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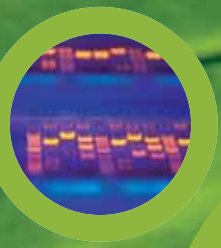

A decade of EU-funded GMO research

(2001 - 2010)

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EUROPEAN COMMISSION

Directorate-General for Research and Innovation
Directorate E — Biotechnologies, Agriculture, Food
Unit E2 — Biotechnologies

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Biotechnologies, Agriculture, Food

2010

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Luxembourg: Publications Office of the European Union, 2010

ISBN 978-92-79-16344-9
doi 10.2777/97784

Printed in Belgium

PRINTED ON ELEMENTAL CHLORINE-FREE BLEACHED PAPER (ECF)

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Introduction

Máire GEOGHEGAN-QUINN
Commissioner for
Research, Innovation and Science
European Commission

Scientific and technological innovation and a fundamental understanding of nature are among the major drivers of progress. The discovery of penicillin by Alexander Fleming in 1928 saved the lives of countless people battling with the number one cause of death in the last century – bacterial infections. Today's advances in the treatment of human disease have been made possible by the discovery of the DNA double-helix structure by Watson and Crick in 1953. And the Green Revolution, a synonym for advances in the agricultural sciences and plant breeding, has saved millions of people from starvation.

The Framework Programmes for research, technological development and demonstration activities are the world's largest international projects for collaborative basic and applied research, and are our means of innovating to tackle the global challenges we face.

These challenges are mainly linked to climate change, food safety and security, limited fossil fuel resources, an ageing population and the fights against disease, poverty and social exclusion. Drawing on the remarkable evolution of biology as a scientific discipline, the concept of the Knowledge-Based Bio-Economy provides many scientific and technical solutions to enhance resource efficiencies while reducing environmental footprints in an economically viable and socially responsible manner.

Biotechnologies could provide us with useful tools in sectors such as agriculture, fisheries, food production and industry. Crop production will have to cope with rapidly increasing demand while ensuring environmental sustainability. Preservation of natural resources and the need to support the livelihoods of farmers and rural populations around the world are major concerns. In order to achieve the best solutions, we must consider all the alternatives for addressing these challenges using independent and scientifically sound methods. These alternatives include genetically modified organisms (GMO) and their potential use.

Global warming and the depletion of fossil fuel resources are already high on the public and political agendas. The development of alternative raw materials for industrial applications such as renewables derived from plants and waste, combined with new industrial techniques that replace thermo-chemical processes by biological ones, have become the focus of our research

activities. The introduction of such advanced processes and materials based on biotechnology and GMOs, has enormous potential not only to enhance quality of life while reducing environmental footprints, but also to improve the competitiveness of European industry.

As with all new technologies, the potential risks and benefits must be identified and quantified. On the basis of the precautionary principle, the EU has developed research programmes and practices to evaluate the risks and benefits to animal or public health and the environment of GMOs. Other international organisations like the OECD follow the same principles, not only to ensure consumer safety, but also to harmonise risk assessment approaches and facilitate the international trade of agricultural commodities and industrial products.

This is the second volume of results studying different aspects of genetically modified organisms. It is part of an initiative of research programmes and activities initiated 25 years ago in response to policymakers' and public concerns regarding the safety of the technology. The reader will note that recent projects dealing with the development of new products and processes based on GMO technology fully integrate safety assessments in their conception, experimentation and development.

I believe that initiatives like this publication align with the principles laid down in the 'Europe 2020' Strategy, where building the Bio-Economy is one of its main targets, aiming to *'... re-focus R&D and innovation policy on the challenges facing our society, such as climate change, energy and resource efficiency, health and demographic change. Every link should be strengthened in the innovation chain, from "blue sky" research to commercialisation.'*

These principles will enable Europe to thrive in an even more competitive and resource-limited global economy, providing education, knowledge, health support and, above all, job opportunities for generations to come.

Foreword

Maive RUTE

Director Biotechnologies, Agriculture and Food

Directorate-General for Research and Innovation

European Commission

The 'Europe 2020' strategy, adopted by the European Council on 17 June 2010, has the mission of giving the European Union a smart, sustainable and inclusive economy, to help it emerge stronger from the financial and economic turmoil of the recent past. The strategy features seven flagship initiatives, one of which is the creation of an 'Innovation Union' with a focus on 'Building the Bio-Economy by 2020'.

The Bio-Economy, as defined by the OECD, refers to economic activities relating to the invention, development, production and use of biological products and processes. Allied with significant advances in the life sciences and biotechnologies, the concept of the Bio-Economy will enhance Europe's competitiveness, increase the efficiency of resource utilisation, reduce the environmental footprint of production processes and products, and stimulate the creation of knowledge-based jobs.

Estimates indicate that the European Bio-Economy is worth EUR 2 trillion annually and accounts for some 22 million employees. The EU already has a very strong research presence in the field, for example in industrial and pharmaceutical biotechnologies, and includes significant know-how on the health-related aspects of the Bio-Economy.

But European researchers and enterprises have to compete at the global level. Countries such as the USA, China, India and Brazil are planning substantial investment in biotechnology, stepping up competition with Europe's leaders. If Europe is to remain at the forefront of research and innovation, clear-cut initiatives are necessary now to support the development of this smart, sustainable and inclusive European economy.

To counterbalance the predicted increase in the world population to up to nine billion people by 2050, and the related implication of climate change, science has to develop technologies that increase yields and productivity in a sustainable way, while lowering the demand for fertilisers and pesticides, and adapting crops to match the effects of changes in the environment.

The main challenges for industry will be to increase resource efficiency, replace the use of finite resources by renewable ones, and develop more eco-efficient products and life cycles.

Biotechnology research can also contribute to the development of new techniques – such as the valorisation of biowaste in biorefineries for the production of biomass and bioenergy, and the development of macro- or micro-algae production plants for the same purpose – while exploiting emerging technologies such as synthetic biology in a safe and acceptable manner. Economic pressure in a globalised world, as well as experience with biotechnology in Europe, should encourage us to pursue the development and application of all available technologies without prejudice, while respecting fundamental safety and ethical principles.

This publication reviews the last ten years of research projects launched under the Framework Programmes for research, technological development and demonstration activities, focusing on safety aspects of GMOs but also taking account of developments in the field over time. More than EUR 200 million has been invested through the Framework Programmes since the year 2001, developing agricultural management techniques for co-existence, tools for detection in and analysis of food and feed, and methods for risk assessment of GMOs, thus responding to the need of farmers, consumers, industry and policymakers.

Sound policy, while needing to take account of a wide range of views, must be based on sound science. Accordingly, we will continue to support science on Biotechnologies, including GMOs, in order to ensure that evidence is available for a constructive debate in our societies.

A decade of EU-funded GMO research (2001 - 2010)

A decade of EU-funded GMO research (2001 - 2010)

Ioannis ECONOMIDIS,
Danuta CICHOCKA,
Jens HÖGEL

In 2001 the Directorate-General for Research and Innovation published the first overview of the accumulated results of 'EC Sponsored Research on Safety of Genetically Modified Organisms (GMOs)[1]'. This publication included work supported over the preceding 15 years from the first to the fifth Framework Programmes for research, technological development and demonstration activities (FP). It featured 81 projects, involving over 400 laboratories, and the results covered a range of subjects: horizontal gene transfer, environmental impact of transgenic plants, plant-microbe interactions, transgenic fish, recombinant vaccines, food safety, and other issues. The 2001 publication attracted the attention not only of the scientific community but also of regulators, public services, non-governmental organisations and other stakeholders.

The European Commission now provides a sequel to this publication, presenting the outcomes and conclusions of studies supported in subsequent Framework Programmes. In addition, the development of the Bio-Economy concept has created significant interest in the follow-up and development of the GMO debate, not only addressing public concerns about the application of genetic engineering to the production of agricultural and industrial commodities, but also offering responses to challenges for which there is currently no solution available.

This new publication presents the results of 50 projects, involving more than 400 research groups and representing European research grants of some EUR 200 million. This figure brings the total Commission funding of research on GMO safety to more than EUR 300 million since its inception in 1982 in the Biomolecular Engineering programme. In addition, many Member States have also launched their own national research initiatives, complementing these coordinated European research efforts.

[1] EC-sponsored Research on Safety of Genetically Modified Organisms, edited by Charles Kessler and Ioannis Economidis, European Communities, 2001, EUR 19884. See also http://ec.europa.eu/research/quality-of-life/gmo/

The 50 research projects can be grouped into the following principal areas:

- Environmental Impacts of GMO;
- GMO and Food Safety;
- GMOs for biomaterials and biofuels – Emerging technologies;
- Risk assessment and management – Policy support and communication.

It is evident from this grouping that many of the research projects have been launched to address not only the scientific unknowns but, more importantly, public concerns about the potential environmental impact of GMOs, about food safety, the co-existence of GM and non-GM crops, and risk assessment strategies. As with the previous publication, this book provides background information and descriptions of the results of the projects for scientists and regulatory communities, as well as for the public. The results and conclusions of these projects increase our accumulated knowledge, enabling the Commission and policymakers in general to contribute to the international debate, and to provide scientific support to regulatory frameworks and initiatives. The main conclusion to be drawn from the efforts of more than 130 research projects, covering a period of more than 25 years of research, and involving more than 500 independent research groups, is that biotechnology, and in particular GMOs, are not *per se* more risky than e.g. conventional plant breeding technologies. Another very important conclusion is that today's biotechnological research and applications are much more diverse than they were 25 years ago, which is also reflected by the current 7th EU Framework Programme.

Due to the its large diversity, biotechnology became the key component of the Knowledge-Based Bio-Economy, a concept applicable in a range of fields extending from primary production to industrial and pharmaceutical applications, and involving emerging technologies such as synthetic biology. Modern biological know-how is used to address major societal challenges, including food and feed security and safety, the development of renewable resource platforms for the production of biomaterials and bio-energy, and pharmaceuticals, while improving environmental sustainability. It is predicted that, whereas the past century was transformed with the commercialisation of personal computers and the development of the Internet, the 21st century will be revolutionised by our growing understanding of the functioning and interaction of biological systems, whether at the molecular or at the ecosystem level.

Biotechnology is not a purely academic exercise: its findings and developments will lead to applications and products essential to society. However, only a structured dialogue with policymakers, stakeholders and the public, based on sound science and empirical evidence, will clear the way for a balanced assessment of the benefits and risks of biotechnology and GMOs within the framework of the bio-economy.

The research described in this volume focuses on possible risks associated with the use of GMOs in different biotechnological applications. Based on a growing body of evidence that biotechnology is not more risky than alternative technologies, today's research projects funded under FP7 are now more carefully integrated and look at the potential technological benefits as well as the risks. A number of stakeholders, such as the European Group on Ethics, have greatly facilitated this approach by providing general reflections and recommendations, for example on the ethics of synthetic biology.

Research efforts in this and other fields of biotechnology will continue, taking due account of environmental, social and ethical concerns while, at the same time, searching for solutions to current and future challenges.

Chapter 1

Environmental Impacts of GMO

Introduction

Marc VAN MONTAGU

Chairman, Institute of Plant Biotechnology for Developing Countries (IPBO)

Ghent University, Belgium

http://www.ipbo.UGent.be
http://www.psb.Ugent.be
http://www.efb-central.org
http://www.pubresreg.org

Undeniably GM technology is an important tool in the fight against global poverty and food insecurity. Farmers all over the world face the challenge of doubling food production to meet the needs of a population that is expected to reach nine billion by mid-century – and all this while maintaining soil and water quality and conserving biodiversity.

The challenge is particularly daunting as it has to be accomplished with decreasing amounts of agricultural land and the unpredictable effects of climate change: mitigation and crop adaptation strategies to prepare today's agriculture for climate change are a pressing issue. Our evolving environment requires the prompt and widespread adoption of more efficient and sustainable agricultural practices to improve food security and, at the same time, reduce the negative effects of intensive agriculture.

The task of enhancing productivity calls for greater innovation, not only in the dissemination of know-how and the development of infrastructure, but also in generating new crop varieties better adapted to specific local environments. Yet the possibilities offered by biotechnology are limitless. GM crops not only have the potential to ensure sufficient availability of food, they can also help domesticate many fast-growing high-biomass crops.

The production of renewable raw materials for industrial use also offers an alternative to traditional chemical manufacturing techniques and has the potential to play an important role in rural income growth and poverty alleviation in developing countries. Nutritionally enhanced GM crops can help improve consumer health and bring environmental benefits, for example by decreasing the need for pesticides and reducing soil erosion.

In spite of the potential benefits, the development and use of GM crops has faced significant opposition, prompted by fears of adverse social implications and health and environmental risks. The latter, which have been debated at length, focus mainly on the concept of modern biotechnology and the genetic engineering techniques used to develop these crops. The fact that humans can 'engineer' a gene from a species of one kingdom to produce a species of another has fuelled imaginations and frightened the public.

The dispersal into the environment of 'foreign genes' (for example the ones coding for antibiotic resistance) through horizontal gene transfer and vertical gene flow – by seed dispersal, pollen flow or simply vegetative reproduction – is a major concern. The potential adverse effect on non-target organisms, e.g. the monarch butterfly in the case of *Bt*-resistant engineered crops, is also frequently mentioned by ecologists.

But can a GM crop become a 'superweed', devastating our habitat in a glimpse? And do we disturb the 'natural biodiversity balance' more when we grow GM crops than when we stay with traditional crops and intensive monocultures where large quantities of chemicals are used? Does the involuntary and irreversible spread of genes really represent such a danger and justify such an alarming scenario?

I admit that, as plant molecular geneticists, we have been reluctant to invest time, finances and energy in evaluating the environmental implications of GM crops. For us, our planet is one large natural genetic pool where all living organisms continuously activate and deactivate genomes in response to perceived environmental stresses. The adaptation, survival and evolution of plants depend on their ability to alter genomes through transposition of the movable elements, accumulation of deletions, insertions, gene amplifications and point mutations.

Genomic studies of the last decade have demonstrated that a genome is not a static entity but a dynamic structure continuously refining its gene pool. So, for a scientist in genetics, the act of splicing to generate a transgenic organism is a modest step when compared to the genomic changes induced by all the 'crosses' and breeding events used in agriculture and husbandry. The molecular biology tools simply add a new precision, speed and reach to this indispensable process of species domestication.

So it was a surprise for many scientists to discover that public opinion did not 'buy into' this line of thought. Some European interest groups even opposed the idea of GM crops with a religious zeal. The Precautionary Principle – which some interpret as saying that, if a course of action carries even a remote chance of irreparable damage, then one should not pursue it, no matter how great the benefits may be – gave Europeans a firm philosophical basis for saying no to GMOs. Political leaders and public servants in the Member States and the EU institutions were ill-prepared for this emotional uproar.

Meeting the challenge to 'prove that GM crops are safe!' is not so easy. It looks like a scientific issue, but it isn't. Science can certify the existence of danger, but not its absence. Moreover scientists will continue to question any negative results that surface, and there will certainly be reward and recognition for the person who finds proof of harm. Expert contention that a 100 % GM variety approved for commercialisation is neither more nor less of a health or environmental problem than its parent crop will not answer the question.

The world of science failed to realise that it was not enough just to say that intensive agriculture causes environmental disruption, independent of whether the crop is GM or not, and that we can in any case develop novel GM varieties that are more environmentally friendly. Society needs step-by-step evidence of studies that support such statements.

Now, after 25 years of field trials without evidence of harm, fears continue to trigger the Precautionary Principle. But Europeans need to abandon this knowingly one-sided stance and strike a balance between the advantages and disadvantages of the technology on the basis of scientifically sound risk assessment analysis.

It is precisely for this reason that I congratulate the Directorate-General for Research & Innovation on initiating, supporting and guiding the research featured in this publication. To identify and motivate the research groups responsible for these results was no mean task; it was essential to ensure that only the best scientific methodology was applied, drawing on molecular research undertaken in close collaboration with experienced field ecologists.

These research projects addressed key aspects of crop improvement, such as resistance to pathogens – from fungi (EURICE) and viruses (TRANSVIR) to nematodes (NONEMA) – nitrogen use efficiency (SUSTAIN), and the use of transgenic microorganisms as biofertilisers (ECOSAFE) and as biosensors for monitoring bioremediation (RHIZOREMEDIATION).

Besides the proof-of-concept innovations in biotechnology, scientists used multidisciplinary approaches to understand the nature and impact of GMOs on the environment. They assessed a wide range of plant crops as well as the different aspects of Environmental Risk Assessment (ERA), from gene flow in a centre of crop diversity (POTATO TRAITS FOR AN) to horizontal gene transfer (CIMES) and effects on non-target organisms (BT-BioNoTa) and soil ecology (ECOGEN, POTATOCONTROL).

Several projects focused on measures to avoid unwanted environmental impact, and significant results were obtained on a new selection system that precludes the use of antibiotic-resistant genes (ECOTUB), on biological containment (CONFLOW) and on environmentally induced epigenetic changes (AENEAS). The coexistence of different types of crops has also been investigated, with recommendations on more cost-efficient coexistence measures (SIGMEA).

The quality of the results shows that the initiative of the Directorate-General for Research & Innovation has met its objectives. The deliverables obtained, including some hundred peer-reviewed scientific publications, will be invaluable to researchers in public institutions as well as to experts in risk assessment and risk management, not only in Europe but elsewhere, especially the less developed countries. Compiling and submitting regulatory dossiers would place an unnecessary burden on both the public sector and start-up companies and would, ironically, favour multinational corporations and hinder the advancement of science and technology.

Currently, studies like these on the environmental impact of GMOs are the only way to decrease the regulatory burden, restore confidence in science and technology, and develop practical applications. Only science, using robust data, can disarm the detractors of this powerful and invaluable technology, demonstrating that GM crops are no more harmful for the environment than any other crop. On the contrary, there are clear ecological benefits when viewed within the framework of the role of agricultural systems in maintaining biodiversity.

The current focus on assessing the environmental risks of GMOs in isolation from other agricultural practices defies logic. Only balanced risk-benefit analyses and pro-active strategies for risk mitigation, if required, can lead to constructive decision-making. The research described in this publication represents invaluable input for the regulatory framework and for coexistence rules. I am convinced that the objective data offered here will help reduce the costs of Environmental Risk Assessment in future GM crop and microorganism release applications, as well as identify the priorities for further studies.

European Rice: Transgenes for crop protection against fungal diseases

Acronym
EURICE

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK5-CT1999-01484

Period
March 2000 – February 2003

Coordinator
Elisabetta LUPOTTO
CRA – Agricultural Research Council
Rice Research Unit
s.s.11 to Torino km 2.5
I-13100 Vercelli
Italy
elisabetta.lupotto@entecra.it

Background and objectives

Rice blast is a major source of crop damage in Europe, caused by the pathogenic fungus *Magnaporthe grisea (M.oryzae)*. It is a highly specialised and extremely virulent fungus on rice, attacking the plant in the vegetative (leaf blast) as well as in the reproductive (neck or panicle blast) stages. In Europe, rice varieties are generally developed taking advantage of *O.sativa* temperate *japonica* ssp., and they are in general sensitive to moderately resistant to blast. Moreover, *M.oryzae* is rapidly evolving in new pathotypes and resistant rice varieties show a rapid breakdown in a few years. Chemical protection and genetic resistances in rice are the most effective tools available to combat the pathogen.

The constitutive expression of antifungal genes in transgenic plants has also proved to be a useful strategy for the production of disease-resistant plants. The outcome varies, but resistance obtained with plant antifungal genes has not supported the realisation of new disease-resistant varieties. The use of genes of non-plant origin, however, results in higher levels of resistance and/or broad-spectrum protection, and constitutive promoters to drive the expression of an antifungal gene may be suitable for proof-of-concept experiments to assess the effectiveness of transgene expression.

However, this approach presents a number of potential drawbacks if used in genetically improved crops. In addition to pathogen-inducibility, the promoter used to direct transgene expression must not be active in edible organs. Instead, the pathogen-controlled production of antifungal compound at the site where and only when the transgene product is needed represents a more desirable strategy for protection against pathogens.

The EURICE Project proposed using a transgenic approach in the control of rice blast to improve and evaluate genes acting as anti-fungal tools during fungal attack, with the aim of reducing chemical use for crop protection. The approach was identified either as a tool for advanced constitution of novel varieties, or as a specific and targeted measure to protect qualitatively élite existing varieties that are difficult to grow without heavy chemical treatment. The rice varieties chosen for this study were representative of the main European rice producers: Italy, Spain, Greece and France.

The approach had the following general objectives:

- to identify and use effective anti-fungal genes of various origins;
- to only have them expressed in appropriate conditions under inducible promoters;
- to validate their effectiveness against *M.oryzae*;
- to make use of the GM plants to monitor gene flow and distribution in the environment.

Overall, the project aimed to identify a valuable and safe GM strategy in terms of crop protection for rice in Europe.

Approach and methodology

Genes expressed upon fungal attack, and previously characterised and cloned from diverse organisms, were chosen as the potential source of resistance. The selected genes were: the *afp* gene, isolated from the fungus *Aspergillus giganteus*, the insect antifungal gene CecA encoding for the antimicrobial protein Cecropin A, and a plant gene, the maize *b32* gene encoding for a RIP (Ribosome Inactivating Protein), an endosperm albumin commonly present in the maize seed.

Transient assays were set up to test the effectiveness of inducible promoters in *gus*-expression cassettes, to identify the most suitable ones. The three genes were first inserted into expression vectors in a constitutive manner to monitor their expression *in planta*. For this purpose, the *CaMV 35S* promoter and the *Ubiquitin-1* promoter were used; their presence was verified in the green tissues of all transgenic rice plants.

The following step was the construction of expression vectors in which the most effective antifungal genes – the *afp* and the *CecA* genes – were placed under the control of inducible promoters derived from barley and maize genes. Transgenic plants were subsequently regenerated, characterised at the molecular level, and brought to homozygosity in three generations of selfing. Homozygous transgenic lines were tested with inoculations with *M.grisea (M.oryzae)* in controlled conditions and in detached leaf assays. Parental rice genotypes were also challenged in the field to establish the best conditions for final field tests. Transgenic rice plants, also carrying the herbicide-resistance gene *bar*, allowing an easy-to-handle *in vitro* selection, were used for field experiments to control gene flow into the environment. The experiments were conducted in Spain and in Italy, in accordance with current legislation.

Partners

David B. COLLINGE
Royal Veterinary and Agricultural Research University (DK)

Michael R. DAVEY
University of Nottingham (UK)

Didier THARREAU
Centre de Coopération Internationale en Recherche Agronomique pour le Développement (FR)

Ulrich SCHAFFRATH
Rheinisch-Westfälische Technische Hochschule Aachen (DE)

Dimitrios NTANOS
NAGREF – Cereal Institute (EL)

Blanca SAN SEGUNDO
Centre for Research in Agricultural Genomics (ES)

Joaquima MESSEGUER
Centre for Research in Agricultural Genomics (ES)

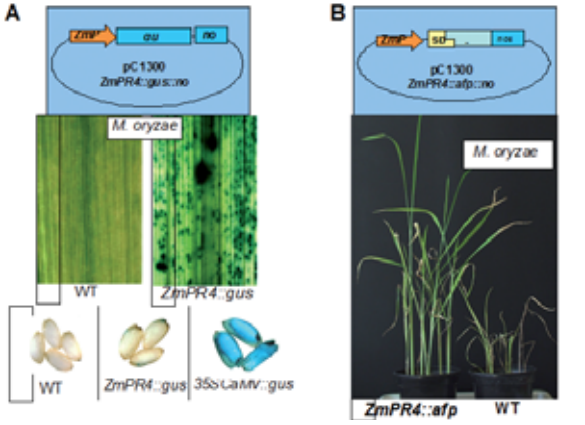
Corrado FOGHER
Plantechno (IT)

Main findings and outcome

Rice protection was verified against fungal diseases caused by *Magnaporthe grisea*, using synthetic codon-optimised antifungal genes expressed at high levels in the plant: the antifungal protein (*afp*) gene from the mould *Aspergillus giganteus* and the *Cecropin A (CecA)* gene from the giant silk moth *Hyalophora cecropia* were the most effective, whereas the *b32* maize gene was active to a lesser extent. The possibility of modulating the defence gene expression by means of inducible promoters, namely maize and barley inducible promoter sequences, was also realised. The maize *ZmPR4* promoter was activated at the sites of pathogen invasion in the rice leaf, whereas this promoter was not active in the endosperm tissue of the mature rice seed. Rice plants expressing the *afp* gene under the control of the maize *ZmPR4* promoter displayed resistance to the blast fungus *M. grisea*. Molecular, biochemical and histological tools for rapid monitoring and evaluation of resistance in rice and GM rice against pathogens (bioassays) were also created. Finally, the risk of gene flow between GM rice and weedy rice in field conditions was assessed, and experimental conditions for an environmentally safe experimental design were developed.

Conclusions

The project reached the planned objectives, and the results obtained and deliverables produced may provide input for research aimed at improving GM plant knowledge, as the basis for regulations and for the development of national co-existence plans in the Member States. Know-how relevant to the future exploitation of defence genes in European GM breeding programmes was also acquired. The effectiveness of defence genes and the availability of a targeted response driven by inducible promoters represent the main outcomes of the project.



A. The maize ZmPR4 promoter is strongly induced in response to fungal infection in rice leaves and is not active in the seed endosperm. Upper panel: construct used for rice transformation. Middle panel: histochemical localisation of ß-glucoronidase (GUS) activity in leaves of wild-type (WT) and ZmPR4::gus rice plants after inoculation with the rice blast fungus Magnaporthe oryzae spores. Lower panel: GUS expression in seed tissues of WT, ZmPR4::gus and 35SCaMV rice plants. Seeds were longitudinally cut before assaying GUS activity.

B. Phenotype of transgenic rice plants expressing the ZmPR4:: afp construct. The construct used for rice transformation is shown in the upper panel. A codon-optimized apf gene was chemically synthesised. The signal sequence from a secreted plant protein, the tobacco AP24 protein, was fused to the mature AFP protein to obtain a chimeric afp gene.

Major Publications

Coca M., Bortolotti C., Rufat M., Penas G., Eritja R., Tharreau D., Martinez del Pozo A., Messeguer J., San Segundo B. (2004). Transgenic rice plants ex pressing the antifungal AFP protein from *Aspergillus giganteus* show enhanced resistance to the rice blast fungus *Magnaporthe grisea*. *Plant Molec. Biol.* 54:245-259.

Zabbai F., Jarosch B., Schaffrath U. (2004). Over-expression of chloroplastic lipoxygenases RC11 causes PR1 transcript accumulation in transiently transformed rice. *Physiol. and Molec. Plant Pathol.* 64:37-43.

Moreno A.B., Penas G., Rufat M., Bravo J.M., Estopà M., Messeguer J., San Segundo B. (2005). Pathogen-induced production of the antifungal AFP protein from *Aspergillus giganteus* confers resistance to the blast fungus *Magnaporthe grisea* in transgenic rice. *Mol. Plant Microbe Inter.* 18:960-972.

Coca M., Penas G., Gòmez J., Campo S., Bortolotti C., Messeguer J., San Segundo B. (2006). Enhanced resistance to the blast fungus *Magnaporthe grisea* conferred by expression of a Cecropin A gene in transgenic rice. *Planta* 223:392-406.

Katsantonis D., Koutroubas S.D., Ntanos D.A., Lupotto E. (2007). A comparison of three experimental designs for the field assessment of resistance to rice blast disease *(Pyricularia oryzae). J. Phytopathol.* 155:204-210.

Acronym
NONEMA

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK5-CT-1999-1501 and
QLK5-2001-02855
(NAS extension)

Period
February 2000 – January 2004

Coordinator
Hans HELDER
Nematology Laboratory
University of Wageningen
PO Box 8123
10, Binnenhaven 10
NL-6701 ES Wageningen
The Netherlands
Hans.Helder@wur.nl

Making plants resistant to plant parasitic nematodes: No access – No feeding

Background and objectives

To guarantee a constant supply of affordable, high-quality food to European citizens, agriculture on a large semi-industrial scale is essential. One of the major unwanted side-effects of production on this scale is its attractiveness or – seen from a different perspective, its vulnerability – to plant pathogens. Most vulnerable are exotic crops, plant species that did not evolve on the European continent. Examples are potato and tomato that originate from South America; sugar beet, a plant that originates from the Mediterranean but is cultivated throughout Europe; and maize, a plant species imported from Central America. In short, large-scale agricultural production is impossible without the proper management of biotic factors that threaten our cops such as fungi, oomycetes, nematodes and insects.

Plant parasitic nematodes constitute a rather problematic category of pathogens as they live mainly in soil among their relatives, the non-plant parasitic nematodes that constitute an important, if not essential, part of the soil food web. Many crops including potato, sugarbeet and a range of vegetables are attacked by plant parasitic nematodes, and these small soil-borne organisms cause substantial primary damage, *viz.* lower yields and lower product quality.

Chemical control using nematicides is a common practice to protect crops. Unfortunately, nematicides *sensu stricto* do not exist, and fumigants used to control nematodes are in fact biocides. Hence, chemical control of nematodes is accompanied by substantial collateral damage, *viz.* killing soil life. In the event that acetyl cholinesterase inhibitors are applied, this damage is restricted to organisms with a nerve system using acetyl choline as a neurotransmitter. In the case of fumigants, there is no restriction at all; all organisms that come into contact with these chemicals are killed. In short, the damage caused by attempts to control nematodes is at least as serious as the primary damage caused by these pathogens.
Restricting the use of nematicides would of course reduce the secondary damage caused by them. However root knot and cyst nematodes in particular are highly successful parasites, and a viable and competitive agriculture requires the control of their populations.

One of the alternatives could be the use of natural host plant resistance genes; several relevant resistance (R) genes have been identified over the last decades, and a few were cloned recently. However transferring resistance from one plant species to the other is far from straightforward because this represents only one initial component from an extensive signal transduction pathway, in most instances resulting in a local hypersensitive response.

The objective of NONEMA, which builds on previous EC-BIOTECH and EC-FAIR projects, has been to identify crucial elements in the compatible interaction between plant parasitic nematodes and plants, and to engineer broad and durable resistance against root knot and cyst nematodes. By blocking multiple specific elements in the interaction between the plant and these nematodes, robust and durable host plant resistance will be available as a deliverable. Because resistance will be based on disrupting basic elements in plant-nematode interactions, NONEMA results will also provide means to design resistance against other plant parasitic nematodes.

Approach and methodology

The 'raison d'être' of the NONEMA project was the fact that chemical soil sterilisation and the use of other unselective pesticides to control plant parasitic nematodes are still a common practice in many European countries, with no realistic alternatives available. Durable host plant resistance will be designed by blocking a number of steps in the life cycle of root knot *(Meloidogyne spp)* and cyst nematodes *(Globodera and Heterodera spp)*. Recent experience with biotechnological approaches has shown that resistance to organisms such as nematodes and fungi is difficult to obtain using a single resistance factor. In many cases the interaction is complex and the pathogen seems to have unexpected escape routes. In this project, resistance will be ensured by preventing plant penetration ('**No access**') as well as feeding ('**No feeding**'). For each of these stages in the life cycle, multiple targets had to be identified and evaluated on their effectiveness in designing durable resistance.

No access. Being endoparasites, young juveniles from both cyst and root knot nematodes have to enter the plant root and migrate to the spot where they form a feeding site. Juveniles are equipped with a protrusible hollow needle – a stylet – that is used to puncture cell walls. In addition, juveniles secrete cell-wall-degrading enzymes such as ß-1,4- endoglucanases. Consequently, knocking out these enzymes results in an incapability to penetrate the root of a potential host.

Partners

John WHITE
Durham University (UK)

Rudy DE KEYSER
Flanders Interuniversity Institut for Biotechnology vzw (BE)

Wlodziemierz KLUCINSKY
Warsaw Agricultural University (PL)

Jean-Michel RABASSE
Institut Nationale de la Recherche Agronomique (FR)

Karin-Barbara SPINDLER
Christian-Albrechts Universität zu Kiel (DE)

Herman VAN MELLAERT
Cropdesign NV (NL)

John HILLMAN
Scottish Crop Research Institute (UK)

The first cellulases from a nematode (and even from an animal) were identified and characterised by Smant *et al.* (1998) in potato and soybean cyst nematodes. The discovery of these highly unexpected endogenous cell-wall-degrading enzymes constituted a starting point for surveys among many other plant parasitic nematodes. All major plant parasitic nematodes appeared to harbour a spectrum of genes encoded for cell-wall-degrading and cell wall-loosening enzymes and proteins. Following penetration of the rhizodermis, root knot nematodes migrate intercellularly whereas cyst nematodes migrate intracellularly to the vascular cylinder. At this stage the plant will start its defence mechanism by producing active oxygen species (AOS) in an attempt to kill the invading organism. Nematodes secrete protective scavenging enzymes (peroxidases, superoxide dismutase) to inactivate plant-produced AOS. Migration of the nematode stops at the moment a cell is encountered that is suitable as a starting point for feeding site formation.

No feeding. Nematode development and reproduction depend on the successful formation of a feeding site. Degeneration of this exclusive food source is lethal to the nematode. Reactivation of the cell cycle in the initial feeding cell is an integral part of feeding cell induction. Root knot nematodes rely on the formation of 2-12 giant cells, each containing 30-60 nuclei. This is the result of nematode-induced acytokinetic mitosis. Using cell cycle inhibitors, it was shown that, for both root knot and cyst nematodes, cell cycle reactivation is not just a facultative feature of feeding cell formation but rather an essential element in this process (de Almeida Engler *et al.* 1999). Resistance based on the ' No feeding I 'concept will be based on a properly targeted expression of cell cycle inhibitors. Cyst nematodes use (or abuse) plant enzymes for feeding cell induction by a highly co-ordinated, local breakdown of cell wall. The result is a syncytium, a conglomerate of fused protoplasts.

Another major goal of NONEMA was the identification of plant cell–wall-degrading enzymes and so called expansins (enzymes), involved in nematode-induced cell wall disassembly. Once it is known which enzymes are employed for syncytium proliferation, resistance will be obtained by inhibition at the RNA or protein level.

Targeting. In addition to target identification, NONEMA aimed to identify promoters that alone or – more likely, in combination – would lead to a very local and targeted expression of anti-nematode genes.

Main findings and outcome

A major research effort was invested in the identification and the functional characterisation of pathogenicity factors from cyst and root knot nematodes. Plant parasitic nematodes employ cell-wall-degrading enzymes to get access to their host plant. After the end of the NONEMA project, one of the partners, INRA Sophia-Antipolis of France, initiated a genome sequencing project for the Southern root knot nematode *Meloidogyne incognita*. This major step towards the understanding of the subtle interaction between root knot nematodes and their hosts revealed the full spectrum of cell-wall-degrading and modifying proteins (# = 81) used by the root knot nematode to exploit its hosts (Table 1).

Plant cell wall component	Nematode	*M. incognita* (plant parasitic)	*C. elegans* (bacterivorous)
Cellulose	GH5 (cel)	21	0
Xylanase	GH5 (xyl)	6	0
Arabinanase	GH43	2	0
Pectinase	GH28	2	0
Pectate lyase	PL3	30	0
Expansin	EXPN	20	0

Table 1.

Overview of cell-wall-degrading enzyme-encoding genes found in the Southern root knot nematode species Meloidogyne incognita as compared to a bacterivorous nematode, Caenorhabditis elegans (based on Abad et al (2008) Nature Biotechnology 26, 909 – 915).

Moreover, expression patterns of a range of cell cycle genes in and around nematode-induced feeding structures were characterised, and plant ß-1,4-endoglucanases and expansins that are recruited by cyst nematodes during syncytium proliferation were identified. In the work package on targeting, deletions studies were done on promoters that had been identified in a preceding EC project (ARENA; BIO4-CT96-0318). This resulted in the identification of nematode-responsive promoter fragments that can be used to drive the expression of a protein or a dsRNA construct that could inhibit nematode invasion or the establishment of a feeding site.

During the project NONEMA research efforts have been gradually shifting from the identification of pathogenicity factors from cyst and root knot nematodes towards the determination of their biological role and the assessment of their relative importance. RNA interference, the degradation of specific mRNAs by exposure to homologous double-stranded RNA, is potentially a useful tool to knock out individual pathogenicity factors in nematodes.

Encouraging progress was made in the development of a protocol to degrade mRNAs that are (mainly) residing in the oesophageal glands of the infective nematodes. For the generation of nematode resistant plant, post-transcriptional gene silencing (PTGS) seems to be a promising time-and-labour-efficient approach. The project partners started PTGS-based plant transformation targeting either the pathogenicity factor from the nematode or plant genes recruited by the invading nematode. In parallel, a number of *Arabidopsis* promoters were identified that show similar expression patterns in tomato.

Major Publications

Abad *et al.* (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology* 26:909 – 915 (NONEMA Partners P2, P1, P2, P4).

Gheysen G. and Vanholme B. RNAi from plants to nematodes. *Trends in Biotechnology* 25(3):89-92 (NONEMA Partner P2).

Qin L., Kudla U., Roze EHA., Goverse A., Popeijus H., Nieuwland J., Overmars H., Jones JT., Schots A., Smant G., Bakker J., Helder J. (2004). Plant degradation: A nematode expansin acting on plants. *Nature* 427:30 (NONEMA Partners P1 and P4).

Finally the proof of concept – that knocking out specific pathogenicity factors results in host plant resistance – was delivered. Potato was transformed with so-called hair-pin constructs encoding dsRNA against two pathogenicity factors produced by infective juveniles of the potato cyst nematode *Globodera rostochiensis*. In both cases, transformed plants looked normal and several lines were selected that are *de facto* resistant to potato cyst nematodes. Host plant resistance was also created by silencing specific plant genes, *viz*. genes that are actively recruited by the invading nematode. In two different approaches the silencing of cell cycle genes and plant cell-wall-degrading enzymes (CWDE) resulted in a significant level of resistance; in the case of the cell cycle genes, a reduction of about 70 %, in the case of the CWDE, the developing females did not contain viable eggs. Hence, NONEMA research efforts identified specific nematode and plant targets that were shown to be effective tools for creating host plant resistance with limited or no undesired effect on the condition of the host plant.

Conclusions

Essentially, there are two targets for genetically engineered -mediated endoparasitic nematode control: the nematode or its feeding site. The anti-nematode strategy aims to interfere with its physiology, development or behaviour in order to block invasion of the plant root, migration, feeding site formation or feeding. Up till now, a range of anti-nematode components had been tested including exotoxins of *Bacillus thuringiensis*, lectins and peptides with acetylcholinesterase-inhibiting properties.

In the field of plant engineering for nematode resistance, the use of protease inhibitors has received most attention. Serine and cysteine protease inhibitors (and derivates therefrom) produced in various plants had been shown to interfere with the digestive proteinases of plant parasitic nematodes. This rather generic anti-feeding approach raised concerns about its environmental safety. Cowgill *et al.* (2002) showed that the risk of cystatins to non-target invertebrates depends on the level of expression. Although recent field experiments in the central Andes with transgenic potato did not detect harm to non-target organisms, gene flow occurred to wild relatives of potato.

Within this research we have identified proteins expressed in the dorsal esophageal glands of cyst and root knot nematodes. These glands are crucial for feeding site induction in the plant root. We have shown that double-stranded RNA-based inhibition of the biosynthesis of some of these proteins can lead to *de facto* host plant resistance (Fig 1.).

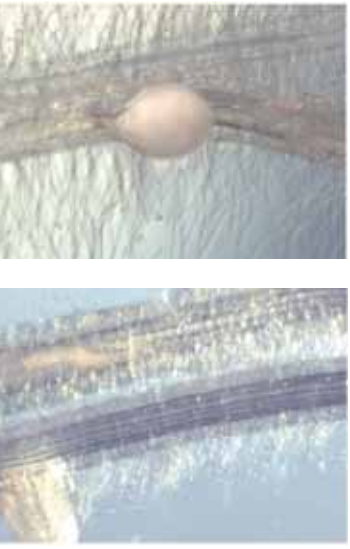
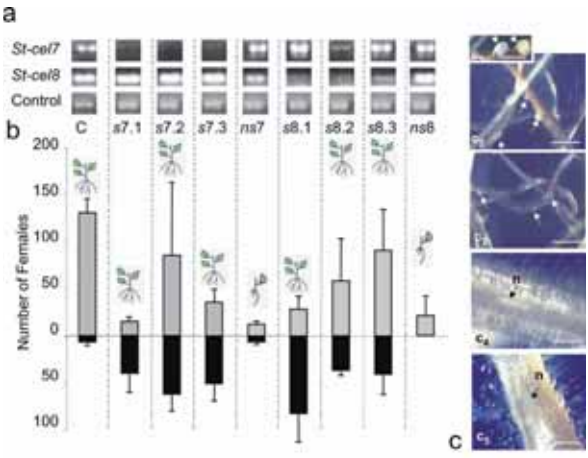


Fig 1.

Effect of dsRNA mediated knock down of a nematode pathogenicity factor delivered via its host plant. Potato plants transgenic for dgl-1 dsRNA 30 dpi (top) compared to empty vector controls (bottom).

Alternatively, feeding cell development can be obstructed by the expression of cytotoxins or by preventing the recruitment of plant genes involved in feeding site induction. For growth and development, sedentary endoparasitic nematodes fully depend on a feeding site and, in the case of cyst nematodes, poor syncytium development will result in a strongly male-biased population and in *de facto* plant resistance. Feeding cell induction and proliferation is accomplished by a dramatic local change in plant gene expression. A substantial proportion of these so-called susceptibility genes is essential for the host plant (*e.g.* genes involved in the reactivation of the cell cycle) and manipulation of such genes will predictably give rise to undesirable off-target effects. However, manipulation of individual members of gene families will in some cases have minor or no effects to plant fitness. Here, we have demonstrated the loss-of-susceptibility of potato plants to potato cyst nematodes, due to the manipulation of two EGases that are apparently essential for syncytium induction, in the absence of an obvious aberrant plant phenotype (Fig 2.).

Hence, it is shown that detailed knowledge about the molecular interaction between a plant and a pathogen can lead to the identification of its Achilles' heel. Double-stranded RNA-based silencing of essential plant or nematode genes can have a dramatic impact on nematode development. This approach illustrates that pathogen control can be obtained without the use of exotic toxic components. We anticipate that our interaction-specific approach could facilitate the public acceptance of genetic engineering-based solutions for pest and pathogen problems.



Major Publications

Karczmarek A., Fudali S., Lichocka M., Sobczak M., Kurek W., Janakowski S., Roosien J., Golinowski W., Bakker J., Goverse A. and Helder J. (2008). Expression of Two Functionally Distinct Plant Endo-2-beta-1,4-Glucanases is Essential for the Compatible Interaction between Potato Cyst Nematode and Its Hosts. *Mol. Plant-Microbe Interact.* 21(6):791-798. (NONEMA Partners P1 and P7).

Wieczorek K., Hofmann Julia, Blochl Andreas, Szakasits Dagmar, Bohlmann Holger and M. W. Grundler Florian. (2008). *Arabidopsis* endo-1,4-b-glucanases are involved in the formation of root syncytia induced by *Heterodera schachtii. The Plant Journal* 53(2):336-51 (NONEMA Partner P.10).

Fig 2.

Silenced potato plants and the corresponding empty vector controls were in vitro inoculated with potato cyst nematodes (10 J2-s per root tip). Five weeks after inoculation, nematode development in EGase-silenced plants was compared to its development on control plants. Two parameters were scored: (1) the total number and (2) the phenotype of adult females. Microscopic observations showed that nematode development stopped in most cases at the J2/J3 life stage (Fig. 2c$_4$, c$_5$). Under normal conditions the posterior part of the female body is spherical and opaque (Fig. 2c$_1$) as it is filled with eggs. Poorly developed females are smaller, and the posterior part of their body is transparent and saccate (Fig. 2c$_2$, c$_3$) as it does not contain eggs. Growth on EGase-silenced potato plants resulted in a sharp increase of the number of poorly developed females; black bars in Fig. 2b). Picture from Karczmarek et al. (2008).

Acronym
POTATO TRAITS FOR AN

Programme Acronym
FP5-INCO 2

Contract number
ICA4-2000-30019

Period
November 2000 – October 2003

Coordinator
Howard J. ATKINSON
Centre for Plant Sciences
University of Leeds
Leeds LS2 9JT
United Kingdom
h.j.atkinson@leeds.ac.uk

Evaluating new traits for potato in the Central Andes with an appropriate poverty focus

Background and objectives

The Nuffield Council on Bioethics has suggested that possible introgression of foreign genetic material into related species in centres of crop biodiversity is insufficient justification for barring GM crop deployment in the developing world. The Council considers that applying the precautionary approach and forgoing possible benefits invokes the fallacy of thinking that doing nothing in itself precludes any risk to the poor. The project studied GM nematode-resistant (GMNR) potato clones that express a cysteine proteinase inhibitor (cystatin) as an example of a beneficial trait for subsistence growers. Part of the study considered aspects of environmental biosafety for GM potato in its main centre of biodiversity, the Central Andes. The aim of the project was to develop the scientific basis on which judgments balancing benefits and concerns can be made.

Approach and methodology

The project studied the environmental effects of cystatins on non-target organisms, but not the food safety issues, because a prima facie case for food safety of cystatins has already been established. It also considered the particular environmental hazard of gene flow from GM potatoes to their wild relatives. The Central Andes is a centre for diversity of the potato crop with about 130 wild potato species recognised in Peru and Bolivia.

Main findings and outcome

The study aimed to underpin the biosafety of future field trials for GMNR-cystatin potato plants in South America. The community-level physiological profile of rhizosphere bacteria at harvest was determined, using methods that have already detected such effects for transgenic potato expressing another transgene. GMNR-cystatin potato cultivars developed for future use in Bolivia imposed no change on this profile, relative to their untransformed parents in glasshouse trials. This lack of effect of the cystatin contrasts with our results for rhizosphere soil recovered from different conventional crops growing in an experimental field plot in Bolivia. Cluster analysis

established such soil associated with native cultivars grouped together but distinct from that in which potato cv 'Desiree' or either broad bean or lupin were growing.

A UK field trial also established that GMNR-cystatin potato do not influence the density or number of soil nematode species beneath a mature potato crop. The GMNR-cystatin potato did not disturb a maturity index based on the proportion of coloniser and persister species in the soil nematode community. This contrasted with the effect of applying a pre-plant nematicide which favoured fast-growing species several months later.

This suggests that soil organism populations would not be harmed at a trial site. It also suggests the needs of the precautionary principle would be met for any subsequent wider uptake of GM plants, when they impose no more impact on the environment than arises from already common agricultural practices.

Concerning the hazard of gene flow, six wild species were selected to establish if gene flow could occur from GMNR-cystatin field trials. Their selection was based on a preliminary survey of four agro-ecological zones in Peru, in order to define examples of wild *Solanum* species that occur close to potato plots. Firstly, it was confirmed that the accessions did cross as reported before in some cases using forced, hand-pollinations between them and *S. tuberosum* cultivar-group Andigenum landraces and improved cultivars. The seedlings from the set seed were analysed using AFLP markers, and hybrids were identified involving all six wild species.

As hand-crossing may not represent natural cross-pollination events reliably, we established five open pollination field trials in different agro-ecological zones at Puno, Junin, Cajamarca and Cusco. AFLP results established that the progeny from each of three wild relatives with accessions in the trial included a few seedlings that were fathered by *S. tuberosum* cultivar-group Andigenum. This confirms that gene flow can occur from a cultivated potato to wild relatives in the field. Assuming that the nearest plant of the male parent identified by AFLP fingerprinting was the pollen donor, their mean distance was less than three plants from the pollen recipient. This is consistent with previous work establishing that only 2% of seedlings were transgenic when the cross-pollination distance from non-transgenic females to transgenic pollen donors was 3m, and 24% when the parents grew next to each other.

Partners

R. VISSER
Wageningen Agricultural University (NL)

J. FRANCO
Promoción e Investigación de Productos Andinos
Cochabamba (BO)

E. CARBONELL
Servicio Nacional de Sanidad Agraria (SENASA)
Lima (PE)

Fig 1.

A farmers' school in Bolivia training growers to recognise potato cyst nematode on their crop (left) and in their soil (centre).

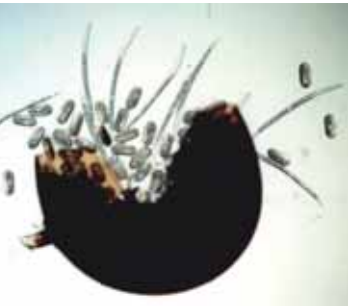


Fig 2.

The cyst is about 600μm diameter and contains the infective juveniles and eggs that remain dormant under potatoes are grown again. This and other nematode pests occur in 91 % of the potato-growing land in Bolivia.

Concurrent flowering of the potato plants and *S. acaule*, *S. bukasovii* and *S. sparsipilum* spanned 52 days. Even the value of 32 days for the early flowering *S. megistacrolobum* provided ample opportunity for cross-pollination by insects. Bumblebees and their relatives are the main pollinators of *Solanum*. Pollen is the only food reward offered by *Solanum* spp., which is only infrequently visited by honey bees seeking nectar. Also the anthers of these plants require sonication by insects to release pollen and so the range of pollinating insects is restricted.

Bumblebees typically forage over 70-631m but pollen from one flower is usually only deposited across a limited number that are subsequently visited. This and factors such as residence time in one crop favour highly localised cross-pollination of plants near the pollen source. According to visits by insects to flowers at five sites in Peru, twelve bee species were recorded but sites differed. It was concluded that the extent of pollen flow between *Solanum* species varies considerably with locality in the Central Andes, and with bumblebee densities and species, their foraging and the location of wild relatives within and near transgenic potato crops.

Introgression of a transgene from GMNR-cystatin potato might confer resistance to nematodes on wild relatives that are currently susceptible to a key potato pest, *Globodera spp.* We found that this nematode did reproduce on five of the six wild *Solanum* species studied, but to a lesser extent than occurs for the much larger root system offered by a cultivated potato plant. The possibility that a stably introgressed transgene for pest resistance might benefit a wild relative growing near a crop species requires examination on a case-by-case basis. Plant invasiveness for both wild and cultivated species of *Solanum* also needs to be considered. The precautionary principle requires such a possibility be precluded, at least until this concern is evaluated.

The Andean cultivar *Revolucion* is of particular interest in meeting this interim need. It is one of the male sterile cultivars arising from crosses between *S. tuberosum* Andigenum and Tuberosum cultivar groups. Therefore it lacks pollen to fertilise eggs of another compatible plant during sexual reproduction. *Revolucion* did not flower abundantly and flower drop occurred at trial sites, so six GMNR-cystatin lines were transformed and developed. Containment trials in the UK established that they provided partial resistance to *G. pallida* relative to ex-tissue-culture untransformed plants of this cultivar. This level of resistance is similar to that reported before

and is sufficient to prevent yield loss due to the nematode within the short-rotation courses that necessarily prevail among subsistence growers in Bolivia. It was proposed that transgenic cv *Revolucion* provides a basis for initial field trials of nematode resistance or other traits of value without gene flow from the potato under trial.

Conclusions

No basis was established for invoking the precautionary principle in order to bar initial GMNR–cystatin potato trials for their impact on non-target soil microbes and fauna or associates of the crop's foliage. Also no basis for harm has been detected, and any effect would be local to the site. By contrast, any gene flow from potato to wild *Solanum* species in the Andes that grow nearby may not remain localised and this requires further study. In the meantime, a barrier to outcrossing can be provided by the male sterile cv *Revolucion*.

This provides a basis for underpinning the development of biosafe genetically-modified field trial regulations for potato in the Andes. Transgenic planting should be limited to male sterile cultivars, while concerns over possible introgression of any given trait are evaluated over several generations of random mating among individuals of a wild species by defined methods. When such information is available, the biosafety of a transgenic, male fertile cultivar for that trait can be evaluated.

Assessing both benefits and concerns provides an informed scientific basis on which to determine whether or not the needs of the poor conflict with the precautionary principle for potato in its centres of diversity.

With regard to potential impact of GM plants on soil organisms, the authors also suggested that the precautionary principle would be respected for any subsequent wider uptake of GM plants by providing proof of them not imposing more impact on the environment than common agricultural practices would do.

Major Publications

Celis C., Scurrah M., Cowgill S.E., Chumbiauca S., Franco J., Main G., Keizenbrink D.T., Green J. and Atkinson H.J. (2004). Environmental biosafety and transgenic potato in a centre of this crop's diversity. *Nature* 432:222-225.

Atkinson H. J., Green J., Cowgill S. and Levesley A. (2001). The case for genetically modified crops with a poverty focus. *Trends in Biotechnology* 19:91-96.

Green J., Fearnehough M.T. and Atkinson H.J. (2005). Development of biosafe genetically modified *Solanum tuberosum* (potato) cultivars for growth within a centre of origin of the crop. *Molecular Breeding* 16:285-293.

Assessment of the environmental and agronomic appropriateness of *Bt* transgenic cotton in small-producer IPM systems in China

Acronym
COTRAN

Programme Acronym
FP5-INCO 2

Contract number
ICA4-CT-2000-10197

Period
November 2001 – March 2005

Coordinator
Derek RUSSELL
Natural Resources Institute (nri)
University of Greenwich
Chatham Maritime
Kent ME4 4TB
United Kingdom

Background and objectives

The overall objective of the project was to enhance the ability of Chinese, European and international research and agricultural extension organisations to take into account the full range of biological, economic and social impacts when assessing the appropriateness for smallholder farmers of cotton plants genetically engineered to produce the *Bt* insecticidal toxin (*Cry 1Ac* from the soil microbe *Bacillus thuringiensis*) for the control of fruit-feeding caterpillars. Much of the work focused on providing the tools that farmers themselves need to make appropriate decisions in relation to the management of *Bt* transgenic cotton. The project worked closely with the six-country EU/FAO Asian cotton IPM Farmer Field school project to help it extend its competence to *Bt* cotton.

Approach and methodology

Prior to the project there was no experience in smallholder farmer assessment of the benefits, weaknesses and management options for *Bt* cotton. A participatory rural appraisal (PRA) was carried out (Deliverable 1- Report on farmers' perceptions of the economic, social and environmental role of *Bt* cotton in their farming system). This identified areas of strength and weakness in farmer understanding of the management of *Bt* cotton and an appreciation of its economic position in cotton production. Farmers perceived bollworm control (at least in the first two-thirds of the season), labour and cost benefits, but were unclear on what to expect from *Bt* cotton and were still spraying an average of 12 insecticides, much of it for bollworm control.

From this improved understanding, a series of farmer participatory exercises were developed, in conjunction with the curriculum development programme of the EC IPM in cotton in Asia programme (2000 - 2004) and presented as a comprehensive workbook for use in Farmer Field School and other IPM training in *Bt* cotton in Asia and beyond (Deliverable 3 – Farmer participatory Workbook to evaluate *Bt* cotton). The methods used formed part of Deliverable 4 – Manual of environmental and economic assessment of *Bt* cotton for smallholder systems.

Main findings and outcome

Of major concern was to improve the understanding of the impact of *Bt* cottons on other parts of the agro-ecosystem. Laboratory and field methods of global applicability were developed to enable the rigorous analysis of impacts on

target and non-target insect pests, parasitoids, predators and soil organisms (written up in published papers and as chapters in *Deliverable 4* – Manual of environmental and economic assessment of *Bt* cotton for smallholder systems). Bollworm control was satisfactory. There were no significant effects on non-target lepidoptera, whiteflies, cotton aphids, or springtail decomposers in the soil. Cotton bollworm, leafworms and cotton aphids could concentrate *Bt* toxin in their tissues while feeding and pass this on to predators. However, no evidence was found for significant disruption of the generalist predator complex in *Bt* cotton and indeed spider diversity was higher in *Bt* than non-*Bt* cotton. No major harmful effects were seen in studies on tritrophic relationships in cotton, including in a simulated field cage system.

The risk that bollworm control by *Bt* cotton would prove unsustainable in farmer systems, where no strategy was in place to reduce the risk of evolved resistance to the toxin, remains a concern in the deployment of these materials, especially as in China now 100 % of its north-eastern cotton growing area is under *Bt* cotton cultivation. Using laboratory-selected *Bt*-resistant strains of the key bollworm, *Helicoverpa armigera*, methods were developed for detecting the presence of resistance genes in field populations and assessing their frequency (>one in a thousand in field populations), phenotypic dominance (around 0.27) in the field and the mechanisms involved (a truncated cadherin toxin receptor in the bollworm gut lining).

These parameters were then used in a simulation model of resistance development in order to explore management options for *Bt* cotton. Resistance is not yet a field control problem. Modelling suggests that imposing additional (non-*Bt*-toxin) mortality on surviving bollworm in *Bt* cotton fields is the single most effective way of delaying resistance development. Results are presented in papers and *Deliverable 4* – Manual of environmental and economic assessment of *Bt* cotton for smallholder systems.

The project worked closely with the national agricultural extension system in China (a partner in the project) to ensure the practical usefulness of the project results and to establish an uptake pathway. This included a dialogue on the EU and Chinese biosafety and approvals processes for GM cotton (*Deliverable 2- Bt* cotton policy discussion document).

A final disemination workshop (*Deliverable 5*), to present these results and deliverables, was held in Beijing in March 2005. It was attended by representatives of all the provinces growing *Bt* cotton, the extension and IPM training organisations, and the national GMO cotton science community.

Partners

Gabor LOVEI
Danish Institute of Agricultural Science (DIAS), Aarhus (DK)

Jean-Michel VASSAL
Centre de Coopération Internationale en Recherche Agronomique pour le Développement, (CIRAD) Montpellier (FR)

Sean MURPHY
CABIO Biosciences, Egham (UK)

Yidong WU
Nanjing Agricultural University (CN)

Fan HAO WAN
Biological Control Institute of the Chinese Academy of Agricultural Sciences (BCICAS), Beijing (CN)

Yang PUYUN
National Agro-Technical Extension and Service Centre (NATESC), Beijing (CN)

An ecologically safe selection system for transgenic crops based on modified plant-tubulin genes

Acronym
ECOTUB

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2000-00060

Period
October 2000 – September 2003

Coordinator

Prof. Dr Peter NICK
Albert-Ludwigs-Universität Freiburg
Institut für Biologie II
D-Freiburg
Germany

Current address:
Botanisches Institut
Karlsruhe Institute of Technology
Kaiserstr. 2
D-76128 Karlsruhe
Germany
peter.nick@bio.uni-karlsruhe.de

Background and objectives

There is substantial public concern about a potential spread of antibiotic resistance genes from transgenic plants into the soil and intestinal bacteria. A selection system for plant breeders to be used during plant transformation that is exclusively based on genetic information already present in the host plant and that does not require antibiotic resistance genes would avoid any such risk.

This project aims to exploit the natural variability of plant tubulins as essential components of cell growth, cell division and stress tolerance in plants. There exists a panel of tubulin promoters that can drive expression either constitutively or, alternatively, in different tissues or in response to different signals. They can be fused with a panel of different tubulin isotypes with distinct physiological features to produce a versatile kit of combinations that will confer distinct properties such as stress or herbicide tolerance to specified target tissues. The result is then used to construct a selection marker based on truncated tubulins lacking the binding site for acrylcarbamate herbicides. The production of the truncated tubulins will be driven by the tubulin's own promoters within DNA fragments devoid of foreign sequences. The suitability of this selection system will be analysed using rice as a model cereal.

Approach and methodology

An ideal selection system should fulfil the following requirements:

- minimal risk by minimal manipulation: undesired side-effects for the environment or consumers are avoided by eliminating everything which is superfluous in terms of function;
- optimal use of natural resources: only DNA already present in the crop plant is used and its potential is exploited simply by recombining genes and promoters in a novel manner.

In combination with the designed coding sequences for plant tubulin that confer tolerance to selective agents targeted to microtubules, it should be possible to obtain a selection

marker that exclusively consists of DNA that is already present in the plant. Throughout this work, the approach will be based on 'clean DNA' technology, which involves avoiding the use of extraneous backbone and vector sequences, thus culminating in the Eco Tub approach, standing for Ecologically safe selection markers based on plant Tubulins.

Main findings and outcome

The versatility of plant tubulins combined with their strong impact on many aspects of plant life are ideal preconditions for biotechnological manipulations that are both subtle and powerful. Resistance based on mutations in the C-terminus of tubulins, where a high natural variability is observed can open a large field for engineering new selection markers. The aryl chlorprophame that have been used extensively as sprouting inhibitors and pre-emergence herbicides bind to the C-terminus of alpha-tubulins.

Moreover, it is possible to discriminate between subpopulations of alpha-tubulins with different affinity for these herbicides. The presence of a carboxyterminal tyrosine that is present in all alpha-tubulins is crucial for the affinity to these herbicides, and the carboxyterminal 13 amino acids can confer the affinity to ovalbumin carrier. These findings imply, that the resistance to aryl carbamates can be generated without interfering with the essential functions of tubulin that are located further upstream from the molecule. In fact, the rice mutant ER31, that is resistant to aryl carbamates, has been found to harbour a tubulin isotype with reduced affinity for these herbicides due to a truncation of the carboxyterminus in consequence of a precocious stop codon. When the mutated tubulin is transferred into calli, these calli will acquire tolerance to otherwise inhibitory doses of the aryl carbamate phenyl urethane. The aryl carbamates and other microtubule-eliminating chemicals bind to the alpha-beta-heterodimers and prevent their incorporation into the microtubule.

In a situation where a wild-type tubulin (which binds to the chemical with high affinity) and a mutated tubulin (not binding to the chemical) coexist, the assembled microtubules would preferentially consist of the mutated tubulin. In fact, the resistance to aryl carbamates has been found to be a dominant-negative trait, indicating that the modified tubulin is progressively trapped in the microtubules. It is therefore possible to use the marker at moderate expression levels.

Partners

Diego BREVIARIO
Istituto Biosintesi Vegetali, Centro Nazionale de Ricercha, Roma (IT)

Paul CHRISTOU
John Innes Centre, Norwich (UK)

Tubulin promoters can provide a great variety of expression profiles that can be modulated in amplitude and tissue specifity or in response to different stimuli. The modulatory function of intron sequences allows further flexibility in the biotechnological use of tubulin promoters for any given tubulin isotype. Pattern and amplitude of expression could be adjusted by the simple substraction (or addition) of its own or cognate intron sequences. This would permit the tuning of expression over a broad range from very weak to high, depending on the requirements of the actual application. Again, it should be noted that this adjustment could be achieved without any DNA sequence that is not already present in the plant of interest.

Conclusions

Promoters, introns and UTR-sequences of plant-tubulins present in any crop plant of interest are versatile enough to generate almost any desired pattern of expression. In combination with the designed coding sequences for plant tubulin that confer tolerance to selective agents targeted to microtubules, it should be possible to obtain a selection marker that exclusively consist of DNA that is already present in the plant.

To fulfil the ECO TUB principles, a gene system is required that carries essential functions (to allow selection after transformation) and a high degree of natural variability. Both preconditions are met by the plant tubulin system. Coding sequences of plant tubulins are modified to generate resistance against tubulin-eliminating chemicals. They are fused to appropriate tubulin promoters from the host plant itself. The desired trait can be achieved without the introduction of alien (cross-species) genetic information. This resistance is then used for selection after transformation. To pinpoint this principle, any extraneous DNA vector backbone sequences are avoided using a clean DNA technology involving transformation with minimal expression units (promoter, open reading frame and terminator, no antibiotic resistance genes, no bacterial origins of replication).

Promoters, introns and UTR-sequences of plant tubulins present in any crop plant of interest are versatile enough to generate almost any desired pattern of expression. In temperate climates, crop yield is limited by low temperatures. Plants owe a high sensitivity of growth to low temperatures. As a consequence, it is the retarded unfolding of leaves that confines productivity during the spring season. However, chilling sensitivity is an important issue outside the temperate regions as well. In fact, in various crops, the cold stability of microtubules has been found to correlate with chilling sensitivity, and disassembly of microtubules by antimicrotubular compounds was observed to increase chilling sensitivity.

It appears that the cold-induced disassembly of microtubules depends on the carboxyterminal region. In addition to arylcarbamate resistance, mutations that truncate the C-terminus of tubulins are therefore expected to produce cold tolerance of microtubules. In the future we therefore plan to generate plants with improved chilling-tolerance by engineering the carboxy terminus of alpha-tubulins.

Major Publications

Nick P., Christou P., Breviario D. (2003). Generating transgenic plants by minimal addition of exogenous DNA – a novel selection marker based on plant tubulins. *AgBiotechNet* 5:ABN 105.

Breviario D., Nick P. (2000). Plant tubulins: a melting pot for basic questions and promising applications. *Transgenic Res.* 9:383-393.

Wiesler B., Wang Q.Y., Nick P. (2002). The stability of cortical microtubules depends on their orientation. *Plant J.* 32:1023-1032.

Abdrakhamanova A., Wang Q.Y., Khokhlova L., Nick P. (2003). Is microtubule assembly a trigger for cold acclimation? *Plant Cell Physiol.* 44:676-686.

Ahad A., Wolf J., Nick P. (2003). Activation-tagged tobacco mutants that are tolerant to antimicrotubular herbicides are cross-resistant to chilling stress. *Transgenic Research* 12:615-629.

Biosafety research directed at more sustainable food production

Acronym
ECOSAFE

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2000-01759

Period
January 2001 – December 2003

Coordinator
Prof. Fergal O'GARA
BIOMERIT
Research Centre
Microbiology Department
UCC
Ireland
f.ogara@ucc.ie

Background and objectives

The question of sustainable development, as it relates to food production and supply, is one that is coming to the fore as we progress into the 21st century. Rapid global population growth, which is estimated to reach to up to 10 billion people by the year 2050, will have to be matched by an equally rapid growth in the ability to produce food throughout the world. If indeed we are to match the rapidly expanding and increasingly urbanised population, then improvements in food production will have to be found. Current practices, associated with the production of higher yields and crop protection, involve the use of agrichemical inputs such as herbicides and pesticides.

Obvious issues of food safety and human health have now prompted the scientific community to focus on the development of novel 'eco-friendly' alternatives. Developments in plant biotechnology, either by conventional breeding or genetic modification, have offered new crop varieties which show favourable traits such as enhanced disease and pest resistance and better nutritional value. However, simply to focus on the plant as a single entity ignores the important role that microbial communities present in the rhizosphere play in promoting the health and productivity of its host. Examples of this include the mutualistic relationship between nitrogen-fixing bacteria and plant roots, thus enabling the plant to utilise reduced nitrous compounds supplied by the bacteria.

More recently certain bacteria, in the presence of plant hormones, have been shown to increase plant root mass, thereby allowing for increased uptake of nutrients. In addition to enhancing the nutrient supply, microbes have also been shown to confer a degree of protection from plant pathogens. To date, little information exists on how these microbes interact with the plant. A greater understanding of this, therefore, could provide new strategies in improving plant health and productivity in an eco-friendly manner.

The core theme of the ECO-SAFE project was the evaluation of the ecological consequences of the use of novel biotechnology products, based on GM plants and microbial inoculants, to meet the goals of sustainability in European agriculture.

This project was established as a cluster of two related component projects, based on a partnership involving industry, an NGO and 15 academic laboratories. Each component project had a principal objective and addressed key problems to be solved. When viewed together, these projects offered an effective strategy for providing the crucial information required for the determination of the ecological impact and safety of GM plants and microbial inoculants and for predicting their fate in the environment.

Approach and methodology

Although *Azospirillum* inoculants are used commercially, there are limitations associated with their use. These bacteria affect plant root growth in response to a secreted plant hormone [Indole acetic acid (IAA)]. By taking advantage of knowledge acquired on the genetic regulation of this hormone, we were able to construct genetically engineered microorganism strains (GMM strains) with increased ability to promote plant growth. The efficiency of these strains, as well as potential impact on indigenous biodiversity, was tested. However, before any GMM strains were tested, it was clear that base-line effects of inoculation with wild-type *Azospirillum* had to be analysed. We undertook extensive field-trials of wild-type inoculants and contained trials of GM inoculants [EU moratorium on approvals for open releases of GMOs during this period] to generate these data.

The second major theme of ECO-SAFE addressed the fact that microbes and plants in biological systems do not always behave as expected. Complex interactions between plant host and microbe take place throughout the region of soil under the influence of the plant. This 'communication' between microbe and plant is mediated *via* signals exuded from the roots of the plant and by signals secreted by the microbe. In bacteria, these signalling events involve the production of extracellular signals [such as homoserine lactones (HSLs)].

Interestingly, it has also been demonstrated that plants can interfere with this signalling process by producing HSL 'mimic' molecules. In view of this the ECO-SAFE project has utilised modern genetic techniques to produce transgenic plants which can produce bacterial signals. These plants have been shown to modulate the gene expression and phenotype of the resident bacterial communities. These plants were evaluated using a range of criteria, including plant health, protection against disease and ecological impact. Due to the importance of these

Partners

Jos VANDERLEYDEN
Centre of Microbial and Plant Genetics, K.U. Leuven (BE)

Marco P. NUTI
Departimento di Chimica e Biotechnologie Agrarie, University of Pisa (IT)

Jose Miguel BAREA
CSIC Granada (ES)

Peter YOUNG
Department of Biology, University of York (UK)

Yvan MOENNE-LOCCOZ
UMRCNRS d'Ecologie Microbienne, Université Claude Bernard, Lyon (FR)

Yaacov OKON
Dept. Plant Pathology and Microbiology, Hebrew University of Jerusalem (IL)

Stewart SMITH
LIPHATECH Inc., Milwaukee WI (US)

Allan DOWNIE
John Innes Centre, Norwich (UK)

Yves DESSAUX
CNRS, Gif-sur-Yvette (FR)

Genevieve DEFAGO
Swiss Federal Institute of Technology, Zurich (CH)

Ben J.J. LUGTENBERG
Institute of Molecular Plant Science, Leiden University (NL)

John BRODERICK
Irish Sugar plc., Carlow (IR)

Tomas RUIZ-ARGUESO
Universidad Politecnica de Madrid (ES)

Rupert FRAY
Division of Plant Science, University of Nottingham (UK)

Dieter HAAS
Laboratoire de Biologie Microbienne, University of Lausanne (CH)

Paul WILLIAMS
Pharmaceutical Sciences, University of Nottingham (UK)

David KING
International Federation of Agricultural Producers, Paris (FR)

signalling systems, it is not unexpected that some microorganisms (both fungi and bacteria) have the potential to interfere with them. Thus, for example, bacteria that degrade these signals have been identified and there is evidence that fungi can also interfere with the signalling systems. Therefore we also undertook fundamental research to establish the molecular basis for cell-cell communication systems.

Main findings and outcome

The engineered *Azospirillum* phytostimulatory strains worked effectively in stimulating root growth and promoting nitrogen uptake. These were more effective than the original wild-type strains. The project demonstrated the importance and value of basic research in determining the underlying biological basis of applied phenomena. Previous EU projects (IMPACT I/II) had funded some of the work that established the molecular pathways for production of IAA by *Azospirillum*. This knowledge was applied in ECO-SAFE to re-regulate the pathways in order to generate improved strains for application. Using wild-type strains in the field and the engineered strains in controlled greenhouse conditions, we assessed the possible negative impacts on microbial flora and biodiversity. The most important finding was that any impact of inoculants on bacterial diversity was less than variations due to natural soil heterogeneity/plant-to-plant variability, seasonal effects and plant age. GM inoculants had no additional effects when compared with wild-type strains.

A major outcome of the work on cell-cell communication was the generation of a large body of data underscoring the central importance of HSL-mediated signalling for ecological function and the role of such signalling in regulating ecological function. A major finding was that genetically modified plants did not have a negative ecological impact. It was also established that other, non-HSL, signalling also occurs between microbes. Significant progress has been made on the study of several other bacterial cell-cell signalling systems.

In subsequent work, which has utilised genomic technologies, we have shown that signals exuded from different plant varieties have differential effects on bacterial gene expression. It has also been shown that a subset of these genes also plays a role in the ability of the bacteria to colonise the plant-root structure. By understanding the genes induced we will obtain an insight into those genes (and the characteristics they encode) that influence the interaction of the bacteria within their environment.

Conclusions

In modern agriculture, the use of nitrogen fertilisers represents a major source of nitrogen contamination in both the atmosphere (as nitrogen oxides) and in surface and ground water (as nitrate). The EU is committed to the reduction of nitrates from agricultural sources. The ECO-SAFE project showed that inoculation with *Azospirillum* demonstrates that it is feasible to switch from heavy N fertilisation to more sustainable N fertilisation levels. By using genetically engineered strains, reduction in application rates of nitrogen fertiliser of up to 25-30 % can be compensated by the presence of *Azospirillum*.

The effect of introduced strains on indigenous microbes is transient, reversible and showed less effects than caused by natural seasonal fluctuations, and can thus be considered environmentally non-detrimental. These developments with *Azospirillum* are the culmination of years of basic research into the biology of the system. The research that was carried out into cell-cell signalling generated a substantial corpus of basic knowledge that will underpin future applications in the biotechnology sector. The ultimate aim of this research is to produce novel biocontrol and biofertiliser bacteria that will reduce our requirements for application of chemical fertilisers and fungicides, by taking advantage of environmentally friendly microbial products.

Major Publications

Morrissey J.P., Walsh U.F., O'Donnell A., Moënne-Loccoz Y. and O'Gara F. (2002). Exploitation of genetically modified inoculants for industrial ecology applications. *Antonie van Leeuwenhoek.* 81:99-606.

Danino VE., Wilkinson A., Edwards A., Downie JA. (2003). Recipient-induced transfer of the symbiotic plasmid pRL1JI in Rhizobium leguminosarum bv. viciae is regulated by a quorum-sensing relay. *Mol Microbiol.* 50:511-25.

Morrissey J.P., Dow J.M., Mark G.L. and O'Gara F. (2004). Are microbes at the root of a solution to world food production? *EMBO Reports.* 5:922-926.

Newton JA., Fray RG. (2004). Integration of environmental and host-derived signals with quorum sensing during plant-microbe interactions. *Cell Microbiol.* 6:213-24.

Somers E., Ptacek D., Gysegom P., Srinivasan M., Vanderleyden J. (2005). Azospirillum brasilense produces the auxin-like phenylacetic acid by using the key enzyme for indole-3-acetic acid biosynthesis. *Appl Environ Microbiol.* 71:1803-10.

Ciliates as monitors for environmental safety of GMO

Acronym
CIMES

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2002-02151

Period
October 2002 – December 2005

Coordinator
Dr. Johannes H.P. HACKSTEIN
Dept. Evolutionary Microbiology
Faculty of Science
Radboud University Nijmegen
Toernooiveld 1
6525 ED Nijmegen
The Netherlands

Background and objectives

Horizontal (or lateral) gene transfer from GMO to organisms that thrive in the surrounding environments has been envisaged as a potential hazard, since it had been shown earlier that foreign DNA in the intestinal tract of mice can be taken up by the host organism. While horizontal (lateral) gene transfer among bacteria is well known and identified as a major source of evolutionary innovation in prokaryotes, virtually nothing is known about the rates and the mechanisms of horizontal (lateral) gene transfer in eukaryotes. This lack of information includes potential bacterial-to-eukaryote transfers, but in particular potential plant-to-animal transfers.

Notably, the presence of some 100 'bacterial' genes in the human genome, which had been suspected after an initial analysis, could not be confirmed after reanalysis. Consequently, it had to be concluded that a bacterial to human gene transfer is extremely rare. This, however, does not exclude the possibility of more frequent horizontal gene transfers to animals, in particular to unicellular, gut-dwelling eukaryotic microorganisms.

A wealth of ciliates – unicellular eukaryotic microorganisms with a peculiar genome organisation – thrives in the intestinal tract of various animals. Well-known examples are the rumen ciliates, which live in the rumen of farm animals such as cattle, sheep and goats, but also the *Nyctotherus* species in the hindgut of cockroaches. All these ciliates take up bacteria and, potentially, also naked DNA; some species of rumen ciliates are able to ingest and to degrade plant material. Their genome organisation, i.e. the presence of a germ line-limited micronucleus and a 'somatic' macronucleus (which encodes the genes required for vegetative growth) should allow not only an unequivocal identification of potential transgenes, but also provide evidence as to whether these genes are expressed or not. Therefore, we postulated that ciliates would be ideal monitors to detect potential lateral gene transfer in the intestinal tracts of animals.

Approach and methodology

Any judgement of a potential horizontal gene transfer from GM plants to eukaryotes requires a solid validation of potential horizontal gene transfers, which occurred in the intestinal ciliates at evolutionary timescales. Therefore, gDNA and cDNA libraries were constructed, randomly sequenced and

analysed bioinformatically. Initially, conventional methods (e.g. BLAST and phylogenetic analysis) were used to identify genes, which might have been acquired by horizontal gene transfer. Also, expression libraries were screened for the presence of fibrolytic genes, which are hot candidates for horizontal gene transfer. In addition, more advanced algorithm-based techniques for the identification of horizontal gene transfer and a detailed codon-usage analysis were developed for the confirmation of the ameliorisation of the codon-usage of transgenes in evolutionary times. Where necessary, the bacterial microbiota in the various gut environments were analysed on the basis of 16S rRNA libraries to identify potential sources of horizontal gene transfer.

To get information about the dimensions of the evolutionary timescales, and the composition of the ciliate communities in the various hosts, 18S rRNA libraries of the intestinal ciliates were generated and analysed phylogenetically. FISH (Fluorescence In Situ Hybridisation) techniques were developed for the identification of particular ciliates in their environments. To identify potential 'real-time' horizontal gene transfers, intestinal ciliates were challenged by feeding *Bt176* maize to the host organisms (e.g. cow or cockroach) or by feeding powdered leaves of *Bt176* maize to selected species of rumen ciliates which were maintained *in vitro* for up to two years.

The macronuclear genome organisation of the target ciliates was analysed to allow the unequivocal identification of potential 'real-time' horizontal gene transfer to intestinal ciliates. Lastly, 'model ciliates", such as *Tetrahymena* and *Euplotes*, were exposed to green-fluorescent protein (GFP)-encoding DNA *in vitro* in order to analyse the potential routes of horizontal gene transfer and to study a potential concentration dependence of DNA in potential DNA-to-ciliate transfers. Alternatively, *Euplotes* ciliates were fed *E. coli* cells carrying the plasmid pEGFP-N3.

In order to test whether selective pressure can enhance LGTs in *in vitro* cultured rumen ciliate, a co-culture of *Entodinium caudatum* and a mercury-resistant (rumen) bacterium was set up and maintained for 16 months with the addition of Hg^{2+} salts to the culture medium.

Main findings and outcome

Analysis of about 5000 ESTs (expressed sequence tags) confirmed that rumen ciliates during their evolution took up bacterial genes by lateral gene transfer at a rate that is unprecedented among eukaryotes. The rate of bacterial to ciliate transfer approximates levels so far known only for bacteria,

Partners

Prof. MARTIJN A. HUYNEN, Ph.D.
Nijmegen Center for Molecular Life Sciences p/a Center for Molecular and Biomolecular Informatics, Nijmegen (NL)

Prof. Dr. Karl-Heinz SCHLEIFER
Lehrstuhl fuer Mikrobiologie – Technische Universitaet Muenchen (DE)

Dr. Johannes FRIED
Lehrstuhl fuer Mikrobiologie – Technische Universitaet Muenchen (DE)

Prof. Dr. C Jamie NEWBOLD
The Institute of Rural Science – University of Wales, Aberystwyth (UK)

Dr. Neil MCEWAN
The Institute of Rural Science – University of Wales, Aberystwyth (UK)

Dr Peter PRISTAS
Institute of Animal Physiology, Slovak Academy of Sciences, Kosice (SK)

Dr. T. MICHALOWSKI
Kielanowski Institute of Animal Physiology and Nutrition, Jabłonna (PL)

Major Publications

Krzysztof Wereszka, Freda M. McIntosh, Tadeusz Michalowski, Jean-Pierre Jouany, Eli Nsabimana, Didier Macheboeuf, Neil R. McEwan, and C. James Newbold (2004). A cellulase produced by the rumen anaerobic protozoan Epidinium ecaudatum has an unusual pH optimum. *Endocytobiology and Cell Research,* 15(2):561-569.

Boxma B., de Graaf R.M., van der Staay G.W.M., van Alen T.A., Ricard G., Gabaldón T., van Hoek A.H.A.M., Moon-van der Staay SY., Koopman W.J.H., van Hellemond J. J., Tielens A.G.M., Friedrich T., Veenhuis M., Huynen M.A. and Hackstein J.H.P. (2005). An anaerobic mitochondrion that produces hydrogen. *Nature* 434:74-79.

Newbold CJ., McEwan NR., Calza RE., Chareyron EN., Duval SM., Eschenlauer SCP., McIntosh FM., Nelson N., Travis AJ., Wallace RJ. (2005). An NAD(+)-dependent glutamate dehydrogenase cloned from the ruminal ciliate protozoan, *Entodinium caudatum. FEMS Microbiol Letts* 247 (2):113-121.

i.e. more than 4 %. The data support the 'you are what you eat' hypothesis of Ford Doolittle (1998); they also show that only genes with an obvious evolutionary advantage have been retained. Moreover, the data analysed so far failed to provide any 'hard' evidence that a significant number of plant genes has been taken up in evolutionary timescales.

A comparable analysis of the ciliate *Nyctotherus ovalis* from the gastro-intestinal tract of cockroaches revealed a much lower LGT with a completely different spectrum of acquired genes.

The bioinformatical analysis of the rumen ciliate data has shown that a scrutinised analysis is rather time-consuming, since the validation of each potential evolutionary horizontal gene transfer requires a sophisticated phylogenetic analysis and a detailed analysis of the codon-usage. The data analysed so far show that genes acquired by horizontal gene transfer exhibit a complete ameliorisation to the codon usage of the recipient ciliate. This observation indicates that these acquisitions of bacterial genes by intestinal ciliates occurred millions of years ago, potentially in adaptation to life in the intestinal tract of their particular invertebrate or vertebrate hosts. This interpretation is supported by the observation that a significant number of genes involved in anaerobic metabolism, and in particular the degradation of plant material, is of bacterial origin. Accordingly, the bacteriovorous ciliate *Nyctotherus ovalis* retained predominantly genes that support its adaptation to anaerobic environments and none involved in the degradation of plant material.

Since the detection of potential evolutionary eukaryote-(e.g. plant)-to-eukaryote transfers might be hampered by a common, albeit ancient, origin of these genes, we analysed the peculiar genetic structure of the macronuclei of the various ciliates, using pulsed field gel electrophoresis (PFGE), the isolation of macronuclei after cellular fractionation, and molecular cloning. While the anaerobic ciliate *Nyctotherus ovalis* possesses gene-sized mini (or micro) chromosomes, the rumen ciliates possess substantially bigger 'midi-chromosomes' which are, however, much smaller than the macronuclear chromosomes of the aerobic model ciliates *Tetrahymena* or *Paramecium*. Moreover, the AT content of *Nyctotherus* and rumen ciliates is rather different, as is the presence and the size of introns. Detailed analysis of these introns might allow the identification of potential eukaryotic donor organisms.

The analysis of *Nyctotherus* cells from the hindgut of cockroaches fed with powdered *Bt176* maize leaves for three years did not provide any evidence for an acquisition of GMO-DNA

from *Bt176* maize. Also the experiments with GFP-DNA challenged aerobic ciliates did not reveal the uptake and expression of GFP genes. Moreover, the experiments aiming to identify the transfer of a mercury-resistance gene under selective pressure were unsuccessful.

Conclusions

Historical horizontal gene transfer played an important role in the evolution of symbiotic anaerobic ciliates, which thrive in the intestinal tract of large herbivorous mammals and cockroaches. Many of the gene transfers seemed to be related to adaptational processes of the symbiotic ciliates, improving their metabolic performance in the anaerobic intestinal environments provided by their co-evolving hosts. The extremely high incidence of historical horizontal gene transfer in rumen ciliates confirms the postulate that intestinal ciliates can be used as powerful monitors for the detection of potential horizontal gene transfer from GMO to eukaryotes.

So far, no evidence has been found for the evolutionary acquisition of 'toxic' genes by ciliates, which, potentially, could harm their hosts. Also, the incidence of acquisition of plant-derived genes in evolution appears to be extremely low – if it occurs at all. The analysis of rumen ciliates challenged with *Bt176* maize *in vitro* for two years, and of *Nyctotherus ovalis* which was exposed to *Bt176* for three years, failed to provide any evidence for the uptake of *Bt176*-related genes.

The data generated for *Nyctotherus ovalis* contributed also to a better understanding of the evolution of cell organelles: the hydrogenosome of *Nyctotherus ovalis* appeared to be a 'missing link' at the subcellular level. The phylogenetic analysis of hydrogenosomal genes revealed that most of them had a ciliate mitochondrial origin; only a handful was obtained by LGT from bacterial sources. Especially for the hydrogenase gene, which had been assembled in evolutionary times from at least two different bacterial sources, an acquisition by horizontal gene transfer could be established unequivocally.

It was shown that horizontal gene transfer is a naturally occurring phenomenon among eukaryotic symbiotic ciliates, allowing co-evolution with their hosts and an adaptation to their anaerobic environment. At the organelle level, the evolutionary acquisition of genes from various sources is a major source of innovation. The potential acquisition of genes, which are unfavourable for the organelle, the symbiont or the hosts, seems to lead to their rapid elimination.

Major Publications

Guénola Ricard, Neil R. McEwan, Bas E. Dutilh, Jean-Pierre Jouany, Didier Macheboeuf, Mitsumori Makoto, Freda M. McIntosh, Tadeusz Michalowski, Nancy Nelson, C. Jamie Newbold, Eli Nsabimana, Nadine A. Thomas, Kaz Ushida, Johannes H.P. Hackstein and Martijn A. Huynen. (2006). Horizontal gene transfer from bacteria to rumen ciliates indicates adaptation to their anaerobic carbohydrates rich environment. *BMC Genomics* 7:22.

Brigitte Boxma, Guenola Ricard, Angela HAM van Hoek, Edouard Severing, Seung Yeo Moon-van der Staay, Georg WM van der Staay, Theo A. van Alen, Rob M de Graaf, Geert Cremers, Michiel Kwantes, Neil R. McEwan, C. Jamie Newbold, Jean-Pierre Jouany, Tadeusz Michalowski, Peter Pristas, Martijn A. Huynen, Johannes H.P. Hackstein. (2007). The [FeFe] hydrogenase of *Nyctotherus ovalis* has a chimeric origin. *BMC Evolutionary Biology* 7:230.

Effects and mechanisms of *Bt* transgenes on biodiversity of non-target insects: pollinators, herbivores and their natural enemies

Acronym
BT-BIONOTA

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2000-00547

Period
October 2000 – September 2003

Coordinator
Dr. Ruud DE MAAGD
Plant Research International B.V.
Business unit Bioscience
P.O. Box 16
NL-6700 AA Wageningen
The Netherlands
ruud.demaagd@wur.nl

Background and objectives

Bt-BioNoTa is an R&D project, which has sought to address important questions regarding the environmental safety of transgenic insect-resistant crop plants expressing *Bacillus thuringiensis* toxins. More specifically, the effects of such crops on the biodiversity of non-target insects were investigated in a multi-disciplinary approach.

Whereas transgenic insect-resistant crops expressing *Bt* toxins continue to grow in importance in countries outside the European Union, inside the EU there is grave concern about the environmental safety of such crops. Specifically, reports on possible negative effects on the biodiversity of non-target insects in and around fields of *Bt* crops have fuelled growing public and political concerns. While the expected reduced input of chemical insecticides for *Bt* crops may well contribute to the EU policy to develop cost-effective, safe and environmentally friendly plant protection, more scientific data are needed to understand the associated environmental risks to support science-based policymaking.

The main general objectives of the project were to:

- to enlarge the body of data in field experiments comparing *Bt*- and non-*Bt* crops;
- to identify the possible effects of *Bt*-crops on non-target insects in laboratory and greenhouse experiments;
- to enhance the understanding of mechanisms of observed effects;
- to improve the prediction and monitoring of effects on the biodiversity of non-target insects in the field.

Field experiments as well as laboratory experiments, using various transgenic crop plants containing different *Bt* toxin genes, were carried out to study the impact of transgenic *Bt*-plants on biodiversity. The experience gained will contribute to the establishment of monitoring protocols for future use. The laboratory feeding experiments will highlight where potential negative effects on biodiversity may occur in the field, supporting the regulatory process in the establishment of field testing and

monitoring protocols. It will also contribute to the understanding of the mechanism and predictability of an observed lethal effect of *Bt* toxin on an important insect predator, the green lacewing. A molecular tool for the monitoring and prediction of such effects has been developed that will increase the speed and effectiveness of monitoring.

Approach and methodology

The workplan of the project was originally divided into six workpackages, in total consisting of 19 'sub-workpackages":

WP1: *Bt* mode of action in green lacewing;
WP2: cDNA microarrays for measuring toxin effects;
WP3: effects of *Bt* transgenes on tritrophic interactions: Parasitoid biodiversity;
WP4: effects of *Bt* transgenes on pollinator biodiversity;
WP5: effects of *Bt* transgenes on tritrophic interactions: Predator biodiversity;
WP6: effects of transgenic *Bt*-crops on biodiversity of non-target insects in the field.

All described experiments and workpackages made use of the same four crop/*Bt*-gene combinations, with their respective target pests:

- corn expressing Cry1Ab toxin, for resistance to European corn borer;
- eggplant expressing Cry3B toxin, for resistance to Colorado potato beetle;
- potato expressing Cry1Ab toxin, for resistance against potato tuber moth;
- canola expressing Cry1Ac toxin, for resistance against diamondback moth and cabbage white butterfly larvae.

Main findings and outcome

Mode of action of *Bt* toxin on the non-target predator Chrysoperla carnea (green lacewing)

The interaction *Bt*-maize/cotton-leafworm/green lacewing larva was investigated, with the following findings:

- *Bt* toxins Cry1Ab (found in *Bt*-maize) and Cry1Ac (found in *Bt*-cotton) did not show specific binding to lacewing larval gut brush border membranes;
- the amount of Cry1Ab toxin ingested by lacewing larvae through cotton leafworm, which had been feeding on *Bt*-maize, is only approx. 1 % of the concentration found to have detrimental effects in direct feeding experiments;
- gene expression in lacewing larvae, and alterations in response to direct or indirect *Bt* toxin exposure were

Partners

Prof. Dr. Stefan VIDAL
Georg-August-Universitaet Goettingen, Institute for Plant Pathology and Plant Protection, Goettingen (DE)

Prof. Dr. Peter EDWARDS
Swiss Federal Institute of Technology, Institute of Integrative Biology, Zürich (CH)

Dr. Maria-Carola FIORE
Metapontum Agrobios s.r.l., Laboratory of Agronomy, Metaponto (IT)

Prof. Dr. Jozsef KISS
Department of Plant Protection, Szent Istvan University, Budapest (HU)

investigated using a custom-made cDNA microarray consisting of 1 440 lacewing cDNA spots: although direct feeding on toxin has a measurable effect on gene expression in lacewing larvae, indirect exposure (through prey) has much less effect and there is little overlap between the two types of response;
- based on these observations with different techniques, we conclude that it is unlikely that green lacewing larvae are directly affected by Cry1Ab in *Bt*-maize.

Effects of *Bt* transgenes on tritrophic interactions: Parasitoid biodiversity

Several crop plant/herbivore/parasitoid systems were studied for effects of *Bt* transgenes on this type of tritrophic interactions. Our findings were:

- no behavioral influences and no adverse effects of *Bt* maize plants on the foraging efficiency and on the development of the parasitoid *Aphidius rhopalosiphi*;
- no behavioral influences and no adverse effects of *Bt* canola plants on the foraging efficiency and on the development of the parasitoid *Diaeretiella rapae*;
- no adverse effects of *Bt* eggplants on the foraging efficiency and on the longevity of the parasitoid *Aphidius ervi*. Parasitation frequency was higher on *Bt* plants;
- no behavioral influences and no adverse effects of *Bt* eggplants on the foraging efficiency and on the development of the parasitoid *Encarsia formosa*;
- different crops showed considerable differences in production of parasitoid-detectable volatiles between *Bt* plants and isogenic lines;
- since few differences were found in the parasitoids, they apparently have considerable flexibility in their behavior towards olfactory cues.

Effects of *Bt* transgenes on pollinator biodiversity

Pollination by insects is an essential step in fruit and seed formation for many crops, so it is important to understand the impact of transgenes on this process. In order to contribute to the understanding of transgene effects on pollinators, pollinator activity and foraging were studied in two of the crops used in this project, canola and eggplant. Overall our results indicate that effects on pollinators of transgenic plants, at least of those that are important food sources and/or are dependent on insects for pollinators, deserve further attention. The examples studied here indicate that pollinator

behavior may be affected, and it was also apparent that external factors such as location (for example distance to commercial bee colonies) can strongly affect pollinator guild composition and thus the outcome of behavioral studies.

Effects of *Bt* transgenes on tritrophic interactions – predator biodiversity

A thorough analysis was made of food webs (specifically for arthropods associated with the crops) in fields of potatoes and eggplants. A number of bitrophic and tritrophic interactions in both crops were recommended as ecologically significant study objects. Effects of *Bt* crop plants on different non-target insect herbivores in these crops lead to the following conclusions:

- cotton leafworm had higher mortality and longer development time on two types of *Bt*-maize (MON810, *Bt*11);
- mortality and development time of Colorado Potato beetle can be negatively affected by *Bt*-potato;
- no statistically significant effect of *Bt*-eggplant on potato tuber moth mortality and development under laboratory and field conditions;
- spider mites showed preference for *Bt*-eggplant.

Different species of predators were fed with activated *Bt* toxins or a *Bt*-free control in bitrophic experiments or with herbivorous prey raised on *Bt* transgenic or non-*Bt* control plants in tritrophic experiments:

- larvae of the predators green lacewing and two ladybird species (*Adalia bipunctata; Hippodamia variegata*) were in several experiments directly negatively affected when fed with activated Cry1Ab or Cry3Bb proteins;
- development of green lacewing larvae on a natural herbivore complex on caged *Bt*-eggplants in the field was slower;
- no signficant effect on specialist ladybirds (*Stethorus punctillum*) feeding on spider mites on *Bt*-eggplant or *Bt*-maize: however prey density of spider mites was higher on *Bt*-eggplant;
- predatory mites had a prey preference for spider mites from non-*Bt* eggplant, but showed no effects on *Bt*- or non-*Bt*-maize.
- *Bt*-maize does not negatively affect a predator spider species, *Theridion impressum*;
- toxin sensitivity and level and frequency of exposure of these predators to *Bt* toxins will have to be taken into consideration when performing risk assesment of *Bt* crops.

Effects of transgenic *Bt*-crops on biodiversity of non-target insects in the field:

- field trials with Cry3Bb-eggplant: three seasons of field trials with eggplant resulted in the following conclusions: *Bt*-eggplant did not affect overall arthropod biodiversity while, as expected, colorado potato beetle density was lower in *Bt* plots and, curiously, aphid populaton dynamics was different between the plots.
- field trials with Cry1Ab-potato: two seasons of field trials with potato led to the following conclusions: *Bt*-potato did not affect overall arthropod biodiversity.
- field trials with Cry1Ac-canola: two seasons of field trials with canola produced the following conclusions: *Bt*-canola did not affect overall arthropod biodiversity. An important non-target herbivore (leafminer) was not negatively affected by *Bt*-canola.
- field trials with Cry1Ab-maize: three seasons of field trials with maize resulted in the following conclusions: *Bt*-maize did not affect overall arthropod biodiversity over two seasons while, in the third season, biodiversity was lower in the trangenic plots. Cotton bollworm is a significant secondary pest and only partially controlled by *Bt*-maize, and therefore should be taken into consideration for resistance management plants. Other specific herbivore and predator species tested were not affected by *Bt*-maize.

Overall, in field trials over several seasons very few negative effects on arthropod biodiversity were found for the *Bt*-crops tested. Naturally the trials and experiments had their limitations in scope, size and duration. The level of certainty required will determine whether more experimentation and trials are necessary for risk assesment, or not. The project final report contains a proposal for a new framework of enviromental risk assesment (ERA) for transgenic plants and attempts to address the shortcomings of the chemicals-derived testing procedures which have been used for transgenic plants in the past.

Major Publications

de Maagd RA. (2004). Biotechnology meets ecology. In: Nap J-P., Atanassov A., Stiekema WJ, eds. *Genomics for Biosafety in Plant Biotechnology*. Amsterdam: IOS Press:117-131.

Kiss J., Szentkirályi F., Tóth F., Szénási Á., Kádár F., Árpás K., Szekeres D. and Edwards C.R. (2003). Bt Corn: Impact on Non-Targets and Adjusting to Local IPM Systems. In: Lelley T., Balázs E., Tepfer M. (Editors): *Ecological Impact of GMO Dissemination in Agro-ecosystems. Facultas Verlags- und Buchhandels AG Wien*, p.157-172.

Rodrigo-Simón A., de Maagd RA., Avilla C., Bakker PL., Molthoff JW., González-Zamora J., Ferré J. (2006). Lack of detrimental effects of *Bacillus thuringiensis* Cry toxins on the insect predator green lacewing: A toxicological, histopathological, and biochemical approach. *Applied and Enviromental Microbiology* 72: in press.

Conclusions

The interaction of toxin expressed in *Bt*-maize has been studied in several ways. All data presented here suggest that, although Cry1Ab at relatively high dose and continuous exposure may negatively affect lacewing larvae, the toxic action of the protein is not likely to be responsible for the negative effect of *Bt*-maize in a tritrophic interaction. More likely, although difficult to prove conclusively, it is the reduced quality of prey (Cry1Ab-exposed cotton leafworm) or another yet uncharacterised interaction between diet and toxin that negatively affects lacewing larvae under those circumstances. Whether the observed negative effect plays a significant role in the field will have to be determined in field trials.

In summary, with one exception, no major differences in parasitoid behavior, parasitation rate and adult longevity were observed between *Bt*-crops and their isogenic lines. Naturally these results have to be judged in the context of their limited scope and duration, which may fail to detect smaller differences or differences that only occur after several generations, or with other species. Nonetheless the single affected interaction that was detected shows the utility of these experiments for identifying putative hazards.

Major Publications

Rovenská GZ., Zemek R., Schmidt JEU., Hilbeck A. (2005). Altered host plant preference of *Tetranychus urticae* and prey preference of its predator *Phytoseiulus persimilis* (Acari: Tetranychidae, Phytoseiidae) on transgenic Cry3Bb-eggplants. *Biological Control* 33:293.

Tóth F., Arpás K., Szekeres D., Kádár F., Szentkirályi F., Szénási A., Kiss J. (2004). Spider web survey or whole plant visual sampling? Impact assessment of Bt corn on non-target predatory insects with two concurrent methods. *Environmental biosafety research* 3:225-231.

Árpás K., Tóth F. and Kiss J. (2005). Foliage-dwelling Arthropods in Bt-transgenic and in Isogenic Maize: A Comparison through Spider Web Analysis. *Acta Phytopathologica et Entomologica Hungarica* 40:347-353.

Impact of three selected biotechnological strategies for potato pathogen control on the indigenous soil microbiota

Acronym
POTATOCONTROL

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2000-01598

Period
January 2001 – December 2004

Coordinator
J. D. VAN ELSAS
Plant Research International B.V.
P.O. Box 16
NL-6700 AA Wageningen
The Netherlands
j.d.van.elsas@rug.nl

Project website
http://www.plant.wageningen-ur.nl/projects/potatocontrol

Background and objectives

Bacterial phytopathogens, in particular those causing brown and soft rots, pose an immense threat to potato cropping in Europe, with severe economic losses reported yearly in several Member States. In recent years, several non-chemical approaches to controlling these phytopathogens have been proposed and developed. These include transgenic potato lines that produce phage T4 lysozyme, and thus ward off invasions by phytopathogens; transgenic potato lines that produce the lytic enzymes cecropin and/or attacin; and the use of avirulent mutants of the pathogen which compete with the wild-type pathogen in the rhizosphere and effectively interfere with its action. None of these approaches has reached commercialisation as yet, and their putative effects on the environment remain unknown. In particular, there are concerns relating to their impacts, if any, on indigenous microbial populations present in agricultural fields or associated with the potato crop.

This project aimed to explore the effects of selected biotechnological strategies to protect potato plants from pathogen attack on the microbial diversity of the phytosphere. The pathogens studied were *Ralstonia solanacearum* and *Erwinia carotovora* ssp. *Atroseptica*, which are the key bacterial pathogens of potato. The main objectives of this study were:

- to assess the effects of three biotechnological strategies for control of bacterial potato pathogens on the structural and functional biodiversity of the microflora associated with potato;
- to assess the effects of the selected biocontrol strategies on the genetic diversity of the pathogen populations targeted in the potato rhizosphere;
- to evaluate the feasibility of the selected strategies to reduce the threat posed upon potato cropping by bacterial pathogens; and
- to recommend, on the basis of profound knowledge about ecological impact, the most viable strategy for the non-chemical control of the bacterial phytopathogens targeted.

To achieve these aims, extensive testing in microcosms, the greenhouse and the field was necessary. For all purposes, a suite of molecular methods at the cutting-edge of technological development needed to be developed and employed, in combination with a range of available traditional methods.

Approach and methodology

The three selected strategies were the use of (1) potato plants containing T4 lysozyme, (2) plants containing cecropin and attacin determinants, and (3) avirulent forms of *R. solanacearum* as 'niche blockers". In a first phase, the tools necessary to assess the expected effects were developed and validated. Detection methods for the pathogens and microbial biocontrol agents, e.g. green fluorescent protein (GFP) marker gene technologies and a specific primer system targeting *R. solanacearum* (fliC), were developed. Further, overall and group-specific molecular microbial community fingerprinting tools were developed or evaluated in order to demonstrate eventual effects related to transgenic plant-released effectors. Clone libraries were constructed from bulk soil DNA samples, in order to establish the most dominant species associated with potato at both locations, Spain and the Netherlands. Activity-based methods were evaluated to explore active microbial fractions in bulk and rhizosphere soils of field plots with transgenic plants. Next, a 'best' strategy to protect potato from pathogen attack was assessed in greenhouse experiments. Unfortunately, permits to apply modified (avirulent) pathogen forms could not be obtained from the competent Member State authorities, preventing field testing of the microbial agents. After the selection of the best strategy, field studies were performed in two member states, Spain and the Netherlands.

Main findings and outcome

The avirulent *R. solanacearum* mutants provided protection against invasion of potato plants by *R. solanacearum* in initial screens, but failed to do so in subsequent soil microcosm experiments. One T4-lysozyme potato line, DL-12, was found to confer optimal protection of the plant against both *R. solanacearum* and *E. carotovora*. Other transgenic potato lines did not confer similar resistance to the applied pathogens, so this technology (i.e. the DL-12 line) was selected for field testing.

First, a baseline study executed in the field in two Member States assessed the dynamics in the natural microflora associated with wild-type potato. A considerable variation was

Partners

Kornelia SMALLA
Biologische Bundesanstalt (BBA), Braunschweig (DE)

André TRIGALET
INRA/CNRS, Toulouse (FR)

Maria MILAGROS LOPES
IVIA, Valencia (ES)

Julian SMITH
CAB International, Surrey (UK)

Janet JANSSON
Södertörns Högskola, Huddinge (SE)

Angela SESSITSCH
ARCS, Seibersdorf (AT)

Fig 1.
Experimental field in Emmeloord (NL) shortly after planting.

found with time, i.e. potato growth phase was a key determinant of plant-associated microflora. Furthermore, site (Spain versus the Netherlands) and potato variety affected the microbial diversity around the plants. Considerable numbers of beneficials, most identified as *Lysobacter* spp. types, were detected and isolated from the field-grown potato plants both from the rhizosphere and from the endosphere. In a second and last phase, field testing of the transgenic line DL12 versus the wild-type (Desiree) was performed in the same two Member States. Unfortunately, the experiment in the Netherlands was destroyed by activists; however part of this experiment could be saved, still yielding useful data. Together, both experiments again indicated that plant growth phase and site (Spain versus the Netherlands) were the most important determinants of plant-associated microbial community structures.

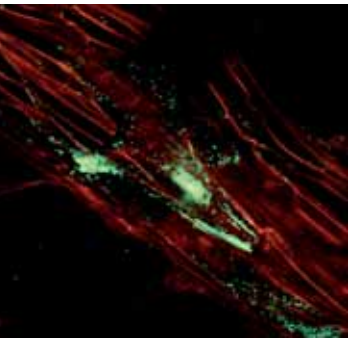


Fig 2.

GFP-expressing R. solanacearum strain 1609::gfp cells in roots of potato plants.

An ephemeral and small difference between the microbial diversity associated with the DL12 and the wild-type line was noted, most notably in the Spain field. However, the effect was minor, and cannot be attributed to the genetic modification *per se*, as the two lines were not strictly isogenic and revealed differences in growth. Other major factors that control these potato-associated communities may also be responsible for observed effects, for instance the origin and age of the seed potatoes. An effect of the transgenic line on the diversity of potential beneficials was not noted.

Fig 3.

Biological control procedure at day + 30 after the challenge inoculation with 1609 Rif'.

The selected biotechnological strategies to control two major bacterial phytopathogens have clear drawbacks in their application. The avirulent strains applied as biocontrol agents showed promise but demonstrating full protection was problematic and further exploration of this approach is needed. Moreover, their ecological competence may be affected by the genetic modification. Also in this study, T4 lysozyme expression in DL12 did not provide full protection against *R. solanacearum* and it must therefore be concluded that the *R. solanacearum* population that infects potato plants is not fully affected by T4 lysozym. Possibly, the *R. solanacearum* cells that invaded the plant tissue are protected from the transgenic product, or the expression of T4 lysozyme is too low to ward off the penetration of R. solanacearum into the roots. Enhanced and tissue-specifically controlled T4 lysozyme gene expression in the transgenic potato lines may be required to further improve the biocontrol in transgenic T4-lysozym lines.

Conclusions

In conclusion, only transgenic potato line with bacteriophage T4 lysozyme showed limited promise as a controller of the bacterial pathogens. Enhanced expression and tissue-specific control of expression of the transgene in the transgenic potato lines may be an important improvement for more effective suppression of the target pathogens.

However, all three proposed strategies aimed to control the selected phytopathogens in potato showed serious drawbacks for applications. Genetic modification in potato with a proposed strong effect on the microbial community structure (T4 lysosyme) did not reveal dramatic changes on the indigenous soil and plant-associated community. Other environmental factors like site and growth phase posed stronger effects on the microbial community structure of these plants. Small observed effects in plant-associated community structures may not be related to the genetic modification *per se*, but may be attributed to small differences in seed potatoes. Seed potatoes must be strictly isogenic and derived from the same source when differences in plant-associated community structures between wildtype and transgenic plants are determined.

Genetically modified avirulent derivatives of major phytopathogens may be successful in combating diseases, however concerns about the possible conversion to virulence and reduced ecological competence limit further exploration of this approach. Moreover, this technology could not be taken to full testing as a result of regulatory constraints. Genetic modification of plants by insertion of phytopathogen-antagonistic genes may be more promising; although it cannot be excluded that broad-spectrum activity of the transgenic product may pose an effect on the antagonistic communities near plants.

Next to the novel detection systems developed under this project, recommendations to EU Member State authorities on the effects of transgenic plants on soil microbiota represent the most important outcome of this project. Screening of microbial diversity and antagonistic populations in and/or near transgenic plants via a culture-independent approach was successfully developed and applied. It is therefore recommended that this (polyphasic) culture-independent microbial screening to test effects of new transgenic constructs on plant-associated communities be accepted as routine procedure in biosafety regulations.

Testing integrated GM-rhizoremediation systems for soil bioremediation

Acronym
RHIZOREMEDIATION

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2001-00101

Period
January 2002 – December 2004

Coordinator
D.N. DOWLING
Institute of Technology Carlow
Carlow
Ireland

Partners

R. RIVILA
Universidad Autonoma de Madrid (ES)

F. O'GARA /J. MORRISSEY
BIOMERIT Research Centre, University College Cork (IE)

U. KARLSON
National Environmental Research Institute (NERI), Roskilde (DK)

H-C PEDERSEN
Danisco Seed, Seed Technology Department, Holeby (DK)

M. BITTENS/A. REIN
University of Tuebingen, Tuebingen, and UFZ Centre for Environmental Research Leipzig-Halle, Leipzig (DE)

T. MACEK
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague (CZ)

S. FRANCISCONI
Armed Forces Institute of Pathology, Washington DC (USA)

Background and objectives

Rhizoremediation is the combined application of plants and rootzone bacteria for the clean-up of polluted soil. The first objective of this project was to develop multifunctional bacteria engineered for *in situ* PCB degradation, to signal the bio-availability of the pollutant, the efficacy of the bioremediation strain, the formation of the intermediate product chlorobenzoates (CBA), and determination of the endpoint of degradation.

The second objective was to develop molecular detection methods for tracking degrader bacteria in the environment in order to quantify the GMO strains under environmental conditions and to monitor effects on microbial community structure in the polluted soil. The final objective was to undertake mathematical environmental modelling of the process and prepare a detailed risk assessment (and guidelines) of the genetically modified organism (GMO) and the bioremediation.

Approach and methodology

Gfp promoter fusions were used to generate biosensor strains that could monitor the end-product of the bioremediations process, chlorobenzoates. Strains were constructed with enhanced expression of the PCB-degrading pathway using nod box promoters and nodD regulatory circuits. Real-time Polymerase-Chain Reaction (PCR) methodologies were used to identify and quantify the GMOs. The impact of introduced GMOs on bacterial populations was monitored through molecular microbial ecology methods such as Thermal Gradient Gel Electrophoresis (TGGE) and screening of group specific genotheques. Plant tissue cultures were assessed for degradation of PCBs and their end-products. The GMOs were tested in realistic mesocosms using real PCB-contaminated soils. Data generated were used in modelling experiments to predict the risk reduction of the GMO-driven process.

Fig 1.
Gm-Rhizoremediation – Inoculation of Willow plants with degrader bacterial to test Rhizoremediation. Willow stems are first rooted in water and then inoculated with the PCB-degrader bacteria. Plants and bacteria are planted in soil contaminated with PCBs.

Main findings and outcome

One of the most exciting aspects of the project was the development of integrated biosensor strains facilitating *in situ* monitoring of PCB remediation. The system developed allowed the detection of bio-available PCBs at levels as low as 100nM. In particular, these tools will allow efficacy monitoring of bioremediation inoculants in real situations. The findings will lead to a better understanding of those measures to be applied for optimisation of bioremediation technologies.

Molecular tools were developed for the identification and unambiguous quantification of genetically modified bacterial strains in the environment. The GMO can be quantified to a level of 1 to 10 cells per gram of soil in approximately 90 minutes. The impact of the inoculant on microbial community composition was shown to only affect the rhizosphere of the inoculated plant and not to significantly spread beyond. Extensive testing of the system in willow plants and other plant species indicated that the GMO significantly increased the removal of total PCBs in the presence of plants, in contrast to plants with autochtonous (indigenous) microbes.

Mathematical modelling confirms the laboratory data and suggests that the use of the GMO process would significantly shorten the clean-up time of contaminated soils. A detailed impact assessment on the use of the GMO strains was carried out. Overall, a general risk reduction would be expected if the process was used in real situations. A set of guidelines was drawn up on the safe use, advantages and limitations of this GMO-based bioremediation technology.

Conclusions

Although working with a model system, the project has generated results of general interest that can be applied to bioremediation and environmental protection. These include:

- characterised rhizosphere/soil strain(s) to degrade PCB: principle can be extended to other target compounds;
- inoculation technologies to introduce strains to the contaminated soil/rhizosphere environment;
- biosensor-functionality of strains for monitoring *in situ* activity: this could be extended to other compounds;
- high-level gene expression system for *Pseudomonas* (based on *Sinorhizobium* nod Box);
- risk assessment – GMOs, metabolic end-products and mathematical modelling.

Major Publications

Aguirre de Carcer DA., Martin M., Mackova M., Macek T., Karlson U., Rivilla R. (2007). The introduction of genetically modified micro-organisms designed for rhizoremediation induces changes on native bacteria in the rhizosphere but not in the surrounding soil. *ISME J.* 1 (3):215-223.

Francova K., Sura M., Macek T., Szekeres M., Bancos S., Demnerova K., Sylvestre M. and Mackova M. (2003). Generation of plants carrying bacterial enzyme for degradation of polychlorinated biphenyls. *Fresenius Environmental Bulletin* 12 (3):309-313.

Hogan J., Sherlock O., Ryan D., Whelan C., Francesconi S., Rivilla R. and Dowling D.N. (2004). Fluorescence resonance energy transfer (FRET) based molecular detection of a genetically modified PCB degrader in soil. *FEMS Microbiology Letters* 236:349-357.

Larrainzar E., O'Gara F., Morrissey JP. (2005). Applications of Autofluorescent Proteins for In Situ Studies in Microbial Ecology. *Annu Rev Microbiol.* 59:257-77.

Marta Villacieros, Clare Whelan, Martina Mackova, Jesper Molgaard, María Sánchez-Contreras, Javier Lloret, Daniel Aguirre de Cárcer, Roke I. Oruezábal, LuisBolaños, Thomas Macek, Ulrich Karlson, David N. Dowling, Marta Martín and Rafael Rivilla. (2005). PCB Rhizoremediation by *Pseudomonas fluorescens* F113 derivatives using a *Sinorhizobium meliloti* nod system to drive *bph* gene expression. Appl. *Environ. Microbiol.* 71:2687-2694.

Acronym
SUSTAIN

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2001-01461

Period
October 2001 – September 2005

Coordinator
Dimah Z. HABASH
Rothamsted Research
Harpenden, Hertfordshire,
AL5 2JQ, UK

Developing wheat with enhanced nitrogen use efficiency towards a sustainable system of production

Background and objectives

Nitrogen use efficiency (NUE) is a new driver for breeding wheat in response to concerns about excessive use of chemical fertilisers, economic costs and environmental pollution. It is in the public interest to support breeding efforts to screen for wheat varieties able to use nitrogen more efficiently by providing the underpinning plant science. Genetic diversity is the material basis for crop improvement and we designed a project to exploit natural and transgenic variation to deliver cultivars with improved agronomic performance. Improved nutrient use efficiency requires novel target traits and we focused on the understanding of plant factors defining nitrogen metabolism and use. Plant markers are needed for key traits that can be used in molecular screening to increase both the level and stability of nitrogen use efficiency to support sustainable agriculture. These markers can then be incorporated into high-yielding varieties using conventional or biotechnological tools in the medium to long term. This project proposed a two-track approach to develop new wheat genotypes using genetic modification and to develop molecular genetics to screen for allelic variation and new molecular traits.

Approach and methodology

A quantitative genetics approach was used to study nitrogen use (NU) in wheat because it is a complex multigenic trait. Linkage analysis was used to dissect component traits, identify new traits and position candidate genes under loci- the first ever study of NU in bread wheat. We used a mapping population of bread wheat and established phenotypes and quantitative trait loci (QTL) relating to physiological, developmental, yield related and biochemical traits. To compare loci and data with rice and maize comparative genomics were used and the enzyme of primary nitrogen metabolism, glutamine synthetase (GS), was mapped to this population.

The latest genetic modification (GM) technology was used to produce new transgenic wheat to test the importance of GS for wheat NU. A number of independent transformed lines in several genotypes, doubled haploid homozygous lines, were produced, transforming a cultivar with construct over-expressing bean GS1 lacking the ampicillin transformation marker. Primary nitrogen acquisition was studies, ammonium transport, inorganic nitrogen status, nitrogen metabolism and photosynthesis of select wheat DHL and transgenic lines. Subsequent screening of the new wheat transgenics for nitrogen use, growth and productivity took place in field trials in Spain.

We cloned the full complement of the wheat GS gene family and compared the findings to rice and maize. The expression and protein profile of the wheat GS gene family during plant development was studied in different tissues, during N starvation and feeding, in plants in the field and related this to growth. We localised some of the GS genes using latest developed immunolocalisation techniques in wheat and maize. We studied the function of cytosolic GS in maize using special mutant lines.

Partners

Betrand HIREL
Institut National de la Recherche Agriculture (FR)

Jens WEYEN
Saaten-Union GmbH (DE)

Jan K. SCHJORRING
The Royal Veterinary and Agricultural University (DK)

Gilles PELTIER
Commissariat à l'Energie Atomique (FR)

Jose L. ARAUS
University of Barcelona (ES)

Major Publications

Jahn T.P., Møller A.L.B., Zeuthen T., Holm L.M., Klærke D.A., Mohsin B., Kühlbrandt W. and Schjørring J.K. (2004). Aquaporin homologues in plants and mammals transport ammonia. *FEBS Letters* 574:31-36.

Quarrie SA., Steed A., Calestani C., Semikhodskii A., Lebreton C., Chinoy C., Steele N., Pljevljakusic D., Waterman E., Weyen J., Schondelmaier J., Habash DZ., Farmer P., Saker L., Clarkson DT., Abugalieva A., Yessimbekova M., Turuspekov Y., Abugalieva S., Tuberosa R., Sanguineti MC., Hollington PA., Aragues R., Royo A., Dodig D. (2005). A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring X SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet. 110 (5): 865-880.

Martin A., Lee J., Kichey T., Gerentes D., Zivy M., Tatout C., Dubois F., Balliau T., Valot B., Davanture M., Tercé-Laforgue T., Quilleré I., Coque M., Gallais A., Gonzalez-Moro MB., Bethencourt L., Habash DZ., Lea PJ., Charcosset A., Perez P., Murigneux A., Sakakibara H., Edwards KJ. and Hirel B. (2006). Two Cytosolic Glutamine Synthetase Isoforms of Maize Are Specifically Involved in the Control of Grain Production. Plant Cell 18:3252-3274.

Main findings and outcome

The genetic studies on the bread wheat mapping population demonstrated the importance of the enzyme glutamine synthetase in controlling aspects of wheat NU. The QTL dataset of 21 traits represents a valuable resource to compare with similar studies in rice and maize and to enable us to uncover generic or species specific regulatory factors that govern nitrogen use. Our results demonstrated genetic variation in NU, particularly GS content and activity, in wheat germplasm and cultivars and this forms a basis to test for alleleic variation in future breeding programmes.

This project highlighted the complexity of genetically modifying enzymes of primary metabolism in wheat for the purpose of modifying a complex quantitative trait. Whilst we produced several transgenic lines, the expressions of the transgenes were complex and prevented us from using this technology to produce wheat lines with higher NU. Unfortunately, the destruction of the GM field trials in the summer of 2004, the first such incident in Spain, disabled us from establishing any definitive phenotype for our transgenic lines in the field environment.

A major deliverable for the project and the scientific community is the cloning, mapping and study of GS gene expression in wheat. This represents the first study of this important enzyme in wheat and we have produced the first detailed profile in a matrix of development, tissue, developmental stage and environment. Comparison with similar studies in rice and maize will enable us to formulate a generic model for NUE in cereals.

Conclusions

This project highlighted the power of using complementary genetic approaches to create and test scientific knowledge in crop science. The use of transgenic technology was not promising in modifying our candidate enzyme of primary metabolism glutamine synthetase and in creating germplasm with improved NUE. This was further exacerbated by the destruction of our experimental transgenic field trials in the summer of 2004 in Spain. The genetical linkage analysis was successful as a complementary study in identifying new genomic regions regulating aspects of N metabolism, assimilation and remobilisation. This should help plant breeders in marker assisted selection and in the search for allelic variation in traits that improve nitrogen use in wheat.

Fig 1.

Destruction of the wheat GM trials of the SUSTAIN project in July 2004.

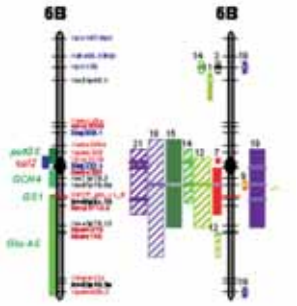


Fig 2.

Chromosome 6B of bread wheat showing quantitative trait loci for yield related traits and candidate genes.

Major Publications

Lopes MS., Cortadellas N., Kichey T., Dubois F., Habash DZ. and Araus JL. (2006). Wheat nitrogen metabolism during grain filling: comparative role of glumes and the flag leaf. Planta 225 (1):165-181.

Habash DZ., Bernard S., Schondelmaier J., Weyen J. and Quarrie SA. (2007). A genetic study of nitrogen use in hexaploid wheat in relation to N utilisation, development and yield. Theor Appl Genet. 114(3)403-419.

Stéphanie M. Bernard, Anders Laurell Blom Møller, Giuseppe Dionisio, Thomas P. Jahn, Marcela Baudo, Marta S. Lopes, Thérèse Tercé-Laforgue, Christine H. Foyer, Martin Parry, Brian G. Forde, Jose Luis Araus, Bertrand Hirel, Jan K. Schjoerring, Dimah Z. Habash. (2008). Gene expression and function of glutamine synthetase isozymes in wheat (*Triticum aestivum L.*). Plant Molecular Biology. 67:89-105.

Acronym
ANGEL

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2001-01657

Period
September 2001 – December 2005

Coordinator
C. VAN DE WIEL
Plant Research International
Droevendaalsesteeg 1
PO Box 16
NL-6700 AA Wageningen
The Netherlands
c.c.m.vandewiel@plant.wag-ur.nl

Project website
http://www.plant.wageningen-ur.nl/projects/angel

Analysis of geneflow from crop to wild forms in lettuce and chicory and its population-ecological consequences in the context of GM-crop biosafety

Background and objectives

The overall objective of this project was to contribute to a framework for assessing the safety of introduction of genetically modified organisms (GMOs) by establishing the primary risks of escape and their consequences for the natural environment. Transgenic plants have a high potential for improving both agriculture and the environment, but their introduction has led to serious public concern about the accompanying risks, amongst others, the consequences for the natural environment. Possible impacts relate to the escape of transgenic plants or transgenes through outcrossing with wild relatives. The fear is that escape will result in the development of noxious weeds that disrupt agricultural systems or vulnerable natural ecosystems.

A series of studies has appeared on crops like oilseed rape, while this project set out to study two model crops from the aster family, chicory and lettuce, on both of which little had yet been published. Chicory is an example of an obligatory outcrosser. Lettuce is regarded as basically a selfing species, but outcrossing is known to occur, though its extent and variability have been little studied. The wild form of lettuce, prickly lettuce, could also serve as a model species (for increased weediness), since it has expanded its distribution enormously in Europe in recent decades.

The research in this project had the following specific objectives:

- to establish the degree of outcrossing under field circumstances in chicory and lettuce;
- to trace evidence of introgression from cultivated to wild chicory and from cultivated lettuce to wild lettuce;
- to study the consequences of gene flow from cultivated to wild forms by assessing fitness effects in field trials and by demographical monitoring of both natural and experimental crop-wild hybrid populations;
- to obtain insight into the recent expansion of the wild form of lettuce by characterising genetic diversity of populations across Europe and by comparing historical

with recent vegetation data for changes in the habitats in which wild lettuce occurs;
- to integrate the results into a model from which recommendations could be derived for handling ecological aspects in procedures for assessing GM-crop biosafety.

Approach and methodology

The degree of outcrossing and gene flow in wild populations of lettuce and chicory was determined by using the co-dominant molecular marker method of microsatellites in lettuce and the dominant multi-locus marker system of AFLP (Amplified Fragment Length Polymorphism) in chicory. In addition, crop to wild outcrossing was studied in field trials in chicory by scoring a colour marker on progeny and, in lettuce, by scoring morphology of progeny and subsequent checking by either a RAPD (Random Amplified Polymorphic DNA) or a real-time PCR (Polymerase Chain Reaction) marker designed from an SSR (Simple Sequence Repeats) locus.

To trace evidence of introgression from crop to wild forms, natural populations were screened by AFLP and SSAP (Stage-Specific Activator Protein), both having the advantage of efficiently generating a large number of markers across the whole genome. To ascertain a representative sampling of the genome, genomic linkage map information was generated, so that introgression of specific parts of the genome could be traced. SSAP is an innovative transposable element-based PCR method. To trace introgression of possibly fitness-related traits, an innovative PCR method was implemented, namely NBS (Nucleotide Binding Site)-directed profiling, which accesses variation around disease resistance genes that are used intensively in breeding.

Possible influences of introgression on fitness were studied by determining fitness-related parameters in experimentally created hybrid and backcross populations under field and greenhouse conditions, and combining these data with molecular marker scores on surviving plants. To put these parameters in perspective, both the experimental hybrid populations and natural populations of lettuce were also demographically monitored, leading to matrix projection models. These models enable identification of crucial stages in the species life cycle where changes in performance will have the largest impact on population growth and fitness.

In an attempt to reconstruct the recent increase of wild lettuce in north-western Europe, genetic variation was screened

Partners

H. DEN NIJS
University of Amsterdam (NL)
Andrew FLAVELL
University of Dundee (UK)
R. BAGGER JØRGENSEN
Risø National Laboratory
Roskilde (DK)
F. FELBER
Université de Neuchâtel (CH)
M. VISCHI
University of Udine (IT)
A. SØRENSEN
Keygene N.V. Wageningen (NL)

on a north-south and an east-west transect through Europe, using co-dominant microsatellites and maternally inherited chloroplast DNA markers. Historic and recent vegetation samples from databases were compared to determine whether wild lettuce is also invading new vegetation types.

Main findings and outcome

In field experiments with chicory, outcrossing rates followed the usual pattern with distance: maximal within the first 20 m and dropping steeply to less than 1 % at 60-90 m. With the basically selfing lettuce, outcrossing rates were found at a significant but mostly not higher level than 5 %, but considerably higher outliers were scored in one of the study years. With natural populations of wild lettuce, too, little heterozygosity was found to study gene flow by pollen. Therefore, seed flow was tested by autocorrelation, which showed most dispersal within ten metres, but evidence for dispersal could be found up to more than 100 m. In natural chicory populations, pollen flow could be established mostly within the first few meters, but some were in the order of 100 metres, the maximum distance tested.

The novel retrotransposon-based SSAP and the resistance gene-based NBS profiling marker methods were successfully developed, and, together with the established AFLP marker method, were applicable in making genomic maps on crop to wild crosses made for the field studies. In chicory, ample indications of introgression between crop and wild were found, the amount of which varied between plants. In the selfing lettuce, introgression could be low and therefore more difficult to trace. Indeed, haplotype analyses to trace evidence of introgression from crop to wild lettuce by searching for genomic segments combining cultivar-specific markers proved to be problematic. The principal problem with allegedly crop-specific haplotypes was in making the distinction between a recent introgression from cultivated lettuce or an origin based on common ancestry from before or around the time of lettuce domestication. Comparison of large microsatellite datasets on both crop and wild lettuce failed to improve this significantly, though rare indications of introgression were found in southern Europe.

In chicory, monitoring of an existing population consisting of a mix of cultivated and wild chicory, and field trials with experimentally produced hybrid lines, showed that there are apparently no barriers to hybridisation (backcrossing) beyond the F1 generation, and that hybrids are are at least as

capable of survival as wild types, depending on environmental conditions. In lettuce, demographic monitoring of natural populations and field trials with experimentally created hybrid lines also showed good survival of hybrid lines in the field, although there is a clear breakdown of vigour over subsequent hybrid generations. However modelling of the results indicated a clear possibility of displacement of wild lettuce or hybrid swarm development, depending on environmental conditions and stochasticity.

An extensive inventory of herbarium and floristic data showed that during the twentieth century, prickly lettuce extended its distribution all over Europe, except for the northern part of the UK and Scandinavia. An association of this expansion with climate warming could be found, but it is not yet clear to what extent other factors may have been involved, such as increased human disturbance of habitats or introgression from the crop. Both chloroplast DNA polymorphisms and nuclear microsatellite diversity indicated several founder events in the spread of prickly lettuce across Europe, but there is no clear geographic pattern in the distribution of its genetic diversity. This is consistent with dispersal being in part related to human activity whereby both short and long distance migrations are possible, on top of a more gradual historical diffusion. Vegetation database analyses clearly show a broadening of prickly lettuce's ecological amplitude towards more shady habitats. This could be indirectly related to climate warming or to the fact that large increases in normal ruderal habitats could increase the seed pressure on other habitats.

Conclusions

The results on chicory show evidence of the occurrence of introgression between crop and wild forms and that hybrids are able to survive and reproduce and thus persist in the field. The results on lettuce also show the possibility of both outcrossing from crop to wild forms and the persistence of hybrids in the field. However, in this mainly selfing species, evidence for introgression actually occurring in the field has been scarce and hard to obtain up till now. The feasibility of modelling for the benefit of risk assessment has been demonstrated with the aid of matrix projection analysis of demographic monitoring results. The broadening of wild (prickly) lettuce's geographic and ecological amplitude in Europe could at least be related in part to climate warming. However, other factors could also still be of relevance, such as increases in ruderal areas by man or micro-evolution, possibly in part influenced by introgression from the crop.

Major Publications

Hooftman D.A.P., Oostermeijer J.G.B., Jacobs M.M.J. & den Nijs J.C.M. (2005). Demographic vital rates determine the performance advantage of crop-wild hybrids in Lettuce. *Journal of Applied Ecology* 42:1085-1095.

Meyer G., Tveit G., Jørgensen R.B., Folker A.F., von Krauss M.K. and Sandøe P. (2005). The factualization of uncertainty: Risks, politics, and genetically modified crops – a case of rape. *Agricultural and Human Values* 22:235-242.

Syed N.H., Sørensen A., Antonise R., van de Wiel C., van der Linden C.G., van 't Westende W., Hooftman D.A.P., den Nijs J.C.M. & Flavell A.J. (2006). A detailed linkage map of lettuce based on SSAP, AFLP and NBS markers. *Theoretical and Applied Genetics* 112:517-527.

Van de Wiel C., Groot M. & den Nijs H. (2005). Gene flow from crops to wild plants and its population-ecological consequences in the context of GM-crop biosafety, including some recent experiences from lettuce. In: Wesseler, J. (ed.) Environmental Costs and Benefits of Transgenic Crops. *Wageningen UR Frontis Series* vol. 7, Springer, Dordrecht.

Van de Wiel C., Flavell A., Syed N., Antonise R., Rouppe van der Voort J. & van der Linden G. (2004). Analysis of gene flow in the lettuce crop-weed complex. In: Den Nijs J., Bartsch D. & Sweet J. (eds.) Introgression from genetically modified plants into wild relatives. *CABI Publishing, Wallingford UK*, pp. 163-171.

Gene flow from transgenic plants: evaluation and biotechnology

Acronym
TRANSBAC

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2001-02242

Period
January 2002 – June 2005

Coordinator
Timothy M. VOGEL
Université de Lyon
Villeurbanne Cedex
France

Background and objectives

The TRANSBAC project goals included the evaluation of gene flow from transgenic plants to environmental bacteria and the development of technology to regulate this gene flow. The transgenic plant used was a transplastomic plant, where the transgene is in the chloroplast genome. The interest in this type of transgenic plant is due to the low probability that pollen will contain the transgene and the greater number of transgenes in the plant. Transplastomic plant pollen is thought not to pose any risk of gene flow through cross-fertilisation. The higher number of chloroplast genomes relative to nuclear genomes is part of the reason transplastomic plants can have larger quantities of the associated protein. Thus, the selected activity of the transplastomic plants can exceed that of nuclear transgenic plants. Yet, these characteristics of transplastomic plants might enhance gene flow to environmental bacteria due to the increased number of transgenes and their presence in chloroplast genomes, which have a greater similarity to bacteria than nuclear genomes.

Approach and methodology

The TRANSBAC project evaluated the barriers to gene flow (or gene transfer) to environmental bacteria by examining several different aspects of gene flow. First, the fate of the transgenes in the dying rotting plant was evaluated. The fate of the transgenes that reached the soil was also explored. Then, several biological models of transgene recipients were studied. For example, the possibility that plant pathogens (e.g., *Ralstonia solanacearum*), which invade the plant and degrade the plant cells, are exposed to the transgenes and might possibly incorporate the transgene in their genomes was considered. In addition, another model bacterium, *Acinetobacter* sp., which has abnormally high transformation frequencies, was used to increase the likelihood of gene transfer. In both cases where model organisms were used, special genetic constructs were implemented to provide homologous sequences to the chloroplast genome with these recipient bacteria. These homologous sequences increased gene transfer frequencies.

Finally, two more natural approaches were attempted using indigenous bacteria as the recipients for the transgene. One was under rotting plant conditions as described above with *Ralstonia solanacearum*, and the other was in soil subjected to lightning. In addition to the different models and systems used to evaluate the potential for gene transfer from transplastomic plants to bacteria, work on developing biotechnology that could help reduce or eliminate this potential transfer was also undertaken.

In order to be able to monitor the gene flow from transplastomic plants to bacteria, plants that had the appropriate transgenes in the chloroplast genome were constructed. These constructions had several different combinations of antibiotic resistance genes and lindane degradative genes between two chloroplast genes (*rbcL* and *accD*). The antibiotic resistance genes used were *aadA* and *nptII*. The lindane (pesticide) degrading gene was *linA*. The advantage of adding the lindane degrading gene is its rarity in most soil systems. The antibiotic resistance genes exist already at relatively high concentrations in the soil microbial community. Finally, some exploration of a fluorescent marker *gfp* was also performed.

All these genes needed to be expressed in a wide range of recipients and to be relatively selective in order to reduce the gene transfer detection threshold. One possible biotechnological offshoot of these plant constructions is the plant containing *linA* and which is capable of degrading lindane. These transplastomic plants were used for the range of experiments on gene flow. The result of this work probably applies to a much wider range of potential transgenes than the model genes used in this project.

Main findings and outcome

Subsequent experiments addressed the immediate fate of the transgenes *in planta* and then in the soil. The possible degradation of the transgene *in planta* was studied with only the plant's nucleases responsible for the transgene degradation and a range of pathogenic simulated conditions including the presence of pectinases and cellulases, and the invasion of *Ralstonia solanacearum*. In general, around 98 % of the transgene was degraded before reaching the soil; however, during the invasion by *Ralstonia solanacearum*, many soil bacteria were able to co-infect the plant and thus come into contact with the plant DNA. In evaluating the possibility of gene transfer *in planta*, the transfer between *Ralstonia solanacearum* and *Acinetobacter* was demonstrated, as was

Partners

Philippe AURIOL
Centre de Génie Electrique de Lyon (CEGELY), Ecully (FR)

Ghyslaine TISSOT
Aventis CropScience, Lyon (FR/DE)

Asa FROSTEGARD
Agricultural University of Norway, Aas (NO)

Jan Dirk VAN ELSAS
Plant Research International B.V., Wageningen (NL)

Renaud NALIN
Libragen, Caluire, Caluire (FR)

Yves DESSAUX
ISV-CNRS, Gif-sur-Yvette (FR)

Christoph C. TEBBE
Bundesforschungsanstalt für Landwirtschaft (FAL), Braunschweig (DE)

Kornelia SMALLA
Federal Biological Research Centre for Agriculture and Forestry (BBA), Braunschweig (DE)

Daniele DAFFONCHIO
Università degli Studi di Milano, Milano (IT)

that between the plant and *Acinetobacter* after infection of the plant by Ralstonia solanacearum and when the recipient *Acinetobacter* contained sequence homologous to *rbcL* and *accD* which were inserted artificially. Lack of these homologous sequences led to the absence of measurable transformants containing the transgene.

Recipient strains	Transformation *in vitro* [1] (frequencies)				Transformation *in planta* [2] (number of transformants)	
	Plasmid pLEP01 [5]	**Pure plant DNA**	**Crushed plant tissues (vein)**	**Crushed plant tissues (foliar)**	**Nuclear transgenic plant** [6]	**Plastid transgenic plant**
Acinetobacter sp. BD413 [3]	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	0	0
Acinetobacter sp. BD413 + pBAB2 [4]	$6 (\pm 3.4) \times 10^{-3}$	$4.1 (\pm 2.3) \times 10^{-6}$	$2.6 (\pm 2.1) \times 10^{-8}$	$6.4 (\pm 2.8) \times 10^{-8}$	0	31

Table 1.

From Kay et al 2002 AEM.

1 Expressed as transformation frequencies: Number of transformants per recipient cell.
2 Expressed as a total number of transformants. For the combination transplastomic plants – *Acinetobacter* sp. BD413 + pBAB2 – experiments were conducted five times and independently. Three of the five plants produced transformants. Out of the 21 independent central leaf veins harvested from the 3 plants, 8 produced 31 transformants.
3 *Acinetobacter* sp. BD413 (without any sequence to promote homologous recombination with plant DNA).
4 *Acinetobacter* sp. BD413 + pBAB2 (harbouring plastid-borne sequences on which homologous recombination could occur).
5 Plasmid pLEP01 cannot replicate in *Acinetobacter* sp. BD413. Transformants resulted from homologous recombination-based integration events only.
6 Based on the use of nuclear transgenic tomato plants pKHG3 and *Acinetobacter* sp. BD413 harbouring or not plasmid pFG4AnptII, homologous recombination occurring potentially on nptII sequences.

Once the plant DNA containing the 2% transgene that survived plant death reached the soil, soil microbial nucleases were able to further degrade the transgene. However, the transgene was in part protected by its adsorption on soil mineral surfaces and in part by the downward movement of rainwater, which drove the transgene out of the microbially-active soil top layer. The long-term fate of the transgene is unknown, but experiments lasting four years still found signs of the transgene in soil, and this becomes susceptible to incorporation by indigenous bacteria and ingestion by soil macrofauna.

These subsequent experiments with transplastomic plant DNA in soil demonstrated the low likelihood of gene flow from plants to soil bacteria. On the other hand, an affirmation of negative

results is difficult. Indigenous bacteria containing sequences that are not too distantly related to the chloroplast flanking sequences, *rbcL* and *accD*, to allow for homologous recombination were screened, but very few existed and, for those that did, the gene transfer mechanism is unknown. In the soil contaminated with transplastomic DNA, other models were used in order to determine whether gene transfer could occur even under artificially favourable conditions. Again, the existence of homologous sequences was needed in order to observe any gene transfer. Since these sequences were always artificially inserted into the recipient bacteria, a screening of indigenous bacteria containing these sequences was made. The number of positive hybridisations was limited but not zero. The likelihood of transfer was not further evaluated.

The apparent dependence of gene transfer from transplastomic plants to environmental bacteria on homologous sequences led to the possibility that metagenomic screening of sequences could identify the chloroplast sequence with the least and also the highest probability of bacterial homologues. This screening could then provide critical information for the construction of transplastomic plants, depending on whether the goal would be to increase or decrease the likelihood of gene transfer. In the case where gene transfer would be minimised, the chloroplast sequences with the lowest number of homologous sequences in soil bacteria would be chosen for the site of transgene insertion.

As mentioned above, many antibiotic resistance gene markers contain genes relatively common in the environment. As a result, experiments meant to examine the soil microbial community after gene transfer failed to observe any differences resulting from the transgenes. On the other hand, the use of *linA* is significant as this not only degrades lindane but also adds acid in the form of HCl (the Cl^-), which comes from the dechlorination of lindane. This gene transfer could lead to significant differences in the microbial community structure.

Conclusions

This work has led to a series of conclusions concerning the potential for gene transfer from transplastomic plants to environmental bacteria. The many barriers to this gene transfer have been well elucidated during this project but, even so, a definitive response is impossible. In any case, the presence or absence of homologous sequences appears to be a major factor in the likelihood of gene transfer.

Major Publications

Kay E., Bertolla F., Vogel T.M. and Simonet P. (2002). Opportunistic colonization of Ralstonia solanacearum-infected plants by *Acinetobacter* sp. and its natural competence development. *Microbiol.Ecol.* 43:291-297.

Kay E., Bertolla F., Nalin R., Vogel T. M. and and Simonet P. (2002). Transfer of Antibiotic Resistance Genes from Transgenic (Transplastomic) Tobacco Plants to Bacteria. *Appl. Environ. Microbiol.* 68:3345-3351.

Kay E., Chabrillat G., Vogel T.M., Simonet P. (2003). Intergeneric transfer of chromosomal and conjugative plasmid genes between *Ralstonia solanacearum and Acinetobacter sp. BD413. Molecular Plant-Microbe Interactions* (IF=3.85), 16:74-82.

Ceccherini MT., Poté J., Kay E., Tran Van V., Maréchal J., Pietramellara G., Nannipieri P., Vogel T.M., et Simonet P. (2003). Degradation and transformability of DNA from transgenic plants. *Applied Environmental Microbiology* (IF=3.35), 69:673-678.

Poté J., Ceccherini MT., Van Tran V., Rosselli W., Wildi W., Simonet P., et Vogel T.M. (2003). Fate and transport of antibiotic resistance genes in saturated soil columns. *European Journal of Soil Biology*, 39:65-72.

Robe P., Nalin,R., Capellano C., Vogel T.M. et Simonet P. (2003). Extraction of DNA from soil, *European Journal of Soil Biology*, 39:183-190.

Acronym
ProBenBT

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2002-01969

Period
November 2002 – April 2006

Coordinator
I. SCHUPHAN
Aachen University-RWTH
Aachen
Germany
Ingolf.schuphan@oc.rwth-aachen.de

Protecting the benefits of *Bt*-toxins from insect resistance development by monitoring and management

Background and objectives

The introduction of transgenic *Bt*-maize into the EU market offers many benefits in increasing pest control while reducing use of insecticides. These benefits could be lost if the targeted pests, European (ECB, *Ostrinia nubilalis*) and Mediterranean (MCB, *Sesamia nonagrioides*) corn borers, develop resistance to the *Bt*-toxin produced by this transgenic crop. To extend the benefits of *Bt*-maize, this joint EU project developed general results by determining susceptibility levels and initial resistance allele frequencies in populations of ECB and MCB collected by the partners involved in this project, and specific results from targeted investigations on aspects of ECB/MCB genetics and *Bt*-resistance. The results provide solid data for sustainable management of *Bt*-maize in order to reduce the risk of evolution of *Bt*-resistance in ECB and MCB.

Approach and methodology

Because the evolution of *Bt* resistance results in a large decline in susceptibility to *Bt*-toxins, the baseline susceptibility in ECB and MCB to *Bt*-toxin before the cultivation of *Bt*-maize should be measured. Baselines were measured for several geographically distinct populations. Bioassays were standardised by using the same reference ECB and MCB strains and the same source of *Bt*-toxin.

The evolution of *Bt*-resistance is significantly influenced by small-scale and long-range movement of the target pests. For each pest, population genetics studies provided indirect estimates of movement over a large geographical scale, and capture-recapture experiments measured small-scale movement.

One of the most critical but difficult parameters to estimate is the frequency of resistance alleles prior to selection when they are still rare. The consortium conducted F2-screens to estimate this frequency in both ECB and MCB populations.

This Europe-wide effort provided an unprecedented opportunity for establishing archival samples that can be used

for retrospective analysis of resistance evolution when *Bt*-resistance genes are cloned. Such cloning will enable development of molecular detection systems. The samples can be used to provide an 'early warning' of the increase of resistance well in advance of field failures, which may provide enough time to alter the resistance management strategy and prolong the use of *Bt*-maize.

Laboratory selection experiments of resistant strains have been performed using discriminating doses of *Bt*-toxin.

ECB and MCB populations can and will evolve resistance to novel challenges such as *Bt*-maize. Fortunately, the genetic and molecular tools available can be used to elucidate the complex mechanisms of resistance and provide needed information for the development of realistic and effective resistance management strategies.

Main findings and outcome

Population genetics: using selectively 'neutral' markers, we showed that ECB and MCB populations were slightly differentiated both within countries (Germany, ECB; Spain, MCB) and within Europe (Germany, Italy, France, Slovakia, Spain, Austria, Bulgaria, Romania and Greece). This suggests a high level of gene flow and movement between populations at small and large scales, which might enable the high-dose plus refuge resistance management (HDR) strategy to effectively delay evolution of *Bt*-resistance.

Small scale dispersal of ECB: this was estimated in Germany, France and the Slovak Republic. The spatial distribution of recaptured moths around the release point suggests that moths probably performed two types of movement. Some of them moved only on a very local scale, while others probably left the area by a different, long-range type of dispersal. At a local scale, resident females mated randomly, i.e. regardless of whether males had experienced a dispersal event beforehand or not. The results also indicated that some moths (~20 % for the females) mated before engaging in any long-range dispersal.

Influence of microsporidia (Nosema pyrausta) infection on Bt-toxin susceptibity: in field studies, we found no relation between the level of maize infestation caused by ECB and the level of infection of ECB larvae by microsporidia. Under laboratory conditions populations showed no differences in susceptibility to Cry 1Ab *Bt*-toxin collected in infected or uninfected localities.

Partners

P. CASTAÑERA
Consejo Superior de Investigaciones Científicas, Madrid (ES)

J. FERRÉ
Universitat de Valencia (ES)

D. BOURGUET
Centre de Biologie et de Gestion des Populations (INRA), Montferrier (FR)

G.C. LOZZIA
Università degli Studi di Milano (IT)

L. CAGAN
Slovak Agricultural University, Nitra (SK)

M. SAVOPOULOU-SOULTANI
Aristotle University of Thessaloniki (EL)

H. RÜDEL
Fraunhofer Institute for Molecular Biology and applied Ecology, Schmallenberg (DE)

D. HECKEL
University of Melbourne (AU)
Max-Planck Institute, Jena (DE)

D. ANDOW
University of Minnesota, Minneapolis (US)

J. JEHLE
Institute of Agriculture, Neustadt (DE)

Baseline susceptibility to Cry1Ab toxin: this was determined for ECB populations collected from Spain, France, Italy, Germany, Slovakia and Greece, and for MCB populations sampled in Spain and Greece. Using overlay, the LC50 (ng/cm^2) for ECB populations varied from 5.7 (in Spain) to 473.06 (in Slovakia). The susceptibly level was not significantly different between populations with respect to the reference strain (lab strain Aachen), except for those collected in Spain; the latter had a higher susceptibly than the German reference strain. Using the diet-incorporation method, the LC50 (µg per ml of diet) values ranged from 8.5 (in France) to 43.50 (in Italy), but were neither significantly different between populations nor significantly higher or lower than the reference strain. The LC50 for MCB ranged from 16.1 ng/cm^2 (in Spain) to 28.02 ng/cm^2 (in Greece).

F_2 Screen: populations of ECB were collected from Germany, Slovakia and Italy, and MCB from Spain and Greece. About 1 345 lines of ECB and 160 lines of MCB were screened on *Bt*-maize tissues. No major resistance alleles to *Bt*-maize were detected. This implies that recessive resistance alleles are sufficiently rare in these European populations to allow the HDR strategy to work effectively.

Field sampling of surviving diapausing larvae in Bt-maize: three main monitoring strategies for detecting *Bt*-resistance were explored within the scope of the ProBenBt project: a) comparing populations with a history of high versus low exposure to the toxin (Spain); b) light-trap cages that attract adults and select larvae (Germany); and c) screening of surviving larvae to establish resistant laboratory populations (Slovakia and Czech Republic). No field-collected insects were able to survive on *Bt*-maize plants.

Laboratory selection of resistant strains to Cry1Ab-toxin: this was performed using discriminating doses of Cry1Ab toxin (high and a low dose strategies). With a low dose for ECB, susceptibility after 23 generations of selection declined by 9-fold (surface treatment) and 16-fold (incorporation method). With a high dose for ECB, susceptibility declined within 4 and 8 generations up to 4- and 10-fold.

Development of genetic tools for detecting Bt resistance genes: a genetic linkage map of ECB was constructed and used to 1) localise *Bt*-resistance genes that have already been identified in other species, 2) position the most likely candidate resistance genes based on knowledge of the mode of action, and 3) identify linkage groups homologous to those identified

in other species that carry mapped, but unknown, resistance genes. The map was based on a cross between two strains from the area of Bonn, Germany; one collected from maize and the other from mugwort. The framework map consisted of more than 200 AFLP markers, covering all 31 linkage groups with a total genetic length of 1 872 cM. Ribosomal protein genes were mapped to provide anchor loci for correspondence to other Lepidopteran genomes. The linkage map is now available as a valuable resource for rapid analysis and identification of ECB *Bt*-resistance genes, if and when they do arise in the field.

Diversity of trypsins in MCB and their interaction with Cry1Ab toxin: four trypsins were purified from midgut lumen of MCB larvae, and a diversity of trypsin-like genes expressed in MCB midgut was identified. The four purified trypsins in MCB belong to the three well-defined phylogenetic groups of trypsin-like sequences detected. Changes in the susceptibility of the trypsin-like activity of midgut extracts from different larval instars to trypsin-type specific protease inhibitors suggest that the relative proportion of the purified enzymes varies through MCB larval development. These changes would be relevant primarily to protease-mediated resistance to *Bt*-maize, were this to develop.

Binding sites analysis of Bt-toxins active against MCB: binding studies were performed with *Bt* toxins Cry1Ab, Cry1Ac, Cry1Ca, and Cry1Fa, found to be active against MCB. Binding assays were performed with ^{125}I- or biotin-labeled toxins and larval brush border membrane vesicles (BBMV). Competition experiments indicated that these toxins bind specifically to the BBMV and that Cry1Aa, Cry1Ab, and Cry1Ac share their binding site. Cry1Ca and Cry1Fa bind to different sites. In addition, Cry1Fa binds to the Cry1A binding site with very low affinity and vice versa (Fig 1.). The presence of distinct binding sites for Cry1Ab and Cry1Fa suggests that pyramiding these two toxins in the same *Bt*-corn plant may provide longer-term control of MCB with minimal risk of cross-resistance.

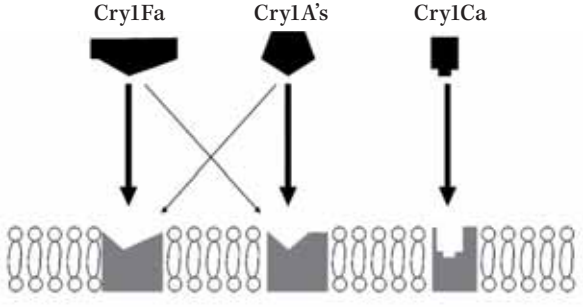


Fig 1.
Model of Bt toxins binding to BBMV from MCB (Sesamia nonagrioides).

Major Publications

Andreadis S.S., Álvarez-Alfageme F., Sánchez-Ramos I., Stodola T.J., Andow D.A., Milonas P.G., Savopoulou-Soultani M., and Castañera P. Frequency of resistance to Bacillus thuringiensis toxin Cry1Ab in Greek and Spanish population of *Sesamia nonagrioides (Lepidoptera: Noctuidae). Journal of Economic Entomology*, 100. (2007). pp. 195-201.

Bailey R.I., Bourguet D., Le Pallec A.H., Ponsard S., Turnover and dispersal of European corn borer in maize field borders. *Journal of Applied Ecology*. 44: pp. 385-394.

Cagán L., Bokor P., Placková A., Microsporidian infection of the ECB populations of Slovakia and Czech Republic. Acta Fytotechnica et Zootechnica, 9. (2007). in press.

Dalecky A., Ponsard S., Bailey R.I., Pélissier C., Bourguet D., Resistance evolution to *Bt* crops: predispersal mating of European corn borers. *PLoS Biology*, 4. (2006). pp.1048-1057.

Díaz-Mendoza M., Farinós G.P., Castañera P., Hernández-Crespo P. and Ortego F. Proteolytic processing of native Cry1Ab toxin by midgut extracts and purified trypsins from the Mediterranean corn borer. *Journal of Insect Physiology*, 53. (2007). pp. 428-435.

Díaz-Mendoza M., Ortego F., García de Lacoba M., Magaña C., de la Poza M., Farinós G.P., Castañera P. and Hernández-Crespo P. Diversity of trypsins in the Mediterranean corn borer *Sesamia nonagrioides (Lepidoptera: Noctuidae)*, revealed by nucleic acid sequences and enzyme purification. *Insect Biochemistry and Molecular Biology*, 35. (2005). pp. 1005-1020.

Active Cry1 Bt-toxins form a pore and interact with leucine cotransport in midgut BBMV from ECB and MCB: the pore forming activity of Cry1Ab, Cry1Fa and Cry1Da toxins and their interaction with leucine transport mediated by the K^+/neutral amino acid cotransporter were studied in brush border membrane vesicles (BBMV) isolated from the midgut of ECB and MCB. The toxins Cry1Ab in ECB-BBMV and Cry1Ab and Cry1Fa in MCB-BBMV reduced leucine uptake in a dose-dependent manner, regardless of the presence of K^+, while no effect was seen with the inactive toxin Cry1Da. Therefore, the inhibition of amino acid transport was strictly connected to the toxic effect *in vivo* and was not related to the channel formed by the toxins.

Binding analyses of Cry1A toxins in resistant and susceptible ECB: Cry1Ab toxin binding analysis was performed to determine whether resistance in laboratory-selected ECB strains is associated with target site alteration. Brush border membrane vesicles (BBMV) were prepared using dissected midguts from late instars of susceptible and resistant strains (Europe-R and RSTT) of ECB (Kansas State University). Immunoblot analysis indicated that three different proteins bound to Cry1Ab toxin and were recognised with an anti-cadherin serum. In a comparison of resistant and susceptible strains, reduced Cry1Ab binding was apparent for all three bands corresponding to cadherin-like proteins in the Europe-R strain, while reduced binding was apparent in only one band for the RSTT strain. In combination, the results of the present work suggest that differences in susceptibility to Cry1A toxins in the Europe-R strain of ECB are associated with altered receptor binding, although the precise nature of this mechanism is still unclear.

Conclusions

The ProBen*Bt*-studies have provided key elements for protecting the benefits of *Bt*-toxins from resistance development in Europe. A genetic linkage map is now available as a valuable resource for rapid analysis and identification of ECB *Bt*-resistance genes, if and when they arise in the field. A diversity of trypsin-like proteases expressed in MCB midgut has been identified.

Competition experiments of different *Bt*-toxins indicate that they bind specifically to the brush border membrane vesicles (BBMV). Pyramiding of Cry1Ab with Cry1Fa in the same plant may give long-term control of corn borers. The inhibition of amino acid transport in BBMV is strictly connected to the toxic effect *in vivo* and is not related to the channel formed by the toxins.

The analyses of resistant strains of ECB indicates that resistance to *Bt*-toxins can evolve as a result of different mechanisms. One strain showed altered binding to the target receptor, which is the most common mechanism of high-level resistance.

ECB and MCB populations have a low level of genetic differentiation both within and between European countries. This suggests a high level of migration between populations, a feature that might foster the efficacy of high-dose/refuge (HDR) resistance management strategy. Still, marked capture-recapture studies show that a fraction of ECB moths could mate locally, a behaviour which might lower the efficacy of the HDR strategy.

Due to the low level of genetic differentiation, only a few ECB populations per country/geographically similar region could be needed as representative populations for susceptibility screening/monitoring.

No major resistance alleles to *Bt*-maize were detected in this study. This both implies that the frequencies of resistance alleles are probably below 10^{-3} in European populations and fosters the efficacy of the HDR strategy.

Samples of different ECB and MCB populations are preserved at -130°C in a specimen bank and are available to subsequent investigators.

Major Publications

Bel Y., Escriche B. Common genomic structure for the Lepidoptera cadherin-like genes. *Gene*, 381. (2006). pp.71-80.

González-Cabrera J., Pérez-Farinós G., Caccia S., Díaz-Mendoza M., Castañera P., Leonardi M.G., Giordana B., and Ferré J. Toxicity and mode of action of *Bacillus thuringiensis* Cry proteins in the Mediterranean corn borer, *Sesamia nonagrioides (Lefebvre). Appl. Environ. Microbiol*. 72. (2006). pp. 2594-2600.

Huarong L., González-Cabrera J., Oppert B., Ferré J., Higgins R.A., Buschman L.L., Radke G.A., Zhu K.Y., Huang F. Binding analyses of Cry1Ab and Cry1Ac with membrane vesicles from *Bacillus thuringiensis*-resistant and –susceptible *Ostrinia nubilalis. Biochem. Biophys. Res. Commun*. 323. (2004). pp. 52-57.

Leonardi G., Caccia S., González-Cabrera J., Ferré J., Giordana B. Leucine transport is affected by Bacillus thuringiensis Cry1 toxins in BBMV from *Ostrinia nubilalis Hb (Lepidoptera: Pyralidae) and Sesamia nonagrioides Lefebvre (Lepidoptera: Noctuidae)* midgut. J. *Membrane Biology*: accepted.

Saeglitz C., Bartsch D., Eber S., Gathmann A., Priesnitz K.U. and Schuphan I. Monitoring the Cry1Ab susceptibility of European corn borer *(Ostrinia nubilalis Hübner) in Germany. Journal of Economic Entomology*, 99. (2006). pp. 1798-1773.

Siqueira H.A.A., González-Cabrera J., Ferré J., Flannagan R. and Siegfried B.D. Cry1Ab binding analyses in resistant and susceptible strains of European corn borer, *Ostrinia nubilalis (Hübner) (Lepidoptera: Crambidae). Appl. Environ. Microbiol*. 72. (2006). pp. 5318-5324.

Acronym
BIOGEM

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2002-02063

Period
January 2003 – January 2006

Coordinator
Tim HART
tim@cybersensebiosystems.com

Project website
http://www.biomatnet.org/secure/FP5/S1694.htm

Development and risk assessment of a field-based portable biosensor using genetically modified bioluminescent bacteria

Background and objectives

Before 2004, there were at least 300 000 positively identified contaminated sites in the EU15 and many more in the EU accession countries. These sites were contaminated with a range of pollutants that included hydrocarbons, heavy metals, chlorinated solvents, pesticides, explosives and various combinations thereof. Cleaning up these sites is justified purely in terms of negating their ecotoxicity and threat to human health; but often the locations are also of economic importance for redevelopment, particularly in inner urban areas. Site assessment and remediation are therefore considered growth industries in Europe, and there is much pressure to employ effective and rapid methods, particularly for monitoring, detection and analysis. Conventional chemical analysis, although accurate, presupposes knowledge of the contaminants present and is time-consuming and expensive.

Biosensors are gaining increasing importance for environmental analysis, providing rapid analytical results useful in land toxicity assessments and remediation operations. A common approach is to use bioluminescent bacteria, which indicate the level of generic bioavailable toxicity in a sample by the reduction in light output. An array of pollutant-specific biosensors can provide a rapid, field-based test for a range of toxins in order to identify hotspots of toxicity and eliminate clean areas from further investigation.

Over the last decade numerous research groups across the world have genetically engineered bacteria isolated from soil to express the luciferase *lux* system, which produces quantifiable bioluminescence that can be induced or inhibited in the presence of specific toxins, including phenols, heavy metals and hydrocarbons. However, Genetically Modified (GM) bacteria cannot currently be used in field-based biosensors due to the risks of release and GM contamination of the environment. In order for this technology to be of real value to the industry, systems must be developed to enable the bacteria to be used onsite. These real-time measurements offer huge time and cost savings to the industry.

Approach and methodology

Within the BIOGEM project various biological and physical methods for ensuring containment of genetically modified bioluminescent bacteria have been developed to allow their safe use in the field. Several parallel approaches have been assessed with the aim of ensuring a high level of containment of GM bacteria:

- design of the biosensor housing with self-sealing components, in order to allow the introduction of aqueous soil extracts of samples for testing but not accidental release of bacteria, and also to withstand breakage under any circumstance in the field;
- engineering of cell death so that bacteria cannot survive outside the unit;
- immobilisation of bacteria in a polyvinyl alcohol matrix.

With regard to biosensor design, the BIOGEM partners have developed and manufactured a multi-chamber sealed unit to ensure the effective containment of GM bacteria, even when a chamber is opened for sample addition. The biosensor design is outlined in Fig 1. Essentially inert freeze-dried desiccated GM bacteria are contained in the lowest chamber, with walls separating them from the rehydration media they require for activation. The upper chamber receives the sample but, even when the upper chamber lid is open, walls still prevent access of bacteria to the environment. The lid of the unit contains an irreversible seal so that, when an operator has added his sample, he seals the complete unit irreversibly. The unit then rotates 90° so that the rehydration media come into contact with the bacteria.

Following an incubation period, a further rotation allows the toxin sample to access the now regenerated bacteria and luminescence can be observed and quantified in light sensing devices such as ROTAS (Rapid On-site Toxicity Audit System).

The biosensor has been successfully tested in the field in Austria and Spain in cooperation with European regulatory bodies and stakeholders. The new biosensor allows rapid high-performance onsite analysis with negligible risk of GM contamination.

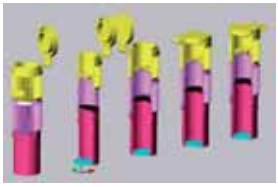


Fig 1.
Final biosensor housing design.

With regard to engineered cell death, an additional containment strategy was developed based on the deletion of the gene *dapB* in relevant biosensor bacteria. The *dapB* gene is required for the expression of diamino-pimelic acid, an essential bacterial cell wall component. When cells are grown in the presence of a supplement, the *dapB* deleted cells grow effectively but, in the absence of diamino-pimelic acid, the cells rapidly die. Thus the addition of a small quantity of diamino-pimelic acid to the rehydration medium effectively rehydrates *dapB* deleted bacteria which produce light when rehydrated but then die in a matter of minutes.

The project also foresaw the immobilisation of cells in polyvinyl alcohol as a rapidly developing field and as one of the most versatile materials able to form a variety of structures, having been used previously in whole-cell biosensors. However, PVA films are hydrophylic and so hydrophobic analytes such as PAHs are largely unable to diffuse into the matrix.

A major objective of the project was the optimisation of PVA gel production by a cryogelation and freeze-drying procedure to encapsulate the bacteria. In the event of accidental breakage any GM bacteria will not disperse over a wide area but will effectively adhere to the biosensor unit. The method is also suitable for storing the bacteria in a freeze-dried state, prolonging shelf life and optimising storage conditions. A variety of PVA gels were tested for their ability to allow access of toxins to the biosensors, as signified by changes in the light output. Research suggests that the shelf life of various biosensor strains can be increased from a matter of days to several weeks.

Fig 2.
Disc PVA bacteria gels loaded onto 24 well plates.

Fig 2. demonstrates the relative size of bacteria containing wafer thin discs loaded onto plates for subsequent light measurement.

Main findings and outcome

In the BIOGEM project a combination of containment methods was utilised to not only minimise the risk of deliberate release of genetically modified microorganisms, but also to provide a high level of confidence for the user and the regulatory authorities. Progress has been positive, with the biosensor housing unit effectively demonstrated as enabling the effective rehydration and reactivation of dormant bacteria when they are exposed to the rehydration media. Further confidence has been developed by the use of *DapB*-deleted bacterial strains, which die rapidly when the minimal supply of supplement contained in the rehydration medium is used up. One of the most significant factors facing the use of biosensors is their shelf-life and performance following prolonged storage. If a user cannot have confidence that the product will give a consistent and high level of performance, he will not utilise the technology. Steps have been taken to increase the shelf-life of bacterial biosensors by immobilising them in PVA matrices. A variety of conditions have been tested and the shelf-life of several biosensor strains has been significantly increased. Further studies are ongoing to maximise the performance and the convenient storage of the bacteria in a manner that makes their use in the field more practical.

Environmental impact assessment of transgenic grapevines and plums on the diversity and dynamics of virus populations

Acronym
TRANSVIR

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2002-02140

Period
January 2003 – June 2006

Coordinator
Marc FUCHS
Department of Plant Pathology
New York State Agricultural Experiment Station
630 West North Street
Geneva, New York 14456
USA
mf13@cornell.edu

Background and objectives

Resistance to viruses in transgenic crops has been a major breakthrough in the successful application of biotechnology to agriculture. Virus resistance can be developed by introducing virus-derived gene constructs, for example the Coat Protein (CP) gene, into susceptible crops, followed by screening for the desired phenotype and horticulture characteristics.

However, since most conventional plants do not contain virus-derived genes, environmental safety concerns have been expressed with the release of virus-resistant transgenic crops. Of major concern is the outcome of recombination between viral transgene transcripts and RNAs from field viruses which infect transgenic plants. Resulting recombinant viruses may have new biological properties such as changes in vector specificity, expanded host range, and increased pathogenicity. Evidence of recombination between viral transgene transcripts and infecting viruses has been obtained mainly with model plants in the greenhouse. Limited information is available on the potential of transgenic plants of agronomic importance to mediate the development of recombinant viruses under field conditions. The project addressed this important issue with CP gene-expressing transgenic grapevines and plums that were established in the field in 1996.

Our objective was to: 1) analyse and compare the dynamics and variability of virus populations in transgenic versus non-transgenic plants; 2) monitor the emergence of recombinant virus species; 3) examine whether transgenic grapevines and plums expressing viral CP genes increase the likelihood of emergence of recombinant viruses beyond that of natural background events; 4) determine the stability of post-transcriptional gene silencing in transgenic plums upon multiple virus infection; and 5) evaluate the translocation of transgene-derived products from transgenic grapevine rootstocks to non-transgenic scions.

Approach and methodology

The test material consisted of transgenic grapevines expressing the CP gene of *Grapevine fanleaf virus* (GFLV), *Grapevine virus A* (GVA) or *Grapevine virus B* (GVB), and transgenic plums expressing the CP gene of *Plum pox virus* (PPV). Conventional grapevines and plums were used as control. Vineyard and orchard sites with transgenic plants were selected in France, Romania, and Spain, and virus-infected conventional plants of interest were selected in Germany, Italy, Slovenia and other parts of Europe, in addition to these countries.

Our experimental approach was based on a comparative characterisation of the biological, serological and molecular properties of viruses in transgenic and non-transgenic plants. The CP gene and other genes of challenging virus isolates were amplified from test plants by immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) or RT-PCR, using appropriate antibodies and primers, and characterised by restriction fragment length polymorphism (RFLP) or single-stranded conformation polymorphism (SSCP), and nucleotide sequencing.

Main findings and outcome

For grapevines expressing the CP gene of GFLV, some transgenic lines exhibited resistance to *Xiphinema index*-mediated transmission of GFLV during a 1996 - 1999 field trial. No characteristics similar to strain F13, which provided the CP transgene, and no statistically significant difference in molecular variability were found for the majority of isolates from transgenic and nontransgenic plants. These results were consistent with the fact that transgenic grapevines did not assist the emergence of viable GFLV recombinants to detectable levels, nor did they affect the molecular diversity of indigenous GFLV populations during the trial period. Analysis of GFLV isolates from different conventional grapevine cultivars and various geographic origins provided the baseline of molecular variability.

Interestingly, GFLV recombinants were identified in conventional plants that were located outside the field sites where transgenic plants were tested. One of these GFLV recombinant isolates had similar biological properties to non-recombinant

Partners

Giovanni P. MARTELLI
Università degli Studi di Bari (UNIBA) (IT)

Pierre PFEIFFER
Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique et Université Louis Pasteur (CNRS-ULP), Strasbourg (FR)

Wilhelm JELKMANN
Biologische Bundesanstalt für Land und Fortwirtschaft (BBA), Braunschweig (DE)

Natasa PETROVIC
National Institute of Biology (NIB), Ljubljana (SI)

Michel RAVELONANDRO
Institut National de la Recherche Agronomique (INRA), Bordeaux (FR)

Mariano CAMBRA
Instituto Valenciano de Investigaciones Agrarias (IVIA), Valence (ES)

Ioan ZAGRAI
Statiunea de Cercetare si Productie Pomicola (SCPP), Bucarest (RO)

isolates. Further, no compelling evidence was found for the translocation of GFLV-derived transgene transcripts and siRNA from transgenic grapevine rootstocks expressing the CP gene of GFLV to non-transgenic grapevine scions. Whether virus infection can trigger such transfer by encapsidation of transgene-derived products and subsequent cell-to-cell and long distance movement was also evaluated. By analysing the genetic variability of virus populations in conventional grapevines that are cross-protected with mild isolates of GFLV and *Arabis mosaic virus*, these studies were expanded to assess whether this virus control strategy has any impact on the emergence of recombinant viruses. It was found that mild protective virus strains did not contribute to the development of recombinants, but that mild protective strains themselves were GFLV/ArMV interspecies recombinant.

Fig 1.

Transgenic grapevines expressing the coat protein gene of Grapevine fanleaf virus in an experimental vineyard.

The impact of transgenic grapevines expressing the CP gene of GFLV on the molecular variability of *Grapevine leafroll-associated virus 1* (GLRaV-1) was investigated by using the diversity of GLRaV-1 isolates from conventional grapevines as reference. Results were consistent with no difference in the population diversity of GLRaV-1 in conventional and GFLV transgenic grapevines. Also, the genome of GLRaV-7 was determined almost to completion and the variability of GVA and GVB isolates infecting conventional grapevines was examined. Transgenic grapevines expressing the CP gene of GVA or GVB have been challenge-inoculated by grafting, mealybug transmission, and heterografting with *Nicotiana species*. Preliminary sequence information did not seem to reveal any significant impact of transgenic grapevines on the genetic variability of GVA and GVB populations.

Fig 2.

Transgenic plum trees expressing the coat protein gene of Plum pox virus in an experimental orchard.

For plums expressing the PPV CP gene, transgenic C5 trees, which display post-transcriptional gene silencing, were highly resistant to graft- and aphid-mediated PPV infection during a 1996 - 2006 field trial. Transgenic C4 trees showed some delay in the onset of disease symptoms, while other transgenic clones and nontransgenic trees were susceptible. These data were obtained in different field sites under various environmental conditions. In addition, no statistically significant serological and molecular differences in the viral RNA 3' end region, i.e. the NIb and CP genes, were found among the majority of PPV isolates infecting transgenic and conventional plums. Therefore, transgenic plums did not alter the diversity and dynamics of PPV populations to detectable levels, nor did they trigger the emergence of recombinant PPV species during the trial period.

Populations of aphids and other insect species were quantified and compared in transgenic vs. non-transgenic plum trees and no significant differences were recorded. To test the effect of heterologous viruses on the stability of post-transcriptional gene silencing against PPV, some branches of transgenic C5 trees were graft-inoculated with *Prunus necrotic ringspot virus* (PNRSV) and PPV, or *Apple chlorotic leaf spot* (ACLSV) and PPV, or *Prune dwarf virus* (PDV) and PPV in the field and in the greenhouse. Across all trials, the engineered resistance to PPV in transgenic plums was stable in the presence of heterologous viruses.

Conclusions

Field experiments provided a direct assessment of the impact of transgenic grapevines and plums expressing viral CP genes on the diversity and dynamics of virus populations, in particular on the emergence of recombinant viruses. They also provided new insights into the environmental impact of transgenic grapevines and plums expressing viral CP genes. Altogether, the studies suggested that transgenic grapevines and plums are not only highly resistant to GFLV and PPV respectively over extended time (3 and 10 years, respectively) but also have no adverse effect, beyond natural background events, on the diversity and dynamics of virus populations via recombination.

Resistance to devastating viruses is of great interest to the European grapevine and stone fruit industries. Given the pressing need for effective and environmentally-friendly virus control strategies, the findings should assist national and international authorities in making scientifically-based regulatory decisions on the release of virus-resistant grapevines and plums, as well as other transgenic crops that contain virus-derived genes and are of agronomic importance.

Major Publications

Capote N., Perez-Panades Monza C., Carbonell E., Urbaneja A., Scorza R., Ravelonandro M. and Cambra M. (2008). Assessment of the diversity and dynamics of *Plum pox virus* and aphid populations in transgenic European plums under Mediterranean conditions. *Transgenic Research* 17:367-377.

Fuchs M., Cambra M., Capote N., Jelkmann W., Laval V., Martelli G.P., Minafra A., Petrovic N., Pfeiffer P., Pompe-Novak M., Ravelonandro M., Saldarelli P., Stussi-Garaud C., Vigne E. and Zagrai I. (2007). Safety assessment of transgenic plums and grapevines expressing viral coat protein genes: New insights into real environmental impact of perennial plants engineered for virus resistance. *Journal of Plant Pathology* 89:5-12.

Vigne E., Bergdoll M., Guyader S. and Fuchs M. (2004). Population structure and genetic diversity within *Grapevine fanleaf virus* isolates from a naturally infected vineyard: Evidence for mixed infection and recombination. *Journal of General Virology* 85:2435-2445.

Vigne E., Komar V. and Fuchs M. (2004). Field safety assessment of recombination in transgenic grapevines expressing the coat protein gene of *Grapevine fanleaf virus*. *Transgenic Research* 13:165-179.

Zagrai I., Capote N., Ravelonandro M., Cambra M., Zagrai L., Scorza R. (2008). *Plum pox virus* silencing of C5 transgenic plums is stable under challenge inoculation with heterologous viruses. *Journal of Plant Pathology* 90:S1.63-S1.71.

Control of flowering time for sustainable and competitive agriculture and forestry

Acronym
CONFLOW

Programme Acronym
FP5-LIFE QUALITY

Contract number
CONFLOW-QLRT-2000-01412

Period
January 2001 – August 2004

Coordinator
Prof. Claus H. ANDERSEN
DLF Trifolium A/S
Hoejerupvej 31
DK-4660 Store Heddinge
Denmark
cha@dm.dtu.dk

Background and objectives

In summer 2000 the EU-funded CONFLOW project brought together ten partners (eight scientific and two commercial) with the objective of developing widely applicable genetic tools that allow the genetic control of flowering for the purpose of containment of transgenes via control of flowering time, as well as the fine-tuning of flowering time in agriculturally important crops and trees.

Biological containment via flowering control is aimed at crops where the vegetative parts are harvested, for example, forage grasses and certain vegetables. The fine-tuning of flowering, either acceleration or delay, allows existing crop varieties to be harvested at the optimal time and exploited in new geographical areas or in response to long-term changes in climatic conditions and land-use policy. The development of new breeding tools for the forest and fruit-tree industry, based on rapid cycling breeding lines with a shortened juvenility phase, accelerates the improvement of woody species. Upon completion of the breeding programme, the dominant transgene can be deleted either by out-crossing or through strategies of controlled recombination, using a proven site-specific recombinase system.

The technologies developed were aimed to be applicable to a wide variety of plants, including both dicot and monocot plants, angiosperms and gymnosperms, and were tested initially in the project in the following application examples: Biological containment aimed to prevent transgene escape from the major European forage grass perennial ryegrass *(Lolium perenne)*; fine-tuning of flowering time focusing on rice (*Lolium*) and birch as model species; and accelerated improvement of woody species using aspen, birch and citrus as models.

Specific objectives were:
Biological containment: the development of methods for the control of flowering in both dicots and monocots conferring a complete biological containment. Non-flowering plants will be developed by control of flowering time genes in combination with a system for inducible reversion of the non-flowering phenotype for the purpose of seed production. The developed technologies are aimed at crops where the vegetative parts are harvested, for example, forage grasses and certain vegetables.

Fine-tuning of flowering time: the development of tools for the positive control and optimisation of the flowering time in agriculturally important crops and trees in order to facilitate flexible and sustainable agriculture and forestry. Small changes in the timing of flowering, either acceleration or delay, in order to optimise the time of seed production, will allow existing crop varieties to be harvested at the optimal time and exploited in new geographical areas or in response to long-term changes in climatic conditions and land-use policy.

Accelerated improvement of woody species: the development of new revolutionary breeding tools for the forest and fruit tree industry based on rapid cycling breeding lines with a shortened juvenility phase. Upon completion of the breeding programme, the dominant transgene will be deleted either by out-crossing or through strategies of controlled recombination using a proven site-specific recombinase system. The novel breeding tools for the temporary conversion of slow-to-mature forest and fruit tree varieties to rapid-cycling breeding lines will revolutionise the ability to improve woody species through conventional breeding techniques.

Partners

Dr. K. K. NIELSEN/
C. H. ANDERSEN
DLF Trifolium A/S,
Heddinge (DK)

Dr. C. DEAN
BBSRC John Innes Centre,
Norwich (UK)

Dr. G. COUPLAND
Max-Planck-Gesellschaft zur
Förderung der Wissenschaften
E.V. Köln (DE)

Dr. T. SOPANEN,
Dr. K. KEINONEN
University of Joensuu (FI)

Dr. M. KATER
University of Milano (IT)

Dr. G. ANGENENT
Plant Research International
B.V., Wageningen (NL)

Dr. J.M. ZAPATÉR
University of Madrid, Consejo
Superior de Investigaciones
Cientificas (ES)

Dr. O. NILSSON
Sveriges Lantbruksuniversitet
Umeå (SE)

Dr. R. BAGGER JØRGENSEN
RISØ National Laboratory,
Roskilde (DK)

Dr. J. NEGUEROLES
FRUTARIA SAT, Zaragoza (ES)

Approach and methodology

A. Work Package 1 – Biological containment
The wealth of data available from work on *Arabidopsis* flowering time control will be exploited in order to manipulate the genetic and physiological pathways that control flowering in other species, such as *Lolium perenne*. Specifically, the main flowering repression pathway (due to the *FRI* and *FLC* genes) will be strengthened by transgenic overexpression whilst a pathway that normally reduces this repression, in response to a winter period of cold temperature (the vernalisation pathway), will be weakened by antisense expression of regulatory key genes (*VRN1* and *VRN2*). This will result in non-flowering plants. For the purposes of seed production, an inducible expression system will be employed in order to obtain flowering plants when desired. Finally, a critical component of this objective will be a risk assessment analysis of the effectiveness and stability of the biological containment through controlled experiments on populations of non-flowering plants under different environmental conditions.

B. Work Package 2 – Fine-tuning of flowering time
The objective will be achieved through two strategies: using known regulatory rice genes (*OsMADS47* and *OsMADS18*) to manipulate the flowering time of rice, and by utilising a genomics approach with *Lolium* to discover novel genes involved in the process of vernalisation. The utility of new genes for manipulation of flowering time will first be evaluated using the well-characterised *Arabidopsis* system. Known and novel juvenility genes will be used in birch trees to delay flowering. Candidate genes will then be tested in rice, *Lolium*, and birch as application examples.

C. Work package 3 – Accelerated improvement of woody species – novel breeding tools
Complementary strategies will be used in order to achieve the objective:
Functional conservation approach: The study will examine the ability of *Arabidopsis* flowering time genes (*LFY*, *API*, *FT* and *SOC1*) to suppress juvenility (age of plant when flowering). In juvenility suppressed transgenic citrus, genes which are up and down regulated will be isolated by use of differential display (DD) and tested for their functionality in juvenile phase suppression in *Arabidopsis* and citrus. The citrus orthologues of these genes will be isolated and tested for their functional conservation by expression in *Arabidopsis*, citrus (and birch where appropriate).

Genomics approach: The discovery of novel genes involved in the flowering process will be undertaken using microarray technologies. Activators and repressors of the floral transition in aspen and citrus will be identified through differential screening of a hybrid aspen EST collection (microarray). The function of isolated genes will be tested as described above.

Genes that have been identified above, and whose expression gives rise to early flowering and fertile flowers that can be used for crosses, will then be tested as breeding tools in breeding programmes. Upon completion of the breeding programme, the dominant transgene will be deleted either by out-crossing or through strategies of controlled recombination using a proven site-specific recombinase system. This will result in a non-transgenic tree that has simply gone through an accelerated breeding programme.

An important part of the work carried out is to ensure that the basic biological knowledge gained from the research is exploited to produce real-world products and solutions that will benefit European companies, consumers and the environment. The project aims to achieve this through application examples and risk assessment analysis.

D. Work package 4 – Environmental risk assessment
Finally, the effectiveness and stability of biological containment in *Lolium* will be subjected to a thorough risk assessment analysis.

Fig 1.

Phenotypes of the transgenic 35S::LpCO co-2 (left) in comparison to the corresponding untransformed co-2 mutant (right). The picture was taken 30 days post-germination and plants were grown in long days. (by P#1 & P#3).

Fig 2.

The effect of 35S::FLC construct in birch (by P#4).

Fig 3.

The effect of overexpression of BpMADS4 in normally flowering birch clone. Fig 2., transgenic plants forming inflorescences (by P#4).

Main findings and outcome

Biological containment

The goal was to work towards biologically containable forage grass by creating non-flowering ryegrass that did not respond to changes in photoperiod or vernalisation.

To achieve biological containment in *Lolium*, knowledge generated in model plants like *Arabidopsis* was used to identify orthologs of known *Arabidopsis* flowering genes and to test different genetic tools to control flowering in *Arabidopsis* as well as in grasses. One important result from this study was that genes from different plant species showing strong sequence homologies can have quite different – and sometimes even opposite – effects on flowering, as seen in model plants. Furthermore, for some flowering genes it was not possible to find the corresponding genes in the investigated crop species by means of sequence homology.

Work with *Arabidopsis* and *Lolium* by partners in this work package quickly established that manipulating flowering time through the factors controlling *FLC* was not ideal for cereals, and a new approach was adopted. This went on to show that there are genes (both endogenous and from *Arabidopsis*) whose overexpression in monocots can significantly delay flowering. Furthermore, the *Lolium* homologue of *Arabidopsis* CO, LpCO, has been shown to be functional in *Arabidopsis* and the monocot barley.

At the end, the necessary genetic tools to control flowering in monocots were identified and non-flowering grasses were generated with achemically inducible switch to induce flowering upon demand for the purpose of seed production. In parallel, a risk assessment analysis was performed of the effectiveness and stability of the biological containment through controlled experiments on populations of non-flowering plants under different environmental conditions.

Fine-tuning of flowering time
The attempts to modify expression levels of specific rice flowering-time regulatory MADS-box genes resulted only in moderate phenotypic changes. An explanation for this pattern could be the known effect of redundancy working with this big family of transcription factors. Results obtained with rice genes in the model plant *Arabidopsis* are not always transferable to the crop plant.

Whereas the investigations dealing with fine-tuning of flowering time in rice gave no clear results with respect to early or late flowering phenotypes, exceptional results in birch were achieved with the generation of both early and late flowering transgenic lines. Furthermore, hybridisation experiments on a developed *Lolium* unigene microarray with more than 1 500 sequences, as well as the generation and use of a micro-array containing 24 000 unique Aspen sequences, identified new important genes involved in flowering and developmental processes in these organisms.

The expression of the *Arabidopsis* flowering repressor FLC in birch resulted in the exact opposite phenotype in this woody species (early flowering), whereas the anti-sense expression of a birch flowering inducers or the over-expression of a *Populus* flowering repressor had the expected effect and delayed flowering in birch. The acceleration of flowering in birch by over-expression of birch flowering inducers was successful and the birch model delivered the best results in terms of fine-tuning or accelerating flowering.

Accelerated improvement of woody species – Novel breeding tools
Functional conservation approach: The objective to develop rapid cycling breeding tools in citrus and sweet orange by over-expression of *Arabidopsis* flowering genes gave very positive results in citrange (hybrid of sweet orange and trifoliate orange). Whereas the specific flowering genes demonstrated different capacities in flowering acceleration in citrange, the positive gene effect could not be transferred to *Citrus sinensis*, at least not within the duration of the CONFLOW project.

In more detail, the effect of *FT* and *SOC1* was demonstrated in citrange and showed that *FT* is much more efficient than *SOC1* in the suppression of juvenility. *FT* also accelerated flowering initiation as efficiently as *API*, but it is not so effective in the suppression of juvenile traits. In addition, genetic materials were generated to test the effect of *API* elimination after juvenile phase suppression in citrange.

In *Citrus sinensis*, transgenic trees were generated but without a clear effect of *API* on the suppression of juvenility and the promotion of flowering. Finally, a set of genes was identified whose expression is increased in citrange as a consequence of *API* overexpression. Their role in the suppression of juvenility and/or floral promotion remains to be tested.

Genomics approach: This approach succeeded in generating early flowering poplar trees that flower after a few weeks or months instead of the normal 10-15 years. The induced flowering is close to normal, forming inflorescences instead of single abnormal flowers. This represents a significant improvement compared to previous results. The technique has been proven to work both for male and female plants. Furthermore, flowering can be induced at will, using inducible constructs. This has never before been demonstrated for trees. Finally, the work, for the first time, characterised the gene expression profiles of almost all poplar genes during the juvenility-to-maturity transition. This work, which identified hundreds of poplar genes that are putative regulators of this transition, will constitute an important basis for further work on the regulation of flowering and maturity in trees.

Conclusions

One important finding of this project is that it is possible to transfer knowledge and genes from model plants like *Arabidopsis* to crop plants, but the outcome in different crops is not always foreseeable. Since this project has involved woody species as well as crops with long transformation and regeneration times, it has not been possible to reach final conclusions on all strategies within the three-year duration of the project.

The prevention of flowering in transgenic crops and forest trees (biological containment) is generally considered to be one of the most important goals for the advantageous use of biotechnology in agriculture and forestry. Biological containment will likely be a prerequisite for the future introduction of genetically modified tree plantations and may facilitate coexistence between genetically modified and conventional crops in the fields. The development of biotechnologically improved plants can lead to a dramatically improved productivity and quality of crops and wood formation, benefiting European farmers and forest industries and helping to relieve the pressure on natural forests.

Early flowering trees will be important tools for conventional breeding of forest trees or citrus trees. It solves an important problem in tree breeding, i.e. the fact that many commercially interesting European trees do not flower until 5-20 years of age. This has severely hampered the development of tree breeding in Europe. Superior, highly bred trees will significantly enhance forest productivity and quality.

The tools developed to fine-tune flowering time will help to make it predictable, even under unforeseeable environmental fluctuations (e.g. weather conditions), and help to adapt new and existing varieties and crops to their location and growing conditions. These benefits will allow existing 'elite' varieties to be grown in locations where they currently cannot be grown, and generally will increase the yields and competitiveness of European agriculture.

Major Publications

Martin J., Storgaard M., Andersen CH., Nielsen KK. Photoperiodic regulation of flowering in perennial ryegrass involving a CONSTANS-like homolog. *Plant Mol Biol*. 56 (2):159-69. (2004).

Levy Y. Y., Mesnage S., Mylne J. S., Gendall A. and Dean C. Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. *Science* 297, 243. (2002).

Mylne J.S., Greb T., Lister C., and Dean C. Epigenetic Regulation in the Control of Flowering. Cold Spring Harbour 69th Symposium: *Epigenetics*. (2004).

Searle I.R. and Coupland G. Induction of flowering by seasonal changes in photoperiod. *EMBO J.* 23 (6): 1217-22. (2004)

Acronym
ECOGEN

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK5-CT-2002-01666

Period
May 2002 – April 2006

Coordinator
Paul Henning KROGH
Deparment of Terrestrial Ecology
National Environmental Research Institute
Aarhus University
P.O. Box 314
Vejlsøvej 25
DK-8600 Silkeborg
phk@dmu.dk

Project website
http://www.ecogen.dk

Soil ecological and economic evaluation of genetically modified crops

Background and objectives

The soil environment is affected by agricultural activities including conventional farming practice elements such as pesticides, tillage practices and crop types. The introduction of genetically modified (GM) crops to the soil environment calls for an ecological and economic assessment, especially as the consequences of this new technology cannot be predicted from previous experience. Soil biodiversity includes a broad range of taxonomic groups of organisms, each calling for specific expertise and experience. These organisms perform a multitude of functions that together create a complex ecosystem. The ECOGEN project included the main taxonomic groups and decomposition of organic matter as its main focus for the ecological investigation of GM cropping systems.

An ecological risk assessment of GM cropping systems and conventional cropping system with regard to soil ecosystems was based on single species tests, multispecies tests and long-term (4 years) field investigations. Existing first-tier ecotoxicity protocols for the testing of chemicals were modified for exposure to GM plant material and validated in a tiered risk assessment system.

When performing ecological risk assessment it has become established practice to analyse the hazard in successive steps or tiers. Due to their lower costs, a low-tier test permits a broader series of questions (i.e. inclusion of many more factors) than higher-tier tests. ECOGEN has approached the risk assessment from a similar perspective, but without requiring a low-tier test result before triggering a higher-tier test. Instead, three tiers were employed, using lower-tier results to support the interpretation of high-tier (field level) observations.

The objective of assessing the ecological and economic impact of GM cropping systems was achieved by establishing a field experimental infrastructure at three locations including GM and non-GM farming systems. They were located in the three geographical European zones corresponding to those in force for authorisation of plant protection products: Zone A – North (Denmark), Zone B – Centre (approximated by a Northern France location), Zone C – South (Mediterranean

South France). Changes in soil biodiversity were measured mainly in relation to tillage practices, soil types, crop type and history, pesticides and the maize variety.

Approach and methodology

The approach taken for evaluation of the costs and benefits of introducing GM crops in Europe was to distinguish between cost and benefits that would be temporary (reversible) and those of a long-term nature (irreversible). In the context of transgenic crops, where concerns are more focused on potentially irreversible costs of the technology, useful information can be obtained from threshold values that indicate the maximum incremental social irreversible costs (MISTICs) that an individual or society in general is willing to tolerate for the sake of the benefits of the technology. So the study also investigated the point at which incremental and irreversible costs would exceed the MISTICs at national, farmers and per capita EU level.

To face the problem of reaching a decision based on knowledge originating from disparate scientific domains, a multi-attribute decision support model is useful, as it inherently has no restrictions on the nature of the knowledge being built into the model – as long as the knowledge about the system can be broken down into attributes and the attributes can be given qualitative symbolic values that influence aggregate properties. The evaluation of cropping systems is suitable for multi-attribute modelling, because it deals with scenarios which need to be evaluated, analysed and compared with each other. And a cropping system can be disaggregated into smaller, less complex elements where the relationships between factors can affect the evaluation of the scenarios. The ecological knowledge from single species tests, multispecies tests, field investigations and economic information from farming practices was conveyed via the domain experts to the computer scientists, building the rule-based model to be used for predicting economic decision-making processes.

The ECOGEN project organisation proved to be successful probably because the elements in the organisation reflected the main goals as broken down into the work packages. The conceptual model of the project organisation combined economics, agronomics and ecotoxicological risk assessment approaches with a modelling enterprise using data mining and decision support. The underlying field studies were conceived with real-world base and infrastructure, upon which the scientific analyses and interpretations were built.

Partners

Jérôme CORTET
ENSAIA-INPL, Nancy (FR)

Bryan GRIFFITHS
Teagasc, Carlow (IR)

Christophe SAUSSE/
Antoine MESSEAN
CETIOM,
Thiverval-Grignon (FR)

Sašo DŽEROSKI
Department of Knowledge
Technologies, Ljubljana (SI)

Justus WESSELER
Environmental Economics
and Natural Resource Group
Wageningen University (NL)

Mathias NEUMANN
ANDERSEN
Department of Agroecology
and Environment
Research Centre Foulum,
Tjele (DK)

Annette GOMOT DE
VAUFLEURY
Department of Chrono-
Environment
UMR UFC/CNRS 6249 USC
INRA, Besançon (FR)

The ECOGEN project addressed soil ecological and economic questions related to the introduction of GM crops to European agriculture. It consisted of:

- soil ecological evaluations of GM maize production systems at three levels of biological organisations: single species, model communities and field ecosystems;
- socioeconomic evaluation of the benefits and costs of Bt-maize and HT-maize at farm level and national level for selected EU Member States; and
- decision support to aid in concomitant economic and ecological factor evaluation by employing a qualitative multi-attribute decision support tool.

A field experimental infrastructure was established to provide the project participants with agricultural management data, experimentally designed comparisons of farming systems and economic data. The field studies were situated in each of the three geographical European zones as used for the authorisation of plant protection products: Zone A – North (Denmark), Zone B – Centre (approximated by a Northern France location), Zone C – South (Mediterranean Southern France).

Main findings and outcome

The existing first-tier ecotoxicity protocols used for the testing of chemicals were modified to fit the exposure to GM plant material. A wide range of taxonomic groups of soil organisms covering *Acari, Oligochaeta, Collembola, Protozoa, Nematoda* and *Mollusca* were investigated for direct sensitivity to Bt-toxin both in a pure chemical form and as part of the plant biomass of maize in first-tier laboratory screening tests. These tests did not reveal effects of *Bt*-toxin and *Bt*-maize, while insecticides selected from the pesticides used in ECOGEN field study sites would have effects on soil invertebrates. The microfauna was, thus, not affected by the pesticides at levels at field application rates and the tier-one overall conclusion is to expect higher impact on soil invertebrates from insecticide application than from *Bt*-maize.

Mesocosm model systems were employed as a middle-tier approach, with the benefits of including soil ecological complexity and greenhouse control of experimental conditions. Field relevant factors such as soil type, pesticides and a range of *Bt*-maize varieties, compared with their near isogenic non-*Bt*, were studied. The largest effects observed were from the soil type and plant growth stage. The GM variety tested had effects on soil populations and processes that were less pronounced than pesticide effects. Comparison between

Bt-maize and near-isogenic *Bt*-maize did not reveal general trends pointing to adverse or beneficial effects of *Bt*-maize on soil organisms. In particular, when comparing a range of varieties of *Bt*-maize and near-isogenic *Bt*-maize, it was demonstrated that there were no detectable differences in the concentration of *Bt*-toxin in plant or soil with any of the *Bt*-expressing varieties. Although soil nematodes and microbial community structure differed between maize varieties, these could not be related to the *Bt*-trait.

An extensive soil ecological field sampling programme was running throughout the project life for soil microorganisms (Bacteria), microfauna (*Protozoa* and nematodes), mesofauna (*Collembola*, mites, enchytraeids) and macrofauna (earthworms). For all major groups of soil organisms no differences were observed with *Bt*-maize that were greater than differences caused by season, soil type, tillage practice or cultivar. Effects observed in the glufosinate ammonium herbicide tolerant maize was interpreted as stemming from the application of the Basta herbicide, which was observed for *Collembola* and earthworms. With regards to the decomposition of organic matter, as studied using a straw-litterbag methodology, *Bt*-maize had no negative effect on the decomposition in any of the three locations.

With herbicide-tolerant (HT) crops, reduced tillage is made agronomically more acceptable because the efficiency of broad spectrum herbicides does not require the assistance of tillage for weed control. Higher faunal abundances were found in reduced tillage both in *Bt*-maize and in HT-maize, however this outcome depends on the specificities of the reduced tillage system used. Dramatic reductions in earthworms were found when the system consisted of omitting autumn ploughing, while retaining vigorous superficial soil cultivation in spring.

The complex of economic and ecological factors involved in assessing cropping systems, including new technologies such as GM crops, was handled and integrated by a qualitative multi-attribute decision support model (Fig 1.). The model was based on domain specific knowledge from ECOGEN experts and was used for assessment at the farm level of GM and non-GM maize crops. Cropping systems were defined by four groups of features: (1) crop subtype; (2) regional and farm-level context; (3) crop protection and crop management strategies; and, (4) expected characteristics of the harvest. The impact assessment of cropping systems was based on four groups of ecological and two groups of economic indicators: biodiversity,

soil biodiversity, water quality, greenhouse gasses, variable costs and production value. The evaluation of cropping systems was governed by rules defined by domain experts and used by modellers to build a rule-based model, aiding the economic decision-making processes and predicting ecosystem behaviour.

When the ecological knowledge from single species tests, multispecies tests and field investigations from maize cropping systems was conveyed via the domain experts to the computer scientists, it was obvious that chemical disturbance, soil fertilisation and physical stress was paramount in determining soil biodiversity. In an overall practical assessment of the ecological and economic outcomes the model ranked cropping systems in the order: organically managed > GM systems including *Bt* and HT traits > conventionally managed maize.

Conclusions

The soil ecological evaluations at three levels of biological organisations, i.e. single species, mesocosm model communities and field ecosystems, produced specific conclusions at each tier of complexity:

- soil organisms held singly in laboratory cultures did not respond negatively to pure Bt-toxin or maize plant material containing *Bt*-toxin;
- mesocosm experimental test systems responded mainly to properties of maize varieties rather than to the *Bt*-toxin and to pesticides; and
- soil biodiversity field responses were detected mainly from tillage practices, soil type, crop type and history, pesticides and the maize variety.

The two upper-tiers did not correspond well with the first-tier, so a triple tier strategy is suggested when assessing the impact of GM plants in order too benefit from the strength of each tier in understanding and predicting field level effects.

Socio-economic evaluation of the benefits and costs of *Bt*-maize and HT-maize at the farm level and national level for selected EU-15 Member States generated crucial conclusions important for EU agricultural policy:

- the economic analyses indicates that EU corn-growing farmers would forego direct economic benefits in the order of EUR 150 million per year by the postponement of a full introduction of *Bt*-maize. Based on the ECOGEN analysis of impacts of *Bt*-maize and HT maize on soil biodiversity, we conclude that the direct economic benefits are likely to be high enough to compensate for possible irreversible costs of full introduction: further delay is therefore not warranted;
- the decision by the European Commission to postpone further approval of GM crops until labelling and tracking systems for GMOs are in place and coexistence rules established can be considered a wise decision, albeit this decision came at a high economic cost;
- from a purely economic perspective there might have been a social gain in waiting to adopt *Bt*-corn until more information was gathered to reduce the uncertainty associated with the reversible net-benefits of private consumers; and
- the introduction of *Bt*-corn and HT-corn only generates minor economic benefits at a per capita level: this might contribute to the reluctance of EU consumers to favour GM crops more.

Major Publications

Bohanec M., Messéan A., Scatasta S., Angevin F., Griffiths B., Krogh P.H., Žnidaršič M., Džeroski S. (2008). A qualitative multi-attribute model for economic and ecological assessment of genetically modified crops. *Ecological Modelling* 215, 247-261.

Kramarz P.E., De Vaufleury A., Gimpert F., Cortet J., Tabone E., Andersen M.N., Krogh P.H. (2009). Effects of Bt-maize material on the life cycle of the land snail *Cantareus aspersus*. *Applied Soil Ecology* 42, 236–242.

Wesseler J., Scatasta S., Nillesen E. (2007). The Maximum Incremental Social Tolerable Irreversible Costs (MISTICs) and other benefits and costs of introducing transgenic maize in the EU-15. *Pedobiologia* 51, 261-269.

Cortet J., Griffiths B.S., Bohanec M., Demšar D., Andersen M.N., Caul S., Birch A.N.E., Pernin C., Tabone E., de Vaufleury A., Ke X., Krogh P.H. (2007). Evaluation of effects of transgenic *Bt* maize on micro-arthropods in a European multi-site experiment. *Pedobiologia* 51, 207-218.

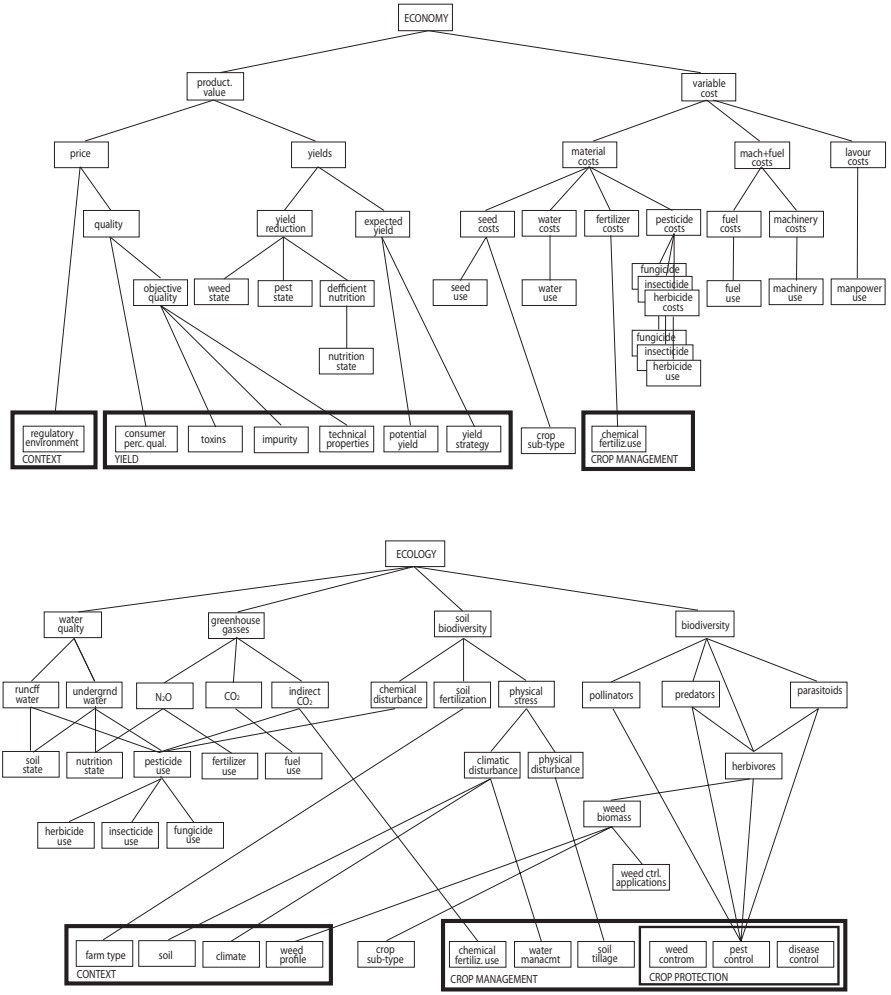


Fig 1.

The hierarchical structure of the comprehensive economy and ecology multi-attribute decision support model for evaluation of cropping systems. The model includes economic, agronomic and ecological knowledge as conveyed via domain expertise of ECOGEN experts.

Major Publications

Griffiths B.S., Heckmann L.H., Caul S., Thompson J., Scrimgeour C., Krogh P.H. (2007). Varietal effects of eight paired lines of transgenic *Bt* maize and near-isogenic non-Bt maize on soil microbial and nematode community structure. *Plant Biotechnology Journal* 5, 60-68.

Krogh P.H., Griffiths B., Demšar D., Bohanec M., Debeljak M., Andersen M.N., Sausse C., Birch A.N.E., Caul S., Holmstrup M., Heckmann L.-H., Cortet J. (2007). Responses by earthworms to reduced tillage in herbicide tolerant maize and *Bt* maize cropping systems. *Pedobiologia* 51, 219-227.

In the Parliament Magazine and European Research & Innovation Review:
Krogh P.H., Cortet J., Bohanec M., Scatasta S., Griffiths B.S., Gomot-De Vaufleury A., Caul S., Birch A.N.E., Andersen M.N., Sausse C., Fernandez S., Wesseler J. (2007). ECOGEN – Soil ecological and economic evaluation of genetically modified crops. *The Parliament Magazine*, p. 54.

Krogh P.H., Munkvold B.E., Bohanec M., Džeroski S., Heymans X., Amaikwu E., Davies M. (2007). Collaborative systems for EU research projects. *Research – European Research & Innovation Review* 1, 4.

Developing efficient and stable biological containment systems for genetically modified plants

Acronym
TRANSCONTAINER

Programme Acronym
FP6-FOOD

Contract number
023018

Period
May 2006 – October 2009

Coordinator
Ruud DE MAAGD
Plant Research International B.V.
Droevendaalsesteeg 1
NL-Wageningen, 6700 AA
The Netherlands
Ruud.deMaagd@wur.nl

Project website
www.transcontaner.org

Background and objectives

EU regulation on genetic modification has so far allowed only one transgenic crop to be grown on European fields. However, the possible release of GM plants was accompanied by a growing public (and scientific) concern that transgenes might be transferred to non-GM crops or even wild relatives where these exist. This concern led to a strong demand for the implementation of technologies that reduce exposure both for environmental reasons (e.g. gene flow between GM crops and wild relatives) and economic reasons (e.g. gene flow between GM and non-GM crops).

Co-existence of GM and non-GM crops could be facilitated by the implementation of biological containment strategies that reduce transgene flow. The TransContainer project, which comprised 13 partners from universities, research and government institutes, SMEs and industry, aimed to facilitate co-existence of GM and non-GM (including organic) agriculture.

The main objectives of the project were:

- to develop stable, environmentally safe and commercially viable biological containment strategies in crops economically relevant for Europe;
- to investigate the socio-economic, environmental and consumer impacts of implementing such strategies in Europe;
- to enhance the understanding and acceptance, by stakeholders and the general public, of co-existence through biological containment strategies, by invoking dialogue with and between these groups, and by facilitating informed policy and public debates on their consequences for co-existence measures.

Approach and methodology

The project was divided into six 'Work Packages' (WPs):

WP1	Management and coordination.
WP2-4	Development of biological containment strategies.
WP5	Environmental and economic technology assessment.
WP6	Dissemination of results.

Work Packages 2-4: Biological Containment Strategies
The goal of these technology-driven WPs was to develop a number of strategies for biological containment of transgenes. Where necessary, these strategies were complemented with controllable switches to restore fertility. Techniques for reversibly blocking flowering or out-crossing in species that are dependent on flowering for propagation and/or for (hybrid) seed production were developed and tested. The crops used were representative for crops grown for their seeds (oilseed rape), for their fruit (tomato and eggplant), or for their vegetative parts (sugar beet, rye grass, red fescue, poplar and birch). For some of these crops, several strategies were tested.

In WP2, 'Chloroplast Transformation', the objectives were the development of efficient plastid transformation protocols for rapeseed and sugar beet, which were to be used to generate herbicide-resistant plants free of antibiotic selection markers. The approach entailed identification of optimal varieties and tissue culture conditions for both species, the production of transformation vectors tailored to the plastome of each species, and the selection and molecular characterisation of transplastomic plants.

In WP3, 'Controllable Flowering', the major objective was to develop non-flowering sugar beet, grasses and trees. The second major objective was to develop systems for restoration of flowering in floral-repressed sugar beet and grasses. Containment strategies relying on floral repression are suitable for plants that are grown for their vegetative parts.

In WP4, 'Controllable Fertility", the objective was to establish biological containment strategies based on transgene mitigation in pollen through nutritional starvation (oilseed rape, [seedless] tomato), transgene mitigation in pollen through interference with basal transcription factors (oilseed rape, eggplant), and transgene mitigation in pollen and seed through cell ablation (grasses and oilseed rape). The work on transgene mitigation through nutritional starvation in oilseed rape was replaced at the end of the second year by a project on transgene mitigation through apomixis (clonal seed production).

Work Package 5: Technology Impact
This WP assessed the economic, environmental and consumer impact associated with implementing biological containment strategies in Europe, with the goal of providing recommendations for the practical use of the transgenic crop

Partners

Ove NILSSON
Sveriges Lantbruksuniversitet, Uppsala (SE)

Giuseppe Leonardo ROTINO
C.R.A. – Istituto Sperimentale per l'Orticoltura (Research Institute for Vegetable Crops) Montanaso (IT)

Alisher TOURAEV
University of Vienna (AT)

Martin KATER
Università degli Studi di Milano (IT)

Philip John DIX
National University of Ireland, Kildare (IE)

Justus WESSELER
Wageningen Universiteit (NL)

Sergio ARCIONI
Consiglio Nazionale Ricerche, Roma (IT)

Detlef Bartsch
Bundesamt fuer Verbraucherschutz und Lebensmittelsicherheit, Bonn (DE)

Ivan MINKOV
University of Plovdiv (BG)

Joseph Petrus MARIA
Schenkelaars Biotechnology Consultancy, Leiden (NL)

Magnus HERZBERG
SweTree Technologies AB, Umeå (SE)

Jensen CHRISTIAN
DLF-Trifolium A/S, Heddinge (DK)

in European agriculture. The economic assessment first considered the incremental economic benefits and costs to be expected from the containment strategy and, secondly, analysed the implications for adoption of transgenic crops under different national rules and regulations governing co-existence. The risk assessment of biologically contained GM crops was performed in conformity with the guiding principles of environmental and health assessment published by the European Food Safety Authority (EFSA).

Work Package 6: Dissemination

The objective of this WP was to communicate the results of the TransContainer project to the public and shareholders and to facilitate informed policy and political debate on the potential of biologically contained GM crops for adoption by the European market. Several public communication activities were carried out including:

1. organisation of three workshops at different locations in the EU;
2. interviews with more than 40 representatives of stakeholder organisations in Western and Eastern Europe;
3. production of a DVD with six stakeholder film portraits and an impression of the final European stakeholder workshop; and
4. provision of information following queries from non-governmental organisations and journalists.

Main findings and outcome

Chloroplast transformation

Two crops were targeted for development of chloroplast transformation protocols: sugar beet and oilseed rape. For sugar beet, a protocol was developed based on particle bombardment (biolistic method) of sugar beet petioles, which resulted in the generation of a number of herbicide-resistant plants (De Marchis *et al.*, 2008) Two transgenes were inserted, a selectable marker, and the green fluorescent protein (*GFP*) gene for visual screening of plastid transformants (Fig 1.). For oilseed rape, both biolistic delivery and PEG-mediated chloroplast transformation were attempted. The latter method successfully yielded homoplastic transformed tissue, however the regeneration capacity in oilseed rape is highly dependent on the variety, and in this respect transplastomic plants have not yet been regenerated in this variety.

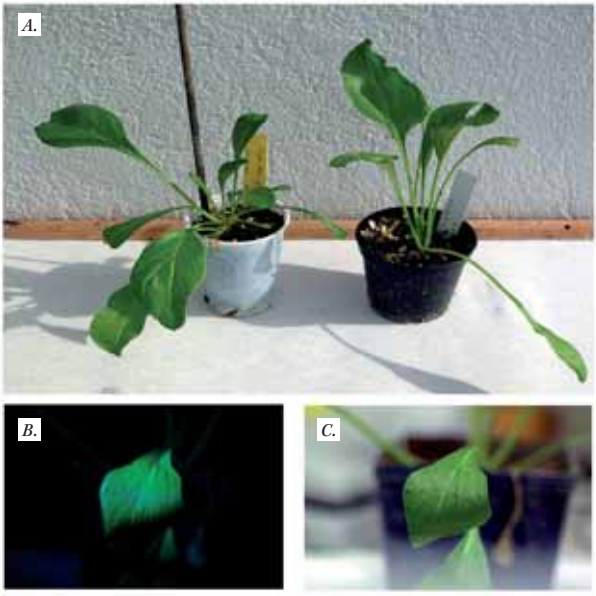


Fig 1.

Transplastomic sugar beet plants (A) growing in the greenhouse. Fluorescence (B) and bright-field (C) images from leaves of a transformed plant. Leaves of the transformed plant fluoresce green in the dark under UV exposure, indicating the presence of the GFP transgene.

Floral repression

Partially overlapping strategies for reduced expression of known flowering activating genes, or for the increased expression of known flowering inhibiting genes, were employed to block flowering in sugar beet, grasses, and hardwood trees. Most strategies were successfully implemented, although in some cases tests are still ongoing. Transgenic non-flowering lines were obtained for sugar beet, perennial ryegrass, tall fescue, hybrid aspen, and poplar. For sugar beet and grasses, where flowering is necessary to allow breeding and propagation by seed, further work was performed to establish a system for inducible restoration of flowering using an ethanol-inducible promoter. This principle has been demonstrated to work in *Arabidopsis* and tall fescue (Fig 2.), and has been introduced in sugar beet as well, but requires further testing and optimisation in all these systems.

Fig 2.

Ethanol induction of tall fescue plants transformed with UBI::LpTFL1 to promote floral repression and AlcA::LpTFL1-RNAi for floral restoration.

Controllable fertility

Various strategies were tested to address transgene transmission through pollen or seed in tomato, eggplant, oilseed rape and grasses. Reversible male sterility by nutritional starvation worked well in tobacco (Ribarits *et al.*, 2007), but less so in tomato. Sterility by down-regulation of basal transcription factors worked well in eggplant and could be reversed using an ethanol-inducible system. Prevention of transgene spread through seeds was obtained by modifying expression of a transcription factor required for chloroplast biogenesis during embryo development (Colombo *et al.*, 2008). This so-called 'Recoverable Block of Function' approach was successfully used for inducible recovery of viable seeds. Apomixis, the asexual production of seeds, was studied as a potential way to ensure true-breeding seed production in male-sterile plants, but the research is still at a fundamental stage.

Technology Impact

The potential impact of implementing biological containment strategies for co-existence in European agriculture and consumer health was assessed. It was concluded that, for many crops, biological containment may not be necessary under current culture conditions, as current co-existence measures would be sufficient (Ansink and Wesseler, 2009). However biological containment may be valuable as an extra level of protection for crops with non-food/feed uses, such as industrial or pharmaceutical-producing plants. As no strategy is expected to be 100 % efficient, a combination of strategies is advisable in such cases. Adverse effects on human health or on the environment resulting from the specific biological containment strategies developed in this project were deemed unlikely, but will require a more detailed analysis, especially for the novel approaches for which there is no precedent n the literature (Koziolek, 2009).

Dissemination

Adequate public communication and stakeholder involvement were important goals in this project. To this end, three one-day workshops were organised and stakeholder interviews, as well as a DVD with film portraits, were undertaken to provide background material for discussions. All results are being published on a public website (www.transcontainer.org) and film portraits are accessible through *www.youtube.com*.

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Conclusions

The Transcontainer project has been successful in achieving many of its objectives. A number of strategies, such as inhibition of flowering, chloroplast transformation and male sterility have been further developed and implemented in several important crops, thus considerably extending the possibilities for obtaining biological containment. The transgenic plants developed in this project may be used for testing the efficiency of containment under greenhouse conditions or in field experiments. Studies on the potential socio-economic impacts and the dissemination activities revealed that our research comes at a rather early stage in the introduction of GM crops in Europe, and that various stakeholder groups are not aware of the different options for biological containment. In this respect our dissemination activities have contributed to growing awareness of this topic.

There has also been some controversy surrounding this project, as certain groups regard the biological containment strategies studied in the project as Genetic Use Restriction Technologies (GURTs), also known as 'terminator technology". Modification of reproductive properties of the plant is inevitable in a biological containment strategy and this aspect of biological containment, whether it is achieved through GM- or non-GM approaches, will remain an issue to be dealt with. In a way, therefore, this project was well timed with regard to raising interest in these issues.

Major Publications

Ansink E., Wesseler J. (2009). Quantifying type I and type II errors in decision-making under uncertainty: the case of GM crops. *Letters in Spatial and Resource Sciences, 1-6.*

Colombo M., Masiero S., Vanzulli S., Lardelli P., Kater MM., Colombo L. (2008). AGL23, a type I MADS-box gene that controls female gametophyte and embryo development in *Arabidopsis. Plant J* 54:1037-1048.

De Marchis F., Wang Y., Stevanato P., Arcioni S., Bellucci M. (2009). Genetic transformation of the sugar beet plastome. *Transgenic Res* 18:17-30.

Koziolek C. (2009). Biosafety assessment and benefits for co-existence of biological contained plants- regulatory assessment in the EU-project 'transcontainer". *Journal fur Verbraucherschutz und Lebensmittelsicherheit* 3:41.

Ribarits A., Mamun ANK, Li S., Resch T., Fiers M., Heberle-Bors E., Liu CM, Touraev A. (2007). Combination of reversible male sterility and doubled haploid production by targeted inactivation of cytoplasmic glutamine synthetase in developing anthers and pollen. *Plant Biotechnol J* 5,483-494.

Sustainable introduction of GM crops to European agriculture

Acronym
SIGMEA

Programme Acronym
FP6-POLICIES

Contract number
FP6-2002-SSP1-501986

Period
May 2004 – December 2007

Coordinator
Jeremy SWEET
National Institute for Agricultural Botany
Huntingdon Road
Cambridge CB3 0LE
United Kingdom
Jeremy.sweet@niab.com

Background and objectives

The cross-disciplinary European SIGMEA Research Project was set up to create a science-based framework to inform decision-makers about the appropriate coexistence and traceability measures that would be needed for GM crop cultivation. To inform the sustainability of GM crops, SIGMEA has: (i) collated and analysed all European data on gene flow and the environmental impacts of the major species concerned by GMOs (maize, rapeseed, sugar beet, rice, wheat); (ii) designed predictive models of gene flow at the landscape level; (iii) analysed the technical feasibility and economic pertinence of co-existence in the principal farming regions of Europe; (iv) developed novel GMO detection methods; (v) addressed legal issues related to coexistence; and (vi) proposed public and private decision-making tools as well as guidelines regarding management and governance.

Approach and methodology

SIGMEA brought together the principal teams and principal programmes studying gene flow in several European countries and a wide range of agricultural systems, including organic cropping. Seven regional case studies were carried out for designing and assessing scenarios for coexistence.

The project collated and synthesised experimental data on gene flow, identified and filled gaps in knowledge by designing and conducting further evaluations, particularly on a landscape-scale or over several years in the cropping sequence. Maize and oilseed rape were the major GM crops targeted for this study – other crops under consideration were sugar beet, rice and wheat.

Available information from past or ongoing field studies on cross-pollination, volunteers, ferals and wild relatives were gathered from 22 SIGMEA partners based on a rigorous quality control process. The database had over 100 data sets, constituting more than 150 'experiment-years'. Around two-thirds of the data are for oilseed rape or close relatives; there is also information for beet and maize and a few data sets for wheat and rice. Data on crops and volunteers each constitute about 35 %, wild relatives 16 % and ferals 6 %. Data on ecological impacts – as distinct from geneflow by seed and pollen – form a small part of the total.

The datasets cover most of the recent relevant information for these crops in Europe (see Fig 1.), especially research at 'field' or landscape' scales. For oilseed rape, the datasets on cross-pollination, volunteers and ferals comprise all but one of the main field experiments carried out by SIGMEA partners (and that one is included in the synthesis). For maize, although two additional major datasets do exist, these data were not available for inclusion but investigations based on these datasets have been published and their findings have been included in the conclusions provided here.

Most of the data sets provide very much more detail than appears in refereed publications and they have been extensively used to provide added value through meta-analysis and data mining. Due to very high replication achieved by combining data from different sites, the crop-specific conclusions in SIGMEA on cross-pollination and seed persistence (see second chapter below) are mostly of very high statistical significance and make it possible to draw general conclusions about given topics, such as pollen dispersal in the case of maize.

In summary, the database, together with information already published, provided a sound basis to enable SIGMEA to reach a set of conclusions specific to each of maize, oilseed rape and beet, as summarised below. Additionally, the database allowed an assessment of three questions about transferability of information: how consistent are measurements at different spatial (or temporal) scales, how consistent are they between agricultural regions in Europe that have different climates and soils, and how consistent are they between species.

Main findings and outcome

1. Gene flow studies inform coexistence in maize, oilseed rape, sugar beet, wheat and rice

Maize. The studies are consistent across numerous sites and indicate (a) a steep decline in cross-pollination over three orders of magnitude (a 1000-fold) over distances up to 100 m from the edge of a field, (b) a strong effect of wind direction and related meterological factors on percentage pollination, and (c) a generally consistent distance-dependence at scales of both plot and field. This latter consistency has been found where donor and receptor fields are well dispersed in the landscape and at a generally low overall density.

Partners

André POUZET
Centre Technique Interprofessionnel des Oléagineux Métropolitains, Paris (FR)

Antoine MESSÉAN
Institut National de la Recherche Agronomique, Paris (FR)

Broder BRECKLING
University of Bremen (DE)

Thomas REIß
Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung eV, München (DE)

Emilio RODRIGUEZ
Joint Research Centre, Brussels (BE)

Saso DZEROSKI
Jozef Stefan Institute, Ljubljana (SI)

Julian KINDERLERER
University of Sheffield (UK)

Joe PERRY
Rothamsted Research, Harpenden (UK)

Geoff SQUIRE
Scottish Crop Research Institute, Dundee (UK)

Howard THOMAS
Institute of Grassland and Environmental Research, Aberystwyth (UK)

Peter STAMP
Swiss Federal Institute of Technology, Zürich (CH)

M.C. CHUECA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid (ES)

Roberto PAPA
Universita Politecnica delle Marche, Ancona (IT)

Enrico BIANCARDI
Istituto Sperimentale per le Colture Industriali, Bologna (IT)

Detlef BARTSCH
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Bonn (DE)

Partners

B. VOSMAN
Plant Research International, Wageningen (NL)

John KILPATRICK
ADAS Consulting limited, Wolverhampton (UK)

Christine HENRY
Central Science Laboratory, London (UK)

Carola PEKRUN
University of Hohenheim, Stuttgart (DE)

Slavomir RAKOUSKY
University of SouthBohemia, Ceske Budejovice (CZ)

Klaus AMMAN
University of Bern (CH)

Marc DE LOOSE
Institute for Agriculture and Fisheries Research, Merelbeke (BE)

Eric TOLLENS
CatholicUniversity of Louvain, Leuven (BE)

Josef SOUKUP
Czech University of Agriculture in Prague (CZ)

Morten GYLLING
Danish Research Institute of Food Economics, Frederiksberg (DK)

Bruno Henry DE FRAHAN
Université Catholique de Louvain, Louvain-La-Neuve (BE)

Soren MIKKLESON
Danish Institute of Agricultural Sciences – now University of Aarhus, Frick (DK)

Otto RICHTER
TechnicalUniversity of Braunschweig, Braunschweig (DE)

Pedro CASTANERA
Consejo Superior de Investigaciones Científicas, Madrid (ES)

Joachim SCHIEMANN
Federal Biological Research Centre for Agriculture and Forestry, Braunschweig (DE)

To sum up, the potential to introduce the adventitious presence of GM material in non-GM maize production is:

- moderate for cross-pollination between fields, which can be managed through separation, discards or buffers where crops are in close proximity;
- low through volunteers, and then mainly in southern Europe;
- low for introgression to landraces from modern crop varieties;
- zero through wild relatives (there are none in Europe).

Oilseed rape. Transfer by seed can be very high, as large seedbanks can survive for several years producing volunteers. Seed can disperse on farm machinery and transport. A range of agronomic practices are deployed to limit volunteers, but the contribution of volunteers to yield is still highly unpredictable and may range from <0.01 % to more than 10 % for the same crop variety in different management, soil and weather. Pollen is carried in the wind and by hive bees, bumble bees and a variety of other insects, and cross-pollination is both greater and much less directional than in maize. Crossing between commercial fields can remain as high as 0.1 % even at distances between 100 m and 1 000 m. The abundance of wild relatives and feral plants differs between regions, but in general the lower fertility and fitness of ferals and wild relatives mean they do not constitute a major route for transmission of traits back to crops.

The potential to introduce GM impurities is:

- moderate for cross pollination between oilseed rape fields, which can be managed through spatial separation and use of buffers or discards where crops are in close proximity;
- high through seed banks resulting in volunteer populations that admix with and pollinate non-GM crops – volunteers are ubiquitous, mobile and commonly in high abundance and are of maximum importance to coexistence over time and, to a lesser degree, in space;
- moderate in the case of wild relatives in localised areas of Europe where they occur in high abundance in fields (e.g., *B. rapa* in Denmark);
- moderate to low through ferals (with some local exceptions) because of their low overall density compared to crops and volunteers in the landscape;
- in addition, the potential for low levels of cross-pollination among crops, volunteers, ferals and wild relatives by insects and wind, up to a long distance in the landscape, may lead to potentially significant background levels to

augment impurities from other sources. Problems of coexistence during the first few years of commercialisation can be reduced by management of cross-pollination (through separation, etc.) and seed purity, but large uncertainties remain about the ability of farming to manage the cumulative, long-term movement and amplification of volunteers and wild relatives.

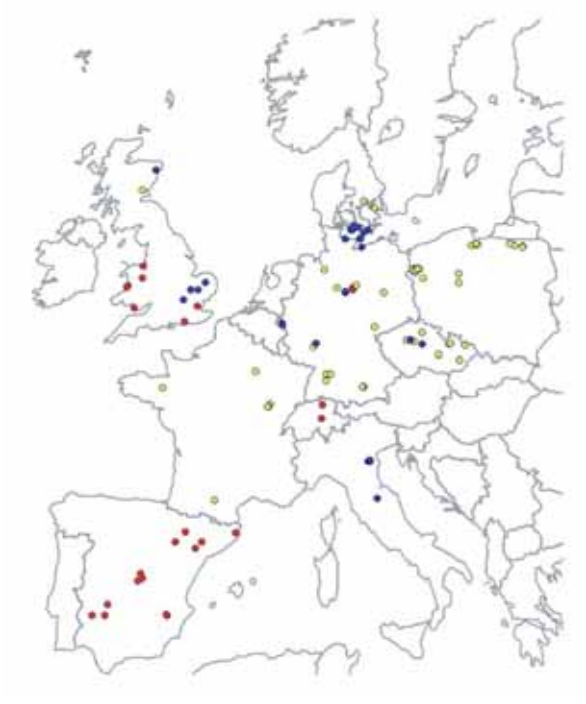


Fig 1.

Locations of main experimental studies available in the SIGMEA database on maize (red), oilseed rape (yellow) and beet (blue). Some locations involved several experiments.

Beet. The main source of impurity is in seed bulked for commercial sowing. To prevent cross-pollination, GM and non-GM seed bulking fields would need to be grown some considerable distance apart, possibly in different regions. The wild form, sea beet, is central to gene movement in the *Beta* complex, and it is important to preserve the diversity of sea beet for conservation of genetic resources and study in its own right. Genetic assessments in SIGMEA, from plants growing along both the Baltic and Adriatic coasts, have confirmed that

Partners

Peter WALKLATE
Silsoe Research Institute, Bedford (UK)

Heike BEISMANN
Technische Universitaet Muenchen (DE)

Julian HUNT
Cambridge Environmental Research Consultants, Cambridge (UK)

Gosta KJELLSON
National Environmental Research Institute, Roskilde (DK)

P.H. GOUYON
University of Paris 11, Orsay (FR)

Rodolphe DE BORCHGRAVE
Arcadia International, Brussels (BE)

Joaquima MESSEGUER
Institut de Recerca i Tecnologia Agroalimentàries, Barcelona (ES)

Rikke BAGGER JORGENSEN
Riso National Laboratori, Roskilde (DK)

Iwano BARTOWIAK
Plant Breeding and Acclimatisation Institute, Blondie (PL)

Michael WILKINSON
University of Reading, (UK)

Daniel BLOC
ARVALIS – Institut du Végétal, Paris (FR)

Klaus MENRAD
University of Applied Sciences of Weihenstephan, Freising (DE)

populations remain highly diverse and distinct from crop varieties. After the annual trait is transferred from sea beet to seed bulking fields, it appears in sugar beet fields as plants that flower in the first year, while the pure biennial sugar beet crop plants are harvested before they flower. If allowed to mature, these annual weedy beets give rise to a seedbank lasting for many years, and during flowering they will exchange genetic material with weed beets in other fields. Traits, whether GM or otherwise, have the potential to spread in commercial production areas, but weed beets are controllable with best farming practice. The spread of such traits constitutes a potential problem for weed control, but not strictly for coexistence provided none of their root material is harvested along with sugar beet.

The potential to introduce GM impurities is:

- low through cross-pollination between sugar beet crops since the harvest is vegetative;
- low through volunteer (weed beet) populations which arise from impurities in sown seed – best management should prevent any root material being harvested from these beets;
- moderate to high, though localised, through ferals and wild relatives through gene movement to seed production crops, especially as male sterile mother plants (readily cross-pollinated) are often cultivated for F1 seed production;
- potentially high between flowering beet seed crops especially as male sterile mother plants are often cultivated for F1 seed production (though there is little direct evidence of the form of decline in cross-pollination with distance between such crops).

Coexistence should be achievable by best management, especially of seed production crops. GM commercial seed production areas need to be sufficiently isolated from non-GM areas and from wild and weedy beet to keep the non-GM seed pure and reduce the spread of genes.

Wheat. The potential for introducing impurities is likely to be:

- low through cross pollination between crops;
- low through hybridisation with wild relatives in those local areas where they occur.

The contribution of volunteers needs to be clarified, but their importance for temporal coexistence is likely to be low or moderate under European conditions;

Further investigations on all aspects of cross-pollination and life cycle dynamics are needed before firm prescriptions can be made on the management of coexistence in wheat.

Rice. The potential for introducing impurities is likely to be:
- low through cross-pollination between crops;
- low through volunteers;
- low to moderate through the weed, red rice, in local areas where it occurs, provided agricultural practices to control this weed are applied.

Coexistence should be achievable through separation of crops, controlling weedy relatives and using high-purity seed.

2. Environmental impacts of GM crops within European cropping systems

The conclusions for the main crops studied are as follows:
- **Maize** (*Bt* varieties, targeted at corn borers). There is no reason on grounds of biosafety to restrict the scale of growing. The most consistent finding is that *Bt* maize in field trials in Europe has so far had no systematic or reproducible effects on any of the invertebrates or soil organisms studied over several years where, over a similar period, other agronomic factors have had large and measurable effects. Resistance development in corn borers should be monitored, as well as the potential effects on certain non-target biotic groups.
- **Oilseed rape** (HT varieties, tolerant to glufosinate ammonium or glyphosate). Adverse impacts occurred where a) the herbicides used in HT cropping caused a systematic depletion of the weed flora and dependent invertebrates, resulting in reductions in biodiversity within fields, and b) the presence of HT volunteers limited future options for use of herbicides and growing certain crops such as beans (in which volunteers are difficult to control). Positive effects may occur as the herbicides used in HT cropping are less toxic to non-weed organisms than most other herbicides and crop protection chemicals. However the ecological effects of HT crops in the same production system are smaller than those resulting from differences between crop species, sowing season or agronomic practices.
- **Beet**. The various types of beet – crop, weed, feral, wild – are in genetic contact through seed and pollen. Wild beet needs proactive conservation, since it is a biologically interesting plant form of restricted habitat, a source of genes for future beet breeding and a source of annual impurities in crop beets. HT beet cultivation could also

deplete biodiversity within fields for the same reasons as discussed for HT oilseed rape, but most effects are smaller than those due to general agronomic operations.

There is an increasing consensus that future assessment of GM crops should consider both negative and positive impacts of GM cropping in a more holistic way. Standards and criteria for environmentally resilient cropping systems are needed, against which GM cropping and its non-GM comparator can be assessed. Setting such environmental standards is now a priority.

3. Analysis of feasibility of coexistence and costs in various European agricultural scenarios

Managing coexistence in practice has been studied at the regional level by assessing the impact of growing GM crops on gene flow under various scenarios. Regional case studies were conducted on three scales: whole regions, small agricultural regions corresponding to homogeneous farming systems, and small landscapes of a few km^2.

Seven case studies were chosen, but the full methodology was implemented only for two of them. The work carried out in Aragon, Aquitaine and Fife aimed at comparing the effect of structural variables on gene flow and the management of critical points between case studies. Simulations were carried out in Switzerland and Schleswig Holstein to illustrate specific problems or phenomena such as the management of boundaries (Switzerland/France) or dilution effects (Schleswig Holstein). Although Beauce and Alsace were the main studies, generic conclusions were drawn for other regions as well.

The work carried out suggested a framework to identify and organise the main factors that could determine the implementation of coexistence in specific contexts:

1. **Structural variables** describing the characteristics of the agroecosystem (cropping systems, landscapes, meteorology, crop management) influencing gene flow.
2. **Organizational variables** of farming operations and grain collection, storage and distribution, explaining how they adapt to certain constraints and rooms for manoeuvre.
3. **Characteristics of the introduction of GMOs**. Coexistence implementation also depends on market conditions (relative prices of GM and non-GM products),

on considered thresholds (which can differ from what is required by regulation, e.g., specific market requirements) and on traits (some traits – e.g. *Bt* traits which require refugia areas – may facilitate or constrain certain types of coexistence measures).

Based on the simulation results obtained in regional case studies, the project identified four major situations, so-called pre-scenarios[4], that local stakeholders may have to deal with:

1. segregation at the silo level is feasible without any specific measures at the field level;
2. curative measures at harvest (selection of non-GM fields or parts of fields) permits meeting market requirements in terms of targeted thresholds;
3. preventive measures at the crop level (e.g., sowing dates) or at the system level (crop rotation, spatial arrangement of crops);
4. coexistence is not possible because, whatever the agronomic measures undertaken at the crop or system level, the targeted threshold cannot be met or requires non realistic measures.

Three processes determine how pre-scenarios may be embedded into global management scenarios:

1. the system and rules for collating and sharing information at the territory level;
2. the framework and procedures describing coordination between actors;
3. and learning processes (both individual and collective).

Based on these findings, contrasting global scenarios may be defined by considering different regulation approaches:

- A 'bottom-up' approach, which freely allows the private actors (collector, farmers) to choose the best way to achieve the objectives of coexistence and to meet regulatory or market-based threshold requirements;
- A 'top-down' approach, based on the strong intervention of public authorities with the implementation of compulsory uniform measures (e.g., isolation distances);
- and a 'third way' approach, which provides a focused response of authorities to lift some constraints on information and coordination between private actors, and allow some flexibility in the measures.

[4] The word 'pre-scenario' is used because the pre-scenarios only cover a component of the overall picture and should then be integrated into overall management scenarios taking into consideration other factors than those affecting farm coexistence (see below).

Each approach has advantages and disadvantages: the 'bottom up' approach allows greater flexibility and lower costs, and may help deal with management problems beyond the scope of the GM regulations, such as specific requirements for the 'Identity Preserved' (IP) market, but it may not prevent public distrust and does not solve all liability issues. The 'third way' takes advantage of both local knowledge from individual stakeholders and the ability of public authorities to collect and share information on a wide scale, in order to cope with practical problems raised by the implementation of coexistence measures.

4. Costs of coexistence

The economic perspective of coexistence of GM and non-GM crops up to the farm gate with specific applicability to oilseed rape (OSR) and maize in different regions of the EU was investigated by SIGMEA. Three levels of coexistence costs were considered:

- costs of compliance with the coexistence measures developed to prevent adventitious presence of GM material as a result of cross-pollination;
- monitoring costs (testing for adventitious presence in non-GM crops);
- costs due to failure of the system (losses due to contamination of conventional crops).

Coexistence costs are calculated with spatial simulation models, taking into account the economic incentives for coexistence. Using a Geographical Information System (GIS) dataset and Arcview® software, a set of simulations of realistic coexistence scenarios was undertaken.

Results showed that coexistence costs depend on the agricultural context (landscapes, cropping systems, climate, practices), the share of GM crop (maize or oilseed rape) in the Agricultural Used Area (AUA) and the willingness of GM and non-GM farmers to cooperate.

Uniform non-flexible coexistence rules might impose a severe burden on GM crop production in the European regions investigated in this study. Indeed, cross-pollination largely depends on structural factors like landscape field patterns, agronomic practices and climatic conditions and, in most cases, small isolation distances would be sufficient to meet the official threshold of 0.9 %. Large uniform isolation distances, as implemented by most European countries, are not flexible and, therefore, not proportional to the actual risk of adventitious presence. In addition, large and/or fixed isolation distance requirements

may lead to a domino effect[5] so that farmers would have few, if any, fields complying with these isolation distances and would be unable to cultivate GM crops. The domino-effect exacerbates the non-proportionality of wide isolation distances by reducing GM crop planting options in the landscape and raising opportunity costs for GM crop adopters.

Flexible measures based on buffer zones or discard zones require a high level of coordination between farmers resulting in additional transaction costs and financial risks. The average per-hectare coexistence management costs, although variable, were relatively independent from the GM adoption rate in moderately dense areas such as Aragon (maize) or Scotland (oilseed rape). There are, however, large differences in the monitoring costs related to GM crop adoption rates: the higher the GM adoption rate, the lower the additional per-ha costs of coexistence[6].

Based on *Bt* maize cultivation experience in Spain, a face-to-face survey was conducted among Spanish commercial maize farmers with the aim both of obtaining data on the agronomic and economic performance of *Bt* maize during three growing seasons (2002–2004) and of comparing the socioeconomic profile of farmers who adopted Bt maize versus those who did not. The survey was conducted in the three leading *Bt* maize-growing regions (Aragon, Catalonia and Castilla-La Mancha), and a province was selected within each region based on the presence of farmers growing *Bt* maize (the provinces of Zaragoza in Aragon, Albacete in Castilla-La Mancha and Lleida in Catalonia).

It was found that *Bt* maize produced variable impacts on maize yields in different provinces, ranging from neutral to 11.8 % yield increase. Regional variability depended mainly on local variations of pest pressure and damage. *Bt* maize seeds were more costly than conventional seeds, but farmers had reduced insecticide use and costs. Yield gains for growers of *Bt* maize were translated into revenue increase and the impact of *Bt* maize adoption on gross margins ranged from neutral to EUR 122/ha per annum. In the survey, the reason most quoted by farmers for adopting *Bt* maize was 'lowering the risk of maize borer damage' followed by 'obtaining higher yields'.

[5] The domino-effect is a dynamic spill-over effect of farmer decisions induced by enforcing wide isolation distances on potential GM crop adopters. It consists in the iterative process of farmers switching their planting intentions from 'GM' to 'IP' crops to comply with isolation distances and hereby restricting planting options of neighbouring farmers.

[6] Monitoring costs of non-GM fields might increase but would be supported by a larger GM acreage.

SIGMEA has also undertaken a large survey to estimate the potential adoption by farmers of three GM crops not yet authorised in the EU: Herbicide Tolerant (HT) oilseed rape, HT maize and Bt/HT maize (combining herbicide tolerance and insect resistance). Preliminary analyses of farmers' responses show that there is high potential adoption of HT oilseed rape and HT maize, as well as Bt/HT maize. On average, 41 % of farmers surveyed in the six countries are prepared to plant these GM crops. This figure nevertheless depends to a large extent on the coexistence measures put in place by EU Member States.

5. Current regulatory regimes of EU and Member States, liability and redress issues

No novel problems are posed at the present time by GMOs in relation to liability and redress. There is a range of established legal tools available to regulate GMOs. Civil regimes, insurance-based regimes, and compensation-based state regimes have all been studied and none shows any particular technical problems. There is, of course, the question for the insurance model of whether a market can be established to make this a viable regime.

The question of responsibility clearly needs resolution before the choice of regulatory regime can be set. It would seem logical that those who encourage the development of the technology, be it state or consumer, actively or passively, bear levels of responsibility for the consequences of those choices. There is no guarantee that the added costs of a system requiring the investigation of proof and blame will be more efficient than a compensation scheme.

Trans-border issues relating to GMOs make the desirability of an EU-wide single legal regime very strong, which could eliminate the likelihood of costly conflicts or legal problems between Member States. However this degree of agreement over GMOs is currently unlikely.

Conclusions

The coexistence between different types of crops is an important issue which must be addressed once GM crops are approved in the EU. SIGMEA has produced a toolbox for addressing GM impact and these tools and outcomes can be combined to assess coexistence at various spatial scales (field, farm or region) and various decision-making levels (farmers, grain handlers, Member States, EU).

It has been stressed that coexistence regimes based on 'uniform isolation distances', as implemented in several Member States, are not optimal, not proportional and may lead to unnecessary additional costs or render coexistence impossible.

SIGMEA recommends that coexistence measures should be as flexible as possible and depend on local climatic, agronomic and environmental factors. Such an approach would lead to more cost-efficient measures. However the current regulatory framework to support such an approach has still to be developed.

To assist in assessing coexistence measures, SIGMEA has developed a series of practical tools: *LandSFACTS*, a landscape generator simulating agricultural landscapes, *LandFlow-Gene*, a practical and dynamic generic gene flow modelling platform, the *Grignon* qualitative multi-attribute model for the assessment of ecological and economic impacts, the *SMAC Advisor* which provides advice to farmers and other decision-makers, and a pre-harvest method for estimating the GM content of conventional maize fields.

Major Publications

In the year 2009:

Messean A., Squire G., Perry J., Angevin F., Gomez M., Townend P., Sausse C., Breckling B., Langrell S., Dzeroski S., Sweet J. (2009). Sustainable introduction of GM crops into european agriculture: a summary report of the FP6 SIGMEA research project. OCL 16(1), 37-51.

Bitocchi E., Nanni L., Giardini A., Buonamici A., Vendramin G.G., Papa R. (2009). Introgression from modern hybrid varieties to landrace populations of maize (*Zea mays* ssp. *mays L.*) in central Italy. *Molecular Ecology*, 18:603-621.

Colbach N., Devaux C., Angevin F. (2009). Comparative study of the efficiency of buffer zones and harvest discarding on gene flow containment in oilseed rape: a modelling approach. *European Journal of Agronomy.* 30:187-198.

For further publications before 2009, please check: http://www.inra.fr/sigmea/publications__1

Acquired environmental epigenetics advances: from *Arabidopsis* to maize

Acronym
AENEAS

Programme Acronym
FP7-KBBE

Contract number
226477

Period
April 2009 – March 2013

Coordinator
Serena VAROTTO
Via VIII Febbraio 2
I-35122 Padova
Italy
serena.varotto@unipd.it

Background and objectives

The 20th century has seen a tremendous increase in crop yield, mainly due to changes in the genetic potential of the crop and to advances in agricultural practices. The increasing yield ability for crops obtained in the last century was due primarily to the use of genetic breeding to improve tolerance of abiotic and biotic stresses, coupled with maintenance of the ability to maximize yield per plant under non-stressing growing conditions. Considering evidence for global climate change, it is clear that improving crop ability to grow in adverse environmental conditions remains the greatest challenge to ensure that the demand side for agricultural produce is matched by the supply side.

Approach and methodology

The 'Acquired Environmental Epigenetics Advances: from *Arabidopsis* to maize' (AENEAS) project explores the environmental-induced and epigenetics-related source of variability as a new, until now largely underestimated, source of variability for crop improvement. Like the classical Aeneas, this proposal aims to 'explore' environmental-induced epigenetics changes as the new frontier of natural and artificial variability. In particular, the study will focus on the exploitation of this source of variability in maize, a crop of high agronomic relevance for Europe (11 Mha of land under maize cultivation) and worldwide (145 Mha).

Environmental cues, particularly stresses and shocks, greatly affect gene and genome activity. The environment, in addition to inducing genetic variability due to mutation of the DNA nucleotide sequence, provokes the formation of stably inherited epialleles, with relevant effects on the phenotype. This occurs by the activation of specific epigenetic mechanisms which, acting on chromatin, add epigenetic marks (DNA methylation, histone modifications etc.) and alter spatial and temporal patterns of gene expression.

A large part of the information on the environmentally related epialleles formations arises from studies carried out on the *Arabidopsis* model plant, but little is known for agricultural crops. In addition, the precise mechanisms of the environmentally induced epialleles formation and, particularly, of their maintenance throughout generations, remain largely unclear. Therefore, the first aim of the proposal is to clarify these phenomena in *Arabidopsis* and to use pre-existing indications for maize, suggesting that mechanisms common to *Arabidopsis* regulate the environmentally related epialleles formations and constitute a so-called 'maize environmental epigenetics' platform. This platform will represent the starting step towards the generation of genetic systems in maize which enhance and optimise the formation and exploitation of the environmentally induced epialleles.

The tools within the 'maize environmental epigenetics' platform will represent the basis for new technologies and strategies to sustain European Union competitiveness in the production of superior maize hybrids with enhanced agronomic performance when grown in adverse environments. The deliverables from the AENEAS project will be the progenitors for the next-generation of breeding programmes, based on the exploitation of the environmentally induced epigenetics variability. The comparative genomics analysis will allow for better understanding the similarities and differences between two evolutionarily distinct plant species in the mechanisms of the epigenome response to environmental cues.

Partners

Vincenzo ROSSI
Consiglio per la Ricerca e Sperimentazione in Agricoltura, Padova (IT)

Jose GUTTIERREZ-MARCOS
The University of Warwick (UK)

Jerzy PASZKOWSKY
Université de Genève (CH)

Caroline DEAN
John Innes Centre, Norwich (UK)

David Charles BAULCOMBE
The Chancellor, Masters and Scholars of the University of Cambridge (UK)

Detlef WEIGEL
Max Planck Gesellschaft zur Foerderung der Wissenschaften e.v., München (DE)

Catherine GUICHARD
BIOGEMMA, Paris (FR)

Chapter 2

GMO and Food Safety

Introduction

Dr. HARRY, A. KUIPER
Formerly RIKILT
Institute of Food Safety
Wageningen University and Research Center

The European Commission has recognized early on the importance of the development of new biotechnology approaches in the context of food policies and consumer protection. From 1985 until present, the Commission has taken important initiatives and provided substantial support for research on biosafety and food/feed safety aspects of genetically modified organisms (GMOs) through a series of EC co-sponsored research programmes. This Chapter focuses on the projects carried out in the area of food safety under the Fifth, Sixth and Seventh EC Framework Programmes for Research.

Safety Assessment

A number of projects (ENTRANSFOOD, GMO CARE, SAFOTEST, NOFORISK, and GMOBILITY) have focused on the development of safety assessment approaches for GM foods/feed. This is an issue of great scientific interest and also of interest for the general public: to examine whether food/feed products derived from GMOs pose particular risks for humans, animals and the environment upon long term exposure and consumption.

The Thematic Network ENTRANSFOOD which consisted of different stakeholders across Europe specialized in development and production of GM crops and derived foods, in risk-assessment, -management, and –communication strategies, and has designed a detailed step-wise procedure for the risk assessment of GM crop derived food and feed. The proposed procedure was an important step forward in risk assessment of the new category of foods, since it added a significant level of detail to the general requirements for the actual safety assessment. The risk assessment approach as developed within ENTRANSFOOD has also been embedded in the risk assessment strategy more recently developed by the European Food Safety Authority, and is in line with international guidelines developed by the FAO/WHO.

The possible occurrence of unintended alterations in the composition of GM food crops as result of the genetic modification, was one of the key issues addressed by ENTRANSFOOD. Detection of unexpected effects in GM food crops relies primarily on a *targeted* approach, i.e. comparative determination in GM and non-GM products of levels of *selected* macro- and micro-nutrients, anti-nutrients, and known toxins. In order to increase the probability of detecting unintended effects,

'*profiling*'- or '*omics*'- techniques have been further developed within the GMOCARE project. These evolving technologies include transcriptomics, proteomics, and metabolomics enable measurement of thousands of metabolic compounds in modified and unmodified plants, which are not defined prior to analysis (*non-targeted approach*). Research has concentrated on various transgenic potato lines modified in their starch, amino acid, or glycoalkaloid metabolism, on transgenic tomato lines with elevated phytosterol or isoprenoid contents, and on various *Arabidopsis* GM-lines, down-regulated in their flavonoid pathway. Application of profiling methods is promising since extensive information is provided on the physiology of GMOs and their non-modified counterparts, but further development and validation is needed, before they can be used in a formalized risk assessment procedure. The classical targeted compositional analysis of GMOs and their conventional non-modified counterparts together with information from the molecular characterization and analysis of agronomical properties of the GMOs, is a sound and robust way to determine possibly whether due unintended effects may have occurred in GMOs possibly due to the genetic modification.

Toxicological testing of newly expressed proteins and of whole GM foods has been examined in the SAFOTEST project. A combination of *in vivo* animal models, *in vitro* toxicological systems, and selected profiling methods was used to characterize GM rice strains containing lectins, or *Bt* protein. Repeated dose studies in rats were performed with diets containing the target proteins, or the GM rice spiked or not with purified lectin. The results demonstrated that the specificity and sensitivity of the 90-day rat feeding study to detect specific compound-related effects and unintended secondary effects is fit-for-purpose, which enables the establishment of the safety of the GM food. This approach provides relevant guidance for future approaches to establish the safety for consumers.

An increasing number of novel foods are generally marketed with claims of benefits to the consumers (functional foods), but these claims are generally poorly underpinned. In the NOFORISK project, an interesting approach was developed to assess the safety, nutritional adequacy and efficacy of such novel foods. Three model foods/food ingredients have been tested: genetically modified and conventionally bred potato tubers with altered contents of glycoalkaloids, a conventionally bred rice line low in phytic acid, and functional food ingredients of natural origin, phytosterol and phytostanol esters.

A comprehensive set of studies has been performed namely chemical studies, *in vitro* and *in vivo* animal studies, gene expression and metabolite profiling, and human exposure scenarios studies based upon probabilistic intake studies. Data from the 90-day feeding study in hamsters suggested that the GM potato was as safe as the non-GM potato. Findings from studies investigating consumer attitudes towards novel foods showed that consumers tended to see only few risks and benefits in the three novel food examples, indicating that novel foods are currently low on most consumers' agendas.

Horizontal gene transfer (HGT) of recombinant DNA from GM crop-derived foods to humans and its consequences for human health is an important issue to be assessed. In particular concerns have been raised about the use of antibiotic resistance genes as marker genes in GM plants. Of concern is a potential transfer of these genes from the plant material to micro-organisms which may lead to an increased level of resistance in micro organisms towards antibiotics, compromising the therapeutic treatment of pathogenic micro-organisms. The GMOBILITY project has studied the effects of food- and gastro-intestinal (GI) tract- environments on the integrity of DNA using various animal models and a newly developed computer-controlled gastro-intestinal simulation model. The digestion process in the GI tract affects rapidly the availability of DNA for transformation, and HGT could be demonstrated via transformation when applying a marker rescue system under optimal conditions, but could not be shown in a natural situation. However transformation of bacteria in the food chain cannot be excluded in particular when homologous sequences are present.

Quality studies of micro-organisms used for food fermentation

There is a continuing search for new bacterial strains to be used in food fermentation to improve the quality, including taste, texture, conservation and possibly the nutritional value and health promoting effects of their products. The project EXPRESS FINGERPRINTS has focused on proteome and transcriptome expression profile analysis of different strains of *Lactococcus lactis*, including natural strains and derivatives constructed by genetic or classical technologies. An important focus of the work was to define the effects of genetic modification on overall gene expression, and to assess whether the methods used to obtain the various strains have an impact on their behavior. The results suggested that gene

modifications induced by molecular biology tend to produce less unexpected events than classical methods.

Detection of GMOs

The intensive public debate on the application of recombinant DNA technology for the production of food/feed has led to stringent regulations on detection, traceability and labeling of GM food and feed. More and more GMOs were approved in the last years in the EU and many new GMOs are available in different parts of the world, underliningthe importance of having available reliable, specific, sensitive , rapid and cost-effective detection methods for GMOs. A number of RTD projects (QPCRGMOFOOD, GMOCHIPS, IMAGEMO) concentrated on the development of detection methods for GM food crop-derived foods with the challenge to design methods able to detect as many GMOs as possible in a single assay.

The QPCRGMOFOOD project focused on the development of quantitative methods for GM food crops , as well as on new strategies for method development and validation, in particular of multiplex methods and specific real-time PCR methods. Also, identification of specific reference genes as essential part of GMO quantification for the most important GM food crops was included. Moreover, attention was paid to what extent the improvement of GMO detection methods might influence consumer confidence in food security, science and risk regulators. The results of these activities formed a fundament for successive work by the European Network of GMO Laboratories (http://engl.jrc.ec.europa.eu) and guidance documents published by the European Commission GMO Community Reference Laboratory (http://gmo-crl.jrc.ec.europa.eu).

In the GMO CHIPS project, a microarray was developed for the screening of GMOs present in the EU market at that time, with a sensitivity of the detection limit below 0.9 %. Further developments have led to an assay which allows the detection and identification of multiple genetic elements potentially present in foodstuffs within a single detection run (DualChip® GMO, developed by Eppendorf Array Technologies, EU project CO-EXTRA, FP 6).

In the IMAGEMO project an alternative approach for the detection of GMOs was developed, namely an immune-detection analyzer for GMO related proteins. An analyzer was developed based on Quartz Crystal Microbalance (QCM) able to monitor the hybridization between nucleic acids complementary strands.

Post market monitoring of GMO derived foods

Post market monitoring (PMM) for GM foods may be considered under certain circumstances to answer questions like: is the product use as predicted, are known effects and side-effects as predicted, does the product induce unexpected side effects. The objectives of the GMSAFOOD project are: to identify and qualify biomarkers for an allergenic prototype GMO for use as a tool for post market monitoring; to relate these biomarker profiles to various developmental stages of test animals, to use biomarkers to identify the movement and effects of GMOs in the food chain, and to establish biomarkers for GMO-induced immunogenicity and allergenicity in animals and humans. This project is still in progress. It should however be stressed that PMM does not substitute a pre-market safety assessment, but rather may complement it.

Consumer attitudes towards GM foods

Societal responses to the application of technological innovations may, besides concerns about possible adverse effects on the environment or human health, be driven by concerns about the impact of the technology on society. In order to understand how people's attitudes and values influence their acceptance or rejection of GM foods, their attitudes towards science and technology have been analyzed within ENTRANSFOOD.

Results of surveys held in 2000 in the EU indicate that Europeans seem to have relatively neutral attitudes towards GM foods as a technology. When compared to the results of the previous survey held in 1996, consumers' attitudes towards GM foods became more negative in terms of usefulness and moral acceptability, but have remained constant regarding risk perception. The project concluded that in case public confidence is to be regained, it is important to explicitly incorporate public concerns into the decision making process. Once public concerns and the values on which they are based are understood, they can be more effectively addressed by appropriate risk management practices.

Conclusion

Extensive research on GMOs, co-funded by the European Commission over the last two decades, has significantly contributed to being able to identify and characterize possible risks associated with foods/feed derived from GMOs. A particular and unique strength of the EU funded research is the multi-disciplinarity of the projects, necessary to address the multi-faceted research issues. The projects under the Research Framework Programmes have significantly contributed developing a robust framework for the food safety assessment of GM foods/feed. These activities provide at least equal assurance of the safety of these foods compared to conventional counterparts, provided these GM products have been approved by the EU and the national food safety evaluation procedures. Moreover, in support of the EU legal requirements, sensitive and specific detection methods for GMOs have been developed, enablingthe traceability of GMO derived products and the enforcement of established threshold levels throughout the whole food chain. This provides an adequate basis for decision-making on market authorization of foods/feed derived from GMOs.

Acronym
GMOCHIPS

Programme Acronym
FP6-GROWTH

Contract number
G6RDCT200000419

Period
January 2001 – October 2004

Coordinator
Dr José REMACLE

Project website
http://www.bats.ch/gmochips

New technology in food science facing the multiplicity of new released GMOs

Background and objectives

European Directives 90/220/EEC and more particularly the later Directive 2001/18/EC, 'Novel Food' and GM food and feed related Regulations (258/97/EC, 1139/98/EC, 49/2000/EC, 50/2000/EC, 1829/03/EC, 1830/03/EC, etc.) imply controlling the presence of GMOs, for labelling and traceability purposes. In the framework of an open European market and more generally of a global market, European national competent authorities express a clear need for standardised GMO detection techniques easily accessible to laboratories.

One objective of the GMOCHIPS project and part of the CO-EXTRA project was to provide an assay for covering all GMOs approved in the EU and at least some not approved in the EU but released in third countries and more particularly in North America. The detection of EU-unapproved GMOs was based on the 'Matrix Approach'. This detection strategy is currently in use in European enforcement laboratories (see the ENGL Working Group on detection of unapproved GMOs). The challenge is to be able to detect as large as possible a panel of GMOs in a single assay, since more and more GMOs have been approved in the last few years in the EU market and many new GMOs are available in various parts of the world (asynchronous approvals).

The European research project (Project G6RD-CT-2000-00419) entitled: 'New technology in food science facing the multiplicity of new released GMO', Acronym: GMOCHIPS was conducted from 2000 to 2005 as part of the FP5 programme. During this project a GMOCHIPS microarray was developed for the screening of 9 GMOs present in the EU market at that time. The assay allowed the identification of Bt11, Bt176, CBH351, GA21, Mon810, RRS, T25, T45 and Topas19/2. The sensitivity of the detection was high enough to achieve detection of reference samples below 0.9 %. The project conclusion was that biochips are suitable as a detection tool for post-PCR assay of different GMOs.

A new assay based on the use of the array was developed by Eppendorf Array Technologies (EAT) which was based on the 'Matrix Approach' for the determination of the GMOs. The assay was then incorporated into the CO-EXTRA EU project for validation at the EU level (FP6 frame, contract 007158, 2005 - 2009).

This assay, DualChip® GMO developed by EAT, allows the detection and identification of multiple genetic elements potentially present in GM events within a single detection run. The assays can identify 30 GMOs, including the 24 plant GMOs which were accepted for commercialisation in the EU in 2006. Also the method indicates the presence of EU-unapproved GMOs as well as identifying plant taxa.

Approach and methodology

The aim of the work performed under the CO-EXTRA programme was to finalise the detection tool and validate the method in accordance with the ISO 5725 standard. This was the first-time example of inter-laboratory validation of a multiplex, microarray based, assay for GMOs. The validation was to assess the performance of the DualChip® GMO as a microarray method for the screening EU-approved GMOs.

The validation was coordinated by the Joint Research Centre (JRC) of the European Commission and organised by Eppendorf Array Technologies (EAT) as part of the European CO-EXTRA project. The validation was performed in accordance with the international ISO 5725 and 17 025 norms. The technology is based on the identification of the genetic elements first amplified by PCR, followed by hybridisation on a predefined microarray, labelling, detection, data acquisition and data analysis.

The assay is a multiplex method based on:

- the detection of 12 screening elements p35S, tNos, pat, cry1Ab (3 gene varieties), cry3Bb1, EPSPS (3 gene varieties), bar, pNos-nptII, used in the Matrix Approach;
- seven plant-species reference systems: maize, soybean, rapeseed, cotton, rice, potato, sugar beet;
- eleven transformation event-specific markers: Bt11, Bt176, GA21, GT73, MON531, MON810, MON863, MON1445, MON15985, RRS, T45;
- a CaMV virus contamination control was also present to determine the presence of Cauliflower Mosaic Virus which may induce false positive results with a P35S screening element.

The results, incorporated into software for GMO identification, are based on the comparison of the different genetic elements found positive in the assay and their presence in the GMOs. The software makes use of the different elements that constitute the GMOs, approved in accordance with Regulation (EC) No 1829/2003 of January 2007, to indicate the putative presence of EU-unapproved GMOs to the analyst.

Partners

Prof. José REMACLE
Laboratoire de Biochimie Cellulaire, Namur (BE)

Dr Yves BERTHEAU
INRA, Unité de Phytopathologie et Méthodologie de la Détection, Versailles (FR)

Dr Pierre BRIGNON
TEPRAL, Brasseries Kronenbourg, Strasbourg (FR)

Dr Neil HARRIS
LGC, Laboratory of the Government Chemist, Teddington (UK)

Dr Philipp SEYLER
Dir. générale de la concurrence, de la consommation et de la répression des fraudes, Laboratoire interrégional, Illkirch (FR)

Dr Pere PUIGDOMENECH
CSIC, Consejo Superior de Investigaciones Cientificas, Barcelona (ES)

Dr O. KÄPPELI
Centre BATS, Basel (CH)

Major Publications

Leimanis S., Hernández M., Fernández S., Boyer F., Burns M., Bruderer S., Glouden T., Harris N., Kaeppeli O., Philipp P., Pla M., Puigdomènech P., Vaïtilingom M., Bertheau Y., Remacle J. (2006). A microarray-based detection system for genetically modified (GM) food ingredients. *Plant Molecular Biology* 61, 123-139.

Hamels S., Leimanis S., Mazzara M., Bellocchi G., Foti N., Moens W., Remacle J., Van den Eede G. (2007). Microarray method for the screening of EUU approved GMOs by identification of their genetic elements. *JRC Scientific and technical reports*. http://bgmo.jrc.ec.europa.eu/home/docs.htm

Hamels S., Glouden T., Gillard K., Mazzara M., Debode F., Foti N., Sneyers M., Esteve Nuez T., Pla M., Berben G., Moens W., Bertheau Y., Audeon C., Van den Eede G., Remacle J. (2009). A PCR-Microarray method for the screening of Genetically Modified Organisms. *Eur. Food Res. Technol*. 228:531–541.

Leimanis S., Hamels S., Naze F., Mbella GM., Sneyers M., Hochegger R., Broll H., Roth L., Dallmann K., Micsinai A., La Paz JL., Pla M., Brunen-Nieweler C., Papazova N., Taverniers I., Hess N., Kirscheit B., Bertheau Y., Audeon C., Laval V., Busch U., Pecoraro S., Neumann K., Rösel S., Van Dijk J., Kok E., Bellocchi G., Foti N., Mazzara M., Moens W., Remacle J. & Van den Eede G. (2008). Validation of the performance of a GMO multiplex screening assay based on microarray detection. *Eur. Food Res. Technol*. 227 (6): 1621-1632.

Bellochi G., Bertholet V., Hamels S., Moens W., Remacle J., Van den Eede G. (2009). Fuzzy-logic based strategy for validation of mutiplex methods: example with qualitative GMO assays. *Transgenic Res*. DOI 10.1007/s11248-009-9293-9.

The validation of the method was based on predefined acceptance criteria. The test samples were provided by EAT to 12 EU validation laboratories as blind samples consisting of DNA reference samples specifically adapted to each GM element. For sensitivity assays, the samples contained GM concentrations (expressed as % of genome copies of the GM-specific and species-specific DNA, as recommended in Commission Recommendation (EC) 2004/787, of 0.045, 0.1, 0.5 and 1 %. The trial involved laboratories in eight countries of the European Union, most of them members of the ENGL and the CO-EXTRA project. The JRC conducted the data analyses and tested a novel fuzzy-based approach (software) for validation of multiplex assays.

The validation was performed on 36 DNA samples. A total of 3 360 PCR reactions and 840 hybridisations were performed for the validation. Data were identified as outliers if their probability of belonging to the same binomial population was lower than 0.01, in accordance with the criteria of ISO 5725 (1994). The accuracy rate criterion was set at 95 %, which is also the threshold used in the ENGL 'Definition of Minimum Performance Requirement for Analytical Methods of GMO Testing' (2005). An indicator, based on the principle of a fuzzy-logic expert system for the purpose of a comprehensive assessment, was used to encompass the ability of the method to detect the full set of targets.

Main findings and outcome

The validation was conducted in order to assess whether the expected overall performance was in line with the criteria of specificity and sensitivity. Data showed that the method was specific and fulfilled the criterion of 95 % confidence at the 0.1 % GM concentration for the GM target elements and at 1 % for the plant targets. Therefore the method was found to fulfil the ENGL requirement in terms of accuracy and limit of detection.

The detection of the GM target elements showed an accuracy rate above 95 % down to 0.1 % GMO concentration for all GM targets (0.1 % corresponds to the cutoff sensitivity level established for the microarray). Seven GM target elements out of nine showed an accuracy rate above 95 %, also at 0.045 %.

For the controls, the CaMV was detected above the accuracy rate of 95 % in all concentrations ranging from 500 to 20 copies. The false positive rate was at 0 % in the non-plant extract in the absence of any plant or GM event, as proposed in the acceptance criteria.

The fuzzy-logic based aggregation analysis confirmed the fulfilment of the expected acceptance criteria down to 0.1 % GM concentration and 1 % for the plant targets.

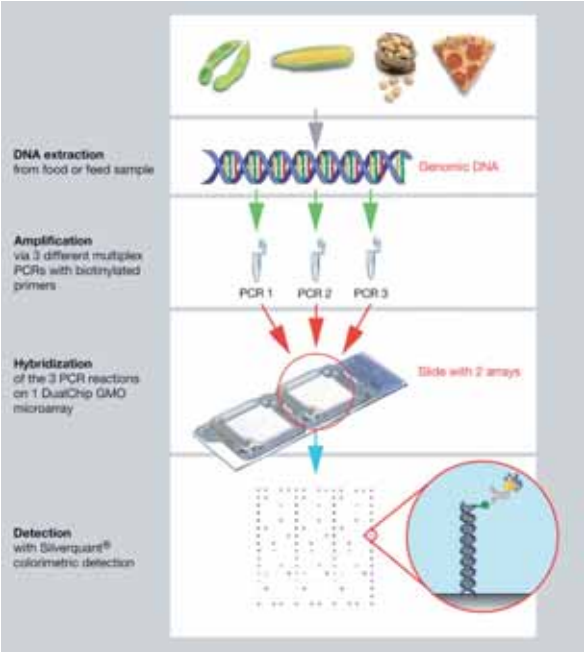


Fig 1.

Principle of the process flow for screening GM elements in a sample using the DualChip® GMO, V2.0 Kit. Once the DNA is extracted from the sample, 31 genetic elements are specifically amplified within 3 multiplex PCR reactions. The resulting amplicons are then merged and hybridised on a single DualChip GMO microarray to be detected specifically by capture probes present on the array. The PCR is performed on small sequences allowing the detection of processed matrices. The DualChip® GMO analysis is part of a complete system including the PCR reactions, microarray hybridisation, Silverquant labelling, scanning and data analysis (Eppendorf AG, Hamburg, DE). The timeframe of the total workflow takes approximately 7 hours to process 24 arrays in parallel.

Acronym
GMOBILITY

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK1-1999-00527

Period
March 2000 – February 2003

Coordinator
Jos M.B.M. VAN DER VOSSEN
TNO Nutrition and Food Research
PO Box 360
NL-3700 AJ Zeist
The Netherlands

Safety evaluation of horizontal gene transfer from genetically modified organisms to the microflora of the food chain and human gut

Background and objectives

Horizontal gene transfer (HGT) as a biosafety issue has been addressed in several studies which have mainly focused on the transfer of antibiotic resistance from genetically modified (GM) plants to soil- and plant-related micro-organisms. HGT to bacteria, in these environments, has been shown by marker rescue experiments via homologous recombination. However, HGT via transformation of bacteria in the food chain cannot be excluded. Free DNA persists in some materials for weeks and, furthermore, some bacteria develop natural/chemical competence to take up DNA from the environment. In addition, in the gastrointestinal tract of man and husbandry animals, DNA may remain stable for some time, particularly in the colon.

The main objective of this project is to quantify the risk of HGT from genetically modified organisms (GMOs), and food derived therefrom, to the microflora of the food chain and the human gut. In addition to analysis of gene transfer frequencies, hazards will be identified, data of exposure to GMO food will be collected, and the impact will be addressed to quantify and precisely define the risks of HGT. Selective conditions are particularly relevant for antibiotic resistance marker genes, therefore the relevance of a particular antibiotic as a therapeutic agent will be dealt with in the risk evaluation. This project also aims to evaluate models which can be used to study these HGT events.

Approach and methodology

To collect data for an evaluation of HGT from transgenic food products, *in vivo* and *in vitro* gastrointestinal tract model systems will be studied, evaluated and validated. Since gene transfer can occur via different mechanisms depending on the genetic background, different donor/recipient systems will be studied. In addition to examining these mechanisms, other parameters relating to the frequency of HGT will be analysed such as survival of bacteria, stability of DNA, development of competence for transformation, possibility of recombination, and selective pressure. The data will be evaluated to quantify the risk of HGT based on the genetic

background, daily intake of transgenic DNA, formulation of the food product, and the nature of marker genes.

Main findings and outcome

DNA integrity food. Food processing has an effect on the integrity of crop DNA. Acidic conditions, heat and irradiation increase the nicking rate of DNA. The acid catalysed depurination was not affected by the food matrix. Food components have an effect on enzymatic degradation of plasmid DNA by DNase I. Compounds such as putrescine and spermine and other polyamines protect the DNA against DNase I activity.

DNA integrity in the GI tract of mammalians. In stomach-derived material, DNA could be detected even one hour after introduction. In the chyme of the small intestine of rat and pigs, DNA is rapidly degraded to a level that it cannot be detected by PCR. This could be mimicked in TNO's *in vitro* intestinal model TIM. In chyme from the rat coecum, DNA remains remarkable integer. Despite the rapid degradation of DNA in the small intestine, some DNA was shown to be transiently present in the GI tract of rats upon the intake of free DNA. There seem to be ways for a limited amount of DNA to escape from the action of nucleases, even in a state where it is not enveloped by a cell wall. Ruminants have a different organisation of their GI tract, compared with monogastric mammalians. In this group of mammalians, DNA released from the ingested plant material is in direct contact with a complex rumen flora. In this flora DNA is degraded by the bacteria. In the rumen model used, the study detected DNA by PCR up to two hours after introduction.

Transformation in the food chain. For studying the transfer of genes from GMO to bacteria associated with food and/or the gastrointestinal tract, a marker rescue system for gram-positive bacteria was constructed and established in *B. subtilis* and *S. gordonii*. The marker rescue system is based on the nptII gene containing a small deletion in the open reading frame, which can be restored by homologous recombination with a complete functional nptII gene. The success of marker rescue was shown to be highly dependent on the size of the donor DNA in *B. subtilis*. The longer the border sequence, the more likely that restoration occurs. However, too much non-targeted DNA present in GM plant material did hamper marker rescue. By using the marker rescue system in B. subtilis, no transformation with genomic DNA of GM potato was observed. Even under optimised laboratory conditions marker rescue was not observed. *Acinetobacter* was also used as a host

Partners

Henk J.M. AARTS
RIKILT-Institute of Food Safety, Wageningen (NL)

Walter P. HAMMES
University of Hohenheim, Institute of Food Technology, Hohenheim (DE)

Margaret DAVIES
BIBRA International Ltd., Molecular Sciences Department, Surrey (UK)

Gerard CORTHIER,
Unité Ecologie et Physiologie du Système Digestif (UEPSD), Jouy-en-Josas (FR)

Birgit NØRRUNG,
Danish Veterinary and Food Administration, Copenhagen (DK)

Alexander HASLBERGER
Institut for Microbiology and Genetics, University Vienna (AT)

Major Publications

Alpert CA., Mater DD., Muller MC., Ouriet MF., Duval-Iflah Y., Corthier G. Worst-case scenarios for horizontal gene transfer from Lactococcus lactis carrying heterologous genes to *Enterococcus faecalis* in the digestive tract of gnotobiotic mice. Environ. *Biosafety Res.* 2003;2:173-180. Erratum in: *Environ. Biosafety Res.* (2003). 2:279.

Bauer T., Hammes WP., Haase NU., Hertel C. Effect of food components and processing parameters on DNA degradation in food. *Environ. Biosafety Res.* (2004). 3:215-223.

Kharazmi M., Bauer T., Hammes WP., Hertel C. Effect of food processing on the fate of DNA with regard to degradation and transformation capability in *Bacillus subtilis*. Syst Appl Microbiol. (2003). 26:495-501.

Kharazmi M., Hammes WP., Hertel C. Construction of a marker rescue system in *Bacillus subtilis* for detection of horizontal gene transfer in food. *Syst. Appl. Microbiol.* (2002). 25:471-477.

for marker rescue experiments. Using DNA of GM transgenic potato, marker rescue transformation in food was observed only when the food matrix permitted growth of the recipient.

Further downstream the food chain, the fate of possible transformants ingested via the food was considered in the GI tract. Ingested bacteria do survive to some extent the conditions in the GI tract as was monitored with TIM. Transformation in the GI tract was studied both in rat, rumen model and TIM. Although *E. coli* was shown to perform well in the small intestine and is a microorganism naturally present in the GI tract of mammalians, transformants could not be obtained with this organism in the GI tract environment. Transformation of *S. gordonii* was extensively tested under GI tract conditions. Those present in oral cavity, colon and rumen displayed a negative effect on the high transformation efficiency found under optimal in vitro conditions. Since the oral cavity is the natural environment of *S. gordonii*, risk calculations were conducted based on the transformation efficiencies in this environment in comparison with transformation of *B. subtilis* in the food environment. *S. gordonii* was shown to be a recipient organism that can more easily be transformed than *B. subtilis*. Under optimised conditions, streptococcal transformation was shown to perform 105 times better.

Nevertheless, under conditions mimicking the oral cavity, marker rescue transformants are expected at a frequency of 10^{-17} transformants/recipient with DNA from processed GM potatoes and 3.2×10^{-9}/recipient for non-processed GM potatoes. With more realistic numbers in the absence of homologous recombination, i.e. illegitimate recombination, these frequencies are hypothesised to drop further by a factor 1.4×10^{-9}, as was determined for *Acinetobacter*. Further downstream, the GI tract transformation of *S. gordonii* could neither be detected in the rumen nor the colon. Even in the presence of high numbers of plasmid DNA for marker rescue, transformants could not be detected.

Conjugation in the GI tract. Recombinant bacteria are currently being developed for intended environmental release as oral vaccines or in fermented foods. *Lactococcus lactis* as a representative of the LAB is by now the first bacterium to be exploited in this way. Morover, bacteria that acquired genes from GM plants or bacteria may also be considered as recombinant bacteria and could transfer the new genetic information to other bacteria in the food chain, including the mammalian gut. This mechanism of horizontal gene transfer

(HGT) was studied with conjugative/mobilisable plasmids and transposons. The model systems used show only HGT if transfer and mobilisation functions are present. A good accessible recipient seems also important for successful conjugation.

Conclusions

It is generally accepted that HGT has contributed to the creation of bacterial diversity. HGT has been described as taking place via transformation, conjugation and transduction mechanisms. Visibly, evidence comes from the analysis of the spread of antibiotic resistances over the last decades as a consequence of the use of antibiotics. Conjugal transfer was shown to be a mechanism that functions in the GI tract of mammalians, when the DNA molecules, the donor organism and the recipient display the appropriate features. Transformation, on the other hand, with plasmid DNA or via marker rescue was shown under optimised conditions. However, when conditions worsen as a consequence of low copy number in the GM plant genome, DNA degradation, components in the food and GI tract, and competing microbial population, transformation via marker rescue becomes a rare event. Even under selective conditions, transformants could not be enriched from GM plants. A further reduction of transformation frequency leads to the fact that illegitimate recombination is far more inefficient than homologous recombination found in our food and GI tract model systems. The spread of antibiotic resistance genes is therefore highly unlikely to occur via HGT from GM plants used as food and feed.

Major Publications

Kharazmi M., Sczesny S., Blaut M., Hammes WP., Hertel C. Marker rescue studies of the transfer of recombinant DNA to *Streptococcus gordonii in vitro*, in foods and gnotobiotic rats. *Appl. Environ. Microbiol. (*2003). 69:6121-6127.

van den Eede G., Aarts H., Buhk HJ., Corthier G., Flint HJ., Hammes W., Jacobsen B., Midtvedt T., van der Vossen J., von Wright A., Wackernagel W., Wilcks A. The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. *Food Chem. Toxicol.* (2004). 42:1127-1156.

Wilcks A., van Hoek AH., Joosten RG., Jacobsen BB., Aarts HJ. Persistence of DNA studied in different ex vivo and in vivo rat models simulating the human gut situation. *Food Chem. Toxicol.* (2004). 42:493-502.

Acronym
ENTRANSFOOD

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK1-1999-01182

Period
February 2000 – January 2003

Coordinator
H.A. KUIPER
RIKILT – Institute of Food Safety
Wageningen University & Research Centre
P.O. Box 230
NL-6700 AE Wageningen
The Netherlands
Harry.Kuiper@wur.nl

European network on safety assessment of genetically modified food crops

Background and objectives

Approaches to the safety assessment of foods derived from genetically modified (GM) crops have been developed over the last two decades by various intergovernmental organisations, and the European Commission has made substantial contributions to research and expert deliberations in this area (see Review of Results of EC-sponsored Research on Safety of Genetically Modified Organisms, 2001, http://europa.eu.int/comm/research/quality-of-life/gmo).

Despite these intensive research efforts, GM food crops have not widely been accepted by the European consumer. Consumer and environmental organisations have voiced their concerns about the safety of these crops, with respect to long-term effects on the environment and human health, consumer freedom of choice between GM-containing and 'GM-free' foods, and the impact the new food production technology might have on society.

The thematic network 'European Network on Safety Assessment of Genetically Modified (GM) Food Crops' (ENTRANSFOOD) was set up in order to address scientific and societal issues related to the introduction GM crops. The network consists of research projects and working groups focused on the development of methods and strategies for safety testing, detection and traceability of GM food crops, and on societal aspects of the introduction of GM foods. It started its activities in February 2000.

The objectives of the Thematic Network are:

- to evaluate the adequacy of current food safety assessment methods and strategies, and to identify possible needs for adaptation for foods derived from GM crops;
- to design and evaluate new alternative (*in vitro*) test methods for the safety evaluation of foods derived from GM crops;
- to provide detailed guidance to notifiers and risk assessors on how to perform the safety assessment of foods derived from GM crops;
- to assess the risks of transfer of recombinant DNA from GM crops to microbes or human cells;

- to examine the fate of GM raw materials and processed products throughout food production chains (traceability);
- to examine new strategies for the detection of GM raw materials, processed products and food ingredients;
- to examine societal aspects and consumer attitudes towards the introduction of foods derived from GM crops.

Approach and methodology

Participants in the ENTRANSFOOD consortium were recruited from academia, research centres, biotech and plant breeding companies, food industries, food retailers, regulatory agencies, and consumer groups across Europe. Altogether, 45 research centres participated in the RTD projects, and 62 experts in the working groups. Many of the working group members are also actively involved in the research projects.

Research was carried out in the context of five EU-funded shared-cost projects:

1. new methods for the safety testing of transgenic food (SAFOTEST);
2. new methodologies for assessing the potential of unintended effects in genetically modified food crops (GMOCARE);
3. safety evaluation of horizontal gene transfer from genetically modified organisms to the microflora of the food chain and human gut (GMOBILITY);
4. reliable, standardised, specific, quantitative detection of genetically modified foods (QPCRGMOFOOD);
5. new technology in food science facing the multiplicity of new released GMOs (GMOCHIPS).

Evaluation and review activities have been carried out in the following five working groups:

1. design of safety assessment strategies for transgenic foods;
2. design of strategies for the detection of unintended alterations in GM food crops due to the process of genetic modification;
3. evaluation of the risks of gene transfer from GM foods to micro-organisms in the human digestive tract or to human cells;
4. evaluation and design of strategies for detection and traceability of GM foods and food components;
5. understanding of societal responses to GM foods.

Partners

J.M. WAL
Service de Pharmacologie et d'Immunologie
Laboratoire Associé INRA-CEA d'Immuno-Allergie Alimentaire, Jouy-en-Josas (FR)

K.H. ENGEL
Department of General Food Technology
Technical University of Munich (DE)

G. VAN DEN EEDE
European Commission
Directorate General Joint Research Centre, Ispra (IT)

M. MIRAGLIA
Istituto Superiore di Sanita
Section of Cereal Chemistry, Rome (IT)

L. FREWER
Marketing and Consumer Group
University of Wageningen (NL)

Fig 1. provides an overview of the way in which activities within ENTRANSFOOD have been structured:

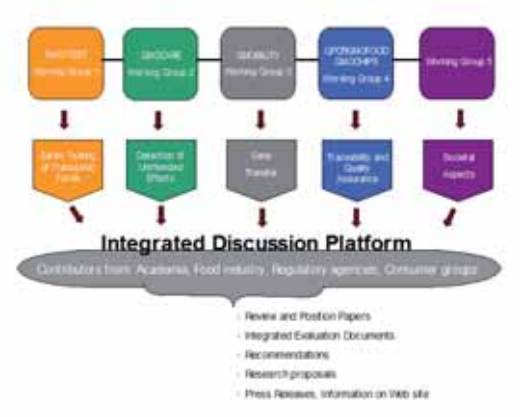


Fig 1.
Structure of ENTRANSFOOD activities.

Main findings and outcome

Each working group of ENTRANSFOOD has generated a scientific review on its particular task. The reviews of all working groups have been grouped together in a special edition of Food Chemical and Toxicology (Volume 42, issue 7, July 2004). This publication should contribute to the current activities on risk assessment and consumer perception. It should help to underpin the European policy on GM crops and form a basis for further discussions and research activities in Europe and also worldwide.

In addition a European Commission-funded overarching paper has been published, written by the ENTRANSFOOD participants, which summarises the main findings of the working groups in order to inform stakeholders, policymakers, consumer groups, and the interested public[7].

An overview of the main conclusions of the working groups is provided below:

Safety Testing of Transgenic Foods (Working Group 1)

The safety evaluation of foods derived from GM crops is carried out in a comparative manner, i.e. differences between the GM crop and the novel or differently conventionally grown

[7] König A., Kleter G., Hammes W., Knudsen I., Kuiper H. (Eds.). Genetically Modified Crops in the EU: Food Safety Assessment, Regulation, and Public Concerns – Overarching Report. The European Network on Safety Assessment of Genetically Modified Food Crops. Office for Official Publications of the European Communities, Luxembourg, 2004.

crop are identified and investigated with respect to their impact on human or animal health (Concept of Substantial Equivalence, see Fig 2.). The basic idea behind this approach is that conventional foods have a long history of safe use.

ENTRANSFOOD Working Group 1 has designed a detailed stepwise procedure to carry out the safety assessment of GM crop-derived foods on a case-by-case basis (Fig 3.). The approach comprises four steps: characterisation of the parent crop which is genetically modified; characterisation of the genetic modification process; toxicological and allergenicity assessment of new gene products (proteins and metabolites); and safety evaluation of the whole GM food crop.

A combination of existing test methods provides a robust test regime and ensures that GM foods that have passed the test procedure are as safe and nutritious as currently consumed plant-derived foods. The designed approach is also applicable to new generations of GM food crops with extensive compositional changes.

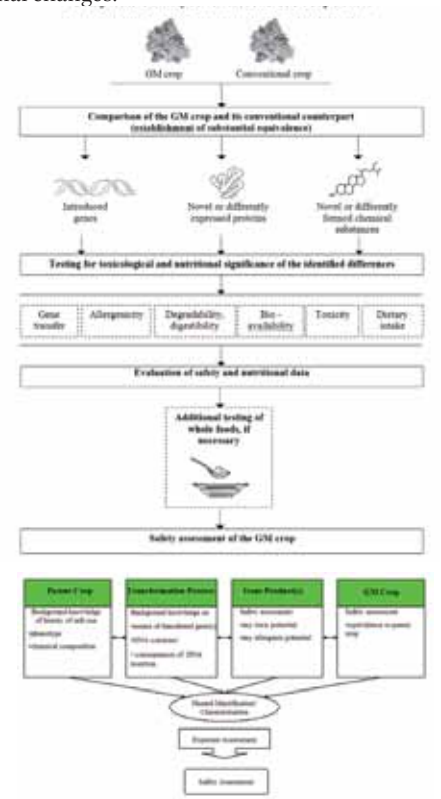


Fig 2.

Comparative Sagety Assesment of GM Crop Plants.

Fig 3.

The elements in the safety assessment of food derived from a GM crop.

Detection of Unintended Effects (Working Group 2)
Unintended alteration in the composition of plants is a common phenomenon that occurs when changing the genetic information of a plant, either by classical breeding methods or by GM technology. There is no indication that unintended effects are more likely to occur in GM crops.

Detection of unexpected effects in plants, which are developed by GM technology, relies primarily on the comparative analysis of levels of selected key nutrients and toxic compounds present in the GM crop and its traditional non-modified counterpart. Identified alterations in composition may fall within the natural range of variations and thus not be of toxicological concern, or fall outside these ranges and then need further toxicological or nutritional investigation. This targeted approach has been shown to be effective in conventional plant breeding in identifying alterations in composition.

In order to increase the probability of detecting unexpected effects, profiling techniques are under development. These methods, based on modern genomic, protein and metabolite detection techniques, are able to provide a 'global' overview of gene expression and chemical composition of the GM and non-GM crop (non-targeted approach).

Gene Transfer (Working Group 3)
Horizontal gene transfer is at the origin of the variety of life itself. The impact of horizontal gene transfer will depend among others on the selective advantage for the bacterial population. There is little scientific reason to assume that the consumption of foods derived from GM crops constitutes a specific risk.

Whereas uptake of ingested recombinant (foreign) DNA by mammalian somatic cells has been demonstrated, there is no evidence that 'consumed' DNA will end up in germline cells. Transfer of antibiotic resistance marker genes from GM plant varieties to the gut microflora of humans and their expression is most probably a rare event, given the low amounts ingested and degradative conditions in the gastro-intestinal tract.

However, since gene transfer cannot completely be excluded, ENTRANSFOOD has classified antibiotic resistance marker genes based on their distribution and therapeutic importance. This risk classification is aimed at contributing to the current debate within the EU regarding the phasing out of

those marker genes that pose a threat to the environment or human health (EU Directive 2001/18/EC).

Traceability and Quality Assurance (Working Group 4)
The recently introduced EU regulations impose requirements of labelling on foods or feed containing ingredients derived from GM food/feed crops or containing traces of GM crops above a certain threshold (>0.9 %).

To facilitate control and to verify labelling claims, and to facilitate product recall when necessary, reliable documentation systems have had to be developed that trace back the origin of the ingredients (traceability system) and analytical methods to detect and quantify the amount of GM derived ingredients.

It is recommended to make detection and/or identification methods available to regulatory authorities and food control laboratories. With more genetically modified organisms (GMOs) being developed worldwide, more efficient GMO detection and identification methods, based on the latest molecular biology methods, need to be developed. In addition, the use of traceability systems for post-marketing applications requires new labelling systems that convey all necessary information on the presence of individual GMO varieties to the reader.

Societal Aspects (Working Group 5)
Risk assessment of GM foods has focused on adverse health effects for humans and the environment, but public concern is much broader, focusing not only on risks, but also on who benefits, what are the needs and how GMOs contribute to sustainable agriculture. It is important to explicitly address public concerns and to develop new methods for stakeholder involvement and public consultation.

For future research projects, it is recommended to address ways to formalise public engagement and consultation into new working procedures, as well as the impact on the risk analysis process, regulatory procedures and the institutions involved.

In addition, this working group considers that there is a need for a general framework for risk analysis of all types of new foods produced by different breeding and production methods, taking scientific, economic and societal aspects into account.

Major Publications

König A., Kleter G., Hammes W., Knudsen I., Kuiper H. (Eds.). Genetically Modified Crops in the EU: Food Safety Assessment, Regulation, and Public Concerns – Overarching Report. The European Network on Safety Assessment of Genetically Modified Food Crops. Office for Official Publications of the European Communities, Luxembourg. (2004).

Kuiper H.A., Kleter G.A., Konig A., Hammes W.P., Knudsen I. (Eds.). Safety Assessment, Detection and Traceability, and Societal Aspects of Genetically Modified Foods. European Network on Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD). *Food Chem. Toxicol.* 42(7, special issue). (2004). pp. 1043-1202.

König A., Cockburn A., Crevel R.W.R., Debruyne E., Grafstroem R., Hammerling U., Kimber I., Knudsen I., Kuiper H.A., Peijnenburg A.A.C.M., Penninks A.H., Poulsen M., Schauzu M., Wal J.M. Assessment of the safety of foods derived from genetically modified (GM) crops. *Food Chem. Toxicol.* 42(7, special issue). (2004). pp. 1047-1088.

Conclusions

ENTRANSFOOD was primarily designed to deal with food safety and consumer concerns related to the introduction of foods derived from GM crops. However discussions during the meetings stressed that consumer concerns regarding a possible negative impact of large-scale cultivation of GM crops on the environment have equal weight. A global risk analysis of the technological innovation in agriculture should include these aspects.

The consortium has been a unique experience, with participants with different disciplines and from different affiliations. This has made it possible to examine a broad range of issues related to the risk assessment, management, and communication of GM crop-derived foods, and to add a significant level of detail to the current guidelines for the scientific pre-market assessment strategies for GM crop-derived foods. Consensus was reached that a rigorous science-based risk assessment of the environmental impact, and of the possible effects on human health of foods derived from GM crops only, is not sufficient to gain public support for the introduction of this new food production technology into society. Appropriate risk management measures and aspects like sustainability, benefits and impact on society must be taken into account.

The consortium has examined existing and evolving methods to detect the possible occurrence of unintended effects on the composition of GM food crops as a result of genetic modification. Detection of such effects should primarily rely on targeted approaches, measuring single compounds in GM and non-GM plants that represent important metabolic pathways. This should continue to be the leading principle in regulatory risk assessment procedures for GM crops and derived foods and feed.

ENTRANSFOOD has examined whether genes coding for antibiotic resistance can be used as markers for the transformation event. The public debate is focused on hazards of transfer of these genes present in GM crop-derived foods to microbes residing in the human gut or to human cells. Although the actual risk of transfer and subsequent spreading of resistance is negligible, we recommend a precautionary approach to the use of these marker genes, since there is still a lack of knowledge on the specific conditions where gene transfer might be possible. The consortium has categorised currently available marker genes into three groups, according to their presence in the environment and the importance of the antibiotic for clinical use. This implies that the presence of certain marker genes like nptII and hpt in GM crop-derived foods does not pose an additional risk to the environment or to human health, therefore the use of these genes as markers can be continued.

Major Publications

Cellini F., Chesson A., Colquhoun I., Constable A., Davies H.V., Engel K.H., Gatehouse A.M.R., Kärenlampi S., Kok E.J., Leguay J.-J., Lehesranta S., Noteborn H.P.J.M., Pedersen J., Smith M. Unintended effects and their detection in genetically modified crops. *Food Chem. Toxicol.* 42 (7, special issue). (2004). pp. 1089-1125.

Van den Eede G., Aarts H., Buhk H.-J., Corthier G., Flint H.J., Hammes W., Jacobsen B., Midtvedt T., Van der Vossen J., Von Wright A., Wackernagel W., Wilcks A. The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. *Food Chem. Toxicol.* 42(7, special issue). (2004). pp. 1127-1156.

Miraglia M., Berdal K.G., Brera C., Corbisier P., Holst-Jensen A., Kok E.J., Marvin H.J.P., Schimmel H., Rentsch J., Van Rie J.P.P.F., Zagon J. Detection and traceability of genetically modified organisms in the food production chain. *Food Chem. Toxicol.* 42. (7, special issue). (2004). pp. 1157-1180.

Frewer L., Lassen J., Kettlitz B., Scholderer J., Beekman V., Berdal K.G. Societal aspects of genetically modified foods. *Food Chem. Toxicol.* 42 (7, special issue). (2004). pp. 1181-1193.

Kuiper H.A., König A., Kleter G.A., Hammes W.P., Knudsen I. Concluding remarks. *Food Chem. Toxicol.* 42(7, special issue). (2004). pp. 1195-1202.

Acronym
QPCRGMOFOOD

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK1-1999-01301

Period
February 2000 – July 2003

Coordinator
Arne HOLST JENSEN
National Veterinary Institute
NO-0106 Oslo
Norway
arne.holst-jensen@vetinst.no

Reliable, standardised, specific, quantitative detection of genetically modified food

Background and objectives

Available methods for the detection of genetically modified (GM) materials can only be used to screen for the presence of potential GM material, or to detect and identify known GM material. Consequently, a potential danger arises from the failure to detect unapproved GM organisms (GMOs). GMO approval covers a specific genetic modification (product of a transformation). Because the genetic construct can be used in more than one GMO, such elements are not reliable identifiers of specific transformations.

The regulatory need for detection methods that can distinguish between approved and unapproved GMOs and quantify GMO content incited this project. Detection methods specific for each transformation event (using the junction between the modified DNA and the part of the host genome where the modified DNA is integrated) were deemed necessary (see Fig 1.). Very few plant species-specific genes that can be used as a reference for quantification of GMO content have been identified. A qualitative analysis that can detect and identify more than a single GMO in a single reaction has clear cost-efficiency potential. Furthermore, DNA extraction methods which can influence the sensitivity and reliability of polymerase chain reaction (PCR)-based detection assays must be optimised. Finally, validation of these methods is critical to assess their reliability. There is also an urgent and growing need for European and international standards for GMO detection.

The major aims of this project were, first, to develop reliable and transformation event-specific tests for qualitative and quantitative detection of genetic modifications in food for at least 12 GMOs. Second, to develop reliable and transformation event-specific multiplex tests for determination of the diversity of genetic modifications in food. Finally, to investigate how improved methods for detection of genetically modified foods might influence consumer confidence in food security, science and risk regulators.

Approach and methodology

This project comprised six work packages. The first involved the identification of the application and limitations of a standard DNA extraction protocol. The effects of various modifica-

tions of the extraction protocol were examined on a variety of product types. PCR-based GMO quantification determines the relative number of a GMO to a single-copy gene specific for the GM host species.

The second work package identified and characterised suitable species-specific reference genes, and developed reference gene-specific primer/probe sets for qualitative and quantitative PCR. This involved screening of literature and DNA sequence databases, sequencing uncharacterised candidate genes, and empirical testing of primers and probes. The target copy number per genome was examined by standard DNA hybridisation techniques.

The third work package involved sequence characterisation of transformation events. Sequence data were requested from biotechnology companies or other sources on a collaborative basis, but generally these requests were unsuccesful. Consequently, DNA fragments containing the junction regions were instead isolated and sequenced from whatever reference material was available.

In the fourth work package, transformation event-specific primer/probe sets were developed and tested. Specific PCR primers and probes were developed for qualitative detection of GMOs in single GMO and multiplex assays, as well as for quantitative detection with real-time PCR.

In the fifth work package, the developed methods were validated in multilaboratory and collaborative trials.

The final work package examined the socio-economic impact of GMO regulation and detection. A questionnaire survey in the UK, Norway and Italy provided information about the impact on consumer confidence of improved analytical methods for GMOs.

Main findings and outcome

In WP1 DNA extraction methods for maize- and soybean-derived matrices were compared, modified, and evaluated, and successively selected protocols were subjected to multilaboratory and collaborative trial validation in WP5. The final report of the workpackage is published on http://www.vetinst.no/eng/content/download/645/5811/file/Deliverable1.pdf

In WP2 candidate reference genes for maize, soybean, wheat, rice, rapeseed (canola), tomato, potato, sugar beet and sunflower were examined, characterised, evaluated and for the majority of the species at least one real-time quantitative PCR

Partners

Askild HOLCK
Matforsk, Norwegian Food Research Institute, Aas (NO)

Yves BERTHEAU
INRA, Institut National de la Recherche Agronomique, Versailles (FR)

Marc DE LOOSE
DvP-CLO, Ministry of Small Enterprises, Traders and Agriculture, Agricultural Research Centre, Gent (BE)

Neil HARRIS
LGC Ltd., Teddington (UK)

Andreas WURZ
Gene-Scan GmbH, Freiburg (DE)

Guy VAN DEN EEDE
DG Joint Research Centre of the European Commission, Institute for Health and Consumer Protection, Food Products and Consumer Goods, Ispra (IT)

Marc VAÏTILINGOM
Tepral-Brasseries Kronenbourg, Strasbourg (FR)

Carlo BULKMANNS
Unilever Nederland, Vlaardingen (NL)

Patrick PHILIPP
DGCCRF-LIR67, Direction générale de la concurrence, de la consommation et de la répression des fraudes, Laboratoire interrégional 67, Illkirch-Graffenstaden (FR)

David ZHANG
Bio-GEVES, Groupe d'Intérêt Public d'Étude et de Contrôle des Variétés et des Semences, Surgères (FR)

Lynn FREWER
IFR, Institute of Food Research, Norwich (UK)

Maria PLA
CSIC, Consejo Superior de Investigaciones Cientificas, Barcelona (ES)

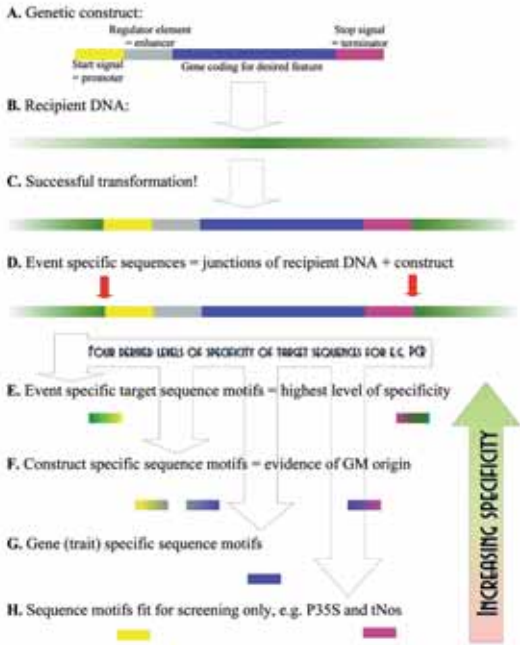


Fig 1.

Relationship between the genetic transformation and the specificity and applicability of detection methods for classes of targets.

A. A gene construct is a synthetic combination of genetic elements containing at least the desired trait gene and a promoter (start) and terminator (stop) element.

B. The objective is to insert this construct into a recipient genome.

C. Succesful transformation will lead to the insertion of a complete copy of the gene construct into the recipient genome.

D. The chimeric sequence motif comprised by a part of the recipient genome and a part of the inserted gene construct is a unique molecular identifier for the transformation event.

E-H. Classes of target motifs for GMO detection, in descending order of specificity.

E. Event specific sequences resulting from the fusion of a host (recipient) genomic sequence and an inserted (transgenic, donor) sequence. These sequence motifs are only found in the descending lines from a specific transformation event, including hybrid stacks from crosses. The motifs are always single copy in the haploid GM genome, and are consequently reliable target sequences for identification and quantitation purposes.

F. These targets correspond to synthetic gene constructs resulting from fusion of naturally occurring elements in a way that does not occur in nature; the synthetic construct may be found in more than one GMO. The presence of these junction motifs is consequently reliable evidence of the presence of GMO-derived material. Their copy number may vary from one GMO to another.

G. Gene specific methods are useful for identification of specific traits only. These targets are often derived from naturally ocurring microorganisms, plants, etc., but may have been modified relative to the original haplotype, in which case they serve as reliable evidence of the presence of GMO derived material. Their copy number may also vary from one GMO to another.

H. Promoters and terminators are useful for screening purposes only. These targets are usually derived from naturally occurring micro-organisms, viruses, etc., and may be found in several different GMOs, in variable numbers of copies, and also in non-GM samples.

method was developed to be used as a reliable reference gene system for GMO quantitation. The final report of the work-package is published on http://www.vetinst.no/eng/content/download/644/5807/file/Deliverable2.pdf.

In WP3 the insert and/or junction between host genome and insert was characterised for GTS 40-3-2 (Roundup Ready) soybean, Bt11, Bt176, Mon810, CBH 351 (Starlink), GA21, DBT418 and T25 maize, and for MS8, Rf3 and GT73 rapeseed. Unsuccessful efforts were made to obtain material for additional events. For approximately half of the events, inferred sequences were conflicting with data provided by the notifiers of the events in the official dossiers. The updated genetic maps consequently amended the information available to risk assessors. The final report of the workpackage is published on http://www.vetinst.no/eng/content/download/643/5803/file/Deliverable3.pdf.

In WP4 the sequence data from WP2 and in particular from WP3 were used to develop single- and multiplex-detection methods for screening, construct and event-specific detection and quantification of GMOs and derived material. Selected methods were subjected to multilaboratory and collaborative trial validation in WP5. The final report of the workpackage is published on http://www.vetinst.no/eng/content/download/642/5799/file/Deliverables4to6.pdf

In WP5 methods developed in WP1, WP2 and WP4 were subjected to multilaboratory and collaborative trial validation, and methods successfully validated in collaborative trials were successively forwarded to CEN/TC 275/WG 11 as proposed annexes to European and International (ISO) standards. Criteria for method evaluation, performance characteristics and acceptance criteria were discussed, including alternative reference materials and modular method validation and implementation. The results of the workpackage activities formed a fundament for successive work by the European Network of GMO Laboratories (http://engl.jrc.ec.europa.eu) and guidance documents publised by the European Commissions GMO Community Reference Laboratory (http://gmo-crl.jrc.ec.europa.eu). The final report of the workpackage is published on http://www.vetinst.no/eng/content/download/641/5795/file/Deliverable7.pdf

In WP6 the questionnaire surveys were unfortunately conducted before the majority of the method development work had been done, and in effect the link between the molecular and the socio-economic research activities was not exploited as much as originally foreseen. However, close dialogue throughout the project duration ensured that relevant information was exchanged, and input from the molecular activities may be exploited more effectively in future socio-economic studies. The final report of the workpackage is published on http://www.vetinst.no/eng/content/download/653/5851/file/Deliverable8a.pdf; http://www.vetinst.no/eng/content/download/640/5791/file/Deliverable8b.pdf

In addition to the activities described above, some activities spanning several of the WPs, including a number of reviews and discussion papers, were realised. The majority of results directly obtained from the WPs are published in peer-reviewed scientific journals and other references.

Conclusions

This project has provided information and methods essential for the detection of GMOs, contributed to shaping the EU GMO Community Reference Laboratory (CRL), including the CRLs method acceptance criteria and approach for method validation. It has also contributed to a better understanding of the impact of knowledge about available detection methods on consumer confidence in stakeholders involved in food production and control, and to confidence in the regulatory framework and food safety.

Major Publications

Berdal K.G. & Holst-Jensen A. (2001). Roundup Ready® soybean event-specific real-time quantitative PCR assay and estimation of the practical detection and quantification limits in GMO analyses. *Eur. Food Res. Technol.* 213: 432-438.

Hernández M., Duplan M.-N., Berthier G., Vaitilingom M., Hauser W., Freyer R., Pla M. & Bertheau Y. (2004). Development and comparison of four real-time polymerase chain reaction systems for specific detection and quantification of *Zea mays L. Journal of Agricultural and Food Chemistry* 52: 4632-4637.

Holst-Jensen A. & Berdal K.G. (2004). The modular analytical procedure and validation approach, and the units of measurement for genetically modified materials in foods and feeds. *J. AOAC Int.* 87: 927-936.

Holst-Jensen A., Rønning S.B., Løvseth A. & Berdal K.G. (2003). PCR technology for screening and quantification of genetically modified organisms (GMOs). *Anal. Bioanal. Chem.* 375: 985-993.

Taverniers I., Van Bockstaele E. & De Loose M. (2004). Cloned plasmid DNA fragments as calibrators for controlling GMOs: different real-time duplex quantitative PCR methods. *Anal. Bioanal. Chem.* 378 (5): 1198-1207.

New methods for the safety testing of transgenic food

Acronym
SAFOTEST

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK1-1999-00651

Period
February 2000 – July 2004

Coordinator
Pr Ib KNUDSEN, DVM
Executive Director
Institute of Food Safety and Toxicology
Danish Veterinary and Food Administration
Mørkhøj Bygade 19
DK-2860 Søborg
Denmark
ik@fdir.dk

Background and objectives

In 1997, the European Union Scientific Committee on Food (SCF) issued a set of recommendations for the safety assessment of novel foods, including food that has been prepared using genetically modified (GM) organisms. The SCF suggested a decision tree approach which, for the toxicological part, may include a demand for separate animal studies to provide the missing information. However, the SCF did not give specific advice on how to carry out these studies, but stated that the design should be determined on a case-by-case basis. As a consequence, in Europe, there is no precise harmonisation of methodologies to assess the safety of transgenic food products, and it is being difficult to use traditional animal feeding studies for toxicological assessments. This clearly raises biosafety issues for the use of GM products in food. *In vivo* and *in vitro* validated nutritional-toxicological testing procedures are urgently required.

The overall objective of this project was to develop and validate the scientific methodology which is necessary for assessing the safety of foods from GM plants, in accordance with the European Union Regulation 258/97 of January 27 1997 concerning novel foods and novel food ingredients. The project was designed to meet the immediate need for a sensitive and specific testing strategy for GM foods in a scientifically valid and economically feasible manner.

Approach and methodology

The project, subdivided into seven different stages, examined a safety testing procedure for food derived from GM plants. In stage I, three strains of GM rice containing introduced genes were developed and characterised. The genes that were introduced encode three potentially insecticidal proteins: the snowdrop lectin GNA (which does not have any known mammalian toxicity, but which interacts with the gut), the kidney bean lectin PHA-E (which has high mammalian toxicity), and a bacterial toxin from *Bacillus thuringiensis* which has no known mammalian toxicity. In addition, recombinant proteins have been expressed and purified for later use as 'spiking materials' for the *in vivo* studies and test material for the *in vitro* studies.

In stage II, the three strains of transgenic rice were grown in sufficient quantities for *in vivo* testing, and their genetic identity and stability have been assured. Stage III involved identification and measurement of the critical nutrients, the critical toxic agents and other critical chemical changes in the transgenic rice strains. In stage IV, a study based on OECD 28-day test guidelines has been performed to ensure optimal diet composition and to measure suspected lectin or *Bt*-sensitive parameters for applications in stage VI. Stage V involved measurement of the effects of lectins and *Bt* toxin in a number of *in vitro* systems, in order to adjust the sensitivity and specificity of parameters which were investigated in the animal study with the transgenic rice species in stage VI. Supplementary mechanistic and dose response studies have also been carried out in vitro to supplement the *in vivo* studies for the final risk assessment. In stage VI, a 90-day OECD toxicity study in rats has been carried out with the three transgenic strains of rice, with and without the relevant test protein. In this study, the measurements of the lectin and *Bt*-sensitive parameters optimised in stages IV and V have been incorporated.

In the final stage, the results as well as the knowledge base acquired from the first six stages were evaluated in order to assess the sensitivity, specificity and efficacy of the safety testing approach.

Main findings and outcome

The results obtained from this project have been presented at workshops, published in international journals (Knudsen and Poulsen, 2007; Poulsen *et al.*, 2007a+b; Schrøder *et al.*, 2007; Kroghsbo *et al.*, 2008) and discussed in international fora, thereby providing a platform from which methodologies and recommendations based on this project can be transformed into internationally accepted guideline tests for safety testing of GM foods.

The SAFOTEST results from the 90-day rat feeding study with 60 % PHA-E rice mixed in the purified diet have demonstrated that the biological/toxicological effects of the inherent PHA-E in the PHA-E rice corresponding to a dosage of 30 mg PHA-E/kg body weight/day can be identified in the 90-day study, especially when the results are compared with the compound specific effects found in the additional test group spiked with 70 mg PHA-E/kg body weight/day (Poulsen *et al.*, 2007a). The results demonstrated, according to the expectation, both the *specificity* of the 90-day rat feeding study to detect specific compound-related effects, and the *sensitivity* of the test design

Partners

Karl-Heinz ENGEL
Technische Universität München (DE)

Mark TAYLOR
Scottish Crop Research Institute, Dundee (UK)

Ad PEIJNENBURG
University of Newcastle (UK)

Harry KUIPER
State Institute for Quality Control of Agricultural Products, RIKILT-DLO, Wageningen (NL)

Qingyao SHU
Zhejiang University (CN)

Illimar ALTOSAAR
University of Ottawa (CA)

to detect the bioavailable PHA-E, inherently formed in the rice (Poulsen et al., 2007a). In this manner the results also allowed the biological distinction between the primary effects caused by the novel gene product and effects of secondary changes.

The complete data set that has arisen from the SAFOTEST approach comprises the data on the parent plant(s), the gene construct(s), the gene product(s), the data from the compositional analyses, the data from the *in vitro/ex vivo* toxicity studies and the data from the 28-day rat toxicity study on the gene product, summed up in the data from the 90-day rodent feeding study with the intact plant food itself. The *hazard characterisation* based upon this data set addressed most precisely the *safety issues* and less precisely the *nutritional properties* of the GM food based on the comparison with its parental counterpart. The experiences gained from SAFOTEST indicated that the nutritional element can be covered as precisely as the safety issue in future studies, but that it is difficult to distinguish between nutritional and safety effects.

Conclusions

It is important that the SAFOTEST approach is not just seen as a 90-day toxicity study confirming the safety of the inherently produced new gene product, but more as a comparative safety study, which establishes the relative safety between the GM food and its traditional counterpart by undertaking a biological screening of health effects of both the intended and unintended changes. In the SAFOTEST approach, this study is the final achievement in a chain of information gathering. The safety assessment in SAFOTEST draws on the knowledge of the identity of the genetic change, as well as the compositional data of the GM food and the 90-day toxicity study on the GM food with and without the spiked material, before the hazard characterisation is concluded. By using the prior data regarding the toxicity of the novel gene product and by spiking the new gene product to the GM food, the 90-day study in SAFOTEST is able to separate unintended safety aspects of the new gene product produced inherently in the GM food from unintended safety aspects derived from the regeneration of the GM plant after the genetic transformation. The biological detection of the unintended nutritional disturbances is refined through compound-directed modifications of the purified diet for the GM groups, so their feed becomes nutritionally equivalent with the feed of the parental group with respect to previously identified differences (Poulsen *et al.*, 2007a).

We interpret the scientific findings of the SAFOTEST project, with the focus on the 90-day PHA-E study, to be strong enough to conclude that:

- the data from a well-designed 90-day rodent feeding study, together with data covering the gene insert, the compositional analysis, and the toxicity of the novel gene product, form the optimal basis for a comparative assessment of the safety of GM food and its conventional counterpart in the pre-market situation;
- the 90-day, rodent feeding study with one high dose level and a dietary design based upon compositional data on the GM food and toxicity data on the gene product is sensitive and specific enough to verify the presence/absence of the biological/nutritional/toxicological effects of the novel gene insert at the level of intake defined in the test and within the remit of the test;
- the 90-day rodent feeding study, with use of spiking on a *case-by-case* basis and with one high dose level and a dietary design as defined above, is sensitive and specific enough to verify the presence/absence of the biological/nutritional/toxicological effects of secondary effects of the genetic changes at the level of intake defined in the test and within the remit of the test;
- the total set of data generated in the SAFOTEST approach will be combined with suitable scenarios for potential human intake to allow a safety assessment of GM foods covering both the safety and nutritional issues normally raised in a pre-market situation.

Major Publications

Knudsen I. and Poulsen M. (2007). Comparative Safety Testing of Genetically Modified Foods in a 90-Day Rat Feeding Study Design Allowing the Distinction between Primary and Secondary Effects of the New Genetic Event. *Regulatory Toxicology and Pharmacology* 49, 53-62.

Poulsen M., Schrøder M., Wilcks A., Kroghsbo S., Lindecrona RH., Miller A., Frenzel T., Danier J., Rychlik M., Shu Q., Emami K., Taylor M., Gatehouse A., Engel KH., Knudsen I., (2007 a). Safety testing of GM-rice expressing PHA-E lectin using a new animal test design. *Food Chem. Toxicol.* 45, 364-377.

Poulsen M., Kroghsbo S., Schrøder M., Wilcks A., Jacobsen H., Miller A., Frenzel T., Danier J., Rychlik M., Shu Q., Emami K., Sudhakar D., Gatehouse A., Engel KH., Knudsen I. (2007 b). A 90-day safety in Wistar rats fed genetically modified rice expressing snowdrop lectin *Galanthus nivalis* (GNA). *Food Chem. Toxicol.* 45, 350-363.

Schrøder M., Poulsen M., Wilcks A., Kroghsbo S., Miller A., Frenzel T., Danier J., Rychlik M., Emami K., Gatehouse A., Shu Q., Engel KH., Knudsen I. (2007). A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis toxin*) in Wistar rats. *Food Chem Toxicol.* 45, 339-349.

Kroghsbo S., Madsen C., Poulsen M., Schrøder M., Kvist PH., Taylor M., Gatehouse A., Shu Q., Knudsen I. (2008). Immunotoxicological studies of genetically modified rice expressing PHA-E lectin ot Bt toxin in Wistar rats. *Toxicology* 245, 24-34.

Acronym
GMOCARE

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK1-CT-1999-00765

Period
February 2000 – July 2003

Coordinator
Dr Hubert P.J.M. NOTEBORN
Dutch Food and Consumer Product Safety Authority (VWA)
Office of Risk Assessment
P.O. Box 51095,
NL-2500 CM The Hague
The Netherlands
hub.noteborn@vwa.nl

New methodologies for assessing the potential of unintended effects in genetically modified food crops

Background and objectives

The introduction of crops produced by genetic engineering methods (GM crops) onto the marketplace were regulated under the Novel Foods Regulation (EC 258/97) which requires a thorough pre-market safety assessment. The assessment process pays particular attention to potential adverse effects that might compromise human and animal health and environmental biosafety. This is not a requirement for the introduction of novel seed varieties bred by conventional breeding, although unintended effects, i.e. effects which go beyond that of the original modification and which might impact primarily on health, may indeed be present in these traditional crops.

The safety of conventionally bred crops is based on a history of safe use. However some extremely rare cases have been reported where unintended effects (DNA rearrangements) have given rise to safety concerns. These were only identified once the crop was already on the market. Characterisation of GM crops is a legal requirement, however. As a result GM crops are better characterised than ever before in the case of conventionally bred crops, including knowledge on the site and nature of the genetic modification.

Approach and methodology

GM crop safety characterisation currently includes animal feeding trials with the whole food and/or compositional analysis of pre-selected nutrients and known toxins, and a comparison is made relative to the composition of conventional crops (targeted analysis). This approach involves the concept of substantial equivalence. Criticisms of this current 'single-analyte' and 'whole food' test strategy are that they are open to bias and will pick up unintended effects only by chance or if anticipated. Related to this, there are gaps such as the availability of: (i) sufficient knowledge of plant biology and metabolic pathway integration and interconnectivity, (ii) a comparator with a similar genetic background, and (iii) 'miracle' compounds to describe the complexity (metabolic networks) of crop plants.

The hypothesis has therefore been formulated that profiling techniques, based on the innovations within the genomics area, may assist in addressing these potential limitations. The new methods envisaged might be necessary for future GMOs, in which the modifications will be far more complex, and for which the current methodology sets boundaries to the safety testing. But the overall aim focuses on demonstrating that the GM crop is as safe as its traditional counterpart, where one exists, and as such does not introduce any additional or new risks to the health of man and animal. In addition, this scientific challenge provides great advantages for all types of breeding programmes, independent of whether they have their roots in traditional, gene-biotech or organic farming.

To that end, the potential value of applying 'profiling' or 'omics' tools as so-called non-targeted, unbiased screening approaches, in order to detect unintended effects due to the insertional mutagenesis process, has been researched. These technologies include metabolomics (parallel analysis of a range of primary and secondary metabolites), proteomics (analysis of polypeptide complement), and transcriptomics (parallel analysis of gene expression). The findings relating to the 'omics' procedures have been compared to results obtained by the conventional 'targeted' analytical approach. This holistic, enlarged comparison shapes the basis on which to focus further toxicological testing, if required.

The work has concentrated on a range of more than 135 (pre-market) GM lines in self contained experiments, such as greenhouse, Tygan netting and polytunnel trials with, for example, transgenic potato lines modified in their starch composition, (defective) glycoprotein processing, polyamine-, sugar-, glycoalkaloid- or lysine and threonine amino acid-metabolism; transgenic tomato lines with elevated phytosterols and/or isoprenoids (carotenoid) content and, transgenic *Arabidopsis* lines impaired in flavonoid contents. Some lines were evidently phenotypically different (e.g. Mal1, SamPAT (over expression) in potato). The crops were continuously characterised on sizes of T-DNA, vector backbone integration, copy number and expression levels. Morphological phenotype (i.e. growth, plant architecture, number, shape and colour of leaves, flowers and tubers, precocity) has been checked and compared to observations from previous cultures. Crops were grown in quantities to enable inter and intra plant variation to be assessed.

Partners

Arjen LOMMEN
RIKILT-Institute of Food Safety, Wageningen (NL)

Howard DAVIES
SCRI, Cellular & Environmental Physiology, Dundee (UK)

Peter BRAMLEY
University of London, Royal Holloway and Bedford New College, School of Biological Sciences (UK)

Geert ANGENON
Free University of Brussels-VUB (BE)

Jan PEDERSEN
Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration (VFA), Aarhus (DK)

Friedrich ALTMANN
University of Renewable Resources and Applied Life Sciences (BOKU), Institut für Chemie Vienna (AT)

Ian COLQUHOUN
Institute of Food Research, Food Quality and Materials Service Division, Norwich (UK)

Jean-Jacques LEGUAY
Départment d'Écophysiologie Végétale et de Microbiologie, Cadarache (FR)

Sirpa KÄRENLAMPI
Institute of Applied Biotechnology, University of Kuopio (FI)

Jean-Michel WAL
Lab Ass INRA-CEA d'Immuno-Allergie Alimentaire, Service de Pharmacologie et d'Immunologie, Jouy-en-Josas (FR)

Main findings and outcome

The results of the targeted chemical analysis did not reveal any unexpected, significant unintended effects associated with specific constructs (traits). Similarly, when individual lines were examined, it was clear that significant deviations from wild-type control values also occur with vector-only transformed controls (no target gene) and also with lines that have been developed using only tissue culture regeneration. This raises the possibility that somaclonal variation (known to occur significantly in, for instance, potato depending on genotype) also introduces significant deviations from the wild-type metabolite levels on a line-by-line, case-by-case basis.

Profiling or fingerprinting techniques such as genomics (e.g. potato microarray, highly enriched for stress-related genes; and a tomato array with genes from different stages of development) and metabolomics provide a 'global' overview of gene expression and chemical composition within the crops, be they GM or non-GM. On the other hand 2D-SDS-PAGE proteomics, including a screening for specific IgE determinants and analysis of N-glycans present in protein spots (glycomics), offer a means of revealing differences in protein profiles. This detection of changes in proteins also improves the ability to do a proper risk assessment by (i) indicating changes in allergenic and/or toxic proteins, and (ii) suggesting changes in the pattern of some metabolites that would not be revealed otherwise. However results also suggest that environmental conditions and genetic differences have a large impact on the expression of many proteins, and this may account for a lot of variation observed in plants grown in field conditions. Moreover, identification of proteins from, for instance, potato and tomato is currently hampered by the limited availability of sequence data and the performance of mass spectrometric structural confirmation.

There were no observations of deleterious effects on the physiology of the GM tomato plants, which were modified in their carotenoid biosynthesis, in which the carotenoid (phytosterol) profiles were significantly changed. But the metabolomics techniques demonstrated that the pleiotropic metabolic engineering of biochemical pathways reveals unknown metabolic linkages (i.e. significant differences from wild-type tomato controls) in GM tomato lines modified in carotenoids, tocopherols (vitamin E), flavonoids or phytosterols, which may be exploited in the design of engineering experimentation or conventional breeding for quality traits. In addition, the degree of phenotype exhibited can change from one progeny to another.

Enormous quantities of data could be generated from these holistic methodologies. However subsequent interpretation of the data is at present rate limiting. For example, microarray analyses of various *Arabidopsis* GM lines, down-regulated in their flavonoid pathway, indicate genes that are differentially expressed, but which are all identified as stress-induced genes that vary tremendously, even when the plants are grown under almost the same conditions. This finding shows that not all genes may be of any value for a comparative safety analysis. It also indicates that much more knowledge about the natural variation of individual genes is needed before any conclusion can be reached in the context of health impacts. Moreover, a natural genotypic variation exists in metabolite levels, such as are found between the fatty acid profiles of the variety Desirée and Record. The capacity to differentiate risk associated with specific transgenic events must therefore depend upon a detailed knowledge (database) of natural variation in the genes, proteins and metabolites measured, probably also under a range of (standardised) environmental conditions.

Ideally, only those parameters which fall outside the range of natural variation will be considered further in a safety assessment. However, there is a lack of information on the natural variation within and between given plant cultivars for all the parameters that can be measured now or in the future. Further research is required to validate the profiling methodologies developed.

Conclusions

The basics are in place for yielding novel tools that are of a generic nature and, therefore, may prove to be valuable for conventional or organic breeding as well. For the time being, the safety assessment of GM crops should focus primarily on the intended novel traits – target gene(s) and product(s). Unintended effects occur in both GM and non-GM crops, however GM crops are better characterised. Possibly the two should be treated in the same way in safety assessments, bearing in mind that these assessments are not required for non-GM crops. Application of a targeted approach still has great value and has resulted in a healthy and relatively safe food and feed package. As regards the stage of maturity of the 'omics' techniques like DNA microarrays, these should still be the leading principle when assessing GM-bred crops.

Although the profiling techniques studied should not yet be an official requirement, their application for instance to GM tomato lines has demonstrated that:

- the perturbation of one component of a biochemical pathway can have effects on the balance of metabolites throughout the pathway;
- perturbations to a specific biochemical pathway can result in unexpected effects on related and unrelated pathways;
- the transformation process can result in unintended effects that appear to be inheritable;
- the expression of a latent non-functional protein can result in unintended effects;
- it is important to perform experimentation on stable homozygous varieties with appropriate azygous controls, as in stable homozygous lines the number of transgene inserts appears to have little effect in contributing to unintended effects.

The novel techniques aim to be unbiased with regard to the choice of analytes to be profiled, whether they are genes, proteins or metabolites. On one hand, these methodologies are still in their infancy, but are rapidly developing. On the other hand, they are not validated and are yet (and may never be) comprehensive. All current profiling approaches are based on comparison of GM materials with selected controls in self-contained experiments. The data generated have a great potential to increase our knowledge of plant physiology and metabolic networks, and will improve the targeted analyses by discovering additional key components. In the case of new

GM plant lines with no appropriate comparator or history of (safe) use, application of the new profiling techniques is of great value for characterisation of their biochemical composition and functions.

Above all, bioinformatic tools need to be developed to extract relevant information from the raw data sets. The development of publicly available databases of crop composition and profiles is an absolute requirement, in order to determine the natural variation of compounds within and between given plant species. As information is gathered, a benchmark (expanding) with which to compare new crops could be envisaged. These databases would also greatly enhance the robustness of targeted analyses.

Finally the new methodology, based on advanced 'cell factory or omics' technology and used in this project, contributed to the development of the 6th Framework Programme research-project SAFE FOODS, where a larger combination of complementary expertise and resources has been established EU-wide.

Major Publications

Cellini F., Chesson A., Colquhoun I., Constable A., Davies H. V., Engel K. H., Gatehouse A. M. R., Karenlampi S., Kok E. J., Leguay J. J., Lehesranta S., Noteborn H. P. J. M., Pedersen A. J. & Smith M. (2004). Unintended effects and their detection in genetically modified crops. *Food and Chemical Toxicology* 42:1089-1125.

Defernez M. I.J. Colquhoun. (2004). NMR approaches to detect unintended effects of genetic modification in plants, in 'Genomics for Biosafety in Plant Biotechnology', Ed. J.-P. Nap, A. Atanassov W. J. Stiekema, *NATO Science Series, IOS Press*, The Netherlands.

Defernez M., Gunning Y. M., Parr J., Shepherd L. V. T., Davies H. V. and Colquhoun I. J. (2004). NMR and HPLC/UV Profiling of Potatoes with Genetic Modifications to Metabolic Pathways. *J Agricultural and Food Chemistry. ASAP Web Release* Date: 10-Sep-2004; (Article) DOI: 10.1021/jf049522e

Dobson G., Griffiths D. W., Davies H. V. & McNicol J. W. (2004). A comparison of the fatty acid and polar lipid content of tubers from two potato species, *Solanum tuberosum and Solanum phureja. Journal of Agricultural and Food Chemistry* 52:6306 – 6314.

Noteborn H.P.J.M., de Wit W. (2004). Scientific Challenges in Risk Assessment. In: Biological Resource Management in Agriculture – Challenges and Risks of GMOs – What Risk Analysis is Appropriate? OECD *Documents*, Paris, 139-141.

Expression profiles as fingerprints for the safety evaluation of new strains, including GMOs used in bioprocessed food

Acronym
EXPRESS FINGERPRINTS

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2001-01473

Period
September 2001 – May 2005

Coordinator
P. RENAULT
Génétique Microbienne
INRA
F-78352 Jouy-en-Josas cedex
France
pierre.renault@jouy.inra.fr

Background and objectives

Lactic acid bacteria are widely used for the production of fermented food, for example cheese, sausage and wine. Fermented food producers are continuously in search of new strains to maintain or improve the quality of their products. Table 1 presents potential sources of new strains and their main features. A series of research projects have been undertaken to decipher genetic traits important for food quality and processes. These findings could improve the quality of products, including taste, texture, conservation and possibly, in the future, nutritional value and health effect. However, these applications are not developed in the EU, due to the suspicions that consumers harbour about genetically modified organisms.

	Source	Genetic characteristics	Acceptability in food
Natural strains	Isolated from a food product or an environmental source. Unknown history until isolation.	Defined as belonging to already used food species. Gene content typically varies 5-25 % between individuals of the same species.	Accepted without further assessment other than their association with an already used species in foodmaking.
Classical variants	Selected in R&D laboratories from strains already used in food processes.	Genetic content modified in an uncontrolled manner (*eg* chemical mutagenesis). Most often this implies punctual mutations, deletions and eventually extensive chromosomal rearrangements.	Accepted without further assessment since considered substantially equivalent to bacteria already used in food processes.
Constructed variants	Constructed in R&D laboratories from strains already used in food processes, with molecular biology techniques that may involve steps realised in different bacteria and the transient use of genetic information.	Genetic content modified in a defined way, but not containing DNA originating from different species.	Varying status depending on country: • US: substantially equivalent to bacteria already used in food processes; • EU: evaluated as genetically modified strains and labelled as such.
Transgenic strains	As 'constructed variant', but with the introduction of DNA from different organisms.	Genetic content modified in a defined way, and containing DNA originating from different species.	Evaluated by specific procedure. Labelled as genetically modified (in EU) or bio-engineered strains (US).

Table 1.
Source and properties of microorganisms used in the fermented food industries.

GMO raises various safety issues, such as dissemination of new genes in the environment, possible impact of these genes on consumer health (for example by potentially increasing allergenicity), and possibly still unknown issues due to the novelty of the technique. It was for example claimed that no test could really ensure the safety of GMOs, because of the complexity of living organisms, hampering any reliable prediction. This issue is linked to the possible deregulation of metabolism and gene expression, following the introduction of new genes. The present work deals with this issue.

Approach and methodology

In recent years, considerable progress has been made in high-throughput technology, allowing global measures of gene expression in the cell, for example by proteomic and transcriptomic approaches. Moreover, the information systems now available for work on living organisms permit the exploitation of the increasing amount of data produced by high-throughput technology and, more widely, by the whole scientific community. In this context, microorganisms are organisms of choice for the development of models, because of their simplicity when compared with higher-order organisms such as plants and animals. *L. lactis*, a food-borne microorganism widely used in the dairy industry was chosen as the model for this programme. The evaluation test set up during this work used high-throughput technologies to develop a methodology applicable to the resolution of safety issues that might result from the deregulation of the organism by the introduction of a new gene as illustrated in Fig 1.

More generally, the objective of the Express-Fingerprint consortium is to provide the EU community with a novel tool for the safety evaluation of new starter strains, including new natural strains and derivatives of known strains obtained by classical methods or biotechnology techniques (often named GMOs). In this respect, the consortium developed:

- expression profiles (proteome and transcriptome) of different strains of *Lactococcus lactis*, including natural strains and derivatives thereof constructed by genetic or classical technology;
- a set of bioinformatics tools to compare globally the results and assess their substantial equivalence or not;
- a biological expertise on the potential effects of genetic modification, especially on effects that were unexpected from preexisting models;
- a first proposal for a risk assessment tool based on expression patterns.

Partners

J. KOK
University of Groningen – Molecular Genetics, Groningen (NL)

P. BESSIÈRES
Mathématique, Informatique, INRA, Jouy-en-Josas (FR)

O. TRELLES
Computer Architecture Dept., Univ. of Malaga, (ES)

Eric JOHANSEN
Genetics and Microbiology, Copenhagen (DK)

Stanislas VEILLET
Vitavaleur, DANONE, Savigny-sur-Orge (FR)

J. BARDOWSKI
Department of Microbial Biochemistry, Torun (PL)

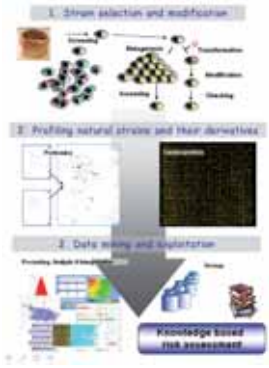


Fig 1.
Evaluation test set-up.

Main findings and outcome

Natural strains and modified bacteria evaluated in the programme:
A collection of 30 different *L. lactis* natural strains have been constituted from strains coming from different products and countries. None of these strains has a similar pattern in pulse field gel electrophoresis, suggesting that the strains collected present a wide genetic diversity. Among the natural strains were derivatives of the *L. lactis* IL594 strain (isolated in Comté cheese in 1955) that lost some of its seven natural plasmids. These plasmids were also sequenced for a better characterisation of their genetic content, since plasmids are often naturally transmitted between bacteria living in the same environment (including strains from the same and other bacterial species). From some points of view, their introduction could be considered as a natural way to produce genetically different bacteria.

Moreover, three types of modified strains have been constructed:
The first set of strains comprises derivatives of an industrial *L. lactis* subsp. *diacetylactis* strain producing an aroma that contributes to the taste of fresh fermented milk products, such as buttermilk, fermented cream and cottage cheeses. Variants of this strain inactivated for the *aldB* gene and encoding alpha-acetolactate decarboxylase, were developed. The modified strains produce more diacetyl, allowing the production of a buttermilk with a more pronounced flavour.

The second set of strains comprises phage-resistant mutants. The GMO was constructed by inserting four extra bases in the gene encoding the phage infection protein (*pip*). These strains are less sensitive to phage attack than their parent, and thus ensure more reliable processes and product quality.

The third set of strains contains a tRNA-based food-grade cloning system. The basis of this system is the restoration of a pyrimidine biosynthesis potential in strains modified by the introduction of an amber stop codon in the chromosomal *pyrF* gene by gene technology. *PyrF* synthesis is restored by the expression of a modified tRNA gene, supD, that can thus be used as a selectable marker. Successful expressions of different genes carried by a *supD*-containing vector were obtained. The system was initially designed for stably maintained plasmids in cell production in milk, since this medium does not contain pyrimidine.

Two types of techniques were used to build these strains:

- chemical mutagenesis, which is a classical technique, not falling under the EU legislation on deliberate release of GMOs or GM food/feed, thus not requiring a in-depth assessment; and
- GM techniques (as defined by EU legislation: 'organisms in which the genetic material has been altered in a way that does not occur naturally by mating or natural recombination"). These strains, if used for production will lead to specific 'GMO labeling' because foreign DNA has been introduced in one stage of the construction.

Detailed phenotypic and genetic comparison of GMOs, chemical mutants and their respective parent strains were carried out, including the sequencing of the mutations in the target genes.

Expression profiles have been performed by proteome and transcriptome analysis. For this purpose, standardised conditions of experimentation were set up by the partners to obtain comparable data in all laboratories. In order to develop a concept of proof, most experiments were carried out from cells grown in a chemically defined medium. However, a procedure has been develop to measure expression profiles from cells grown in milk, the natural medium for food fermentation of *L. lactis*.

DNA microarrays containing more than 2 000 PCR probes corresponding to annotated *L. lactis* IL1403 genes have been designed and DNA microarrays produced with the same set of amplicons are now commercially available. Software has been developed to allow (i) data mining and (ii) high-speed processing of the extensive data obtained from expression profiles and parallel comparison of transcription and statistical analysis of data.

Comparison of traditional and GM technologies to produce variants:

An important focus of the work was to further define the effects of genetic modifications on overall gene expression, and to assess whether the means used to obtain strains has an impact on their future behaviour. Proteome and transcriptome profiles were performed on the three sets of strains described above.

The results of our work show that:

- the extent of modification of the proteomic and transcriptomic profiles is always equal or more important in 'classical mutants' than in their GM counterparts. This result strongly suggests that the way modification has been produced may influence the transcription pattern, and that molecular biology techniques are producing less side-effects than classically used techniques;
- mutations generated by either technique do not systematically produce perturbation in the expression of the other genes of the cell. In particular, no significant changes were observed in the expression patterns of *aldB* and *pip* mutants. In such cases, it might be proposed that the modified strains obtained by either technique are substantially equivalent, although the EU legislation does not allow the application of this concept for strains obtained by molecular techniques;
- modification of gene expression following genetic changes could often be simply explained by good biological expertise. This is the case for (a) one of the two chemical mutants for which iron metabolism was modified, and (b) *pyrF* strains containing only one copy of the *supD* non-sense supressor or which the expression system was not fully restored.

Taken together, these data support the idea that molecular biology techniques are valuable tools for obtaining simple variants of natural strains and do not pose a risk when compared with classical methodologies.

Assessment of natural gene exchange effect on expression profiles:

The expression profiles of the natural strain IL594 were compared to those of its plasmid-devoid derivative IL1403. Among plasmid genes, we characterised genes potentially inherited from horizontal gene transfer from bacteria living in the same ecological niche, such as *Lactobacilli* and *Enterococci*. Expression profiles showed that half of the 151 plasmidic genes were expressed in the IL594. Moreover, a significant number of chromosomal genes where found repressed or induced, such as genes involved in sugar metabolism, amino acid catabolism, and prophages. These results show that the introduction of genes by natural means could significantly change the expression of cellular genes, and thus cell metabolism.

Natural diversity of expression profiles:
The last focus was on the comparison of gene expression variability in natural strains used in food, compared to that obtained by genetic modifications. Proteomic and transcriptomic profiles of 12 natural strains were compared among themselves and then with the profiles of the variants previously studied. The results of this work showed that profiles in natural strains differ substantially from one another and that, in most cases, biological expertise could not provide simple explanations of the observed differences. These data were further analysed to expertise the level of equivalence of variants obtained by different technologies and natural strains. This showed that, irrespective of the techniques used for their construction, profiles of variants are much closer to that of their respective mother strain than they are to any other natural strain or than most natural strains are to each other.

Conclusions

In this work, we have developed a concept of proof for risk assessment of new strains, with *L. lactis* as a model. This tool is based on the measure of expression changes induced by genetic modifications produced by any means in a set of strains. It generates lists of genes whose expression is modified and links genes to the knowledge database. This allows linking genetic changes to cell function that may be perturbed and thus directing expertise for further safety assessment if necessary. Similar procedures could be set up on any microorganism whose sequence is known. The scientific results obtained on real case studies strongly suggest that gene modifications performed by molecular biology tend to produce less unexpected events than classical methods. In this respect, EU legislation that emphasise the way organisms have been constructed in order to classify them as GMO may appear scientifically not relevant and potentially misleading for consumer choice.

Major Publications

Renault P. Genetically modified lactic acid bacteria: applications to food or health and risk assessment. *Biochimie*. (2002). Nov,84(11):1073-87. Review.

Pedersen MB., Iversen SL., Sorensen KI., Johansen E. The long and winding road from the research laboratory to industrial applications of lactic acid bacteria. *FEMS Microbiol. Rev.* (2005). Aug,29(3):611-24. Review.

Jan Kok, Girbe Buist, Aldert L. Zomer, Sacha A.F.T. van Hijum and Oscar P. Kuipers. Comparative and functional genomics of lactococci. *FEMS Microbiol. Rev.* (2005). Aug,29(3): 411-433. Review.

van Hijum SA., de Jong A., Baerends RJ., Karsens HA., Kramer NE., Larsen R., den Hengst CD., Albers CJ., Kok J., Kuipers OP. A generally applicable validation scheme for the assessment of factors involved in reproducibility and quality of DNA-microarray data. *BMC Genomics*. (2005). May 20,6(1):77.

Barriere C., Veiga-da-Cunha M., Pons N., Guedon E., van Hijum SA., Kok J., Kuipers OP., Ehrlich DS., Renault P. Fructose utilization in *Lactococcus lactis* as a model for low-GC gram-positive bacteria: its regulator, signal, and DNA-binding site. *J Bacteriol.* (2005). Jun,187(11):3752-61.

Development of a capacitance-based immunodetection analyser for the assay of GMOs in foods

Acronym
IMAGEMO

Programme Acronym
IMAGEMO

Contract number
QLK3-2002-02141

Period
January 2003 – January 2006

Coordinator
T. ALBANIS
University of Ioannina
P.O. Box 1186
GR-45110 Ioannina
Greece
talbanis@cc.uoi.gr

Project website
http://users.uoi.gr/imagemo

Background and objectives

The intensive public debate on the application of genetic engineering to the production of food has led to a high degree of controversy, both among consumers and within industry. There are two methods in general commercial use today for detecting GMOs in field crops. One method involves the detection of proteins that have been produced through biotechnology in the crop; the other is based on the detection of DNA sequences inserted in the crop. ELISA and Lateral Flow Strip methods are used for protein assay and PCR (Polymerase Chain Reaction) and Southern Blot for DNA.

The aim of this project was to develop a generic capacitance-based immunodetection analyser for GMO-related proteins. CP4 EPSPS (CP4-synthase) and Cry1(A)b-protein were employed as relevant model targets, corresponding to 'Roundup Ready (RR)-Soy' and '*Bt*-Maize' GMOs respectively. To achieve the overall objective, the production of monoclonal antibodies (MAbs) specific to the target proteins, the design and fabrication of a portable analyser able to monitor interactions between biomolecules, and the development of different impedimetric electrode architectures were conducted in parallel.

Considering problems associated with the detection of the target proteins in highly processed foods (e.g. extensive changes in conformation to the antigens), the development of antibodies for characteristic peptide sequences – as well the development of an analyser based on Quartz Crystal Microbalance (QCM) capable of monitoring the hybridisation between nucleic acids' complementary strands – were also decided on during the early meetings of the project. In addition, studies on PCR-based methods and the use of new primers were included in the objectives of the project.

Approach and methodology

To achieve these objectives, development concentrated on different types of capacitive immunosensors based on metal/metal oxide electrodes, on mixed self-assembled monolayers onto gold electrodes and on antibody/amphiphile Langmuir –Blodgett films deposited onto hydrophobic surface of 1-octadecanethiol (ODT) modified silver electrodes. The develop-

ment of MAbs specific to the target proteins was attempted by following different immunisation strategies:

- antigens were extracted and purified from crude plant material containing CP4 EPSPS and Cry1Ab antigens, by applying consecutive purification schemes consisting of ion exchange and size exclusion chromatography;
- specific peptides, which describe different families of Cry-toxins and CP4-synthase were synthesised and coupled to neutravidin as an immunogenic carrier protein;
- in addition the synthesis of recombinant antibodies against GMO peptides was pursued. The rational behind this last approach was the availability of single-pot libraries and that no immunisation procedures were required, which essentially saved time.

The design, construction and optimisation of a stand-alone, low-cost electronic device able to monitor interactions between biomolecules that may change the capacitance of an electrode was also carried out. Particular attention was paid to reducing or eliminating the constant (dc) component of non-capacitive current flowing through the cell, which is observed in electrodes characterised by a dielectric layer of relatively low resistance.

For the development of a QCM-based method for probing DNA interaction, a commercial QCM analyser, μ-Libra, was employed and the method set up was performed using the Cry1A(b) gene. Using the Bt-176 DNA sequence as template, a 200-bp fragment of the Cry1A(b) gene was amplified and purified. The yield of the PCR fragment was further cloned using the pGEM®–T Easy Vector System. After the vector digestion (EcoRI) and fragment purification, the Cry 1A (b) gene was labelled with biotin, and used as a probe to functionalise the quartz surface.

Main findings and outcome

The resultant capacitive immunosensors were tested with pilot antibodies, showing sensitivity with a limit of detection of ng/mL of the pilot antigen. To reduce the cost per sensor and in order to create a geometry suitable for in-field measurements in combination with the palm-sized analyser, the so-called Multipulser, inter-digitised screen-printed gold electrodes arrays were designed and fabricated.

The applicability of the sensors to GMO-specific proteins was restricted to the low affinity of the antibodies, which had so far been produced.

Partners

B. HOCK
Technische Universität München (DE)

C. MARTELET
Ecole Centrale de Lyon (FR)

P. MILLNER
University of Leeds (UK)

C. EFSTATHIOU
University of Athens (EL)

T. FRELINK
ECO CHEMIE B.V., Utrecht (NL)

M. PIGHINI
TECHNOBIOCHIP S.c. a r.l., Latina (IT)

Th. MICHAS
SKYLAB-MED S.A., Athens (EL)

D. KORAKI
ENGENE S.A., Athens (EL)

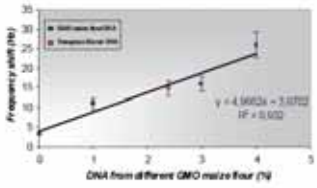


Fig 1.

Calibration curve for the QCM-based DNA sensor.

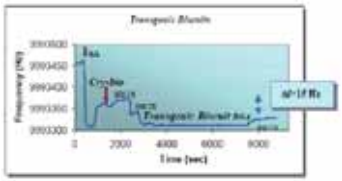


Fig 2.

Real-time hybridization between biotinylated Cry1A(b) fragment and Transgenic Biscuit DNA.

The operation of the Multipulser is based on the repetitive charging of the electrochemical cell capacitance by applying a predetermined number of short-duration, low-amplitude voltage pulses (perturbation pulses). All packets of charge are accumulated in an analogue integrator whose output voltage is proportional to the cell capacitance. The Multipulser features three user-selectable operating modes, each of them characterised by the shape of the applied perturbation pulses. 'Mode 3' seems particular interesting for electrodes characterised by a dielectric layer of relatively low resistance, as it effectively reduces or eliminates the contribution of this dc current component to the measured signal. The Multipulser was successfully used to monitor the binding of biotinylated molecules on two different avidin-modified electrode assemblies, one based on a thiol SAMs on gold and another based on a Ti/TiO_2 semiconductor. Measurements conducted in parallel with a commercial frequency response analyser gave similar reaction patterns.

The analytical performance of the QCM-based DNA analyser was evaluated using IRMM-413 certified reference materials. Total DNA extracted from IRMM products of dried maize powder with different mass fractions (from 0.1 % to 5 %) of genetically modified MON 810 maize flour have been analysed by DNA-sensor. The resonance frequency shift versus the different % of GMO maize flour DNA is shown in Fig 1. The applicability of the analyser in real samples was tested by extracting total DNA from transgenic cookies (certified genetically modified MON 810 maize flour (Fig 2.).

In the context of this project, a PCR-based method for GM soy and maize was also developed and validated. For this purpose a 35S primer set was designed, and the successive procedures of sample extraction, preparation and analysis were validated. Validation experiments were performed in various Certified Reference Materials and commercial samples. Finally, commercial samples were screened for the presence of GM Soya and GM Maize. Samples positive for the presence of 35S were analysed by real time PCR (TaqMan™) for quantification of GM percentage.

Conclusions

This project provided information on the development of capacitive immunosensors, novel instrumentation for the monitoring of interactions between biomolecules, and methods based on QCM and PCR for the detection of GMOs.

Major Publications

Hou Y., Tlili C., Jaffrezic-Renault N., Zhang A., Martelet C., Ponsonnet L., Errachid A., Samitier J., Bausells J. Study of mixed Langmuir-Blodgett films of immunoglobulin G/amphiphile and their application for immunosensor engineering *Biosensors and Bioelectronics*, 20 (6). (2004). 1126-1133.

Chaker T., Hou Y., Youssfi H. K., Ponsonnet L., Martelet C., Errachid A., Jaffrezic-Renault N. Impedancemetric probing of mixed amphiphile-antibody films transferred onto silver electrodes *Sensors Letters* 2. (2005). 1-6.

Mantzila A.G., Prodromidis M.I. Development and Study of Anodic and Thermal Ti/TiO2 Electrodes. Potential Use as Impedimetric Immunosensors, *Electrochimica Acta* in press. (2005).

Mantzila A.G., Prodromidis M.I. Performance of Anodically Formed Ti/TiO2 Based Architectures as Impedimetric Biosensors, *Electroanalysis* 17 (20):1878. (2005).

Bolis S.D., Charalambous P.C., Efstathiou C.E., Mantzila A.G., Malamou C.A., Prodromidis M.I. Monitoring of the Avidin – Biotinylated Dextran Interaction on Au and Ti/TiO2 Electrode Surfaces Using a Charge Integrating Device, *Sensors and Actuators* in press. (2005).

Hays H.C.W., Millner P.A. Prodromidis M.I. Development of Capacitance based Immunosensors on mixed-seld-assembled monolayers, *Sensors and Actuators* in press. (2005).

Passamano M., Pighini M. QCM DNA sensor for GMOs detection. (Submitted to *Sensor & Actuators* B).

Prodromidis M.I. Development of a kit for the construction of Voltametric Electrodes, Patent GR200401001172.

Quantitative risk assessment strategies for novel foods

Acronym
NOFORISK

Programme Acronym
FP6-FOOD

Contract number
506387

Period
April 2004 – March 2007

Coordinator
Morten POULSEN
Danish Institute for Food and Veterinary Research (DFVF)
Mørkhøj Bygade 19
DK-2860 Søborg
Denmark

Background and objectives

The overall objective of this project is to develop and validate the scientific methodology necessary for the safety assessment of novel foods in accordance with the EU Regulation 258/97 of 27 January 1997 concerning novel foods and novel food ingredients, and to communicate the importance of the approaches to key end-users, including consumers.

The purpose of any safety testing protocol is to enable a pre-market assessment which provides assurance that the novel food is safe to eat and nutritionally adequate at the anticipated level of consumption, in respect to its contents of toxic substances and nutritionally important compounds. An increasing number of novel foods, particularly the so-called 'functional foods', are generally marketed with claims of benefits to consumers. The nature of such claims can be broad, but the efficacy of such benefits needs to be determined.

This project addresses the scientific challenge of developing state-of-the-art approaches to assessing the safety, nutritional adequacy and efficacy of such novel foods in a comprehensive and interlinked set of studies.

The novel approaches deployed are tested on three model examples which are either already on, or may be introduced to, the market. The models selected are:

- genetically modified (GM) and conventionally bred potato tubers with altered content and balance of inherent toxicants (glycoalkaloids);
- a conventionally bred rice mutated line low in an anti-nutritional constituent (phytic acid); and
- functional food ingredients of natural origin (phytosterol and phytostanol esters).

The project summary will primarily focus on the GM potato tubers with altered ratio of the glycoalkaloids solanine and chaconine.

Approach and methodology

In order to undertake a quantitative probabilistic risk assessment, the GM potatoes were developed, grown and bulked up

in Scotland, while the pure solanine and chaconine were purchased from commercial companies. Comparative genomic, proteomic and metabolomic studies of the genetically modified and parental potato lines were performed by several contractors. Compositional comparisons of the GM and non-GM potatoes on the contents of proteins, fats, carbohydrates, fibres, major vitamins and minerals, as well as analyses for pesticide residues and accidental contaminants, were undertaken as well.

In vitro toxicity studies of the potato glycoalkaloids and genomic studies of samples from the *in vitro* and *in vivo* studies were designed to examine the feasibility of gene expression profiling (transcriptomics) and metabolite profiling (metabolomics), together with *in vitro* cell culturing for the hazard identification of glycoalkaloids and potato extracts containing glycolalkaloids. For this purpose the human cell lines Caco-2 and HepG2, derived from intestine and liver respectively, were used.

For the animal studies, the hamster was chosen as the best animal model for glycoalkaloid toxicity testing. A 28-day toxicity study was carried out with female Golden Syrian hamsters given up to 33.3 mg of total glycoalkaloid/kg body weight in ratios of solanine and chaconine, which can be compared to the ratios observed in a parental and a gene-modified potato respectively. A 90-day study was performed feeding female Syrian Golden hamsters a purified diet containing 20, 40 and 60 % of either the GM and non-GM potato. To increase the sensitivity of the study, pure glycoalkaloids were spiked to the diet of one group to a total of 33 mg glycoalkaloid/kg body weight/day.

Human exposure scenarios for potatoes and solanine/chaconine were developed based upon probabilistic intake studies using human consumption data. Intake estimates of solanine, chaconine and total glycoalkaloids were performed using consumption data collected within Italy and Ireland. 'Recipe factors' and 'water loss factors' were used to express food consumption data into Raw Agricultural Commodities. Recipe factors were used to calculate the amounts of potatoes consumed within mixed dishes, whereas the water loss factors were used to reflect changes in food weights, due to moisture gains and/or losses, during food preparation. Exposure to solanine, chaconine and total glycoalkaloids was first assessed by using a deterministic approach.

Partners

Ad PEIJNENBURG
RIKILT-Institute of Food Safety, Wageningen (NL)

Derek STEWART
The Scottish Crop Research Institute, Dundee (UK)

Michael GIBNEY
The Provost Fellows and Scholars of the College of the Holy and Undivided Trinity of Queen Elizabeth near Dublin (IR)

Karl-Heinz ENGEL
Technische Universität München (DE)

Angharad GATEHOUSE
University of Newcastle (UK)

Qingyao SHU
Zhejiang University (CN)

Joachim SCHOLDERER
The Aarhus School of Business, Aarhus (DK)

Catherine LECLERCQ
Istituto Nazionale di Ricerpa per gli Alimenti e la Nutrizione, Rome (IT)

Ingmar WESTER
Raision Bencol Ltd (Raisio), Kuopio (FI)

Dieter LÜTJOHANN
University of Bonn (DE)

Wout SLOB
National Institute of Public Health and the Environment, Bilthoven (NL)

The consumer perception of food-related risks and benefits in general and in relation to the GM potato was examined. The usefulness of the NOFORISK data for probabilistic assessments of risk and benefits of the GM potato were also considered. Finally the overall results from the study were presented for discussion at a stakeholder/expert workshop in order to develop conclusions and recommendations from the work performed.

Main findings and outcome

With regard to the GM potato, southern blot analyses demonstrated the presence of two insertion events of the transformation cassette into the genome of the transgenic potato line. The detailed compositional analysis of the GM and parental line used in the animal studies showed relatively few significant differences. The microarray data performed as part of the in vivo studies with pure glycoalkaloids indicated that these, particularly in the ratio present in the non-GM potato, increase the expression of many genes involved in cholesterol synthesis.The most prominent finding from the microarray experiments was the up-regulation of many cholesterol biosynthesis genes in Caco-2 cells. Thus cholesterol biosynthesis genes may serve as potential early biomarkers of glycoalkaloid toxicity.

In the 28-day hamster study, doses of up to 33 mg glycoalkaloid/kg body weight, in the same ratio as present in the wild-type potato, were more potently toxic than the ratio of the GM potato. A significant increase in distension of stomach and intestines, with a clearly dose-response, was determined upon macroscopic examinations. Data from the 90-day study in hamsters suggest that the GM potato is as safe as the non-GM potato, when fed for 90 days mixed in a purified diet in concentrations from 20-60 %. Various parameters showed differences between animals fed 0 or 20 %, compared to animals fed 40 or 60 % potato. These differences were the result of the concentration of potato in the diet and not potato type or glycoalkaloid ratio.

The levels of consumption for different groups of 'potato based food products' are reported for Italy and Ireland. In general, exposure scenarios were found to be below the threshold for human toxicity. Findings from the studies investigating consumer attitudes towards novel foods showed that consumers tended to see only a few well-defined risks and benefits in the three novel food examples. Interestingly, consumers were often much more concerned about issues

outside the scope of current legislation. Moreover, expert participants tended to define risk and benefit in terms of detailed chains of cause-effect relationships between variables, for which clear definitions and measurement rules exist. Such a level of detail could not be found in the consumer data.

The consumer studies indicated furthermore that, compared to other societal issues, novel foods are currently very low on most consumers' agendas. Of the three novel food technologies investigated in the survey, mutation breeding was evaluated most negatively by consumers. Genetic modification was evaluated slightly negatively, and food enrichment neutrally. At the same time, consumers felt least certain about their attitudes towards mutation breeding and considerably more certain about their attitudes towards genetic modification and food enrichment, suggesting that subjective uncertainty about a novel food technology may be seen by consumers as a risk unto itself.

Conclusions

In general, NOFORISK was a very ambitious project both in terms of concepts and methodologies, complexity and work to be done in a short time. The work has however led to quite a high number of peer-reviewed scientific publications and doctoral theses. However it was not possible to fully address safety, nutritional adequacy and/or health efficacy in a single animal study and, secondly, to combine the outcomes of the hazard/efficacy assessment and exposure assessment in an overarching probabilistic derived quantitative risk/benefit evaluation. The answers to these questions are still important in a pre-market safety assessment and also of great importance for consumer attitudes to the societal introduction of novel foods in the marketplace as can be seen from the results of the consumer studies.

Major Publications

Fricke CB., Schrøder M., Poulsen M., von Bergmann K., Wester I., Knudsen I., Mortensen A., Lütjohann D. (2007). Increased plant sterol and stanol levels in brain of Watanabe rabbits fed rapeseed oil derived plant sterol or stanol esters. *British Journal of Nutrition* 98:890-899

Langkilde S., Schrøder M., Stewart D., Meyer O., Conner S., Davies H., Poulsen M. (2008). Acute toxicity of high doses of the glycoalkaloids, solanine and chaconine, in the Syrian Golden hamster. *Journal of Agricultural and Food Chemistry* 56:8753-8760.

Hearty AP., Duffy E., Gibney MJ. (2009). Intake estimates of naturally occurring phytosterols using deterministic and probabilistic methods in a representative Irish population. *International Journal of Food Sciences and Nutrition*. 60(6):533-546.

Langkilde S., Mandimika T., Schrøder M., Meyer O., Slob W., Peijnenburg A., Poulsen M. (2009). A 28-day repeat dose toxicity study of steroidal glycoalkaloids, α-solanine and α-chaconine in the Syrian Golden hamster. *Food and Chemical Toxicology*, 47:1099-1108.

Hagemann KS., Scholderer J. (2009). Hot Potato: Expert-Consumer Differences in the Perception of a Second-Generation Novel Food. *Risk Analysis*, 29(7):1041-1055.

Biomarkers for post-market monitoring of short- and long-term effects of genetically modified organisms on animal and human health

Acronym
GMSAFOOD

Programme Acronym
FP7-KBBE

Contract number
211820

Period
August 2008 – July 2011

Coordinator
Herbert HÖNIGSMANN
Medizinische Universitaet Wien
Spitalgasse 23
A-Wien 1090
Austria
Herbert.Hoenigsmann@meduniwien.ac.at

Project website
http://www.gmsafood.org

Background and objectives

The function of post-market monitoring is to evaluate potential nutritional and health effects of authorised GM foods on mixed populations of human and animal consumers. Currently, there is little known about predictability of adverse effects following market release of GM foods. Our approach is to identify biomarkers, which could be used to predict harmful GMO effects after product authorisation. Of particular interest is the speculation by some stakeholders that the increasing prevalence of allergic diseases in recent decades might be related to the allergenic potential of new GM foods. For this reason, the partners decided to focus on the allergic potential of GMOs in their approach.

The objectives of the GMSAFOOD project are:

- to identify and qualify biomarkers for an allergenic prototype GMO for use as a tool for post-market monitoring of GMOs;
- to relate these biomarker profiles to developmental stages including gestation, growth, maturation, and adult-life;
- to use biomarkers to identify the movement and effects of GMOs in the food chain; and
- to establish biomarkers for GMO-induced immunogenicity and allergenicity in animals and humans.

Approach and methodology

To achieve these objectives, the partners are using a prototype allergenic α-amylase inhibitor (AAI) GM-pea (*Pisum sativum L.*) that expresses the AAI gene normally found in the common bean (*Phaseolus vulgaris L. cv. Tendergreen*). The inserted α-amylase enzyme protects the peas from damage by the pea weevil (*Bruchus pisorum*), which is one of the major pests of pea crops. Importantly, this GMO differs from most commercialised GM plants because the transgenic protein accumulates in the seed at high concentrations. This new approach to GMO production may result in the production of transgenic proteins that are handled differentially in the digestive tract and by the immune system of animals and humans. Many GM crops being

currently developed with pharmacological biologicals, such as immunoglobulin, are using this type of methodology and little is known about their safety. An authorised GMO, namely *Bt* corn, will also be tested in this project.

The project aims to extrapolate biomarkers that correlate potential GMO effects during gestation, growth, and maturation in various animal models with those of humans. It is studying GMO-fed pigs, salmon, rats, and mice during critical states in the lifecycle, in addition to the indirect effects of GM feeding in the food chain by comparing rats fed with GMOs to rats fed with fish and pork that were fed GMO. To extrapolate the data to humans, the partners have established a comparative database with antigenic epitopes and antibody crossreactivity in legume allergic patients and human-mouse chimera, in which a human immune system is transplanted into a mouse lacking an immune system. These experiments will yield data on general health with a specific focus on allergy and immunology and will establish the basis for an approach for post-market monitoring for authorised GMOs.

Partners

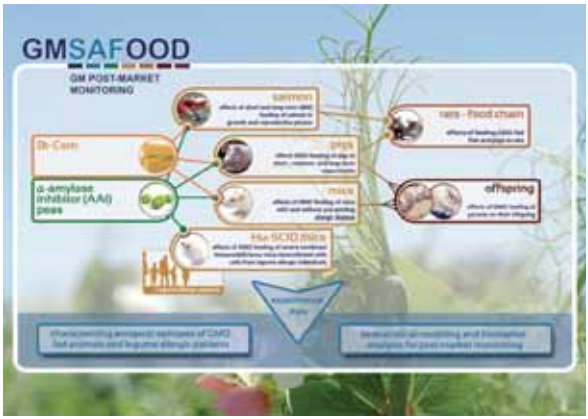
Helen BARKER
Commonwealth Scientific and Industrial Research Organisation, Canberra (AU)

Knut BØRVE
Norges Veterninaerhogskole, Oslo (NO)

Mark RYAN
Teagasc – Agriculture and Food Development Authority, Carlow (IE)

Erzsébet NÉMEDI
Kozponti Elelmiszer Tudomanyi Kutatointezet, Budapest (HU)

Nuri AKKAS
Troyka Makine Gida Sanayii Danismanlik Ve Pazarlama Ltd Sirketi, Ankara (TR)



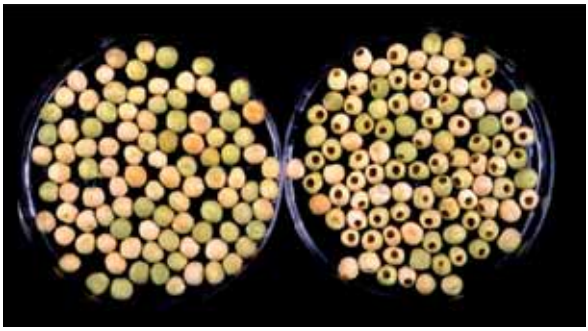


Fig 1.

Bt-corn and alpha amylase inhibitor peas will be fed to salmon, pigs, mice, rats and human-mouse chimera in addition to experiments to assess effects in the food chain and during reproduction. Antigenic epitopes will be determined and all data will be collected for biomodeling and biomarker identification for post market monitoring.

Chapter 3

GMOs for biomaterials and biofuels – Emerging technologies

Introduction

Lene LANGE
Professor and
Dean of Research
Aalborg University
Denmark

Europe has for a considerable length of time discussed crop improvement based on GMO technology. The skepticism towards this technology has been more pronounced in Europe than in any other part of the world. Consumer acceptance of putting GMO food on the dinner table has remained limited. However, mainly two factors, namely green house gas emissions, threatening our climate and near depletion of the easily accessible oil reserves, have accentuated the need for more environmentally benign and sustainable solutions, based on biological products and processes, providing new input into the discussions on biotechnology and GMOs in Europe. Interestingly, the Danish Board on Technology documented, through a Consensus Meeting approach, that the Danish people remain unwilling to accept GMO plants for food, but were however, more open to accept in the future GMO plants, providing for more sustainable and economic production of pharmaceuticals and improved materials for industrial purposes.

The FP7 portfolio of projects within the field of Biomaterials and emerging technologies constitutes a new chapter of European research in emerging technologies applied in plant research. It focuses on production of human therapeutics in plants and on providing new plant materials, optimized for use as a basis for more sustainable biological solutions, substituting fossil based materials or providing alternatives for other scarce natural resources. And importantly, in some instances the objective can be met without designing a GMO plant construct!

The urgent need for improved crops for feeding and fuelling the soon 9 billion people on earth along with increased molecular understanding of plant biology has led to a new focus in plant breeding: Can we use GMO as a research tool, applied to produce new crops which are not GMOs but a result of normal breeding? The two projects, MEIOSYS and RECBREED both build on using advanced molecular biology (e.g. studies of homologue recombination and gene targeting – of both somatic and meiotic cells) to generate diversity which can be exploited in crop improvement. This could serve to accelerate traditional plant breeding processes, where the end result is not a GMO, as no foreign DNA was inserted.

The FP7 project on production of new drugs in plant systems have focused on the large group of plant metabolites, the terpenoids. TERPMED has the objective of solving the three most important obstacles for development and upscaling of plant terpenoids for human health: poor availability of the plant material holding the terpenoids in demand; too low concentration of the compounds in the plant materials; and inherent difficulties in obtaining pure compounds from the plant materials. They focus in this work on two types, sesquiterpenes and phenolic diterpenes (both groups with high biological activity). The objective is to improve the production technologies and also making new discoveries for future evaluation as novel drugs for human health. The project SMART CELL focuses on terpenoids for both cancer and malaria and has the objective of developing new upscaled amounts of the terpenoids by production in cell and tissue cultures of periwinkle and in new traits of GMO tobacco plants.

The project PLAPROVA has the objective of improving the heterologue protein expression in plants. More specifically they want to refine the transient expression technologies, enabling cloning and expression of milligram amounts of novel compounds within only 2-3 weeks. Such technology would facilitate screening and characterization of series of novel, hitherto not evaluated products. The compounds in focus are proteins forming polypeptides complexes/virus like particles, possibly opening for new lead vaccine candidates.

The FP7 portfolio of new plant materials for industrial use spans from bioenergy over substitutes for classical rubber to new production systems for wax esters. RENEWALL has the objective of identifying traits which give plant cell walls with increased digestability and hereby optimized saccharification levels and therefore also increased biofuel potentials. ENERGYPOPLAR aims at identifying improved easy to process plant materials, with high level cellulose combined with low level lignin. EnergyPoplar expands the focus to also ensuring good agricultural robustness, and possibly suitable for being grown on marginal land.

The FP7 project EU PEARLS will transform naturally occurring plants, which are not used in the food production, to be able to act as starting material for production of rubber and latex. The increased need for such materials, when fossil based substitutes for rubber will have to be phased out is obvious. The rubber tree will be overexploited and access to the amounts needed, will be severely restricted. The ICON project is based on the hypothesis that known industrial oil crops can be modified, through insertion of just two enzymes, to produce wax esters instead of the normal triaglycerols. No risk of spread to food crops is inherent.

As it appears, the FP7 portfolio of plant science projects within the field of new materials for industrial and pharmaceutical use holds potentials for creating real value for society: providing the basis for biological products and biological processes, thereby contributing to delivering solutions to important problems.

The portfolio builds not only on advanced plant science. It constitutes a project portfolio where biologists work together with engineers as well as medical doctors. Further, it builds on close collaboration between plant scientists and microbiologists, using in parallel both plant (*Arabidopsis*) and fungal (*Sacharomyces cereviciae*) model species. Further, and most importantly, it clearly shows that genomics, metabolomics and GMO technologies can be used as research tools where the end product will not necessarily, and only if needed, be a GMO. The main focus of the activities lies in applying the most appropriate technology, which could solve the problem, not on using technology per se. For the entire portfolio bioethics, safety and sustainability aspects have been taken into account in an integrated manner, a prerequisite for being considered for funding. It also indicates that Europe is moving into a new phase where more sustainable solutions to important problems will have to come from plant materials.

Industrial crops producing added-value oils for novel chemicals

Acronym
ICON

Programme Acronym
FP7-KBBE

Contract number
211400

Period
March 2008 – February 2013

Coordinator
Sten STYMNE
Swedish University of Agricultural Science
Arrheniusplan 2C
PO Box 7070
SE-Uppsala – Sweden
sten.stymne@ltj.slu.se

Project website
http://icon.slu.se/ICON/

Fig 1.
The non-food oil crop Crambe abyssinica will be used for the production of added-value industrial seed oils.

Partners

Cecilia VAG
Axel Christiernsson International AB, Nol (SE)

Peter DENOLF
Bayer Crop Science, Geel (BE)

Baungquan HUANG
Hubei University, Wuhan (CN)

Antoni BANAS
University of Gdansk, Gdansk (PL)

Johnathan NAPIER
Rothamsted Research Station, Harpenden (UK)

Margit FRENTZEN
Aachen University of Technology, Aachen (DE)

Background and objectives

Replacing fossil oil with renewable resources is perhaps the most urgent need and the most challenging task that human society faces today. Cracking fossil hydrocarbons and building the desired chemicals with advanced organic chemistry usually requires many times more energy than is contained in the final product. Thus, using plant material in the chemical industry not only replaces the fossil material contained in the final product but can also save substantial energy in the processing.

Plant oils are of particular interest for the chemical industry since their chemical structures are very similar to the fossil oil. Plant oils show a great variation in their composition between different plant species. Many of the oil qualities found in wild species would be very attractive for the chemical industry if they could be obtained at moderate cost in bulk quantities and with a secure supply. Genetic engineering of vegetable oil qualities in high-yielding oil crops could generate such products – and in a relatively short timeframe. The ICON project aims at developing such added value oils in dedicated industrial oil crops. Thus the outcome of the project will save energy, protect the environment and improve rural economies. Since the GM technologies to be used in the project are still met with great scepticism in Europe, the keywords of communication strategies will be openness and appreciation of public concerns.

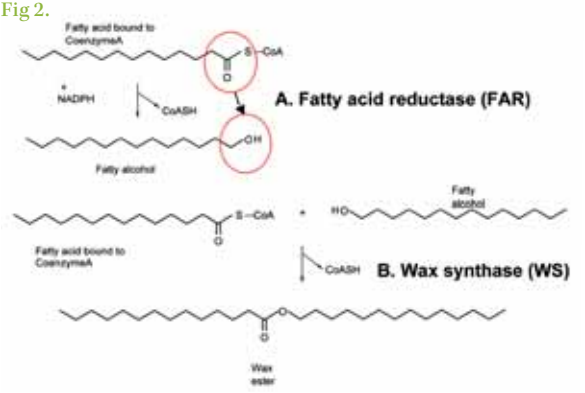
Approach and methodology

ICON will redesign seeds to produce wax ester oils instead of common triacylglycerol oils. The wax esters are much more resistant to high temperatures and pressures than triacylglycerols (commonly called triglycerides), making them suitable for lubrication applications where other natural oils decompose. ICON will develop a range of different wax ester qualities with different melting points and other features to improve their uses as lubricants.

In order to minimise the risk of inadvertent mixing with seeds for food purposes, and to prevent genes for industrial oil qualities from crossing into oil crops intended for food, the project will not use any food crops in its activities. The partners will use two non-food oil crops, *Crambe abyssinica* (Fig 1.) and *Brassica carinata* as vehicles for industrial qualities. Neither of these crops is used for human consumption due to the very high percentage of erucic acid in the seed oil. They also do not easily intercross with related *Brassica* food crops, such as *canola* (rapeseed) or *Brassica* vegetables. The project will introduce into these oil seeds, by genetic engineering, an enzyme named FAR (Fatty Acid Reductase) that converts some of the fatty acids to fatty alcohols (reaction A in Fig 2.). A second introduced enzyme, named WS (Wax Synthase) will link the produced fatty alcohol to other fatty acids, producing wax esters instead of triacylglycerols in the seeds (reaction B in Fig 2.).

FAR and WS enzymes occur in nearly all types of living organisms, such as bacteria, fungi, plant and animals. They can have widely different specificities, *i.e.* they handle different kinds of fatty acids and fatty alcohols. By using genes for enzymes with specificities for the kinds of fatty acid and fatty alcohol required, while also altering the kinds of fatty acids produced in the seeds, the partners will develop a wide range of functionally different wax esters suited to various applications.

Fig 2.



Partners

Ivo FEUSNER
University of Göttingen, Göttingen (DE)

Brigitte THOMASETTE
Centre National de la Recherche Scientifique, Paris (FR)

Peter J. EASTMOND
University of Warwick, Coventry (UK)

Eibertus Nicolaas VAN LOO
Plant Research International, Wageningen (NL)

Randall J. WESELAKE
University of Alberta, Alberta (CA)

Allan GREEN
Australian Commonwealth Scientific and Industrial Research Organization, Canberra (AU)

Owen ROWLAND
Carleton University, Ottawa (CA)

Jan JAWORSKI
Danforth Center, Saint Louis (US)

Robert MULLEN
University of Guelph, Guelph (CA)

Basil NIKOLAU
Iowa State University, Ames (US)

Mark SMITH
NRC-Plant Biotechnology Institute, Saskatoon (CA)

Xiao QIU
University of Saskatchewan, Saskatoon (CA)

John OHLROGGE
Michigan State University, East Lansing, Michigan (US)

John DYER
United States Department of Agriculture, Washington (US)

Ljerka KUNST
University of British Columbia, Vancouver (CA)

Peter DÖRMANN
University of Bonn, Bonn (DE)

Edgar CAHOON
University of Nebraska, Nebraska (US)

Mikael GÄLLSTEDT
Innventia (former STFI-Packforsk), Stockholm (SE)

Enhancing poplar traits for energy applications

Acronym
ENERGYPOPLAR

Programme Acronym
FP7-KBBE

Contract number
211917

Period
March 2008 – February 2012

Coordinator
Francis MARTIN
INRA
Rue De L'Universite 147
F-75338 Paris
France
fmartin@nancy.inra.fr

Project website
http://www.energypoplar.eu/

Background and objectives

With the world's growing energy demand, liquid fuels derived from lignocellulosic biomass offer an important alternative to conventional fossil fuels. Biofuels, such as bioethanol, can minimise energy import dependence, reduce greenhouse gas emissions and assist agricultural development. Bioethanol can be produced from cellulose, a sugar present in the cell wall of woody plants. Trees, and in particular poplars (*Populus*), are attractive 'second generation' bioethanol crops that can grow with reduced costs on marginal soils unsuited to food crops.

ENERGYPOPLAR aims to develop new poplar trees with both desirable cell-wall traits and high biomass for use as an efficient, renewable and sustainable source of lignocellulosic feedstock for industrial production of bioethanol. To achieve this goal, this project will:

- study the genetic mechanisms a) determining optimised yield in *Populus* and b) controlling the synthesis of cell wall polysaccharides and lignin to produce 'high cellulose/low lignin' poplars for an improved saccharification potential (the conversion of lignocellulosic biomass into fermentable sugars is called saccharification);
- develop high throughput assays for lignocellulosic quality and lignocellulose saccharification potential;
- establish a platform for rapid gene discovery and testing to identify novel genes controlling traits of interest;
- create a delivery pipeline for improved poplar trees carrying traits of interest and start the process of commercialisation;
- set up tools for environmental sustainability assessments of SRC *Populus* growing systems with respect to soil microbial diversity, greenhouse gas mitigation, water and other inputs relevant to a changing climate;
- actively promote transfer of technology and biological materials for commercialisation to bioenergy companies, plant breeders, the European forest-based sector, scientists, policymakers and consumers.

Approach and methodology

The ENERGYPOPLAR project will establish field trials under short rotation coppice culture (SRC) with existing transgenic poplars that contain less lignin. SRC is a cultivation method in which the trees are planted at high density to maximise biomass production (~15 000 trees /ha). Since lignin is the main factor limiting saccharification, the partners have chosen transgenic poplars with reduced lignin content. These trees carry silenced or anti-sense down-regulated genes involved in the lignin biosynthesis pathway, such as CAD, COMT, CCR and CCoAOMT.

The project will also develop new transgenic poplars combining genes that improve yield with genes that reduce lignin. An increase in biomass and modifications of lignin polymer can be achieved independently, following single gene manipulations in transgenic poplars. New transgenic lines are being obtained by gene-stacking. The genes used are CAD, F5H, 4CL, CCR (lignin modification) and GS, CEL1, GA20-oxidase (increased biomass).

Finally the project will identify novel genes involved in wood formation in order to study their biological role by altering their expression in transgenic poplar and to investigate their potential for wood quality improvement.

As result, ENERGYPOPLAR will:

- improve understanding of cell wall assembly and develop 'low lignin/high cellulose' trees with increased nutrient-use efficiency;
- develop poplar as a SRC crop system suitable for large-scale deployment in Europe in areas unlikely to be used for food agricultural production;
- promote environmental sustainability of novel energy plantations in Europe.

Partners

Ingrid RAGNARSDOTTER
Sveriges Lantbruksuniversitet, Uppsala (SE)

Klaus PALME
Albert-Ludwigs-Universitaet Freiburg, Freiburg (DE)

Wim GOEMAERE
Flanders Interuniversity Institute for Biotechnology, Zwijnaarde (BE)

Richard TROWBRIDGE
University of Southampton (UK)

Raffaele TESTOLIN
Associazione Istituto di Genomica Applicata, Udine (IT)

Ute KROEKEL
Georg-August-Universitaet Goettingen Stiftung Oeffentlichen Sechts, Göttingen (DE)

Magnus HERTZBERG
Swetree Technologies ab – stt, Umea (SE)

Brooke ALASYA
Imperial College of Science, Technology and Medicine, South Kensington (UK)

Luc OZANNE
INRA Transfert s.a., Paris (FR)

Rational design of plant systems for sustainable generation of value-added industrial products

Acronym
SMARTCELL

Programme Acronym
FP7-KBBE

Contract number
222716

Period
January 2009 – December 2012

Coordinator
Kirsi-Marja OKSMAN-CALDENTEY
VTT Technical Research Centre of Finland
Vuorimiehentie
PO Box 1000
Finland
kirsi-marja.oksman@vtt.fi

Project website
http://www.smart-cell.org/

Background and objectives

SMARTCELL is a four-year large collaborative project that aims to build on understanding of secondary metabolism in plants and use this knowledge to create plants that produce valuable molecules such as pharmaceuticals in a sustainable manner. More than a quarter of all the active ingredients in our current medicines originate from plants, and in many cases they are still extracted from plant material because they are too complex for chemical synthesis. Unfortunately, the most useful ones tend to be synthesised in tiny amounts among many similar molecules, so they are difficult and expensive to isolate and purify from cultivated plants.

Approach and methodology

The SMARTCELL project will focus on one particular metabolic pathway – the terpenoid pathway – which gives rise to a wide range of useful molecules, including the anticancer drugs paclitaxel, vinblastine and vincristine, and the antimalarial drug artemisinin. By studying how the enzymes and corresponding genes in the terpenoid pathway are controlled, SmartCell aims to establish novel ways to engineer plant cells and develop them into Green Factories producing specific valuable molecules. To achieve these aims, the project will develop novel gene discovery, multigene transformation and cloning methods and will transfer combinations of genes into plants to equip them for the production of specific terpenoids.

The engineering component of the project will be complemented by an analytical component, which will use sophisticated methods to evaluate the engineered plants and determine which metabolites are produced. The project will focus on two species: periwinkle and tobacco. Periwinkle plants (*Catharanthus roseus*) already produce useful terpenoids such as vinblastine and vincristine, but at such low levels that tonnes of biomass must be collected to isolate a single gram of product. Here the aim will be to use cultured cells and tissues (e.g. hairy roots, which grow indefinitely in culture) to increase the production of these valuable molecules and develop large-scale cultures that meet the needs of industrial manufacturing.

Fig 1.
Periwinkle (Catharanthus roseus) plant in culture.

Tobacco (*Nicotiana tabacum*) does not produce the same terpenoids as periwinkle, but the plant grows rapidly and produces large amounts of biomass in a short time, making it useful for large-scale production. Here the aim is to introduce genes that confer upon tobacco the ability to produce new terpenoid molecules, and then exploit its prodigious growth to make production economic and sustainable. Tobacco is also very amenable to genetic transformation and grows well anywhere in the world, making it a highly versatile and transferrable platform.

As well as taking metabolic engineering in plants well beyond the state of the art, SMARTCELL will also generate a comprehensive secondary metabolism knowledge-base which will enhance the competitiveness of European R&D by generating a positive environment for the accumulation of new intellectual property. SMARTCELL has also set aside project funds to encourage dissemination and outreach activities in order to promote the project and an interest in science generally. The outreach activities include an open-access website (http://www.smart-cell.org), and initiatives involving the SAW Trust (http:// www.sawtrust.org), which promotes science in schools by teaching children to appreciate science through art and writing.

Fig 2.
Hairy root culture of tobacco.

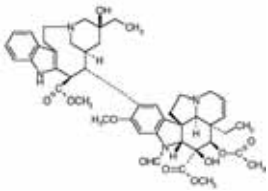


Fig 3.
Chemical structure of anti-cancer compound vincristine obtained from periwinkle.

Partners

Alain GOOSSENS
VIB Gent (BE)

Robert VERPOORTE
The Institute of Biology Leiden (NL)

Josep M. SENTIS SUÑÉ
Universidad de Lleida (ES)

Stefan SCHILLBERG
Fraunhofer IME Forschung (DE)

Enrico MARTINOIA
University of Zurich (CH)

Marc BOUTRY
Université Catholique de Louvain (BE)

Denis FIX
Centre National de la Recherche Scientifique, Paris (FR)

Regine EIBL
Zurich University of Applied Sciences (CH)

Anne OSBOURN
John Innes Centre, Norwich (UK)

Barbara Ann HALKIER
Copenhagen University (DK)

Alisdair FERNIE
Max Planck Institut fur Molekulare Pflanzenphysiologie, München (DE)

Lee SWEETLOVE
University of Oxford (UK)

Harro J. BOUWMEESTER
Wageningen University (NL)

Frédéric BOURGAUD
Plant Advanced Technologies SAS, Vandoeuvre (FR)

Jan PEN
SoluCel Ltd, Espoo (FI)

Markus KLEIN
Philip Morris Products SA, R&D, Neuchatel (CH)

Ronald MACIAK
Dow Agroscience LLC, Indianapolis (US)

Systematic analysis of factors controlling meiotic recombination in higher plants

Acronym
MEIOSYS

Programme Acronym
FP7-KBBE

Contract number
222883

Period
July 2009 – June 2014

Coordinator
Dr Sue ARMSTRONG and
Prof Chris FRANKLIN
University of Birmingham

Project website
www.meiosys.org

Fig 1.
The model plant Arabidopsis thaliana.

Background and objectives

Cereals and other plant crops have played a major role in meeting mankind's food demands for the last ten millennia. Today, plants are at the heart of the European food industry with an annual turnover of more than a trillion euro. Intensive breeding has boosted plant yield, quality and resistance to stress, but current predictions suggest that, with population growth and climate change over the next 50 years, we will have to produce more food than was created over the last 10000 years. To achieve this, we will need to adopt ever more novel approaches to plant breeding, including developing crops matched to individual world populations. One key to success may be more effective exploitation of natural genetic diversity.

Meiosis, which underpins sexual reproduction in plants and animals, is essential for the accurate transmission of chromosomes from one generation to another. We believe developments in genomics, bioinformatics and systems modelling, in conjunction with functional analyses, provide a prime opportunity for a step-change in understanding how meiosis is controlled in the model plant *Arabidopsis* and applying this knowledge to crop species.

The MEIOSYS project is the first fully integrated combinatorial approach to the analysis of the genes/proteins that underpin plant meiosis. Bringing together nine European participants, it aims to enable crop improvement programmes, domestication of new crops and industrial innovation by developing – without genetic modification – the most advanced strategy yet to accelerate traditional breeding.

Approach and methodology

MEIOSYS intends to build a comprehensive, predictive model of the gene/protein networks responsible for the controlled distribution of homologous recombination events during meiosis in the model plant *Arabidopsis thaliana*. This data will form the bedrock for manipulating genetic crossover (CO) frequency and distribution in barley and brassica – two of the most important crop species in the EU.

Using an integrated approach based on the complementary expertise of the consortium members, we aim to elucidate the gene/protein networks that underpin meiotic recombination and its control through a combination of systems biology and genomic approaches. Our scientists will determine the functional role of novel meiotic proteins and their interactions with the components of the recombination machinery. The project will investigate the pattern of recombination hotspot distribution and establish its inter-relationship with chromosome organisation to achieve a comprehensive, systems-level model of the gene/protein network controlling the frequency and distribution of meiotic crossovers in plants. Members will validate predictions arising from the network modelling in Arabidopsis in the important EU crops of barley and brassica. Crop lines showing modified recombination will then be generated.

The MEIOSYS project intends to deliver the technology necessary to provide an efficient system for modulatiing recombination in crop plants. The benefits of this will include increasing the competitive edge for crop breeders and European research, while generating and disseminating higher scientific understanding of meiotic recombination. Providing a sound basis for future research in this arena, the project will also produce significant technological, social and environmental benefits.

Fig 2.
The MEIOSYS project holds promise for increased future production of the EU crop of barley.

Partners

Peter SCHLÖGELHOFER
University Vienna (AT)

Karl MECHTLER
Research Institute of Molecular Pathology, Vienna (AT)

Dr Raphael MERCIER/
Dr Christine MEZARD
Institut National de la Recherche Agronomique, Versailles (FR)

Marie-Pascale DOUTRIAUX
Université Paris XI (FR)

Juan L SANTOS
Universidad Complutense de Madrid (ES)

Clara CONICELLA
Consiglio Nazionale delle Ricerche – IGV (IT)

Tom GERATS
Radboud Universiteit Nijmegen (NL)

Robbie WAUGH
Scottish Crop Research Institute (UK)

Acronym
RECBREED

Programme Acronym
FP7-KBBE

Contract number
227190

Period
March 2009 – February 2013

Coordinator
Holger PUCHTA
Universität Karlsruhe
Kaiserstrasse 12
D-76131 Karlsruhe
Germany
holger.puchta@bio.uka.de

Project website
http://recbreed.eu

Recombination: an old and new tool for plant breeding

Background and objectives

Plant breeding has always relied on homologous recombination (HR) for generating the genetic variation needed to develop new varieties. The objective of the current proposal is to improve plant breeding by enhancing HR rates in somatic and in meiotic cells. The expected outcomes of such enhancement are to establish an efficient gene targeting (GT) technology for precise engineering of plant genomes and to increase the rate of meiotic recombination between homologous or homeologous chromosomes.

Approach and methodology

There are many factors of the HR machinery that are common to somatic and meiotic cells. This enables both objectives to be addressed in a synergistic way. HR can be divided into different steps: the initiation, by double-strand breaks (DSBs), followed by chromatin remodelling, invasion of the homologous sequence and the resolution of the recombination intermediates. Each of these stages contains a bottleneck of HR that we will address here.

Work Package (WP) 1 will aim at enhancing GT and meiotic HR through targeted DSB induction. DSBs will be induced by zinc-finger nucleases that can be custom-designed for target sequences anywhere in the genome.

In WP 2, we will test the influence of HR factors involved in homologue invasion or modulating chromatin structure on GT and meiotic HR. This includes RAD51 homologues, genes that affect cytosine methylation, chromatin structure and mismatch repair, which influences recombination between divergent sequences.

In WP 3 we will concentrate on factors involved in crossover promotion and resolvases such as Mus81, Xpf/Ercc1 and Xrcc3-Rad51C homologues.

In WP 4 the combined effect of bottlenecks removal as found in the first three WPs will be studied. Most experiments will be performed with the *Arabidopsis* plant model and implemented by the industry partners into crops such as tomato and corn to guarantee quick applicability for breeding.

Partners

Avraham LEVY
The Weizmann Institute of Science, Rehovot (IL)

Charles WHITE
CNRS, Clermont-Ferrand (FR)

Jerzy PASZKOWSKI
University of Geneva (CH)

Paul HOOYKAAS
Leiden University (NL)

Michiel DE BOTH
Keygene, Wageningen (NL)

Wyatt PAUL
Biogemma, Clermont-Ferrand (FR)

Pnina DAN
OSM-DAN Ltd, Rehovot (IL)

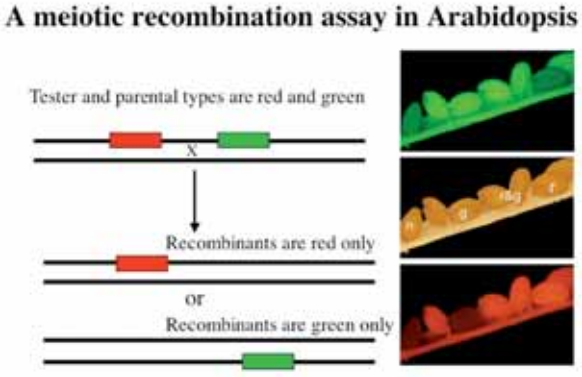


Fig 1.

Meiotic testers were developed to determine the rate of crossover in Arabidopsis WT and mutants. GFP and RFP expressed under strong seed promoters were transformed into the Arabidopsis genome and mapped. Testers were prepared containing both GFP and RFP linked in cis. In crosses with these testers, progeny can be of the parental type, i.e. seeds fluoresce in both red and green (r&g on right panel), or do not fluoresce (n) or are of recombinant types, i.e. seeds that fluoresce only in red (r) or only in green (g).

Plant terpenoids for human health: a chemical and genomic approach to identify and produce bioactive compounds

Acronym
TERPMED

Programme Acronym
FP7-KBBE

Contract number
227448

Period
June 2009 – May 2013

Coordinator
Albert FERRER
Universitat de Barcelona
Gran Via de les Corts
Catalanes 585
ES-08007 Barcelona
Spain
albertferrer@ub.edu

Project website
http://www.terpmed.eu/

Background and objectives

Plant secondary metabolites are the source of a large number of biologically active compounds, in fact many of the therapeutic drugs used today are derived from plants or developed from a plant lead compound. The TERPMED project is devoted to plant terpenoids, which are the largest and chemically most diverse group of plant natural products with over 25 000 compounds identified so far (Fig 1.). This huge chemical diversity is however hardly exploited for the development of new drugs due to several reasons such as poor availability of the source plant material, too low concentrations in the plant material, and difficulties in isolating pure compounds. This project is aimed at providing solutions to overcome these difficulties for two classes of plant terpenoids bearing specific chemical functional groups, sesquiterpene lactones and phenolic diterpenes. These two classes of terpenoids have been chosen because of their high potential as novel human drugs for treating cancer and central nervous system disorders, and there is evidence that the γ-butyrolactone and phenolic groups are responsible in part for the biological activity of these compounds.

Approach and methodology

The development of genomic and metabolomic technologies has now made it possible to bring the field of plant natural products into the 21st century and replace serendipitous and haphazard finding by rational design and discovery. The TERPMED

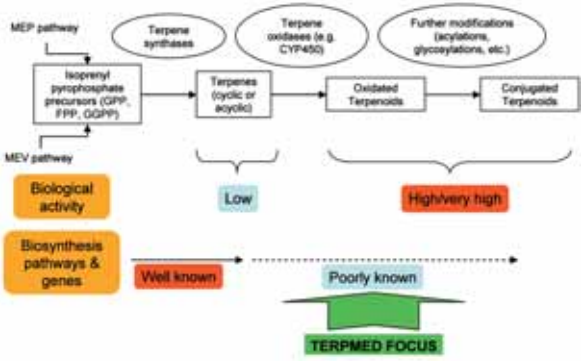


Fig 1.
Major steps of terpenoid biosynthesis. Knowledge is relatively poor on steps downstream of terpene synthases.

project will develop the technology required to achieve this goal. By using a combination of comparative metabolomics and genomics (Fig 2.) the project will contribute to the understanding of the biosynthetic pathways leading to the synthesis of the above-mentioned classes of terpenoids. To this end, the partners will develop efficient and robust analytical screening methods to detect and identify terpenoids bearing the γ-butyrolactone and phenolic functional groups.

Extraction and purification procedures will be set up at laboratory scale to isolate these compounds for subsequent structural and functional characterisation. These function-based screens will lead to the establishment of a comprehensive library of compounds from a subset of selected plant species and varieties. The most representative compounds will be tested for biological activity and the most active molecules will be selected. In parallel, large-scale sequencing of cDNAs from producing and non-producing tissues of a number of plants selected for their ability to produce the targeted compounds will be achieved. High throughput cDNA sequencing, coupled to the comparative analysis of the metabolic profiles of targeted species, will be used for elucidation of the biosynthetic pathways leading to these compounds.

The genes discovered will be used to increase the production of compounds containing the γ-butyrolactone and phenolic functional groups, but also to generate new functional molecules through combinatorial biosynthesis. Innovative plant production platforms using secretory organs such as the trichomes and organ *in vitro* cultures will be tested for the efficient production of the most promising compounds identified. Extraction and purification procedures at semi-industrial scale will be developed to isolate the bioactive compounds from these production platforms. In summary, the TERPMED project will lead to the improved production of known biologically active molecules and the discovery of new bioactive molecules. The project will also generate the technology to produce and isolate these compounds from genetically modified heterologous plant hosts.

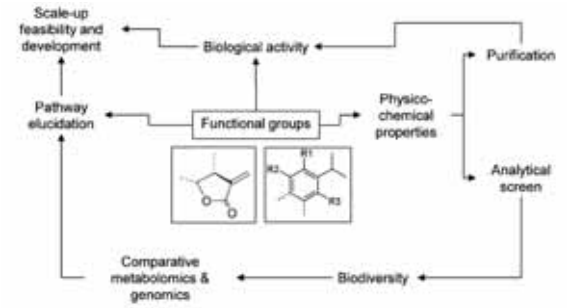


Fig 2.
Overall strategy of the TERPMED project.

Partners

Harro BOUWMEESTER
Wageningen Universiteit (NL)

Christof KERSCH
Prisna bv, Weesp (NL)

Milica DJORDJEVIC
Institut Za Bioloska Istrazivanja, Belgrade (RS)

Christina BESTA
Aristotelio Panepistimio Thessalonikis (EL)

Jeffrey A. JOHNSON
University of Wisconsin-Madison (US)

Leny VERSTEEG
Plant research international b.v., Wageningen (NL)

Improving plant cell walls for use as a renewable industrial feedstock

Acronym
RENEWAL

Programme Acronym
FP7-KBBE

Contract number
211982

Period
August 2008 – July 2012

Coordinator
Simon MCQUEEN-MASON
University of York
Heslington GB-York, YO10 5DD
United Kingdom
sjmm1@york.ac.uk

Project website
http://www.york.ac.uk/org/cnap/Renewall

Background and objectives

The need for renewable energy and raw materials for industry and society has become a pressing concern. The dependence of a burgeoning and rapidly industrialising world population on fossil fuels is clearly unsustainable, due to dwindling reserves and the impact of greenhouse gas emissions on global climate. Industrial economies are also increasingly concerned about the stability and security of fuel supply. Transportation fuels derived from petroleum account for more than 30 % of EU energy consumption. Liquid biofuels such as bioethanol (produced by fermentation of plant-derived sugars) and biodiesel (produced from plant or animal oils) offer a renewable alternative for liquid transportation fuels and have the potential to lessen the dependence of EU Member States on oil imports. Bioethanol production is increasing worldwide, but expansion of the current production from sugar cane, sugar beet or cereal grain can put a strain on world food supplies and prices. Development of 'second generation' biofuels that can be made from low-input non-food plant biomass can alleviate dependence on oil in a sustainable carbon-neutral way without placing further stress on food supply.

Plant biomass (or '*lignocellulose*') is one of the greatest untapped reserves on the planet and is mostly composed of cell walls. Energy-rich polysaccharide polymers make up about 75 % of plant cell walls and these can be broken down to produce sugars that can be fermented to produce bioethanol and other products. Even greater value can be added by using integrated processing systems that allow multiple products to be produced from the same biomass – the biorefinery concept. However, the complex structure of cell walls, consisting of a network of cellulose microfibrils and matrix polysaccharides encrusted by the phenolic polymer, lignin, makes them very resistant to degradation. Improving the ease and yield of cell wall saccharification (conversion to sugar) represents the major technological hurdle to overcome before the full vision of the plant-fuelled biorefinery can be realised.

Approach and methodology

The aim of RENEWALL is to make breakthroughs in our understanding of plant cell walls and to use this knowledge to

develop new strategies for breeding added-value plants with modified wall properties that are better suited for biorefining. The project will do this by identifying the molecular barriers to digestibility, and the genes that can be manipulated to lower these barriers: these may be plant genes involved in cell wall biosynthesis or other (often microbial) genes that can modify wall properties or degrade wall polymers when expressed in plants. These genes can be directly used in GM approaches to breed improved plant feedstock for biorefining, or used as markers to improve crops by conventional breeding approaches.

The project brings together leading plant cell wall scientists, along with experts in the area of microbial enzymes for plant cell wall deconstruction, and crop breeders and genetic engineers with interests in biomass crops. Between them, the cell wall scientists bring a depth of experience and resources in the area of cell wall structure and biosynthesis, which has not previously been focused on the aim of understanding and improving cell wall deconstruction.

During year 1 the project has developed a set of new analytical tools with which to measure the digestibility of plant cell walls. These include a high-throughput robotic system to identify plants with altered digestibility in large populations of plants, and a reactor system for the more detailed analysis of plants with altered digestibility. The partnership has produced a wide range of plant materials for digestibility analysis, and preliminary data have already identified plants with modified lignin content that exhibit greater than double the ease with which sugars are released.

Partners

James HOUSTON
University of Dundee (UK)

Anna HALDRUP
Kobenhavns Universitet (DK)

Ake ERIKSSON
Kungliga Tekniska Hoegskolan, Stockholm (SE)

Herman HÖFTE
Institut National de la Recherche Agronomique, Paris (FR)

Amanda GREGORY
University of Newcastle Upon Tyne (UK)

Henrik WREM
Aarhus Universitet (DK)

Deborah GOFFNER
Universite Paul Sabatier Toulouse III (FR)

Dawn BARKER
The Chancellor, Masters and Scholars of the University of Cambridge (UK)

Kerry CHANTREY
The University of Manchester (UK)

Ingrid RAGNARSDOTTER
Sveriges Lantbruksuniversitet, Uppsala (SE)

Win GOEMACIE
VIB, Zwijnaarde (BE)

Hinrich HARLING
KWS SAAT AG, Einbeck (DE)

Adam WALKER
Bioniqs Limited, York (UK)

Magnus HERTZBERG
Swetree Technologies Ab, Umea (SE)

Thomas DIDION
DLF- Trifolium A/S, Roskilde (DK)

Martha STEINBOCK
United States Department of Agriculture, Washington (US)

Kimberly HAYES
Cornell University, Ithaca (US)

EU-based production and exploitation of alternative rubber and latex sources

Acronym
EU-PEARLS

Programme Acronym
FP7-KBE

Contract number
212827

Period
April 2008 – March 2012

Coordinator
Hans MOOIBROEK
Agrotechnology and Food Innovation BV
Bornsesteeg 59
NL-Wageningen 6708 PD
The Netherlans
hans.mooibroek@wur.nl

Project website
http://www.eu-pearls.eu/UK/

Background and objectives

Natural rubber is a widely used raw material essential to industry, medicine, transportation and defence whose major source, the rubber tree *Hevea brasiliensis*, is currently both sustainable and environmentally beneficial. However, increased worldwide demand for natural rubber and latex means that alternative sustainable sources are urgently required. In order to meet this challenge, the partners have created a network that links all stakeholders involved in the development and sustainable use of *Parthenium argentatum* (guayule) and *Taraxacum koksaghyz* (Russian dandelion) as alternative rubber and latex sources in the EU. The technical performance and economic potential of latex and rubber extracted from these plants will be evaluated by producing specific prototypes, such as surgical gloves and tyres.

Approach and methodology

To guarantee the sustainable development and exploitation of both crops throughout the value creation chain, the project includes research into the collection and creation of new germplasm, biochemistry and genetics, breeding, agronomy, processing and product development. The entire rubber biosynthetic pathway will be analysed, and potential bottlenecks will be identified and bypassed through targeted conventional breeding. Genes involved in rubber biosynthesis will be mapped, helping to accelerate breeding strategies in order to generate plants with commercially viable rubber yields. Such plants will be tested for efficient growth and rubber production in the field under different climatic and edaphic conditions in Europe.

Since it is foreseen that the improvement of multiple traits will be required to ultimately obtain economically viable production systems for both crops, the project includes classical crossing and novel genomics-based marker-assisted breeding and targeted mutagenesis (TILLING variant) strategies, rather than the production of transgenic field-grown crops. Because of high deregulation costs and EU policy, transgenics will not be used for the improvement of European rubber crops. This contrasts with the USA rubber crops programme.

However, because Russian dandelion can be hybridised with various other dandelion species, transformation of rubber biosynthesis genes into these species is a form of *cis*-genesis. *Cis*-genesis is thus an option for future improvement when EU-regulations for *cis*-genesis become less stringent. Traits to be addressed at some point during the entire crop improvement programme and expected to last for a longer period than the four years project's life-time include:

- growth vigour for improved overall biomass production;
- agronomical performance;
- root morphology (Russian dandelion, rubber in roots only, improved harvestability);
- latex and rubber contents;
- down-regulation of competing pathways (e.g. sterols or unwanted terpenes);
- rubber polymeric properties and size distribution;
- absence of latex allergenic proteins;
- overcome the necessity of cold-induction of rubber synthesis (guayule);
- salt tolerance (guayule);
- cold tolerance (guayule).

To support and speed up breeding strategies and to promote the understanding of rubber biosynthesis the model plant *Arabidopsis thaliana* and Baker's yeast *Saccharomyces cerevisiae* are part of the programme to identify and assess the roles of key genes involved in acetyl-CoA accumulation, in determining the flux through the mevalonate pathway and, finally, in the (initiation of) polymerisation of IPP and GGPP units to long-chain (poly)-isoprenes. DNA sequences concerned will be used to support breeding strategies for guayule and Russian dandelion.

Fig 1.

Taraxacum koksaghyz (Russian dandelion, left) and Taraxacum officinale (common dandelion).

Partners

Andries KOOPS
Plant Research International b.v., Wageningen (NL)

Peter VAN DIJK
KEYGENE NV, Wageningen (NL)

Katharina STEINBERG
Westfaelische Wilhelms-Universitaet Muenster (DE)

Yves POIRIER
Université de Lausanne (CH)

Jeffrey MARTIN
Yules Corporation, Maricopa (US)

Jaroslav NOVOTNY
Botanicky Ustav AVCR, Pruhonice (CZ)

Enrique RITTER
Instituto Vasco de Investigacion y Desarrollo Agrario Neiker SA, Vizcaya (ES)

Kamila MAGZIEVA
Uchrezhdenie Centr Ekologicheskaya Rekonstruktsiya, Almaty (KZ)

Jacques NOLIN
Centre de Coopération International en Recherche Agronomique pour le Développement, Paris (FR)

Berend BLOKHUIS
Vredestein Banden Bv, Enschede (NL)

Peter SCHEEPERS
Stramproy Contracting BV, Stramproy (NL)

Plant production of vaccines

Acronym
PLAPROVA

Programme Acronym
FP-KBBE

Contract number
227056

Period
January 2009 – December 2011

Coordinator
Mary ANDERSON
John Innes Centre
Norwich Research Park
Colney
GB-Norwich NR4 7UH
United Kingdom
mary.anderson@bbsrc.ac.uk

Project website
http://www.plaprova.eu/

Background and objectives

The use of plants as bioreactors for the production of pharmaceutical proteins stands at a cross-roads. On the one hand, the past five years have seen considerable advances in the technologies for expressing proteins and extracting them in an active form from plants. This culminated in 2006 in Dow Agroscience obtaining regulatory approval for a plant-expressed vaccine against Newcastle disease in poultry. On the other hand, most of the recent successes have concerned the production of well-characterised antigens and antibodies which have already been produced using previously established methods such as mammalian cell culture.

From a scientific standpoint, this approach clearly made sense as it was important to establish the principle that plant-produced pharmaceuticals are comparable in safety and efficacy to their conventionally produced counterpoints. Furthermore, given the timelags associated with the production of lines of stably transformed plants, it was essential that proteins with previously characterised pharmacological properties were expressed, as only a few candidates could be examined. However, the downside is that the plant-expressed proteins which are most highly developed and, in some cases, undergoing clinical testing will be in direct competition with existing products.

It is now clear that for plants to fulfil their potential as a means of producing pharmaceutical proteins, it is imperative that methods are developed for the rapid production and characterisation of plant-expressed proteins. This is particularly important for cases for which it is not currently possible and/or practical to produce the protein by other means. In these cases, there is likely to be far less pre-existing information available about the efficacy of the final product, and it will be essential that the properties of a number of variants can be quickly assessed to determine which are the most suitable for further development. Stable genetic transformation (either nuclear or plastid) is unsuitable for these studies in view of the time taken to obtain expression.

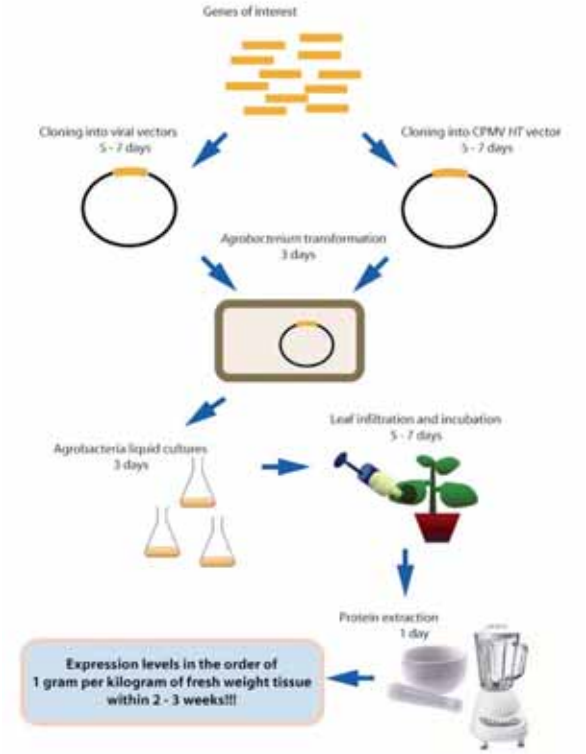
Approach and methodology

It is one of the major aims of this project to refine transient expression technologies so that they can produce sufficient

material in a sufficiently short timeframe to allow pharmacological studies of a large number of vaccine candidate variants to be undertaken. This permits optimisation of the methods of antigen presentation of a variety of different potential immunogens. A particular advantage of the transient approach is that yields can easily reach 10-30% of TSP. Furthermore these high levels of expression can be achieved in the timeframe of a few days.

Using transient expression systems, the consortium can go from cloning of constructs to the expression of milligram amounts of candidate immunogens within 2-3 weeks (Fig 1.). This speed means that a wide range of vaccine candidates can be screened in a short time. Thus the PLAPROVA consortium is able to evaluate potential vaccines against a number of diseases of great and increasing importance to both the EU and Russia.

The consortium is concentrating on the expression of proteins which form polypeptide complexes or virus-like particles. Due to their immunological properties, these are particularly suitable as candidate vaccines.



Partners

Neil HATTERSLEY
Scottish Crop Research Institute, Dundee (UK)

Ruddie JULLENS
Wageningen Universiteit (NL)

Carlos Manuel ABAD RUIZ
Consejo Superior de Investigaciones Científicas, Madrid (ES)

Concetta MOTTURA
Consiglio Nazionale Delle Ricerche, Rome (IT)

Ivan MINKOV
University of Plovdiv (BG)

Sue CUSTERS
University of Cape Town (ZA)

Nikolay RAVIN
Centre Bioengineering of the Russian academy of sciences, Moscow (RU)

Joseph ATABEKOV
Moscow Lomonosov State University, Biological Faculty, Moscow (RU)

Liudmila TSYBALOVA
Research Institute of Influenza of Russian Academy of Medical Science, Saint-Petersbourg (RU)

Vladimir BORISOV
FGI Federal Centre for Animal Health, Vladimir (RU)

Chapter 4

Risk assessment and management – Policy support and communication

Introduction

Prof. Dr. Joachim SCHIEMANN
Julius Kühn Institute (JKI)
Federal Research Centre for Cultivated Plants
Head of the Institute for Biosafety of Genetically Modified Plants

Since the publication of the first 'EC-sponsored Research on Safety of Genetically Modified Organisms' book in 2001, the EU legal framework on Genetically Modified Organisms (GMO) has changed substantially. Currently, it mainly comprises of legislation covering the deliberate release of GMO into the environment as well as GM food and feed and their traceability and labeling respectively. As part of the legal initiatives, the European Food Safety Authority was founded in 2002, which today is the body responsible for conducting the environmental and health risk assessment of any GMO to be marketed within the EU. In parallel to these important changes, considerable efforts were made and are continuously being made in the EU to ensure that research addresses the main public concerns regarding GMOs. The projects within this section provide insight and tools to tackle challenges related to – amongst others – the introduction of GMOs in EU markets, which, in principle, could serve as a basis for future policy making.

The projects described here, deal with biosafety research; risk analysis; coexistence and regulatory issues; consumer choice as well as risk/safety communication. In addition, the 7th Eurobarometer survey of 2010 on biotechnology has received financial support.

Since virus-resistant GM plants have already been commercially released worldwide (e.g. Papaya resistant to Papaya ring-spot virus), it is important to provide the necessary basis for risk assessment of virus resistance traits before their commercial release in Europe. Concerning their potential ecological impact, the project VRTP IMPACT studied two forms of gene flow, either from plant to virus by recombination or from plant to plant by outcrossing. Based on their previous work, using approaches that include laboratory, greenhouse and field studies, the participants in the project have made considerable progress in providing the knowledge that will allow assessment of potential risks linked with GM virus resistance traits.

In order to promote food safety, a new integrated risk analysis approach has been developed as part of the SAFE FOODS project. By combining the skills of several natural and social scientists, representing a broad range of institutions, countries, and disciplines, the project aimed to contribute to the restoration of consumer trust in the food chain through the development of a new integrated risk analysis approach for

foods. The development of an improved Governance Framework for foods produced by different agricultural and food processing methods and practices changed the focus of decision making on food safety from single risks to considering foods as baskets of risks, benefits and costs that are associated with their production and consumption, also taking into account the social context in which decisions are made. The major outcome of the project is a new Risk Analysis Approach for foods that integrates assessment of human health aspects, consumer preferences and values, as well as impact analysis of socio-economical aspects. The findings of the multidisciplinary research approach can contribute to moving the risk analysis model forward by translating them into best-practice recommendations and using them to amend the classical risk analysis model.

Meanwhile, more than 40 countries (including 27 EU Member States) have introduced traceability and labeling regulations mainly following either the EU or USA regulatory patterns, which may differ according to the decision whether labeling is mandatory or voluntary, and whether there is a tolerated threshold for the adventitious presence of GMOs. Coexistence of supply chains incorporating GM products is one of the issues faced by European agriculture and downstream processing chains within a frame of different constraints. In line with these developments, several research projects have been funded under the fifth and sixth European Framework Programmes for research, focusing on traceability (QPCRGMOFood, GMOCHIPS) and coexistence (SIGMEA, TRANSCONTAINER, CO-EXTRA).

The CO-EXTRA project participants represent a broad expertise in several aspects of coexistence and traceability of GM and non-GM supply chains. The project examined the practices of supply chains from seed production to retailers' shelves with practical implementation (e.g. documentary and analytical) tools, addressing coexistence issues by multidisciplinary teams embracing supply chains as a whole. The methods, strategies, tools, and models developed in CO-EXTRA for GM and non-GM supply chains' coexistence and traceability might be used in the management of other supply chains, value added and niche markets, and for detecting and excluding harmful products. The main results of the project were disseminated at the biennial Genetically Modified Crops Coexistence Conferences (GMCC-07/09) in Seville and Melbourne, the only international forum focused on coexistence between GM and non-GM agricultural supply chains.

Public research in modern biotechnology aims at solving constraints in agricultural production, with regard to health care and environmental protection. Developments in public sector research in agricultural biotechnology are closely dependent on the design and implementation of the regulatory frameworks for GM crops at the national, regional and global level. Modern biotechnology can contribute to human well-being only if the regulatory frameworks are science based, predictable, transparent and balanced. To achieve this, it is crucial that policy makers, regulators and the general public are better informed about the objectives and progress in life sciences in agricultural biotechnology. Conversely, the public research sector itself needs to be informed about and involved in regulations relating to modern biotechnology and the implications for research, so as to be better aligned with broader policy developments for food security, environmental protection and sustainable development. Therefore, the SCIENCE 4 BIOREG project has been funded aiming to involve public sector scientists working in biotechnology research in international negotiations and policy discussions and extending the activities of the Public Research and Regulation Initiative (PRRI). As a result of the project, public researchers have become a well-known entity in the international arena and the role of science in general and of public research in particular have clearly been set on the negotiating agendas.

Following the 2004 adoption by the EU of compulsory labeling of all food products containing GM in any ingredient, it was uncertain how rapidly such products would appear on the shelves of retail grocery stores. Therefore, the EU funded project CONSUMERCHOICE asks 'Do European consumers buy GMO foods?' Several public opinion polls and some focus groups explored public attitudes to GM-containing food products which showed that a majority of the European public were somewhat antipathetic to the technology being applied in agriculture. It is questionable, however, whether attitudes expressed can be taken as a proxy for behavior. The studies performed lead to the conclusion that only a small number of GM labeled products were on sale and being purchased in several European countries. As the number of GM products available after the introduction of labeling has declined significantly due to considerations by large food suppliers and manufacturers, it can be concluded that European consumers are restricted in their choice with respect to their purchases of GM-foods. The findings also suggest that most people are actually neither really interested in, nor very alert to, the presence of GM ingredients or products, although actually being present in the foods purchased. The observations

underline the fact that what people say differs from what they do. Though the data obtained are not sufficiently extensive, it may reasonably be concluded that (i) most people do not actively avoid GM foods while shopping, suggesting a divergence between their opinions and actual shopping patterns; (ii) linking purchasing data with answers to questionnaires is a more reliable way to establish attitudes than relying on opinion polls only.

In the foreword of the report of the European Commission on the EC-sponsored research on safety of GMOs published in 2001, J.E. Beringer cited some comments of opponents of GMOs: 'GMOs are unsafe and must never be released into the environment.' 'We don't know enough about GMOs to risk releasing them – what is being done about this?' 'Why doesn't someone do something to understand what the risks of GMOs are?' According to Beringer, 'they have encouraged and exploited public unease very effectively because most people are unaware that biosafety research is being done and, with the exception of GM vaccines and other medical uses, there has been very little direct public benefit to counteract perceived risks.

In this earlier publication, Beringer stated that 'a decade of research has been done and millions of Euros have been spent, but the anti-GM lobby's agenda has hardly changed at all. They profess still to be concerned that we have insufficient knowledge and that no GMO should be released until we can predict with certainty what it, and the cloned genes within it, will do in the environment.' How far are we ten years later? Still, the results and even the existence of GMO biosafety research are often ignored in the public debate on the biosafety of GMOs. As a consequence, the already established strong basis for a science-based discussion on GMO biosafety is not fully explored in Europe or worldwide. In line with the complex public debate on the use of genetic engineering in agriculture and food production, the European Commission has been funding projects supporting science-based political decisions and improving the communication on 'green genetic engineering'. The four projects described in this chapter supported a European initiative to enhance communication regarding GMO biosafety research (GMO RES COM), established a Biosafety Research Communication Network (BIOSAFENET), performed a pilot study on innovative approaches to public communication of life sciences and biotechnology by students and young researchers (BIOPOP), and provided a GMO communication and safety evaluation platform (GMO-COMPASS).

Although the EC and the EU Member States have invested considerable means in research on the potential impact of GMOs, it is clear that these research activities need to be accompanied by greater efforts to improve communication, both within the scientific community concerned, and between scientists and other stakeholders. Therefore, the project GMO RES COM supported the participation of EU scientists in the biennial International Symposia on the Biosafety of GMOs (ISBGMOs), launched an international peer-reviewed scientific journal entirely devoted to GMO biosafety research, and created a database of GMO biosafety research projects. For these three communication tools regarding GMO biosafety research, efforts were made to ensure that they will continue to function in the future. In the case of the ISBGMOs, the symposium series is clearly well established, and there can be no doubt about their long-term continuation. Concerning the peer-reviewed scientific journal on Environmental Biosafety Research (EBR), there is also little reason to fear for the journal's future, since the number of manuscripts submitted has increased significantly since its first years. The future of the Biosafety Research Database is also assured, since, under the name 'biosafe.res database', it is now part of the toolkit of web-based GMO biosafety resources of the International Centre of Genetic Engineering and Biotechnology (ICGEB).

The major aim of the BIOSAFENET project was to promote scientific involvement and its broader perception in discussions of, and decision making in, the biosafety of GMOs. Therefore, the activities covered information tools, scientific meetings and logistic support for networking, also strengthening the position of European researchers in the international arena by exploiting platforms provided by the International Society for Biosafety Research (ISBR). To achieve this, the active participation of European scientists at two ISBGMOs, which are the only internationally recognized symposia in this important field of research, and networking activities of Scientists in the New Member States and associated countries were supported. Besides the direct support of networking, additional communication tools enhanced the flow of information on biosafety research results. Target groups were scientists, regulators, decision makers, the media and the broader public. These activities addressed specific demands of each group, from information about scientific projects, compilation of knowledge for the evaluation of critical (up-coming) biosafety issues and presentations of results from biosafety research to the general public.

The project BIOPOP was a mixture of the innovative ideas and the enthusiasm coming from young scientists, aiming at setting a new format in science communication and public participation, by creating a special environment where the next generation of scientists and citizens can actually meet, and where they can effectively establish a dialogue and a long-lasting communication. The entire project was developed in close contact with professionals from the media. A complex evaluation system was designed to assess and follow-up the interaction established between the young scientists and the public and especially to understand the impact of the new communication model on both the citizens and the researchers.

The GMO-COMPASS project demonstrated its ability to support Europeans in understanding GMO policy and regulations and to inform them of new developments, benefits and the public debate on GMO issues. The consumer has been reached by offering science-based information aligned with the expectations of laypeople and embedded in a balanced reporting including counter-expertise and views of non-scientists. Thus, GMO-COMPASS successfully complemented the existing information supply of regulators, policy developers, lobby groups and scientific institutions. The broad interest in the online discourse shows that such tools are highly suitable for fostering a serious public debate on GMO issues within Europe.

In line with the complexity of the public debate on biotechnology, especially on the use of genetic engineering in agriculture and food production, the European Commission has not only funded projects dedicated to biosafety research as a prerequisite for competent risk assessment and risk management, but also projects supporting science-based decision-making as well as improving communication on 'green genetic engineering'. The know-how developed in the EU during the last ten years could result in substantial benefits for the environment, consumers and societies at large. In addition, it strengthened the role of European scientists and regulators in the international debate on the safety of GM plants as well as the coexistence of GM and non-GM agricultural supply chains and supported building bridges between different interest groups including those that normally are not involved in the scientific process.

Virus-resistant transgenic plants: ecological impact of gene flow

Acronym
VRTP IMPACT

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-2000-00361

Period
December 2001 – March 2004

Coordinator
Mark TEPFER
Laboratoire de Biologie Cellulaire
INRA-Versailles
F-78026 Versailles cedex
France
mark.tepfer@versailles.inra.fr

Project website
http://www.inra.fr/vrtp-impact/

Background and objectives

Virus resistance was among the very first agronomically useful traits to be introduced into plants by genetic transformation, and several virus-resistant transgenic cultivars have already been commercially released in the USA. In order to provide the necessary science-based risk assessment of such plants before contemplating commercial release in Europe, it is of use to clarify several points concerning potential ecological impact. The most important potential impacts could result from two forms of gene flow, either from plant to virus by recombination, or from plant to plant by sexual outcrossing.

Approach and methodology

VRTP IMPACT addressed these two outstanding questions concerning the potential ecological impact of virus-resistant transgenic plants:

- will recombination with an infecting virus lead to the creation of novel virus genomes?
- will transmission of a virus resistance gene to wild relatives of the modified crop species confer a fitness advantage on the wild plants?

In the studies of recombinational plant-to-virus gene flow, the particular targets were two groups of important plant viruses, the cucumoviruses and the potyviruses. The plant-to-plant gene flow studies were carried out with two major crop plants that are known to be able to outcross with wild relatives, beet and oilseed rape. Since it is known that gene flow between the crop and wild relatives can occur with these species, the work focused not on the incidence of gene flow, but on its outcome. In all cases, potential impact was assessed relative to studies of these two forms of gene flow in the absence of transgenic plants.

Main findings and outcome

There is considerable evolutionary evidence for recombination between related viruses that have hosts in common. Thus, only if recombinants are observed in transgenic plants that are different from those in non-transgenic plants will there be any reason for concern about recombination in transgenic plants being a source for emergence of novel virus genomes. In order to compare the recombinant viruses appearing in transgenic and non-transgenic plants, extremely sensitive molecular tools were developed for detection of recombinant viral genomes, even when they represent extremely minor parts of the population of viral RNAs. They were applied to both doubly-infected non-transgenic plants, and to singly-infected transgenic plants expressing a viral coat protein gene. These studies have made it possible to characterise in detail the recombinant cucumoviruses present in plants infected with Cucumber mosaic virus and the closely related *Tomato aspermy virus*. This analysis of recombination sites on RNA3 in plants infected by two cucumoviruses has made it possible to identify a variety of hot spots for recombination. This provided the essential baseline information with which the study of recombination in transgenic plants expressing a coat protein gene was compared, in order to determine if novel recombinant viruses occur in the transgenic plants. Studies of the biological properties of the recombinant viruses observed has shown that the majority of them are deficient to varying degrees, and would not be expected to survive in nature.

One of the key limiting factors in virus recombination is that the viral RNAs must be in the same cells at the moment that viral RNAs are replicated. In order to determine the extent to which two related viruses cohabit the same cells in plants infected with both viruses, novel viral genomes encoding an additional fluorescent protein were created. When this strategy was applied to potyviruses (*Plum pox virus* and *Tobacco vein mottling virus*), a novel cellular exclusion phenomenon was observed; related viruses were present only in a small subfraction of infected cells, at the interface between patches of cells infected with only one virus. Since in principle the viral transgene sequences that could recombine are present in all infected cells in transgenic plants expressing such sequences, the likelihood of virus cohabitation at the cell level in non-transgenic plants is a key piece of baseline information.

Partners

Mireille JACQUEMOND
INRA, Avignon (FR)

René DELON
Institut du Tabac, Bergerac (FR)

Ervin BALÁZS
Agricultural Biotechnology Center, Gödöllö (HU)

Edgar MAISS
University Hannover, Hannover (DE)

Detlef BARTSCH
Aachen University of Technology RWTH, Aachen (DE)

Fernando García ARENAL
ETSI Agronomos Ciudad Universitaria, Madrid (ES)

Peter Palukaitis
SCRI, Dundee, Scotland (UK)

Ian COOPER
NERC, Oxford (UK)

Erkki TRUVE
NICPB, Tallinn (EE)

Little is known about the prevalence of recombinant viral genomes in nature. This is why molecular studies of viruses found in the field were carried out. Viruses that have resulted from recombination between two strains of either *Cucumber mosaic virus* or of the potyvirus *Watermelon mosaic virus* were found to be clearly part of natural virus populations. This confirms that recombination is a normal occurrence, and that recombinant viruses are part of the natural variability of virus populations.

Plants encounter numerous types of stress that can affect their ability to grow and reproduce. Virus infection can be considered to be an important biological source of biotic stress. This is why understanding the impact of virus infection on plant fitness (growth and reproduction) is important in evaluating the potential impact of a virus resistance gene on wild relatives of virus-resistant crops. The work carried out suggests that virus infection can affect the fitness of wild beet (the same species as sugar beet), and certain wild *Brassica* (close relatives of oilseed rape). Although this suggests that the transmission of a virus-resistance gene from transgenic or non-transgenic crops to their wild relatives can affect their fitness, the results also showed that the impact on fitness is highly dependent on the genotypes of both the host plants and viruses, as well as on the environment.

Conclusions

The two key biosafety issues regarding virus-resistant transgenic plants are the impact of plant-to-virus gene flow by recombination and plant-to-plant gene flow by outcrossing. Based on their previous work, using approaches that include laboratory, glasshouse and field studies, the participants in the VRTP IMPACT project have made considerable progress in providing the knowledge that will allow assessment of these two potential risks. Additional work presently underway in these labs will further clarify the impact of these two forms of gene flow.

Fig 1.

Expression of a viral coat protein transgene confers virus resistance. The non-transgenic susceptible tobacco plant (right) showns typical mosaic symptoms when infected with Potato virus Y. The plant expressing the coat protein of Lettuce mosaic virus (left) is resistant to Potato virus Y.

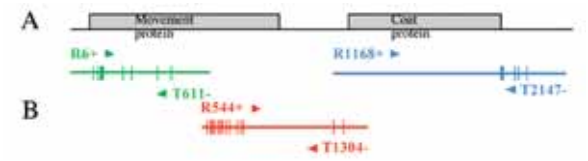


Fig 2.

When non-transgenic plants are infected with both Cucumber mosaic virus and the related Tomato aspermy virus, recombined RNA 3 molecules can be detected by RT-PCR. A: the structure of RNA 3, showing the position of the movement protein and coat protein genes. B: sites of recombination, shown by vertical bars on the horizontal lines representing the positions of the RT-PCR amplification products. (de Wispelaere et al., 2005).

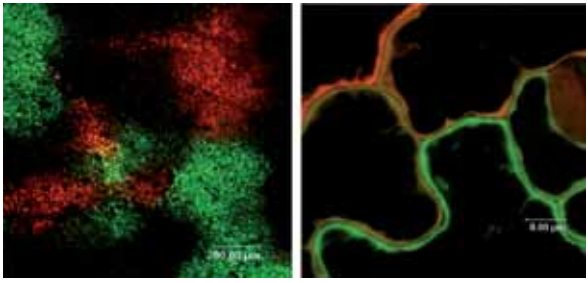


Fig 3.

When plants are simultaneously infected with Plum pox virus expressing either a GFP or DsRed gene, the viruses infect separate patches of cells, which appear green or red in confocal microscopy (left). Where the patches are contiguous, only a few cells are infected with both viruses (right). (Dietrich and Maiss, 2003).

Major Publications

Bonnet J., Fraile A., Sacristán S., Malpica JM., García-Arenal F. (2005). Role of recombination in the evolution of natural populations of Cucumber mosaic virus, a tripartite plant virus. *Virology* 332:359-368.

Dietrich C., Maiss E. (2003). Fluorescent labelling reveals spatial separation of potyvirus populations in mixed infected *Nicotiana benthamiana* plants. *J. Gen. Virol.* 84: 2871–2876.

Pierrugues O., Guilbaud L., Fernandez-Delmond I., Fabre F., Tepfer M., Jacquemond M. (2007). Biological properties and relative fitness of inter-subgroup cucumber mosaic virus RNA3 recombinants produced in vitro. *J. Gen. Virol.* 88: 2852-2861.

Raybould A., Cooper I. (2005). Tiered tests to assess the environmental risk of fitness changes in hybrids between transgenic crops and wild relatives: the example of virus resistant *Brassica napus*. *Environ. Biosafety Res.* 4: 127-140.

Turturo C., Friscina A., Gaubert S., Jacquemond M., Thompson JR., Tepfer M. (2008). Evaluation of the potential risks associated with recombination in transgenic plants expressing viral sequences. *J. Gen. Virol.* 89: 327-335.

Results and perspectives on the coexistence and traceability of GM and non-GM supply chains

Acronym
CO-EXTRA

Programme Acronym
FP6-FOOD

Contract number
007158

Period
April 2005 – September 2009

Coordinator
Yves BERTEAU
Institut national de la Recherche Agronomique
147, rue de l'Université
F-75138 Paris
France
info@coextra.eu

Project website
http://www.coextra.eu/contactus.html

Background and objectives

Genetic engineering of plants was developed in 1983 by three different groups, one in Belgium, and two in the USA. Since that time, plant biotechnology has expanded enormously and is now the basis of a major multinational industry. Genetically modified plants for human consumption or animal feed are mainly grown in the USA and Canada, with increasing production in Brazil, Argentina and China. Europe cultivates only a small amount of GM crops (mainly GM maize grown in Spain), though this is likely to increase in the future.

Worldwide regulations on GMOs can mostly be divided into two completely different types. The first, typified by the USA, relies on the principle of substantial equivalence and asks the question whether or not GMO-derived products are substantially the same as their generally accepted non-GMO counterparts. If this is judged to be so, then little further regulation, other than the food safety requirement, is required. The second viewpoint, initiated by Europe, concentrates on the method of production, arguing that GMOs are produced by a different technology or production process, and thus require special regulation. Extensive EU legislation, including GMO detection, traceability and labelling, has been introduced to support this viewpoint. More than 40 countries (including 27 EU Member States) have introduced traceability and labelling regulations. These mainly follow either the EU or USA regulatory patterns and may differ according to whether labelling is mandatory or voluntary, and to whether there is a tolerated threshold for the adventitious presence of GMOs.

The European frame recognises both the freedom of production for producers and the right of consumers to choose the products they wish, be they issued from GMO, conventional or organic agriculture. Accordingly, coexistence of supply chains incorporating those products is one of the issues faced by the European agriculture and downstream chain within a frame of different constraints such as those of the WTO.

Several programmes have been launched into the FP5 and FP6 European research frames on traceability (QPCRGMOFodd, GMOCHIPS) and coexistence (SIGMEA, TRANSCONTAINER, CO-EXTRA). CO-EXTRA (contract 007158, www.coextra.eu) has been (2005 - 2009) the largest EC-funded project on coexistence and traceability of GM and non-GM supply chains.

Approach and methodology

The CO-EXTRA project addresses for the first time the whole issue of coexistence of GM and non-GM supply chains, examining the practices of supply chains from seed production to retailers' shelves with practical implementation tools such as documentary and analytical systems supporting the coexistence of supply chains. This coordinated and fruitful way of working was possible only due to the size of this kind of programme (Integrated project). In this way, the launch of such large research projects should be favoured; small and fragmented research projects should be avoided where possible.

Numerous results show that the coexistence of GM and non-GM supply chains, even at the farm level, cannot be addressed by studying separately the different components of these chains. Coexistence issues have to be addressed by multidisciplinary teams embracing supply chains as a whole.

CO-EXTRA conducted experimental work on corn, rapeseed, sunflower and tomato, as well studying pollen flow models over large fragmented landscapes.

Partners

Joachim SCHIEMANN
Federal Biological research Centre for Agriculture and Forestry, Braunschweig (DE)

Morten GYLLING
The Danish Research Institute of Food Economics, Frederiksberg (DK)

Julian KINDERLERER
The University of Sheffield (UK)

Stephen LANGRELL
Joint Research Center, Ispra (IT)

Christina GRUDEN
National Institute of Biology, Ljubljana (SI)

Arne HOLST-JENSEN
National Veterinary Institute, Oslo (NO)

Ivan ATANASSOV
AgroBioInstitute, Sofia (BG)

Esther KOK
Rikilt Institute of Food Safety, Rikilt (NL)

Kristina SINEMUS
Genius Biotechnologie GmbH, Darmstadt (DE)

Hermann BROLL
Bundesinstitut fur Risikobewerbung, Berlin (DE)

Steven BENTLEY
NIAB, Cambridge (UK)

William MOENS
Scientific Institute of Public Health, Brussels (BE)

Ann VAN GYSEL
Flanders Interuniversity, Institute of Biotechnology, Gent (BE)

Klaus MENRAD
University of Applied Sciences of Weihenstephan, Freising (DE)

Matthias STOLZE
Forschungsinstitut fur biologischen Landbau, Frick (CH)

Pere PUIGDOMÈNECH
Consejo Superior de Investigaciones Científicas NC, Madrid (ES)

Partners

Mia EECKHOUT
Centre for Agricultural Research, Gent (BE)

Jose M. GIL
CREDA, Barcelona (ES)

Peter BRODMAN
Biolytix AG, Witterswill (CH)

Andreas WURZ
Geescan Analytics GmbH, Freiburg (DE)

Christine HENRY
Central Science Laboratory, Defra (UK)

Gilbert BERBEN
Centre Wallon de Recherches agronomiques, Gembloux (BE)

Patrick PHILIPP
Laboratoire de la Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes de Strasbourg, Illkirch-Graffenstaden (FR)

Laurence TISI
Lumora Ltd, Cambridge (UK)

Askild HOLCK
Matforsk AS, Norwegian Food Research Institute Ltd, Aas (NO)

David ZHANG
Groupement d'intérêt public, Groupe d'Etudes et de contrôle des Variétés et des Semences, Guyancourt (FR)

Christophe VAN HUFFLE
Eppendorf Array Technologies SA, Namur (BE)

Giorgos SAKELLARIS
National Hellenic Research Foundation, Athens (EL)

Jorge ARENAS VIDAL
Vitalia Consulting S.L., Madrid (ES)

Jan WISSE
Schuttelaar &Partners Ltd, The Hague (NL)

Xavier PINOCHET
Centre Technique Interprofessionnel des oléagineux métropolitains, Paris (FR)

Main findings and outcome

Biocontainment techniques such as corn cytoplasmic sterility and rapeseed cleistogamy might be used for reducing pollen flow and thus facilitating coexistence at field level. The final admixture rate is dependent on conditions and the varieties used. Accordingly, the long-term accuracy and stability (for instance some corn CMS types are also highly susceptible to plant disease) of those biocontainment systems should be reassessed or, in some instances, still have to be developed. In the event that a full containment of plants is required, particularly when considering plants dedicated to non-food/non-feed GMO cropping, stacking of biocontainment measures will be necessary. Plastid transformation could be used for biocontainment, but will impact on analytical methods. However, the question of compatibility between the duration necessary for the development of varieties integrating biocontainment and the schedule of European coexistence implementation has to be raised. Moreover, the commercial availability of such biocontainment methods, already used for producing hybrids, is questionable.

The partners confirmed earlier observations that stakeholders are using a practical contractual threshold of ca. 0.1 %, well below the 0.9 % European labelling threshold. This practical threshold was explained by the sampling (no consensus sampling plan available) and measurement (the inter-laboratories variability is ca. 200 % around a value). Using practical thresholds below a quality or safety threshold is a very common practice in all sectors dealing with a threshold. This practical threshold is easily reached in third countries with large fields. Its sustainability in small-to-medium European fields will depend on future GMO pressure in the EU. The observed pollen dissemination over large distance, and sampling and measurement uncertainties, imply that the coexistence of open pollinated crops is only possible by using either large distance of isolation or production of GM and non-GM products in dedicated areas, as determined by the models developed in the EC-funded SIGMEA project. The technical and legal definitions of such dedicated production areas remain open.

In the absence of pollen dissemination, the final GMO content is dependant on the initial GM seeds. Seeds purity is thus a crucial basic factor of coexistence. Any seed threshold should be largely lower than the labelling to leave enough leeway to make coexistence possible at the field level. However

gene stacking, a growing trend in GMO production, will reduce by the additional content of GM DNA the ability of farmers to comply with downstream supply chain requests. Finally, coexistence cannot exist without sustainable availability of low-cost non-GM seeds incoorporating the latest genetic improvements. Farmers using farm-saved seeds should benefit from the same protection as seeds producers under contracts with seed companies. In all cases, good information systems between farmers should be in place.

Supply chain coexistence on downstream farms is not a new issue, as segregation between different products, such as waxy corn or erucic rapeseeds, is already in place. However, there is little experience in the EU of coexistence between GM and non-GM products. Empirical studies of several supply chains were carried out on corn, wheat, soybean and rapeseed to determine the critical points and develop supply chain management models. Elevators were identified as the main source of unintended admixture. Where GM and non-GM coexist, strategies for handling coexistence are different between food and feed sectors, due to the current absence of labelling products issued from animals fed with GMOs.

CO-EXTRA underlined several basic economic and legal facts. In particular, co-existence cannot exist without an economic valorisation of the whole supply chain which could imply, for instance, labelling of animals fed with and/or without GMOs.

Three kinds of segregation strategies are possible: plant specialisation, production line specialisation and time specialisation. Due to the differences in companies' size and structure, the choice of a specific strategy should be taken on a case-by-case basis and will probably be driven by market demand. A modelling approach of vertical relationships along the supply chain was carried out (i) to represent current strategies, (ii) to identify changes in context and mitigation measures that would make it easier to handle GM/non-GM segregation and (iii) to discuss such models or derived decision-support systems with stakeholders. Such models can also be combined with models of farm outcomes such as MAPOD, a spatially explicit gene-flow model. The generic model considers admixture risks between GM and non-GM products and the resulting consequences, in terms of product compliance with a regulatory labelling threshold. The simulation model, based on the example of the starch corn supply chain, simulates physical flows from fields to processing. It uses three kinds of batch controls: simple traceability, automatic downgrading and PCR testing.

Partners

Daniel BLOC
ARVALIS-Institut du végétal, Paris (FR)

Peter SAMP
Swiss Federal Institute of Technology, Zurich (CH)

Roger JAQUIERY
Delley Samen und Pflanzen AG, Delley (CH)

Boy FEIL
Agro-Projektmanagement Dr Feil, Kronprinzenkoog (DE)

Ralph BOCK
Max Planck Society for the Advancement of Science, Munchen (DE)

Marie Angèle HERMITTE
Centre national de la recherche scientifique, Paris (FR)

Neil HARRIS
LGC Limited, Teddington (UK)

Nelson MARMIROLLI
Universita di Parma (IT)

Marina MIRAGLIA
Istituto superiore di Sanita, Roma (IT)

Vladimir MEGLIC
Agricultural Institute of Slovenia, Ljubljana (SI)

Bao DIEP
INRA Transfert SA, Paris (FR)

Andreas WÄSCHE
Fraunhofer Gesellschaft zur Förderung der angewandten Forschung, München (DE)

Paola VECCHIA
Centro Ricerche Produzioni Animali CRPA SpA, Reggio Emilia (IT)

Huguette NICOD
ADRIANT SAS, Nantes (FR)

Konstantin SKRYAGIN
Center Bioengineering RAS, Moscow (RU)

Norma Ana PENSEL
Instituto Nacional de Tecnologie Agropecuária, Buenos Aires (AR)

Victor PELAEZ ALVARES
Instituto Tecnologia do Paraná, Curitiba (BR)

Conclusions

Results show there is a threshold effect and that, from an economic viewpoint, there is a tradeoff between the level of compliance of the final product and the number of downgraded non-GM batches. This tradeoff depends on both the penalty incurred as a consequence of non-compliance and the non-GM premium in the marketplace. Downstream farm operators use stewardship, for instance sampling methods, involving practical contractual thresholds of GMO content, for instance through GAFTA agreement, independent of national legislations. The practical threshold of ca 0.1 % used in these stewardships drastically impacts field outcome content and production organisation, but also the value of the future European seed thresholds for fortuitous and technically unavoidable content of approved GMO.

The CO-EXTRA project's empirical and modelling results can apply to most supply-chains with quality and/or safety requirements. The methods, strategies, tools, models developed in the project for GM and non-GM supply chain co-existence and traceability will be used in the management of numerous other supply chains, value-added and niche markets, and for detecting and excluding harmful products such as allergens and mycotoxin-producing organisms or pathogens. Traceability (on both analytical and documentary viewpoints) is a major segregation tool for coexistence. Documentary traceability is very largely used by companies and only a few controls (analytical traceability) are effectively carried out.

Traceability has been studied from a regulatory viewpoint and also for its economic and social function: allowing trust to be established between actors and on activities posing risks on admixture. CO-EXTRA showed that, at the intersection of knowledge and risk, legal systems are trying to establish confidence in a society that links the two.

Several general societal questions can be raised from such facts or from basic questions reminded by CO-EXTRA, for instance should the sustainability of non-GM seeds only be market-driven or should public institutes be involved in non-GM varieties production? The same may apply to the availability of biocontainment methods, which may appear necessary, probably in a stacked way, for increasing the security of field co-existence but are all owned by companies and will probably not be easily made available to farmers, except in a few cases such as containment of small scale fields devoted to non-food production such as pharmaceuticals. Other general societal questions or proposals have been made by CO-EXTRA such as extending liability without fault and compensation schemes, already in place for farmers in some Member States, to the whole supply chains thus downstream from the farms.

The putative costs of coexistence measures have to be quantitatively and accurately measured and their distribution assessed to impede unfair charging to some supply chains and consequently to consumers. Cost benefits of supply chain coexistence and traceability should be better assessed by taking into account the application of European Directives and Regulations impacting on coexistence and traceability. Generally speaking, the socio-economic aspects of coexistence, from seeds to shelves, need to be better assessed.

The detection methods for analytically tracing GMO are more and more accurate and able to provide reliable information to end-users and consumers. Due to the development of numerous multiplexed detection techniques and the important use of documentary traceability by operators, the economic impact of analytical controls is estimated not to increase final costs. However these improvements do not solve the inter-laboratories reproducibility issues calculated as being between 50 and 200 % of calculated sample GMO content, depending on sample grinding, DNA extraction and the PCR detection methods used, as well as staff training. This inter-laboratories reproducibility range explains in part, with sampling issues, the difference observed between the practical contractual thresholds (ca 0.1 %) used by operators and the labelling threshold of 0.9 %.

Some issues – such as (i) how to deal with 'botanical impurities' in routine analyses (in relation to LLP issues) and (ii) how technically, economically and legally to manage coexistence in the field with large isolation distance or dedicated production areas – are still pending and should be further researched from the scientific, technical, economic and legal standpoints. CO-EXTRA is thus recommending:

- that such large integrated research work on supply chain coexistence should be continued as all coexistence issues are interrelated and cannot be addressed separately;
- such integrated work on coexistence and traceability should embrace more global issues, not only EU-related ones;
- more generally speaking, coexistence and traceability of supply chains, from seeds to shelves, with less specific focus on the GM aspect be studied;
- coexistence at the field level integrates biotech and seeds area structures and strategies and their impacts on commercial availability of usable tools, such as biocontainment tools;
- greater in-depth study of the dispersal of viable pollen over large distances on fragmented landscape for several cropping plants, thus not restricted to the currently approved GM species (maize) and corresponding models:
- retrieval in a GIS-based (web-interfaced) central repository system, preferably operated by the European JRC, of all coexistence data, including organic farming sustainability in GMO production regions resulting from, for instance, cropping in Spain;
- the preparation of a GIS-based (web-interfaced) central repository system, preferably operated by the European JRC for Post-Market Environmental Monitoring, for both Case Specific Monitoring and General Surveillance, and able to integrate data and increase transparency for citizens;
- the ability to have either large isolation distances between GM and non-GM crops, or to develop dedicated production areas;
- the ability to maintain sources of non-GM seeds, integrating the latest genetic improvements;
- the ability to stack different biocontainment methods for both food- and feed-dedicated GMOs and GMOs developed for non-food purposes.

It is clear that it is necessary to rapidly determine future research, but probably mostly expertise frames from the fields for rapid implementation of coexistence from seeds to shelves. However, before launching new research or expertise actions, it would be helpful that Competent Authorities took decisions and make the framework less uncertain: such as determining the GMO fortuitous presence level(s) in seeds, guidance on individual vs regional coexistence measures and harmonization, provision for seed production, etc. Some other facts should also be considered such as the preferred hypothesis of dedicated production areas *versus* coexistence using large isolation distances, public funded tools to facilitate non-GM seed provision and maybe biocontainment publicly available methods before any additional work on coexistence is done. It means that is necessary that decisions and harmonisation of coexistence rules should be taken and proposed to Member States in order to restrict to a minimum the expertise or research fields to be further launched. Several decisions taken in advance by European Competent Authority should pave the way to coexistence in a rapid, time- as well as in a cost-efficient way. Keeping too many open issues clearly hampers the ability to rapidly find solutions to coexistence issues.

Acronym
GMO RES COM

Programme Acronym
FP5-LIFE QUALITY

Contract number
QLK3-CT-2001-30037

Period
March 2002 – February 2005

Coordinator
Mark TEPFER
ICGEB Biosafety Outstation
Via Piovega, 23
31056 Ca' Tron di Roncade (TV)
Italy
E-mail: Mark.Tepfer@icgeb.org

A European initiative to enhance communication regarding GMO biosafety research

Background and objectives

Although the European Commission (EC) and the EU Member States have invested considerable means in research on the potential impact of genetically modified organisms (GMOs), it is clear that these research activities need to be accompanied by greater efforts to improve communication, both within the scientific community concerned, and between scientists and other stakeholders. The objective of the GMO RES COM project was to contribute to improvement of communication in this area. This was implemented primarily through three tools: 1) support for participation of EU scientists in the biennial International Symposia on the Biosafety of GMOs (ISBGMOs), 2) launching an international peer-reviewed scientific journal entirely devoted to GMO biosafety research, and 3) creation of a database of GMO biosafety research projects.

Approach and methodology

The EC, along with the United States Department of Agriculture (USDA), was at the origin of the most important ongoing series of international conferences on GMO biosafety research, the International Symposia on the Biosafety of GMOs (ISBGMOs), which are now organized by the International Society for Biosafety Research (ISBR: http://www.isbr.info/). GMO RES COM has served to enhance the role of European scientists in the ISBGMOs that were held in 2002 and 2004.

As has been evident over the past decades, one of the particular features of GMO biosafety research is its highly interdisciplinary nature. Also, certain types of important research data, such as those showing no effect of a GMO, were difficult to publish in existing scientific journals. For these reasons, with the support of the GMO RES COM project, a new international interdisciplinary journal in this area was created, Environmental Biosafety Research (EBR: http://www.ebr-journal.org/).

It was also recognized that it was difficult to have an overview of the current state of GMO biosafety research. In addition to published volumes of project summaries, it was clearly desirable to set up a public-access web-based database that would present the current state of research in a dynamic fashion. This was also achieved by the GMO RES COM project.

Main findings and outcome

The International Symposium on the Biosafety of GMOs (ISBGMO)
Since the first in 1990, an ISBGMO has been held biennially, in order to address the scientific basis for biosafety issues associated with GMOs. The Symposium series is designed for scientists, policy makers, regulators, non-governmental organizations, and industry representatives involved in GMO development and relevant biosafety studies.

Participation in the 7th ISBGMO, 10-16 October 2002, Beijing, China
The 7th ISBGMO was held in China, in recognition of the increasingly important role that China is playing in GMO biosafety research. Thus it was important for the European research community to be well represented. GMO RES COM provided travel and subsistence support to all of the public sector European scientists who were members of the organizing committee, chaired a session, or presented a paper in a plenary session.

The presence of an important European delegation has also helped to ensure that European scientists continue to play a central role in the activities of the International Society for Biosafety Research (ISBR). For instance, thanks to the European delegation at the Beijing ISBGMO it was decided that the 8th Symposium would be held in France in 2004.

Organization of the 8th ISBGMO, 26-30 September, 2004, Montpellier France
The 8th Symposium, held 26-30 September 2004 in Montpellier, France, under the responsibility of the ISBR, was attended by approximately 250 participants. As during the previous editions, four days of the symposium were devoted to presentation of research covering selected major themes relevant to GMO biosafety, with a particular effort made to project from the current state of knowledge into the future. The entire symposium proceedings can be downloaded at the ISBR web site (http://www.isbr.info/). In addition to the usual four days of sessions, the 8th symposium included two novel elements. One of these was a special evening event for better contact between scientists and the public, which was in the form of a public presentation of the work of the symposium, including an opportunity to ask questions of participating scientists. The second was a special North-South workshop. Here the purpose was to mobilize the GMO biosafety research community to identify the GMO-related biosafety research

Partners

Ervin BALÁZS
Agricultural Research Institute, Martonvásár (HU)
Decio RIPANDELLI
Biosafety Unit, Trieste (IT)
Joachim SCHIEMANN
Inst. Plant Virology, Microbiology, Braunschweig (DE)

that countries in the South will need in order to evaluate the development of GMOs for their own uses, and to create better contacts between researchers in North and South.

Fig 1.

A poster used to advertise the 8th International Symposium on the Biosafety of GMOs, held in Montpellier (France), September 26-30, 2004, which was supported by the GMO RES COM project.

Environmental Biosafety Research (EBR)

A brief history of Environmental Biosafety Research

Environmental Biosafety Research (EBR), the official journal of the ISBR, was born directly from the ISBGMOs. The idea to launch a new journal reflecting the highly interdisciplinary nature of the symposia was first discussed at the 5th symposium (1998). This was followed by several years of discussion and planning, before the first issue of EBR was released at the end of 2002. The editorial office, was set up at INRA-Versailles, and the journal was run initially with three editors-in-chief, Mark Tepfer (France), Klaus Ammann (Switzerland) and David Andow (USA), and an editorial board composed of six European members, six from the USA, and three from developing countries. For further information, see http://www.ebr-journal.org/. Particularly during the first years of EBR, considerable effort was made to enhance its visibility. This included creating and distributing fliers at numerous scientific meetings, and a real effort was made to have the journal indexed in the most important online indexing services, such as the ISI Master Journal List and Medline.

Overview of the first years of publication of EBR

EBR has appeared quarterly since the end of 2002. As seen below, up to the end of 2004, a total of 49 articles were published, for a total page count of 575. In the years since the end of the GMO RES COM project, EBR has continued to publish a similar number of papers.

	2002 Volume 1 1 issue	2003 Volume 2 4 issues	2004 Volume 3 4 issues	Totals
Regular articles	4	9	17	30
Review articles	1	5	1	7
Editorials	2	3	2	7
Book reviews		1	3	4
Others		1		1
Totals	7	19	23	49

Thanks to the online version of EBR, the journal has a good level of perception by the scientific community. At last count, the most downloaded article had received more than 1 000 hits.

The GMO RES COM Biosafety Research Database

Creation of the GMO RES COM Biosafety Research Database

This database was designed to become an up-to-date source of information, fulfiling needs of several stakeholder groups. It facilitates creating new consortia of research groups for carrying out biosafety research is an excellent source of expertise in the various fields of GMO biosafety, and it also provides greatly enhanced public access to GMO biosafety research. The database created during the GMO RES COM project was further improved thanks to support by the BiosafeNet project (http://www.gmo- safety.eu/en/biosafenet_navigator/562.docu.html), and is presently housed at ICGEB (http://www.icgeb.org/~bsafesrv/).

Conclusions

As described above, the execution of the GMO RES COM project has resulted in the full development of tools for communication regarding GMO biosafety research. Efforts were made to ensure that they will continue to function in the future. In the case of the ISBGMOs, the symposium series is clearly well established, and there can be no doubt about their long-term continuation. Concerning EBR, there is also little reason to fear for the journal's future, since the number of manuscripts submitted has increased significantly since its first years. The future of the GMO RES COM Biosafety Research Database is also assured, since, under the name 'biosafe.res database', it is now part of the toolkit of web-based GMO biosafety resources of the ICGEB (http://www.icgeb.org/~bsafesrv/).

Fig 2.

Covers of the journal, Environmental Biosafety Research (EBR), launched with support from the GMO RES COM project.

Acronym
SCIENCE 4 BIOREG

Programme Acronym
FP6-FOOD

Contract number
043012

Period
November 2006 – January 2010

Coordinator
Dr Zuzana KULICHOVA
Delft University of Technology
Julianalaan 67
NL-2628BC Delft
The Netherlands
z.kulichova@tudelft.nl

Global involvement of public research scientists in regulations of biosafety and agricultural biotechnology

Background and objectives

The SCIENCE4BIOREG project addresses the growing gap between life sciences on the one hand and regulatory policies and public perceptions on the other.

Public research in the modern biotechnology field aims at solving constraints in agricultural production, healthcare and environmental protection. Developments in public-sector research in agricultural biotechnology are closely dependent on the design and implementation of the regulatory frameworks for genetically modified crops at the national, regional and global levels. Modern biotechnology can contribute to human wellbeing only if the regulatory frameworks are science-based, predictable, transparent and balanced. Not only the risks that may accompany any technological innovation need to be addressed, the benefits also need to be taken into account in ensuring good governance. To achieve this, it is crucial that policymakers, regulators and the general public are better informed on the objectives of and progress in the life sciences of agricultural biotechnology. Conversely, the public research sector itself needs to be informed about and involved in regulations relating to modern biotechnology and the implications for research, so as to be better aligned with broader policy developments relating to food security, environmental protection and sustainable development.

The objective of this project, focused on public-sector scientists working in biotechnology research, is to involve them in international negotiations and policy discussions that pertain to biotechnology. The SCIENCE4BIOREG project extends the activities of the Public Research and Regulation Initiative with the aim of ensuringe that the public research sector will be at least minimally represented at all relevant meetings.

Approach and methodology

Successful involvement of life scientists in negotiations and discussions on biosafety regulations requires that they have an understanding of a regulatory background. For this purpose the project management regularly organises regional preparatory meetings where scientists can learn about existing and planned international and regional regulations. In addition, scientists are supported in participating in international discussions and expressing their opinions on regulatory developments. The target regulations of the project include the Cartagena Protocol on Biosafety (CPB), the Convention of Biological Diversity (CBD), the Aarhus Convention, and EC Directives and Regulations.In addition to participation in and organisation of international meetings, the SCIENCE4BIOREG project informs governments, organisations and other stakeholders on ongoing public research in modern biotechnology and the concerns of public researchers regarding specific regulatory developments.

Partners

Kim MEULENBROEKS
Stichting Publiek Onderzoek en Regelgeving (Foundation for Public Research and Regulation), Delft (NL)

Pieter-Jan VAN DER MEER
Horizons sprl, Lasne (BE)

Sylvia Dina Frida BURSSENS
Ghent University, Ghent (BE)

David John BENNETT
Cambridge Biomedical Consultants Ltd, Cambridge (UK)

Monty P. JONES
Forum for Agricultural Research in Africa, London (UK)

Atanas ATANASSOV
Black Sea Biotechnology Association, Sofia (BG)

Yaroslav BLUME
Institute of Cell Biology and Genetic Engieering, Kiev (UA)

Desiree M. HAUTEA
University of the Philippines Los Baños, Laguna (PH)

Charles MUGOYA
Association for strengthening Agricultural research in East and Central Africa, Entebbe (UG)

Katharina KUMMER PEIRY
Kummer EcoConsult, Villar-sur-Glane (CH)

George SAKELLARIS
National Hellenic Research Foundation, Athens (EL)

Oda LEILA
Associação Nacional de Biossegurança, Rio de Janeiro (BR)

Joachim SCHIEMANN
Federal Biological Research Centre for Agriculture and Forestry, Braunschweig (DE)

Lionel GIL
Faculty of Medicine, University of Chile, ICBM, Santiago (CL)

Main findings and outcome

During the project duration the following outcomes were achieved:

- 40 public sector scientists from 21 countries participated in the Third Meeting of the Parties (MOP3) to the Cartagena Protocol on Biosafety;
- 40 public sector scientists from 21 countries participated in the Fourth Meeting of the Parties (MOP4) to the Cartagena Protocol on Biosafety;
- 10 public sector scientists participated in the 8th and 9th Conference of the Parties (COP8 and COP9) to the Convention on Biological Diversity;
- 10 public sector scientis ts participated in the Conferences of the Parties to the Aarhus Convention;
- numerous public sector scientists participated in the many intersessional preparatory meetings of all MOPs and COPs;
- public-sector scientists participated in the various meetings on biotechnology organised by the European Commission, Member States, EFSA, and other European Organisations as COST;
- six international and regional meetings were organised to inform public-sector scientists on existing and planned international and regional regulations pertaining to biotechnology;
- statements on specific items on the agendas of MOPs and COPs, such as risk assessment and socio-economic considerations, were prepared in consultation with PRRI members and submitted to the negotiating parties;
- the framework for an online database including public sector research in biotechnology worldwide was created;
- guidance materials for compliance with biosafety regulations were prepared and made available to public researchers;
- project activities were presented during international meetings;
- newsletters on the outcome of international meetings and negotiations relevant to biotechnology and biosafety were disseminated to public-sector scientists worldwide;
- letters explaining the relevance of public-sector research in modern biotechnology were sent to governments and international organisations.

Details of these activities can be found at www.pubresreg.org

Conclusions

Public researchers have become a well-known entity in the international arena and the roles of science in general, and of public research in particular, have been clearly established on negotiating agendas.

Experience from the project indicates that a continuous dialogue between life scientists and policymakers can contribute to the establishment of biotechnology regulatory frameworks. Transparent, science-based and predictable regulations are the first prerequisite for liberating the potential of modern biotechnology from research pipelines and delivering its benefits to farmers' fields.

Fig 1.

'Participants of the 3rd Meeting of the Parties to the Convention on Biological Diversity in Curitiba, Brasil, March 2006'.

Acronym
SAFE FOODS

Programme Acronym
FP6-FOOD

Contract number
506446

Period
April 2004 – June 2008

Coordinator
Dr Hans J.P. MARVIN
RIKILT – Institute of Food Safety
Akkermaalsbos 2
NL-6708 WB Wageningen
The Netherlands
hans.marvin@wur.nl

Project website
http://www.safefoods.nl/default.aspx

Promoting food safety through a new integrated risk analysis approach for foods

Background and objectives

The governance of food safety has long been regarded as the domain of 'experts' and professional risk managers, with minimal input from other interested parties, such as consumers. However, a number of food safety incidents in Europe (GMOs, BSE, dioxins...) have had a negative impact on public trust in food safety regulation and management and have exposed the need for improvements in the current approach to food risk analysis.

The EU project SAFE FOODS (2004 - 2008) aims to contribute to the restoration of consumer trust in the food chain through the development of a new integrated risk analysis approach for foods. Combining the skills of over 100 natural and social scientists, coming from 37 institutions in 21 countries, the project is integrating a broad range of disciplines to refine risk analysis practice for food safety.

The major objective is to develop an improved governance framework for foods produced by different agricultural and food processing methods and practices. The objective of this framework is to change the focus of decision-making on food safety from single risks to considering foods as baskets of risks, benefits and the costs that are associated with their production and consumption, taking into account the social context in which these decisions are made.

Questions that have been addressed are:

- the applicability of new informative profiling methods for identification of emerging risks in food production;
- how information about risk assessment should be communicated to the public, and how public concerns can be incorporated into this process;
- how effective communication and inclusive public participation in risk management and science and technology policy can be developed; and
- the role of institutions involved in risk assessment and management in the light of a broader risk analysis framework taking into account socio-economical risk-benefit issues and the consequences of introducing foods and new production methods.

The major outcome of the project is a new risk analysis approach for foods that integrates assessment of human health aspects, consumer preferences and values, as well as impact analysis of socio-economic aspects. The strengths of the model are the transparent and novel method of risk identification and assessment, and the inclusive approach to risk management with active involvement of all stakeholders, taking a broad range of ethical, social and economic factors into account.

Approach and methodology

SAFE FOODS consists of a number of research projects (work packages) with a high degree of coherence bringing together multidisciplinary teams of dedicated experts from academia in many EU countries, including the new Member States:

1. comparative Safety Evaluation of Breeding Approaches and Production Practices Deploying High- and Low Input Systems;
2. early Detection of Emerging Risks Associated with Food and Feed Production;
3. quantitative Risk Assessment of Combined Exposure to Food Contaminants and Natural Toxins;
4. consumer Confidence in Risk Analysis Practices Regarding Novel and Conventional Foods;
5. investigation of the Role of Regulatory Institutions in Risk Management;
6. design of a New Integrated Risk Analysis Approach for Foods.

WP1. The primary objective of Work Package 1 was to develop comparative safety assessment methods for foods produced by different breeding approaches and production practices. The methods selected were designed to facilitate large-scale analysis of gene expression, protein expression and metabolite content using transcriptomic, proteomic and metabolomic techniques linked to relevant frameworks for the statistical analysis of data. These methods were used to assess sources of compositional variation in maize kernels and potato tubers, as examples of important European crops. To do so effectively required that field trials were performed and analysed over four growing seasons. In total more than 3 000 samples were analysed.

Partners

Dr Esther KOK MSC
DLO-RIKILT – Institute of Food Safety, Wageningen (NL)

Prof. Howard Davies SCOTTISH
Crop Research Institute, Dundee (UK)

Prof. Karl HEINZ ENGEL
Technical University Munich (DE)

Prof. Sirpa KÄRENLAMPI
University of Kuopio (FI)

Dr. Ewa ZIMNOCH–GUZOWSKA
Plant Breeding and Acclimatization Institute, Blonie (PL)

Dr. Marina MIRAGLIA
National Institute of Health, Rome (IT)

Prof. Jane MORRIS
Council for Scientific and Industrial Research, Pretoria (ZA)

Dr. Jim MCNICOL
Biomathematics and Statistics Scotland Research Institution, Edinburgh (UK)

Prof. Yu LI
Institute of Crop Germplasm Resources, Beijing (CN)

Dr. Declan BOLTON
National Food Center, Dublin (IE)

Prof. Aldo PRANDINI
Catholic University of Piacenza, Milano (IT)

Dr. Olga ORLOVA
Latvian Food Center, Riga (LT)

Prof. Diána BÁNÁTI
Central Food Research Institute, Budapest (HU)

Dr. Peter BOS
National Institute of Public Health and the Environment, Bilthoven (NL)

Dr. Juliane BRAEUNIG
Federal Institute for Risk Assessment, Berlin (DE)

Dr. Beat BRÜSCHWEILER
Swiss Federal Office of Public Health, Bern (CH)

Partners

Dr. Leif BUSK
National Food Administration, Uppsala (SE)

Prof. Yongning WU
National Institute of Nutrition and Food Safety, Beijing (CN)

Dr. Ib KNUDSEN
Danish Institute for Food and Veterinary Research, Soeborg (DK)

Dr. Jiri RUPRICH
National Institute of Public Health, Prague (CZ)

Prof. Lynn FREWER
Wageningen University (NL)

Dr. Gene ROWE
Institute of Food Research, Norwich (UK)

Prof. Peter SANDOE
Royal Veterinary and Agricultural University, Frederiksberg (DK)

Prof. Ortwin RENN
DIALOGIK gGmbH, Stuttgart (DE)

Dr. George CHRYSSOICHOIDIS
Agricultural University of Athens (EL)

Dr. Andy STIRLING
University of Sussex, Brighton (UK)

Prof. Ellen VOS
University of Maastricht (NL)

Dr. Asa BOHOLM
University of Göteborg (SE)

Prof. Ragnar LÖFSTEDT
King's College London (UK)

Dr. Anna VÁRI
Institute of Sociology at the Hungarian Academy of Sciences, Budapest (HU)

Prof. Vittorio SANTANIELLO
Centre for International Studies on Economic Growth (CEIS), University of Rome (IT)

Mrs. Laura SMILLIE
European Food Information Council, Brussels (BE)

Prof. Wout SLOB
Institute for Risk Assessment Sciences, Utrecht (NL)

WP2. This Work Package's aim was to explore and develop methods and tools for the early identification of emerging hazards, with particular focus on issues that may be associated with agricultural production methods, namely high- or low-input agricultural production systems. The following techniques were used: review of existing early warning systems and methods; review of literature on emergence of microbial and chemical food safety hazards; establishment of a transfer point where experts can exchange information and of an EU-wide expert database; review of the potential linkage between food safety and climate change on a global and European scale, providinge capacity building for European risk assessors and risk managers on the issue of emerging hazard identification.

WP3. This Work Package had three main aims:

- development of an electronic platform of food consumption and residue databases, all linked to probabilistic software via the Internet. This platform will facilitate pan-European modelling of exposure in which national and international exposure calculations are performed in a harmonised way;
- development of a probabilistic integrated risk model in which exposure to compounds via food is directly linked to information on possible detrimental health effects. In this way, more refined and realistic quantitative risk assessments can be performed, compared to the methods currently applied;
- the use of this model in situations where consumers are exposed simultaneously to more than one chemical and in which the risk manager has either to balance the effect of the risk (or benefit) of one compound against another or consider cumulative effects of compounds with the same mode of action.

WP4. The main purpose of this Work Package was to understand food risk management perceptions in Europe. A series of studies was conducted in various EU Member States which attempted to identify the psychological determinants of good institutional food risk management. The perceived effectiveness of food risk management practices was explored in a qualitative study using focus groups comprising members of the public and food safety experts. The foundations for a model of the underlying psychological factors that affect consumer confidence in food risk management practices were provided by the results of these explanatory studies, which

were tested quantitatively using structural equation modelling. The quantitative study involved an Internet-based survey conducted in four European Member States – Germany, Greece, Denmark and the UK – and a telephone survey in Slovenia. In order to validate the model of consumer perceptions of food risk management quality, a series of case study interviews was conducted to provide 'proof of principles' against past food safety incidents.

WP5. The main objective of this Work Package was to outline and explore some of the major challenges for EU food safety governance and relate them to potential procedural and institutional responses. The first phase of the research work was devoted to investigating some of the major recent institutional rearrangements and procedural reforms in European food safety regulation and to outlining the legal and policy bases for these changes and reform efforts. The second phase involved identifying certain issues that emerge as essential to the task of changing food safety governance for the better and to suggest ways of addressing these issues. This was done by referring to the imperatives identified in the major legal and policy documents and as emphasised by key stakeholders in the field on the basis of experience since the changes were introduced.

WP6. One of the main objectives of this Work Package was to integrate the outcomes of the other Work Packages (1-5) into the new integrated SAFE FOODS risk analysis framework. Besides the innovations in risk assessment that were realised in Work Packages 1, 2, and 3, the new approach took stock of the recommendations for improved risk communication and institutional organisation on food safety governance that had resulted from the research of Work Packages 4 and 5 respectively. The new risk analysis framework aims at enhancing the transparency of the risk analysis procedure, as well as ensuring stakeholder involvement. Besides risks to human health associated with food consumption, this Work Package explored the possibility of assessing the associated benefits and of including socio-economic factors such as the social, economic, and environmental impacts, as well as the ethical implications, in this assessment. Retrospective case studies helped determine the added value of an improved approach to risk analysis. A discussion document describing the new framework was submitted to a broad range of stakeholders during two workshops, in order to obtain feedback which was then used to refine the approach further.

Main findings and outcome

Innovative profiling technique. In recent decades, producers have been experimenting with different methods (e.g. GM, conventional, organic) to ensure staple crops, but risk assessors have had a limited understanding of how the different cultivation methods affect crop composition. Using an innovative 'omics' technique, SAFE FOODS analysed the composition of potatoes and maize of different genetic backgrounds and grown under different production practices. The project found that 'omics' technology can allow scientists to do unprecedented analysis of crop composition by measuring thousands of parameters at once (including genes, proteins and metabolites). These capabilities allow scientists to determine whether inserting a new gene in a maize kernel changes its composition in an unintended way. Although 'omics' approaches are not currently required in risk assessments, evidence shows that the approach provides a much greater insight into crop composition than the tools now in use. The new technology is useful in cases where it is imperative for risk managers to understand how the crop's cultivation process changes the nutritional value of the food on a consumer's plate (e.g. nanotechnology, GM, organic).

Holistic approach to emerging risk. It is standard practice to track food and feed alerts and to maintain an early warning system which focuses on hazards. Today's early warning systems are largely reactive, catching the hazard after it has occurred. SAFE FOODS explored the potential of applying trend analysis to reports from the rapid alert system for food and feed, and of combining it with additional data on factors affecting the food and feed supply chain (e.g. size of farms, climate, imports/exports, and regulatory change). The project has identified case-specific and generic indicators and data sources for detecting microbial and chemical hazards, and will propose methods to utilise this information for early identification of food safety risks. This holistic approach, looking at the internal and external influences affecting the development of a hazard in the food chain, has the potential to help risk assessors identify emerging risks proactively. The importance of the growing number of global factors impacting on food safety has been recognised and the holistic approach is currently being explored in the Netherlands.

Probabilistic modelling tools. Countries collect data on what people eat and on the toxins to which people are exposed via food. SAFE FOODS explored the potential of using probabilistic modelling tools to combine consumption and food residue databases with the aim of allowing risk assessors to project the exposure of a population to food contaminants over a long period. Risk assessors can then forecast, for example, whether vegetarians have a higher exposure to pesticides than non-vegetarians and at what point their exposure reaches a critical level. This tool has the potential to facilitate a more realistic exposure assessment than current practices, because it allows risk assessors to identify vulnerable groups within a population.

European consumer views on risk analysis. Consumer perspectives of risk analysis have been explored previously at the national level, but the differences in questions and the methods used to gather responses have made it difficult to see how cultural values influence food risk perceptions. An understanding of how these cultural differences can influence risk analysis is of crucial importance in multicultural societies. SAFE FOODS bridged this gap by conducting a series of surveys, interviews and focus groups in EU Member States that were regionally representative. The project's consumer research provides a view of how European consumers would optimise the food risk analysis process.

Comparative analysis of food risk governance in Europe: Food risk governance is structured differently at national and European levels. For example, in the UK, risk management and risk assessment are dealt with by separate bodies, whereas in Hungary these responsibilities overlap. At the European level, where these tasks are separated, authorities involved in food safety recognise that they can send clearer messages to consumers by optimising collaboration. SAFE FOODS analysed the division of responsibilities for food risk governance in five Member States and at the European level. The project extracted best-practice examples from these country comparisons to contribute to the optimisation of interaction between risk managers and risk assessors, identifying when such interaction is helpful.

Fig 1.

The Work Package 2 training in Moscow, February 2008.

Fig 2.

Framework developed by SAFE FOODS.

A new integrated risk analysis framework for food safety. Further extending the risk analysis models previously developed by *Codex alimentarius* and the European Commission, SAFE FOODS developed a new integrated risk analysis framework, visualised as a cyclic process involving various stages including framing, assessment, evaluation, management (decision-making, implementation, and monitoring), and review (see Fig 2.). Novelties featured within this approach include the explicit distinction of framing and evaluation stages, increased stakeholder involvement, and the inclusion of other factors besides risks in the scientific assessment stage.

Conclusions

The common thread throughout SAFE FOODS' multidisciplinary research was to ensure that the findings contribute to moving the risk analysis model forward. The project translated the findings above into best-practice recommendations and used these recommendations to amend the classical risk analysis model.

The basic structure of the SAFE FOODS model builds on the three-phase structure developed by the FAO/WHO. It evolves the classical model into a five-stage process involving framing, risk-benefit assessment, evaluation, risk management, and review.

The main objectives in developing this framework for optimal food risk analysis were to:

1. take benefits into account whenever possible and explore ways of balancing risks and benefits;
2. broaden the aspects of risk to consider ethical, economic, social and environmental impacts; and
3. ensure formal stakeholders involvement.

Major Publications

Shepherd T., Dobson G., Verrall SR., Conner S., Griffiths DW., McNicol JW., Davies HV. & Stewart D. (2007). Potato metabolomics by GC-MS: what are the limiting factors? *Metabolomics* 3(4):475-488.

Special issue of *Food and Chemical Toxicology* Early Awareness of Emerging Risks to Food and Feed Safety, Volume 47, issue 5, May 2009; Guest Editors: Harry A. Kuiper and Gijs A. Kleter.

Special issue of *Food and Chemical Toxicology* Probabilistic risk assessment of dietary exposure to single and multiple pesticide residue or contaminants, Volume 47, December 2009, guest editors: Hans J.P. Marvin, Polly E. Boon and Andy Hart.

Van Dijk H., Houghton J., Van Kleef E., Van der Lans I., Rowe G., & Frewer L. (2008). Consumer responses to communication about food risk management. *Appetite*, 50(2-3),340-352.

Vos E. and Wendler F. (eds.), Food Safety Regulation in Europe. A Comparative Institutional Analysis, *Series Ius Commune*, Vol. 62, Intersentia, Antwerp, 469 p. (2006).

Special issue of Food Control featuring articles on the new integrated SAFE FOODS risk analysis model, of which the publication is foreseen for 2010; Guest Editors: Harry A. Kuiper and Gijs A. Kleter.

Acronym
CONSUMERCHOICE

Programme Acronym
FP6-FOOD

Contract number
518435

Period
May 2006 – October 2008

Coordinator
Dr Vivian MOSES
Strand
GB-London, WC2R 2LS
United Kingdom
v.moses@qmul.ac.uk

Do European consumers buy GMO foods?

Background and objectives

Following the 2004 adoption by the EU of compulsory labelling of all food products containing GM in any ingredient, it was uncertain how rapidly such products would appear on the shelves of retail grocery stores. It appeared that, at the end of 2005, labelled GM foods of one sort or another were on sale in various countries in Europe.

During the past decade there have been numerous debates and campaigns focusing on genetically modified crops and their food products. Several public opinion polls and focus groups exploring public attitudes to GM-containing food products showed that a majority of the European public was somewhat antipathetic to the technology, with views ranging from some who were vigorously opposed, a proportion enthusiastically in favour, while most people were essentially disinterested.

These surveys were hypothetical in asking 'what would you do if you had the opportunity of buying GM-products?' since it is questionable whether attitudes expressed can be taken as a proxy for action. There were a few small-scale experiments in which limited numbers of consumers were offered a choice between identical products with a GM label and without, but with a price differential in favour of the GM option. However, no exploration was made of what consumers actually choose when shopping for food in familiar stores offering food labelled as containing or being derived from GM ingredients.

The prime strategic objectives of these studies were therefore to:

- determine the discrepancy between measured attitudes of European consumers towards GM foods and their actual purchases when they were given the opportunity to choose between GM and non-GM;
- record GM products offered for sale, how customers are informed by labelling, price and supplementary information, and product position and prominence on the shelves;
- supplement the findings with specific opinion polls and focus groups;
- provide reliable evidence of genuine consumer GM food choices to food chain stakeholders in order to help them in their future planning.

The CONSUMERCHOICE project 'Do European consumers buy GM foods?' conducted a series of studies which included the exploration of purchasing choices in the Czech Republic, Estonia, Germany, Greece, The Netherlands, Poland, Slovenia, Spain, Sweden and the United Kingdom.

Approach and methodology

In order to make a general assessment of GM-labelled products on the market, the first study used a series of random shop visits to determine which supermarkets offered these products over an extended period. In parallel with these visits, a media analysis of GM issues was made to ensure a general understanding of the situation in each country, applying a commonly used method of content analysis to establish the tone of media coverage. This was supplemented by the identification of political viewpoints by tracking policy statements on GM issues of the political parties.

Two quantitative studies using questionnaires were also carried out to identify:

1. whether Polish and British citizens now living in the United States were aware that GM products do not have to be labelled in the US;
2. whether they were buying these products; and
3. how they felt about this situation.

A third quantitative study compared peoples' actual purchases with their perceptions and attitudes towards GM-labelled products. This was achieved by comparing purchase data of GM products with consumers' answers to a short questionnaire. This data was collected in identical ways in each country, using questionnaires translated into the national language using the services of the GfK (Growth for Knowledge) company. The questionnaire posed questions about respondents' level of knowledge on the labelling of GM products, and their perceptions and attitudes towards GM foods. The data was interpreted using Statistical Package for Social Sciences (SPSS) software, version 16. Chi-square tests were used to compare answers given by buyers and related non-buyers.

Finally, a qualitative study was carried out in four European countries: Sweden, the United Kingdom, Spain and The Netherlands. This was done through focus group discussions, using standardised guidelines for group size, set-up, questioning and interpretation. The study also further explored consumers' views on GM-labelled products and on their willingness to buy these products, together with the implicit value premises and assumptions underlying their arguments.

Partners

Siglinde FISCHER
BioAlliance AG, Frankfurt (DE)

Jaroslav DROBNIK
Association Biotrin, Prague (CZ)

Tomasz TWARDOWSKI
Agencya Edytor, Kiekrz (PL)

Raivo VOKK
Tallinn University of Technology, Department of Food Processing, Tallinn (EE)

Giorgos SAKELLARIS
National Hellenic Research Foundation, Athens (GR)

Peter RASPOR
University of Ljubljana, Biotechnical Faculty, Ljubljana (SI)

Victoria WIBECK
Linköping University, Linköping (SE)

Louis LEMKOW
Universitat Autonoma de Barcelona (ES)

Patricia OSSEWEIJER
Delft University of Technology (NL)

George GASKELL
London School of Economics and Political Science (UK)

Hugo SCHEPENS
EuropaBio, Brussels (BE)

Steve MARINKER
Citigate Dewe Rogerson Ltd, London (UK)

Barbara GALLANI
Bureau Européen des Unions de Consommateurs, Brussels (BE)

Main findings and outcome

During the period of the project the public debate on GM issues in Europe was generally relatively subdued, although markedly more active in some countries at particular times, such as in the UK in the summer of 2008 and in France earlier in the year. The tone of the media coverage changed during the period from negative-neutral to neutral-slightly positive.

The preparedness of supermarket managers to discuss the GM issue varied between individual companies as well as between countries. Most large supermarket chains were not willing to provide sales data on GM-labelled products. Small shopkeepers usually were unaware of the transgenic provenance of some of the products in their stores. However, none of them, large or small, reported any consumer reactions whatsoever.

We determined that GM-labelled products were on sale in Estonia, Poland, the Czech Republic, Spain, The Netherlands and the United Kingdom. In Slovenia, Greece, Germany and Sweden no GM-labelled products were found on the market during the period of the project. In those countries where GM-labelled foods were on sale, most were oils from GM soya or GM maize sold either as cooking oil or incorporated into other products such as margarine and crisps. We established that the number of GM-containing products on offer was considerably lower than before the introduction of the labelling regulation.

The results of the two questionnaires to Europeans living in the US showed that most of them (92 %) said they knew what GM food was and more than half were aware that unlabelled GM foods are for sale in the US. However this knowledge failed to prompt most of them (73 %) to make any effort to identify these products in order to avoid them.

For all the countries with GM-labelled products on sale, 75 % of respondents claimed to know that these have to be labelled by law. Nearly 60 % said they did not know how to distinguish a GM-containing product from a conventional one. Although not everyone read the detailed ingredients list before they bought a particular food item, 54.1 % of respondents said they did. There was no significant difference between buyers and non-buyers in the answers to these three questions. More than half the respondents said they were not careful in avoiding GM-labelled food.

Comparison of respondents' actual behaviour with their perceptions revealed no significant difference between buyers and non-buyers. Half the respondents (49.8 %) said they *did not* buy GM-labelled food. Interestingly, 48 % of GM buyers thought they *did not* buy GM-labelled food. Conversely, almost 23 % of non-buyers thought they *did* buy GM-labelled food. A remarkably high number of respondents (30 %) claimed not to know.

Focus group studies showed that GM food is not high in people's minds when discussing food purchasing habits. Labelling was demanded by participants, yet few of them actually looked at the labels when buying food. Sceptical arguments were more dominant than consideration of benefits but it seems likely that, in the future, climatic and population restraints on food availability may lead to greater acceptance of GM foods.

Conclusions

Overall these studies lead us to conclude that only a small number of GM-labelled products are for sale and purchased in various European countries. As the number of GM products available since the introduction of labelling has declined significantly, we can conclude that European consumers are restricted in their choice of purchases, reflecting the lack of availability of these products in the stores.

That three in four people claim to know that GM-food has to be labelled, and that two-thirds say that they cannot distinguish GM from non-GM products, may reflect the fact that fewer than 50 % of respondents bothered to read labels before buying a food item. Alternatively, it may mean that the information on the label is misunderstood or misinterpreted. Another reason may be that people are simply not interested: this seems to be confirmed by the finding that only 30 % of respondents are careful never to buy foods with GM ingredients.

Our findings, including the studies of Polish and British residents living in the United States, suggest that most people are neither really interested in, nor very alert to, the presence of GM ingredients or products. Opinion polls elsewhere have shown a low and declining level of interest in the GM issue when respondents are asked unprompted to list their concerns about food. It is only when GMOs are brought specifically to their attention that they show antipathy. This is also confirmed by the results of the focus group discussions.


NL Slasaus.
Ingrediënten: water, plantaardige olie (bevat genetisch gemodificeerde sojaolie), suiker, azijn, gemodificeerd zetmeel, tarwezetmeel, zout, mosterd (water, mosterdzaad, azijn, zout, specerijen, kruiden), eigeel, verdikkingsmiddel (E412), voedingszuur (E330), conserveermiddel (E202), kleurstof (E160a), antioxidant (E385).
Geproduceerd in: Nederland. Koel en droog bewaren. Schudden voor gebruik.

BE/LUX Salad dressing.
Ingredients: water, vegetable oils (contains genetically modified soyabeanoil), sugar, vinegar, modified starch, wheat starch, salt, mustard (water, mustard seed, vinegar, salt, spices, herbs), egg yolk, thickener (E412), acids (E330), preservatives (E202), colours (E160a), antioxidant (E385).
Produced in: The Netherlands. Store in a cool, dry place. Shake before use.
750 ml e
Ten minste houdbaar tot: zie etiket../
Best before: see label.

By and large, consumers continue to display a negative attitude towards genetically modified ingredients in food products and gene technology in particular. When asked whether they would buy GM foods, supposing such benefits as lower prices, healthier or tastier products, or production under 'environmentally-friendly' regimes, most people remained negative. This is not reflected in the focus group results, where people seemed more positive about GM foods with specific benefits. The focus group study leads us to conclude that genetically modified ingredients are not an issue that people consider seriously while shopping. Care for the environment or quality in proportion to price are more important. It would be interesting to explore the reasons for such differences further.

The fact that GM-labelled products are available and actually bought shows that there is indeed a market for such products. Our results may suggest that this market might be even larger than believed, as 20 % of non-buyers thought they were already buying GM-foods, and around 30 % did not even know whether or not they were doing so. Interestingly, the data showed no significant differences between buyers and non-buyers.

Our observations underline the fact that what people say differs from what they do. When asked whether they had bought GM food, half of our respondents said they had not. Yet the barcode analyses of their purchases showed that half of them were wrong and that they had indeed bought such products. Perhaps they did not know what they had bought. Some people also thought they had bought GM food when, in fact, they had not. Our data is not sufficiently extensive to probe more deeply into the minds of the shoppers but we may reasonably conclude that:

- most people do not actively avoid GM food, suggesting that they are not greatly concerned with the GM issue;
- linking purchasing data with answers to questionnaires is a more reliable way to establish attitudes than just opinion polls.

Country	Type of labelling	Nr. of barcodes
Czech Republic	GM	8
The Netherlands	GM	18
Poland	GM	1
Spain	GM	7
United Kingdom	GM	27
Estonia	GM	13
Germany	GM	29
Sweden	GM	22
Slovenia	GM	13
Greece	GM	0

Table 1.

Number of GM labelled products (barcodes) per country found in supermarkets in 2007.

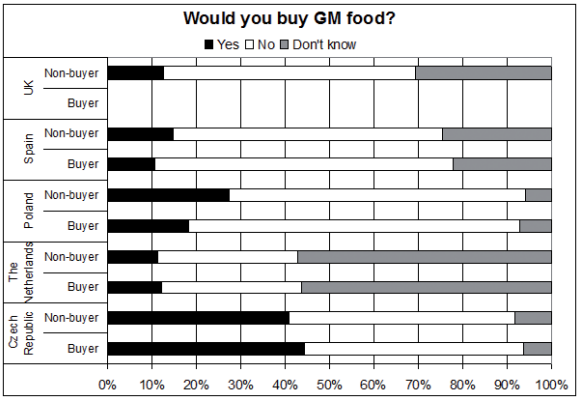


Fig 1.

Answers to the question 'Would you buy GM food?' in percentages differentiated between buyers (n=500) and non-buyers (n=500). A buyer is a consumer who bought at least one GM-labelled product per year (2007).

Major Publications

John G. Knight, Damien W. Mather, David K. Holdsworth & David F. Ermen. (2007). Acceptance of GM food — an experiment in six countries. *Nature Biotechnology,* 25 5:507-508.

Charles Noussair, Stephane Robin and Bernard Ruffieux. (2004). Do consumers really refuse to buy genetically modified food? *The Economic Journal*, 114 1:102-120.

Marks L., Kalaitzandonakes NG. and Vickner S. (2004). Consumer purchasing towards GM foods in The Netherlands, in Consumer Acceptance of Genetically Modified Foods by R E Evenson, Economic Growth Center, Department of Economics, Yale University, Connecticut, USA.

Alexa Spence & Ellen Townsend. (2006). Examining Consumer Behavior Toward Genetically Modified (GM) Food in Britain. *Risk Analysis*, 26 3: 657-670.

Osseweijer P., Kinderlerer J. and Ammann K. (2009). Societal issues in industrial biotechnology. Chapter in Soetaert, Vandamme (eds): *Industrial Biotechnology. Sustainable Growth and Economic Success*. Wiley. In print.

Biosafety Research Communication Network

Acronym
BIOSAFENET

Programme Acronym
FP6-FOOD

Contract number
043025

Period
September 2006 – February 2009

Coordinator
Prof. Dr. Joachim SCHIEMANN
Julius Kühn Institute (JKI)
Federal Research Centre
for Cultivated Plants
Head of the Institute for
Biosafety of Genetically
Modified Plants
Erwin-Baur-Strasse 27
D-06484 Quedlinburg
Germany
joachim.schiemann@jki.bund.de

Background and objectives

In the public debate on the biosafety of genetically modified organisms (GMOs), the results and even the existence of GMO biosafety research are often ignored. As a consequence, the already established and solid basis for a science-based discussion on GMO biosafety is not fully explored in Europe or worldwide. The major aim of this project was to promote scientific involvement and its broader perception in discussions of and decision-making on the biosafety of GMO. As such, BIOSAFENET was not a research project but a networking initiative for biosafety research.

The activities covered information tools for scientists, decision-makers, stakeholders and the general public, and scientific meetings and logistic support for networking. A main aim was to strengthen the position of European researchers in the international arena. To achieve this, the active participation of European scientists at the International Symposia on the Biosafety of GMO (ISBGMO), the only internationally recognised symposia in this important field of research, as well as the networking activities of scientists in the new Member States and associated countries, were supported. The platforms provided by the International Society for Biosafety Research (ISBR) were exploited in order to enhance the role of European experts in the international biosafety debate. Besides networking, other communication tools enhanced the flow of information on biosafety research results. Target groups were scientists, regulators, decision-makers, the media and the broader public. These activities addressed specific demands of each group, from information about scientific projects, compilation of knowledge for the evaluation of critical (upcoming) biosafety issues, and presentations of the results of biosafety research to the general public.

BIOSAFENET activities were coordinated in the project 'Global involvement of public research scientists in regulations of Biosafety and Agricultural Biotechnology (Science4BioReg)'. Exchange with other EU-funded projects conducting research on GMO was also established via the Advisory Board.

Approach and methodology

The project was structured into four work packages and the management section (Fig 1.):

- **WP1**. This comprised support for European scientists to participate actively in the 2006 and 2008 ISBGMO symposia on biosafety, as well as the organisation of expert seminars on key issues of biosafety research. Internationally recognised scientists were invited to co-organise six expert seminars on prominent biosafety issues. BIOSAFENET financed the seminars and organised the venue(s) and logistics, with project partners participating actively in these seminars or co-organising them. Recommendation reports were/are published on the issues discussed in the seminars.
- **WP2**. The project established an interchange with the International Society for Biosafety Research (ISBR), the organisers of the ISBGMO symposia, in two respects. The programme committee and BIOSAFENET coordinated invitations and the activities of European scientists at the symposia. Since ISBR also provides an international science-based platform for the discussion and promotion of biosafety issues, BIOSAFENET together with ISBR established general communication tools involving European specialists, i.e. a website and a newsletter. In addition to the link with the ISBR, WP 2 supported scientific networking activities in the Balkans and Turkey, including meetings with representatives of other EU-funded projects such as Science4BioReg, CO-EXTRA, TransContainer and PharmaPlanta. Another activity was the reconstruction of a biosafety research database (formerly GMO RES COM), now under the umbrella of ICGEB and renamed *BiosafeRes*. The exchange with other EU-funded projects was fostered via the Advisory Board.
- **WP3** had the task of establishing an Internet platform for the dissemination of biosafety- related information to all groups of stakeholders and to the public.
- **WP4** was created to inform the media and journalists on biosafety issues. Information on biosafety research topics was compiled and edited for distribution to the media.

Partners

Mark TEPFER
International Centre for Genetic Engineering and Biotechnology, Trieste (IT)

Ervin BALÁZS
Agricultural Research Center of Hungarian Academy of Sciences, Martonvasar (HU)

Kristina SINEMUS
Genius GmbH, Darmstadt (DE)

Giorgos SAKELLARIS
National Hellenic Research Foundation, Athens (GR)

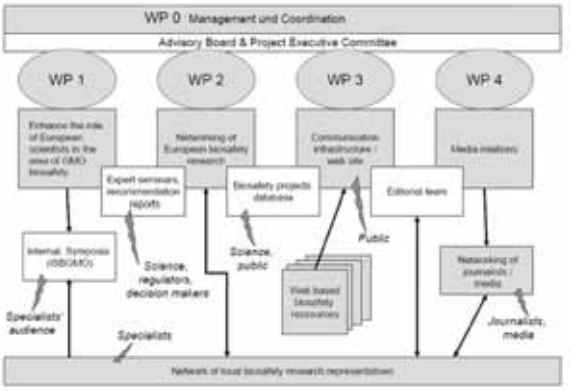


Fig 1.

Organisation of BIOSAFENET. WP = work packages; italics refer to target groups identified for different activities.

Main findings and outcome

In September 2006 the project started by selecting scientists for the European delegation to the 9th International Symposium on Biosafety of Genetically Modified Organisms (ISBGMO) in Jeju, Korea. Eleven scientists (from six EU countries) were supported in participating in the symposium as speakers, chairs or in the organising committees. In 2008 the 10th ISBGMO took place in Wellington, New Zealand, and the participation of 16 scientists (from 10 EU countries) was funded by the project. Abstracts and reports on both symposia are available on the ISBR website (www.isbr.info) or will be published in *Environmental Biosafety Research*.

Six expert seminars were organised during the course of the BIOSAFENET project on the following topics:

1. plant viruses and genetic engineering;
2. fitness of transgenic crops;
3. experience on monitoring of *Bt*-maize;
4. statistics for field trials regarding risk assessment;
5. transgenic insects; and
6. GM plants and abiotic stress tolerance.

BIOSAFENET invited scientists with expertise in their respective fields to explore the subjects in an open-minded atmosphere with a group of up to 20 scientists, in a 2-3 day meeting. The discussions and conclusions of each seminar were or will be published as Commentaries in *Environmental Biosafety Research*. Several seminars were held back-to-back with meetings of EFSA GMO Panel working groups, providing a direct and supporting link between biosafety research and environmental risk assessment. The concept of expert seminars will be adopted by ISBR for further activities.

BIOSAFENET reconstructed a database on biosafety research projects. The new ICGEB-managed database, now called *BiosafeRes* (http://www.icgeb.org/~gmores/prod/index.php), provides worldwide and free public access to descriptions of past and current research projects on GMO biosafety. Target groups range from scientists to journalists and the general public. The database will also support researchers in developing countries anxious to extend their contacts with European scientists and develop collaborative projects with them.

The website www.gmo-safety.org, edited by a partner, was supported in part by BIOSAFENET as it provides a forum for disseminating information on biosafety research to a broader public. Its services include the *Biosafenet Download Centre* for freely accessible papers and publications, and the *Biosafenet Navigator* which provides links to relevant websites. The number of visitors between 2007 and June 2009 rose from 16 000 to approximately 25 000 per month.

In addition, media sets and news on highlighted issues such as 'herbicide resistant plants and biodiversity', 'the soil ecosystem' and '*Bt* and butterflies' were prepared and distributed to media and journalists. During the course of the project, 21 media sets were compiled and a list of more than 800 European/international journalists established to receive further information on biosafety research.

Several activities of BIOSAFENET also established direct contacts with target groups and stakeholders. In May 2008, Science4Bioreg and BIOSAFENET organised an information booth and several side events at the 4th Conference of the Parties to the Cartagena Protocol, 12-16 May 2008, in Bonn, Germany. Scientists from Science4Bioreg, BIOSAFENET and other EU-funded projects introduced their biosafety research topics, as well as the GMO risk assessment process and sources of related information, to an international audience.

European scientists working in the field of biosafety were contacted directly at various conferences and seminars to participate in scientific discussions on biosafety. In addition to the active participation of BIOSAFENET partners in regional and international conferences, seminars and workshops on risk assessment and biosafety focused on the new Member States, the Balkans region and Turkey (17 countries), and a list of 90 experts from these regions was compiled. A continuous exchange with ISBR, the *Pannonian Plant Biotechnology Association* (PPBA) and the *Black Sea Biotechnology Association* was maintained during the project.

The BIOSAFENET project closed with a public conference held on 29 June 2009 in Berlin. Project partners and representatives from cooperating institutions, such as ISBR and PRRI/Science4Bioreg, reported on their projects, joint activities and conclusions. The conference concluded with a panel discussion by scientists, journalists, industry and NGO representatives on the biosafety research communication, taking public concerns into account.

Conclusions

The Advisory Board of BIOSAFENET, recognised European biosafety experts and managing members of other EU-funded projects, evaluated the activities halfway through and at the end of the project. The Board highlighted the flexible tools developed and the overall record of the project, rating the format and content of expert seminars and recommendation reports as convincing tools for resolving specific biosafety problems. The seminar concept will be adopted by ISBR for future events. The Board also recommended maintaining the database (*BiosafeRes*) and website activities (www.gmo-safety.eu), and even translating them into additional EU languages. This is essentially in line with the feedback from the side event at COP/MOP 4, where participants stressed the need to gain access to scientific and general information in a 'one-stop-shop' manner. ICGEB will continue to manage *BiosafeRes*. The continuation and extension of the website and related activities, such as editing of collated information for the media, will depend on future project funding at the national or European level.

Exchange within the scientific community was most effectively realised through support from existing networks of organisations like ISBR, PPBA or the *Black Sea Biotechnology Association*. In addition, seminars on GMO risk assessment and biosafety for regional researchers were well accepted and may increase awareness of the topics discussed. A regional communication structure reflecting regional needs would improve involvement, but is not yet well institutionalised in most of the new Member States or associated countries.

BIOSAFENET facilitated the initial contacts and meetings of researchers and interested experts in these regions, but supporting initiatives will be needed to establish routine meetings and foster international integration.

Major Publications

Teycheney PY., Tepfer M. (2007). Possible roles of endogenous plant viral sequences and transgenes containing viral sequences in both virus resistance and virus emergence. *Environmental Biosafety Research*, 6 (4):219-222.

Wilkinson MJ., Tepfer M. (2009). Fitness and beyond: Preparing for the arrival of GM crops with ecologically important novel characters. *Environmental Biosafety Research*, 8 (1):1-14.

Perry JN., ter Braak CJF., Dixon PM., Duan JJ., Hails RS., Huesken A., Lavielle M., Marvier M., Scardi M., Schmidt K., Tothmeresz B., Schaarschmidt F., van der Voet H. (2009). Statistical aspects of environmental risk assessment for GM plants. *Environmental Biosafety Research*, 8 (2):65-78.

Wilhelm R., Sanvido O. , Castanera P., Schmidt K., Schiemann J. (2009). Experience from monitoring commercial cultivation of *Bt* maize in Europe – conclusions and recommendations for future monitoring practice. *Environmental Biosafety Research*, 8, in press.

BIOSAFENET (2007). Strengthening the voice of European biosafety research. *The Parliament Magazine*, 256, 26 November 2007, 32.

BIOSAFENET (2009). Driving biosafety research on GM plants. *eStrategies – Projects*; April 2009, 68-69.

Acronym
BIOPOP

Programme Acronym
FP6-FOOD

Contract number
007086

Period
January 2005 – December 2006

Coordinator
Mr Francesco LESCAI
Associazione Nazionale
dei Biotecnologi Italiani
Via della Beverara 123
I-40131 Bologna
Italy
Francesco.lescai@yebn.org

Project website
http://www.biopop-eu.org/

Pilot study on innovative approaches to public communication of life sciences and biotechnology by students and young researchers

Background and objectives

Take two important cities in different parts of Europe, Italy and The Netherlands. Imagine, on the one hand, a group of young scientists freed from their laboratory environment and anxious to discuss their work and, on the other hand, a group of men-and-women-in-the-street keen to share their views and contribute to new roles in science. That's BIOPOP!

This project is a remarkable mix of the enthusiasm and the innovative ideas of five young scientist organisations of the Young European Biotech Network, led by the Association of Italian Biotechnologists together with the BtS, Amicale, GeNeYouS, TU Lodz ASSB, Aethia and Observa Science in Society.

BIOPOP addresses the big issues present at the 'science and society' interface. Recognising this, the European Commission agreed to fund this source of fresh ideas, thereby setting a new record: the youngest age-group of researchers and students ever awarded a grant within the FP5 framework programme for research and technological development.

By evoking the words 'BIOtechnology' and 'POPular", associating them with pop music and the pop movement, the project aims to establish a new format for communication and public participation in science, creating a special environment where the next generation of scientists and citizens can actually meet, and where they can effectively establish a dialogue and long-term communication.

One of the BIOPOP events was the placement of a specially designed tent in the main squares of these two important cities, where researchers and passers-by had the opportunity to meet and establish a unique relationship between society and science.

The project was developed in close collaboration with media professionals organised in both countries in 'media contact groups – 'activist' journalists and communicators who discussed, compared and reviewed the ideas of these young scientists from various standpoints.

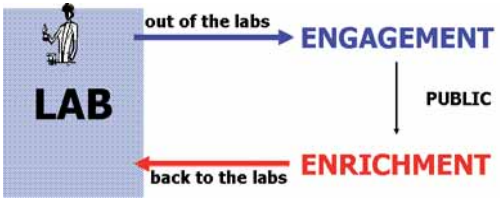
The first event was held in Bologna in October 2005 and the second in Delft in April 2006. Four thousand people in Bologna and more than 2 000 in Delft contributed to an extraordinary experience that touched on 'hot' issues such as cancer therapy, stem cells, GMO and food safety, and patents in life.

These young scientists discussed such issues openly with the people they met, shared their feelings and emotions, listen and gathered reactions, challenging themselves on their own research topics. At the same time, members of the public were able to express their views on regulatory initiatives and on the scientific fields that deserved better funding.

A complex evaluation system was used to assess and follow up the exchanges between the young scientists and the public, and to measure the impact of this new communication model on both citizens and researchers.

Approach and methodology

The project produced a communication format structured to match the people participating and the approach they had been trained in. This was a tailored training, based on a specifically designed model to ensure communication and using a new content management system to ensure the young scientists applied the model correctly.



People

The people involved in BIOPOP were the teachers or animators but researchers and students in life sciences. The process was therefore *sui generis*, in the sense that the participants came from their labs to meet the public and, after their involvement, returned to their daily work in science; they underwent the special experience of interaction with the public while remaining active in science. The aim was to provide an enrichment process, with expected impact not only, or not mainly, on the public but on the scientists themselves.

Partners

Gianpaolo PEREGO
AETHIA SRL, Colleretto Giacosa (IT)

Federico NERESINI
Observa, Vicenza (IT)

Amelie CHABOUREAU
Amicale des Elèves ingénieurs de l'école de Biotechnologie de Strasbourg (FR)

Holger NICKEL
Biotechnologische Studenteninitiative, Köln (DE)

Sylwia GORLACH
Politechnika Lodzka, Lodz (PL)

Terry VRIJENHOEK
Genomics Network for Young Scientists, Wageningen (NL)

Approach
Compared to previous interaction experiences, the model developed requires scientists to meet the public in a neutral (i.e. not role-based) environment, in order to share viewpoints rather than present positions. The model aims to create peer-to-peer interaction where it is not a question of scientists meeting the public, but of a person-to-person interaction where each has his or her own 'baggage' of experience to exchange.

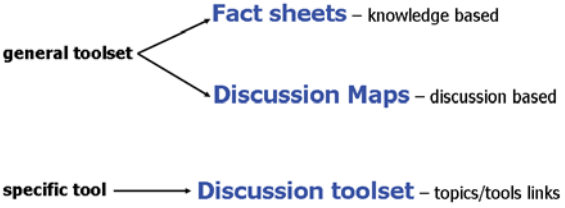
Training
In order to ensure these features in the communication model, a special set of training modules was developed with the specific aim of:

1. deconstructing a role-based and categorised view of the public;
2. developing an approach to the public based on experience-sharing;
3. training in a different contents/knowledge management system, specifically developed under the project.

Context
The main features of the environment in which the communication was established were:

- youth-based, i.e. inevitably the interaction was going to be essentially with young people;
- informal, i.e. the meetings, discussions and all the interactions during the event were open and shaped by participant contributions;
- science-based: despite the presence of young people and the informality, it was evident that this was an opportunity to discuss science with scientists. Each experience was essentially scientific.

Content Management
Specific tools were developed to manage the information exchange during the event and, thus, establish fruitful interaction avoiding the so-called 'deficit model'.



The tools developed linked scientific knowledge to daily life, thus establishing a process of knowledge management that was more complex than any based only on facts, and closer to the common way of linking different levels of information during discussion. Moreover, some of the discussion-based tools were designed to ensure that every exchange was open throughout, rather than lead to 'conclusions' or to fixed positions.

Main findings and outcome

A novel approach to the media was established: journalists and media organisations not only distributed information about the events, but were actively involved in the development and realisation of the communication model and in all the dissemination activities (press releases, advertisement, website).

The innovative communication model worked well and was readily accepted by all involved. The positive response of the public to 'bottom-up' participation is a sure sign that these methods are appropriate: the rare opportunities to participate in such activities therefore tend to be fully taken up by the most active people, regardless of the level of their daily involvement in scientific topics. Together with the set-up of the event, a specific framework was developed to evaluate scientifically the impact of the model on people: the design was planned *ad hoc* to measure parameters never measured in previous experiences based on the 'deficit model'. This original evaluation scheme is another concrete result of the project.

Events like these appear to be very successful, especially at the level of interpersonal communication between members of the public and researchers. An active public looking for information and willing to engage in individual discussions on biotechnology seems to be well served with an event like this. The non-mediated and human interaction provides the possibility of creating an authentic two-way communication process, a dialogue between scientists and members of the public.

This 'rediscovery' of open interpersonal communication could lead to more trust and less cynicism, maybe more than with other kinds of mediated interaction on science and technology in the mass media or in museums. It seems vital that the communicators are researchers themselves, in particular young researchers, and no PR or information officers are involved.

The young age of the scientists involved is a major factor in facilitating communication, because it makes the dialogue more informal, in comparison with the relationship between well-known scientists and the public. The open-laboratory sections in the tent, the relatively open communication message in the 'big head exhibition', and the combination of stereotyped and strongly non-stereotyped images produced a successful environment for interpersonal communication. Together, all these aspects provided the opportunity to preserve, or restore, a certain degree of complexity to the communication, as is the reality in scientific research.

This kind of communication does not reduce science to black-and-white statements or true-or-false statements about reality. It not only demonstrates scientific results but also the process of biotech research (despite this being a very small part of the whole) and the societal and ethical questions surrounding this kind of research. The comparison between the two experiences provided useful hints in ascertaining the success of the communication approach launched in the BIOPOP project.

Conclusions

On the one hand, if we conclude that this is an innovative and successful communication model, we invite institutions and media to exploit the material and methods that BIOPOP has now made available. On the other hand, however, some unsolved issues remain from the BIOPOP experience. Without exception, each of the 150 scientists from all over the Europe that participated in the initiative experienced the same difficulties in engaging with the project. Since one of the key features was to involve young scientists willing to remain active in science, the main obstacle for them was to devote part of their time to communication and training.

It was not only a matter of the time materially available for these activities. *It was also a matter of the cultural acceptance of their engagement within their labs*. In almost all cases, lab directors, and colleagues as well, considered even a small amount of time devoted to public engagement as a waste of that time! Instead of being an added value, it was considered detrimental to benchwork and, in some cases, even as detrimental to the person's career prospects.

This is the main paradox: at every level, European and national institutions claim a higher social responsibility by scientists and stress the need for a commitment to the public as part of the researcher's duties, especially when activities are funded from public grants. Committing the scientific community to a different vision depends by definition on young scientists, since this implies training on one side (training on communication and on participatory models) and a cultural evolution on the other, accompanied by a change in public perceptions. Thus, the picture emerging on one side is that:

1. institutions call for greater engagement;
2. young scientists should be the main actors involved in this process,

but on the other side:

1. the culture of the established scientific community doesn't favour this engagement;
2. there are no incentives or rewards for those scientists engaging with the public while remaining active in science;
3. both key issues impact especially on young scientists, since they're less autonomous and often hold insecure positions.

It is evident that there is a need to find feasible proposals to remove these obstacles, in order to offer incentives to researchers and their institutions, as well as provided added-value to careers in the short-to-medium term, and produce a shift in the cultural dimension in the long term. While the cultural shift can be partially left to generation change, different methods and solutions have to be experimented with in the other key aspects.

The second issue emerging from the BIOPOP project is more closely related to the experiences of scientists while engaging with the public. It relates to the impact of the public on scientists' activities when they return to their labs.

Public engagement is just a performance? How do we deal with the epistemological issue that the debates on biotech highlight, i.e. the need for a more complex and participative governance of research and technological development? These are questions for which, in order to provide serious answers, Europe's young scientists will need concrete incentives.

GMO communication and safety evaluation platform

Acronym
GMO-COMPASS

Programme Acronym
FP6-FOOD

Contract number
FOOD-CT-2004-06914

Period
January 2005 – February 2007

Coordinator
Kristina SINEMUS
Genius Biotechnologie GmbH
Robert-Bosch-Str.7
DE-Darmstadt, 64293
Germany
ksinemus@genius-biotech.de

Project website
www.gmo-compass.org

Background and objectives

European consumers expect safe, high-quality foods. However, they seem to doubt that GMO products can fulfill this requirement. Polls make clear that the majority of European consumers regard gene technology in agriculture and food products with some scepticism. In a Eurobarometer poll in 2005, only 27 % of Europeans expressed a positive attitude to GM food.

It is also evident that accurate information on GMOs is the key to ensuring the consumer's informed choice on topics related to GM products. National and European polls indicate that many European consumers have yet to form an ultimate opinion on the topic. According to a Eurobarometer poll in 2007, the 'use of genetically modified organisms in farming' is the second most common topic for which European consumers cite a clear deficit of information (34 % of respondents).

Science-based information on the use and safety of GMO products, as well as on the approval procedure, often fails to fully reach its target group, the consumer and the general public. Likewise, research outcomes concerning safety aspects of GMOs are hardly known to the public. The public perception of these facts is selective: signs of safety concerns evoke a much larger reaction than science-based explanations. This intensifies a feeling of uncertainty for many consumers.

Approach and methodology

The Internet platform www.gmo-compass.org was established within the EU's 6th European Research Framework Programme under Priority 5: Food Quality and Safety from 2005 - 2007. The main objectives were:

- a stronger presence and awareness of science-based facts about food safety and the potential of GM crops in public debate;
- transparency in the GMO safety evaluation regulatory practice thus fostering more public trust in GMO food safety;
- comprehensible information source for knowledgeable customers;
- a dialogue platform for the discussion of green biotechnology.

Disseminating and facilitating access to science-based information on GMO issues is one of the main objectives of the GMO-COMPASS information portal. Information offers need to adapt better to demand – this means matching expectations, needs, interests and the knowledge background of consumers. Considerable effort is needed to deliver scientific information on GMO safety aspects and the foundations of state control to the public in order to ensure the appropriate perception. This is where GMO-COMPASS contributes by providing appropriate concepts.

GMO-COMPASS features a consumer-orientated platform which offers easily comprehensible information on GMO products, their use and related issues of safety evaluation. Information on specific GMOs and GMO products is linked to background information and general topics of relevance. Political, national, legal and socio-economic aspects along the food chain are closely linked and presented in the form of condensed information packages.

Fig 1.
Home page of GMO-Compass.org

Partners

Gerd SPELSBERG
Transgen, Aachen (DE)

Matthias POHL
TÜVNORD EnSys Hannover GmbH & Co. KG (DE)

Science-based information: The editorial concept indicates that the website is the work of science journalists who exercise journalistic freedom in the selection and presentation of website content. This ensures credibility, which is an essential success factor when presenting GMO-related topics. Using a balanced approach, information is compiled and transmitted in a way that is factual and relatively easy to understand. Users who want to learn more can find links to relevant full-text sources on each page of the website. All texts are written journalistically and presented in an appealing and concise design with multiple presentation styles including reports, interviews, animations, photos and diagrams.

Public dialogue: The GMO-COMPASS website was also tested as a tool for dialogue processes on GMO issues. In 2006, GMO-COMPASS initiated an online discourse on 'The Future of GM Crops in Europe – Coexistence with Conventional and Organic Farming'. Online discourses serve as a means for gathering information and preparing for decision-making. They were to offer consumers and stakeholders a fair opportunity to express their needs and wants (information demands). The discourse was limited to a timeframe of four weeks and ended on October 8th, 2006. To encourage debate, the team of moderators came up with three different fictitious scenarios concerning the implementation and results of coexistence practices in the year 2016.

Main findings and outcome

User statistics of GMO-COMPASS.org have grown continuously, so that it has become one of Europe's most used independent information portals on GMO issues. During the project period, the website was visited by approximately 250 000 people who read around 2.6 million pages. At the end of the EU-funded project period (February 2007), visitor numbers reached an average of some 1 000 visits per day (approx. 30 000 visitors per month). Visitors came from a wide range of countries (approx.130) within Europe and beyond.

Right from the start, GMO-COMPASS aimed to improve the dissemination level by cooperating with international news services in the area of agro-biotechnology. This work has led to extensive publicity for the website, which is also reflected in the high number of web pages referring to GMO-COMPASS (around 8 000 external links).

Conclusions

The rapid increase of user numbers is the most important indicator of broad public acceptance and usefulness of this website and the information it offers. The GMO-COMPASS website has been kept alive until today and its visitor numbers have continued to grow, almost tripling from 2007 to 2009, now reaching around 80 000 users per month.

The GMO-COMPASS web project demonstrates that it can help Europeans understand GMO policy and regulations and inform them of new developments and benefits and of the public debate on GMO issues. The consumer has been reached by offering science-based information aligned with the expectations of lay persons and embedded in balanced reporting that includes counter-expertise and the views of non-scientists. By this means, GMO-Compass successfully complements the existing information supply and transparency measures of institutions and state bodies, as well as the websites of lobby groups and scientific institutions.

The great interest in the online discourse shows that such tools are very suitable for fostering a serious public debate on GMO topics and issues within Europe. For future online discourses, it is recommended to consider local issues such as the debate on GM-free zones or local field trials. Such topics are believed to add to the interest for the lay person. Experience suggest that consumers tend to be more active if they are directly affected by local topics.

Major Publications

Frank-Olaf Brauerhoch, Christoph Ewen, Kristina Sinemus. (2007). Talking biotech with the public. *Biotechnology Journal*, Volume 2 Issue 9, p 1076-1080.

Klaus Minol, Gerd Spelsberg, Elisabeth Schulte, Nicholas Morris. (2007). Portals, blogs and co.: the role of the Internet as a medium of science communication. *Biotechnology Journal*, Volume 2 Issue 9, p 1129-1140.

Kristina Sinemus, Marc Egelhofer. (2007). Transparent communication strategy on GMOs: Will it change public opinion? *Biotechnology Journal*, Volume 2 Issue 9, p 1141-1146.

Europeans & Biotechnology in 2010 Findings from Eurobarometer 73.1

Acronym
STEPE

Programme Acronym
SiS

Contract number
SiS-CT-2008-217815

Period
August 2008 – April 2011

Coordinator
George GASKELL
London School of Economics
& Political Science
Houghton Street
London, WC2A 2AE
United Kingdom
g.gaskell@lse.ac.uk

Background and objectives

This was the seventh in a series of Eurobarometer surveys on biotechnology conducted in 1991, 1993, 1996, 1999, 2002, 2005 and 2010. This latest survey was based on a representative sample of 30 800 respondents from the 27 Member States, plus Croatia, Iceland, Norway, Switzerland and Turkey.

Issues such as regenerative medicine, production of Genetically Modified Organisms (GMOs, both transgenic and cisgenic), biobanks, biofuels and other innovations such as nanotechnology and synthetic biology were investigated. In addition broader issues, such as the governance of science and the engagement of citizens, were considered. The survey stands as a contribution to the public and policy debate. This summary emphasises the findings dealing with GMOs.

Approach and methodology

The questionnaire for this Eurobarometer survey included key trend questions designed to assess the stability, or change, in aspects of public perceptions. It also included new questions to capture opinions and attitudes on emerging issues in the field of biotechnology. And, as in 2005, there were questions on nanotechnology, partly because this has been heralded as the next strategic technology and partly because of its close links with biotechnology. There were also new questions on the emerging field of synthetic biology.

Main findings and outcomes

The portrait of European citizens painted in the 2010 survey, in comparison to earlier surveys, shows that the crisis of confidence in technology and regulation that characterised the 1990s (as a result of BSE, contaminated blood and other perceived regulatory failures) is no longer dominant. Today, there is a much greater focus on the technologies themselves: Are they safe? Are they useful? There is no rejection of the impetus towards innovations and commercialisation, and Europeans are in favour of regulation to balance the market, reflecting their desire to be involved in decisions about new technologies when social values are at stake. Overall the majority of the European public remains optimistic about biotechnology and genetic engineering.

Major Publications

Gaskell George, Nick Allum, and Sally Stares. (2003). Europeans and Biotechnology in 2002. (Europa.eu.int/comm/public_opinion/archives/eb/ebs_177_en.pdf)

Gaskell George et al. (2007). Europeans and Biotechnology in 2005: Patterns and Trends (http://ec.europa.eu/public_opinion/archives/ebs/ebs_244b_en.pdf).

Gaskell G., Allum N.C., Wagner W., Hviid Nielsen T., Jelsoe E., Kohring M., Bauer M.W. In the public eye: representations of biotechnology in Europe in *Biotechnology 1996-2000: the years of controversy* Gaskell G., Bauer M., Eds. (Science Museum Publications, London, 2001).

Gaskell, George, Martin Bauer, John Durant and Nick Allum. (1999). Worlds Apart: the receptions of GM foods in Europe and the United States. Science, 264, 5426, 384-387.

The Eurobarometer survey, however, indicates that some controversies persist, and GM food remains the Achilles' heel of biotechnology. Concerns about safety remain paramount, followed by the absence of perceived benefits. GM food is seen as unnatural. Interestingly, when the technology is explained in more detail in the context of apple production and a comparison is made between transferring genes from different species (transgenics) and those from naturally crossable varieties (cisgenics), the picture becomes more complex. The majority of the European public considers the cisgenic technology to be safe and, given the advantage of reduced pesticide use, this approach is seen as acceptable. Even transgenic approaches receive a somewhat more positive reception than GM food itself, possibly because of the potential benefits of lower pesticide use. Thus the survey indicates that objections to GM food are related to concerns about safety seen in the context of a lack of perceived benefit, and these are objections which may wane if new varieties offer clear benefits.

The European public is even less supportive of animal cloning for food products, which is seen as unbeneficial, unsafe, inequitable and worrying. The similarities between perceptions of animal cloning and GM food suggest that the combination of biotechnology and food is an unpalatable recipe.

However, there is also a clear disparity of opinion across Europe. The survey indicates that there is no majority in any country in favour of encouraging GM food: the respondents who think it should be encouraged vary from 44 % in the UK to only 10 % in Greece and Cyprus, and an even lower level (7 %) in Turkey. However, breaking down the technology into transgenic and cisgenic approaches gives strikingly different results. When asked if cisgenic technology should be encouraged, Cyprus now comes out highest with 76 % in favour, while Luxembourg is most sceptical at only 35 %.

Conclusions

The survey shows that European public opinion need not be seen as a constraint to technological innovation. While GM food is still opposed overall, Europeans favour sustainable innovation – for example technologies allowing reducing pesticide use, and the cisgenic rather than the transgenic approach. There is now also greater trust in industry and in the regulatory authorities. And while the European public expects the appropriate regulation, in preference to leaving issues to market forces, it wants its views to be taken into account when technology and values collide.

Partners

Paul CASTRO
Centro de Investigação e Intervenção Social (PT)

Wolfgang WAGNER and Nicole KRONBERGER
Department of Social and Economic Psychology, Johannes Kepler University, Linz (AT)

Jürgen HAMPEL
University of Stuttgart (DE)

Claude FISCHLER and Jocelyn RAUDE
Ecole des Hautes Etudes en Sciences Sociales and Centre National de la Recherche Scientifique, Paris (FR)

Helge TORGERSEN
Institute of Technology Assessment, Austrian Academy of Sciences, Vienna (AT)

Agnes ALLANSDOTTIR
The Centre for Law and Biotechnology, University of Siena (IT)

Gemma REVUELTA
Universitat Pompeu Fabra, Barcelona (ES)

Alexandre QUINTANILHA
Instituto de Biologia Molecular e Celular, Porto (PT)

Maaris RAUDSEPP and Andu RAMMER
The Environmental Psychology Research Unit, Tallinn University (EE)

Nick ALLUM
Department of Sociology, University of Essex (UK)

R Yilmaz ESMER
Department of Political Science and International Relations, Bahcesehir University (TK)

Niels MEJLGAARD
Department of Development and Planning, Aalborg University (DK)

European Commission

EUR 24473 — A decade of EU-funded GMO research (2001 - 2010)

Luxembourg: Publications Office of the European Union

2010 — 264 pp. — format, 14.85 x 21 cm

ISBN 978-92-79-16344-9
doi 10.2777/97784

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KI-NA-24473-EN-C

This publication summarises the results of 50 selected research projects on genetically modified organisms (GMO), co-funded by the European Commission under the Framework Programmes for Research and Innovation, and conducted in the period 2001 - 2010, including a summary of the latest Eurobarometer survey 2010 on Life Sciences and Biotechnology.

The projects presented here address areas of major public concern in relation to GMO: environmental impact of GMOs, food safety aspects, risk assessment of GMOs and risk management and finally aspects of genetic modification of plants and/or microorganisms related to biomass and biofuel production. The publication is complemented by a number of highly valuable contributions of distinguished scientists, renowned experts in their respective fields.

Publications Office

ISBN 978-92-79-16344-9