



# Crops of Uncertain Nature?

Controversies and Knowledge Gaps Concerning Genetically Modified Crops  
An Inventory

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## Preface

This literature study aims to provide an inventory of gaps in current knowledge of environmental effects of genetically modified agricultural crops. Greenpeace Netherlands has commissioned the report but Plant Research International (Wageningen University and Research Centre) is the sole author and Greenpeace did not place any constraints on the substance of this literature review.

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## Summary

This study has identified several areas of controversial, fragmentary or missing knowledge concerning the design, functioning and use of genetically modified (GM) crop plants, from a standpoint of the natural sciences. These areas concern the biological and agronomical sciences which are discussed here, while philosophical, ethical, social-economical and legal scientific areas are indicated to stress their relevance for the public debate on GM (food) crops. Controversies and knowledge gaps appear to be present at all levels of biological organization ranging from the levels of DNA and cellular metabolism to organism and ecosystem levels.

Controversial issues, generally involving knowledge gaps, are:	section
• Precision of genetic modification (GM) and predictability of its effects	3.2.2, 3.3.2
• Differences between classical breeding en GM	3.2.3
• Effects of GM crops on (agro)biodiversity	3.4.2
• Interactions between neighbouring GM and GM-free agro-ecosystems	3.4.3
• Consequences of the use of GM crops for the environment	3.4.4, 3.4.10
• 'Genetic pollution' of ecosystems	3.4.6
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• Long-term effects of GM crops on human and animal health	3.5.3
• Consequences of the occurrence of horizontal gene transfer in the field	3.5.4
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In addition, knowledge gaps identified concern:	
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• Reliability of annotations in genome databases and quality control	3.2.4
• Regulation of gene expression: transgenes in their 'xeno-genomic' environment	3.2.6, 3.3.5
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• Irrevocable and unprecedented effects on natural ecosystems	3.4.5
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• Gene stacking	3.4.11
• Prediction and testing of allergenic properties of (transgenic) proteins.	3.5.1
• Separation and isolation of GM and GM-free food chains	3.5.5

These findings demonstrate the fragmentary nature of current knowledge of genome structure and function and regulation of gene expression in general, and the limited understanding of several physiological, ecological, agronomical and toxicological aspects relevant to present-day and planned genetic modifications of crops. Whether and in which case this limited understanding can be judged as relevant to the public debate on GM and as sufficient for adequate risk assessment are questions requiring further interdisciplinary study.

## Keywords

*agro-ecosystem, allergenic proteins, controversies, conventional breeding, crops, ecological interactions, food safety, genetic engineering, genetic modification, genetics, genome structure and function, knowledge gaps, metabolic pathways, pest resistance, physiological functioning, regulation of gene expression, toxicology.*



## Samenvatting

Deze studie identificeert diverse gebieden met controversiële, fragmentarische of ontbrekende kennis van ontwerp, functioneren en toepassing van genetisch gemodificeerde (GM) gewassen, vanuit een natuurwetenschappelijk gezichtspunt. Van deze gebieden worden de biologische en agronomische wetenschappen hier met name besproken, en worden aspecten op andere relevante wetenschapsgebieden, waaronder filosofische, ethische, sociaal-economische en legale, aangegeven wegens het grote belang daarvan voor het maatschappelijk debat over GM (voedsel)gewassen. De genoemde controversen en kennishiaten blijken aanwezig te zijn op alle biologische organisatieniveaus van DNA en cellulair metabolisme tot organismaal en agro-ecosysteem niveau.

Controversiële onderwerpen, veelal met kennishiaten, zijn:	sectie
• Precisie van genetische modificaties (GM) en voorspelbaarheid van effecten	3.2.2, 3.3.2
• Verschillen tussen klassieke veredeling en GM	3.2.3
• Effecten van GM gewassen op (agro)biodiversiteit	3.4.2
• Interacties tussen naburige GM en GM-vrije agro-ecosystemen	3.4.3
• Gevolgen van het gebruik van GM gewassen voor het milieu	3.4.4, 3.4.10
• 'Genetische vervuiling' van ecosystemen	3.4.6
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Deze resultaten tonen de fragmentarische aard van de huidige kennis van de genoomstructuur en – functie en de regulatie van genexpressie in het algemeen, en de beperkte kennis van verscheidene fysiologische, ecologische, agronomische en toxicologische aspecten die relevant zijn voor de gerealiseerde en geplande genetische modificaties van planten. Of en in welke geval deze beperkte kennis beschouwd kan worden als relevant voor het maatschappelijk debat over GM en als voldoende voor adequate risicobeoordingen zijn onderzoeksvragen die een interdisciplinaire aanpak vereisen.



***'We don't know as much as we think we know'***  
**Socrate**

# 1. Introduction

## 1.1 Background

In a recent extensive study by the Nuffield Council on Bioethics in the UK, Ryan et al. (1999) observe that the introduction of genetically modified (GM) crops into the environment and the food chain has become highly controversial in the United Kingdom, parts of Europe and in other parts of the world. These authors note a contrast with the near-indifferent attitude of consumers in the US and Canada with regard to GM crops, which is seen differently by others (e.g. Lacy et al. 1991). The ethical and social issues dealt with in the study by Ryan et al. (1999) are highly relevant to the mentioned controversy but beyond the aim of the present report, which mainly deals with unresolved life-science issues related to GM crops.

Through the use of genetic modification (GM), new organisms (GMOs) have been and are being created which would not likely have been formed otherwise through spontaneous or selection-driven evolution. GMO is defined as an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (involving several techniques which are listed in annexes of EU Directives 90/219, 220; see web site 14 in section 5.2).

This uniqueness of GMOs, even on an evolutionary time scale, might be based on scientific arguments dealing with the following characteristics of present-day GM (c.f. Gilissen & Nap 1999; Lewin 1990; Maessen 1997; Travers 1993):

1. random insertion by illegitimate recombination of a well-defined DNA construct into an intact well-functioning genome of a species, leading to one or more insertions of complete or partial DNA constructs, thus creating 'insertion mutations', possibly involving a loss or addition of one or more functions of the GM-plant under some conditions;
2. such a construct generally contains several specified, often alien, genes and regulatory elements, i.e. DNA sequences often originating from various, phylogenetically distinct taxons; transfer of this DNA to the recipient species is occasionally referred to as 'breaching species barriers';
3. among the genes in the construct are some that encode the desired new trait (called 'gene of interest'; e.g. a new enzyme) under the control of promoters which may or may not target the enzyme's activity to a certain place (e.g. the leaves) at a certain developmental time (e.g. during flowering);
4. other genes facilitate the selection of the transgenic organism (e.g. selection markers, often conferring antibiotic or herbicide resistance, recently Novartis's *manA* gene, and reporter genes).
5. common sources (or donors) of such genes are species of bacteria (e.g. *Escherichia coli*), yeasts (e.g. *Saccharomyces cerevisiae*), and plants.

Today, about 14 years after the first field introduction of a GM crop, concerns exist among parts of the nearly world-wide society about some of these very basic characteristics of GMOs, especially fundamental aspects like random genome changes, 'breaching species barriers', and their potential environmental effects like decrease of biodiversity, and effects on health via food safety and antibiotic resistance (e.g. Van Gool 1999, 2000). It is mainly these new unprecedented aspects which appear to be fundamental to public concerns about the unpredictability of the outcome of interactions of GMOs with agro-ecosystems (e.g. Website 1). Regarding the possibility of the occurrence of unexpected and unintended side-effects of GM-crops, uncertainties and controversies exist among various groups in society, also among scientists (e.g. Lacy et al. 1991; Ewen & Pusztai 1999; Mae-Wan Ho 2000; Van Dommelen 1999, Van Erkelens 1999; De Lange 1999, Voormolen 1999). Such uncertainties and

controversies also exist among regulators of EU member states and the European Commission (web site 15). Illustrative examples are the discussions on the 'precautionary principle' (e.g. Pape 1999) and the concept of 'substantial equivalence', e.g. Millstone et al. 1999; Trewavas 1999; Trewavas & Leaver 2000). Apart from these issues in the area of natural sciences, bases of concern have been identified in other scientific areas which are outside the scope of the present study, but nevertheless may be just as or more important in the public debate (e.g. ethical aspects; Ahuja 1997; Banner et al. 1998; Bruce & Bruce 1998; Ryan et al. 1999).

Greenpeace Netherlands has requested more information on the availability of knowledge relevant to potential decisions on field-introduction of genetically modified crops, in the form of an inventory of scientific knowledge gaps and controversies. Greenpeace is interested in biological-scientific arguments for raising fundamental questions that should be answered when considering field-introduction of genetically modified crops.

This study aims to identify gaps in and borders of the present knowledge of the functioning of transgenic crop plants at the various interacting levels of biological organisation, from molecular to agro-ecosystem levels, focussing on genetic modification, genome functioning, biochemistry, physiology, toxicology, ecology and agronomy. We do not attempt to answer the question whether the observed knowledge gaps and controversies are serious to the extent that responsible decisions about the field-introduction of (specific) GM crops can or cannot be made, because this requires a wider interdisciplinary study which is outside of the scope of this report.

## **1.2 Aim, starting-point and scope**

This inventory aims to list unresolved issues regarding design, creation, testing, breeding, field-introduction and use of GM crops, by identifying knowledge gaps and controversies based on the current state of biological and agronomical sciences on these subjects.

While we recognize the need for interdisciplinary studies on (the acceptability of) the use of GM crops, we need to stress here, that our starting-point is strictly the natural sciences arena. Within this area, the scope of the study includes unresolved issues in a range of areas, from transgenic crop design and molecular biology to functional genomics, physiological and ecological interactions, and toxicological aspects. Where evident, we indicate the reasons for controversies and the relevance of unresolved questions for the current social debates on uses of GM crops. An extensive, detailed and methodological analysis of the causes of knowledge gaps and controversies requires additional, interdisciplinary research.

## **1.3 Subjects outside the scope of this report**

The scope of the present study is an inventory of unresolved questions relevant to field introduction of GM crops, based on identification of knowledge gaps and controversies. Some subjects and approaches that we consider highly relevant to current debates on the use of GM crops, were necessarily outside the scope of the present study:

- First, biological or other scientific arguments are not the sole basis of decision-making processes regarding the use of GM crops. Emotions, claims, beliefs and philosophies of life also have their impact on the opinions of 'competent civilians' and politicians (HJ Achterhuis in: Spiertz & Dons 2000), but such aspects had to be largely ignored here, except in some cases when indicating the relevance of the identified questions for widely accepted risks.

- Second, an extensive methodological analysis of the controversies identified (cf. Van Dommelen 1999), while highly relevant to the ongoing debates, is a next step towards improving these debates, and is outside of the scope.
- Third, this study does not discuss approaches that may be successful alternatives to GM and conventional breeding as (sustainable) solutions to pest and abiotic stress problems in crops. For example, crop damage by insects has been successfully reduced by an ecological approach, the so-called habitat management approach, involving mixed-cropping systems with insect-repelling and insect-attracting plant species (e.g. Khan et al. 1998; ICIPE web-site, 8). Such and other alternative approaches are not assessed and evaluated here, and also their comparison with GM approaches is outside the scope of this study.
- Fourth, this study is not a risk assessment, although it may provide basic information to that end. The inevitable involvement of (non-scientific) valuation in risk assessments (e.g. Douglas & Wildavsky 1982) goes beyond our present aim. For further information, the reader is referred to extensive reviews on risks of biotechnology (e.g. Kasanmoentalib & Longino 1996; Marks et al. 1998; McNab 1998; Rubbiani et al. 1999; Scholderer et al. 1999; Van Dommelen 1996, 1999) and general literature on dealing with risks (e.g. Douglas & Wildavsky 1982; Klingholz 1992).
- Fifth, this study is based on the literature available in June 2000. Presented conclusions will have to be reconsidered sooner or later, since scientific knowledge may be subject to change by new findings as long as research will continue.
- Sixth, this report is not written for average civilians, but rather for specialists familiar with the general topic of GM crops. While the need for a layperson's version is recognised, this had to be outside the scope of this study.

## 1.4 Outline

In the following, an inventory is given of knowledge gaps, controversies and fundamental questions on the topic of agro-ecological risks of (field introduction of) GM crops by reviewing the literature available in June 2000, following the structure shown below. According to Van Dommelen (1999) 'all research depends on the questions raised'. This may be so for the research process, however, it does not necessarily apply to all research outcome, because of the important element of serendipity. There is in research practice more 'trial and error' and 'unexpected success in error' than many may want to admit.

### Structure

From a natural science stand point, this study deals with biological and agronomic issues, and is structured according to the the different levels of biological organisation. Molecular, cell and ecosystem-aspects are strongly related and intertwined (Fig. 1). Examples are: antibiotic resistance ('selectable marker' tool in GM) & human health (increased antibiotic resistance in pathogenic organisms); farmers' interest in better pest control & more rapid resistance development to Bt-toxins in pest insects due to continuous exposure to the transgenic crop Bt-toxins; e.g. Van Dommelen 1999, 33). Effects of GM may occur at all levels of biological organisation, each affecting the potential final use of a GM crop. Two phases of GM crop development can be distinguished, a lab phase and a field phase. The various - interactive- steps in the process from gene construct design to GM crop product, or, paraphrasing, 'from DNA to food' are:

## Levels of biological organisation

Molecular to Organism level (mostly laboratory-phase):

1. Design of DNA constructs and GM crop plants
2. genetic modification (GM), selection and breeding
3. testing physiological and biochemical characteristics of GM crops
4. Population to Agro-ecosystem level (mostly field-phase):  
evaluating ecological and agronomic interactions of GM crops
5. testing toxicological and allergenic properties of (products of) field-grown GM crops.

As these five aspects are interrelated by nature, the choice for this structure should not be taken to imply a unidirectional or step-wise flow of information. Indeed, each of these aspects is affected by and has consequences for each of the other aspects (Fig. 1). For instance, a change in a biochemical pathway (biochemistry/physiology) by introducing a new enzyme may potentially affect crop yield and quality (agronomy), but may also lead to emergence of new allergenic proteins (toxicology) or affect (agro) biodiversity.

An advantage of this -largely disciplinary- systematics includes a distinction between two phases in GM crop development. These phases differ in their level of control of risks: the laboratory phase with nearly complete risk control, and a field phase with virtually no risk control (e.g. Goewie 2000).

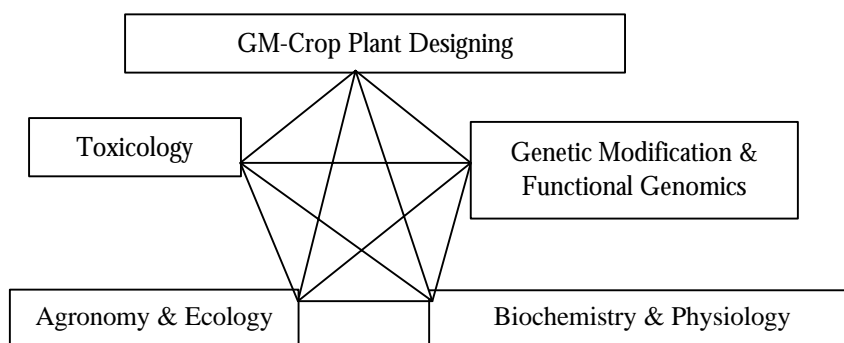


Figure 1. *Interacting natural-science aspects of the developing and functioning of GM crops.*

## 1.5 Terminology

The terms genetic manipulation (e.g. Harlander 1991) and genetic engineering were the first to be coined in the scientific literature to indicate direct anthropogenic transfer of DNA between living organisms as early as the seventies. Since then other terms have been used for the same purpose, including gene technology, genetic transformation and genetic modification.

Since the present report is a literature overview, we have decided to reflect as much as possible intentions of authors by clinging to their terminology. We think that it is the only real option in a review such as this, covering various disciplinary fields, each with its own historically developed jargon. A shortlist of some essential terms not explained in the text is presented at the end of this report.

For more extensive and detailed information, the reader is referred to excellent recent overviews on various aspects of GM crops which include extensive glossary lists, descriptions of genetic engineering concepts and techniques, and terminology definitions (e.g. Bruce & Bruce 1998; Kahl 1995; Lotz et al. 2000; Ryan et al. 1999). Definitions of terms like GM and GMO are also available on the Web (sites 14 to 17, section 5.2); for instance, definitions within the Directive 90/219/EEC are listed by the Belgian Biosafety Server (website #14).



‘Asking the right question is more important than a quick answer’  
Socrate

## 2. Methodology

### 2.1 General

In the view of Van Dommelen (1999: 37) the biosafety debate is in need of a more basic interpretative framework to be able to analyse the contested claims and to evaluate the persisting controversies.

Identifying knowledge gaps and controversies is to a large extent a scientific enterprise. Whether such unresolved questions are considered relevant to (widely accepted) problems, however, is determined by the ‘window of concern’ of the actor involved (Van Dommelen 1999). Definition of a problem is subjective and individual. To make this explicit Van Dommelen (1999) has proposed the concept of ‘set of relevant questions’ (SRQs) as a practical analysis tool for weighing opposing arguments and claims. While we see this approach as highly valuable for analysing controversies, we must stress here, that such an analysis is outside the scope of the present study.

Given the objectives of this study, it is clear that no one scientist will be sufficiently competent in all of the disciplinary fields. In part, this problem was solved by consulting internal and external specialists, within budgetary and time limitations. Where possible, unfounded claims and theoretical possibilities were distinguished from sound conclusions derived from empirical data and observations of practical situations. Furthermore, the rules of logic were applied to lines of reasoning, to identify improper conclusions. For example, frequently found conclusions like ‘no proof of risk’ and ‘no evidence’ are not equivalent to ‘proof of no risk’, and ‘absence of evidence’, respectively, in the absence of a statistically adequate number of reliable risk assessment studies. It is interesting to note that the order of the EU concerning novel foodstuffs Number 258/97 requires ‘a proof of no risk’ to the consumer of genetically modified products (Hammes & Hertel 1997).

Reproducibility and statistical significance are important scientific rules. Therefore, single, unique studies are not acceptable as conclusive evidence, but may merely indicate a gap in knowledge, a result requiring independent confirmation.

### 2.2 Technical

The present review is based on information from various sources. These included computerised literature databases (see list below; use of search profiles), internet web sites (see section 5 ‘Sources’ for a list of hyperlinks), newspapers and other periodicals (not available via literature databases or the internet, personal communications, as with participants of national and international meetings and symposia (e.g. New Frontiers in Biotechnology, April 2000, Toulouse). The data for this review were collected mainly during the period of March-June 2000. The larger part of the resulting collection was incorporated into a relational database using MS-Access, in order to facilitate the categorising of references according to various criteria related to specific subjects or issues.

*Literature databases used:*

BA 1996-2000/03;

CAB 1996-2000/04;

AGRICOLA 1998-2000/03;

TROPAG & RURAL 1998-1999/12;

FSTA 1990-2000/06;

Current Contents Search 2000/01-2000/06.

## 2.3 Approach

Unresolved research questions have been formulated on the basis of data in the literature indicating knowledge gaps or controversies. As a basis for structuring the present report we have chosen the level of biological organisation of life, from the level of molecule and gene to agro-ecosystem (field) and food.

For analysing controversies, Van Dommelen (1999) has proposed the adoption of 'sets of relevant questions' (SRQs), and has characterised controversies as competing SRQs and associated 'windows of concern'. Such an analysis of controversies is outside the scope of this study, since identifying the boundaries of knowledge is our main objective. However, in a few cases we provide arguments for the relevance of the listed questions for the identification of potential hazards.

This study deals with unresolved questions which may be pertinent to various aspects of GM crops, from design and creation, to selection, screening, field-testing and market-introduction. Controversies or knowledge gaps have been identified preferably based on data in scientific journals and other peer-reviewed publications, in some cases based on non-peer-reviewed papers, and extended by expert opinions and commentaries. Therefore, these three types of publications have been separated in the list of references.

General scientific criteria for relevance of research questions cannot be given. The perspective of the person raising the question plays a major role (cf. 'window of concern', Van Dommelen 1999). Relevance has been based on widely recognized or debated, current public concerns, notions and opinions, often presented in media other than scientific journals. General notions among the public regarding issues like health, food safety, environmental pollution, biodiversity, etc. are widely recognised to be associated with, for instance, the right of free choice, rejection of unsubstantiated claims and unclear argumentation, fear for the unknown, association of randomness with uncertainty and risk, suspicion of utopian predictions by scientists, and the consideration of 'breaching species barriers' as unnatural.

In the section 'Results', we list a number of subjects which have raised questions that evidently have not been answered unequivocally (controversy) or have not been receiving (adequate) attention in the literature (gap), but are likely to be relevant to decisions regarding field introduction of GM crops. The criteria for these qualifications are described below.

Whether the occurrence of such knowledge gaps and controversies constitute risks is in some cases scientifically well established. In such cases, qualifications like 'risk' or 'benefit' are also assigned to an open question. Following an unresolved question, a conclusion is given regarding the current state of knowledge, followed by data reported on the subject (from the literature, consultation or other source). It should be noted that questions and issues concerning genetic modifications are often proposed to be evaluated on a case-by-case basis (e.g. Gilissen & Nap 1998), which may indicate in itself a lack of knowledge for developing general theories and models.

In order to clearly distinguish conclusions in the literature from our own conclusions, it should be stressed that text presented under 'literature data' generally does not include our interpretations, conclusions or opinions, unless printed in italics.

## 2.4 Criteria for classification of (scientific) issues

The main criterion for in- or exclusion of references was the scientific quality of the information, as reflected by independent reproduction of data (i.e. result demonstrated at least twice) and a peer-reviewing process. However, the relatively large number of anonymously authored reports, generally not published in regular scientific journals, could not be ignored. Such information is evident from the list of references, which has been split up into three parts: 1. Peer-reviewed scientific publications, 2. Reports and proceedings (may have been peer-reviewed), and 3. Non-peer-reviewed articles, opinions and commentaries. More specifically, in drawing conclusions from such literature data the following criteria were used for classification into the categories 'Controversy' and 'Gap in knowledge':

- **Controversy** over an issue is defined by the presence of an ongoing debate in the scientific literature, and/or by conflicting data or conclusions being published (without published signs of a debate); such an issue may be a research outcome, or a research question (or SRQ; Van Dommelen 1999). Controversy generally involves knowledge gaps.
- **Gap in knowledge** is defined by absence of (reproduced) answers to a question or data on an issue (to the best of our knowledge), i.e. issues dealt with in less than a few reports in the scientific literature.

The term 'risk' has been defined by most authors as 'the product of hazard and chance of its occurrence (e.g. Van Dommelen 1999, 50; a hazard is an unwanted, unintended or undesirable (by whom?) effect that has been demonstrated (more than once) , with a chance being  $>0$ . 'Benefit' may be defined as an event and its desirable effects (to whom?), which have been demonstrated to occur (chance  $>0$ ).



*'We cannot predict the future, but we can prepare it'*  
*Ilya Prigogine, Physics Nobel laureat*

### 3. Results: Account of present controversies and knowledge gaps

Non-biological, non-agronomic areas

Controversies and knowledge gaps concerning GM crops have been noted world-wide, since the early years of field introductions, especially in the USA (e.g. Lacy et al. 1991) and for a wide range of issues. Most of these issues are not strictly of biological nature, and are therefore not within the scope of this review. These controversies on the use of GM crops are of ethical, social, economic, or legal nature and are merely noted here, in order to avoid the impression that controversies and knowledge gaps would be limited to biological issues.

Such other areas of controversies include: bioethics (Ahuja 1997; Banner et al. 1998; Bruce & Bruce 1998; Ryan et al. 1999), GM-products trade negotiations (Zach 1999), general economic aspects (Bogdanovic 1993), regulatory policies regarding genetically engineered foods (McCullum 1997), intellectual property rights and the patenting of biotechnological innovations such as improved varieties (Anonymous/FAO 1995; Baumann et al. 1996; I. Kroes in: Spiertz & Dons eds 2000), legal constraints on biotechnological research (Tucker et al. 1993), legal obligations of producers (Michael 1999), legal aspects of GM food (Schubert et al. 1990; Baumann et al. 1996; Law 1997), and risk assessments (e.g. Kasanmoentalib & Longino 1996).

Biotechnologists have particular concerns about controversies. Most of GM scientists (>90%) appear to see the present public concerns as a threat to their competitive edge in the field of GM (Rabino 1992).

The problem of proper communication between all the actors involved in the public debate on GM crops (Scholderer et al. 1999) has received attention for a long time in both Europe and North-America: e.g. Lacy et al. (1991) mention that 'Biotechnology [in the USA] is fraught with concern and controversy within both the scientific community and the broader public'; the first passage of the Genetic Engineering Bill through the Bundesrat in Germany showed that a great number of fundamental and detailed issues are controversial (Schubert et al. 1990). The latter authors concluded that sustained discussion is necessary so that satisfactory results are ultimately achieved. This last concern is indeed receiving more attention nowadays, e.g. with regard to respecting the public as 'competent civilians' rather than as ignorant consumers (HJ Achterhuis in: Spiertz & Dons eds 2000; cf. Baumann et al. 1996), as neglected for instance by Penman (1994) and Reynolds (1997), and to avoiding making unbalanced utopic predictions (HJ Achterhuis in: Spiertz & Dons eds 2000).

Differentiating between opinions and experimentally established scientific data.

In the following, all controversies and knowledge gaps regarding genetic modification (GM) are presented in the form of questions, with answers listed in two parts:

1. **Conclusion** regarding current state of knowledge in the literature (by present authors).
2. **Literature data** found on the subject, as presented by other authors, forming the basis of the conclusion.

*Our interpretations and conclusions are printed in italics, to assist the reader in separating them from experimental data.*

### 3.1 Design of DNA constructs and GM crop varieties

The phase of design of a GM crop is considered to be crucial, not only in dealing with potential benefits and risks, but also in exploiting all of the relevant knowledge available on the functioning of plants, crops, ecosystems and food webs. In the design phase of a GM crop, theoretical and experimental analyses, risk and technology assessments and feasibility studies may be performed in order to deal with those aspects. Such assessments should cover all relevant levels of biological organisation, from the level of DNA to the level of (agro-) ecosystem. A literature review on such assessments is beyond the scope of the present study. The next sections of this study deal with the various levels of biological organisation, from DNA to GM food.

#### Conclusion

Important biological limitations to successful design of GM crops include the lack of knowledge of biochemistry and physiology (e.g. signalling pathways and gene regulation, metabolic regulation, pest and stress tolerance), ecology (e.g. biotic interactions), agronomy (e.g. pest management), and toxicology (food safety; allergies), and a lack of knowledge exchange between these areas.

#### Literature data

Several authors stress the importance of the design phase of GM crops for increasing the chances to successfully achieve goals regarding genetic, physiological and agronomic performance and the ecological and toxicological interactions of the GM crop, e.g. Arndt & Rank (1997; genome structure and regulation of gene expression); Ebinuma et al. (1997; selection markers); Kapley et al. (1999; bioremediation); Hum-Musser et al. (1999); Lotz et al. (2000; discuss interaction of scenarios and designs); Nguyen et al. (1997; stress tolerance); Tucker (1993); Zhang et al. (1999; drought resistance). Other aspects requiring attention in this phase include social, economic, legal, and ethical aspects (see first part of section 3).

### 3.2 Unknown Mechanisms of genetic modification (GM) and breeding

General information regarding current practise of genetic modification may be found in several extensive reviews and other studies (e.g. De Maagd et al. 1999; Dunwell 2000; Ellstrand et al. 1999; Franck-Oberaspach & Keller 1997; Hilder & Boulter 1999; Maessen 1997; Ryan et al. 1999; Tucker 1993).

Current practise of GM mainly involves the following techniques of transformation: *Agrobacterium tumefaciens*; Micro-particle bombardment ('biolistics'); Electroporation.

Characteristics of GM include:

- Transformation through recombination of the recipient genome with some or all of the following components:
  - selectable markers, usually from microbial and/or virus origin
  - a number of tool-box-genes usually from microbial and/or virus origin
  - main gene(s) of interest (goi) which may (transformation) or may not (xeno-transformation) be originating from another species; such a goi is missing in 'empty vector' constructs.
- Precise knowledge about the sequence of the genes (DNA) and about the primary functioning of the genes in the donor species *before* insertion/transformation; however, much less knowledge about the sequence, location, (partial) copy number and functioning of genes *after* the transformation event;
- Uncertainties regarding the site and number of successful inserts, i.e. random insertion of the donor DNA construct into the genome of the recipient, and an a priori unknown number of copies, which may not be complete; uncertainties about associated changes in genome structure and

function, and difficulties with creating desirable types of regulation of gene expression. New methods avoiding random insertion are being developed, including site-specific recombination-mediated and homing endonuclease-mediated chromosome modifications; however risks associated with these methods have been identified (Gilissen & Nap 1999).

3.2.1 Does the interspecific (or xeno-) transformation in GM differ from the classical transformation that occurs naturally between closely related species?

#### **Conclusion: Gap**

There is a gap in knowledge of scientific-fundamental differences between interspecific (or xeno-) transformation (as in genetic modification) and intraspecific transformation.

This question is relevant to public concerns about GM leading to 'breaching of species barriers' of which subject very little (experimental) knowledge is available.

#### **Literature data**

Interspecific transformation will be referred to as xenotransformation, in analogy to xenotransplantation. Xenotransformation is one of the prime features of genetic modification (GM), constituting a 'breaching of species barriers', if not in principle (e.g. because of viral DNA transfer, and actions of transposons) then likely with regard to its current scale, occurrence and nature (e.g. involving genes of various origin, including hazardous antibiotic resistance genes; Van Dam & Schenkelaars 2000). It should be noted that the classical (bacterial) transformation is not in any way related to conventional or classical breeding of crop plants.

Unlike modern GM, classical transformation occurs naturally between closely related species of bacteria (Schlegel 1972). In the literature, little attention is paid to mechanistic differences of xenotransformation as opposed to transformation between related species. There is, however, a scientific basis for distinguishing xeno-transformation from the classical transformation.

The term transformation has been -and is being- used in microbiology to indicate the transfer of genetic information between *closely related* species, especially bacteria, resulting in surviving cells displaying the newly transferred characteristic in an inheritable way (Schlegel 1972). This still is the general rule, with only very few exceptions (e.g. the first demonstration of conjugal plasmid transfer from *Escherichia coli* to *Bartonella henselae* has been reported by Dehio & Meyer in 1997). This transformation contrasts with the anthropogenic transfer of DNA between organisms of widely different phylogenetic origin, as is common practise in GM (see also 3.5.4). In the light of recent findings on differences in structural and functional aspects of genomes between -for instance- micro-organisms and eukaryotes, it is necessary to make a distinction between transformation and xenotransformation. In analogy, the terms transplantation and xenotransplantation are used in the medical literature to indicate transfer of organs between individuals of the same and different species, respectively.

Characteristic for different species are their differences in functionalities, based on often minor but significant differences in genome structure (e.g. non-coding regions or 'Junk' DNA, Fransz et al. 2000), DNA structure (e.g. Shimizu et al. 1997); enzyme structure & function and corresponding gene sequence and regulation.

These different functionalities are associated with (e.g. Lehninger 1982):

- Differences in cellular compartmentation between bacteria and higher plants, associated with protein targeting by signalling sequences;
- Differences in genome structure and function, and in mechanisms of regulation of gene expression between micro-organisms (prokaryotes) and plants (eukaryotes);
- Large differences in secondary metabolism between higher plant genera.

Interspecific transformation has been referred to as 'Breaching species barriers' (e.g. Ryan et al. 1999; Van Gool 1999). Some examples of unintended side-effects, potentially related to species-specific differences in regulatory characteristics are discussed here for the case of aluminium tolerance (see also paragraph 3.2.1).

*Examples of trial and error in GM: improving aluminum tolerance in plants*

Aluminum soils are unproductive for most crop varieties, while common in the world, especially in the tropics (e.g. Herrera-Estrella 1999). Biotechnologists have attempted to solve the problem of Al-toxicity in crops by overexpression of a bacterial gene coding for citrate synthase (CS) in tobacco plants. The choice of CS was based on the outcome of physiological research showing increased immobilisation of Al by organic acid exudates from roots of Al-tolerant plant species (de-la-Fuente-Martinez & Herrera-Estrella 1999; Sasaki et al. 1996). This approach was successful in generating Al-tolerance in tobacco.

*This is an example of adequate integration of physiological and biotechnological knowledge, and one important step forward. However, there are more steps needed towards a properly functioning crop. Overexpression is still a primitive way of gene regulation often leading to unintended side-effects (e.g. Winicov 1998; Delhaize et al. 1999). It remains to be seen whether the bacterial enzyme can be properly regulated in the plant, preferably only in the roots, and induced by aluminum (gap). Agronomic performance, ecological interactions and toxicological effects of the new crop were not evaluated.*

In another case, a plant cDNA was selected on its ability to confer Al-tolerance in yeast, i.e. TaPSS1 encoding a phosphatidylserine synthase (PSS; [CDP-diacylglycerol-serine O-phosphatidyltransferase]) from wheat, *Triticum aestivum* (Delhaize et al. 1999). When the TaPSS1 gene was overexpressed in *Arabidopsis* and tobacco (*Nicotiana tabacum*), the plants showed necrotic lesions on leaves, associated with accumulation of large amounts of phosphatidylserine (PS) at the expense of other phospholipids in the cells. These phenomena indicated poor regulation of PSS activity (Delhaize et al., 1999).

*Whether this poor regulation was caused by species-specific differences in regulation of PSS activity, i.e. between wheat (donor) and Arabidopsis and tobacco (acceptors), was not clear.*

Ezaki et al. (1999) isolated eleven aluminium (Al) stress-induced genes derived from plants (wheat, *Arabidopsis* and tobacco) and introduced these genes into *Saccharomyces cerevisiae* (yeast) to test if expression would confer Al-tolerance. Al sensitivity tests showed that expression of two (out of eleven) genes, either an *Arabidopsis* gene for blue copper binding protein (BCB), or a tobacco gene for the GDP dissociation inhibitor (NtGDI1), conferred Al-tolerance. Determinations of total content and localization of Al ions in these transformants suggested that the BCB gene product functions in restricting Al uptake, while expression of the NtGDI1 gene promotes release of Al ions after uptake (Ezaki et al. 1999).

*It remains unclear what the reasons were for absence of Al-tolerance in nine out of eleven transformants. Also, no study was conducted to test for normal growth and development in the two Al-tolerant transformants.*

Another question, raised by findings in organisms other than higher plants, concerns the impact of changes in GC content of the plant genome (e.g. Mouchiroud et al. 1997; Ricroch & Brown 1997).

3.2.2            Are there predictable, unintended effects of random insertion of DNA constructs on gene expression and genome functioning and stability?

**Conclusion: Controversy & Gap**

The question is relevant to general concerns among the public about random changes to well-functioning organisms. This is a conspicuous issue of many unknowns and fundamental problems. There is



hardly any report of systematic experimental studies comparing the functioning of crop plants originating from several different (xeno)transformation events; the few reports found are conflicting.

Genome structure and function are important for gene expression (regulation); yet, there are many fundamental unknowns, including the nature of the differences between bacteria, yeast, plants and animals in structure and functioning of the genome in relation to other differences like cellular compartmentation.

Indirect, non-specific effects of (random) DNA insertions are known. Any currently used technique of genetic transformation is accompanied by an insertion mutation, that may or may not occur in a coding region of the genome (De Jong et al. 1999; Kahl et al. 1995; Von Wettstein 1995), OR, if within a non-coding region, may occur in a DNA-folding-instruction region (c.f. Fransz et al. 2000).

Such effects are evident from 'empty vector' transformants, i.e. transformants without a 'gene of interest'. Empty vector effects, mechanistically related to insertion mutations, are well known, but seldomly described in the literature (Gap), highly variable effects depending on the transformation event, even without any involvement of a gene of interest. The origin of such 'empty-vector' effects is generally not known.

#### Literature data

Gilissen & Nap (1999) support the relevance of the above question, and show the absence of adequate and safe methods circumventing the problem. They confirm that current methods of GM of plants result in insertions of DNA into the host genome at random and often multiple sites, and that, associated with this, position effects, copy number differences and multigene interactions make gene expression highly variable and the occurrence of desired phenotypes less predictable. These authors pose the question, whether the tools that are presently being developed to avoid such problems by insertion of DNA copies at predetermined sites in the plant genome, have risks associated with them (Gilissen & Nap 1999). These tools involve enzyme systems that are site-specific recombination systems and homing (rare-cutting) endonuclease systems. In an attempt to identify and evaluate possible biosafety issues related to applications of these systems, Gilissen & Nap (1999) conclude that, without technological improvements, endonuclease-mediated targeting is unlikely to become a popular method for agronomically relevant gene targeting in plants, because of various unpredictable changes in the host genome. Also they conclude that GM plants with high constitutive expression of a recombinase gene require special consideration when aimed at field and market introduction, and that the occurrence of homing endonuclease system phenotypes with effects on plant fitness and toxicological characteristics cannot be excluded. They argue that plants expressing highly constitutively a transgenic endonuclease gene, deserve special consideration in biosafety assessment. Also, chromosome modifications from transgenic homing endonuclease activity at an introduced transgenic recognition site will not cause ecological and toxicological effects other than possible effects caused by the nature of the incoming DNA and the associated illegitimate recombination events (Gilissen & Nap 1999).

Maessen (1997) notes that the level of gene expression varies with the site of integration. Since the positioning of the integration is a random, unpredictable process, this means that gene expression is unpredictable for each transformation event. Also, instability of inheritance generally becomes visible within a few generations, but once it is stable it is supposed to remain so provided the environment does not change dramatically. Transposons (TEs) affect the functional stability of the genome (cf. Lonig & Saedler 1997). However, the frequency of transposing in most crop species is not known (Maessen 1997).

Gaps in the knowledge of the control of gene expression in (transgenic) plants are also illustrated in the following studies:

Arndt & Rank (1997) review the well-documented use of complementary RNA sequences and argue that despite the simplicity of this approach, the technique usually results in only partial suppression of gene expression and, in some instances, even fails to regulate the gene of interest.

The authors expect that recent advances in the understanding of the global architecture of the nucleus, chromatin structure, and RNA metabolism provide useful and necessary information for designing novel approaches to improving antisense RNA and ribozyme regulation. Available data suggest that the position of genes within the nucleus is not random and that transcripts produced from these genes follow specific tracks in migrating to the cell cytoplasm.

Random insertion will have (unknown) consequences for the structure of DNA and thus, for DNA-protein interactions and genome functioning and regulation of gene expression (cf. Travers 1993). This author demonstrates the importance of role of dynamic interactions between proteins and nucleic acids, which is increasingly being recognized as an important regulator of gene expression, with differs between eubacteria and eukaryotic organisms like plants.

Confirming the notions of Travers (1993), Strahl & Allis (2000) observed distinct modifications of post-translational histone (DNA binding protein), on one or more tails of the molecule, and propose that these are acting to form a 'histone code' that is read by other proteins to bring about differential regulation of gene expression.

*Gap: DNA methylation, gene expression and genome functioning*

An intriguing process that is playing a role in controlling (the timing of) gene expression is DNA (de)methylation.

DNA (de)methylation plays a role in gene regulation (e.g. Regev et al. 1998). Tatra et al. (2000) observed that lower levels of cytosine methylation are associated with low red/far-red ratios of light incident on ecotypes of *Stellaria longipes*. These data led them to suggest that DNA demethylation (=gene activation) is involved in the shade-avoidance response of these plants. An important question that remains is: what is the role of DNA (de)methylation in genome functioning and gene expression in (transgenic) crop plants? (Shimizu et al. 1997).

To our knowledge, little information is available on differences in methylation between taxons or species (however, cf. Shimizu et al. 1997). While data on higher plants are scarce, some interesting studies exist on animals. Monk (1995) reviewed data on changing patterns of DNA methylation and the regulation of gene expression in mouse embryonic development. Global demethylation of the DNA occurs from the eight-cell stage to the blastocyst stage in preimplantation embryos, and global de novo methylation begins at implantation. Monk raises the question whether methylation could be causal to gene inactivation, as well as being involved in its maintenance, and presents a picture of the inheritance of methylation imprints. On a more general level, an hypothesis of evolution by 'adaptive epigenetic/ genetic inheritance' is considered. This proposes modification of germ line DNA in response to a change in environment and mutation at the site of modification (e.g., of methylated cytosine to thymine). Epigenetic inheritance could function to shift patterns of gene expression to buffer the evolving system against changes in environment. If the altered patterns of gene activity and inactivity persist, the modifications may become 'fixed' as mutations; alternatively, previously silenced gene networks might be recruited into function, thus appearing as if they are 'acquired characteristics.' An extension of this hypothesis is 'foreign gene acquisition and sorting' (selection or silencing of gene function according to use). 'Kidnapping' and sorting of foreign genes in this way could explain the observation that increased complexity in evolution is associated with more 'junk' DNA.

Adaptive epigenetic/genetic inheritance challenges the 'central dogma' that information is unidirectional from the DNA to protein and the idea that Darwinian random mutation and selection are the sole mechanisms of evolution.

3.2.3 Are there differences in mechanisms between classical, or conventional, breeding (CB) and genetic modification (GM)?

### **Conclusion: Controversy & Gap**

There is a clear controversy over potential differences between CB and GM. While no systematic research on (crop) plants has been found on this subject, only one study on yeast and one on wheat were found where a comparison is made between organisms with one particular new trait created by either CB or GM. The question is highly relevant to the debate on the use of GM crops, because it deals with the potential uniqueness of GM crops, which, when confirmed, should have consequences for political decisions and regulations.

### **Literature data**

Regarding the relevance of the question: In the perception of the public, breaching species barriers is often seen as a source of potential risk to health and environment (e.g. Van Gool 1999, 2000; Franck-Oberaspach & Keller 1997). How important are such differences between CB and GM for assessing risks and benefits in ecophysiological functioning? Many biotechnologists seem to be convinced of a negative answer. However, there are no data available for an adequate evaluation of the question.

The question may be considered from various standpoints other than 'natural-science' (cf. Lammerts van Bueren et al. 1998, 1999). For instance, socio-economically, several reports indicate considerable differences, e.g. in the required levels of capital investment (Nguyen et al. 1997; Vasil 1998), knowledge infrastructure (Burnell & Dowds 1996, Hum-Musser et al. 1999, Tucker 1993).

The issue of a (fundamental) difference between CB and GM is controversial within the area of natural science, since both the answers 'no' and 'yes' to the question are found in the literature. For instance: Some authors consider a comparison of CB and GM as incorrect or irrelevant, arguing that it is not the breeding technique by which a plant was produced that should matter, but the characteristics of the plant produced (e.g. Gilissen & Nap 1998).

Others see no difference between CB and GM: Trewavas (Univ. of Edinburgh) and Leaver (Univ. of Oxford) in *Nature* of January 6, 2000: '...all the food we eat has been continuously engineered by natural phenomena in ways that do not differ in any fundamental way from the current GM technology'. Ryan et al. (1999) present a similar opinion, although with some reservation: 'Although techniques required to create GM crops are recent and relatively sophisticated, genetic modification is in most respects an extension of what has been happening for ten thousand years'. With regard to the dominating influence of the 'genotype x environment'- interactions Brennan et al. (1999) see no difference between CB and GM in the level of disturbing the outcome of breeding efforts. The authors note that genotype x environment fluctuations in fruit quality exist, potentially reducing the quality in the genotypes developed by both classical and biotechnological means.

On the other hand, differences have been mentioned by other authors, with viewpoints outside (Lammerts van Bueren et al. 1998, 1999) and within the natural sciences, e.g. Franck-Oberaspach & Keller (1997), while an implicit 'yes' to our question is evident in articles by other authors, e.g. Von

Wettstein (1995), Honee (1999) and Daniell et al. (1998). Their opinions are presented in more detail because of the wealth and variety of arguments.

GM differs from CB according to Franck-Oberaspach & Keller (1997) discussing the consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity. The first food products derived from transgenic plants that are resistant to diseases, insects or viruses are now reaching the market and there is growing public concern about problems of allergenicity and toxicological changes in such transgenic food plants. The source of the transgene is of great importance for the application of immunological assays. Several 'self defence' substances made by plants are highly toxic for mammals, including humans. The source of the transgene is of no relevance in assessing the toxicological aspects of foods from transgenic plants. Food safety can also be severely influenced by invading pathogens and their metabolic products.

The authors conclude that a proper comparison may be a trade-off between 'nature's pesticides' produced by transgenic plants or by varieties from traditional breeding programmes, and synthetic pesticides and mycotoxins or other poisonous products of pests.

An implicit 'YES' to the question of difference between GM and CB is also found in other papers. Daniell et al. (1998) see GM as an alternative to CB: 'In the past, cotton fibre quality has been improved by classical plant breeding; however, this approach is limited by species incompatibility and available traits. An alternative approach is to introduce foreign genes to confer desired traits into cotton via genetic engineering. Protein-based polymers (PBPs) are available in nature as materials with extraordinary mechanical properties, such as spider webs composed of silk threads tougher than steel and elastin. Therefore, introducing this PBP into cotton fibre should increase fibre strength, water absorption, thermal characteristics and dye binding.'

The view that GM differs from CB, is also implicitly evident from a paper by Honee (1999) who notes that genetic engineering has provided strategies in an addition to other strategies like those based on CB; most of the GM strategies are based on the overproduction of one component of the plant's own defense response. These approaches to increase resistance to fungi have been successful under laboratory conditions. Incorporation of these strategies in resistance breeding programs of agriculturally important crops will depend on the results obtained from field experiments.

Burnell and Dowds (1996) argue that the methodologies of classical genetics and genetic engineering (GM) can both be used, in their specific case for the genetic improvement of entomopathogenic nematodes (EPNs) and their symbiont bacteria. However, they see a greater need for basic knowledge in the case of GM. They conclude that much basic research is needed for any progress to expect. The authors conclude that there is a lack of basic information on the genetics and biochemistry of the characteristics that might be altered by transgenic methods in EPNs, and their bacteria.

As a beneficial difference with CB, GM is often suggested to be faster in producing new improved crop varieties (Boerjan et al. 1999; Jung & Wyss 1999; Nguyen et al. 1997; Renard et al. 1997). *However, no experimental data have been found to support this claim.*

For instance, a specific advantage of GM is seen in the case of tree-breeding. Boerjan et al. (1999) discuss the genetic improvement of trees which is a slow process in comparison to that of annual crops. Tree breeding though is important, given the ever increasing demand for wood and wood products. The authors intend to show that the classical genetic improvement of trees by breeding and selection can be assisted and accelerated by the application of molecular biology tools that have been developed over the last decade. They give two reasons. First, it is now possible to develop a set of diagnostic markers that predict the characteristics of new hybrids soon after they have germinated, thus long before the traits are displayed. Second, genetic engineering allows the modification or addition of a

given trait that would be difficult or impossible to obtain by conventional breeding. Case studies in both fields, with respect to disease resistance and wood quality, are presented.

A fair comparison of CB and GM may be difficult, because GM is a relatively young technique (e.g. in barley breeding: 6 yrs) as opposed to e.g. selection (since 5000 yrs) and the use of induced mutation (since 1927). Von-Wettstein (1995) reviews the history of breeding of barley which has been bred for food, feed and beverages by selecting for spontaneous mutations and random hybrids. Genetic transformation of barley has been a routine procedure since 1994 and permits the introduction of tailored genes for adding quality value to the grain. The author argues that it complements, but does not replace, existing efficient breeding methods, and that radiation- and chemically induced mutations, as well as genes introduced by transformation, have to be fitted into the genome, which may take 50 years of breeding effort and testing for agronomic and industrial performance.

#### *Insufficient direct experimental evidence*

Only ONE example of an experimental study has been found where a comparison was made between new organisms of one species with identical acquired traits, while originating from either CB or GM: this concerned a yeast utilizing melibiose (Vincent et al. 1999), with similar success reported in both ways of breeding.

*However, no long-term risk assessments were made, nor was any genome structure analysis undertaken to investigate potential differences.*

A study on crops coming close to a proper comparison, is that of Baga et al. (1999), on increasing levels of unbranched starch in wheat, by GM or CB (see next page).

*However, the authors do not present a proper comparison, and are unclear about the actual quantitative aspects of the changes in unbranched starch levels brought about by GM (BE enzyme levels are no conclusive evidence; cf. Fell 1997), while reporting large effects achieved by CB (>95% unbranched starch).*

#### *Breaching species barriers and 'tampering with nature'*

What evidence is there to support the term 'tampering with nature' as a characteristic of GM more than of CB (van Gool 1999)? This question cannot be answered from our standpoint in the present study, which is strictly in natural-science. The discussion on 'natural' vs 'unnatural' is of a philosophical nature, and outside of the scope of our study (however, see for a discussion, Visser & Verhoog (1999); Dr. H. Verhoog has started an NWO funded project on this subject at the Louis Bolk Institute, Driebergen, Netherlands).

However, one aspect of this question that is within our 'window of natural-science expertise' concerns the so-called 'breaching of species barriers'. This topic is discussed in section 3.2.1.

Regarding the distinction between natural (CB) and unnatural (GM), Ryan et al. (1999) state: "The question of how to decide whether GM crops are 'unnatural' to an unacceptable degree is more difficult to address'. These authors mention a theoretical (natural-scientific) argument for their conclusion 'no difference': i.e. 'because the same gene complement is achieved by CB and GM'. This is surprising, since it is known that in practise 'the same gene complement' may only be achieved at present at a certain degree, and has not yet been reported in the literature (knowledge gap). A clear definition of the term 'same gene complement' will be required before any further discussion of this issue. This should be part of a methodological analysis identifying the various 'windows of concern' and 'sets of relevant questions' (cf. Van Dommelen 1999) of the participants in the debate.

*How different are the DNA and genomes of distantly related species?*

Also, Ryan et al. (1999; 1.34) give the impression to deny the very existence of differences between CB and GM, by stating: 'In what way is a gene that is found in a fish and which might be very similar in structure and function to one found in a micro-organism, plant or animal, a 'fish' gene? Some would say that it is no more than a defined stretch of DNA in a fish cell. But that does not seem to help. What lies behind such concerns?'

*Comment:*

*In order to throw some light on this issue: indeed, the literature provides some evidence for such concerns. First, the assumption 'which might be very similar in structure and function' is purely theoretical. In practise, even a highly conserved gene like the one encoding the wide-spread respiratory enzyme cytochrome oxidase (ca. 104-108 amino acids; with a similar function) varies greatly in structure, e.g. by about 20 to 30% between yeast and fish (Vogel & Angermann 1971). Secondly, in the light of current knowledge about the complex mechanisms of regulation and control of metabolic pathways and modern control analysis of (allosteric) enzyme activities (Fell 1997), such structural differences are bound to have profound effects on the functioning of the enzyme in the transgenic organism (cf. Dressler & Potter 1992). Also, protein trafficking differs greatly between bacteria and eukaryotic organisms (signalling sequences, etc.).*

Fell (1997) argues that the lack of understanding of metabolic regulation has been revealed by poor results from attempts to increase the rates of selected metabolic pathways by genetic engineering techniques. Current biochemical theory predicts that it is relatively easy to decrease the activity of a metabolic pathway, as opposed to increase it (Fell 1997).

**Reported differences between CB and GM, mainly associated with 'breaching species barriers' and with 'poor gene regulation' and 'random insertion, c.q. multiple and/or partial insertions'**

- a. The genetic code is largely universal, exceptions have been demonstrated. Differences exist between bacteria and eukaryotes, between plants and other eukaryotes, and between mitochondrial and nuclear DNA (Lewin 1990)
- b. Inserted gene-DNA structure: this must be different from other genes in the recipient genome, to avoid gene silencing, which is generally caused by additional identical copies of genes; also, differences in DNA-signalling sequences exist between species (address labels for protein trafficking, related to compartmentation of functional proteins), and differences in base methylation, cf. Shimizu et al. (1997)
- c. Neighbouring DNA structure on both sides of gene-construct-DNA: certainly different between species also, flanking DNA regions are known to affect gene expression, see Maessen 1997; Fransz et al. 2000)
- d. Gene origin: generally (most tool-box genes in DNA constructs are of bacterial or viral origin)
- e. Expression regulation mechanisms: in contrast to natural gene regulation, the promoters used to regulate gene expression in present GM crops are often not-inducible, not organ specific, constitutive, leading to overexpression (see sections 3.2.5 & 6, and 3.3.3)
- f. Genomic location (positioning of new DNA where and on which chromosome) differs between CB and GM; reliable targeting techniques in GM are not yet available (Gillissen & Nap 1999)
- g. Partial DNA inserts: evidence is increasing for the presence of multiple and partial copies of inserted constructs in transgenic crop plants, for instance in Roundup-Ready Soybean of Monsanto (cf. Van der Horst 2000); avoidance or removal of such partial DNA inserts are unexplored techniques, while needed to prevent unintended side-effects like production of new, unknown proteins (e.g. risk of new allergens)
- h. Post-transcriptional modification, translation, and regulation of enzyme activity: most likely, because of
- i. Speed: increasing breeding speed has been claimed and disputed; controversial issue; Renard et al. (1997) and Boerjan et al. (1999) argue that the use of genetic transformation combined with classi-

- cal breeding techniques allows faster development of new varieties and rapid selection. Also, Jung & Wyss (1999) and Nguyen et al. (1997) suggest a quicker breeding process by the use of GM.
- j. GM is more knowledge-intensive than CB (cf. Tucker 1993; Burnell and Dowds 1996)
  - k. GM is more capital-intensive than CB. For instance, Vasil (1998) stresses the importance of long term investment in addressing the challenges of biotechnology in trying to feed the burgeoning world population: 'International efforts to transfer biotechnology to developing countries have been largely ineffective due to a lack of scientific and industrial infrastructure. Long term investment in these areas will enable developing countries to make full use of biotechnological advances'. However, a potential role of GM in world food security is debated (e.g. Waelbers 1998). The main arguments involved in this debate are of a socio-economic nature and highly relevant (e.g. Anonymous 1996, FAO/WHO: 'World food scarcity is caused by poverty, not by lack of technology') have to be outside the scope of this study.

- 3.2.4            How reliable are present-day genome databases and annotations?  
                          Is there an adequate proofreading system operative?

**Conclusion: Gap & Risk**

While widely recognized as a risk, few reports of a systematic experimental study are available; there is now increasing attention for bioinformatics.

The question is relevant to the debated issue of the 'precision' of GM practise.

**Literature data**

Genome databases and annotations are 'Not sufficiently reliable; not operative in a well-organized manner', according to Claverie (2000) and Rouzé (2000). Many errors have been detected in existing genome databases, in preliminary studies, especially in annotations.

These questions are relevant to the level of predictability of genetic modifications, which is generally claimed by biotechnologists to be higher than in classical breeding (e.g. Harlander 1991; De Jong et al. 1999).

- 3.2.5            What is known about DNA & chromosome structure and function?

**Conclusion: Gap**

The term '**Junk DNA**' reflects the obvious gap in knowledge of molecular biologists, illustrated by recent evidence for a function of a non-coding DNA stretch in chromosome folding, up to then identified as junk DNA (Fransz et al. 2000). The question is relevant to the debated issue of the 'precision' of GM practise.

**Literature data**

*The following example illustrates the poor knowledge of genome structure and function, and how to deal with these in genetic engineering.*

The term 'Junk DNA' has been poised to indicate stretches of DNA without a known function, that happen to constitute the major part, often more than 80%, of large genomes. Recently, this 'junk DNA' was shown to have an –obviously unexpected- function, i.e. in the folding of a chromosome, by Fransz et al. (2000) in one of the leading scientific journals, Cell.

These authors started correlating the DNA sequence with the structure and function of the chromosome, which is required for elucidating the role of heterochromatin (condensed, non-expressing regions) in the regulation of gene expression in eukaryotic organisms. Their object of study was the short arm of chromosome 4 (4S) of *Arabidopsis thaliana*, a cosmopolitan plant species with a relatively simple structure of its chromosomes (Fransz et al. 2000) and a relatively small genome containing small amounts of 'junk DNA'. According to Maessen (1997), its genome size is only 1.1% of the size of the wheat genome. Thus, it must be clear that we have only just started to uncover the role(s) of 'junk DNA', with undoubtedly many surprises ahead of us, when exploring the larger genomes.

Genome sizes in higher plants vary by three orders of magnitude (a factor 1000). Large genomes, which contain more non-expressed heterochromatin or 'Junk DNA', may be expected to allow a higher (relative) incidence of inactive transgenes, although evidence is lacking. Any insertion may result in a destabilized genome and lower viability (Maessen 1997). According to Ryan et al. (1999), such unexpected effects do not necessarily mean that the GM technology used is unsafe, as plants showing such side-effects are generally discarded in the subsequent breeding process (cf. Maessen 1997), but they do point to the need for vigilance in the regulatory procedure (Ryan et al. 1999). However, in practise, several examples are now available of unexpected side-effects of primary GM modifications, including physiological disorders (e.g. Delhaize et al. 1999; Gertz et al. 1999; Murray et al. 1999).

*The very fact of naming the unknown 'junk', whether or not exemplifying the attitude of biotechnologists towards nature, may reflect an interesting aspect of the communication problems that some scientists have in the current debate on GMOs.*

Kahl et al. (1995), in an article entitled 'Junk DNA - not so junky after all', presented the various experimental functions of a particular species of 'Junk DNA', i.e. microsatellites, simple sequence repeats. Their exploitation in DNA profiling techniques has expanded the repertoire of useful sequences for plant biologists. Some old and new techniques involving microsatellites are presented, such as microsatellite primed polymerase chain reaction (PCR), anchored microsatellite primed PCR, random amplified (microsatellite) polymorphic DNA (RAPD) analysis, the generation of microsatellite fingerprints in RAPDs and the production of sequence tagged microsatellite sites.

Schmidt & Heslop-Harrison (1998) suggest that it is now possible to obtain a better understanding of the structure and functioning of plant genome. They show that plant species from wide taxonomic groupings have similar genes and ordering of genes along the chromosomes. However, the repetitive DNA, much of no known function and often constituting the majority of the genome, varies extensively from species to species in absolute amount, sequence and dispersion pattern. Despite this, it is known that families of repeated DNA motifs each have a characteristic genomic location within a genus, and that there are different constraints on the evolution of repetitive DNA and genes.

The authors conclude that there are now enough data about different types of repetitive DNA, from sequencing, Southern analysis and in situ hybridization, to build a model of the organization of a typical plant genome, and apply it to gene cloning, evolutionary studies and gene transfer.

3.2.6 Do we understand the mechanisms of regulation of (trans)gene expression in higher plants?

#### **Conclusion: Gap**

The gap in present knowledge of the regulation of gene expression is evident from the large number of failures and unintended side-effects reported in the literature (see also 3.3.3).

This subject is relevant to the debated issues of 'well-defined' changes and the 'precision' of GM practise.



## Literature data

At present, the answer must be that knowledge on the control of gene expression in higher plants has increased dramatically but nevertheless often appears to be inadequate for successful application. This means that at present it is generally not possible to control the expression of transgenes in an adaptive way in time and space, i.e. under the proper conditions and at the desired developmental stage and in the target plant part. In a review on mechanisms underlying stability of gene expression, Maessen (1997) noted that it is not possible to draw final conclusions about the stability of transgene expression because of limited and preliminary data, identifying a number of gaps in knowledge. While there are no indications that the introduced GM trait is less stable than the non-modified trait, insufficient data are available about the mechanisms underlying the high variability of transgene expression, about the randomness of transgene integration, about the mechanism of the (positive) effect of MAR (matrix-associated regions of DNA) elements on gene expression, and about the utility of MAR elements in other species than tobacco.

Other recent developments support the above notions. One of the major challenges in genetic modification is the development of adequate controls of gene expression. An example of one of the first steps taken in this direction is the use of a leaf senescence-associated promoter by Gan and Amasino (for a recent report, see Jordi et al. 2000). Other recent attempts include the use of inducible promoters (e.g. Salter et al. 1998; Goddijn & Van Dun 1999) and the use of a cascade of chimaeric genes (including toxin and anti-toxin genes) to correct for promoter leakage (Mouradov et al. 1998). However, the general picture is, that properly adaptive transgene regulation is not yet achievable.

*'Our ability to interfere with an organism's genetics has far outstripped our ability to predict the effects on its metabolism'*  
(Fell, 1997)

### 3.3 Genetic and physiological performance of GM crops

The questions below are considered to be relevant to debated issues like 'unintended effects on crop performance under specific environmental conditions', 'the need for GM', 'alternative approaches to GM', 'stability of GM crop characteristics', 'feasibility and risks of gene-pyramiding, and 'predictability of (unintended) effects of GM on crop performance' and 'effects on (agro)ecosystems and food webs'.

In most areas not enough is known about plant physiology and biochemistry to provide clear-cut designs for GM crops with improved performance. Metabolic control analysis theory predicts and has successfully predicted that in biotechnological applications, to ensure a significant increase in a metabolic flux without a marked change in metabolite concentrations, it is necessary to simultaneously activate many, even most of the pathway enzymes (Fell 1997). This view is supported by others. Effects of GM on secondary metabolism is an open and not researched aspect according to Richard D. Firn and Clive G. Jones, Secondary metabolism and the risks of GMOs, Nature 400, 13 - 14 (1999).

For instance, Zhang et al. (1999) mention current controversies on effective mechanisms of drought resistance. Plant water deficit is a component of several different stresses, including drought, salinity and low temperature, which severely limit plant growth and crop productivity. Genetic modification of plants to allow growth and yield under unfavourable conditions is an important component of the solution to problems of environmental stress. While disagreement and even confusion may characterize some of the discussions on what constitutes a significant and an effective mechanism of drought resistance in crop plants, osmotic adjustment (OA) is receiving increasing recognition as a major mechanism. The paper starts with the review of OA functions, genetic variation and inheritance, and theories and principles involved in commonly used protocols for quantifying OA. Emphasis is placed on a summary of current molecular strategies and advances in the improvement of plant stress resistance

through manipulating OA. Zhang et al. include a genetic engineering approach and a QTL mapping approach. They suggest that future promising strategies for improving drought resistance lie in molecular technology that allows genes or QTLs controlling OA to be tagged and isolated, these genes to be expressed in transgenic plants, and efficiency of breeding via marker-assisted selection to be improved, combined with physiological research.

3.3.1 Are present GM techniques, in combination with conventional breeding (CB), more successful than CB alone in exploiting genetic variation?

#### **Conclusion: Gap & Benefit**

There is a insufficient information on the subject, while both risks and benefits of GM have been described concerning issues like pesticide use, stress tolerance, and productivity and quality.

This question is relevant to issues like 'the need for GM' and 'alternative approaches to GM'.

#### **Literature data**

There are few studies which enable a proper comparison of CB with GM. Some of these mention both positive and negative results of GM, on insect resistance (e.g. Bowen et al. 1998) and aluminum tolerance breeding. The latter example is discussed below.

Aluminum tolerance has been bred into various crop plants by both classical breeding (CB) techniques and by a combination of CB and GM. Aluminum soils are unproductive for most crop varieties, while common in the world, especially in the tropics (e.g. Bouton et al. 1997; Herrera-Estrella 1999). Biotechnologists have attempted to solve the problem of Al-toxicity in various ways: one based on a bacterial gene coding for citrate synthase (de-la-Fuente-Martinez & Herrera-Estrella 1999), an other based on a plant gene, i.e. a cDNA (TaPSS1) encoding a phosphatidylserine synthase (PSS; [CDP-diacylglycerol-serine O-phosphatidyltransferase]) from wheat (Delhaize et al. 1999), and yet another dealing with testing eleven Al-stress induced genes derived from higher plants (wheat, *Arabidopsis* and tobacco) for conferring Al-tolerance to yeast; Ezaki et al. 1999). The first approach was successful (de-la-Fuente-Martinez & Herrera-Estrella 1999), the second was not, due to leaf necrosis (Delhaize et al. 1999), while in the last case (Ezaki et al. 1999) only two out of eleven types of transformants showed increased Al-tolerance. Ofcourse this may have been pure coincidence. Alternatively, the rate of success may have been largely determined by available knowledge of the physiology of aluminum tolerance.

Duncan et al. (1995) provide an example of successful breeding for multiple stress tolerance using biotechnological techniques without the use of GM, i.e. in vitro cell selection and somaclonal variation. A field selection protocol was developed for the three soil stresses and inter-stress evaluations were conducted in an effort to find multiple, stress-tolerant genotypes of *Sorghum bicolor* which is generally quite sensitive to salt and acid (high aluminium) soil stresses, but quite tolerant of drought stress. They report a variant frequency of 0.1 to 0.2% for stress tolerance and acceptable agronomic traits among the surviving somaclones. The authors conclude that the stress-tolerant regenerants had superior acid soil and drought stress tolerance to that of the donor parents, their yield capabilities under stress were superior to their parents, and their stress tolerance attributes were transferred in hybrid combinations.

*Most authors propose a combined approach, using both GM and CB, e.g. Santos et al. 1997) and:*

Morpurgo et al. (1997) discuss the problems associated with plantain (*Musa paradisiaca*) and banana (*Musaceae*) breeding and development of novel biotechnological techniques to overcome these

problems. Aspects considered include: limitations of conventional breeding methods for improving banana quality and yield; need for an increased variety of banana crops with good quality and yield, disease resistance, storage and shelf life characteristics; development of techniques aimed at facilitating banana and plantain breeding (etc., they give an extensive list of required types of research and facilities, etc); examples of traits that have been introduced by genetic engineering (herbicide, virus, insect and fungus resistance, genes for production of essential amino acids and for delaying fruit ripening); and the need to combine classical and novel approaches for continued development of improved crops.

3.3.2 Can physiological characteristics of transgenic plants be predicted for relevant environmental conditions?

### **Conclusion: Controversy & Risk**

There is a controversy regarding the level of predictability of characteristics of transgenic plants growing under various environmental conditions; some findings suggest risks (e.g. for farmers).

The physiological functioning of plants is found to be more complex than expected, as is evident from reported shortcomings of GM crops. The question is relevant to the claim of high precision of GM.

### **Literature data**

Several authors suggest that the performance of transgenic plants is highly predictable, e.g. Harlander (1991), and De Jong et al. (1999).

However, there are data indicating the opposite. For instance, some recent field crop and experimental laboratory observations indicate unexpected (and unintended) responses of GM soybean with glyphosate resistance based on a modified EPSPS (CP4 EPSPS; Gertz et al. 1999; discussed by Coghlan 1999b). These transgenic glyphosate-resistant soybean plants show splitting stems and yield reduction (up to 40%) under growth conditions of non-exceptional, high soil temperatures (ca. 45 °C), and about 20% higher lignin levels at normal temperatures (25 °C). Gertz et al. (1999) hypothesize that the addition of glyphosate-resistant EPSPS in these varieties might have altered the product distribution in the shikimate pathway which leads to aromatic amino acids, lignin, some vitamins and other secondary metabolites.

*The absence of a timely discovery of such an unintended side effect seems remarkable in the light of the existing extensive knowledge on lignin biosynthesis (for a review: e.g. Boudet et al. 1996) and effects of high lignin levels (Sasaki et al. 1996). Such knowledge and an interdisciplinary approach might have enabled a proper and timely prediction of such a side-effect of glyphosate resistance based on the modified EPSPS.*

Other examples of unexpected side-effects (pleiotropy), described in other sections, are: necrotic lesions on leaves of plants overexpressing phosphatidylserine synthase (Delhaize et al. 1999; 3.3.1), phytotoxicity of glucose oxidase expression conferring resistance to fungal infection (Murray et al. 1999).

- 3.3.3 Are pleiotropy and polygenic characteristics a barrier towards designing and predicting traits of GM crops like pest and stress tolerance, productivity and food quality?

#### **Conclusion: Gap & Risk**

There is a gap in present knowledge of the biochemistry and physiology of quantitative, multigenic traits of crops, like stress tolerance and food quality, increasing the risk of unexpected side effects of GM.

#### **Literature data**

Predictability of characteristics of GM crops is seen as a problem by many among the public, and appears to be a key-issue undermining public trust in GM (e.g. Website 1; Van Gool 2000; Schalk 2000). This adds to the relevance of the question.

Most authors provide a positive answer to the question. Tucker (1993) confirms the above-mentioned gap, in a review on the application of recombinant DNA technology to quality and processing properties of tomatoes, discussing genetic manipulation of pectolytic activity in tomato fruit (action of pectinesterase, reduction of polygalacturonase activity) and the control of ethylene synthesis. The author concludes that application of genetic engineering to improvement of food crops has great potential, but is limited by a sparsity of biochemical knowledge regarding the traits to be manipulated.

Also, Hum-Musser et al. (1999) see gaps in present knowledge of the physiological mechanisms of thermotolerance, while noting potential prospects for applying GM in improving heat tolerance.

Winicov (1998) stresses the present lack of knowledge on the physiological mechanisms of salt and drought stress, hampering molecular approaches to improving salt tolerance in crop plants. Improvements to salt and drought tolerance in crop and ornamental plants have been elusive, partially because they are quantitative traits and part of the multi- (or poly-) genic responses detectable under salt/drought stress conditions.

However, Winicov (1998) also stresses the great potential of GM and proposes new strategies of transgenic manipulation more sophisticated than the rough type of overexpression accomplished till now. The author sees many practical limitations of overexpressing all of the genes required for stress tolerance in a plant in a tissue specific manner that would maintain developmental control as needed. New approaches are being developed towards being able to manipulate expression of functionally related classes of genes by characterization of signalling pathways in salt/drought stress and characterization and cloning of transcription factors that regulate the expression of many genes that could contribute to salt/drought tolerance. Transcription factors that regulate functionally related genes could be particularly attractive targets for such investigations, since they may also function in regulating quantitative traits. Transgenic manipulation of such transcription factors should help us understand more about multigene regulation and its relationship to tolerance.

In a review on transgenic approaches to crop improvement, Dunwell (2000) observes a trend towards genetic modification of more complex agronomic traits such as growth rate and increased photosynthetic efficiency.

### 3.3.4 Are Genetic modifications in crop plants stable during several generations?

#### Conclusion: Risk

There is a risk of trans-genome instability, i.e. leading to loss of desirable traits, but this is usually restricted to the plant breeding phase where instable traits are lost or discarded in the selection process.

#### Literature data

It seems that instability is generally not seen as a great problem?; Maessen 1997 (see 3.2.5); Metz et al. (1997). Welin et al. (1996) review ways for improving plant cold acclimation, examining the genetics of cold acclimation and frost tolerance (classical genetics, low temperature (LT)-induced gene expression, signal transduction), the function of LT-induced proteins (dehydrins, antifreeze proteins, cryoprotection), and genetic engineering of freezing tolerance.

Several other areas of genome functioning have been identified where knowledge is fragmentary or absent. Maessen (1997) mentions the following knowledge gaps in relation to possible factors influencing stability of genes and genomes:

- Actual transposing of transposons (or transposable elements, TEs) in other higher plants than the few species investigated, including maize, *Anthirrhinum*, *Petunia*, *Arabidopsis* and *Nicotiana*.
- GM of large genomes may be prone to relatively more inactive transgenes because of their large fraction of non-coding chromosome regions ('Junk DNA')
- Are the recombination mechanisms different between the commonly used transformation techniques using *Agrobacterium* (single-stranded DNA insertion) and particle gun bombardment (normally double stranded DNA insertion).
- Complete randomness of the site of integration or is there any preference for sites on chromosomes?
- It is unclear whether the non-Mendelian inheritance observed with some (transgenic) traits, depends on the DNA construct used.
- For higher plants, little information is available on the site of integration of the DNA, and on the effect and mechanism of action of MARs (matrix-associated regions, often stabilizing gene expression)
- Mechanisms of variability of gene expression, including variable promoter activity, environmental factors during plant growth, and developmental stage.

### 3.3.5 Are pyramidal (trans)gene systems feasible, such as proposed in future stress tolerance and pest resistance management?

#### Conclusion: Controversy & Gap

There is a controversy over whether there are limitations to inserting large numbers of genes which are supposed to be required; also a general lack of knowledge exists, especially in the areas of biochemistry and physiology (regulation, control and interactions of metabolic pathways).

#### Literature data

*There are presently limitations to inserting large numbers of properly regulated genes via GM (although a novel technique 'multiple co-transformation' may expand the present possibilities to groups of about 20 genes by an unknown mechanism (Ryan et al. 1999)), an important limitation being the lack of knowledge of the nature and control of metabolic and signaling pathways and their encoding genes involved in polygenic plant traits. Current biochemical theory predicts that it is relatively easy to decrease the activity of a metabolic pathway, as opposed to increase it (Fell 1997).*

See also Winicov 1998: there are practical limits to gene stacking. Alternative strategies involve genetic engineering of signalling pathways, thereby changing gene expression of families of functionally related genes in a coordinated way. Another example of such a new strategy involves modification of trehalose metabolism in higher plants (Goddijn & Van Dun 1999).

An example of un-successful gene pyramiding (+ strategy ideas):

Santos et al. (1997) tested transgenes for insect resistance using *Arabidopsis*. One possible strategy to delay the selection of resistant insect populations is the pyramiding of multiple resistance genes into a single cultivar. However, the transformation of most major crops remains prohibitively expensive if a large number of transgene combinations are to be evaluated. *Arabidopsis thaliana* is a potentially good plant for such preliminary evaluations. The authors determined that four major agricultural pests, the four caterpillar species *Spodoptera exigua*, *Helicoverpa zea*, *Pseudoplusia includens*, and *Heliothis virescens* grew as well when feeding on 'Landsberg Erecta' *Arabidopsis* as they did on plants of 'Cobb' soybean. Landsberg Erecta was then transformed with either a synthetic *Bacillus thuringiensis* cryIA(c) gene, or the cowpea trypsin inhibitor gene. Transformed plants were crossed to produce plants transgenic for both genes. Following quantification of transgene expression, the four caterpillar species were allowed to feed on wild-type plants, plants expressing either cryIA(c) or the cowpea trypsin inhibitor gene, or plants expressing both. Both genes reduced growth of the species tested, but cryIA(c) was more effective in controlling caterpillar growth than the cowpea trypsin inhibitor gene. The resistance of plants with both transgenes was lower than that of plants expressing the cryIA(c) gene alone, but higher than that of plants expressing only the CpTI gene. This could be due to a lower concentration of Cry protein in the hemizygous F1 plants. Thus, if the cowpea trypsin inhibitor had any potentiation effect on cryIA(c), this effect was less than the cryIA(c) copy number effect. Alternatively, expression of the trypsin inhibitor gene could be antagonistic to the function of the cryIA(c) gene. Either way these results suggest that the combined use of these two genes may not be effective.

An example of successful gene pyramiding:

Singh & Munoz (1999) found that common bean (*Phaseolus vulgaris* L.) is highly susceptible to common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. High levels of cultivar resistance would minimize yield losses, reduce bactericide use and production costs, and facilitate integrated disease-and-crop management and the production and distribution of pathogen-free seed. The authors aimed to (i) assess the levels of CBB resistance of different *Phaseolus* species in the tropics, (ii) determine the CBB reaction of resistant cultivars and advanced breeding lines, and (iii) report on CBB resistance of lines developed from *P. vulgaris* x *P. acutifolius* (teparty bean) hybridization and gene pyramiding at CIAT. Between 1994 and 1998, the authors evaluated, in the field, 162 accessions of tepary, scarlet runner (*P. coccineus*), lima (*P. lunatus*), and common beans, 119 CBB-resistant cultivars and advanced breeding lines of common bean, and six lines recently developed by interspecific hybridization and gene pyramiding. For inoculation, the authors used aspersion, surgical blades, and/or multiple needles. The highest levels (scores of 1.2-2.0) of CBB resistance were found in *P. acutifolius* accessions, G40029 and G40156, followed by *P. lunatus* (scores of 4.2-6.2), *P. coccineus* (scores of 4.8-5.5), and *P. vulgaris* (scores of 4.5-6.4). Resistance available in *P. coccineus* and *P. vulgaris* landraces has already been transferred to common bean. But resistance transferred from *P. acutifolius* was much lower (scores of 3.8-4.5) than those available. Gene pyramiding produced lines with high CBB resistance (scores of 1.5-2.4), and is thus, a suitable method for developing CBB-resistant cultivars of different market classes.

A positive opinion on the potential of gene pyramiding is presented by Burrows et al. (1998) who propose gene pyramiding as a strategy towards improving nematode resistance in crops. Plant-derived enzyme inhibitors and lectins for resistance against plant-parasitic nematodes in transgenic crops. Due to increasing restrictions on the use of toxic and expensive nematicides, there is now a greater than ever need for crop cultivars that are resistant to plant-parasitic nematodes. Genetically engineered nematode

resistance is not as well developed as other engineered traits but, even so, the first genetically modified plants with enhanced nematode resistance have been produced and tested. Plant-derived enzyme inhibitor and lectin genes are being evaluated for their ability to confer broad-spectrum nematode resistance in transgenic crop plants. Early indications are that these are likely to be effective. Gene pyramiding has potential to increase field durability and to widen the spectrum of nematodes controlled by any one transgenic line.

In general: most authors agree on multidisciplinary approaches, mobilizing geneticists, physiologists and agronomists; e.g. Nguyen et al. (1997) discuss drought stress which is a major constraint to rice (*Oryza sativa* L.) production and yield stability in many rainfed regions of Asia, Africa, and South America. The genetic improvement of adaptation to drought is addressed through the conventional approach by selecting for yield and its stability over locations and years. Because of low heritability of yield under stress and inherent variation in the field, such selection programs are expensive and slow in attaining progress. The objective of this paper is to review the current knowledge in physiology and molecular genetics which have implications in breeding for drought resistance in rice. The ability of root systems to provide for evapotranspirational demand from deep soil moisture and capacity for osmotic adjustment are considered major drought resistance traits in rice. However, selection for these traits still entail extensive investments in field nurseries or greenhouse facilities and is prone to problems of repeatability because of environmental variability. Recent development of molecular linkage maps of rice and other advances in molecular biology offer new opportunities for drought resistance breeding. Molecular markers linked to root traits and osmotic adjustment are being identified, which should lead to marker-assisted selection. Transgenic rice plants having tolerance to water deficit and osmotic stresses have been reported. Work on genetic engineering of osmoprotectants, such as proline and glycine betaine, into the rice plant for drought tolerance improvement is in progress. Close collaboration between molecular geneticists, plant physiologists, and breeders is needed to critically assess the contribution of specific genes and application of molecular genetics to breeding for drought resistance in rice and other crops.

### 3.4 Ecological and agronomic interactions of GM crops

In this section, about a dozen questions are formulated indicating knowledge gaps and controversies on (agro-) ecological effects of GM crops. This is an area where a rapidly expanding literature deals with potential risks and benefits of a large and increasing number of genetic modifications of crop plants. The 'case-by-case' approach, often proposed for risk assessment (e.g. Gilissen & Nap 1998, 1999), appears to reflect the absence of sufficient knowledge and adequate models, which are the common scientific tools for making predictions. In general we conclude that there is insufficient (comprehensive) understanding of the functioning of ecosystems to enable the prediction of potential unintended side effects of GM crops.

The questions listed below indicate specific areas of controversy and lack of knowledge, relevant to widely accepted problems like gene spread, increased weediness ('super weeds'), deleterious effects on non-target organisms, food webs and biodiversity, increased rate of resistance development of pest organisms, 'genetic pollution', and decreasing sustainability of agriculture.

#### 3.4.1 Can GM-DNA be contained within experimental field plots?

##### **Conclusion: Risk**

There is, depending on the case, an widely accepted risk of gene spread, for instance in oilseed rape. Much less is known about other crops. Whether serious hazards are involved is determined by the specific properties of the GM crop.

### Literature data

The answer depends several factors, the most important being the GM-plant species and location. Most authors propose a case-by-case analysis (cf. Gilissen & Nap 1998, 1999), although international consensus appears not to be complete (e.g. debated by Miller; discussion in Nature, 2000).

Thus, for oilseed rape, containment of field-grown plant material was concluded to be impossible (Timmons et al. 1995; Metz et al. 1997) with ample opportunities for outcrossing with wild relatives in the Netherlands (De Visser et al. 1999).

Other authors present optimistic views concerning potential risks (e.g. Trewavas 1999), or potential (theoretical) solutions to risks (Gressel 1999). The latter author proposed (hypothetical) strategies for mitigating introgression of transgenes from rice to weede rices, including genetic placement (e.g. on mitochondrial or chloroplast) or transgenic mitigation using tandem constructs with genes deleterious to weedy rices.

In case of experimental limitations, modelling is an attractive technique for studying gene spread according to. Cruywagen et al. (1996) who modelled the risk of spread of a genetically engineered population. They used a classical Lotka-Volterra competition model with diffusion. It examines the spread of organisms introduced into a heterogeneous environment, with emphasis on the risk of spread and the feasibility of containment strategies. The authors were able to define conditions that lead to invasion.

Whether modelling results have been compared with experimental data is not known.

3.4.2. Do GM crops pose a threat to maintaining biodiversity? (E.g. by outcrossing, running wild, weediness, gene spread, effects on non-target pests, etc.)

### Conclusion: Controversy & Gap

There is a controversy over the potential effects of GM on biodiversity. Both positive and negative effects are predicted; depending on the case, there appears to be more speculation than experimental evidence.

### Literature data

Gilissen & Nap (1998) give an overview of the various published views on biodiversity, distinguishing ecology-, resource- and gene-centered views. The various definitions of biodiversity share the element of 'the variability among organisms from all sources', but differ in the particular aspects they emphasize.

Opinions on this question vary widely. While, for instance, Ellstrand et al. (1999), O'Reilly (1996) and Serratos Hernandez (1996) mention the possibility of unintended and undesirable effects of GM crops on biodiversity, others like Sriwatanapongse (1997) expect that GM will contribute to reducing loss of biodiversity and environmental degradation. This might, according to the latter author, be achieved by the development and use of bio-fertilizers, bio-insecticides, crops with built-in pest resistance, and GM organisms for treating industrial wastes through bioremediation and biodegradation. Neubert & Knirsch (1993) question the use of herbicide resistant crops in developing countries. With regard to insect resistance management, Li-WenBin et al. (1999) argue that delaying pest-insect resistance (to Bt-toxins) by refuges alone is not acceptable.



### **Social, legal and moral aspects of dealing with biodiversity**

Baumann et al. (1996) presents a critical analysis of ways of biodiversity conservation, including economic and social arguments. The countries in the South, with most of the world's biodiversity, are used as reservoirs of biological and genetic resources to develop new products such as crop varieties, drugs, biopesticides, oils and cosmetics, without much concern for local stakeholders. New approaches to biodiversity conservation should be explored, away from a top-down, blueprint approach towards a bottom-up, people-centered, process-oriented approach, requiring outside institutions to help local people to plan, implement and manage their own projects. On-farm conservation is presented as an example of a more participatory, process-oriented approach to the management of biological resources.

### **GM-Pest resistance a threat to biodiversity**

Hilder & Boulter (1999) review the genetic engineering of crop plants for insect resistance: 'Genetically engineering inherent crop resistance to insect pests offers the potential of a user-friendly, environment-friendly and consumer-friendly method of crop protection to meet the demands of sustainable agriculture in the 21st century. Work to date has concentrated on the introduction of genes for expression of modified *Bacillus thuringiensis* (Bt) toxins. Impressive results on the control of Bt-susceptible pests have been obtained in the laboratory and the field, and the first commercial Bt transgenic crops are now in use. A main alternative approach exploits plant-derived insect control genes. Enhanced resistance to a wide spectrum of pests has been demonstrated in laboratory trials of transgenics expressing various protease inhibitors, lectins, etc. and some promising field trials have been carried out, but the scale of effects produced by plant-derived insect control genes has not been deemed convincing enough to lead to serious attempts at commercialization. Both classes of compounds have limitations: there have been serious failures in resistance to targeted pests in Bt cotton; most plant-derived resistance factors produce chronic rather than acute effects; and many serious pests are simply not susceptible to known resistance factors. The authors have analysed the characteristics which would be desirable in an ideal transgenic technology: these include being environmentally benign, relatively inexpensive to develop, with a potentially wide spectrum of activity (although targetable at pests and not beneficials), generated by a flexible technology that allows any insect site to be targeted and readily adaptable so that alternatives can be produced as required. The authors are developing such a technology based on the expression of single-chain antibody genes in crop plants which would be compatible with the likely trends in pesticide discovery using biology-driven target-based methods. The importance of a changed, more socially responsible attitude in this sector is emphasised as is the need for much improved presentation of the benefits and need for responsible deployment of genetically engineered crops.'

Mullin & Bertrand (1998) argue that the release of new genetic materials into forest ecosystems, regardless of the method used to develop them, should be done in an environmentally responsible manner. Canada is participating with the OECD in efforts to harmonize regulatory control of products derived from biotechnology, including forest trees. Prepared under contract to the Canadian Forest Service, the purpose of this document is to facilitate a discussion within the forestry community, leading to improved direction of research and contributing to the harmonization of regulatory oversight of engineered forest trees. While the focus of the paper is on transgenic trees, many of the issues raised are equally applicable to all novel products from tree breeding.

The fixation of heterosis by apomixis (a process by which some seed plants produce clonal, asexual seeds) is of great interest for plant breeding (Maheshwari et al. 1998; Van Dijk and Van Damme 2000). The prospect of changing sexual crop species into apomictic crop species by genetic engineering - apomixis technology - has recently caused a boom in apomixis research. According to evolutionary biological theories, a dominant apomixis gene will rapidly become fixed in an outcrossing sexual population. Therefore, in theory, apomixis transgenes could have unconditional advantages that could result in the uncontrollable spread of the transgenes. By contrast, 'classic' transgenes might only have conditional advantages (Van Dijk and Van Damme 2000). In their review on apomixis technology, these

authors point out that the fact that sexual reproduction and not apomixis is common in nature, is no guarantee that apomixis transgenes will be ecologically safe because there could be essential differences between natural and transgenic apomicts.

3.4.3 Are there (unintended) interactions between neighbouring GM- and GM-free agro-ecosystems?

**Conclusion: Controversy & Risk**

Problems in separating these systems have been noted; There is a controversy regarding the success of management strategies (often as yet unavailable).

**Literature data**

Riggin-Bucci & Gould (1997) and Stewart et al. (1999) present several agronomic complications. The need of adequate management strategies is generally acknowledged (James et al. 1999; All et al. 1999; Brennan et al. 1999). Specific studies are available concerning various aspects, e.g. pest management (Lewis et al. 1997; Roush et al. 1997; Whalon & Norris et al. 1996), weed-control (Marshall 1998).

3.4.4 To what extent are the effects on agro-ecosystems of introduction of GM crops into the field (un)predictable?

**Conclusion: Risk**

There is evidence of unintended effects of field introduction of GM crops; several of these effects have not been predicted by scientists

**Literature data**

Unpredictability of ecological and environmental effects of GM crops is seen as a problem by many among the public, and appears to be a key-issue undermining public trust in GM (e.g. Website 1; Van Gool 2000; Schalk 2000). Recent, well-known, and unpredicted effects are the toxic effects of pollen of Bt-maize on the larvae of the Monarch butterfly; toxic effects of insect-pest predators like ladybirds (Birch et al. 1999; Hilbeck et al. 2000 – see also 3.4.7). On the other hand predictable effects like gene spread by dispersion of pollen and seeds (Van Raamsdonk & Schouten 1997) are seen as a risk because of a lack of control of containment. Potential unintended effects in the area of herbicide resistance have been found in the field, but scientific analyses have been largely limited to scenario studies (e.g. Lotz et al. 2000).

3.4.5 Are effects of field introduction of GM crops irrevocable and unprecedented?

**Conclusion: Gap**

This question is difficult to answer because of insufficient relevant information.

**Literature data**

O'Reilly (1996) suggests irrevocable effects. However, no relevant studies have been found dealing with the question above.

- 3.4.6 Does introduction of GM crops in the field lead to 'genetic pollution' as the outcome of gene flow, i.e. gene establishment?

#### **Conclusion: Controversy & Gap**

There is a controversy and knowledge gap, not so much about the occurrence of gene flow, but more about the definition and valuation of 'genetic pollution'. Gene flow by itself may not be a new phenomenon in specific cases. However, the nature of the genes spread should be the main issue of concern.

#### **Literature data**

Depending on the case, c.q. GM crop, gene flow may be inevitable, as in oilseed rape (Van Raamsdonk & Schouten 1997; Bonny 1998; Ellstrand et al. 1999). Whether the effect is valued as pollution depends on both appraisal and estimation; this issue is beyond the aim of the present study. These and various other aspects of gene flow in agriculture are discussed in Lutman (1999). In these proceedings, Daniels & Sheail define 'genetic pollution' as the loss of identity of wild plant species as a result of transfer of genes from crop plants, with engineered fitness genes causing special concern. These authors argue that gene flow may have its greatest effect on weed species which are closely related to crop plants, taxonomically, ecologically and in their reproductive biology, in which case there is already a history of gene exchange.

Experimentally, many data are obtained by computer simulations, while many field experiments are still under way (e.g. in Lutman 1999). Ahmad M & Roush R (1999) Estimation of Allele frequencies for *Bacillus thuringiensis* resistance in diamondback moth, *Plutella xylostella* and cotton bollworm, *Helicoverpa armigera*: an isofemale line (F<sub>2</sub>) approach. 'Resistance to Bt toxins in field populations of DBM has already been documented from many parts of the world' - (3 refs).

These authors hypothesize that transgenic Bt-cotton may exert higher selection pressure in target insect pests due to continuous expression of insecticide proteins. Fast selection of resistance would eliminate transgenic Bt cotton as valuable pest control tool.

An important problem with estimating frequencies of resistance genes, is that these genes are predominantly recessive; a resistance gene frequency of 1% requires >300 ex sample size; <0.1% would require thousands for a reasonable chance of detection.

Further work needs to be done to evaluate the risk of transgene spread from *Brassica napus* to related species (*B. rapa* & *B. juncea*). The persistence of a herbicide tolerance gene in the absence of herbicide selection pressure is undetermined (Pinder et al., p. 275-80 in Lutman 1999).

- 3.4.7 Is there evidence showing that GM-insecticide production *in planta* ('Bt crops') differs in its effects on agro-ecosystems from crop applications of the same insecticide?

#### **Conclusion: Controversy & Risk**

There is evidence of unintended side-effects of Bt crops, i.e. toxicity to non-target organisms and rapid development of Bt resistance in target organisms; there was for some time a controversy over the preferred application system. Some authors consider the development and use of the present types of Bt crops a 'dead-end road' (e.g. De Maagd et al. 1999; see also next section).

## Literature data

This is a controversial issue, see also Hilbeck et al. (2000) who showed several limitations and shortcomings in many of the available experimental studies testing for toxicity of Bt toxins to non-target organisms (in both field and lab). We did not find direct experimental evidence relevant to the question. However, chronic exposure to insecticide (as in Bt crops) is more likely to give rise to resistance of pest insects than acute exposure as in Bt application systems (Hilder & Boulter 1999; see also 3.4.2).

Arpaia (1996) found no effect of Bt-additions to nectar on honey bees or their larvae; however, no evidence was presented of actual intake of Bt. To assess the possible hazards for honey bees of CryIIIB protein from a *Bacillus thuringiensis*-derived gene, the toxin was supplied, mixed in supplemental syrup to *Apis mellifera* colonies. Two different toxin concentrations were used at levels of about 400 and 2000 times higher than the expected protein content in pollen from Bt-transgenic plants. Hives were sampled every week to record larval survival and pupal dry weight. Frames of bees were counted at the beginning and the end of the experiment as an index of colony strength. No toxic effects on larvae were observed. Pupal weight was not significantly affected by diet regime. The author concludes that the results indicate that transgenic crops producing CryIIIB toxin may represent a suitable environment for pollinators.

Saxena et al. (1999) have demonstrated stability in soil of Bt-toxins in root exudates of transgenic Bt corn plants resistant to the corn borer. This finding points to the possibility of toxic effects of such Bt plants on non-target organisms in soil.

*One of the major disadvantages of Bt-toxins seems their lack of specificity, thereby affecting biodiversity in a negative way.*

Some insects show avoidance if given choice, according to Stapel et al. (1998). These authors observed that *Spodoptera exigua* (*Lepidoptera: Noctuidae*) larvae showed behavioural avoidance of Bt-diets in choice tests. The behaviour of *S. exigua* larvae on *Bacillus thuringiensis* (Bt)-containing and Bt-free food substrates was investigated in artificial diet tests and tests on leaves of transgenic cotton using dual-choice test arenas. The commercial Bt formulation MVP and one of the transgenic cotton lines (C 1076) caused behavioural avoidance of Bt diets in *S. exigua* larvae in situations where both Bt-containing and Bt-free diets were present. Even though developmental time was longer for larvae in MVP choice situations, weight of pupae was not different from the weight of control pupae.

However, high mortality, low pupal weight and longer developmental time were observed in no-choice situations with Bt-treated diets, suggesting that *S. exigua* is able to survive and complete larval development in a dual-choice toxic environment by behavioural avoidance. In neonate drop-off tests, the mean percentage of neonates found after 24 h was significantly lower on MVP-treated plants than on control plants. After 48 h, a concentration-dependent correlation was observed. The application of Bt-formulations and transgenic crops to pest management are briefly discussed.

Specificity of Bt toxins has been reported, e.g. by Yu et al. (1997). They present methods for testing the effects of toxins or small molecules (*Bacillus thuringiensis* subsp. *kurstaki* delta-endotoxins HD-1 CryIA(b) and HD-73 CryIA(c)) in plants (cotton and potato) on 2 nontarget soil arthropods, a collembolan, *Folsomia candida*, and an oribatid mite, *Oppia nitens*. Time to oviposition, egg production and final body length were unaffected when *F. candida* were fed residues of transgenic cotton lines <hash>81 or <hash>249. Total production of *O. nitens* adults and nymphs was unaffected by feeding on leaves of both transgenic cotton lines. With *B. thuringiensis* subsp. *tenebrionis* CryIIIA in potato, no differences were seen in the 3 indices for *F. candida*. Overall, transgenic toxin sources had no negative effects, but similar tests with cadmium as a positive control showed a concentration-rate response of decreased reproduction.

Specificity of Bt-toxins may be absent due to their transfer to insect predators. Birch et al. (1999) discuss tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. They report adverse effects on ladybird fecundity, etc). Transgenic crops genetically engineered for enhanced insect resistance should be compatible with other components of integrated pest management for the pest resistance to be durable and effective. An experimental potato line of cv. Desiree was previously genetically engineered to express an anti-aphid plant protein (snowdrop lectin, GNA), and assessed for possible interactions of the insect resistance gene with a beneficial pest predator. Plant contained a gene construct where the GNA coding sequence from clone LECGNA2 was expressed using the CaMV35S promoter. Adverse tri-trophic interactions involving a lectin-expressing transgenic crop, a target pest aphid and a beneficial aphidophagous predator were demonstrated. When adult 2-spot ladybirds (*Adalia bipunctata*) were fed for 12 days on peach-potato aphids (*Myzus persicae*) colonising transgenic potatoes expressing GNA in leaves, ladybird fecundity, egg viability and longevity significantly decreased over the following 2-3 weeks. No acute toxicity due to the transgenic plants was observed, although female ladybird longevity was reduced by up to 51%. Adverse effects on ladybird reproduction, caused by eating peach-potato aphids from transgenic potatoes, were reversed after switching ladybirds to feeding on pea aphids from non-transgenic bean plants. These results demonstrate that expression of a lectin gene for insect resistance in a transgenic potato line can cause adverse effects to a predatory ladybird via aphids in its food chain. The authors conclude that the significance of these potential ecological risks under field conditions needs to be further evaluated.

In a review of several lab- and field- studies dealing with effects of Bt-plants on non-target organisms, Hilbeck et al. (2000) conclude that testing procedures should be expanded to include multi-trophic interactions during more than one generation, and chronic-lethal and sublethal toxicological parameters in addition to the common practise of testing short-term, acute toxicity. These authors also argue that large variability severely constraints statistical power, leading to effects of 10-20% which may be statistically not significant, but nevertheless can be of great ecological significance, especially in the long run. This is another example of a case where the rules of logic (cf. section 'Methodology') are often neglected.

3.4.8 Will (pest) insects develop resistance against Bt-toxins faster in GM-Bt systems than in Bt-application systems?

#### **Conclusion: Gap & Risk**

There is little experimental information available concerning a comparison of the two types of Bt-application systems; several authors seem to confirm the question.

#### **Literature data**

The first generation of insect –resistant transgenic plants are now in large-scale use in agriculture (De Maagd et al. 1999). At the moment, resistance development is indeed seen as realistic problem, as is also evident from the discussion of this problem, e.g. by Daniell (1999), mentioning potential solutions like high dosage, gene pyramiding, tissue specificity.

Similarly, Bhau & Koul (1998) anticipate resistance problems; they propose the transfer of Bt (*Bacillus thuringiensis*) endotoxin genes (Cry genes) to plant species, as a tool in breeding for resistance to pest insects, and the use of Bt proteinase inhibitors as a biopesticide spray are reviewed. The structure of Bt toxins, mode of action, development of Bt resistance in insects, and strategies to avoid such resistance are discussed. Evidence is presented by Jelenkovic et al. (1998), reporting that insect exposure increases occurrence of Bt resistance.

The consequences of these findings for the overall effect on ecosystems (biodiversity, etc.) are unclear.

Meyer & Reiter (1998) examine the question whether GM insecticide resistance can be considered a long-term, stable approach to plant protection in developing countries. They discuss the use of the delta endotoxin gene from *Bacillus thuringiensis* (Bt) to control *Scirpophaga incertulas*. They expect that the large-scale use of plants with Bt will lead to widespread Bt resistance in pests. The authors conclude that the prospects for the application of transgenic Bt pest resistance in crops are slim. Similarly, De Maagd et al. (1999) consider the present, first generation of Bt transgenic crops as a 'dead-end road'. Alternatives to Bt are currently being investigated (e.g. Bowen et al. 1998; Holtorf et al. 1998).

The continuous exposure to GM-Bt contrasts with the temporary susceptibility of insects to Bt, thus probably increasing chances of resistance development. Huang-Fang Neng et al. (1999) provide evidence that Bt susceptibility varies with developmental stage of insect. Bt susceptibility varies also with type of leaf/leaf age, as is shown in a study by Harcourt et al. (1996). They tested the effectiveness of purified *Bacillus thuringiensis* Berliner insecticidal proteins in controlling three insect pests of Australian eucalypt plantations. First instars of the chrysomelid leaf beetles *Chrysophtharta bimaculata* and *Chrysophtharta agricola* (Coleoptera: Chrysomelidae) were fed eucalypt leaves coated with purified protein encoded by the CryIIIa gene of *Bacillus thuringiensis* var. tenebrionis. The CryIIIa protein was highly effective against *C. bimaculata* when presented on adult *Eucalyptus nitens* leaves, but not when leaves of the preferred host *Eucalyptus regnans* were used. The difference between tree species may be the result of differences in the amount of endotoxin ingested or to interactions between the CryIIIa protein and eucalypt allelochemicals, (e.g., leaf oils). CryIIIa was not effective against *C. agricola* fed on juvenile *E. nitens* leaves coated with CryIIIa protein. Purified protein encoded by the CryIA(b) gene of *B. thuringiensis* var. kurstaki was also tested in laboratory feeding trials against 1st instars of *Mnesampela privata* (Lepidoptera: Geometridae). The CryIA(b) protein was highly effective against *M. privata* when presented on juvenile *E. nitens* leaves. These results indicate that CryIIIa and CryIA(b) proteins could be used to control 1st instars of *C. bimaculata* and *M. privata*, respectively, in some commercial eucalypt plantations when either sprayed as microbial formulations or present in transgenic plants.

Filho et al. (1998) report specificity of Bt-toxins, also mentioning, much variation in susceptibility. Whalon & Norris (1996) discuss resistance management for transgenic *Bacillus thuringiensis* plants. *Bacillus thuringiensis* (Bt) toxin genes have been genetically engineered into dozens of plant species, protecting them from some insect pests without farmers resorting to pesticide sprays. The 4 key strategies of resistance management: diversification of mortality sources, reduction of selection pressure, the use of refugia, and prediction and monitoring of resistance, are discussed. The mixing of Bt toxins with other toxins, the level of toxin expressed in the plant and understanding of the local ecological and agricultural conditions are advocated for delaying rapid resistance development. Transgenic cultivars can be problematic in those countries where wild native plants may acquire Bt genes from cross-pollination, as is the case with transgenic rice. Regulatory options for Bt transgenic plant deployment are provided by these authors.

As alternatives to Bt, new biopesticides are being developed (e.g. Morin 1998).

3.4.9 Does the use of transgenic herbicide-resistant crops lead to unintended and unavoidable continuation of herbicide use and accumulation in the environment?

#### **Conclusion: Controversy**

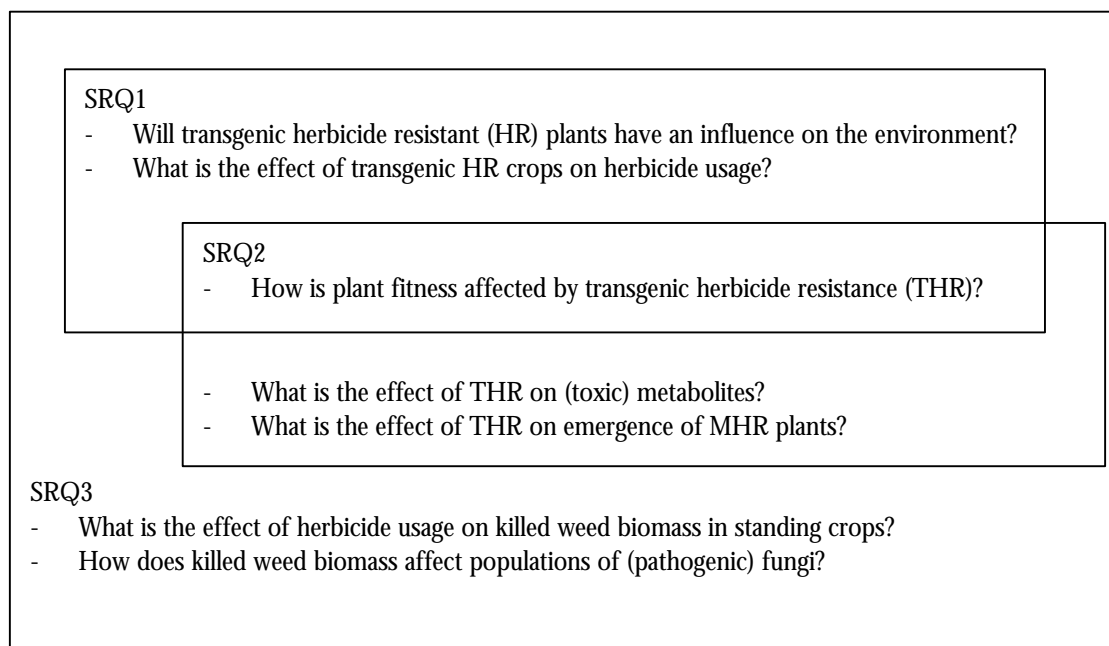
There is a controversy over the overall effects of transgenic HR crops on the environment.

#### **Literature data**

Meyer & Reiter (1998) examine the question whether genetically engineered herbicide resistance can be considered a long-term, stable approach to (sustainable) plant protection in developing countries. As a result of the introduction of herbicides and the widespread adoption of direct sowing, wild rice (*Oryza*

*rufipogon*) and *Echinochloa frumentacea* have become important weeds in rice. Looking at the potential for genetically engineered herbicide resistant rice lines, they expect that wild rice will develop the same resistance in a few years time. They conclude that the prospects for the application of transgenic pesticide resistance in crops are slim.

While several authors present optimistic views (e.g. Marshall 1998; Morpurgo et al. 1997; Renard et al. 1997; Anonymous 2000), others provide information suggesting a near-zero balance of risks and benefits of transgenic herbicide resistant crops (e.g. Lotz et al. 2000).



*Figure 2* Sets of relevant questions (SRQs) concerning potential effects of large-scale application of transgenic herbicide resistant (HR) crops, following the methodology proposed by Van Dommelen (1999). SRQs 1 and 2 were derived from Bergmans (1994; Web site #13); SRQ3 is extended to include questions raised in a recent study by Lotz et al. (2000).

Marshall (1998) notes the special value of the integration of GM crops in minimum tillage situations, maintaining of a wide range of herbicides with different modes of action to provide a variety of opportunities for weed-control management. Associated with these special [GM] crops are a series of environmental issues which at present-limit the rate of commercial development in Europe. Unlike the successful performance of herbicide tolerance in the crops, these strategic issues are much more difficult to resolve for technical, political, ethical and moral reasons. The primary concerns are the feasibility of controlling the volunteer crop and the opportunity for indiscriminate introgression of the herbicide-tolerance gene into agricultural and natural ecosystems. The author thinks it unlikely that these questions will be resolved without scaling-up field experiments to include the detection of herbicide-tolerance genes. The author suggests that economic and management implications of herbicide-tolerant crops require special consideration in view of the necessity to integrate conventional and transgenic crops in new cropping systems.

### 3.4.10 Can GM plants safely be used for the purpose of fighting pollution, as in phytoremediation? (e.g. hydrocarbons, heavy metals)?

#### **Conclusion: Gap & Benefit**

There is some controversy, although some authors report mainly on (anticipated) benefits in fighting pollution.

#### **Literature data**

Practical applications of pollution tolerant transgenic plants to remediation of contaminated soils have been described for some pollutants, including mercury (Rugh et al. 1998), methylmercury (Heaton et al. 1998 and Bizily et al. 1999), and toxic hydrocarbons (Hollowell et al. 1999). While hydrocarbons are potentially oxidised to water and carbon dioxide, heavy metal-enriched plants may not be so easily disposed of, an issue not receiving attention in these heavy-metal studies. Also, little attention is paid to the question whether such plants offer better opportunities for remediating polluted soils than non-transformed plants.

Monciardini et al. (1998) propose to use plants with the GUS reporter, under the control of heavy metal inducible promoters, as biomarker for monitoring heavy metal pollution. Environmental monitoring can benefit from the application of molecular and biochemical techniques as biomarkers. In principle, molecular biomarkers may have some advantage over more classical, organismal level markers because of their sensitivity, efficiency and reproducibility. Nevertheless, some disadvantages such as the cost, resource requirements and lack of specificity have limited their widespread use. Molecular genetics and recombinant DNA technologies, however, allow to improve simplicity and cost, together with reliability, efficiency and sensitivity, of molecular biomarkers. The stress inducible promoter of the barley gene *Hvhsp 17*, which is induced by a number of environmental stress (including some heavy metals), has been fused to the reporter gene GUS in a plasmid. This plasmid was used for stable transformation of tobacco. Some of the transgenic plants obtained after selection showed enhanced GUS expression after exposure to some heavy metals. Maintenance of the plants for three generations, and the analysis of the response to treatments, suggest that these transgenic plants may be utilised as an affordable biomarker for monitoring heavy metal pollution.

Since these applications have only been proposed, little is known about risk-benefit assessments of the use of transgenic plants as biomonitors of pollution.

Removal of hydrocarbons may also be possible in marine environments, as suggested by trials with micro-organisms of Kapley et al. (1999). These authors report on transformed micro-organisms showing capability to grow under hyper-saline conditions. Some degradation capacity of the engineered consortium was found using a designed model petroleum mixture. However, little information appears to be available on ecological risk assessments of the use of such transgenic micro-organisms.

Giddings (1998) reviews several aspects of the release of genetically engineered micro-organisms and viruses into the environment, including consumer-protection, risk-assessment, effects on nontarget-organisms, biological-control-agents, microbial-pesticides.

On the subject of bioremediation the National Institute of Public Health and the Environment (RIVM, NL) has published reviews (e.g. Mulder et al. 1998; see contributions by Eijsackers, Notenboom and Posthuma in: Van Straalen en L. kke 1997). According to Mulder et al. (1998) the potential for bioremediation as a treatment technique for PAH-contaminated soils is mainly determined by the mass transfer dynamics of PAHs.

*This suggests that the potential for bioremediation is limited by physical rather than biological factors. If so, it is not clear how bioremediation of PAH-contaminated soils could benefit from GM plants or micro-organisms.*



### 3.4.11 Are there unintended effects of 'gene stacking' on (agro-)ecosystems?

#### **Conclusion: Gap & Risk**

There is a gap on the possible spontaneous occurrence of gene stacking in the field, although volunteer crops with multiple herbicide resistance have been found.

#### **Literature data**

Gene stacking (or gene pyramiding) is often required, as in the case of properly changing quantitative traits like yield (e.g. Khush 1997; Morpurgo et al. 1997), pest resistance (e.g Santos et al. 1997; Singh & Munoz 1999) and stress tolerance (drought: Nguyen et al. 1997, Zhang et al. 1999). On the other hand, it may also be undesirable under some conditions, as in the case of multiple herbicide resistance (Kempenaar & Lotz 1999; Marshall 1998; risk of emergence of weeds that are no longer sensitive to available herbicides), or impractical, c.q. too complex (Hum-Musser et al. 1999; Winicov 1998; cf. previous section, 3.3.5).

Theoretically, the chance that unintended side-effects of GM occur increases with the number of changes brought about in a plant's metabolism. Indeed, gene stacking is seen as a potential source of problems, and is avoided by various new GM approaches, for example by marker-free selection.

#### *Avoiding unnecessary gene stacking by marker-free selection:*

Ebinuma et al. (1997) present a method for marker-free selection of transgenic plants using the isopentenyl transferase gene: they have developed a new plant vector system for repeated transformation (called MAT for multi-auto-transformation) in which a chimeric *ipt* gene, inserted into the transposable element *Ac*, is used as a selectable marker for the transformation. Selectable marker genes conferring antibiotic or herbicide resistance, used to introduce economically valuable genes into crop plants, have three major problems: (i) the selective agents have negative effects on proliferation and differentiation of plant cells; (ii) there is uncertainty regarding the environmental impact of many selectable marker genes; (iii) it is difficult to perform recurrent transformations using the same selectable marker to pyramid desirable genes. The MAT vector system containing the *ipt* gene and the *Ac* element is designed to overcome these difficulties. When tobacco leaf segments were transformed and selected, subsequent excision of the modified *Ac* produced marker-free transgenic tobacco plants without sexual crosses or seed production. In addition, the chimeric *ipt* gene could be visually used as a selectable marker for the transformation of hybrid aspen (*Populus sieboldii* x *Populus grandidentata*). The chimeric *ipt* gene, therefore, is an attractive alternative to the most widely used selectable marker genes. The MAT vector system provides a promising way to shorten breeding time for genetically engineered crops.

This method could be particularly valuable for fruit and forest trees, for which long generation times are a more significant barrier to breeding and genetic analysis.

Another alternative to antibiotic resistance markers is Novartis's *manA* marker gene, enabling plants to digest a simple sugar called mannose-6-phosphate, which most plants cannot utilize as an energy source (Coghlan 1999a).

### 3.4.12 Will sustainable agriculture be supported and promoted by GM?

#### **Conclusion: Controversy & Gap**

Accepting the Brundtland Report-definition (UN) of 'sustainability', there is controversy over and limited knowledge of how the use of GM crops will affect sustainable agriculture.

#### **Literature data**

A generally accepted definition of sustainable agriculture does not exist. In the UN-Brundtland Report, cited by Goodland (1995) and Hueting and Reijnders (1999), the concept of sustainability is linked to the issue of intergenerational equity and sustainable development is thus defined as 'development that meets the needs of the present without compromising the ability of future generations to meet their own needs'. Useful descriptions of the concept of sustainability are provided by Goodland (1995) and Hueting and Reijnders (1999). The latter authors define sustainability as 'the use of the vital functions (possible uses) of our biophysical surroundings in such a way that they remain indefinitely available'. They argue that sustainability is an objective concept to the extent that natural science is objective. Goodland (1995) distinguishes social, economic and environmental sustainability. Each of these can be argued to contribute to sustainable agriculture. Surprisingly, in much of the literature on GM crops and sustainable agriculture, it remains unclear how the authors define 'sustainable agriculture'.

Nevertheless, authors appear to disagree on the influence of GM on sustainable agriculture. Some authors have reasoned that the use of GM crops will not contribute to sustainable agriculture (Ahmed et al. 1992; Meyer & Reiter 1998). On the other hand, others advocate GM as positively affecting the sustainability of agriculture, e.g. Anonymous (2000; see 3.5.3), Hilder & Boulter (1999; insect resistance, see 3.4.2), Rogers (1997; on integrated pest management), and Trewavas (1999). Remarkably, GM is not mentioned by Lewis (1997) in a review on sustainable pest management.

Gap: A prerequisite for evaluating effects on sustainability is a proper way of monitoring it. There appears to be a gap in knowledge of how to measure and monitor sustainability, although progress has been made (e.g. Verschoor & Reijnders 1991; Udo de Haes et al. 1991; see also contributions by G. Yohe and R. Costanza in Munasinghe & Swart 1999), including the use of earthworms as indicator organisms (Paoletti 1999). The latter author proposes a role of earthworms in assessing different environmental impacts such as tillage operations, soil pollution, different agricultural input, trampling, and industrial plant pollution.

*Is there a need for use of GM? The following study indicates that the current rate of rice improvement by classical breeding of rice should be sufficient until the year 2025:*

Khush (1997) reports that there are two cultivated and twenty-one wild species of genus *Oryza*. Human selection and adaptation to diverse environments has resulted in numerous cultivars. It is estimated that about 120000 varieties of rice exist in the world. After the establishment of International Rice Research Institute in 1960, rice varietal improvement was intensified and high yielding varieties were developed. These varieties are now planted to 70% of world's riceland. Rice production doubled between 1966 and 1990 due to large scale adoption of these improved varieties. Rice production must increase by 60% by 2025 to feed the additional rice consumers. New tools of molecular and cellular biology such as anther anther culture, molecular marker aided selection and genetic engineering will play an increasing role in rice improvement.

#### *Plant pathology*

Lewis et al. (1997) present a total system approach to sustainable pest management. A fundamental shift to a total system approach for crop protection is urgently needed to resolve escalating economic and environmental consequences of combating agricultural pests. Pest management strategies have

long been dominated by quests for 'silver bullet' products to control pest outbreaks. However, managing undesired variables in ecosystems is similar to that for other systems, including the human body and social orders. Experience in these fields substantiates the fact that therapeutic interventions into any system are effective only for short term relief because these externalities are soon 'neutralized' by countermoves within the system. Long term resolutions can be achieved only by restructuring and managing these systems in ways that maximize the array of 'built-in' preventive strengths, with therapeutic tactics serving strictly as backups to these natural regulators. To date, man has failed to incorporate this basic principle into the mainstream of pest management science and continues to regress into a foot race with nature. The authors establish why a total system approach is essential as the guiding premise of pest management and provide arguments as to how earlier attempts for change and current mainstream initiatives generally fail to follow this principle. They then draw on emerging knowledge about multitrophic level interactions and other specific findings about management of ecosystems to propose a pivotal redirection of pest management strategies that would honor this principle and, thus, be sustainable. They discuss the potential immense benefits of such a central shift in pest management philosophy.

### **3.5 Toxicological and allergenic properties of (products of) GM food crops**

Food safety of GM crops is one of the major issues of concern among the public. Aspects receiving much attention include transfer of antibiotic resistance from GMO's (including plants) to pathogenic micro-organisms by horizontal gene transfer, and allergenicity and (long-term) toxicity of new plant proteins and products. Below we deal with six questions, all on issues where lack of knowledge is making it impossible at the moment to adequately assess potential risks of GM food in general and in specific cases. Food safety assessment encompasses the following main issues (Kuiper, personal communication):

1. Assessment of the safety of new products of gene expression.
2. Tracing and characterising unintended effects of GM.
3. Assessing the safety of the genetically altered food.
4. Gene transfer and potential risks for humans and animals
5. Allergies.

Over the past ten years these aspects have received much attention by organisations like FAO, WHO and OECD. A report of a recent 'Expert Consultation' can be found on web site nr 17 (section 5.2). Main topics include substantial equivalence, allergenicity, long-term safety of GM crops, and identification and characterisation of unintended effects of GM.

Antibiotic resistance is now widely recognized as an undesirable and unnecessary trait in GM crops, because of the risk of resistance-gene transfer to (human) pathogenic micro-organisms. Regarding the risk of gene transfer to pathogens it should be noted, that micro-organisms do not have to be pathogenic (cf. section 3.5.4.) to have environmental effects (Jäger & Tappeser 1996). For a sound risk assessment, knowledge is required of type of hazard and exposure time. Such basic knowledge often appears to be absent in the case of allergenic proteins.

Representative examples of common statements on GM food from the perspective of many biotechnologists, are provided by Casey (1997) and Harlander (1991). The latter author wonders: 'Why the current controversy over food?', mainly focussing on the potential and benefits of biotechnology: 'These technologies allow targeting of genetic manipulation of plants, animals, and microorganisms in less time and with greater precision, predictability, and control, than is possible with traditional methods. Biotechnology provides a new set of tools for improving variety, productivity, and efficiency of food production. Benefits to the agricultural sector encompass food processing applications (fermented foods, processing aids, food ingredients, rapid detection systems, and biosensors), waste management

and value-added technology.' Similarly, others like Casey (1997) mainly emphasize the production of novel plant foods, and the benefits of the use of genetic modification for food production. However, there has been too little attention given to the food quality of GM crops and their products. For instance, Jäger & Tappeser (1996) state: 'Molecular politics do not help to engender public trust and good faith. Only good science and proper risk assessment procedures can provide reliable knowledge. Democratic and responsible decisions can be founded only on the background of such knowledge'.

More recently, however, industry is taking consumer concerns about GM food more seriously, stressing the need for dialogue between dairy industries and consumer groups (e.g. Mortensen 1999; RTR Hiel, in: Spiertz & Dons eds 2000).

The potential of GM in contributing to world food security and sustainable agriculture is advocated by several authors. For instance, Vasil (1998) addresses the challenges in trying to feed the burgeoning world population on less per capita land, with less water and under increasingly challenging environmental conditions, and proposes that crop genetic engineering is the only way these increases in production can be achieved in time, by supplanting traditional breeding methods.

### 3.5.1 Are theoretical models available predicting successfully the allergenicity of proteins in general and of proteins from GMOs?

#### **Conclusion: Gap & Risk**

There is a gap in knowledge of the prediction of allergenicity of proteins; therefore, risks of unexpected allergenicity cannot be excluded at present.

#### **Literature data**

Wal (1997) states: 'At the present time, there is no evidence to suggest that GMOs are either more or less allergenic than the corresponding traditional organisms.' *However, this is a misleading remark, since there was hardly any literature available at the time dealing with a proper search for such evidence.*

Most authors agree that possibilities for predicting of allergenicity of new (GM) foods are insufficient and that experimental assessment is generally required (Gilissen & Nap 1997; Lehrer & Reese 1997; Wal 1997). Kuiper et al. (1999) are of the opinion that adequate methods are available for such testing. Lehrer & Reese (1997) and Gilissen & Nap (1997) conclude that present knowledge is insufficient for unequivocal prediction of allergenicity of a given protein. Kleter et al. (2000) confirm this conclusion, while extending it to other secondary effects (see website 18). Gilissen & Nap (1997) discuss decision-tree approaches to assessment of potential allergenicity of GM foods, providing an overview of relevant literature till 1997.

Proteins may keep their (non-)allergenic properties irrespective the nature of the (recipient) transgenic plant; for instance, Nordlee et al. (1996) found that an allergenic, S-rich protein from Brazil nut is also allergenic when expressed in GM soybean. However, not enough data are available for establishing a general model.

### 3.5.2 Can the concept of 'substantial equivalence' be used as a reliable and adequate guideline in toxicological studies of GM-crop products?

#### **Conclusion: Controversy & Gap**

This concept appears to be controversial; also, scientific information seems scarce.

### Literature data

Recently, several authors mingled in a discussion in the journal *Nature*. According to Millstone et al. (1999) the concept of 'substantial equivalence' has no scientific basis. Schalk (2000) adds another aspect by posing the question *to whom* it is equivalent. Several other authors, including Trewavas & Leaver (2000) and Kearns & Mayers (1999) defend the concept. Presently, experimental studies dealing with (aspects of) the question are scarce (cf. Ewen & Pusztai 1999ab). Also, a generally accepted theory of substantial equivalence or other criterion for food safety assessment appears to be nonexistent.

It appears that the apparent controversy over the concept of substantial equivalence has not been resolved. In a recent report of a joint FAO/WHO Expert Consultation in Geneva (see website 17) it is recommended that 'consensus documents are developed to facilitate uniform application of the concept of substantial equivalence'. The Consultation concluded that the key message to be conveyed is that substantial equivalence is a concept used to identify similarities and differences between the GM food and a comparator with a history of safe food use which in turn guides the safety assessment process.

- 3.5.3 Are there specific (long-term) effects of new GM crops on human and animal health? Is adequate knowledge presently available concerning the toxicological and allergenic aspects of GM food?

### Conclusion: Controversy & Gap

There is a controversy over the long-term safety of GM food and methods of evaluating it, and insufficient information, if any, on long-term evaluations of GM food.

### Literature data

While these questions are too general, and a case-by-case analysis is generally considered as proper, such generalizations are nevertheless found in the literature (e.g. Anonymous 2000; Ryan et al. 1999). Some authors express reservations regarding the former question and doubts about the latter (e.g. Kuiper 2000; supported by Schauzu 1999, Van Dam & Schenkelaars 2000). Ewen & Pusztai (1999ab) were the first to present some evidence for an unintended effect of GM itself (GM-lectin potato) on the health (growth and development) of young rats. It must be clear that such a first indication requires further independent research to provide conclusive evidence on this subject.

Dixon & Steele (1999) highlight positive aspects of food quality, noticing that flavonoids possess some antioxidant activity with potential benefits for human health, which has encouraged research into plant flavonoids, commonly found in foods such as fruit, vegetables, green tea and wine. Their review deals with biosynthesis and metabolic engineering of bioactive plant flavonoids and isoflavonoids, and includes biological activities of flavonoids that impact on plant and animal health, metabolic engineering of flavonoid compounds through manipulation of pathway genes, regulatory genes and enzymes, and future prospects for regulatory gene expression.

However, it should be noted that still major gaps exist in the knowledge of the plant metabolism of phenolics, including flavonoids (Stafford 1998), making it difficult to predict the effects of genetic on the concentration and chemical composition of the various (iso)flavonoids and many related compounds in the resulting GM food.

This knowledge gap is relevant to the possibility of unintended side-effects of increasing (iso)flavonoid contents of foods, such as increased toxicity of such foods by associated increases in alkaloids. This is not unlikely, since (iso)flavonoids and alkaloids are products of the same (i.e. isoprenoid pathway) or metabolically related pathways (Conn 1995). Therefore, in view of such risks and gaps in knowledge,

we conclude that the questions we formulated here, can be considered as unresolved for the case of genetic modification of (iso)flavonoid production in food plants.

A backgrounder prepared by the US Institute of Food Technologists (Anonymous 2000) reviews scientific and policy issues concerning foods derived from genetically modified organisms (GMO), highlights the benefits of rDNA technology (mentioning increased biological resistance to pests/diseases, adaptability to harsh growing conditions, herbicide tolerance, desirable functional characteristics) and argues against safety issues (lack of evidence to indicate hazardous effects of transfer of genes between unrelated species, endorsement of GMO safety by the World Health Organization, indications of equal safety of genetically modified (GM) and traditionally bred crops). The authors of this backgrounder conclude that rDNA technology has huge potential, is likely to pose little risk to the environment and consumers, may reduce the need for chemical pesticides, and is compatible with environmental conservation and sustainable agriculture. They suggest that further public debate is required.

3.5.4 Will horizontal gene transfer (HGT) possible from GM crops or micro-organisms to (pathogenic) micro-organisms? [Relevant to: hazardous gene spread; antibiotic resistance of commensal and pathogenic bacteria; food safety]

#### **Conclusion: Gap & Risk**

HGT does occur, also between plants and micro-organisms. Risks include spread of antibiotic resistance genes to pathogenic micro-organisms. Present knowledge gaps concern especially the nature and presence of selection forces and conditions that promote growth and spread of such organisms in food chains.

#### **Literature data**

It becomes increasingly evident, that horizontal gene transfer (HGT; no parent-to-offspring transfer of genes) is far more common in nature than previously thought, and can occur also between plants and micro-organisms. Especially many bacteria are well known to be able to import and integrate external DNA, and to show expression of the foreign genes. DNA may remain stable in soil long enough for HGT to occur (Saxena et al. 1999). Various types of HGT phenomena have been observed, mainly in soil (e.g. Nielsen et al. 1998) but also in microbial ecosystems like bovine paunch, and –most recently– HGT from transgenic oilseed rape pollen to bacteria and yeasts in the intestines of larvae of honey bees (Kaatz et al. 2000).

*If the latter finding is found to be reproducible, it constitutes a potential link with the human food chain. Although the probability of HGT is very low (Nielsen et al. 1998, 2000), HGT will occur inevitably. Therefore, in risk analyses, it is not so much the (chance of) occurrence of HGT that counts, but rather the selective forces and environmental conditions leading to proliferation and spread of the new organisms (Nielsen et al. 1998), and the (hazardous) nature of the transferred genes, for instance antibiotic resistance (Schauzu 1999; Van Dam & Schenkelaars 2000).*

Other reports supporting our conclusion of ‘Risk’ and ‘Knowledge gap’:

Lee-GeonHyoung et al. (1999) studied the transformation and survival of donor, recipient, and transformants of *Bacillus subtilis* in vitro and in soil; they conclude that HGT (Bacillus transformation) does occur and should be taken into account in risk assessments of the release of genetically modified microorganisms to soil. Ulrich-A (1998) gives consideration to evolutionary aspects of horizontal gene transfer and examines the risk of horizontal gene transfer from genetically modified organisms, such as transgenic plants, to other organisms within the ecosystem.

In a review on horizontal gene transfer (HGT), Nielsen et al. (1998) present data suggesting that HGT from transgenic plants to terrestrial bacteria can occur but may be a rare event: 'As new and unique characteristics are frequently introduced into GMPs, risk assessment has to be performed to assess their ecological impact. The possibilities of HGT from plants to microorganisms are frequently evaluated in such risk assessments of GMPs before release into the field. In this review the authors indicate why putative HGT from plants to terrestrial (soil and plant associated) bacteria has raised concern in biosafety evaluations. Further, the authors discuss possible pathways of HGT from plants to bacteria, outline the barriers to HGT in bacteria, describe the strategies used to investigate HGT from plants to bacteria and summarize the results obtained. Only a few cases of HGT from eukaryotes such as plants to bacteria have been reported to date. These cases have been ascertained after comparison of DNA sequences between plants and bacteria. Although experimental approaches in both field and laboratory studies have not been able to confirm the occurrence of such HGT to naturally occurring bacteria, recently two studies have shown transfer of marker genes from plants to bacteria based on homologous recombination. The few examples of HGT indicated by DNA sequence comparisons suggest that the frequencies of evolutionarily successful HGT from plants to bacteria may be extremely low. However, this inference is based on a small number of experimental studies and indications found in the literature. Transfer frequencies should not be confounded with the likelihood of environmental implications, since the frequency of HGT is probably only marginally important compared with the selective force acting on the outcome. Attention should therefore be focused on enhancing the understanding of selection processes in natural environments. Only an accurate understanding of these selective events will allow the prediction of possible consequences of novel genes following their introduction into open environments.'

Gebhard & Smalla (1999) deal with field releases of transgenic rhizomania-resistant sugar beet (*Beta vulgaris*) plants which were accompanied by a study of the persistence of DNA from transgenic sugar beet litter in soil and of horizontal gene transfer of plant DNA to bacteria. The transgenic sugar beets contained the marker genes nptII and bar under the control of the bidirectional TR1/2 promoter conferring kanamycin (Km) and glufosinate ammonium resistance to the plant. Long term persistence of transgenic DNA could be shown under field conditions (up to 2 years) and also in soil microcosms with introduced transgenic plant DNA. No construct-specific sequences were detected by dot blot hybridizations of bacterial isolates. The experimental limitations of detecting horizontal gene transfer from plants to bacteria under field conditions are discussed.

*It is likely that this might have been caused by relative insensitivity of the detection technique used.*

### 3.5.5 Is it possible to separate GM and non-GM food chains?

#### **Conclusion: Gap**

This is a generally recognized question, with gaps like tracking & tracing, management.

#### **Literature data**

##### **The issue of (labelling of) GM-free food:**

Reavell (1999) argues that increasing consumer demand for natural and healthy foods and concerns about food safety (residues, microbiological quality, GM fears) and ethical issues (animal welfare and health) have led to the development of a new 'wholesome food' market category (organic products, GM-free items, functional foods). The author discusses aspects of the development of 'wholesome foods': e.g. growth in organic food sales within the UK and other European countries; consumer concerns (resistance to genetic modification, interest in healthy eating, demand for quality, public awareness of ethical issues); the need for quality assurance schemes to support claims made by 'wholesome food' manufacturers is also emphasized.

Michael (1999) discusses the labelling of products as genetically modified (GM)-free with reference to: setting an upper limit for permissible presence of GM ingredients in products labelled GM-free; testing products for GM ingredients; and legal obligations of a producer to ensure products labelled GM-free are totally free of any traces of GM ingredients.

3.5.6 In testing for toxicological side-effects, is it sufficient to test the isolated gene-product rather than the whole plant part?

#### **Conclusion: Controversy & Gap**

Great benefits for food quality have been predicted by several authors (e.g. nutritional value, improved shelf life, reduced levels of anti-nutritional factors), but controversy exists about methods of testing for the safety of GM food.

#### **Literature data**

There is a study suggesting a negative answer (Ewen & Pusztai 1999b), which has however been criticised (e.g. Kuiper et al. 1999). Attempts to reproduce the experiment of Ewen & Pusztai (1999b) are under way. General frameworks illustrating approaches to quantitative food safety risk assessment have been published (e.g. McNab 1998; Kuiper 1998; Kuiper et al. 1999).

The literature discussed in section 3.5.3 supports the notion that –in view of the numerous linkages between metabolic pathways- modification of (part of) one specific metabolic pathway may well affect the activities of other pathways. In each specific case, such unintended effects should be investigated in relation to the above-mentioned question, especially in cases –like that of (iso)flavonoids and the metabolically related, toxic alkaloids- where the existence of risks is well-established.



‘For the most part, the future will be what we make of it, even if ...its fabric consists of uncertainty, change and unpredictable creation’  
*Federico Mayor, UNESCO Director-General ‘87-’99*

## 4. General conclusion

This report identifies about thirty unresolved questions on the present nature and (potential) use of current genetically modified crops, including a dozen controversial issues, as seen from the standpoint of natural science.

Our findings demonstrate the fragmentary nature of current knowledge of genome structure and function and regulation of gene expression, and the unbalanced and limited understanding of the physiological, ecological, agronomical and toxicological effects of present-day genetic modifications of crops.

Our inventory of the available literature leads us to conclude that much ‘trial and error’ is still involved in the design and creation of genetically modified crops, and that several unintended side effects were not predicted, apparently because of lack or neglect of knowledge. On the other hand, some examples are presented where available interdisciplinary knowledge has been exploited, leading to a better understanding of potential implications of the use of genetically modified crops.



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3. <http://www.foundation.novartis.com/publications.htm>  
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4. <http://www.cgiar.org/isnar/fora/biotech>  
ISNAR Information and Discussion Forum on Policy and Management Issues in Agricultural Biotechnology.
5. <http://www.icgeb.trieste.it/>  
The international centre for genetic engineering and biotechnology (ICGEB): an international organisation dedicated to advanced research and training in molecular biology and biotechnology, with special regard to the needs of the developing world.
6. <http://www.icgeb.trieste.it/biosafety/bsfdata1.htm>  
ICGEB Biosafety database on biosafety studies featuring records from CAB ABSTRACTS[tm] from CABI Publishing.
7. <http://www.aphis.usda.gov/biotech/>  
Detailed information on how the USDA Animal and Plant Health Inspection Service (APHIS) regulates the movement, importation, and field testing of genetically engineered plants and microorganisms through permitting and notification procedures.
8. <http://nbo.icipe.org/agriculture/stemborers.html>  
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10. <http://vm.cfsan.fda.gov/~lrd/biotechm.html>  
U. S. Food and Drug Administration FDA, Center for Food Safety and Applied Nutrition (CFSAN); Biotechnology Information.
11. <http://www.rivm.nl/csr/bggo.html>  
Het Bureau Genetisch Gemodificeerde Organismen (Bureau GGO) is een onderdeel van het Centrum voor Stoffen en Risicobeoordeling (CSR) van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM).
12. <http://www.environment.detr.gov.uk/acre/index.htm>  
Department of the Environment, Transport and the Regions; Advisory Committee on Releases to the Environment ACRE.
13. <http://www.greenpeace.org/~geneng/index.html>  
Greenpeace International; genetic engineering; search news, press releases, background.
14. <http://biosafety.ihe.be/>  
[http://biosafety.ihe.be/GB/Dir.Eur.GB/Cont.Use/98\\_81/98\\_81\\_T.html](http://biosafety.ihe.be/GB/Dir.Eur.GB/Cont.Use/98_81/98_81_T.html) (definitions; Directive 90/219/EEC) Belgian Biosafety Server; Report on 'Safety-considerations of herbicide-resistant plants to be placed on the European market'; Institute of Hygiene and Epidemiology, Service of Biosafety and Biotechnology, Rue J. Wytsmanstraat 14, B-1050 Brussels. Workshop Report, Brussels, January 26th, 1994.
15. <http://www.tec.open.ac.uk/cts/bpg.htm/>  
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16. <http://www.kluyver.stm.tudelft.nl/efb/home.htm>  
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  17. <http://www.fao.org/es/esn/biotech.htm/>  
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## 6. Explanatory notes

### 6.1 List of definitions

A shortlist with some general terms is in prep (e.g. transformation, random insertion, transgenic, genetic modification, annotation, classical breeding, pleiotropy, polygenic trait, pyramidal gene system, sustainability, biodiversity, phyto-remediation, etc.); for a comprehensive collection of terminology relating to gene technology, see Kahl (1995). The EFB (web site 16, [kluiver.stm.nl](http://kluiver.stm.nl)) provides definitions of terms like Biotechnology; Biotechnologist; Code of Conduct; Ethics; Technology assessment; Constructive technology assessment; The principle of precaution.

Definitions according to the Directive 90/219/EEC (web site 14, Belgian Biosafety Server):

- (1) 'organism' is any biological entity capable of replication or of transferring genetic material;
- (2) 'genetically modified organism (GMO)' means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Within the terms of this definition (see also below):

- (1) genetic modification occurs at least through the use of the techniques listed in Annex I Part A;
- (2) the techniques listed in Annex I Part B are not considered to result in genetic modification;
- (3) 'deliberate release' means any intentional introduction into the environment of a GMO or a combination of GMOs without provisions for containment such as physical barriers or a combination of physical barriers together with chemical and/or biological barriers used to limit their contact with the general population and the environment;
- (4) 'product' means a preparation consisting of, or containing, a GMO or a combination of GMOs, which is placed on the market;
- (5) 'placing on the market' means supplying or making available to third parties;
- (6) 'notification' means the presentation of documents containing the requisite information to the competent authority of a Member State. The person making the presentation shall be referred to as 'the notifier';
- (7) 'use' means the deliberate release of a product which has been placed on the market. The persons carrying out this use will be referred to as 'users';
- (8) 'environmental risk assessment' means the evaluation of the risk to human health and the environment (which includes plants and animals) connected with the release of GMOs or products containing GMOs.

## Annex I

### Part A

Techniques of genetic modification referred to in [Article 2\(b\)\(i\)](#) are, *inter alia*:

1. Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.
2. Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism including micro-injection, macro-injection and micro-encapsulation.
3. Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

## Part B

Techniques referred to in Article 2(b)(ii) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs made by techniques/methods other than techniques/methods excluded by Annex II, PART A:

- (1) *in vitro* fertilisation;
- (2) natural processes such as: conjugation, transduction, transformation;
- (3) polyploidy induction.

## 6.2 Glossary of some relevant acronyms

(see for extensive lists, e.g. Kahl 1995; Paula 1999)

Bt, *Bacillus thuringiensis*

CB, classical (or conventional) breeding

DNA, deoxyribonucleic acid

GM, genetic modification (genetic engineering)

GM(M)O, genetically modified (micro-) organism,

HGT, horizontal gene transfer

ELSI, ethical, legal and social issues

GDB, genome database

GENET, European NGO Network on Genetic Engineering

IPR, intellectual property rights

mRNA, messenger RNA

NBV, Netherlands Society for Biotechnology

NIABA, Netherlands Industrial and Agricultural Biotechnology Association

PCR, polymerase chain reaction

NGO, non-governmental organisation

RNA, ribonucleic acid

RIVM, national institute of public health and environment protection (NL)

TA, technology assessment

VROM, ministry of housing, physical planning and the environment (NL)

WHO, world health organisation