were formulated *inter alia*

> *To ensure the coherence of scientific data which will form the basis of risk assessment, and in particular to avoid unnecessary duplication of testing between various sectors.*

BRIC was asked to prepare an inventory of Community regulations in the biotechnology field[6]. BRIC summarized results from several meetings and evaluated other events that occurred at that time (e.g. Denmark as the first European country adopted the Gene Technology Act and OECD issued the report "Recombinant DNA Safety Considerations"). This document drawn up by BRIC[7] reflected on the possible introduction of GM technology to agriculture:

> *On the field release: It was accepted that the scientific basis for prediction of effects was inadequate, and research for this purpose should be reinforced; in the meantime, there should be notification and case-by-case consideration before approval.*
>
> *While the desirability of Community framework of regulation was generally agreed there was some reticence expressed. Some States, particularly the U.K., France and the Netherlands, seemed inclined to view existing legislation as a basic requirement to which countries might add further requirements relevant to their particular situation – geographical, climatic or regional;*

It is obvious that regional requirements of individual countries was considered as early as 1986.

In November 1986, BRIC prepared a document "A Community Framework for the Regulation of Biotechnology"[8] giving the target."....*to draft proposals for legislation on genetically engineered organisms to be presented to the Council by Summer 1987.*" It was decided to divide responsibility within BRIC: DG V was responsible for the drafting of the Council Directive 89/391 "On the introduction of measures to encourage improvement in the safety and health of workers at work", which also included biological agents. The document regulating the contained use of genetically modified organisms

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6 The European Community and the Regulation of Biotechnology: An Inventory. European Commission 1986, BRIC/1/86.

7 Biotechnology Regulation: Meeting of Commission Staff with Member State Officials Brussels, 29-30 April 1986. European Commission 1986, BRIC/2/86.

8 Communication from the Commission to the Council COM (86) 573, 4 November.

(GMOs) was jointly prepared by DG XI and DG III and the draft regulations for the deliberate release of GMOs into the environment was the sole responsibility of DG XI.

The BSC meeting held in July 1988 was the last one because the new Vice-President Pandolfi, who took office in 1989, did not resume its function. BRIC thus took over the biotechnology regulation agenda and after ten meetings it published two Council Directives on the contained use of GM microorganisms and the deliberate release of GMOs in May 1988. The definition of GMO was principally taken from the 1978 UK regulations and the techniques in the deliberate release directive were very vaguely defined in Annex I. After several modifications, the two Directives were submitted to the EU Parliament (rapporteur Gerhard Schmid) and adopted by the Council of Environment Ministers on 23 April 1990.

All EU proceedings on GMOs were then heavily influenced by the pressure exerted by the "Greens" that were perceived by the scientific community as a burden to science, industry and the international market[9]. The 40th meeting of the Council of EMBO on the 1st October 1988 discussed the proposal of the Directives and came unanimously to the opinion that

> *....any legislation should focus not on the technique but on the safety or otherwise of the products generated with it. ...Over the last 15 years, experience has shown that recombinant DNA methods, far from being inherently dangerous, are an important tool both for understanding properties of life and for developing applications valuable to humankind and the environment. EMBO strongly believes that there is no scientific justification for additional specific legislation regulating recombinant research per se. Any rules or legislation should only apply to the safety of products according to their properties, rather than according to the methods used to generate them.*

This statement was presented to the European Parliament on 16 May 1989 by Max Binstiel, then the head of EMBO Council, and by Lennart Philipson, the Director General of EMBL. Two days later, 16 European Nobel Laureates in Medicine and Chemistry addressed a supportive open letter to the President of the European Parliament, the EC Council and the Commission.

The Nobel Laureates wrote another letter before the second Parliament reading on 8 February 1990. However, science lost the battle and the paradigm "method is risky and transgenesis is the only one of this sort" was accepted. Twenty years have now passed since the formulation of the first Directives. This is a very long period of time in such a rapidly developing field. Millions of hectares have been planted with GM crops globally and millions of tons of GM crops have been harvested and consumed without any negative consequences. The methods used for the production of GM crops have been improved to eliminate some of the potential (never seriously proved) risks. Nothing from this development, however, has moved the politicians to amend the fossil regulation paradigm.[10] Ironically, in the EU, over three thousand radiation

9 Young F. E., Miller H. I. (1989) „Deliberate releases" in Europe: over-regulation may be the biggest thread of all. Gene 75, 1-2 (Editorial).

10 McHughen A. (2007) Fatal flaws in agbiotech regulatory policies. Nat Biotechnol 25, 725–727.

mutants with unknown changes to their genome have been deployed without any restrictions, but keeping the glowing zebra fish in an aquarium in a living room presents a "serious jeopardy to European nature and human health" with a fine up to 50 000 €.[11] Such a system is officially called "scientific" but in fact it is a flout to science.

2.2 RATIONAL RULES FOR ASSESSING THE RISKS OF BIOTECHNOLOGIES

With an unbiased approach we have to assume that the deployment of GM crops may bring, as other technologies, certain benefits but it may also damage the environment and human and animal health. Only a scientific evaluation of the benefits and risks can set the level of acceptable risk as a basis for a wise decision of the acceptance or refusal of the technology in any given situation. Proper assessment of the benefits and risks must be done by comparison with alternative technologies that serve the same general purpose. For example, by comparing farming of a pest-resistant GM crop with the cultivation of similar non-GM varieties that are protected against pests by insecticide treatments or by the application of bioagents. Risk assessments are worthless if done without appropriate controls under the assumption that the current "standard" ("conventional", "traditional", etc.) methods pose no risks. Economics should also be taken into account, in particular in evaluations of the long term use. For example, since the seeds of insect-resistant GM crops are more expensive than the non-GM counterparts, farmers will plant the non-Bt cultivars when pest infestations are low.

Several types of genetic manipulations are employed to produce GM crops. The method of **gene silencing** was used to produce the Flavr-savr tomato, which was treated as a "risky" intervention leading to a compulsory change of the genotype. However, the mix of two different genomes achieved in the breeding of triticale cereal is a much more profound intervention and yet, the product proved safe, is widely accepted, and does not require regulation. **Gene transfer from wild relatives** is also objected to by the opponents of GM, despite the fact that similar but less pronounced effects were obtained through classical breeding procedures. For example, classical breeding produced potato varieties with partial resistance to the blight caused by *Phytophthora infestans*. The cause of resistance is unknown but there are no special regulations on the use of such potatoes, in contrast to the GM potato that carries a defined gene transferred from the wild *Solanum bulbocastanum*. Additional objections to gene transfer are raised when the gene is combined with **antibiotic resistance**. Genes encoding resistance to certain antibiotics were used in the first phase of transgenic crop development for purely technical reasons. Later they were replaced by genes providing herbicide tolerance, but are eliminated in later steps of GM crop breeding. However, certain very useful crops still include genes conferring antibiotic resistance. The consumption of such a gene from a GM crop represents a negligible addition to similar genes that we receive from bacteria present in our daily diet. Thus the public should be better informed about the amount of bacteria in the food and feed consumed and about the level of antibiotic resistance they already carry.

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11 Der Spiegel, March 20, 2007.

2.2.1 Principles of the risk assessment process

A) General premises

1. Any new variety by definition must exhibit at least one new stable and uniform trait. Since any trait has a genetic basis, it can, in the majority of cases, be transferred to related plants.
2. Any agriculture, silviculture, etc. brings about disturbances to natural ecosystems.
3. Any new crop and variety alters the current state of the community of organisms.
4. The impacts mentioned above can be positive or negative; there is no reason to classify them à priori as negative and the probability of their occurrence as a risk.
5. Farmers having a free choice will prefer planting the most productive and commercially most successful variety and thereby reduce the diversity of varieties grown.

B) The concept of risk

1. Risk is given by the probability of damage resulting from exposure to a hazard. There is no human activity in the field of ecology with zero risk.
2. As the risk scale does not start with zero, only relative risk can be assessed by comparison with alternative human activities.
3. Acceptable risk must be defined based on the ratio benefit/risk, implying that risk assessment must be complemented by benefit assessment.
4. It should correctly be distinguished between the particular risk of a crop and the complex risk of all agrotechnologies linked to the crop planting (use of machinery, chemicals, timing of planting and harvest, etc.).

C) The position of GMOs

1. There are no scientific data showing the exceptional position of plants expressing a trait based on transgenesis. Thus there is no ground for their regulation to be any different to plants obtained by traditional breeding methods.
2. However, the EU Commission issued on 2 February 2000 "Communication on the Precautionary Principle"[12] The point v) of this states: Decision-makers need to be aware of the degree of uncertainty attached to the results of the evaluation of the available scientific information. Judging what is an "acceptable" level of risk for society is an eminently political responsibility. However, according to point vi): Where action is deemed necessary, measures based on the precautionary principle should be, inter alia:

    a) *proportional* to the chosen level of protection,
    b) *non-discriminatory* in their application,
    c) *consistent* with similar measures already taken,

12 Communication from the Commission on the precautionary principle. COM(2000) 1, Brussels, 02. 02.

d) *based on an examination of the potential benefits and costs* of action or lack of action (including, where appropriate and feasible, an economic cost/benefit analysis),
e) *subject to review*, in the light of new scientific data, and
f) *capable of assigning responsibility for producing the scientific evidence* necessary for a more comprehensive risk assessment.

3. Risk is given by the probability of damage; since the term probability, by definition, expresses an uncertainty (the fact that certain information is not available), the risk assessment includes the "precautionary principle". The request to include this principle in the risk assessment is superfluous.

2.2.2 Steps in the risk assessment process

Risk deduction. The first step usually includes analysis of the nature and magnitude of possible risk, i.e. evaluation of known facts and experience concerning probability of damage connected with a particular situation. If there are sufficient reasons to expect that actual risk is higher than the acceptable risk ($R_{ex} > R_{acc}$), experimental testing of the risk must follow.

Experimental and control testing. Experimental testing is performed by established procedures, e.g. feeding tests, allergy induction assays, field experiments, etc. Since the tests are expensive and are funded by the tax payers/consumers, any decision on testing should be taken in a responsible manner. Unfortunately, some demands for testing are not based on facts but on unfounded catastrophic scenarios generated by certain pressure groups referring to the precautionary principle. At the same time, much higher risks that are not linked to GMOs are neglected. For example, possible risk of the bacterial enzyme CP4 EPSPS introduced into some crops to induce tolerance to glyphosate has been subjected to repeated experimental testing (see, e.g. the report by the Scientific panel of EFSA 2007[13]), including allergenicity tests[14]. None of these studies considered the fact that human food (and even more animal feed) contains millions to tens of millions of bacteria per gram. Most of them are soil bacteria that contain the EPSPS enzyme. This protein is a standard component of food and feed and some of the excessive and expensive tests were superfluous.

A similar situation concerns the *nptII* gene that confers resistance to kanamycin. Many papers have been published on the possible transfer of this gene from genetically modified plants to intestinal pathogens[15]. No one, however, has experimentally performed a simple estimation on the amount of this gene ingested daily with our food.

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13 Safety and nutritional assessment of GMO plants and derived food and feed. The role of animal feeding trials. Report of the EFSA GMO Panel Working Group on Animal Feeding Trials (2008), Food Chem Toxicol 46, S2-S70.

14 Hoff M. et al. (2007) Serum testing of genetically modified soy beans with special emphasis on potential allergenicity of the heterologous protein CP4 EPSPS. Mol Nutr Food Res 51, 946-955.

15 Courvalin P. : Transgenèse et résistance aux antibiotiques. Conférence de consensus organisée par le Ministère de l'Alimentation, de l'Agriculture et de la Pêche du Danemark le 12 Novembre 1997 à Copenhague, consacrées aux risques liées aux plantes modifiées génétiquement.

Bacteria-to-bacteria transfer of genes (horizontal gene transfer) is easy whereas the transfer from plant-to-bacteria has never been convincingly proven. The conclusion of EFSA panel[16] reflected this situation, but in 2007 Commissioner Stavros Dimas used the presence of *nptII* gene as a pretext for banning the approval of the potato industrial variety Amflora for planting. This decision was based on personal believes and not on scientific facts.

Interestingly, the number of radiation mutants introduced into the environment has reached 3000[17], but for political reasons they are automatically regarded as being below the acceptable risk. This is because "Mutagenesis" is excluded from "genetic modification" in the Annex I B of the Council Directive 90/220/EEC[18] as well as in all other Directives and Regulations that follow:

> *Techniques of genetic modification to be excluded from this Directive, on the condition that they do not involve the use of GMOs as recipient or parental organisms, are:*
> *(1) mutagenesis,*
> *(2) cell fusion (including protoplast fusion) of plant cells where the resulting organism can also be produced by traditional breeding methods.*

This statement neglects the fact that even stabilized radiation mutants contain, in comparison with the parent plant, more new proteins than the varieties obtained by the GM technique[19]. It is also well known that even a single amino acid substitution, what is a frequent result of radiation mutagenesis, may decrease protein digestibility and/or glycosylation patterns. Both these changes are important factors in protein recognition by the immune system and may cause allergic reactions. This possible risk is described here to emphasize the absurdity of GMO regulations, not as a call for the application of these time-consuming and expensive bureaucratic procedures to be extended to radiation mutants.

Testing of the risks to health. All tests acceptable by the scientific community must include appropriate controls[2] but their selection is often difficult. The parent (near isogenic line; near identical to the GM except for expression of the desired trait) may not always be the most appropriate choice due to unintended effects that may occur during transformation (an effect that also occurs during conventional plant breeding). However, such effects are usually mitigated by using several different transformation events and also GM plants transformed with the empty vector (i.e. gene construct containing the same marker genes etc., but devoid of the target gene).[10] It is also recommended

16 Opinion of the Scientific Panel on genetically modified organisms [GMO] on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. Adopted date: April 2, 2004. EFSA J 48, 1–18.

17 Nuclear Science for Food Security. IAEA report, Vienna, December 2, 2008. Available at: <ftp://ftp.iaea.org/dist/adpi/PressCampaign/PressRelease/FoodSecurityPressRelease.pdf>.

18 Council Directive of April 23, 1990 on the deliberate release into the environment of genetically modified organisms (90/220/EEC), OJ L 117, May 8, 1990, p. 15.

19 Batista R., Saibo N., Lourenço T., Oliveira M. M.: Microarray analysis reveal that plant mutagenesis may induce more trancriptomic changes than transgene insertion. Proc Natl Acad Sci US 1005, 3640–3645.

to use varieties produced by radiation (if available), but this naturally further increases the cost of testing. During tissue culture stress related genes may be switched on, however, such effects disappear in subsequent generations. It is for this reason that testing should not be carried out on "primary" transformants but, if possible, on homozygous plants.

Experimental testing of the environmental risks. The inclusion of proper controls is particularly costly in field tests. They are usually formulated as "Study of a GM crop impact on non-target organisms" or "The study of the impact of herbicide tolerant crops on biodiversity", etc. In addition to the intended target effects these studies may reveal differences between a GM and a non-GM crop variety, but do not assess differences from the standard crop cultivation that includes protective measures against insect pests, weeds, etc. The controls must therefore include plots subjected to the standard agrotechniques and, if possible, cultivars with similar properties as the GM cultivars but obtained by other breeding methods. For example, herbicide-tolerant GM plants are unlikely to affect biodiversity but the use of herbicides associated with their planting may have an effect. Herbicide-tolerant varieties developed by other breeding techniques should be studied as controls.

Conclusion. Studies of the various effects of GM crops are meaningless without properly designed controls and cannot be accepted as a basis for decision making processes. Unfortunately, reports presented in the public media often lack or ignore proper controls and provide background for the disinformation of the policy-makers and general public.

2.2.3 The precautionary principle

The precautionary principle (PP) has been a subject of numerous analyses[20]. A very comprehensive study was published by Sabrina Shaw a Risa Schwartz as a report of the United Nations University.[21] These two WTO officials tried to correlate the policy based on the PP and executed by EU with the patterns of rational decisions. They found common points indicating that the PP could be consistent with scientific risk evaluation – provided that the rules set in the "Communication on the Precautionary Principle" (see 2.2.1) in 2000 were strictly observed. However, the precautionary principle is very often misused to justify unrealistic catastrophic scenarios and consequent political conclusions. For example, the current requirement of strict labelling and continuous tracking of the GMO ignores 14 years of safe use of almost one billion tons GM soya for food and feed. The requirement therefore violates the rule that regulations should be reviewed in the light of new scientific data (point e, p. 16). An illustrative example of improper risk assessment (point d*)* is represented by the bans invoked by several Member states as a "Safeguard clause", Article 23 of Directive 2001/18/EC. The sloppy form of the recent French ban of GM maize cultivation[22] indicates that it is a purely political move having

20 Kogan L. A.: Monograph Documents Advance, Impact of Europe's "Risk-Free" Regulatory Agenda. Washington Legal Foundation, November 4, 2005; and Kogan L. A.: Precautionary Preference: How Europe's New Regulatory Protectionism Imperils American Free Enterprise. Institute for Trade, Standards and Sustainable Development, Inc., Princeton, July 2005.

21 Shaw S., Schwartz R.: Trading Precaution: The Precautionary principle and the WTO. United Nations University, Institute of Advanced Studies. November 2005.

22 Arêté – suspendant la mise on culture des variétésde semences de maïsgenetiquement modiffié (*Zea mais* L. lignée MON 810). Ministére de l'agriculture et de la pêche.

nothing to do with science[23]. It is unacceptable that references (not correctly copied) of scientific papers were presented and referred to as "new information".

2.3 BENEFITS OF GENETICALLY MODIFIED CROPS

2.3.1 Insect resistant crops

All commercial insect resistant GM crops carry and express one or more genes from *Bacillus thuringiensis* and are therefore referred to as Bt crops. Introduced genes encode crystalline (Cry) toxins, each of which acts very specifically on a narrow range of insect or nematode species. The spores or *B. thuringiensis* or partly purified Cry toxin preparations are commonly used by organic farmers as biopesticide without any objections. However, the much more spatially controlled toxin application via the GM crops has raised concerns about possible effects on non-target organisms. A well cited case by opponents of the technology is that of the "Monarch butterfly (*Danaus plexippus)*" published in 1999 where unrealistic doses of Bt expressing maize pollen where shown to have a deleterious effect in laboratory studies[24]. The opponents of GM crops neglected subsequent studies published in the special issue of the Proceedings of the National Academy of Sciences, where overwhelming evidence was presented for GM crop safety (e.g., by David S. Pimentel and Peter H. Raven[25]). Another set of independent data was presented by several researchers (e.g. by M. Sears - University of Guelph; G. Nively - University of Maryland; and R. Hellmich - USDA) at the Monarch Butterfly Symposium in Chicago on the 7th November 1999. The evidence showed that the pollen distribution patterns and subsequent deposits on milkweed plants within and outside the corn fields are at levels that are highly unlikely to affect caterpillars which feed on these plants. All reports on the beneficial effects of Bt crops[26] were ignored by opponents of GM crops. These benefits include reduction of insecticide use (this implies savings on manpower, fuel consumption, less damage caused to soil by the heavy machinery), more effective control of the pests, and, consequently, higher yields. Indirect benefits include reduced contamination of the soil and waters by the chemicals used for crop protection and in some situations reduced crop contamination by mycotoxins.

2.3.2 Herbicide tolerant (HT) crops

Selection of weeds resistant to a particular broad-spectrum herbicide is the most frequently mentioned risk of HT crops. HT weeds could evolve through (a) gene transfer to the weeds that successfully hybridize with the GM crop (for example oil seed rape can cross with some wild plants from the same family) or (b) spontaneous mutation followed by selection under herbicide pressure. Two sets of data must be considered to evaluate the second possibility:

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23 Editorial, Le Figaro (France), February 11, 2009.

24 Losey J. E., Rayor L. S., Carter M. E. (1999) Transgenic pollen harms monarch larvae. Nature 399, 214.

25 Proc Natl Acad Sci US 97, 8198–8199.

26 Hanley A. V., Huang Z. Y., Pett W. L. (2003) Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella.* J Apicul Res 42, 77–81.

(I) List of herbicides used with the non-GM varieties, assessment of the current weed resistance to the herbicides, level of herbicide residues in the soil, and the regime of herbicide application (timing, type of machinery, etc.). The initial status of weed resistance is particularly important. For example, *Polygonum sp.* and *Chemopodium sp.* in the fields of Czech Republic have already acquired resistance to up to 5 different herbicides before the introduction of any HT crop.

(II) The use of a broad-spectrum herbicide independent of the HT crop plantation. This use is likely to be many fold higher than the use associated with a HT crop deployment. The current situation in the Czech Republic (no HT crop is grown) is reviewed in Table 1. Planting of herbicide tolerant soya on 10,000 ha would increase the application of glyphosate only by about 2%.

Table 1. The use of glyphosate and glufosinate (kg) in Czech Republic

YEAR	Glyphosate	Glyphosate-IPA	Glyphosate trimesium	Total Glyfosate	Glufosinate NH_4
2000	53 674	272 151	83 183	409 008	5 133
2003	51 272	281 944	122 908	456 124	5 529
2004	62 931	381 748	57 497	502 086	3 394
2005	64 267	465 034	104 231	633 532	3 045
2006	87 504	647 631	93 281	828 416	3 553
2007	108 635	787 088	124 382	1 020 205	3 610

These data put into proper perspective the concern of the French authorities published during their EU Presidency[27]:

> *The risks associated with the use of herbicidal products required for the cultivation of certain GMPs which are tolerant to such products are also largely unknown and poorly assessed over the medium and long term.*

It should be noted that HT crops derived by other breeding methods are cultivated (e.g. Clearfield crops tolerant to the imidazolinone herbicides) without any regulations[28].

The Farm-scale field trials in Great Britain[29] clearly documented that the impact of GM crop cultivation on biodiversity might be either positive or negative and always depended upon the agriculture system as a whole, and not on the GM crop.

27 GMO Paper made by Fr Presidency at Informal Environment Council Meeting on July 4, 2008.

28 Coghlan A.: Conventional crop breeding may be more harmful than GM. New Scientist. February 4, 2009.

29 Squire G.R. et al. (2003) On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Phil Trans R Soc Lond B 358, 1779–1799.

2.4 ADVERSE CONSEQUENCES OF EU LEGISLATION

There are many analyses of the **economic impact** of the restrictive versus rational GMO policy[30]. The most complex one was worked out for the UK Prime-minister Tony Blair based on information available in 2002[31]. The study by Food Navigator-Europe[32] warning that

> *"Europe's opposition to genetically modified ingredients will significantly increase producers' costs over the next three years as it becomes ever harder to secure GM-free supplies."*

is often quoted as a serious assessment of the GMO policy impact on the economy and competitiveness of the whole of Europe. A recent review presents the summary of ten years[33] Socio-Economic and Environmental Effects.

Restrictive regulations reduce the innovation potential of Europe because biotechnology companies or their R&D divisions are leaving,[34] as was shown in an EU document published in 2003.[35] The withdrawal of research is accompanied by the **brain-drain** of scientists that emmigrate overseas.[36]

Perhaps the most serious social impact of the EU paradigm on GMOs is the support of irrational claims about the dangerous nature of GMOs. The effect on **public perception of GMOs** is documented in Table 2 (next page).

European legislation exerts an unfortunate **influence on the developing countries**. On 20 February 2007, Connie Hedegaard, the Danish Minister for the Environment, announced that she was concerned if Europe had a negative effect on countries in the developing world by imposing its own standards on the rest of the world with regard to the regulation of GMOs. As a follow-up to this event, plant researchers from the developing world met in Brussels at a meeting organised by the European Action on Global Life Sciences (EAGLES). The purpose of the meeting was to discuss how European regulation on GM foods influences legislators in the developing world. The former Head of the DG Research at the European Commission and Head of the biotechnology unit of OECD, Mark F. Cantley, stated: "We have painted ourselves into a corner in Europe, from which we shall not easily escape, and from which we have a malign influ-

30 Kalaitzandonakes N. (2007) Compliance costs for regulatory approval of new biotech crops. Nat Biotechnol 25, 509–511.

31 Field Work: Weighting up the Costs and Benefits of GM Crops.

32 Mercer Ch.: EU's anti-GM stance is unsustainable; available at: <http://www.foodnavigator.com/Financial-Industry/EU-s-anti-GM-stance-is-unsustainable-says-study> .

33 Brooks G., Barfoot P. (2008) Global Impact of Biotech Crops: Socio-Economic and Environmental Effects, 1996-2006, AgBioForum, 11, 21–38.

34 de La Hamaide S.: Biogemma threatens to leave France after GM attack. Reuters, July 17, 2003.

35 Lack of information and public scepticism on agricultural biotechnology contribute to biotech companies leaving Europe. March 14, 2003. DN: IP/03/387.

36 Sample I., Meikle J.: Britain: Brain drain threatens GM crop research**.** The Guardian, September 25, 2003.

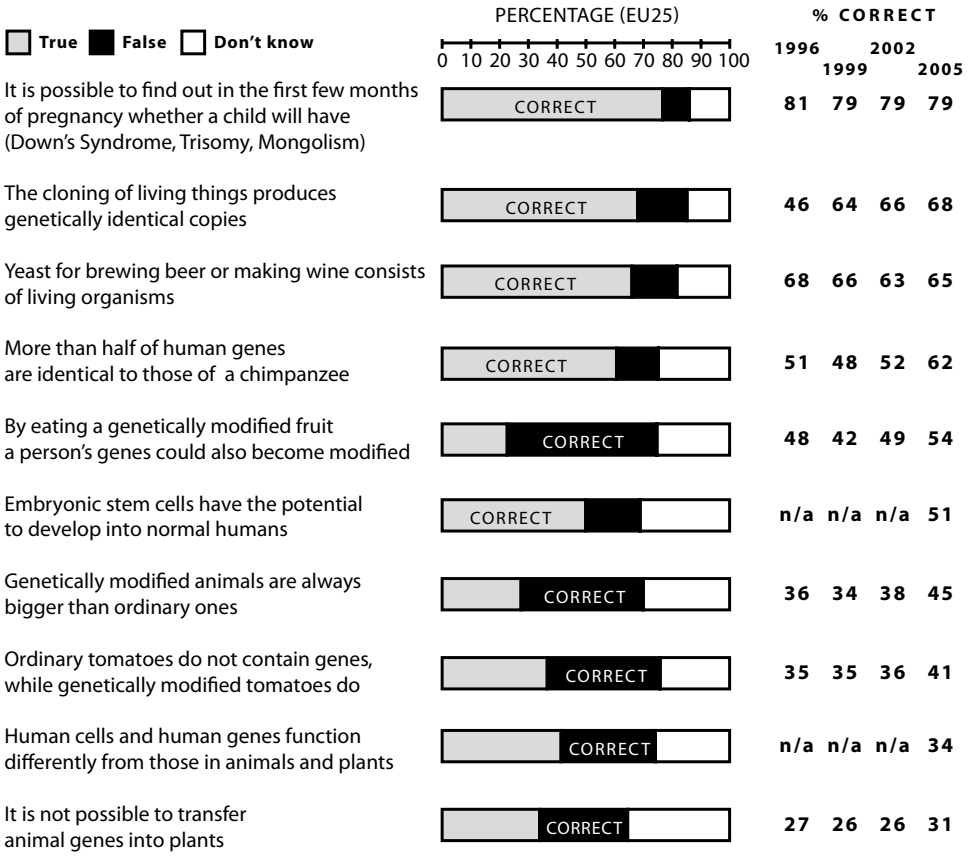

Table 2. Europeans and Biotechnology in 2005: Patterns and Trends, Eurobarometer 64.3 May 2006

ence on poor countries all over the world". Marc van Montagu, a distinguished Professor of Ghent University and President of the European Federation of Biotechnology (EFB), concluded the meeting with the following comment: "A sustainable agriculture and a less-polluting industry badly need the GM-technology and the transgenic plants developed, worldwide, over the last ten years. Exactly in the same period, well-intentioned regulators in the EU set up an unnecessary and very costly application of the regulatory system. No small or medium enterprise, public research centre, charity or foundation can afford to open a file for approval through the established system. It is a crying injustice towards the developing world, towards nearly 85 % of the world population."

EU legislation on GMOs is restrictive and inconsistent. The ignoring of benefits, which are referred to in the precautionary principle document, is the most apparent case. ACRE (an independent group composed of leading scientists whose main function is to give statutory advice to UK Government Ministers on the risks to human health and the environment from the release and marketing of genetically modified organisms) clearly stated:[37]

37 Report of the ACRE Sub-Group on Wider Issues raised by the Farm-Scale Evaluations of Herbicide Tolerant GM Crops. Revised after public consultation May 3, 2007, available at: <http://www.defra.gov.uk/environment/acre/fsewiderissues/pdf/acre-wi-final.pdf>.

In recent years, it has become apparent that there are inconsistencies in the regulatory assessment of the environmental impact of GM crops in comparison with other agricultural crops and practices. The EU Directive 2001/18, which covers the release of genetically modified organisms, requires an environmental risk assessment of possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GM plant as part of a rigorous approval process. Non-GM crops and other changes to agricultural management do not require similar risk assessments.

Directive 2001/18 also makes no provision for assessing both potential environmental risks and benefits. By contrast, environmental benefits are now a major focus in the introduction of a number of other novel crops (e.g. energy crops) and agricultural management practices in the UK. There is no regulatory requirement to assess potential environmental costs in a fashion similar to GM crops. Environmental benefits (or side-effects) are also the focus of the most recent round of EU and national agricultural policy reforms.

2.5 POLITICAL BACKGROUND OF CURRENT EU BIOTECH REGULATIONS

2.5.1 Bans on Bt maize cultivation

The **ban on Bt maize in France** is a typical example of a politically motivated move. The bill, which will regulate the cultivation of GM crops in France, was passed in May 2008 by 289 to 221. During the parliament session the delegates had to consider 479 amendments to the original wording, which provoked heated debate. An amendment to severely restrict the use of GM plants met with majority approval: whilst the original draft required only that they be grown with "consideration for the environment and public health", transgenic plants may now be cultivated only with due consideration for agricultural structures, regional ecosystems and GM-free production lines.

The French ban on Bt maize is specified in a document signed by Minister Barnier.[38] This document was prepared extremely superficially. For example, whilst it cites (Icoz et Stostky, 2007) and (Ipoz, Stotsky, 2007) it not only miss-references the paper by Isik Icoz and Guenther Stotzky[39], entitled "Cry3Bb1 protein does not persist or accumulate in soil and is degraded rapidly. Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil", but also misinterprets/misrepresents the data claiming that the study provides evidence of the risk of Bt maize for soil fauna (earthworms, etc.)!! France used its Presidency of tha EU in attempt to design a new approach to whole-European GMO regulation.

No one from the French authorities would address the question as to how the area of 22 000 ha covered in 2007 with Bt maize would be protected from pests after the ban.

38 Note des Autorites Francaises. Ministère de l'agriculture et de la pêche, NOR: AGRGO8O34664, 07 FEV 08.

39 Transgenic Research, September 13, 2007.

It turned out that it would require 8,800 litres of insecticides and 30 000 litres fuel. In spite of this undiserable environmental impact, the ban was justified by the claim that Bt maize presented an ecological danger. This justification was not accepted by EFSA[40]. Similar bans on Bt maize cultivation were submitted by **Austria, Hungary and Greece**. All were disproved by EFSA on the ground that they were supported neither by new scientific data nor by the experience with the cultivation of GM crops.[41] In respect to France, the Scientific panel of EFSA concluded:

> *Having assessed the information package provided by France in support of its safeguard clause and having considered all relevant publications on the subject, the GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information package does not present new scientific evidence that would invalidate the previous risk assessments of maize MON810. Therefore, no specific scientific evidence, in terms of risk to human and animal health and the environment, was provided that would justify the invocation of a safeguard clause under Article 23 of Directive 2001/18/EC and an emergency measure under Article 34 of Regulation (EC) No 1829/2003.*

The Commission will now take the EFSA´s findings into consideration and are likely to order France to lift its ban. If the Commission does request the removal of this ban, France could decide to challenge the Commission´s decision by a) providing more information to justify the ban, or b) appeal to the European Court of Justice.

In the past, the EU's highest court fined France 10 million Euros for failing to update the country's laws on genetically modified crops and foods. The law in question was issued in 2002 and concerned rules for the planting of GM in areas where conventional crops were also grown. The Official statement of the European Court of Justice read: "unlawful conduct repeatedly engaged in by France in the GMOs sector is of such a nature as to require the adoption of a dissuasive measure, such as a lump sum payment".

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40 Request from the European Commission related to the safeguard clause invoked by France on maize MON810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation No 1829/2003/EC1. Scientific Opinion of the Panel on Genetically Modified Organisms (Question No EFSA-Q-2008-077). Adopted on October 29, 2008. EFSA J 850, 1-45 (2008).

41 Request from the European Commission related to the safeguard clause invoked by Hungary on maize MON810 according to Article 23 of Directive 2001/18/EC1; Scientific Opinion of the Panel on Genetically Modified Organisms (Question No EFSA-Q-2008-316). Adopted on July 2, 2008. EFSA J 756, 1-18 (2008). Request from the European Commission related to the safeguard clause invoked by Greece on maize MON810 according to Article 23 of Directive 2001/18/EC1; Scientific Opinion of the Panel on Genetically Modified Organisms. (Question No EFSA-Q-2008-313). Adopted on July 3, 2008. EFSA J 757, 1-12 (2008). Request from the European Commission related to the safeguard clause invoked by Austria on maize MON810 and T25 according to Article 23 of Directive 2001/18/EC1; Scientific Opinion of the Panel on Genetically Modified Organisms. (Question No EFSA-Q-2008-314). Adopted on December 4, 2008. EFSA J 891, 1-64 (2008).

2.5.2 Dismissal of the Amflora potato placement on the market

Amflora potato with elevated amylopectin content was developed for industrial use. Field tests of this potato variety started in the Czech Republic in 2006[42], following similar tests in the Netherlands[43], Sweden[44] and Germany[45]. Based on positive results of the tests, application for its commercial use was submitted in 2003. Approval was expected at the end of 2007 because of the positive EFSA statement[46]:

> *In conclusion, the GMO Panel considers that the information available for the potato EH92-527-1 addresses the outstanding questions raised by the Member States and considers that the potato EH92-527-1 is unlikely to have an adverse effect on human health or the environment in the context of its proposed uses.*

The political situation in France encouraged speculations concerning the possible transfer of the *nptII* gene conferring kanamycin resistance from the potato to bacteria dwelling in the intestine[4]. No one took the trouble to check the level of kanamycin--resistance genes present in regular food and feed. The daily uptake of bacterial genes with an easy bacteria-bacteria transfer is usually in the region of 10^7 (in accordance with EFSA reasoning[47]). Consumption of a gene from *Amflora* potato at a similar level is practically excluded. In addition, the plant-bacteria transfer is highly improbable[5]. The voting of the EU Council on the *Amphora* potato occurred on July 16, 2007, with the following outcome:

For approval: Germany, Belgium, Finland, Estonia, United Kingdom, Slovakia, The Netherlands, Lithuania, Sweden, Slovenia and Czech Republic;

Against: Austria, Ireland, Italy, Latvia, Luxemburg, Greece, Cyprus, Denmark, Poland and Hungary;

Abstaining: France, Bulgaria, Malta, Spain, Portugal.

In such cases the Commission has to make the final decision. In a debate on May 7, 2008, most commissioners acceded approval but this was disabled by the veto of Mr. S. Dimas[48]; the danger concerning the transfer of the *nptII* gene was given as the official reason for rejection.

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42 Notification Number B/CZ/05/642; Notification Number B/CZ/06/05, available at: <http://gmoinfo.jrc.ec.europa.eu>.

43 Notification Numbers B/NL/03/9,10 and 11.

44 Notification Numbers B/SE/95/30 and 32, 96/513, 98/1105, 00/1020 and 03/1946.

45 Notification Numbers B/DE/03/153, 154, 162 and Notification Number B/DE/08/197.

46 Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (Reference EFSA-GMO-UK-2005-14) for the placing on the market of genetically modified potato EH92-527-1 with altered starch composition, for production of starch and food/feed uses, under Regulation (EC) No 1829/2003 from BASF plant science [Published: 24 February 2006; Adopted on December 7, 2005.

47 EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. Adopted on April 2, 2004. EFSA J 48, 1-18.

48 Editorial, Nature 450, 921 (December 13, 2007).

Conclusions:

a) The voting of ministers is unrelated to the factual risk/benefit status of the given variety. It reflects the ideological views of each minister and the political climate in his/her country.

b) The veto by a single commissioner overruled the opinion of a statutory EU organisation EFSA, that is best qualified to provide scientific assessment. The veto was evidently not based on scientific knowledge but reflected the sympathies of Mr. Dimas with GM opponents (he is known to have paid from EU budget more than 7 millions euro to anti-GMO lobbyists).

2.5.3 The weak points of EU regulations

European legislation imposes **uniform measures across Europe** from the north of Sweden to the south of Sicily. This is in sharp contrast not only with the climate, geography, geology, but also with the tradition and the social structure of the European population. Specific conditions are considered only when a Member state asks for a ban on GMOs. This is a rational approach providing that the real reason for the ban is not on the grounds of ideology, business, or populism. Specific conditions should also be considered when a Member state asks for planting of a GM crop that has not been approved for the whole EU. The industrial potato *Amflora* is a good example. Its cultivation in the hilly land of the Czech Republic would not bring risk to Greece or Luxemburg that objected it.

During its Presidency, France suggested the inclusion of **social-economic factors** in the scientific risk assessment of GMOs[49]. A working document prepared on 5 September 2008 for the Ad hoc Working Party on Genetically Modified Organisms (GMO) presented *inter alia:*

> *Genetically modified plants (GMPs) are the subject of public controversy because their advantages for society in general and for agriculture in particular are disputed. Could a better analysis of these socio-economic aspects clarify these points in the public's perception?*
>
> *The GMPs now being cultivated have been created to provide direct benefits to farmers and in some cases to processors or users of the resulting products. These benefits may consist in facilitating or decreasing the work associated with producing crops, particularly as regards combating pests. However, a study of the advantages of using GMOs cannot be limited to an assessment of the individual benefits to particular users, but must also consider the collective benefits for society as a whole and the environment. In addition, while such an assessment must take into account the benefits (decreased mycotoxin content, farmers' health, etc.), it must also assess the costs arising from the use of GMOs, such as those associated with checking or preventing the adventitious presence of GMOs in products from "non-GMO" channels.*
>
> *Hence "non-GMO" channels (i.e. conventional or not using GMOs) could be faced with additional costs when GMOs are grown over large areas. Use of GMOs may also*

49 Working document from Presidency to Ad hoc Working Party on Genetically Modified Organisms (GMO); AGRILEG ENV, DS 751/08 Brussels, July 24, 2008 (30.07).

lead to changes in agricultural practices, both at micro-economic level (at the level of the individual farm or agricultural system) and on a larger scale.

The factors quoted in this chapter are of a political nature, far from the scientific analysis of the situation. Such an analysis would include comparison of benefits versus the inherent costs and the extra costs imposed by EU regulations. These additional costs hamper EU competitiveness in agricultural production[50]. The rigidity and irrational basis of EU regulations may cause a collapse of animal production[51].

2.5.4 Conclusions concerning the EU regulation of GM crops

Legislation is the responsibility of policy makers. Scientists are not qualified to suggest the formulation of laws. However it is their duty to make clear statements that:

- EU regulation is largely based on prejudice and political calculus;
- GMO regulation similar to that of narcotics, poisons and explosives sends a message to the public that the products of biotechnology pose a comparable level of danger to human and animal health;
- Current GMO regulation restricts the farmers and restrains agricultural productivity, decreases EU competitiveness in the global market, and in the long run endangers the environment.

It has been suggested[52] that GMOs should be treated as drugs and the decision on their use should be strictly based on scientific evaluation and not on voting by insufficiently informed politicians. This does not mean that the public should be left out of the decision making process. However the involvement of citizens ought to be proportional to their knowledge of the issue. The current level of ignorance of its citizens over such issues, is to Europe's shame.

The scientific community cannot understand why the EU provides financial support[53] to pressure groups who disseminate nonsense such as the proposition of a parallel between transgenes and prions, or worse still, the denial of the presence of genes in traditional crops. The damage caused by these groups in the European population may be greater than that imposed by political extremists whose activities are restrained by law. It is naive to suppose that scientists can neutralise professional propaganda executed by these pressure groups who do not care about providing evidence as required of the scientists.

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50 Gómez-Barbero M., Rodríguez-Cerezo E.: Economic Impact of Dominant GM Crops Worldwide: a Review. EUROPEAN COMMISSION DG JRC-IPTS, Sustainability in Agriculture, Food and Health Unit. December, 2006.

51 Economic Impact of Unapproved GMOs on EU Feed Imports and Livestock Production. Directorate General for Agriculture and Rural Development, Brussels, July 19, 2007. Available at: <http://ec.europa.eu/agriculture/envir/gmo/economic_impactGMOs_en.pdf>.

52 Seehofer H.: Less politics, more science. Debate on the authorisation procedure for GM crops in the EU. Available at: <http://www.gmo-safety.eu/en/news/599.docu.html>.

53 Cox S., Radio 4 (BBC), December 6, 2007. Available at: <http://news.bbc.co.uk/1/hi/world/europe/7127182.stm>; The Telegraph (UK), August 17, 2007.
Available at: <http://www.telegraph.co.uk/news/main.jhtml?xml=/news/2007/08/17/weu217.xml>.

Careful attention should be paid to ensure that the public is truthfully informed. Biotechnology is a complex field and it is not easy to understand its principles and interactions with the ecology, economy and political issues. However, a basic understanding should be achieved for the sake of the future of the EU. The rejection of scientific evidence coupled with the support of pressure groups may foster the political career of a non-responsible individual, but in the long term it is a crime to the European electorate.

3. THE CZECH EXPERIENCE

3.1 INVOLVEMENT OF SCIENTISTS IN BIOTECH ISSUES

3.1.1 Research

Annotations of selected research results are presented in part 4 of this book; major research directions are reviewed here.

Genetic transformations of model organisms have been routinely performed in several Czech laboratories in parallel with the advance of bioengineering methods in other EU countries. Basic research on transgenic plant models has been systematically developed in the Department of Plant Physiology, Faculty of Science, Charles University (Prague), in the Institute of Molecular Biology of Plants (České Budějovice) and the Institute of Experimental Botany (Prague) of the Academy of Sciences, and elsewhere.

Successful examples of applied research include development of a GM potato line with increased metabolism of reducing sugars in the tubers. The gene *Lbpfk* from *Lactobacillus bulgaricus* coding for phosphofructokinase was introduced to plants together with the gene *nptII* coding for kanamycin as a selectable marker by means of *Agrobacterium tumefaciens* mediated transformation. Lines expressing the construct predominantly in tubers were selected. Examples of successful insertion mutagenesis in flax include introduction of the *bar* gene to enhance herbicide tolerance, of a gene for a serine protease inhibitor to increase resistance to microbial pathogenes and/or insect pests, and of genes for metallothionenine αHMT1A or the CP peptide to promote heavy metal accumulation.

Effects of transgene expression on plant properties have been examined in both model and commercial GM crops under controlled environmental conditions. Some unexpected phenotype changes were observed in certain GM potato and tobacco lines, as well as in other plant species. These changes were traced to genetic or epigenetic effects connected with the site of foreign gene insertion or with "somaclonal and proclonal variations" that are based on natural instability of plant genomes combined with an increase of mutability by the transformation/regeneration process.

Investigations on the **environmental impact** of GM crops have been spurred on by their commercialization. Effects on non-target species are a major focus of attention. For such studies a 3-tier test is performed: laboratory tests on small groups of organisms followed by greenhouse experiments mimicking outdoor conditions, and finally field experiments. Investigations performed on the first two levels include pilot studies with the non-commercial model crops aimed at elucidating mode of action of the transgene product. Such studies have included potatoes expressing *Galanthus nivalis* agglutinin (GNA) or serine proteinase inhibitors derived from genes of insect origin. Laboratory based studies with commercial GM crops included comparison of the effects of a given GM crop with those caused by the purified transgene product added to the artificial diet of test insects. Field studies have been done with several crops (Table 3) to monitor variability in transgene expression, expected effects (pest control, weed tolerance, etc.), and environmental impact.

Table 3: Numbers of field tests of GM crops in the Czech Republic

YEAR	Maize	Potato	Flax	Plum-tree	Rape seed
2002	Bt 5, HT 1	1	1	--	HT 3
2003	Bt 3, HT 3	1	1	1	--
2004	HT 5	1	1	1	--
2005	HT 5	2	1	1	--
2006	HT 5,	4	1	1	--
2007	HT 5, Bt+HT 1	4	1	1	--

It must be emphasized that all investigations with GM crops are performed according to strict rules, must be approved by the Ministry of Environment, and are subject to relatively frequent monitoring. Experiments can be performed only in certain areas by appropriately trained personnel, and all GM material must be destroyed. These stringent conditions make experiments with GM plants more costly than comparable tests with standard cultivars or tests with pesticides and bioagents. The permit required for a field test is relatively costly in itself. The fields must clearly be marked.

3.1.2 International activities

Since 1993, the Czech and Slovak Academies of Sciences have alternated in organizing, biennially, an International Symposium on "Recent Advances in Plant Biotechnology" (the next, 8th Symposium, is due in 2009, and will take place in Hungary). The Biotechnology Institute of the Charles University (BtICU) collaborated with New York University in organizing a Conference "Biotechnology and Business" in Prague in November 1993. Representatives of BtICU have participated as observers in the OECD Working Party on Biotechnology since January 1994. The Director of BtICU was a member of the OECD group on Regulatory Oversight and the Scientific Secretary of BtICU spent three months in the OECD Environment Directorate in 1995 (Czech Republic became OECD member in 1995). Furthermore, the BtICU Director was also a member of the Czech delegation at the 6th Meeting of the Ad hoc Open-ended Working Group on Biosafety in Cartagena (February 14-22, 1999) and participated in the European Commission public consultation on "Life Sciences and Biotechnology – A strategic vision" on September 27-28, 2001. BtICU in cooperation with the Ministry of Environment organised a Conference of UNEP in Prague. Staff members of BtICU have worked in organisations such as UNIDO, UNEP and EFB.

Other international activities include organization of seminars and workshops by the Institute of Plant Molecular Biology of the Academy of Sciences with the Biotechnology Platform of EPSO (European Plant Sciences Organization) and ESF (European Science Foundation). In September 13-15, 2001 an international ESF workshop aimed at the "Assessment of the Impact of Genetically Modified Plants" was held in České Budějovice. Nearly 120 scientist from Europe and overseas took part at this event. A landmark workshop "Ecological Impact of the Genetically Modified Organisms", at which the IOBC working group "GMOs in Integrated Plant Production" was established (see *IOBS wprs Bull.* 27 (3) 2004), was hosted by the Entomological Institute of the Academy of Sciences in Prague in November 26-29, 2003. The working group of about 100 scientists from the whole of Europe has subsequently met in different

countries every year and has become an authoritative scientific body for the environmental risk assessment of GM crops in Europe. Czech scientists also participated in the founding of IOBC WPRS "GMOs in Integrated Plant Production" and the "European risk assessment consortium" (secretariat in Agroscope Reckenholz-Tänikon Institute in Switzerland) that prepares a generally acceptable methodology for assessing the impact of human activities on ecosystems. The activities of Czech scientists have been paralleled by government undertakings. For example, in October 16-20, 2006, Prague hosted a FAO workshop "Utilization and Management of Biotechnology in Crop Production."

Since 1992, Czech Republic has been represented in the COST (Cooperation in Science and Technology) programme of EC with active participation in several projects. During the past ten years, both basic and applied research on GM crops have advanced the priorities of "Agriculture, Food Science and Biotechnology" – present Domain "Food and Agriculture". To foster research and to disseminate knowledge on the risks and prospects of GM crops, an exploratory workshop on GM technology and safety was organized by the National representatives of this Domain from Ireland, Germany, Italy, Belgium and the Czech Republic. The workshop was held as a satellite symposium "What role for GM technology in the future competitiveness of the European agri-food sector?" of the First European Food Congress in Ljubljana, November 2008. The spectrum of invited speakers included both top scientists as well as EFSA and OECD representatives. All types of risks and benefits, including ecological ones, were discussed. The conclusion was clear – there are no "biological", only political, obstacles limiting the proper application of GM technologies throughout the world – these political obstacles significantly handicap Europe in comparison with American and the more developed Asian economies.

Czech scientists have been involved in several Framework projects concerning GMOs. This White Book has been prepared as part of MOBITAG project (7FP Research Potential) supported by the Biology Centre of the Academy of Sciences.

3.1.3 Legislation

After the restoration of democracy in 1989, a voluntary group of scientists from the Czechoslovak Academy of Sciences agreed to prepare rules for the conducting of experiments involving recombinant DNA techniques and harmonize them with practices in the more advanced countries. The group later cooperated with the Ministry of Environment and formally became the Czech Committee for the Use of Genetically Modified Organisms and Products (GM Committee) when the national GMO law came into effect in 2001. The work on the legal framework of modern biotechnology was initiated in 1991-1992 when the Biotechnology Institute of the Charles University (BtICU) began negotiations with the Ministries of Health, Environment and Agriculture. The Czech government designated the Ministry of Environment as a competent authority in this regard. Between 1993 and 1995, BtICU was asked to compile information on the situation relating to GMOs in other countries and particularly on the relevant ECC Directives so as to prepare a technical base for the GMO law. In 1996, BtICU prepared a proposal for the GMO law in collaboration with the School of Law of the Charles University and the three participating ministries. The Law was drafted during 1999,

published on 10 May 2000 as No 153/2000, and came into force on January 1st, 2001. The "GM Committee" specified by this law includes 10 scientists, one representative from each of the three ministries, NGOs, and regulatory bodies. An advisory team of 18 to 20 scientists can be invited to review particular issues for GM regulation.

3.2 AGRICULTURE

Biotechnology was positively accepted by the Czech farmers. In cooperation with the research institutes (e.g. Research Institute of Crop Production in Prague, Agriculture Research Institute in Kroměříž, Biology Centre ASCR in České Budějovice), breeding institutions (e.g. Potato Research Institute in Havlíčkův Brod) and companies (e.g., AGRITEC in Šumperk), the farmers participate in the testing of GM crops. Conducted tests are reviewed in Table 3. Maize infestation by the European corn borer (*Ostrinia nubilalis*) has increased substantially over the last 10 years and currently this pest has to be controlled on approximately 20,000 ha of maize. Farmers welcomed the deployment of Bt maize MON 810 (the only GM crop approved in the EU!) as an alternative to insecticide application. The acreage of Bt maize has increased year-on-year since 2005 (Table 4).

Table 4. Bt maize cultivation in the Czech Republic

YEAR	AREA ha	NUMBER OF FARMERS
2005	270	52
2006	1290	85
2007	5000	131
2008	8300	171

The Western corn rootworm, *Diabrotica virgifera virgifera*, has spread in the last few years through the eastern part of the Czech Republic and represents a serious threat to the maize-growers. While Austria and Hungary still prefer to combat this pest with chemical insecticides, many scientists, including those from the Czech Republic, welcome the use of Bt maize that is partly resistant to this beetle and its larvae. To this end they are carrying out large-scale field trials (2009–2011) to thoroughly compare the performance, and any possible non-target effects, of the GM maize alongside maize grown conventionally, i.e. non-GM maize treated with an insecticide.

3.3 TEACHING AND PUBLIC INFORMATION

3.3.1 University courses and textbooks

University courses on GM technology have been organized and associated teaching texts issued since the 1990s. For example, in1997 BtICU published, with support from a PHARE programme, the course texts "Safety of Biotechnologies" and "Gene Engineering of Plants". Agricultural University of J.G. Mendel in Brno edited a course text "The Principles of Plant Gene Engineering" in 2000. The textbook "Plant Transgenosis" was published by the Academia publishers in 2002. The number of teaching materials published at different Czech universities is probably in excess of twenty. In 2002,

the University of South Bohemia issued a set of posters "Research of Genetically Modified Organisms in Czech Republic.". The students of biology, agriculture, food chemistry, and related fields receive sufficient information on GMOs. It is estimated that up to 50 PhD students graduate every year from the Czech universities in areas that include GMOs.

3.3.2 Informative brochures, web portals, and TV films

The bulletin BIOPROSPECT, published in 1990 by the Biotechnology Society, was the first periodic newsletter focused on GM technologies. In 1997 the Association Biotrend (BIOTRIN) initiated a series of regular monthly reports. The edition began with a "White Book on genetic modifications", followed by the bulletin "Biotrend-In". It was later replaced by the web portal www.biotrin.cz that published monthly "News" on global biotechnologies in English and reviewed in Czech the most important news in the "Media review" page. These presentations were replaced in 2006 by regular internet bulletin "World of Biotechnology" (in Czech). South Moravia Innovation Centre (JIC) distributes a weekly magazine Gate2Biotech (parallel to the web www.gate2biotech.com) and an annual "Czech Biotechnology Report" that includes addresses and web pages of the Czech institutions active in the biotechnology field.

The community of farmers and the general public are informed by means of printed materials and TV films. The university teaching texts prepared by BtICU were edited by the Institute of Agro and Food Information for laypeople, and published in 2002. In 2006, the association BIOTRIN prepared for publication in Academia a Czech version of the brochure "Biotech Guide" obtained from the University of Ghent.

In 1999 the first Czech film on GMOs was also prepared by BIOTRIN in collaboration with the Czech TV. The film (35 minutes) "Genes of Controversy" shows the use of GM crops in USA and demonstrates the biased and often false nature of the anti-GMO propaganda executed by Greenpeace[54]. In 2003, a 20 min. film "What Mendel never dreamed of", which was prepared by the Agritec Co. with support from the Ministry of Education, Youth and Sports, shows the technology of pea breeding, including genetic engineering. In 2005, the Institute of Entomology of the Academy of Sciences, in collaboration with the FATE film studio and with financial support from BIOTRIN, prepared a 22 min film "Life in the Corn Field". The film documents environmental safety of the Bt corn MON810. A more general film "Who is Afraid of Genes" was prepared at the request of, and in collaboration with, several institutes of the Academy of Sciences.

3.3.3 Public seminars, meetings and media

Probably all universities, which are accredited for PhD studies that include genetic engineering, also organize seminars for the general public within the frame of programme such as University of the Third Age, University of Free Time, and Regional European University (ESF Project). A few (usually weekly) courses for teachers and also for high school students are organized across the country on an annual basis. According to our experience, professional information on the new GM technologies are very positively

54 The film is available at <http://www.youtube.com/user/BIOVIDIN>.

accepted by the audience, irrespective of age and educational background, providing that the discussion is fair and open to any objections and clearly distinguishes between "science" and "ideology". Seminars have been organized with active participation of both supporters and opponents of GM crops to address the potential problems of co-existence of conventional, organic (ecological), and "biotechnological" farming. Objections by some organic farmers persist, but continuation of non-militant dialog is likely to lead to a consensus. It is important to note that educational activities are also executed by the Ministries of Agriculture and Environment, by the Agricultural chamber, etc.

The authors insist on the view that there is no scientific/biological reason for the incompatibility between eco/organic farming technologies and the GM procedures. Existing animosity and/or fear of organic farmers is based on, or at least encouraged by, the scientifically unsubstantiated legislative. When examined without prejudice, some of the GM technologies should be welcomed by the eco/organic farmers because they comply with the requirement to produce "healthy and natural food/feed" with minimal negative impact on the "natural environment". Modern "biofortified" crops could be included in organic farming.

Good relations with journalists were established by providing them with proven facts and helping them to discriminate between true and false information. Scientists occasionally contribute to the news in the radio, TV, and internet.

4. ANNOTATIONS OF SELECTED RESEARCH RESULTS

4.1 NEW METHODS AND NEW TRANSGENIC CROPS

A JAGGED CUTTING EDGE OF TRANSGENIC TECHNOLOGY?[55]

Fischer L.

Insertion of transgenes into the genome of an organism is regarded by the scientific world as a precise technology for modification of genetic information. In principle, it is certainly a very accurate method; a selected gene is equipped with proper regulatory sequences to ensure production of the respective protein in certain organs and/or under certain conditions. However, as a consequence of the site of insertion in the genome, the transgene activity does not always meet our expectations. Though the site of insertion cannot be predicted and influenced in advance, it can be analyzed after transformation to exclude lines with any unwanted changes in essential or potentially harmful genes. Similarly, only lines with suitable transgene activity are selected for further application. However, further changes can occur thereafter, making it difficult to be sure what we have in our hands. This can be demonstrated by examples from our studies on transgenic potato plants and tobacco cell lines.

Transgene activity is influenced not only by the number and sites of transgene insertion, but it differs also among genetically identical cells or plants (with transgene inserted in the same genome location) due to epigenetic changes randomly occurring in the inserted DNA (Nocarova and Fischer 2009). Moreover, activity of transgenes is often unstable as documented during our long-term study of vegetatively propagated potato plants. The introduced gene can be silenced as late as one or more years after transformation. Our results also document that silencing of different transgenes arranged in tandem can be coordinated, probably due to spreading of compact DNA (chromatin) arrangement from one transgene to another (Nocarova et al. 2009). As this spreading can in principle continue further to adjacent plant genes, some delayed phenotypic changes might occur in transgenic plants.

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Plants are generally very flexible, having the possibility to meet certain targets through multiple routes (and usually also some detours). Thus, for instance, complete inactivation or over-activation of many (seemingly essential) plant genes has often no visual effect on the phenotype (Fischer, Dvořáková, unpublished). On the other hand detailed analysis of transgenic potato plants exhibiting strong spontaneous tuberization proved that their phenotype was not related to inserted DNA designed to randomly activate genes at the site of insertion. The inserted DNA was localized in the non-coding region, which indicates that the phenotypic change was caused by mutation associated with either the transformation process or with somaclonal variation – transgenic plants are regenerated from differentiated somatic cells which can already harbour mutations or whose genetic information can be modified during the regeneration process.

In accord with results presented, we can assume (in contrast to frequent claiming of die-hard supporters of GM technology), that the technology of targeted genetic modifications is not absolutely precise in respect of introduction of unintended changes to the genome of the target plant. However, the frequency of these changes is still much lower than that introduced by chemical or physical mutagenesis used in classical breeding. Considering that plant genomes are still very dynamic and full of mobile genetic elements, which can occasionally change their positions and insert into other places in the genome, current genotypes of crop plants have a "safe history of use" in naturally occurring processes of genetic modification. If natural or human-induced mutation of these plants had not resulted in a "dangerous crop", we can not expect that possibility to arise as a result of transgenic technology itself. Thus introduction of properly selected safe transgenes, together with reasonable testing of the final GM plant and products is a very efficient and reasonable way of targeted and quick improvements of plant features. The general discussion should not always be directed towards the technology itself, but should instead address the proper selection of genes and applications for this technology.

ALTERNATIVE SELECTABLE MARKER GENES FOR TOMATO AND POTATO TRANSGENESIS[56]

Bříza J.

Traditional and frequently used plant selectable marker systems include genes confering resistance to antibiotics or to herbicides. Unfortunately, antibiotic resistance markers are not appropriate for all plant species, for example for monocots, and they are usually not accepted by the public in the same manner as the markers based on herbicide resistance. In addition, the selective agent often adversely affects the transformed plant cells bringing about a decrease in the regeneration of transformed cells by the accumulation of toxic compounds from killed non-transformed cells. Moreover, when introduction of several genes into a single transgenic plant is necessary the development of further types of selectable markers may be desirable.

To date, a number of marker genes have been employed for development of alternative selection methods without use of either antibiotics or herbicides. Phosphomannose isomerase (*pmi*) gene was first used as a selectable marker by Joersbo et al. (1998) for transformation of sugar beet. In the following years, PMI was shown to be a useful marker in the transformation of a number of plant species like cassava, maize, *Arabidopsis*, wheat, durum wheat, rice, sweet orange, hemp, pearl millet, bentgrass, papaya, sorghum, almond, onion, cucumber, Chinese cabbage, tomato, flax, sugarcane, apple, plum, and citrus.

The pNOV2819 vector from Syngenta Seeds AG, Basel, Switzerland was supplemented with the *nptII* coding sequence from plasmid pGA472 by insertion into a polylinker sequence near the right border of the binary vector pCB3160. The *pmi* gene was driven by a short version of the cestrum yellow leaf curling virus promoter (CMPS), the *nptII* gene by the nopaline synthase promoter and both were accompanied by the nopaline synthase terminator (tNOS). The pCB3160 was transfected into *Agrobacterium tumefaciens* strain LBA4404.

In the tomato variety Moneymaker, the highest transformation rate (4.2 %) of cotyledon explants on mannose-selection medium was obtained when the mannose/sucrose concentration in the regeneration medium was 5/15 g/L. The best transformation efficacy with the commonly used concentration of 100 mg/L kanamycin as a selection agent was 9 %. In the potato variety Bintje, the highest transformation frequency was 53.3 % when the mannose concentration in the regeneration medium was 5 g/L during the first 3 weeks after transformation and 10 g/L thereafter. The optimum concentration of sucrose was 20 g/L. The transformation efficiency using kanamycin as a selection agent at a concentration 100 mg/L was 33.3 % with potato. Our results demonstrated that the transformation efficiency using mannose selection was 1.6-fold higher for potato and about 2 times lower for tomato compared with the standard protocol using kanamycin. Alternative selection methods for potato cv. Bintje and tomato cv. Moneymaker transgenesis are now available.

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DEVELOPMENT OF TRANSGENIC PEA (*PISUM SATIVUM* L.) LINES FOR IMPROVED TOLERANCE TO INSECT PESTS AND FUNGAL PATHOGENS[57]

Griga M., Švábová L., Sehnal F., Hanáček P., Reinöhl V., Horáček J.

In principle, plant resistance to insect pests can be obtained by expression of various genes, such as Bt-toxins, proteinase inhibitors, α-amylase inhibitors, lectins, enzymes, secondary metabolites, and/or by direct introduction of resistance genes. Gene *gmspi2* (*Galleria mellonella silk proteinase inhibitor 2*) isolated from *Galleria mellonela* has been modified and expressed in the microbial expression system *Pichia pastoris* to prove its high effectiveness to bacterial and fungal proteases. A new construct containing the sequence for the SPI2:GFP fusion protein was prepared to enable non-destructive detection of transformed tissues and to study the cellular localization of the gene product. As the entry cassette, a pUCA7-TX with the proteainase inhibitor gene *gmspi2* fused to the sequence for GFP under the control of 35S (triple X) promoter and OCS terminator into the pGREENII 0229 (John Innes Center, UK) was constructed, and the plasmid pWell09 obtained was tested by restriction analysis. Its efficacy was demonstrated via transformation of tobacco leaf discs.

A set of Czech pea cultivars was transformed by *Agrobacterium*-mediated transformation (4, 5) using three regeneration systems with differences in regeneration potential, frequency of rooted shoots, and potential to produce fertile plants. T0 plants were histochemically tested for GUS expression, and putative transformants tested using PCR. From total of ca 300 regenerated T1 lines, 150 samples of putative transformants were tested by PCR and more than 60 of them gave positive results. The efficiency of transformation ranged between 1-5% from initially established explants.

The main objective of transforming pea with the *gmspi2* gene was to increase inherent resistance to insects (*Bruchus pisorum*) and possibly also to some fungal pathogens (complex of leaf anthracnoses and root diseases). Bioassays to investigate the efficacy of this approach will be carried out on T2 plants, and following seed generations, in both the glasshouse and field.

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THE USE OF TRANSGENIC PLANTS IN THE DISSECTION OF HORMONAL REGULATIONS OF PLANT GROWTH[58]

Hejátko J.

Cytokinins are one of the plant growth regulators (phytohormones) that play essential roles in many aspects of plant growth and development, including cell division, shoot (the aerial part of plant) initiation, apical dominance, vascular development, leaf senescence and crop productivity (Mok and Mok, 2001; Ashikari et al., 2005). Cytokinin signalling is mediated via a so called two-component phosphorelay. The signalling pathway consists of the membrane-located sensor histidine kinase. Upon cytokionin interaction with the extracellular domain, autophosphorylation of the His in the kinase domain triggers the phosphorelay that leads to the phosphorylation of HPt proteins (AHP1-5 in *Arabidopsis*) that transmit the signal to the nucleus (To and Kieber, 2008).

In our lab, we use a combination of functional genomics, molecular biology, biochemistry and proteomic approaches to study the molecular aspects of cytokinin signal transduction. An intrinsic and inseparable procedure of all of those approaches is the generation of transgenic plants. The major model plant for our studies is *Arabidopsis thaliana* (Thale cress). All transgenic lines are cultivated in controlled facilities (greenhouse and phytotrons) dedicated to safety work with GMOs (pollen grain filters, underpressure, UV-based decontamination of waste water, etc.).

In collaboration with the lab of Prof. Ildoo Hwang (Korea) we have recently found that cytokinin signalling is an important regulatory mechanism driving vascular tissue formation in *Arabidopsis*. Cytokinins exert this effect by regulating activity of procambial cells, the stem cells of vascular tissue (manuscript in a preparation). Importantly,

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meristematic activity of procambium and vascular and interfascicular cambium strongly affects biomass production in plants. Thus, our findings are of high potential commercial interest for plant biomass production. A Czech patent has been issued and an international patent application submitted under the Patent Cooperation Treaty (PCT) (Hejatko et al., 2009a and 2009b) to protect the commercial use of our results in the plant biotech field.

Another example is the use of transgenic *Arabidopsis* lines for the analysis of interactions of cytokinin with another plant hormone, auxin. Recently, we have shown that cytokinins modulate the distribution of auxin during root growth (Kuderova et al., 2008). Using the transgenic lines carrying transgenes for identification of gene expression (both transcriptional and translational fusions of the promoter and/or the gene of interest, respectively), recombinant DNA for inducible transgene expression and constructs allowing monitoring of intracellular hormone levels, we have found that auxin, but not cytokinin is able to induce *de novo* organogenesis in plants. Auxin-induced endogenous cytokinin production leads to changes in the expression of auxin transporters, thus affecting the intracellular auxin distribution (Pernisova et al., 2009). Further, we have found that these regulations take place *in planta* in the regulation of root growth and development (Kuderova et al., 2008).

Taken together, these examples clearly show that the use of transgenic plants is the only effective way to explore the molecular mechanisms of plant growth and development. The ability to modulate plant growth and thereby plant biomass production via modifications of plant hormonal signalling pathways is a very promising way to lessen our recent dependency on mostly non-renewable energy resources.

RNA INTERFERENCE IN THE FUNCTIONAL GENOMICS OF TICKS[59]

Kopáček P.

Ticks transmit a wide variety of pathogens to humans and domestic animals. The European species *Ixodes ricinus* is the vector of tick borne-encephalitis virus and the spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease. Since genome sequencing of the closely related American deer tick *Ixodes scapularis* is about to be completed, we enter the postgenomic era also with *I. ricinus*. The hard ticks have rather complicated life cycles and genetic manipulation of tick is not feasible. The method of RNA interference (RNAi) is at present the only reliable tool to study the function of tick genes. The injection of double-stranded RNA (dsRNA) into the animal results in degradation of cytoplasmic mRNAs containing the same sequence as the dsRNA trigger. Gene translation to the protein is essentially prevented. RNAi just changes the phenotype without altering the genetic information (genome) (no transgenic animals are produced). We use RNAi in a reverse genetics approach (from genes to proteins and their function). Our research is focused on three areas that are promising in respect to rational control of the vector and/or tick-borne pathogens.

Innate immunity of ticks and its link to pathogen transmission: Attention is paid to the thiolester protein - α_2-macroglobulin and plasmatic lectins since their orthologs in malaria mosquitoes turned out to determine vector competence.

Proteins involved in iron metabolism of ticks: Ticks ingest 100-times their own weight of host blood but are not adversely affected by the excessive amounts of toxic heme or iron from digested hemoglobin. We have characterized three key molecules of tick iron metabolism – the cytosolic and secreted ferritin and iron regulatory protein. Using RNA interference, we have disclosed their function in iron storage, transport and regulation. The secreted ferritin 2 has great potential to be used as an efficient anti-tick vaccine.

Tick digestive machinery: Ticks digest host blood intracellularly in the acidic endo/lysosomal vesicles of gut cells. We have found that this key physiological process is based on an evolutionary conserved system of cysteine and aspartic peptidases, very similar to the digestive network of blood-flukes, but different from insect blood-feeders. The components of tick digestive cascade/network are of prime interest as candidates for the rational design of another type of an efficient "anti-tick" vaccine.

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FUNCTIONAL GENOMICS OF *TRYPANOSOMA BRUCEI*[60]

Lukeš J.

Trypanosoma brucei is the causative agent for human African sleeping sickness, and its close relatives are responsible for other serious diseases such as Chagas's disease and leishmaniasis. The number of people succumbing to these diseases is in the millions annually.

T. brucei belongs to the phylum Euglenozoa, which in turn belongs to the eukaryotic supergroup Excavata. Importantly, it is the most genetically tractable member of this supergroup, i.e. amenable to genetic modification (transgenesis). Furthermore, it represents one sixth of all extant eukaryotic diversity on the planet.

Thus, *T. brucei* has become an important model organism. In hundreds of studies, its versatility for various methods of forward and reverse genetics has been exploited. Moreover, it became one of the first organisms in which the revolutionary approach of RNA interference (RNAi) was successfully applied. We decided to use extensively RNAi for functional analysis of the mitochondrial genes of *T. brucei*. At the same time, we have developed numerous assays for screening mitochondrial functions of the genetically modified cells.

To this end, our laboratory has generated about 50 strains of *T. brucei* procyclic and bloodstream stages, in which one or two different genes have been ablated. In numerous studies we have presented detailed analyses of phenotypes which, in several cases, led to breakthroughs in our understanding of how the mitochondrion of these important pathogens operates.

We have overexpressed selected proteins in *T. brucei* and, perhaps even more importantly, inserted genes from other organisms in their cells under regulatable promoters and/or selectable markers. Using this revolutionary approach for this protist, we were able to investigate the function of selected genes from the human pathogen *Trichomonas vaginalis*, which itself is not amenable to functional genomics. Similarly we were able to investigate the function of selected genes from the ecologically extremely important diatom *Thalassiosira pseudonana*, for which the same limitation applies. Very recently, we have studied the function of the human protein, frataxin, in trypanosomes, the deficiency of which causes lethal and currently untreatable disease called Friedreich's ataxia. Using the advantages of the trypanosome model, we were able to resolve several questions related to the specific features of the processing of human frataxin, as well as to address its putative functions in iron-sulfur cluster assembly. Importantly, we were also able to show that despite an evolutionary distance of over one billion years, processing of human and trypanosome frataxin is virtually identical.

In summary, we are using numerous cutting edge approaches of reverse genetics to study the functions of selected *T. brucei* proteins with the intention to use these in novel drug design against serious human diseases caused by this and related pathogens.

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PREPARATION OF GENETICALY MODIFIED PLANTS WITH ENHANCED METAL TOLERANCE AND ACCUMULATION[61]

Najmanová J., Kotrba P., Macková M., Macek T.

Metal concentrations in soils might be locally high, and their content is still increasing due to many human activities, leading to elevated risk for health and the environment. Although phytoremediation may offer a viable solution to this problem, the presence of heavy metals may inhibit plant growth and the concentration of metals could be limiting for the application of phytoremediation. Therefore one option is to genetically engineer fast-growing plant species to improve their metal tolerance and metal-accumulating capacity. γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS) are key enzymes in glutathione (GSH) biosynthesis. GSH is not only the direct precursor of phytochelatins (PCs), but glutathione itself is also believed to play important roles in the detoxification of many heavy metals. The aim of the present study is to overexpress *Saccharomyces cerevisiae gsh1* gene for γ-ECS and *gsh2* gene for GS in both *Nicotiana tabacum*, as a model plant, and *Linum usitatissimum*, as the target species, to obtain plants with enhanced cadmium (Cd) accumulation and tolerance; this target species is an annual plant widely cultivated in temperate climates.

Several vectors were designed for *Agrobacterium* mediated transformation, pNOV1 and pNOV2 contain *gsh1* gene and *gsh2* gene respectively, whilst pNOV12 contains both *gsh1* and *gsh2* genes. Each gene was flanked by Rubisco small subunit light-inducible promoter RbcS from *Asteraceous chrysanthemum* and at 3' end by the RbcS transcriptional terminator. A special vector was constructed for promoter expression studies harboring the *gus* gene for β-glucuronidase. Transient expression with tobacco leaves was carried out using this vector and demonstrating promoter activity. A method was designed for determination of glutathion and phytochelatins using RP-HPLC. For this purpose aseptically grown flax was stressed for 14 days using different concentrations of $CdCl_2$. Analysis of leaf extracts indicated the presence of GSH, PC2, 3, 4 and 5.

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TRANSGENIC POTATOES WITH A DECREASED REDUCING SUGAR CONTENT IN TUBERS[62]

Navrátil O.

Storage of harvested potato tubers at low temperature brings many advantages including natural control of sprout growth, minimization of physiological weight loss, and reduction of losses caused by storage diseases. It also provides the possibility of whole year processing of tubers to some products (e.g. chips and crisps). However, potatoes display the phenomenon of low temperature sweetening whereby the harvested tubers accumulate reducing sugars and this accumulation steadily increases during cold storage. Accumulated reducing sugars affect flavour and colour of fried potato products. This problem was addressed by engineering the glycolytic pathway in the potato tubers.

For our transformation studies we used the bacterial gene coding for phosphofructokinase from *Lactobacillus bulgaricus.* Phosphofructokinase from *L. bulgaricus* (*Lbpfk*) differs substantially from some other bacterial enzymes so far used in potato plant transgenosis. It lacks an allosteric inhibition and functions in a "hybrid" conformational state. With a steady decrease in temperature we demonstrated that activity of this bacterial enzyme declined more slowly than potato *pfk*.

The transgenic potato plants expressing *Lbpfk* were tested in field trials and their tubers cold-stored for more than three months to verify the effect of *Lbpfk* under natural conditions. Reducing sugar content and frying colour of chips were also measured. Initially we used two different Czech cultivars. As expected, some transgenic lines displayed lower reducing sugar content compared to non transformed control plants, as well as improved frying colour; these traits remained stable throughout the duration of the study which was carried out over several years.

For further investigation we prepared a new variant of the gene *Lbpfk*. The sequence adjacent to the initiation codon was changed to the consensus sequence of dicots and some rare codons at the 5´ end of the gene were replaced by frequently used plant codons. Two other Czech potato cultivars were transformed and introduced into field trials. The beneficial effects of transgene expression were again established. The stability of the phenotype in these new cultivars will be further investigated.

The observed decrease in reducing sugar content for the best performing lines at the end of cold storage was 33 % (Korela cv.), 62 % (Kamýk cv.) and 80 % (Vladan cv.) compared to tubers from nontransgenic plants. In summary, the use of a transgenic approach to modify the glycolytic pathway in cold-stored potato tubers led to the stable performance of some lines over several years of cultivation.

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INCREASE OF PHYTOREMEDIATION ABILITIES BY INTRODUCING GENES FOR BACTERIAL DIOXYGENASES TO PLANTS[63]

Nováková M., Macková M., Chrastilová Z., Prokešová J., Sylvestre M., Macek T.

The target of this work is to increase biodegradation of polychlorinated biphenyls (PCBs), toluene and other organic pollutants by transgenic plants. For this reason several bacterial genes, selected on the basis of their environmental importance, were cloned into tobacco plants. The *todC1C2* genes were chosen since they encode oxygenase ISP_{TOL} (with histidine tail), a component of bacterial toluene dioxygenase that can oxidize toluene and other organic pollutants.

TodC1C2 genes cloned into plasmid pQE31 with histidine tail were amplified with two pairs of primers to amplify either *todC1* gene or *todC2* gene. *TodC1* gene was amplified together with the histidine tail. With respect to different expression systems in prokaryotic and eukaryotic cells, *todC1* and *todC2* gene were then inserted separately into plant vector pGreen with 35S cassette. Prepared vectors were transformed into *Agrobacterium* C58-C1 (pCH32) by electroporation together with plasmid pSoup.

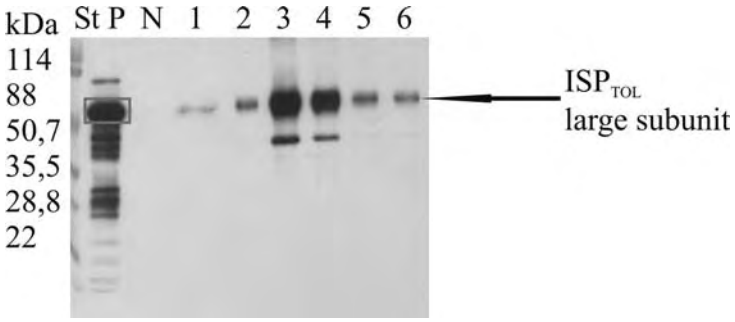

Fig. 1: Detection of ISP_{TOL} after transient expression in *Nicotiana benthamiana* by Western blot. St- protein standard Low Range Prestained SDS-PAGE, P – bacterial ISP_{TOL}/His, N – elution of wild type *N.benthamiana*, 1-6 – elutions from Ni-NTA isolation from *N. benthamiana* transiently expressed ISP_{TOL}

The presence of plant vectors was demonstrated by PCR. Prepared strains of agrobacteria were used in a mixture culture for transient expression in *Nicotiana benthamiana* to verify the possible expression in a plant system. Expressed ISP_{TOL} was isolated by

63 References:

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the method described in 2.4 and detected by immuno assay by Western blot, using commercial antibody against the histidine tail (figure 1).

Although transient expression is a fast method to verify possible protein expression, there is also a need to create transgenic plants where the genes are transferred to subsequent generations. Therefore the next step involved the generation of stably transformed plants; the vector used contained both genes (*todC1*/His and *todC2*) in separate cassettes with their own promoter (figure 2).

While checking the sequences of cloned genes, mutation was shown to have occurred within the *todC1* gene (Met101 to Thr101). This was corrected by *in vitro* mutagenesis using PCR amplifying the total plasmid. *Agrobacterium* C58-C1 (pCH32) containing the desired vector was then prepared and the presence of *todC1*/His and *todC2* gene was demonstrated.

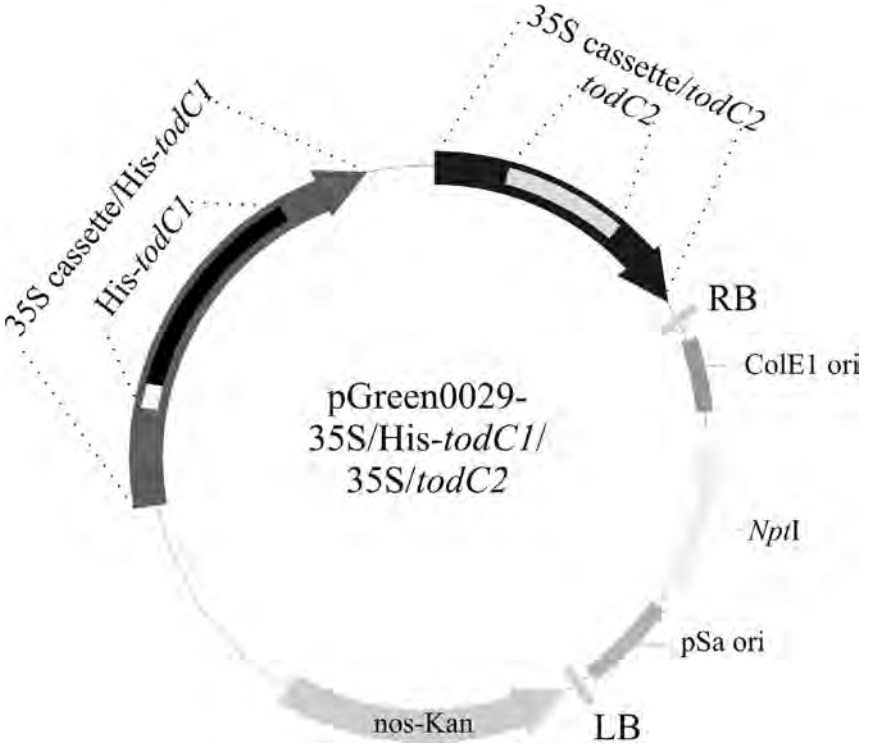

Fig. 2: Plant vector containing genes *todC1*/His and *todC2*.

Nicotiana tabacum plants are now in the process of being transformed. Subsequently they will be analysed for expression of the transgenes and the ability of these transgenic plants to degrade toluene and other organic pollutants.

CLONING THE BACTERIAL *BPHC* GENE INTO *NICOTIANA TABACUM* TO IMPROVE THE EFFICIENCY OF PCB PHYTOREMEDIATION[64]

Nováková M., Macková M., Chrastilová Z., Viktorová J., Szekeres M., Demnerová K., Macek T.

The aim of this work is to increase the efficiency of the biodegradation of polychlorinated biphenyls (PCBs) by the introduction of bacterial genes into the plant genome. For this purpose, we selected the *bphC* gene encoding 2,3-dihydroxybiphenyl-1,2-dioxygenase from *Pseudomonas testosteroni* B-356 to be cloned into tobacco plants. The dihydroxybiphenyldioxygenase enzyme is the third enzyme in the biphenyl degradation pathway, and its unique function is the cleavage of biphenyl. Three different constructs were designed and prepared in *E. coli*: the *bphC* gene being fused with the β-glucuronidase (*GUS*) gene, with the luciferase (*LUC*) gene, and with histidine tail in three separate plant cloning vectors. The *GUS* and *LUC* genes were selected because they can be used as markers for the easy detection of transgenic plants, while histidine tail better enables the isolation of protein expressed in plant tissue. The prepared vectors were then introduced into cells of *Agrobacterium tumefaciens*. The transient expression of selected transgenes was first studied in cells of *Nicotiana tabacum*. Once this had been succesfully achieved, model tobacco plants were transformed by agrobacterial infection with the *bphC/GUS*, *bphC/LUC* and *bphC*/*His* genes. The transformed regenerants were selected on media using a selective antibiotic, and the presence of the transgenes and mRNA was determined by PCR and RT-PCR. The expression of the fused proteins BphC/GUS and BphC/LUC was confirmed histochemically by analysis of the expression of their detection markers. Western blot analysis was performed to detect the presence of the BphC/His protein immunochemically using a mouse anti-His antibody.

64 References:

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Support: MŠMT Centrum 1M06030, MŠM 6046137305, MŠMT BIOAROM, ME 09024.

TRANSGENOSIS IN ORNAMENTAL PLANTS[65]

Pavingerová D.

A method of somatic embryogenesis from the leaf segments of *Dendranthema grandiflora* Tzvelev., cultivars 'Yellow Spider', 'White Snowdon', Orange Westland' and 'Mistletoe', was used for transformation of these cultivars by *Agrobacterium*. Two different *Agrobacterium tumefaciens* strains were used: the wild type B6S3 and the disarmed strain carrying in its T-DNA the β-glucuronidase gene and the neomycine phosphotransferase II gene as a selectable marker gene. Non-chimeric transformed plants were obtained. The cultivar 'White Snowdon' has previously been shown to be susceptible to *Agrobacterium* transformation. Regenerated plants showed stable octopine synthase or β-glucuronidase (GUS) expression during vegetative propagation.

Phenotypic deviations in transgenic plants of cv. 'White Snowdon' carrying either whole pTiB6S3 T-DNA or the GUS gene Construct were evaluated. Morphological differences from the controls were observed not only in regenerated transgenic plants carrying *Agrobacterium tumefaciens* B6S3 T-DNA, but also in transgenic plants carrying the *gus* gene. The phenotypic changes were stable through several cycles of clonal propagation.

Five *Rhododendron* cultivars, 'America', 'Catawbiense grandiflorum roseum', 'Madame Carvalho', 'Mars' and 'Nova Zembla' were used for transformation by *Agrobacterium tumefaciens* carrying T-DNA with the *gusA* gene encoding β-glucuronidase (GUS) gene and the neomycine phosphotransferase II gene as a selectable marker gene. The GUS reporter gene was successfully transferred into all five cultivars as indicated by fluorimetric staining, polymerase chain reaction (PCR) and Southern blot analysis. Some primary transformants appeared to be chimeric as both GUS expression and GUS nucleotide sequences were lost during vegetative propagation.

Micropropagation and *Agrobacterium*-mediated transformation were developed in *Kalmia latifolia* cv. Ostbo Red. The transformation of *Kalmia latifolia* plants was carried out by an *Agrobacterium tumefaciens* strain containing the *nptII* and *gusA* genes in its T-DNA. Shoots were regenerated on kanamycin selection medium and the expression of the *gusA* reporter gene was verified by fluorogenic β-glucuronidase (GUS) assay in positive transformants after regeneration. The presence of the *gusA* gene in regenerated kanamycin resistant plants was detected by polymerase chain reaction (PCR).

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EXPERIENCE OF GM OILSEED RAPE FIELD TRIALS: LONG-TERM MONITORING IN THE CZECH REPUBLIC[66]

Rakouský S., Hraška M., Čurn V., Bříza J., Hrubý J., Psota B.

In the Czech Republic the first field trials with genetically modified (GM) herbicide-tolerant (HT) oilseed rape (OSR) were established at the end of the 1990s. Based on the results from these trials, the Czech national authorities (Ministry of the Environment and Ministry of Agriculture) initiated the steps necessary to achieve environmental safety in future wide-scale OSR cultivation and its co-existence with non-GM production. Important among these steps was support for post-harvest crop biosafety research, part of which is referred to here together with the results obtained during our participation in the 6. RP EU project SSPE-CT-2004-501986-SIGMEA.

Our research activities were focused mainly on the following areas: 1) A survey of three selected experimental field locations for HT OSR transgene identity and homogeneity. 2) Post-harvest monitoring of HT OSR volunteers grown from the soil seed bank and carried out in eight locations in which experiments had been carried out, with a special emphasis on volunteer dynamics and possible persistence under different conditions of crop rotations and agronomy treatments. Its objective was also a limited study of gene transfer via pollen to non-GM OSR and related species. 3) The composition and dynamics of OSR volunteer populations at the border area of an experimental plot and field margin. As a major part of the study, long-term monitoring was carried out on the GM OSR incidence of volunteers and their possible impact on gene transfer in areas of former experimental fields. Plant samples were collected (OSR, wild related species) as well as data on the agronomy practices applied. Samples were analysed for the presence and expression of the HT transgene.

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Summary and Implications

A huge OSR seed bank is present in fields following harvest - furthermore, GM rape seeds can persist in the soil for many years. The frequency of volunteers is influenced to a great extent by the post-harvest treatments applied. This study confirmed previous findings of a substantially decreased frequency of GM winter OSR volunteers during the first years of post-cultivation monitoring if appropriate agronomy measures were taken. One of the most effective steps in controlling GM OSR volunteers is to allow seed germination directly on a field immediately after the harvest; skimming(s) and/or herbicide treatment (if used) should be postponed until the OSR plant is at its most sensitive developmental stage. Only a low level of incidence or complete absence of OSR volunteers (GM and non-GM) was found to be present in fields and closed areas five years after experimental cultivation. Proportions of GM volunteers among all OSR samples collected in successive years were stable. There was no evidence of HT gene transfer to feral and wild-related species in the limited number of other related plant (9) species studied. The cultivation of high-density- or competitive crops (e.g. cereals, alfalfa) in the following years helps to further suppress the incidence of volunteers. In principle, the safe wide-scale cultivation of HT OSR is feasible if appropriate measures and conditions are observed, taking into account proper geographical and biological factors, as well as local agriculture practice.

NEW BIOTECHNOLOGICAL APPROACHES FOR NEPOVIRUS RESISTANCE CREATION IN GRAPEVINE ROOTSTOCK CULTIVARS[67]

Pavingerová D.

The goal of the project is to develop new biotechnological approaches to enhance resistance to nepovirus GFLV (Grapevine Fanleaf Virus) in grapevine rootstock cultivars. The project will involve the optimisation of genetic transformation methods for grapevine rootstock, followed by insertion of virus genes.

67 NAZV QH91214, 2009–2011

SELECTABLE MARKERS IN PLANTS?[68]

Řepková J.

A wide range of genes are used as selectable markers to facilitate the process of *in vitro* selection of transgenic cells both during insertional mutagenesis (T-DNA or transposon) and in the subsequent selection of transgenic plants. While insertional mutants are confined to the laboratory, genetically modified (GM) crops are intended for environmental release and processing. The content of antibiotic or herbicide resistance genes in the GM plants is frequently used as argument against their deployment. There have been concerns about horizontal gene transfer from transgenic plants to bacteria to render them antibiotic resistant. The *Neomycin phosphotransferaseII* (*nptII*) gene, which can confer kanamycin resistance in transgenic plants, represents an invaluable tool for plant engineering and belongs to a class of antibiotic resistance genes acceptable for commercial release (EFSA 2004).

Nevertheless, elimination of selectable markers in transgenic plants would improve public perception of plant genetic engineering. Some genes are considered to be a good alternative to antibiotic resistance genes. Mentewab and Stewart (2005) characterized a plant gene *Atwbc19* encoding an *Arabidopsis thaliana* ATP binding cassette (ABC) transporter which conferred antibiotic resistance to transgenic plants. The mechanism of resistance is novel, and the levels of resistance are comparable to those attained through expression of bacterial antibiotic-resistance genes in transgenic tobacco using the CaMV 35S promoter. ABC transporters are endogenous to plants; therefore, the use of *Atwbc19* as a selectable marker in transgenic plants may provide a practical alternative to current bacterial-derived marker genes.

Rosellini et al. (2006) used a *Synechococcus* gene encoding glutamate 1-semialdehyde aminotransferase (GSA-AT) as a selectable marker for alfalfa genetic transformation. This gene has been shown to confer resistance to gabaculine (3-amino-2,3-dihydrobenzoic acid) in tobacco. Gabaculine is toxic to plants through the potent inhibition of the synthesis of tetrapyrrole compounds via binding to GSA-AT. GSA-AT is present in all plants and the encoded protein is about 73% identical to the bacterial protein.

In summary, there are some promising alternatives to the use of antibiotic-resistance genes of bacterial origin for use as selectable markers in GM plants; further, there is also potential for their removal prior to commercialization. In addition, genetic transformation using T-DNA insertion into chloroplast DNA results in the absence of any marker genes being present in the pollen, thus removing any potential for "gene flow".

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TRANSGENIC PEA WITH IMPROVED TOLERANCE TO PEA ENATION MOSAIC VIRUS AND PEA SEED-BORNE MOSAIC VIRUS[69]

Švábová L., Griga M., Navrátil M., Šafářová D., Hanáček P., Reinöhl V., Horáček J., Smýkal P.

Pea Enation Mosaic Virus (PEMV) and Pea Seed-borne Mosaic Virus (PSbMV) are the most common pea viruses in the Czech Republic (Šafářová *et al*. 2008). They decrease seed yield by 10-25% and present serious risk to the pea growers. Genetic engineering enables development of virus tolerant or resistant plants by means of a pathogen derived resistance mechanism, via blocking disassembly of the infecting virus due to the presence of transgenically expressed coat protein, or by inducing post-transcriptional gene silencing.

During a three-year field screening (2003-2005) project, a number of PSbMV and PEMV isolates were collected from the naturally-infected pea plants at different locations of the Czech Republic, and *cp* genes (coding for viral coat protein) of the most virulent types were sequenced. Virus resistant/tolerant transgenic pea lines were obtained by introgression of the full length or the fragments of the PEMV and PSbMV coat protein cDNAs that were cloned in sense and antisense orientations into pGreenII plasmid (JIC, Norwich, UK). The resulting pGreenII based vectors contained the appropriate *cp* cDNA, a *2x35S::uidA* reporter gene, and the *nos::bar* gene encoding phosphinothricin acetyltransferase. Plasmids were inserted into the hypervirulent *Agrobacterium tumefaciens* strain EHA 105 together with the helper plasmid pSoup.

Transformation of pea plants was carried out using standard protocols (Švábová *et al*. 2005, 2008) and the presence of transgenes in T_0 – T_2 plants was confirmed by PCR detection (*cp*, *gus, bar*) and Southern blotting (*cp*, *gus*). The T2 progeny were screened in the glasshouse (mechanical inoculation of transgenic and control non-transformed plants with natural PEMV/ PSbMV isolates) and shown to exhibit various levels of virus tolerance combined with phenotypic differences (normal growth x growth depression, normal or delayed flowering, PEMV or PSbMV symptoms). Virus replication was monitored by timed DAS-ELISA. In T3 transgenic pea lines expressing PSbMV coat protein fragments the concentration of virus RNA after 14 days was transiently enhanced (in average up to 125%), but after 28 days it droped to 37% of the control. Similar response was found in transgenic lines expressing PEMV coat protein fragments. Several tens of transgenic pea lines were chosen for the preparation of seeds for field studies and feeding tests with monogastric animals.

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DEVELOPMENT OF TRANSGENIC TISSUE LINES OF SPRUCE (*PICEA ABIES*) SHOWING HIGH TOXICITY TOWARDS BARK BEETLE (SCOLYTIDAE) SPECIES[70]

Vlasák J.

Norway spruce (*Picea abies L.*) is a beautiful and highly productive tree species. In our country, spruce forests represent more than 50% of all wooded areas. Unfortunately, over the last few decades, bark beetles (*Scolytidae*) attack have significantly encreased and cause major damage in the whole country. It is a big problem; the amount of attacked wood now stands at 15 millions cubic meters of wood and large wooded areas may be completely destroyed in the near future.

Various strategies were developed to control this pest, with little success, up to now. Experiments with *Bacillus thuringiensis* delta endotoxin, a well known and highly successful biological insecticide, failed, too, because of problematic application of commercial preparations on larvae developing in the bark layer and low sensitivity of bark beatles to common kinds of *B. thuringiensis* toxin.

It is our aim to overcome problems with delta endotoxin application against spruce bark beatles using the methods of molecular biology. *Bacillus thuringiensis* var. tenebrionis delta-endotoxin gene Cry3A was completely reconstructed for high expression in spruce and synthesized. Cleavage sites were introduced in specific positions in the protein molecule, where cleavage occurs in sensitive insects. Modified versions of cry3A genes are now expressed in *E. coli* and toxicity is currently beeing tested in Forestry Institute *in vivo* on Ips typographus larvae.

Also, modification of endotoxin protein domain II, loop 1 and loop 3 amino acids that should increase toxicity to Scolytidae species, are performed at present.

We will use spruce embryogennic lines developed and maintained in the Forestry and Game Management Research Institute for spruce transformation with optimized and efficacious endotoxin constructs. Both *Agrobacterium*-mediated transformation and biolistic transformation are beeing used. Transgenic plants will be regenerated and characterized. Especially, toxicity of plant extracts to bark beetle larvae will be tested. Lab experiments only are proposed in this project.

70 NAZV, QH71290, 2007-2011

PLANT PRODUCTION OF HUMAN PAPILLOMAVIRUS PROTEINS AND IMMUNOGENIC PROPERTIES OF TRANSGENIC PLANT TISSUES[71]

Vlasák J.

Advances in plant biotechnology have made the production of recombinant proteins in plants feasible. Common crop plants, such as potato, tomato, lettuce and carrot transformed with foreign genes have become accepted as potential production systems for various recombinant proteins for industrial, agricultural, veterinary and pharmaceutical uses. Transgenic crops are inexpensive to grow, and postharvest handling and crop processing are well-established. Sometimes they can be used directly with minimal processing as nutrient additives or so called "edible vaccines".

One of the most attractive prospects for this technology is a **plant produced HPV vaccine**. Genital infection caused by human papillomavirus (HPV) is the most common sexually transmitted viral disease worldwide. Oncogenic HPVs have been found to be associated with high-grade cervical lesions and carcinomas that are the leading cause of death from cancer in developing countries, where control of the disease through screening programmes is not performed. Development of HPV vaccines has therefore become the major goal of HPV research at present. In developing countries, low cost and oral delivery are the key factors that determine successful adoption of vaccination, and these requirements could be met particularly well by vaccines produced in transgenic plants.

Therapeutic DNA vaccines against oncogenic infection with HPV are mostly targeted against viral oncoproteins E7 and E6. To adapt the E7 oncoprotein for DNA immunization, we have previously reduced its oncogenicity by modification of the Rb-binding site and enhanced immunogenicity by fusion with the 5'-terminus of the gene encoding *E. coli* glucuronidase (GUS; Vlasák et al. 2003, 2006). We have shown that the fusion is more immunogenic than any other known E7 fusion, protecting all DNA-vaccinated mice against challenge with E7-tumor TC-1 cells (Šmahel et al. 2004). Transgenic potatoes and tomatoes containing the E7/GUS fusion gene

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(E7GGG/GUS), and a modified version of the fusion with a shortened E7 gene have been produced (Bříza et al. 2007). Unfortunately, the steady-state level of the E7GGG/GUS gene product is 100x lower than the shortened fusion where the most important antigenic epitopes are deleted.

New fusions of the E7 oncogene with *E. coli* GUS were constructed and tested in human as well as plant cells in subsequent experiments. Additionally, fusions using synthetic E7GGG genes optimized for human or potato cells were also constructed. Expression level of the gene constructs, stability of the products and immunogenicity on mice were tested. It was shown that fusion protein stability correlated with the steady-state levels of proteins accumulated in human cells, and with the resulting production of E7-specific antibodies. Surprisingly, higher production of fusion proteins could not enhance cell-mediated immunity. The addition of a signal sequence was the only modification that induced stronger cell mediated immunity (Šmahel et al. 2008). Immunogenic fusions of E7 with GUS and their use in therapeutic HPV vaccine development have been carried out (Vlasák et al. 2006).

Potato and tomato were transformed with various E7GGG gene fusions with GUS, using *Agrobacterium*-mediated transformation. Chloroplast vectors for the integration into three intergeneric loci in chloroplast genome were constructed and used for the biolistic transformation of tobacco chloroplasts with E7GGG/GUS gene fusions. In all cases, transgenic plants were regenerated, characterized and cloned. Unfortunately, these plants did not accumulate intact fusion protein; it was shown that the E7 part of the fusion protein is degraded in plant cells during isolation and storage.

At present, new E7 fusions are being constructed with potential for higher stability in the plant cell.

DEVELOPMENT OF A NOVEL RECOMBINOGENIC TECHNIQUE FOR CHLOROPLAST TRANSFORMATION[72]

Vlasák J.

Chloroplast transformation is an environmentally friendly approach to plant genetic engineering that minimizes out-crossing of transgenes to related weeds or crops and, at the same time, can enable substantial increase of transgenic protein production. The plastid genome is highly polyploid and transformation of chloroplasts permits the introduction of thousands of copies of a foreign gene per plant cell, often generating extraordinarily high levels of the foreign protein. Chloroplast transformation vectors carry two targeting sequences that flank the foreign genes and insert them at a precise, predetermined location in the organelle genome via homologous recombination. This results in uniform transgene expression among transgenic lines and eliminates the "position effect", often observed in nuclear transformants. Foreign proteins found to be toxic in the cytosol are sometimes nontoxic when accumulated within transgenic chloroplasts. In addition, gene silencing, frequently observed in nuclear transgenic plants, has not been reported in genetically engineered chloroplasts.

Little is known about the mechanism of homologous recombination in chloroplasts that drives successful transformation. Very likely, double-strand-breaks (DSB) or long single stranded stretches in the vector or target sequence are required to start vector integration. Presumably, stalled replication forks of two head-to-head oriented chloroplast origins of replication are involved, providing a single-stranded substrate for a chloroplast protein similar to the RecA recombination protein. Nuclear encoded homologues of this conserved recombination protein are known to be transported into chloroplasts. Nevertheless, no systematic study of homologous recombination in chloroplasts has been published. The project aims to compare tobacco, tomato and *Chlamydomonas reinhardtii* chloroplast transformation with circular, linear ends-in, and linear ends-out dsDNA and ssDNA vectors. Each type of vector should show a different transformation efficiency as a result of the involvement of different recombination mechanisms.

Plasmid vectors with 2 kb long chloroplast target sequences flanking gene cassettes containing E7GGG, GFP, or GUS genes with suitable chloroplast-active promoters, and their fusions with the selectable *aadA* gene have been constructed. Linearised versions with a unique cut in the homology region (ends-in vectors) and PCR-amplified linear vectors with synthetic-flanking sequences of at least 70 bp (ends-out vectors) will also be prepared. Chloroplast cassettes for transient expression of bacteriophage lambda Beta, Exo, and Gam recombination proteins will be constructed and co-transformed with linear vectors. We assume that free DNA ends will promote homologous recombination, and lambda Red proteins will support recombination in chloroplasts as observed in *E. coli*, mediating production, protection and annealing of recombinogenic single-stranded overhangs.

We plan to use the human papillomavirus E7 oncogene, which has large potential for therapeutical vaccine design, for chloroplast transformation. Chloroplast expression may be an important source of HPV protein for vaccine production.

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72 GA AV ČR IAA500960903, 2009-2012

FLAX (*LINUM USITATISSIMUM L.*) TRANSFORMATION WITH HEAVY METAL BINDING PROTEIN GENES[73]

Vrbová M., Horáček J., Smýkal P., Griga M.

Flax (*Linum usitatissimum* L.; also referred to as linseed) is a fibre crop which has been utilized in a variety of industrial applications and recently found to have a potential to extract heavy metals from polluted soils. Screening of commercial varieties of flax, as well as germplasm resources showed significant differences in the uptake and accumulation of Cd, Pb, Cu or Zn between genotypes. However, the potential of flax for heavy-metal uptake is not high enough for large-scale industrial use in phytoremediation technology. Thus, introduction of genes connected with heavy metal tolerance/transport/detoxification represents an alternative approach to improve the phytoremediation ability of flax.

Various modifications of published *Agrobacterium*-mediated transformation protocols of flax were tested, using flax cv. Jitka and linseed AGT-0916, with the aim of improving the efficiency of the available methods. Modifications included removal of epidermis (peeling) in initial explants (hypocotyl segments), changing the duration of the cocultivation period, or application of various cocultivation additives. *A. tumefaciens* strain EHA 105 containing the *nptII* gene as a selectable marker and several genes of interest (heavy metal binding peptides – *αmt* and *cp* fused to *uidA/gus* gene) were used in these experiments. The transformation efficiency was measured by GUS expression in explants after 3 weeks on selection medium (MS medium + 500 mg Timentin, 200 mg Augmentin, 150 mg Kanamycin, 1 mg/l BAP, 0,02 mg/l NAA). The expression of a ß-glucuronidase (*uidA/gus*) gene served as a reporter allowing early determination of transformed shoots, and estimation of transformation efficiency (using the Image Analysis DIA application to quantitatively assess transgene expression).

Removal of epidermis enhanced the transformed tissue ratio by 11.75%. The optimum conditions for transformation were 10-15 min. cocultivation time, and 100 mg/l acetosyringone and 200 mg/l cellulase as cocultivation additives. Putative transformants were screened by kanamycin selection and expression of GUS. GUS-positive T0 transformants were assayed for insertion of genes of interest by PCR. Segregating T1 progeny of selected T0 transformants, covering various transformation events, have been analysed for gene copy number, gene expression and phenotypic behaviour under Cd treatment.

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4.2 RISK ASSESSMENT STUDIES

THE EFFECT OF BT-CORN ON SOIL INVERTEBRATES, THE SOIL MICROBIAL DECOMPOSERS COMMUNITY, AND DECOMPOSITION RATES OF CORN POST-HARVEST RESIDUES UNDER FIELD AND LABORATORY CONDITIONS[74]

Frouz J., Elhottová D., Helingerová M., Nováková A., Kocourek F.

Effects of Bt corn on post-harvest residue decomposition, soil microflora, and soil fauna were studied in two field experiments in the Czech Republic. At each location, Bt corn and a non-Bt conventional corn hybrid with a similar genetic background were each planted on half of a field. This experimental design was repeated at both locations for three years. Field microcosms containing litter bags of Bt or non Bt corn post-harvest residues and matching field soils were exposed in field plots in completely randomized blocks and sampled after 3, 6 and 18 months. Decomposition of litter bag content, microbial biomass, PLFA profile, and abundance of soil fauna in whole microcosms were determined. No significant effects of Bt corn on the investigated parameters were recorded. In the laboratory, either fresh post-harvest residues or post-harvest residues exposed to soil for 90 days from the above field experiment were used to study the effect of Bt corn on population growth of *Enchytraeus crypticus* (Oligochaeta: Enchytraedae). Significant reductions (approx. 30%) of *E. crypticus* population growth in fresh Bt corn litter in comparison with non-Bt corn were observed. However, this was not observed in litter exposed to soil for 90 days. In conclusion, Bt corn may have a deleterious effect on decomposers in the laboratory, but this effect was minor and restricted to the initial stages of decomposition, and was undetectable in long-term field experiments.

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FIELD ASSESSMENT OF CROSS-POLLINATION RATE IN PEA (*PISUM SATIVUM* L.)AS A BACKGROUND FOR RELEASE OF GENETICALLY MODIFIED PEA INTO THE ENVIRONMENT[75]

Dostálová R., Smýkal P., Seidenglanz M., Griga M.

We have recently developed transgenic pea lines with improved resistance to PEMV (Pea Enation Mosaic Virus) and PSbMV (Pea Seed-borne Mosaic Virus). To assess possible risk of uncontrolled spreading of the transgene in the environment, we examined the frequency of outcrossing between several distinct non-GM commercial peas. It should be emphasized that in Central Europe there are no naturally occurring wild *Pisum* species crossable with the cultivated pea (*P. sativum* L.). Cultivated peas also do not cross naturally with other cultivated legumes (genera *Phaseolus*, *Vicia*, *Glycine*, *Lathyrus*) or wild European species of the family *Fabaceae*. Only gene flow between the commercially grown non-GM and GM pea cultivars may be expected. Our study included monitoring pollinators. An inventory of insect taxa occuring in pea revealed frequent occurrence of three species whose adults may invade closed flower buds and thus potentially transfer foreign pollen. The species include thrips *Kakothrips pisivorus*, and *K. flavus*, and the pea weevil *Bruchus pisorum*. Two pea cvs. differing in flower colour, cotyledon and seed coat colour and whole plant habit were grown in close proximity in 2001-2004. Dry-seeded cv. Zekon with recessive traits (afila type, AT; white flower, WF; green cotyledons, GC; colourless seed coat, CssSC) served as a trap variety, and fodder cv. Arvika with dominant traits (normal leaf type, NT; violet flower, VF; yellow cotyledons, YC; coloured seed coat, CoSC) as a pollen donor. Flowering periods of the cvs. overlapped. All seeds of the trap variety were sown to monitor the incidence of dominant traits in the F_1 generation. In this experimental series (approx 40 thousand F_1 plants screened each year) we did not find any plants with dominant traits in the progeny of the trap variety (0% outcrossing).

In the second experiment (2004-2007), we extended the number of pea cvs by dry-seeded pea cv. Adept (NT; WF; GC; CssSC), canning pea cv. Radim (NT; WF; GC; CssSC), canning pea line B99/112 (NT; WF; GC; CssSC; resistant to powdery mildew), and fodder pea cv. Racer (AT; VF; YC; CoSC). Using the same strategy as in the first experiment, we recorded cross-pollination frequencies of 0.70% in 2005, 0.57% in 2006 and 0.72% in 2007. The hybrid nature of phenotypically different F1 plants was confirmed by genotyping, using microsatellite marker AD-372 and retrotransposon marker RBIP 281-R44, whose combination enabled exact discrimination of potential parental components.

Based on these field trials, supported by molecular analyses, we conclude that the outcrossing rate in commercial peas grown in Central Europe does not exceed 1%.

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RESULTS OF A FOUR-YEAR STUDY OF THE IMPACT OF BT MAIZE ON ARTHROPOD COMMUNITIES[76]

Habuštová O., Hussein M. H., Doležal P., Spitzer L., Růžička V.

Maize cultivar YieldGard®, which expressed Cry1Ab toxin from *Bacillus thuringiensis* (Bt), and the non-transgenic parental cultivar were each grown on 5 plots of 0.5 ha for four years. The plots were distributed checker-wise in a 7.6 ha field in 2002 and in a 14 ha field in 2003-2005. Bt cultivar contained about 1 ppm Cry1Ab in the leaves and stem and undetectable amounts in the flowers and ears. Due to the presence of the toxin, the Bt maize proved fully resistant to *Ostrinia nubilalis*. Toxin residues in the plant pieces left in the field decreased gradually during autumn and winter and became undetectable in March of the next year. To increase the field load of Cry toxin, maize of the waxy ripening stage was shredded into small pieces that were ploughed into the soil. The aim of our study was to detect possible impact on arthropod communities in the field. Samples of arthropods dwelling on the plants and of epigeic species caught in the pitfall traps were collected at 2-week intervals.

The analysis of arthropods included taxonomic determination of about 50 thousand specimens. Highly represented taxa such as spiders, aphids, predatory bugs, thrips, ground beetles (Carabidae), and rove beetles (Staphylinidae) were evaluated with statistical methods. Species diversity and abundance on different plots were compared with Canoco statistical software and their dependences on the plot position, year, and Cry presence were assessed with the Monte Carlo permutation test. Considerable variability in species diversity and abundance proved dependent on the year and occasionally on the plot position, but NOT on Cry expression (data summed up for the four years of study are showed in the tables). We conclude that the presence of Cry1Ab toxin has no adverse effect on the ecosystem.

Abundance of several taxa dwelling on the plants – sum of data for 2002-2005

Maize type/insects	Thrips (2 species)	Aphids (3 species)	Predatory bugs
Non-Bt maize	14862	10790	647
Bt-maize	14315	10731	604

Numbers of species and specimens of dominant arthropod taxa caught in the pitfall traps

Maize type/ arthropods	Spiders		Carabidae		Staphylinidae	
	Species	Specimens	Species	Specimens	Species	Specimens
Non-Bt maize	100	15079	131	16630	102	1977
Bt-maize	87	15494	129	16353	101	1916

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GENETICALLY MODIFIED MAIZE AS A BARRIER TO *DIABROTICA* SPREADING IN EUROPE: CHECKING POSSIBLE IMPACT ON OTHER ARTHROPODS[77]

Habuštová O., Sehnal F.

The Western corn rootworm, *Diabrotica virgifera virgifera* (WCR) has been monitored in Czech Republic with the aid of pheromone traps by the State Phytosanitary Administration since 1999. The traps are set up at observation points each month from 1 July to 30 September and checked every 1–2 weeks. The monitoring began in the districts adjacent to Austria and Slovakia and after WCR invasion had shifted North and West. WCR adults were first detected in the Hodonín District in July 2002. Since that time, nearly all Moravia (Eastern part of the country) has been infested and a mandatory programme of insecticide use had to be introduced.

WCR significantly hampers maize production by damaging the root system and the flowers. The eggs are laid in the soil during autumn and hatch in spring. Larval feeding on the corn roots causes direct physiological yield loss and also secondary harvesting losses due to plant lodging. The emergence of adults begins in early July and continues through the late summer. The adults preferentially feed on the female flowers (silks) and soft kernels. The economic losses are particularly high in regions where maize is grown on the same field repeatedly. To reduce the losses, fields in the WCR infested zone of Czech Republic, which are used for maize cultivation for 3 consecutive years, must annually be treated with an insecticide. Currently there are no biological control agents that will consistently reduce WCR populations and associated injury below economically damaging levels. Genetically modified (GM) maize expressing *Bacillus thuringiensis* toxin Cry3Bb1 is resistant against this pest, but it has not been deployed in Europe because of fears of environmental side effects. In 2009 we start a 3-year study to examine environmental impacts of GM maize 88017, which is *Diabrotica*-resistant and herbicide-tolerant, on the biodiversity and abundance of arthropods that live on the maize plants (aphids, thrips, predators and parasitoids) or in the soil (spiders, Carabidae and Staphylinidae beetles and nematodes). GM maize will be compared with the isogenic cultivar with or without the standard insecticide protection and with 2 reference maize cultivars. Each variant will be tested on five 0.5 ha plots distributed in a 14 ha field from the 6-leaf stage until the waxy ripening stage when the plants will be shredded and fermented to produce biogas. Enzyme Linked Immunosorbent Assay will be applied to quantify the products of both transgenes in the 3rd leaf from the top in plants of the 6-leaf stage, at the time of flowering, and at the end of the experiments. Additional measurements will be done in the flowers and kernels. The results will reveal if the GM maize or the insecticide treatment is more damaging to the environment. Special attention will be paid to the effect on potential natural enemies of WCR.

77 NAZV QH91093, 2009–2011

EFFECT OF MON 810 BT TRANSGENIC MAIZE DIET ON STORED-PRODUCT MOTHS (LEPIDOPTERA: PYRALIDAE)[78]

Hubert J., Kudlíková-Křížková I., Nesvorná M., Zemek R., Stará J., Stejskal V.

The transgenic corn hybrid MON 810-YieldGard® was developed to protect maize against herbivorous Lepidoptera larvae in the field. Although the hybrid kernels contained levels of Cry1Ab toxin 20 times lower than in leaves, they have been shown to be toxic to some stored product pests, indicating a protective effect of Cry1Ab during maize storage. Characterization of the resistance level and benefits of expression of Cry1Ab toxin in kernels during their storage is still incomplete. In this study, we compared the insecticidal effects of diets obtained from the MON 810-YieldGard® hybrid to four species of stored product moths: *Ephestia kuehniella*, *Ephestia elutella*, *Cadra cautella* and *Plodia interpunctella*. The diets, which were produced from kernels obtained from two different experimental fields, contained the same concentration of Cry1Ab (0.35±0.056 μg g^{-1}). They caused 100% mortality in *E. ellutella*, *C. cautella* and *P. interpunctella*, and 65% mortality in *E. kuehniella*. Comparisons of LD^{50} (time when 50% individuals died) and larval relative growth rate (RGR) among the tested species revealed that *P. interpunctella* was the most sensitive species followed by *E. elutella*, *C. cautella* and *E. kuehniella*. The lowest toxic concentration of Cry1Ab in the diet of *E. kuehniella* larvae was determined by mixing diets from hybrid kernels containing Cry1Ab with diets from control kernels without Cry1Ab. The mortality of *E. kuehniella* larvae decreased with decreasing Cry1Ab concentration, and the LD_{50} (concentration when 50% individuals died) was 0.20 μg Cry1Ab g^{-1} of diet. Similarly, the larval RGR decreased with decreasing logarithmically transformed concentrations of Cry 1Ab in the diet. These results show that MON 810-YieldGard® hybrid kernels are protected during their storage against feeding by stored product moths. Insecticidal cocktails containing sublethal doses of Cry1Ab toxin (0.011 to 0.091 μ Cry1A g^{-1} of diet) with added soybean trypsin inhibitor (STI) + chitinase as a secondary compound significantly decreased RGR of *E. kuehniella* larvae. It is hypothesized that the protease inhibitor (STI) protects both chitinase and Cry1Ab proteins from endogenous proteases in the larval midgut and prolongs their insecticidal activities. The application of insecticidal cocktails could enhance the control of *E. kuehniella* by Cry1Ab.

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DIVERSITY OF CARABID BEETLES (COLEOPTERA: CARABIDAE) UNDER THREE DIFFERENT CONTROL STRATEGIES AGAINST EUROPEAN CORN BORER IN MAIZE

Kocourek F., Saska P., Řezáč M.

We compared three control strategies against the European corn borer (*Ostrinia nubilalis* Hubner) in maize with respect to the beneficial epigeal carabid beetles. The impact of the focal treatment (insect resistant Bt-maize) was compared with the conventionally farmed and with *Trichogramma*-treated plots on two sites (Praha-Ruzyně and Ivanovice na Hané) and replicated in three cropping seasons (2002–2004). Carabid beetles were sampled using pitfall traps. Observed species richness (S_{obs}) was calculated for each plot, sampling date and year, and Chao 1 index (±SD) was used to estimate the true species richness (Chao 1987; Biometrics 43, 783–791). The rarefaction curves were produced by repeatedly re-sampling the pool of N individuals and Q samples at random for 50 times (Colwell, 2005; EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. User's Guide), and rarefied species richness was plotted ("re-scaled") against rarefied number of specimens collected; this standardization eliminates the effect of sample size on observed species richness. The composition of carabid assemblages was compared between fields using the estimated abundance-based Chao-Jaccard similarity index (Chao et al., 2005; Ecology Letters 8, 148–159).

The sampled assemblages were poor in species, which were unevenly distributed; the assemblages were dominated by 7 (Ruzyně) or 3 (Ivanovice) species. No differences were found in species richness or species composition between treatments, seasons or sites, suggesting no effect of planting transgenic insect resistant maize on the communities of carabid beetles in the study fields.

Species richness estimators. N ind – number of collected individuals, S obs – number of observed species, S est (Chao 1) – estimated species richness using Chao 1 index ±S.D.

	Bt			*Trichogramma*			Control		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
Ruzyně									
N ind	749	967	433	822	991	461	760	809	467
S obs	23	16	18	19	19	18	24	24	16
S est	34±10	22±8	34±17	44±31	25±7	24±7	32±8	27±17	18±2
Ivanovice									
N ind	535	349	12	366	388	225	466	673	222
S obs	14	10	5	13	18	9	12	15	12
S est	15±2	12±4	5±1	14±1	48±29	14±7	22±10	18±3	21±10

IS THE TITER OF ADIPOKINETIC PEPTIDES IN COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA*) FED ON GENETICALLY MODIFIED POTATOES INCREASED BY OXIDATIVE STRESS?[79]

Kodrík D., Krishnan N., Habuštová O.

The level of adipokinetic hormones (AKHs) (Peram-CAH-I and -II) in the corpora cardiaca (CC) and the haemolymph of *Leptinotarsa decemlineata* was increased up to 10-fold and more than 6-fold respectively, in adult insects fed on genetically modified (GM) potatoes containing either GNA lectin or Cry 3Aa toxin. This increase is indicative of increased oxidative stress in gut tissues, and similar significant enhancement of the AKH titer was observed when insects were injected with paraquat, which evokes oxidative stress. The increases caused by paraquat (1.9 fold in CC and 2.7 fold in haemolymph) were recorded within 4 hours post injection, while control insects injected with saline and intact insects (no intervention) maintained original levels of AKH in both CC and haemolymph. Feeding on the GNA and Cry 3Aa-expressing potato cultivars significantly increased the content of protein carbonyls in insect gut tissues after feeding for 6 days, with a 1.9 fold increase recorded in the GNA-fed, and a 2.4 fold increase in the Cry 3Aa-fed insects. The protein carbonyl content in the gut tissue of Cry 3Aa-fed insects was 1.3 times higher than in GNA-fed ones, indicating that Cry 3Aa, at least at the level of expression in transgenic potatoes, was more toxic to *L. decemlineata* than GNA. In agreement, insects exposed to the cultivar expressing Cry3Aa did not survive beyond 8 days feeding. Paraquat injection of 6-day old *L. decemlineata* fed on normal potatoes resulted in a significant increase in protein carbonyl levels, and a sharp and significant fall in the reduced glutathione level in the haemolymph within 4 hours post injection. However, combination of paraquat with Peram-CAH-II elicited a significantly lower increase of protein carbonyls than paraquat alone, and levels of GSH in the haemolymph similar to the control groups, and significantly greater than in the insects injected with paraquat alone.

These observations indicate that there is feed-back regulation between the action of oxidative stressors and the level of AKH in the insect body, and that AKHs might be involved in the activation of an antioxidant protection mechanism. These results are, to our knowledge, the first evidence for the involvement of AKHs in oxidative stress mitigation, in addition to a plethora of other roles.

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MYCOTOXINS AND GENETICALLY MODIFIED MAIZE[80]

Nedělník J., Moravcová H., Rotrekl J., Cholastová T.

Feeding ration is one of the crucial factors affecting the health of animals, their efficiency as producers, and the quality of livestock products. Feedstuffs may contain harmful substances that negatively affect animal health as well as the safety and acceptability of the products. These harmful contaminants may engender during the production, preservation and storage of feedstuffs, or during their technological processing. Common contaminants include fungi and their toxins. The occurrence of fungi in maize harvested for silage, which has higher stubble remaining after cutting, is increased when the upper part of the ear is contaminated by the European Corn Borer (*Ostrinia nubilalis*). Corn borer damage facilitates fungal contamination which can subsequently spread to other parts of the plant. Harvesting of severely contaminated and older maize plants with high dry matter content is often a source of high silage contamination with *Fusarium* toxins that decrease silage digestibility, and negatively influence animals' health. The occurrence of fungi and their mycotoxins can also be expected if silage is not produced in accordance with proper standards. Especially problematic are slow and interrupted ensiling, contamination with the soil, failure to cover the material, leakage of rainwater, insufficient sealing against air, etc. These factors lead to greater contamination with undesirable bacteria and fungi that might cause secondary fermentations and not only result in loss of nutrients but also put at risk the health and physiological functions of the animal consuming the silage.

Mycotoxin contaminants are derived from: 1) maize cultivation; 2) maize harvesting and silage production; and 3) feeding process, including failure to remove dangerous contaminants from the feedstuffs. Under the Czech Republic's soil and weather conditions, the main mycotoxin producers are soil fungi of the genus *Fusarium*, sometimes referred to as "field fungi". Attention is focussed on growers' interventions that may decrease the contamination of plants by these pathogens. From the spectrum of technological possibilities, the most important are: the cultivar and its type, dry matter

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content, and phytopathological and biotechnological steps toward reducing damage to plants by the corn borer.

Damage to plants by *O. nubilalis* promotes infection by fungal pathogens and is one of the factors that increase the possibility of contamination by mycotoxins. Experiments carried out over several years have compared protection of maize against *O. nubilalis* using a genetically modified Bt-hybrid, traditional protection using insecticides, biological protection using wasps of the genus *Trichogramma*, and a control variant (isoline to Bt-hybrid). These experiments have demonstrated very low or no contamination of Bt maize by *O. nubilalis*. A 60–70% effectiveness was achieved using insecticides. The effectiveness of biological approaches was strongly dependent upon the weather conditions, but the average effectiveness was less than that of chemical protection. Subsequent analysis of *Fusarium* mycotoxin showed a correlation with insect resistance, i.e., mycotoxin content in the GM material was lowest compared to the highest content in the control, untreated maize. It should be noted that the mycotoxin content in GM material was not always zero. Even if the maize was not attacked by *O. nubilalis*, the material could still be contaminated by fungi (genetic modification protects maize against *O. nubilalis* and it does not directly increase its resistance to fungal pathogens). Notwithstanding all the questions that are related to the use of genetically modified plants, cultivation of GM maize can be recommended from the viewpoint of decreasing mycotoxin contamination. During the preparation of this report, the Ministry of Agriculture published information that Bt maize was grown in the Czech Republic in 2008 on 8,300 ha, making this country the second-largest grower of Bt-maize in the EU after Spain with about 70,000 ha. The Minister of Agriculture stated that the Czech Republic will continue to support sensible use of Bt-maize. From the viewpoint of mycotoxin contamination, one can welcome this recommendation. Cultivation of Bt maize is particulary important in locations with a high occurrence of *O. nubilalis* and in case of later-maturing maize hybrids that are exposed to this pest for a longer period of time.

A separate problem relates to the harvesting of maize, its quality, subsequent speed and quality of its ensiling, and the quickest possible sealing of the silage against air. Recommendations are provided for increasing the quality of silage and prevention of secondary contamination of the ensiled material by "storage fungi". The authors have analysed a wide range of samples collected during the ensiling processes from individual locations of trench silos, as well as samples that have been taken from the face of the silage during its loading for feeding. The results confirmed that if the ensiled material contains a greater amount of mycotoxins, they occur across the entire profile of the final silage. If mycotoxins are present during the period of silage fermentation, they are also present at the final opening of the silo. The ensiling process does not decrease the amount of mycotoxins present in harvested maize because these compounds exhibit high thermal and chemical stability.

Results reported here were obtained in research on the project "Production of good-quality and safe grain products using various strategies for protecting maize and stored products" (1B53043), supported by the Ministry of Agriculture of the Czech Republic, and the project "Genetic-breeding and technological aspects of sustainable fodder crops production" (MSM2629608001), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

IMPACT OF BT POTATOES ON NON-TARGET ARTHROPODS[81]

Nedvěd O., Spitzer L., Kaluškov P.

Potato cultivar Superior Newleaf® producing Cry 3Aa Bt toxin is resistant to Colorado Potato Beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), a serious defoliator of potato plants. The cultivar has been tested in 2000–2001 in Bulgaria, in field plots about 1 ha large. We monitored species composition and abundance of several dominant non-target arthropod taxa: Aranea, Carabidae and Coccinellidae.

Samples of arthropods dwelling on the plants were collected by sweeping net and the epigeic species were caught in the pitfall traps, both at 2-week intervals through the growing season. The species diversity and abundance on the two plots, Bt and control, were compared using multivariate statistics (Canoco), and their dependence on the field type, spraying by insecticides, and collection date was tested by the Monte Carlo permutation test.

The analysis of epigeic arthropod communities showed either no difference between the Bt and control fields, or a positive effect of Bt cultivar in comparison with the field sprayed with insecticides. There were 34 species (2645 individuals) of carabid beetles collected in the Bt-field, while only 25 species (1313 individuals) were collected on the sprayed field. The distribution of species in the abundance classes was more equitable in the Bt field. The abundance of beetles on the conventional field was similar to that in the Bt field in June but the abundance decreased after spraying and the decrease persisted to the end of the season. Epigeic spiders were similarly distributed in both field types, suggesting that they were less sensitive to the insecticides or more easily dispersed to the field after insecticide treatment.

Insecticides caused significant decrease in the abundance of aphidophagous coccinellids. Laboratory experiments revealed that Bt potatoes had no effect on a non-target pest, the aphid *Myzus persicae*, and that two differentially fed groups of aphids provided to the seven spot ladybird, *Coccinella septempunctata*, had no effect on their larval development and mortality.

We conclude that the presence of Cry 3Aa toxin was very effective against the target pest, Colorado Potato Beetle, and had no adverse effect on non-target taxa of arthropods. Seasonality had always the largest effect on the community structure and abundance of monitored organisms.

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FIELD TRIALS WITH GM FLAX AND LINSEED – NEW CHALLENGES TO CROP BREEDING[82]

Rakouský S., Tejklová E.

Projects of the Grant Agency of the Czech Republic (GA 521/97/1135) and Ministry of Youth, Education and Sports (ME 210) have established the necessary basis for producing and testing of genetically modified (GM) flax plants under conditions of contained use in the Czech Republic (CZ). Based on a positive opinion to the results of the risk assessment (RA), approval for field trials (release to the environment under letter B of the Directive 2001/18 EC) was granted by the Ministry of Environment in 2001. Since that time nearly two and half thousand different genotypes (independent strains and lines) derived from insertional mutagenesis based on transformation with both model and target genes (*gfp*, *bar*) have been tested on experimental field plots of the Agritec, Research, Breeding and Services, Ltd. company. The method of GM insertional mutagenesis of flax was developed during co-operation between the Institute of Plant Molecular Biology, AS CR, and the Agritec breeding company. It was developed for two flax cultivars, and was later applied to a further twenty varieties and breeding lines of flax and linseed (in collaboration with the University of South Bohemia). Progeny of the mutated plants were evaluated in field trials. Linseed cultivars and breeding lines included both classical (high-linolenic content – HL) and modern (low-linolenic acid – LL) seed oil types. Each genotype of T2- and following generations was evaluated for its morphological, physiological and health characteristics on 0.36 and 0.6 m^2 plots. Selected forms were sown repeatedly during the next few years (up to T_7 generation) to confirm stability of traits, heritability and to study other aspects (projects ME 434, ME 703, 1P05ME800).

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Tejklová E., Rakouský S. (2002) Transgenoze ve šlechtění lnu (In Czech). Úroda 12, 30-31.

Tejklová E., Rakouský S. (2005) Biotechnology in flax and linseed breeding in the Czech Republic. Proc 11th International Conference for Renewable Resources and Plant Biotechnology NAROSSA 2005. Poznan, Poland (CD document).

Based on the results of field trials numerous new forms of flax and linseed of interest to plant breeders have been derived. For instance, among progenies of bast flax lines intermediate forms showing common characteristics of flax and linseed were identified, e.g. those with enhanced ball numbers and seed yield, elevated seed oil content but still preserving high fiber content in stems as well genotypes differing in stem length, starting period of flowering and seed colour. We have found also some linseed genotypes with important shifts of the flowering period, e.g. in cv. Areco (newly identified late- HL type), cv. Atalante (longer growth period) or NLN line (early flowering LL type). Some other flax lines were less prone to the attack of fungal pathogens to stems and seeds compared to the control. Another genotype of cv. Areco had larger seeds with slightly changed colour. Finally some sterile and semi-sterile forms of flax have been produced.

Selected lines were propagated (1.2 m^2) and finally grown on 8 or 10 m^2 plots (up to 18 genotypes annually) for at least 2 years, to determine stability of their performance, uniformity, yield, oil and fiber content and other breeding parameters. Some of the flax lines have shown higher content of stem fiber than the original cultivars. An example of the results obtained during analysis of the seed yield is shown below.

Insertional mutagenesis by GM has been shown to be a valuable tool for creation of genetic variability in flax of interest to plant breeders. During the 8 year period of field experiments with different GM flax lines no changes in plant fitness have been found. Also no indications of possible negative impacts on human and animal health, or the surrounding environment, have been observed. The GM flax modification tested so far has not suggested any safety concerns.

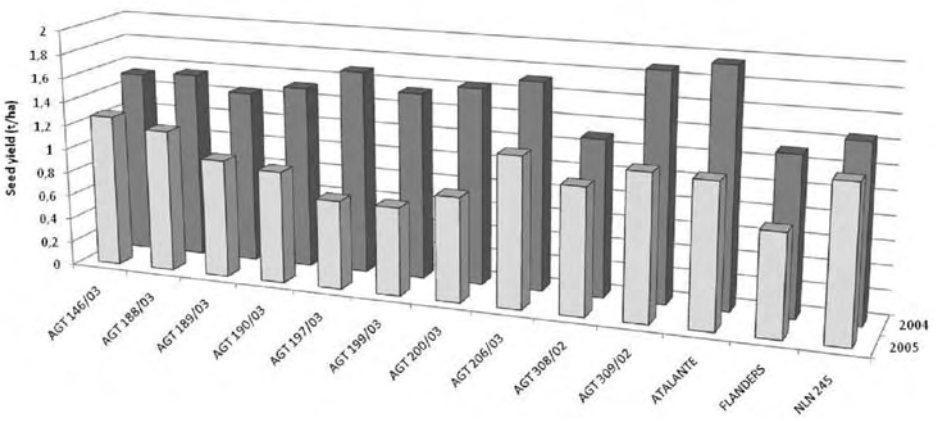

Fig. 1. An example of seed yield analysis performed in two years (2004-5) on 10 m^2 plots with progenies of the Agritec linseed breeding lines (AGT) obtained by insertional mutagenesis using *Agrobaterium tumefaciens* vectors. Non-modified cvs. Atalante and Flanders served as controls .

THE EFFECT OF CONTROL STRATEGIES AGAINST EUROPEAN CORN BORER ON EPIGEIC SPIDERS (ARANEAE) AND HARVESTMEN (OPILIONES) IN MAIZE[83]

Řezáč M., Pekár S., Kocourek F.

European corn borer, *Ostrinia nubialis* Hubner, is one of the most important pests of maize in central Europe. Several methods have been used to control *O. nubialis*: pesticide applications, ploughing, and more recently development of transgenic insect resistant maize. The use of pesticides led to dramatic changes in the composition of non-target organisms. In this respect, the use of transgenic crops that carry a gene from *Bacillus thuringiensis* responsible for production of the endotoxin Cry1A, appear to be "safer". But as the endotoxin can be released to the environment it can affect non-target organisms. One of the most important non-target group of organisms in various crops, including maize, are the spiders.

In this study we monitored effects of two different strategies used to control *O. nubialis* on the abundance and diversity of epigeic spiders and harvestmen in maize, over a three year period. The two strategies were (1) transgenic insect-resistant maize and (2) biological control by *Trichogramma* parasitoid wasps on an isogenic maize hybrid. These were compared with a conventional system (isogenic maize hybrid). The investigation was performed in two localities (Ivanovice na Hané and Praha-Ruzyně) in the Czech Republic from 2002 to 2004. Spiders (Araneae) and harvestmen (Opiliones) were collected by means of pitfall traps.

We found that the annual abundance and diversity of epigeic arachnids on plots with the two strategies were not significantly different from a conventional system. There was no difference in the species, family and guild (hunters versus web-builders) composition between strategies and the conventional system. Similarly, no adverse effects on arachnofauna were found in the use of Bt maize investigated in several different countries in Europe: the Czech Republic, Hungary, Germany and Italy. In agreement with other studies the main differences observed in this study were explained by geographic location and temporal variation. The epigeic fauna of arachnids is, however, quite similar, dominated by linyphiid spiders, irrespective of the geographic position. An overall decrease of abundance observed during the study is presumably a result of a population fluctuation.

It can be concluded that Bt maize strategy had no adverse effect on epigeic arachnids, which is in agreement with previous investigations of Bt maize in Europe.

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BT-MAIZE, *TRICHOGRAMMA* WASPS AND SELECTIVE INSECTICIDES – THREE CONTROL STRATEGIES USED AGAINST EUROPEAN CORN BORER (*OSTRINIA NUBILALIS*) IN CENTRAL EUROPE[84]

Stará J., Kocourek F.

The efficacy of Bt maize against European corn borer (ECB) was tested during 2002–2008 in field trials. Four types of ECB control were tested: (1) Bt-maize hybrid MON 810 DKC3421YG (producing cry1Ab toxin from *Bacillus thuringiensis*), (2) *Trichogramma* wasps, (3) chemical control (only in 2005-2008), (4) untreated control. The maize hybrid Monumental DKC3420 was tested as non-transgenic counterpart of Bt-maize hybrid M0N 810. Each treatment was tested on 0.25 ha plot. In the chemical control treatment, Mospilan 20 SP (acetamipid), Integro (methoxyfenozide) and Steward (indoxacarb) were applied. The biological efficacy of Bt maize on reduction of plant injury by larvae of ECB was always 100%. The biological efficacy of Integro and Steward was comparable and ranged from 70% to 96.3%. Efficacy of Mospilan proved very low, ranging from 4.1% to 12.8%. The biological efficacy of *Trichogramma* varied greatly, according to the conditions in a given year, and ranged from 0% to 81.2%. It was found that the number of tunnels caused by ECB in kernels increased linearly with percentage of injured plants (R=0.74). In contrast to this, an exponential relationship was found between percentage of injured plants and the number of tunnels per plant (R=0.94). No relationship was found between the incidence of *Fusarium* sp. in kernels and percentage of injured plants. The incidence of *Fusarium* sp. in kernels was influenced strongly by weather conditions in a given year.

84 Grant Nr. 1B53043

EVALUATION OF RISK ASSESSMENT ASSOCIATED WITH RELEASE OF GENETICALLY MODIFIED FLAX (*LINUM USITATISSIMUM L.*) INTO THE ENVIRONMENT IN THE CZECH REPUBLIC[85]

Tejklová E., Seidenglanz M., Griga M.

Model situations of possible transgene escape in flax (*Linum usitatissimum* L.) were simulated over the period 2001–2007. The situations considered were (1) uncontrolled cross-pollination between GM and non-GM flax including the possible role of insect pollinators; (2) uncontrolled interspecific hybridization between *L. usitatissimim* and *L. flavum*, the only wild *Linum* species in the Czech Republic potentially crossable with cultivated flax/linseed; and (3) possible survival of GM flax/linseed in the environment as a result of escape of transgenic seeds released during the transport and processing of mature plants.

Three flax lines with contrasting traits and corresponding flowering period were used, namely NLN 14C2 line (obtained via T-DNA insertional mutagenesis) exhibiting recessive yellowish shoots in the period from germination to flowering and dominant blue petals, and line XC1 (or cv. Atalante) with standard dominant green shoots and recessive white petals. In experiment 1, flax lines NLN 14C2 and XC1 were grown alternately in 1 m rows, 10 cm between rows, 120 seeds per row in nine replicates. In this experiment, pollen transfer between lines could occur through insect pollinators, or by flower touch. The seeds of both lines were harvested separately and sown in separate plots in the next year. Green F_1 plants in NLN 14C2 line and blue-flowering plants in XC1 line were selected and the outcrossing rate was determined. In experiments 2 and 3, the maximum distance of pollen transfer was assessed with cv. Atalante (as a pollen donor) and line NLN 14C2 as a trap variety (pollen recipient). The pollen donor plot (1,3 × 10 m) was located close to the trap variety plot (1.3 × 200 m in exp. 2; 1,3 × 20 m in three replicates in exp. 3). Samples of 100 matured plants were collected at discrete distance intervals; 1200 seeds per sample were sown in the greenhouse and cross-pollination rate was determined as a frequency of green seedlings. Experiment 1 gave estimates for maximum outcrossing rates of 0.36 % in line XC1 and 1.99 % in line NLN 14C2. The maximum distance of pollen transmission (from exp. 2 and 3) was 440 cm, with no hybrid plants resulting from cross-pollination detected beyond this distance.

Plants of *L.usitatissimum* (cv. Atalante, lines 1118/05, 1159/05, 1196/05) were grown in close proximity with *L.flavum* either in pots (open area not protected from insect pollinators) or under field conditions. Flowering in *L.flavum* starts earlier and finishes later than in *L. usitatissimum*. No hybrids were detected within progeny both of *L. usitatissimum* and *L. flavum* in observations over three years. Artificial pollination

85 References:

Griga M., Tejklová E., Seidenglanz M. (2008) Assessment of possible risks associated with release of genetically modified flax and linseed (*Linum usitatissimum* L.) into environment of the Czech Republic. In: 10th Int Symp Biosafety of Genetically Modified Organisms, Symposium Handbook: Biosafety Research of GMOs: Past Achievements and Future Challenges. Wellington, New Zealand, p. 105.

of *L.flavum* with pollen of *L. usitatissimum* and *L. flavum* was then carried out by the standard technique used in flax breeding, which routinely yields 90% hybrid efficiency. No hybrid seeds were obtained after interspecific crossing, while pollination of emasculated flowers of *L. flavum* with its own pollen resulted in fertile capsules with seeds. Thus, the probability of unintended crossing between GM flax and its wild relative *L. flavum* may be considered as extremely low or zero.

Insects able to transfer pollen which were abundant and regular in occurrence in flax during the flowering period were thrips (Thysanoptera: Thripidae: *Thrips linarius*), bees and bumble-bees (Hymenoptera: Apoidea: *Apis mellifera, Bombus terrestris, B. agrorum* and other species of the genus). The distance of pollen transfer in the case of thrips is probably very limited (maximum several meters). Some members of Heteroptera species may also be considered as potential pollinators. Here the most frequent species are: *Lygus rugulipennis, L. pratensis, Calocoris norvegicus, Leptoterna dolobrata*, and *Notostira erratica*. A detailed list of insect species occurring in flax fields is provided.

Experiments with spring and winter flax survival from seeds lost during harvest have been described in detail. Based on these results it can be concluded that uncontrolled spreading of flax without specific human intervention is not possible. Standard agrotechnology is sufficient to eliminate all surviving flax plants – no spring and winter flax plants were detected in two successive years after "classical" flax harvest. Monitoring of composted capsules containing germinating seeds from flax over a two year period showed that composting may be used for safe destruction of seeds of the genetically modified flax plants.

INTERACTIONS BETWEEN TRANSGENIC BT CROPS, SPIDER MITES AND THEIR NATURAL ENEMIES[86]

Zemek R.

Plants that contain gene(s) for a crystal (Cry) type protein from *Bacillus thuringiensis* are rendered resistant to certain insects or nematodes. Wide adoption of this new technology for crop protection requires risk assessment based on research into possible unintended side effects on the diversity, abundance, and ecological functions of the non-target organisms. Our work focused on spider mites (Acari: Tetranychidae) that are important pests, and the beneficial predatory mites (Acari: Phytoseiidae), many of which are widely used as biocontrol agents.

In the first study, a transgenic eggplant variety resistant to the Colorado potato beetle due to Cry3Bb expression, and the corresponding non-transgenic variety were used as host plants. Adult females of the spider mite *Tetranychus urticae* were individually placed on leaf discs (2 cm diameter; one half transgenic and one half control) and observed for five days. Females were present more frequently and laid more eggs on the transgenic halves (Wilcoxon signed rank test, P=0.029 and P=0.016, respectively). To investigate the effect of Cry3Bb-eggplant fed prey on the feeding preference of the predator *Phytoseiulus persimilis*, 8 spider mite females from the transgenic and 8 from the isogenic eggplants were offered to well-fed females of *P. persimilis*. Numbers of consumed spider mites were registered and new spider mites were provided six times at 12 h intervals. Predatory mites consumed significantly less Bt-fed than control-fed spider mites (Wilcoxon signed rank test, P<0.0001). Hence, Cry3Bb-eggplants are preferred over control plants as food for spider mites but spider mites feeding on control plants are preferred by the predatory mites. This finding may have practical consequences for the biological control of spider mites.

In the second study we investigated whether feeding pollen of Bt maize var. MON 810, which expresses Cry1Ab, affects the predatory mite *Typhlodromus pyri*. Various life history parameters were measured in laboratory experiments. Survival analysis revealed no significant differences in the longevity, several reproduction parameters, and developmental rate of the progeny between mites fed the control and Bt pollen. We concluded that a detrimental effect of Cry1Ab-maize pollen on this phytoseiid mite species is unlikely.

In summary, no acute toxicity but an effect of Cry3Bb plants on the behavior of mites was found. It is unknown if the change in feeding behavior of *T. urticae* and *P. persimilis* was due to the toxin or to unknown changes in plant physiology or prey quality. Presence of Cry1Ab did not affect the life table parameters of *T. pyri*.

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86 References:

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4.3 CONTRIBUTIONS TO REGULATORY ISSUES

TIME TO RELAX GMO REGULATION IN EUROPE[87]

Drobník J.

Sufficient experience has been gained during ten years of genetically modified (GM) crop deployment to seriously evaluate the ratio of risk to benefit and reduce the existing regulation in Europe. The present regulatory regime does not compare the benefits and risks of GM to alternative situations when GM crops are not used. The precautionary principle is applied only to applications of GM crops, and never to alternative strategies for agricultural practices such as pest control. The Eurobarometer 2005 shows how propaganda inseminates public opinion with shameful nonsense.

Voices asking for change of this policy come not only from the European Parliament, British ACRE, EuropaBio, scientists and other European sources, even from the Commission, but also from Africa and other developing countries.

87 Presented at: The 7th symposium in the series "Recent Advances in Plant Biotechnology". Impact on High Quality Plant Production, Stara Lesna, June 10–16, 2007, High Tatras, Slovak Republic, Plant Cell Tiss Organ Cult (2008) 94, 235–238.

PLANT BIOTECHNOLOGIES: EDUCATION AND PR STRATEGY[88]

Opatrný Z.

Undoubtedly, political decisions concerning both research and the subsequent application of GMOs reflect to various extents public attitudes towards potential accompanying problems, such as ecological risks and co-existence with the main types of agriculture (conventional, organic farming, biotechnological production). The value of relevant enquiries, however, is naturally affected by the objectivity of the information available and its accessibility to "public" groups of varying age, level of education and professional orientation.

In contrast to the well organized, sponsored and massive long term anti-GMO campaigns of various GMO opponents (mainly based incertain NGOs), the systemic education of the general public in modern biology, ecology and similar relevant disciplines is lacking both in CR and globally. The number of specialized lectures focused on the elucidation and confrontation of the results/knowledge of plant biology/biotechnology is insufficient. Not surprisingly, the superficial arguments put forward by the GMO opponents are accepted by a considerable portion of insufficiently informed public.

For more than 10 years the Faculty of Science, Charles University, Prague, mainly through the Department of Plant Physiology, has conducted fundamental and applied research in the field of transgenic plants and has also provided a broad-based education in: plant genetics, cytology, molecular biology, biotechnology – including GMO "risks and profits". Lectures and courses have been organized for Bc/Ms and PhD students, secondary school students, as well as senior citizens (University of the Third Age, University of the Free Time). We use up-to-date knowledge to demonstrate that there are no convincing differences in the properties of "classically bred" crops and GM crops; that there are no qualitative new "types of risk" associated with reasonable GMO application; that the biology of "organic" and "GM" plants is based on the same principles; that close co-existence of all types of agriculture (and, in particular, of "organic" and "biotechnological" farming) is not only possible, but even mutually beneficial – and ultimately inevitable.

In general, the public response to the arguments we have put forward in support of GMOs has been very positive, very understandable – reflecting an historically based high standard of the natural science disciplines in the CR. Furthermore, it resonates with the public who have a desire for "life-long" education and are deeply interested in the problems connected with their health and life quality – as well as quality of the environment. They recognise that the proper application of GM strategies provides means for improving the quality of life and protecting the environment.

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Opatrný Z.: Plant biology, organic farming, GM crops and human health. Abstr, p. 45, In: Proc Int Conf. Biologický výzkum pro lidské zdraví, XIX. Biologické dny ČSBS (Biological Research for Human Health, XIX. Biological Days CSBS), Ed. M. Červinka, Hradec Králové, 29–31 October, 2008, Publ Tribun EU, Brno 2008, ISBN 978-80-7399-545-4.

5. RESEARCH ON GM CROPS IN OTHER COUNTRIES[89]

Research results obtained with GM crops in the Czech Republic are consistent with data generated in other EU countries, as well as overseas, most notably in the USA. The amount of thoroughly analyzed data is overwhelming and fully sufficient for rational regulation of GM crops in the EU. Unfortunately, the preparation of EU legislation seems to be more affected by unjustified and biassed opinions, than by the expert statements of EU advisory bodies, such as EFSA, and by the results of scientific studies conducted in part on the request of the EU Commission. Several projects supported by the EU analyzed the pros and cons of the GM crops and none of them found serious negative GM effects. This is a likely reason why they have not been considered in the formulations of EU regulations, but it is also possible that the data were not disseminated enough. Policy makers cannot be expected to study hundreds of individual reports. To facilitate their orientation in the subject, we cite here just a few most recent reviews in which crucial information can be found in concise form. This approach will hopefully promote consideration of scientific data in the revisions of EU regulations of GM crops. We trust that EU leaders are open-minded, pragmatic, and interested in an objective assessment of GM crops.

The questions of risk assessment related to the environmental safety of GM crops were reviewed on the basis of available data by Sanvido et al. (2007). This paper should be consulted when procedures of the risk assessments are discussed. The information available for GM crops expressing a toxin(s) from *Bacillus thuringiensis* was summarized and subjected to statistical meta-analysis by Naranjo (2009). The author demonstrates environmental advantages of Bt crops in comparison with the insecticide treatment. It is estimated that cultivation of Bt maize and cotton from 1996 to 2006 was associated with a 29.9% reduction of the insecticide use, corresponding to 136.6 million kg chemicals! The deployment of Bt crops in Europe would certainly promote the much desired curtailment of insecticide application in most agricultural regions of the continent.

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Naranjo SE (2009) Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition, and Natural Resources 4, No, 011 (see <http://www.cababstractsplus.org/cabreviews/>)

Sanvido O, J. Romeis J, Bigler F (2007) Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. Adv Biochem Engin/Biotechnol 107: 235–278

Sharma HC (2009) *Biotechnological Approaches for Pest Management and Ecological Sustainability*. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA; ISBN 978-1-56022-163-0; 530 pp.

Only a small number of papers reported negative effects of GM crops. To the best of our knowledge, all such claims have been disproven in the follow-up critical investigations. For example, a study showing a negative effect of Bt maize on the lacewing (Hilbeck et al. 1998), which was carried out in an artificial laboratory setup, was convincingly disproved by several later thorough investigations, most recently by Li et al. (2008). Interestingly, GM oponents consistently quote singular reports on the undesired effects of GM crops and neglect their disproval.

Two comprehensive treatise on the environmental impact of GM crops appeared at the beginning of 2009. According to the publisher, the book "**Environmental Impact of Genetically Modified Crops**" (edited by N. Ferry and A. Gatehouse) "addresses the major concerns of scientists, policy makers, environmental lobby groups and the general public with regard to this contraversial issue. While the main focus is on environmental impact, food safety issues for both humans and animals are also considered. The book concludes with a discussion on the future of agricultural biotechnology in the context of sustainability, natural resource management and future global population and food supply." The book should be used as reference by all who want to comment on the GM crop deployment in Europe and is highly recommended to policy makers responsible for EU legislation on GM crops.

Another book on GM crops entitled "**Biotechnological Approaches for Pest Management and Ecological Sustainability**" was written by H.C. Sharma. This book deals in detail with various methods of crop protection against insect pests. The author describes the mechanisms of natural plant resistance to pests and explains the mechanisms of enhanced resistance in the GM crops. Various aspects of the environmental impact of insect-resistant GM crops (effect on the non-target organisms, management of potential emergence of pest resistance to the GM crop, etc.), and the safety of food and feed are also considered. Finally, the author considers applications of GM techniques to entomopathogenic microorganisms and insectivorous arthropods, and provides his vision of the genetic modifications of crops in the near future. The book is a valuable source of information for those who want to gain a deeper insight into GM directed to plant protection against the insects.

6. REMARKS ON EU LEGISLATION DOCUMENTS CONCERNING GM CROPS

The 2912th Environment Council Meeting (Brussels, 4 December 2008) endorsed EFSA to prepare a review of the current EU regulations of GMOs and to submit its recommendations no later than March 2010. Competent scientific bodies of the Member States were invited to participate, and put forward their opinions on current EU guidelines on the environmental risk assessment to EFSA and other relevant EU authorities. The Czech academic community seized this opportunity to review data obtained in the country in various studies concerning GM crops. The experience of Czech researchers proved to be fully consistent with the results of scientific investigations conducted elsewhere in the EU. The scientific evidence for the safety of GM crops is overwhelming and calls for a critical evaluation of the current GMO regulations that were designed in the eighties of the last century and have become obsolete. The following text tackles the major problems in the EU legislation that is summarized on the web page http://ec.europa.eu/environment/biotechnology/index_en.htm. The Introduction specifies the subject of regulation in the following way:

> *A genetically modified organism (GMO) is defined in the relevant European legislation as any organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.*

This definition correctly implies that radiation and chemical mutagenesis and other breeding methods based on external interventions are man-made genetic modifications and should be treated in the same way as the targeted interventions such as transgenesis. However, genotypes obtained by enforced mutagenesis are not included in the Directives and Regulations and are therefore exempted from the following rule:

> *It is important to ensure that all use of GMOs accords with the precautionary principle in order to protect human health and the environment.*

Strangely, EU regulation concerns only the targeted genetic manipulations that are clearly defined and can easily be monitored. Anybody can grow plants with unknown genotype modifications but researchers or farmers (notifiers) interested in cultivating GM plants with a defined gene intervention must apply for permission for the deliberate GMO release into the environment in accordance with the *Directive 2001/18/EC*[90] or, when crop cultivation is linked to its subsequent use for food or feed, in accordance

90 DIRECTIVE 2001/18/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of March 12, 2001, on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities L 106/1, April 17, 2001.

with the *Regulation (EC) No. 1829/2003*.[91] The GMO legislation also includes *Directive on the contained use of GM micro-organisms (90/219/EEC)*, the *Regulation for traceability and labelling of GMOs (1830/2003)* and the *Regulation on the trans-boundary movement of GMOs (1946/2003)*. Rules for the trans-boundary movement of GMOs comply with provisions of the *Cartagena Protocol*. DG Environment shares the responsibility for implementing the GMO legislation with DG SANCO. In the following text we demonstrate inconsistencies and erroneous statements in the principal EU document. We consider that the document is inherently flawed in that no benefits of GMOs are considered; in fact, the term "benefit" does not occur on any of the 26 pages of the *Directive 2001/18/EC*. The evaluation of risks in agriculture not using GMOs is mentioned only marginally. Environmental impact assessment of GMOs is compulsory but without controls.

6.1 DIRECTIVE 2001/18/EC

6.1.1 Whereas

As for other documents of this kind Directive 2001/18/EC starts with "Whereas" where the basic idea and the purpose of the document are explained. "Whereas" states *inter alia:*

> *(4) Living organisms, whether released into the environment in large or small amounts for experimental purposes or as commercial products, may reproduce in the environment and cross national frontiers thereby affecting other Member States. The effects of such releases on the environment may be irreversible.*

This statement represents an incorrect generalization and violates the principle of case-by-case assessment. For example, maize cannot spontaneously reproduce in the environment of the Czech Republic to affect other Member States. Climatic conditions of Europe are zoned and plants have different requirements. Legislation should respect these factors.

> *(5) The protection of human health and the environment requires that due attention be given to controlling risks from the deliberate release into the environment of genetically modified organisms (GMOs).*

This is a highly biased statement. No comparisons of GMOs and the products of other breeding methods (e.g. listed in Annex IB of the Directive) were published to demonstrate that GMOs are the only newly developed varieties that represent risk to human health and/or environment. A large review of feeding trials by EFSA[92] containes no data supporting this assumption in the legislation. The directive implies

91 REGULATION (EC) No 1829/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of September 22, 2003 on genetically modified food and feed. Official Journal of the European Union L 268/1, October 18, 2003.

92 Safety and nutritional assessment of GM plants and derived food and feed. The role of animal feeding trials. Food Chem Toxicol 46 (2008), 1–70.

that breeding method rather than traits is the source of risk and that transgenesis is the only breeding method accompanied by a risk. Since every new variety represents a certain change in the genome, the following points should be applied to all of them.

(8) The precautionary principle has been taken into account in the drafting of this Directive and must be taken into account when implementing it.

Not true; the precautionary principle as defined by the Commission[93] asks for evaluation of the benefit and risk but the benefit of GMOs is never mentioned in the Directive and the risk of agriculture without GMOs is not addressed. In fact the Directive violates the precautionary principle as defined by the Commission.

(9) Respect for ethical principles recognised in a Member State is particularly important. Member States may take into consideration ethical aspects when GMOs are deliberately released or placed on the market as such or in products.

"Ethical aspects" are not defined but they probably condemn human intervention with the natural state. It should be remembered that other breeding techniques (listed in Annex IB of the Directive), including radiation and chemical mutagenesis, protoplast fusion, aneuploidy induction, androgenesis, dihaploid formation, distant crossing, etc., also alter the natural state of the organisms and, in contrast to the GM modification, in an uncontrolled way that is in many cases harmful to the plant. Strict observation of ethical aspects defined in this way would prevent us from growing some traditional crops, for example many of the wheat cultivars, that represent a much greater interference with the original genotype than transgenesis or other modern and controlled methods.

(17) This Directive should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record.

This is a rational statement but it has never been applied by the Commission. For example, the RR soybean (genetically modified) has been planted since 1997, and the amount consumed by humans and animals has reached about a billion tons without any adverse effects. In spite of this, the Commission insists on testing, labelling and monitoring – all expensive measures. More than 10 years of safety record should be sufficient for lifting these requirements. On the other hand, annual reports of IAEA[94] document that new radiation mutants (e.g. halotolerant rice) with unknown genotype changes are introduced into the environment and included in the food chain every year after very short periods of testing. No "long term safety record" is required for these mutants.

93 EU Commission Communication on the Precautionary Principle [COM (2000) 1], February 2, 2000.

94 IAEA: Food and Agriculture. Programme Objective – year 1999, 2000, 2001, etc. Available at: <http://www.iaea.org/>.

(62) A report to be issued every three years by the Commission, taking into account the information provided by Member States, should contain a separate chapter regarding the socioeconomic advantages and disadvantages of each category of GMOs authorised for placing on the market, which will take due account of the interest of farmers and consumers.

No such report evaluating advantages/disadvantages is available.

(63) The regulatory framework for biotechnology should be reviewed so as to identify the feasibility of improving further the consistency and efficiency of that framework. Procedures may need to be adapted so as to optimise efficiency, and all options which might achieve that should be considered.

No such evaluation based on facts has been performed.

6.1.2 Comments on Directive Articles

Article 1

...the objective... to protect human health and the environment when:
carrying out the deliberate release into the environment of genetically modified organisms, — placing on the market genetically modified organisms as or in products within the Community.

This statement implies that GMOs are potentially dangerous whereas organisms modified by other techniques are risk-free to human health and to the environment, irrespectively of their traits. The statement is wrong.

Article 7

Differentiated procedures
1. If sufficient experience has been obtained of releases of certain GMOs in certain ecosystems and the GMOs concerned meet the criteria set out in Annex V, a competent authority may submit to the Commission a reasoned proposal for the application of differentiated procedures to such types of GMOs.

Rational procedure proposed in Article 7 has never been applied. Fundamentalist ideology prevailed over the scientific evidence when RR soya, the industrial potato Amflora, and some other crops were subjected to "differentiated procedure".

Article 9

Consultation of and information to the public 1. Member States shall, without prejudice to the provisions of Articles 7 and 25, consult the public and, where appropriate,

groups on the proposed deliberate release. In doing so, Member States shall lay down arrangements for this consultation, including a reasonable time-period, in order to give the public or groups the opportunity to express an opinion.

The public should be correctly informed about the nature of recombinant DNA technology and ecology but the Directive neglects this crucial step and emphasizes consultation. It is obvious that consultations are useless if the principles are not understood.

Article 20

2. If new information has become available, from the users or other sources, with regard to the risks of the GMO(s) to human health or the environment after the written consent has been given, the notifier shall immediately take the measures necessary to protect human health and the environment, and inform the competent authority thereof. In addition, the notifier shall revise the information and conditions specified in the notification.

It is clear that the Directive does not consider that new information can reveal benefits or considers such type of information unimportant. No provisions for positive information leading to the liberalisation of regulation are anticipated.

Article 24. Information to the public

The article deals only with information about the legislation and the restriction-type measures. No information on the nature of GMOs is provided.

Article 28

Consultation of Scientific Committee(s)

1. In cases where an objection as regards the risks of GMOs to human health or to the environment is raised by a competent authority or the Commission and maintained in accordance with Article 15(1), 17(4), 20(3) or 23, or where the assessment report referred to in Article 14 indicates that the GMO should not be placed on the market, the relevant Scientific Committee(s) shall be consulted by the Commission, on its own initiative or at the request of a Member State, on the objection.

2. The relevant Scientific Committee(s) may also be consulted by the Commission, on its own initiative or at the request of a Member State, on any matter under this Directive that may have an adverse effect on human health and the environment.

Consultation of scientists is obviously requested only when it can demonstrate an adverse effect, implying that GMOs by principle cannot have positive effects whereas conventional agriculture is devoid of the negative effects. EFSA is undoubtedly the most "relevant Scientific Committee" of the EU but its opinions are disregarded.

6.1.3 Comments on the Annexes

ANNEX I B. Exceptions to the Regulation

The following techniques/methods of genetic modification yielding organisms to be excluded from the Regulation are:

> 1. *mutagenesis,*
> 2. *cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.*

This is a scientifically unfounded assumption that the two techniques do not represent any risk to human health and/or environment.

ANNEX II. Principles for the environmental risk assessment

A.) Objective

> *The objective of an e.r.a. is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have. The e.r.a. should be conducted with a view to identifying if there is a need for risk management and if so, the most appropriate methods to be used.*

The objective is clearly biased because it does not consider any GMO benefits and comparison of their risks with the "standard" agriculture.

B). General Principles

> *In accordance with the precautionary principle, the following general principles should be followed when performing the e.r.a.:*
>
> *- identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations;*
>
> *- the e.r.a. should be carried out in a scientifically sound and transparent manner based on available scientific and technical data;*
>
> *- the e.r.a. should be carried out on a case by case basis, meaning that the required information may vary depending on the type of the GMOs concerned, their intended use and the potential receiving environment, taking into account, i.a., GMOs already in the environment;*
>
> *- if new information on the GMO and its effects on human health or the environment becomes available, the e.r.a. may need to be readdressed in order to:*
>
> *- determine whether the risk has changed;*
>
> *- determine whether there is a need for amending the risk management accordingly.*

Some of the General Principles are rational but are not applied in the Directive. It is disturbing that no benefits of GMOs are considered, which contradicts the precautionary principle. Nevertheless, in this chapter a comparison with the risk of "standard" agriculture is indicated for the first time. Indirectly the risk of standard agriculture might be interpreted as a benefit of GMOs. However, particular evaluation of such risk at comparable level to the assessment of risk connected with GMOs is not requested. The possibility of relaxing ("amendment") the regulation is also indirectly touched upon, however it is constantly called "risk management".

In drawing conclusions for the e.r.a. referred to in Articles 4, 6, 7 and 13 the following points should be addressed:

1. Identification of characteristics which may cause adverse effects: Any characteristics of the GMOs linked to the genetic modification that may result in adverse effects on human health or the environment shall be identified. A comparison of the characteristics of the GMO(s) with those of the non-modified organism under corresponding conditions of the release or use, will assist in identifying the particular potential adverse effects arising from the genetic modification. It is important not to discount any potential adverse effect on the basis that it is unlikely to occur.

Only adverse effect, no "identification of characteristics which may cause benefit effect" is the objective of evaluation. More than 80 pieces of information are needed to characterize a GMO and an additional 18 points are required in relation to the interaction of GMOs with the environment.

ANNEX V. Differentiated procedure

CRITERIA FOR THE APPLICATION OF DIFFERENTIATED PROCEDURES (ARTICLE 7)

The criteria referred to in Article 7(1) are set out below.

1. *The taxonomic status and the biology (for example mode of reproduction and pollination, ability to cross with related species, pathogenecity) of the non-modified (recipient) organism shall be well-known.*
2. *There shall be sufficient knowledge about the safety for human health and the environment of the parental, where appropriate, and recipient organisms in the environment of the release.*
3. *Information shall be available on any interaction of particular relevance for the risk assessment, involving the parental, where appropriate, and recipient organism and other organisms in the experimental release ecosystem.*
4. *Information shall be available to demonstrate that any inserted genetic material is well characterised. Information on the construction of any vector systems or sequences of genetic material used with the carrier DNA shall be available. Where a genetic modification involves the deletion of genetic material, the extent of the deletion shall be known. Sufficient information on the genetic modification shall also be available to enable identification of the GMO and its progeny during a release.*

5. *The GMO shall not present additional or increased risks to human health or the environment under the conditions of the experimental release that are not presented by releases of the corresponding parental, where appropriate, and recipient organisms. Any capacity to spread in the environment and invade other unrelated ecosystems and capacity to transfer genetic material to other organisms in the environment shall not result in adverse effects.*

This differentiated procedure has never been applied. See the example of the RR soy beans. On the other hand, information listed under 4) is never known for the radiation mutants. Why should they be excluded from the Directive?

ANNEX VII. Monitoring plan

The objective of a monitoring plan is to:
- confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the e.r.a. are correct, and
- identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the e.r.a.

Only the GMO is the subject of monitoring. No monitoring of "standard" agriculture (e.g., using pesticides or "conventional" herbicides) is requested, although it is well known that no scientific study should be done without valid controls. Data collected without appropriate controls are useless. Objective monitoring must consider both the adverse and the beneficial effects.

6.2 EU REGULATION 1829/2003: FOOD AND FEED

6.2.1 Whereas

(3) In order to protect human and animal health, food and feed consisting of, containing or produced from genetically modified organisms (hereinafter referred to as genetically modified food and feed) should undergo a safety assessment through a Community procedure before being placed on the market within the Community.

All food and feed should be safe and therefore subject to safety assessment. The Regulation opens the door to political and ideological decisions about safety.

(32) It is recognised that, in some cases, scientific risk assessment alone cannot provide all the information on which a risk management decision should be based, and that other legitimate factors relevant to the matter under consideration may be taken into account.

This phrase also invites decisions based on interests other than food and feed safety.

In order to provide a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed, ...

Such a statement implies that the high level of protection of human and animal health is strictly linked to the most restrictive regulation of GMOs. This is unfair to EU citizens who deserve truthful information about the cause and purpose of the restrictive regulations. In-stead, the citizen can read:

Requirements arising from this Regulation should apply in a non-discriminatory manner to products originating in the Community and imported from third countries.

6.2.2 Regulation

Like the Directive 2001/18/EC, the Regulation 1829/2003 is based on the paradigm that transgenesis generates specific kind of risks that are absent in other genotype modifications. The inherent danger of GMOs to human and animal health and welfare is emphasized. Article 1 is cited here as an example, there is no need to analyze the following ones because they all reflect similar biased opinions:

The objective of this Regulation is to provide the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed, whilst ensuring the effective functioning of the internal market;

7. SUMMARY

Regulation of agricultural biotechnology has both immediate and long-lasting socio-economic consequences and affects the sustainability of agroecosystems. Policy-makers are responsible for formulating the regulations while scientists must provide data necessary for prudent decisions.

All human activities bear a certain risk; a zero risk does not exist but relative risk can be estimated when two following conditions are observed:

Risk is a probability of damage; the probability term by definition expresses the uncertainty, i. e., reflects the fact that certain information is not available. The adherence to the "precautionary principle" reflects unwillingness to consider, or incompetence to perform a fair risk evaluation.

Risk assessment must be accompanied by benefit assessment performed under the same conditions and with identical methodology. The ratio benefit/risk is essential for the identification of acceptable risk as crucial information for decision making.

The risks and benefits of GM crops can be assessed only by comparison with conventional non-GM varieties grown with the use of standard procedures, including applications of insecticides, herbicides, etc. Absence of adequate control renders the data obtained for GM crops meaningless.

Agriculture has inevitably converted natural, diversified ecosystems to monoculture-based agroecosystems that are sometimes exploited to the point of irreversible damage. Evaluation of the environmental impact of new technologies is dictated by the need to mitigate this damage for the sake of agriculture sustainability. GM crops should be scrutinized as any other technology in respect to possible effect on the communities of organisms in the ecosystems, in particular on species that are either essential for "ecological services" (control of pests, soil aeriation, humus formation, etc.) or serve as indicators of the maintenance of biodiversity.

New cultivars bring to the ecosystem a new genetic setup; possible transfer of the introduced or modified genes to sexually compatible plants should be examined in all of them.

Care should be paid to discriminate between the impact of plant varieties and that of agriculture as such, i.e. including methods of field management, applications of chemicals, crop selection and rotation, etc.

Impact of new technologies can be either positive or negative; there is no reason to classify some technologies *à priori* as negative and risky. Numerous scientific studies have been performed with GM crops and no adverse effects exceeding those of standard agriculture were found. GM crops were recommended for the organic farmers.[95]

Scientific data are neglected in the regulations of GM crop deployment. The attitude of policy makers to GM crops depends on their personal ideological opinions and is affected by political trade-offs, provisions like taxes and subsidies, economic outputs and

95 Ammann K. (2008) Feature: Integrated farming: Why organic farmers should use transgenic crops. New Biotechnology 25 (2): 101–107; available at <http://www.botanischergarten.ch/New-Biotech/Ammann-Integrated-Farming-Organic-2008.publ.pdf>.

inputs of national agriculture, the level of unemployment, assessment of international trade with agricultural commodities, mood of the electorate, etc.

There are no scientific data showing an exceptional position of GM plants in respect to "classical" breeding techniques. Separate regulative measures for GM crops were possibly justified by the novelty of this technique a decade ago, but have now become obsolete.

The European regulation of GMOs is comparable to that of toxic chemicals, explosives and narcotics; this implies to the general public and many politicians that GMOs present a similar level of danger. The public should correctly be informed about the nature of various breeding methods, as well as about the principles of ecological science. Only properly educated citizens are able to contribute to the discussions concerning safety measures and GM crop deployment.

Scientifically unjustified bans on the deployment of GM crops slows down agricultural output, deprive farmers of the right to chose what they want to grow, reduce EU competitiveness in terms of global trade, and indoctrinates EU citizens with the opinion that new technologies should better be avoided. This is a very dangerous legacy to future generations.

The socio-economic factors affecting GM crop deployment include pressure of various interest groups. All these issues are very volatile and hard to control. Decisions based on these factors should be clearly declared as political, and should not pretend to have a scientific basis.

The deployment of GM crops has spread rapidly outside Europe. Cooperation with developing countries in agricultural research should be expanded with a focus on the risk assessment of newly deployed technologies.

This Declaration summarizes suggestions of Czech scientists that have practical experience with genetic modifications (GM) applicable, or already exploited, in agriculture.

8. A CALL FROM CZECH SCIENTISTS

Scientific evidence and long experience with the cultivation of GM crops have demonstrated their safety to the environment and human health, but EU legislation petrifies unjustified opinions and neglects the current situation. We therefore appeal to the EU and national policy makers to consider the following rules:

- Decisions concerning genetic modifications should not contradict scientific evidence.
- Breeding techniques, including genetic modifications, should primarily be evaluated in respect to the outcome rather than the process itself.
- The precautionary principle should be replaced by serious and robust risk/benefit assessment applied to all innovations in agriculture.
- Risk assessments should always include the benefits of a technology and comparison of parallel technologies with all their components (e.g. GM crop deployment, standard agriculture with pesticides, and organic farming with permitted plant protection measures).
- Economic assessment should also be done by comparison with parallel technologies.
- If Member states are allowed to ban technology permitted elsewhere in the EU, they should also be allowed to use a technology that has not yet been approved by the EU, provided that it does not impinge on the other Member states.

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