Transgenic plants

UNIT 9

European Initiative for Biotechnology Education

Contributors to this Unit

Vic Damen (Unit Co-ordinator)
Cathrine Adley, Fred Brinkman,
Dorte Hammelev, Margareta Johansson, Marleen van Strydonk.

The European Initiative for Biotechnology Education (EIBE) seeks to promote skills, enhance understanding and facilitate informed public debate through improved biotechnology education in schools and colleges throughout the European Union (EU).

EIBE Contacts

AUSTRIA

- Rainhart Berner, Höhere Bundeslehr- und Versuchsanstalt für Chemische Industrie Wien, Abt. für Biochemie, Biotechnologie und Gentechnik, Rosensteingasse 79, A-1170 WIEN.

BELGIUM

- Vic Damen / Marleen van Strydonck, R&D Groep VEO, Afdeling Didaktiek en Kritiek, Universiteit van Antwerpen, Universiteitsplein 1, B-2610 WILRIJK.

DENMARK

- Dorte Hammelev, Biotechnology Education Group, Foreningen af Danske Biologer, Sønderengen 20, DK-2860 SØBORG.
- Lisbet Marcussen, Biotechnology Education Group, Foreningen af Danske Biologer, Lindevej 21, DK-5800 NYBORG.

EIRE

- Catherine Adley / Cecily Leonard, University of Limerick, Plassey, LIMERICK.

FRANCE

- Gérard Coutouly, LEGPT Jean Rostand, 18 Boulevard de la Victorie, F-67084 STRASBOURG Cedex.
- Laurence Simonneaux, Ecole Nationale de Formation Agronomique, Toulouse-Auzeville, Boîte Postale 87, F-31326 CASTANET TOLOSAN Cedex.

GERMANY

- Horst Bayrhuber / Eckhard R. Lucius / Regina Rojek / Ute Harms / Angela Kroß, Institut für die Pädagogik der Naturwissenschaften, Universität Kiel, Olshausenstraße 62, D-24098 KIEL 1.
- Ognian Serafimov, UNESCO-INCS, c/o Jörg-Zürn-Gewerbeschule, Rauensteinstraße 17, D-88662 ÜBERLINGEN.
- Eberhard Todt, Fachbereich Psychologie, Universität Gießen, Otto-Behaghel-Straße 10, D-35394 GIEßEN.

ITALY

- Antonio Bargellesi-Severi / Stefania Uccelli / Alessandra Corda Mannino, Centro di Biotechnologie Avanzate, Largo Rosanna Benzi 10 , I-16132 GENOVA.

LUXEMBOURG

- John Watson, Ecole Européenne de Luxembourg, Département de Biologie, 23 Boulevard Konrad Adenauer, L-1115 LUXEMBOURG.

THE NETHERLANDS

- David Bennett, Cambridge Biomedical Consultants, Schuystraat 12, NL-2517 XE DEN HAAG.
- Fred Brinkman, Hogeschool Holland, Afdeling VP&I, Postbus 261, NL-1110 AG DIEMEN.
- Guido Matthée, Hogeschool van Arnhem en Nijmegen, Technische Faculteit, HLO, Heijendaalseweg 45, NL-6524 SE NIJMEGEN.
- Liesbeth van de Grint / Jan Frings, Hogeschool van Utrecht, Educatie Centrum voor Biotechnologie, FEO, Afdeling Exacte Vakken, Biologie, Postbus 14007, NL-3508 SB UTRECHT.

SPAIN

- Maria Saez Brezmes / Angela Gomez Niño, Facultad de Educación, Universidad de Valladolid, Geologo Hernández Pacheco 1, ES-47014 VALLADOLID.

SWEDEN

- Margareta Johanssen, Föreningen Gensyn, PO Box 37, S-26800 SVALÖV.
- Elisabeth Strömberg, Östrabo Gymnasiet, PO Box 276, Kaempegatan 36, S-45181 UDDEVALLA.

THE UNITED KINGDOM

- Wilbert Garvin, Northern Ireland Centre for School Biosciences, NIESU, School of Education, The Queen's University of Belfast, BELFAST, BT7 1NN.
- John Grainger / John Schollar / Caroline Shearer, National Centre for Biotechnology Education, The University of Reading, PO Box 228, Whiteknights, READING, RG6 6AJ.
- Jill Turner, Department of Science and Technology Studies, University College London, Gower Street, LONDON, WC1 6BT.
- Paul Wymer, The Wellcome Centre for Medical Science, The Wellcome Trust, 210 Euston Road, LONDON, NW1 2BE.

EIBE Co-ordinator

Horst Bayrhuber, Institut für die Pädagogik der Naturwissenschaften an der Universität Kiel, Olshausenstraße 62, D-24098 KIEL 1, Germany. Telephone: + 49 (0) 431 880 3137 (EIBE Secretary: Regina Rojek). Facsimile: + 49 (0) 431 880 3132.

Transgenic plants

UNIT 9

European Initiative for Biotechnology Education

CONTENTS

Contents

World Wide Web

Few areas are developing as rapidly as biotechnology. So that they can be revised and kept up-to-date then distributed at minimum cost, the EIBE Units are published electronically.

These pages (and the other EIBE Units) are available throughout Europe and the rest of the world on the World Wide Web. They can be found at:

http://www.reading.ac.uk/NCBE

All of the EIBE Units on the World Wide Web are Portable Document Format (PDF) files. This means that the high-quality illustrations, colour, typefaces and layout of these documents will be maintained, whatever computer you have (Macintosh - including Power PC, Windows, DOS or Unix platforms).

PDF files are also smaller than the files from which they were created, so that it will take less time to download documents. However, to view the EIBE Units you will need a suitable copy of the *Adobe Acrobat*® Reader programme.

The latest *Acrobat*® Reader programme is available free-of-charge. It can be downloaded from the EIBE Web site or from:

http://www.adobe.com/

With this software, you can view or print the EIBE Units. In addition, you will be able to 'navigate' around and search the documents with ease.

Contributors to this Unit

- **Vic Damen (Unit Co-ordinator) & Marleen van Strydonck**
  Universiteit Antwerpen, R&D Groep VEO, Afdeling Didactiek en Kritiek, Universtteitsplein 1, B-2610 Antwerpen, Belgium.
- **Catherine Adley**
  University of Limerick, Plassey, Limerick, Ireland.
- **Fred Brinkman**
  IDO/VU, Vrije Universiteit Amsterdam, De Boelelaan 1115, NL-1081 HV Amsterdam.
- **Dorte Hammelev**
  IMFUFA
  University of Roskilde, Denmark.
- **Margaretta Johansson**
  Svalöv Science Centre, Svalöv, Sweden.

Design, illustration and typesetting: Caroline Shearer, NCBE, The University of Reading, The United Kingdom.

Acknowledgements

Dr F. Folmer D. Eriksen from the Danish Ministry of Food, Agriculture and Fisheries Institute of Toxicology has been extraordinarily helpful throughout the preparation of this Unit.
Dr Holger Petersen and Dr Juliane Alberg from the Danish Ministry of Environment and Energy, and the Danish Environmental Protection Agency, have provided useful information and advice.

© Copyright

EIBE Secretariat
c/o IPN
Universität Kiel
Olshausenstraße 62
D-24098 KIEL 1
Germany
Telephone: + 49 (0) 431 880 3137
Facsimile: + 49 (0) 431 880 3132
E-Mail: rojek@ipn.uni-kiel.de

About EIBE Units

These materials have been devised by practising teachers and educationalists from several European countries, brought together with financial support and encouragement from DGXII of the European Commission, under the auspices of EIBE, the European Initiative for Biotechnology Education. The EIBE materials have been extensively tested in workshops involving teachers from across Europe.

The views expressed in this Unit and the activities suggested herein are those of the authors and not of the European Commission.

About this Unit

This Unit consists of up-to-date information on transgenic plants and their use in society today. It is intended to increase understanding and provide background information as a basis for classroom discussions about the role of transgenic plants in the modern world

This Unit consists of:

1. Problem-oriented introductory text.
2. The essential scientific principles and technologies that are involved in the making of a transgenic plant.
3. The importance and implications of field trials.
4. Information about risk assesment and EU regulations.
5. Background information on selected vegetables and crops: tomatoes; potatoes; soya beans and rape seed oil, which are in the forefront of transgenic plant research.
6. Suggestions for reflections about the benefits and the problems that are foreseen with the generation and world-wide use of transgenic plants.
7. An evaluation of students' understanding of the concepts of plant, gene and the expression of genetic traits (Questionnaire: Appendix 2).

How can this Unit be used?

The students do not need extensive previous knowledge of transgenic plants or DNA technology. They should have a basic knowledge of genetics and if possible of some basic elements of genetechnology. To obtain an impression of students' understanding of the concept of plant, gene and the expression of genetic traits, the questionaire (Appendix 2) can be used. The form should not take more than 10 minutes to complete. It is important not to give any hints and the students should be encouraged to answer the questions even if they are not sure about the answers.

The Unit can be used in a traditional way in science classes in order to develop the concept of transgenic plants and the societal isssues accompanying the use of these plants.

Objectives: the students can

- decribe the different techniques used to make a transgenic plant;
- explain the low success rate, i.e. the few successful results and the instability of the transgenic plant under non-optimum circumstances;
- explain that introducing transgenic plants in western society is preceeded by research, field trials, in-depth risk assesment and is controlled by extensive regulations;
- balance the benefits and disadvantages of the use of transgenic plants, using arguements from biological, economic and sociological perspectives.

The Unit can also be used in a problem-oriented approach. The introductory text will then act as a starter to analyse a specific problem that arose from the application of the transgenic cotton plant. The Unit provides supporting information to be used by the students to obtain answers to their questions. As a result of these activities, students can discuss the advantages and disadvantages of the use of different transgenic plants.

In addition to the objectives stated above, the students can also develop skills of problem-analysis and search for information to develop an insight into the problems identified in the introductory text.

INTRODUCTION

Introduction

★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★

In the summer of 1996 headlines about genetically modified cotton filled the newspapers. In Mexico and the southern states of the USA, cotton plants genetically modified to be resistant to caterpillars had been found in some areas, in their second season, to have failed to show resistance. Caterpillars had damaged the crop in the usual way by eating the seed capsules. 800 thousand hectares of this particular cotton plant had been grown. The resistance introduced into the plants should have caused the larvae to be poisoned on eating the plants.

The gene for the poison comes from *Bacillus thuringiensis*, known as the Bt-bacterium. It is common on leaves and its poison has been used for many years as a pesticide spray against different types of caterpillars. Such spray is relatively environmentally friendly because it decomposes fast and only a selected group of animals—some caterpillars and larvae—are killed. The poison does not harm other animals living on or around the cotton, neither is it poisonous to human beings. Cotton-growing has for many years demanded very intensive use of insecticides. It was therefore an advantage to grow the new insect-resistant plants with the consequent reduction in the quantity of chemical sprays needed.

It is unfortunate that these particular genetically-modified cotton plants, grown in Mexico and the Southern United States, have been attacked by three different species of caterpillars all of which were supposed to be poisoned when eating the plants.

The question for the scientist and the farmer is now whether the larvae have developed resistance to the Bt poison or are there other possible explanations? Organic farmers have already demanded that the new cotton should be removed as the Bt bacteria are used by them as a biological protection against harmful caterpillars of various kinds, and they greatly fear the development of resistant caterpillars.

A alternative explanation for the extremely high number of caterpillars in the cotton fields could be that the growth rate has been much higher due to an extremely hot and dry summer. It is also known that stress, such as very high temperatures, can effect gene expression in different tissues.

This case is interesting for us here in Europe because at the moment (late 1996) three different maize plants are being considered by European Commission for permission to market. All three have an added Bt gene construction similar to that used in the cotton. One of the reasons for the delay at the European Commission is a careful consideration of the possibility of the development of insect resistance to the existing Bt spray and of its effect on the environment.

Definition

Gene modified, gene spliced or transgenic plants are defined as plants that have had one or more genes from a different plant or organism, or a gene or genes that have been altered or specially assembled, inserted into their genome.

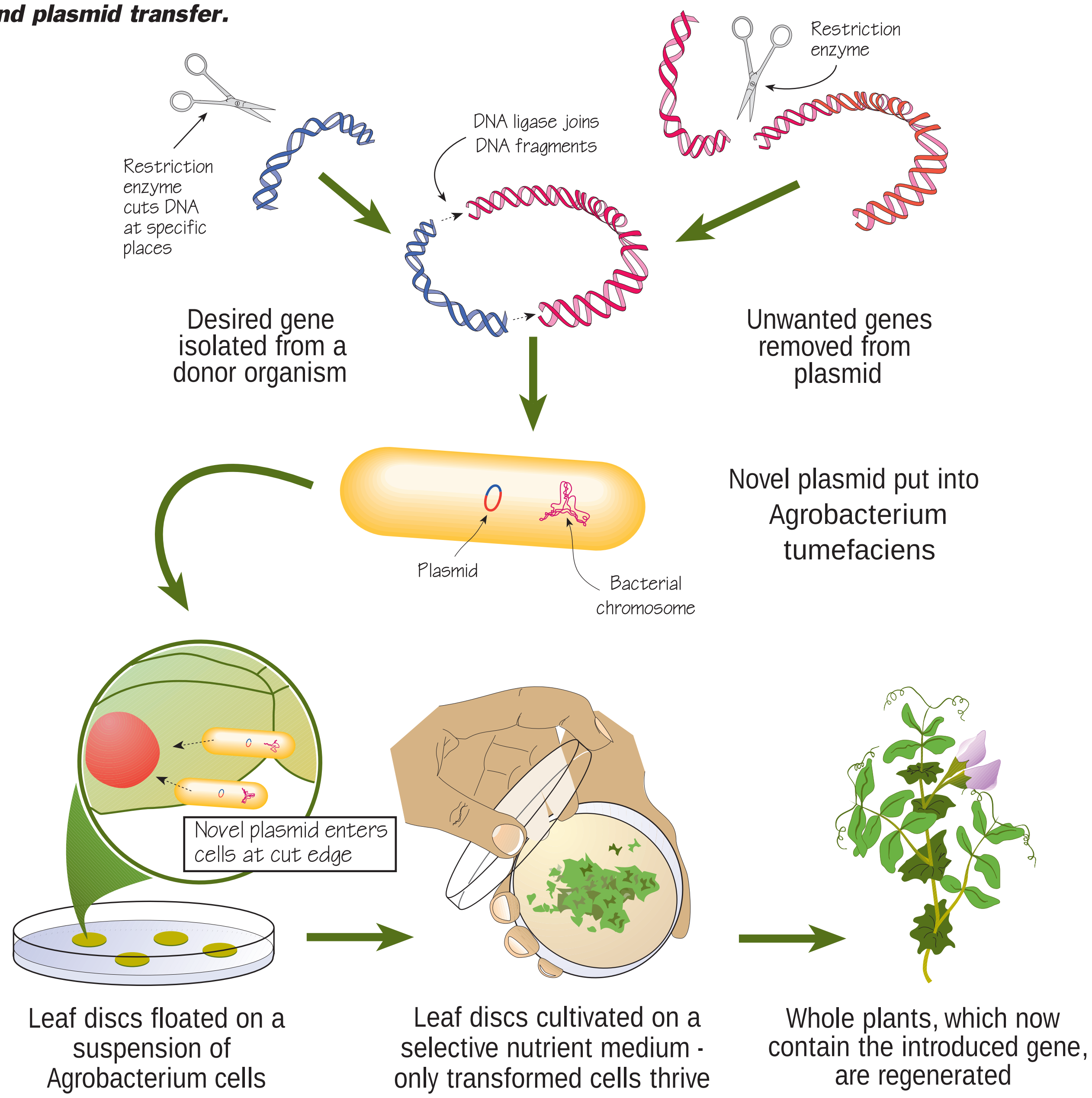
How a transgenic plant is made

Laboratory techniques

***Agrobacterium tumefaciens* method**

The first transgenic plants were created in the early 1980s, when the ability of a bacterium, *Agrobacterium tumefaciens*, to transfer genetic material into plants was discovered. Other methods are now

***Figure 1:* Agrobacterium tumefaciens and plasmid transfer.**



available but this first technique to be developed is still widely used.

A. tumefaciens is a soil bacterium which contains, as well as its chromosome, an extra, circular mini-chromosome called the tumour-inducing (Ti) plasmid. This piece of DNA contains genes that are resposible for the crown gall disease of plants. It is possible to remove the genes that cause the tumours and replace them with selected genes, making the Ti plasmid a vector to transfer new genes into the plant (Figure 1). This method is now a standard procedure and further details can be found in many text books.

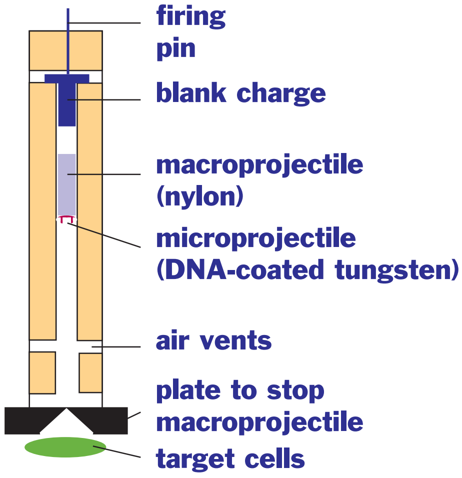
In vivo, infection requires wounding of the plant tissue. *A. tumefaciens* attaches to plant cell walls activated by compounds from the wounded cells (compounds that activate the bacteria are also produced by the wounded cells). Part of the Ti plasmid (the T-region) is then transfered into the chromosomes of the host plant where it becomes integrated (T-DNA). Several gene loci on the bacterial chromosome and a set of virulence (vir) genes located on the Ti plasmid code for functions involved in plant cell recognition and attachment as well as for the excision, transfer and integration of T-DNA into the target genome.

Although this is a very effective transformation method, it works better for some plants than others. A successful transformation depends both on the ability of *A. tumefaciens* to infect the cells and incorporate its T-DNA into the plant genome before it is destroyed by the plant cell and on whether the transformed cells can be cultured to form to a whole plant. Plants from the Solanaceae family such as tobacco, tomato and potato have given the best results. At the other, negative end of the scale are the monocotylodons, including the four species of grain, rice and maize which *A. tumefaciens* does not readily infect. It has proved more difficult to transform these plants, all with great nutritional and commercial value, using the *Agrobacterium* method. A new, more aggressive type of *A. tumefaciens* has recently proved successful in preparing transgenic maize plants.

The gene canon method

Plant geneticists have, however, come up with several alternative methods. In one of these, the gene canon, minute metal beads coated with DNA are 'shot' directly into plant cells. The plant cells repair the wounds quickly and in some cells DNA is incorporated into the plant chromosomes.

Figure 2: The Gene Cannon



The rate of success

Whether the *Agrobacterium* or the gene canon method is used, the transformation success rate is rarely over 1:10 000 per cell.

It is not possible to determine where the new gene (or perhaps several copies of it) are going to be incorporated. This problem is currently under investigation but satisfactory methods have not yet been developed. On the other hand it is possible to find those plants with more than one copy of the desired gene, these are then removed as multiple copies of the same gene often inhibit expression. The mechanism of this is not yet understood.

Faster and more precise plant breeding

The production of transgenic plants has to be seen in connection with traditional plant breeding, where humans since prehistoric time have selectively bred particular wild plants with good characteristics. Qualities such as strength, yield, resistance against noxious organisms and the ability to withstand wind and weather were improved by crossing the best individuals with each other.

It takes 10–15 years to develop a new plant type using traditional breeding methods. Gene tranfer techniques can reduce this time by a half and make it possible selectively transfer genes so that it is possible to know exactly which characteristics have been introduced. Plant breeding using modern gene technology also gives the potential to introduce genes from non-related species.

'Synthetic' genes

Sometimes 'synthetic' genes genes are used in gene transfer, where the base sequence of the DNA in the gene to be introduced has been changed. In most cases the last base in a triplet codon can be changed without changing the amino acid that it codes for. Before the bacterial Bt gene is introduced into a plant it is changed to make the CG:AT ratio similar to that of plants. These changes are necessary for a satisfactory expression of the gene in the plant cells.

Antisense and partial sense

One of the factors involved in fruit softening is an enzyme, polygalacturonase or PG, that breaks down pectin in the cell wall. When producing the *Flavr Savr*® tomato (which does not go soft when ripe) the tactics of the scientists working for *Calgene* in the USA were to make a 'reverse copy' of the PG gene and then to introduce this into plant cells. The RNA produced from both the original gene and the introduced one complement each other. The expression of the PG gene in the tomato is reduced and consequently the softening process is delayed. The *Flavr Savr*® tomato was sold only in the USA but has been withdrawn from the market for the time being.

In the United Kingdom, *Zeneca Plant Sciences* have developed a tomato in which a slightly different technique has been used to reduce softening. A shortened PG gene has been inserted which, in some way that is not completely understood, reduces the production of polygalacturonase. It is thought that the RNA produced from the two genes interfere with each other.

Several biotechnology companies are trying to alter the contents and the amount of starch in potatoes. Using the antisense technique, a Danish company is trying to inhibit the α-amylase gene that causes conversion of starch into sugar during the storage of the potatoes. The formation of sugar is a natural process and a pre-condition for germination, but is undesirable when potatoes are in storage. If the company succeeds, they will have a potato that stores much better without deterioration. At the same time they will have gained an improved potato for making crisps or chips, because the lower sugar levels will reduce the tendency of the potato to burn when deep-fried.

Marker genes

Marker genes are genes introduced with the aim to identify and isolate the cells that have been transformed from those that have not taken up the desired gene. Marker genes in bacteria are often antibiotic resistance genes. In plant cells the marker gene is often a gene that gives tolerance to a herbicide e.g. glyphosate. A frequent concern during risk assessment is whether it is possible for a gene from a transgenic plant to transfer into a bacterium. The gut of an animal or a human being would be a good environment for such an event, where, during digestion, the plant DNA is exposed in the presence of millions of bacteria. Expert opinion is that this type of transfer is extremely unlikely. Nevertheless at present the genes preferred for this purpose confer resistance to antibiotics that are not used in human medical treatment. The kanamycin resistance gene is therefore one of those considered acceptable. It is not used in medical treatment, and many soil bacteria are already resistant to it. The use of the gene conferring ampicillin resistance is for the same reason less acceptable as a marker gene because ampicillin *is* used in medical treatment. Further work is underway on the use of alternative genes for metabolic enzymes as genetic markers.

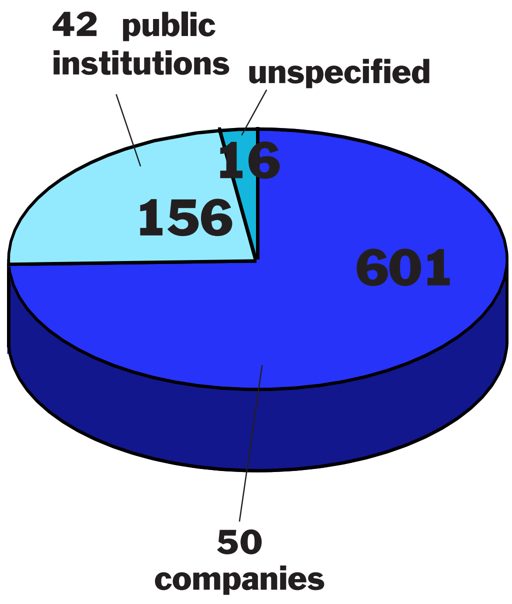
The use of transgenic plants

Field trials and regulations

Economy and Research

In the 'old days' (which, in this field, means 10 to 15 years ago) there were many small plant breeders in each country. However, many of these small companies have been bought up or amalgamated and the market is now dominated by a few big multi-national companies. The use of biotechnological techniques to speed up plant breeding is not cheap: the necessary manpower and the materials and methods used are expensive. The use of biotechnology in plant breeding has therefore only been possible because companies have been large enough to carry this investment in research and development. Today, research in this area

Figure 3: Number of field test applications for transgenic plants by type of applicants.



Source: Agro Food Industry Hi tech, Italy, Vol. 2 5 March/April 1994

paid for by public money is small compared to the research undertaken by multinational companies.

The large input of research from industry has had the effect of reducing both the variety of basic research topics under investigation and the time lapse between research and its commercial application. Figure 3 gives details of applications for field tests of transgenic plants in Europe.

Field trials

Transgenic plants are produced and tested all over the world, as can be seen from the information in Figure 4. The numbers do not indicate the number of new varieties of plants being developed as many have several trials. China is reported to have established its own regulations for field tests, but little is known of work there.

Risk assessment

The principal concerns of a risk assessment of transgenic plants can be summarised:

- the possibility of transfer of genetic materials to other organisms;
- the environmental consequences;
- effects on human and animal health.

Figure 4: World-wide field trials of transgenic plants, 1986–1994.

Country	Number of Trials
Europe	
Belgium	81
Denmark	11
Finland	10
France	168
Germany	6
Hungary	4
Italy	14
Netherlands	84
Norway	1
Portugal	4
Spain	16
Sweden	17
Switzerland	2
United Kingdom	78
Asia	
Australia	26
Japan	8
China	30
New Zealand	15
Thailand	2
North America	
Canada	358
USA	1,031
Africa	
Egypt	1
South Africa	9
Middle East	
Israel	4
Latin America and Caribbean	
Argentina	20
Belize	4
Bolivia	4
Chile	13
Costa Rica	5
Cuba	9
Dominican Republic	1
Guatemala	1
Mexico	15
World Total	**2053**

Source: The Gene Exchange, Vol. 5, No. 3, December 1994

Investigations are made case-by-case using model systems gradually increasing in complexity to include organisms others than the one investigated. Artificial systems are well defined making experiments easy to repeat. These progress to more natural ecosystems. A lot of experimental problems are encountered in the more complex systems and it is important to stress that for risk assessment, information from all systems may be useful.

EU regulation

A necessary condition prior to marketing a transgenic plant in EU is that the plant has been field tested without any unforeseen effects, especially with respect to interbreeding with other crops or wild relatives. Permission for field tests is given by the authority in the country of the application. Authorities in other EU countries can challenge a field test application within 30 days (for more details see Appendix1).

A licence for the sale of a transgenic variety or product is granted by one EU country and permission obtained in one country will automatically mean permission in all EU countries. A licence can be questioned by authorities in other member states within 60 days. In Denmark the legislation allows interested organisations and green groups to participate in the discussion and 10 different associations participate regulary. One of these is The Association for Danish Biologists (an organisation of Danish secondary school biology teachers) thus giving a good opportunity for biology teachers and their students to understand and follow current cases.

Which plants?

Which Traits?

Around the world, researchers are working with many different types of genes. As much of this work is commercially sensitive, information about it is not available. It is only when the application for a field test is made that the work is made public and the trends become apparent.

In a review of field tests (Figure 5) it can be seen that the largest group of tested plants were modified to be tolerant to different herbicides such as *Roundup*® and *Basta*. This reflects the world wide pattern. Herbicide tolerant genes were the first to be successfully transferred into crop plants.

Figure 5: The top five plants and their modification traits from 1986 to 1994.

Trait	Number of Field Trials* with				
	Potato	Oilseed rape	Tobacco	Maize	Tomato
Herbicide tolerance	16 (5)	94 (7)	29 (6)	54 (3)	21 (5)
Quality improvement	31 (9)	57 (5)	13 (4)	15 (2)	39 (3)
Virus resistance	60 (12)	2 (2)	24 (7)	10 (4)	20 (9)
Insect resistance (Bt)	34 (4)	3 (3)	19 (3)	24 (2)	16 (1)
Marker gene	23 (7)	17 (5)	28 (9)	8 (4)	4 (3)
Fungal resistance	9 (7)	5 (4)	9 (4)	2 (1)	
Multiple traits	8 (7)	2 (1)	4 (3)		
Bacterial resistance	9 (3)	1 (1)			
Unspecified	3	1	5	5	3

*The number of different properties introduced into the crop is shown in brackets.

Source: P.Ahl Goy and J.H.Duesing, From Pots to Plots: Genetically Modified Plants on Trial. 1995 Biotechnology Vol. 13. May, 454-458.

Figure 6: Transgenic plants for which field trial applications have been made worldwide (1994). The list is only complete for the tests applied for in the EU. Not all plants on the list have actually been field tested. As a curiosity it can be mentioned that the herbicide resistant oilseed rape was not trialled as planned in Germany in 1994 because the test area was blocked by activists during the growing season. Trials have now taken place in various countries. Many of the plants listed for EU trials have also been tested outside the EU.

Plant	EU trial	Non-EU trial
Vegetables, fruits and other foods:	Apples, Carrot, Cauliflower, Chicory, Lettuce, Maize, Melon, Potato, Squash, Strawberry, Tomato,Wheat, Wine	Asparagus, Cucumber, Kiwi, Papaya, Rice, Plum and Walnut trees
Animal feeds & Non food use:	Alfalfa, Beetroot, Cotton, Oilseed rape, Soyabean, Sugar beet, Sunflower, Tobacco	Flax
Flowers:	Chrysanthemum, Petunia, Marigold, Dianthus	Gerberra
Trees:	Birch, Eucalyptus, Poplar	

Sour

Which crops?

Work is being done all over the world on a large variety of plants. Obviously not all this work progresses to field test applications,yet these are the main source of information about the crops for which transgenic varieties are being developed (see Figure 6).

Which transgenic plants are for sale?

When considering genetically modified foods that have actually gained approval for sale, the list (Figure 7) is shorter. China also grows several different transgenic plants for sale such as virus resistant tobacco, tomatoes and sweet peppers, but there is no information available on possible monitoring programmes or field tests of these (Prosamo Report).

Figure 7: Transgenic plants approved for market production (by late 1996).

In the European Union
(to February 1997)

Herbicide tolerant:
Oilseed rape, (Basta, only as seeds);
Maize, (released 24/1/97 for seeds, food and feed);
Tobacco (Bromoxynil, the marketing permission is not used);
Soyabean (Basta, not grown in EU)
Also:
Red Chicory salad (male sterile, permission to produce and sell seeds only, new permission would be needed for food and feed marketing)

In USA, Mexico and Canada
(to November 1996)

Herbicide tolerant:
Oilseed rape, (Basta); Maize, (Basta); Soyabean, (Basta, Roundup); Cotton, (Round Up); Oilseed rape, (Roundup); Cotton, (Bromoxynil)
Insect resistant:
Cotton, (Bt gene, 3 companies); Potato, (Bt gene) ; Maize, (Bt gene, 3 companies)
Also:
Virus-resistant Squash;
Oilseed rape, altered fatty acid composition
Tomatoes with altered softening and ripening characteristics:
Flavr Savr® Tomato (antisense PG gene, now withdrawn); Zeneca Tomato (partial sense PG gene,sold only in UK); Endless Summer Tomato (less production of ethylene); Cherry Tomato; Tomato (delayed ripening)

INFORMATION BOX

Herbicide tolerance

Glyphosphate is one of the most potent broad spectrum **herbicides** known. It is marketed under the trade name *Roundup*®. *Roundup*® works by inhibiting the action of an enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSP synthase). This enzyme is necessary for the production of the aromatic amino acids tyrosine, phenylalanine and tryptophane, essential amino acids for plant growth. Animals acquire these amino acids in their diet and do not have the enzyme EPSP synthase so they are unffected by glyphosate. The gene for the enzyme EPSP synthase was isolated and modified, using genetic engineering techniques, to be able to make large amounts of EPSP synthase. This was then inserted into crops such as tomato, soyabean, cotton and oilseed rape in order to give them tolerance to glyphosphate at levels that could be used to control weeds.

Insect resistance

Genes from ***Bacillus thuringiensis*** (Bt) are the only **insecticide** genes in use at the present time. The cytoplasm of bacterial cells does not contain the complex organelles such as mitochondria and chloroplasts found in plant and animal cells. However some bacterial species contain ‘structures’ in the cytoplasm e.g. the endospore and, in the case of *B. thuringiensis*, a crystalline parasporal body.
The parasporal body contains a toxic protein, the crystal (cry) protein. In *B. thuringiensis* the toxin genes are carried on large plasmids. There are several cry variants and each are poisonous to a very specific group of moths.
The most common variants are:

Kurstaki	δ-endotoxin* type I	caterpillars
Kurstaki	δ-endotoxin* type II	caterpillars, beetles
Tenobrionis, San Diego	δ-endotoxin* type III	Beetles
Israelensis, Morrisoni	δ-endotoxin* type IV	Diptera (mosquitoes and flies)
Thuringiensis	β-exotoxin**	Flies and others

* δ-endotoxins are accumulated in the bacteria as crystals containing precursors for the true toxin. Most of the sensitive insect species have alkaline stomach juices which dissolve the crystals; they also have enzymes for the conversion of the toxin precursors to the active toxin. Type I–IV can be further sub divided.
**β-exotoxin is excreted from the bacteria. Its function is to block mitosis, its use is prohibited in Europe and the USA because of its potential to change chromosomes and its toxic effects on embryos of higher animals. Bt strains with β-exotoxin are produced and used in the former Soviet Union.

Together they can kill over 100 species of moths, but they are harmless to spiders and many other insects, higher animals and humans. This is due to three factors:

- the crystal dissolves when ingested by moths due to the alkaline conditions in their gut,
- a specific protease is produced in the gut
- the gut cells are especially effective in taking up the poison.

The toxins break down quickly in the environment and leave no harmful residues.

Case studies

CASE STUDIES

Oilseed rape

In 1995 the herbicide-resistant (*Basta*-resistant) oilseed rape was approved for use in the EU, for the production of seeds. It was not unanimously accepted. Denmark voted against its approval, referring to the fact that oilseed rape can cross with bird rape, a wild relative often present as weed on fields. Crosses could also occur with other varieties of oilseed rape growing nearby, giving a risk that other oilseed rapes and wild relatives will become resistant. This could result in varieties of oilseed rape becoming persistent weeds, especially as seeds from oilseed rape can remain in the soil for many years and still retain their ability to germinate.

The intended benefit is to enable weed control to be achieved with a few sprayings of *Basta*, a relatively environmentally friendly agent for weed control, but if resistance spreads to other varieties it could ultimately lead to the need for extra sprayings with less environmentally friendly agents, thus defeating the original purpose of the herbicide tolerent plants. As a curiosity it can be mentioned that investigations in Denmark, during trials for a *Roundup*®-resistant sugar beet, revealed a lot of hybrid plants between sugar beets and wild sea beet. Investigations of natural populations of wild sea beet showed that genes from sugar beets had been incorporated.

Maize

Certain varieties of maize, grown in the USA have now been genetically modified to be resistant to the pest, the European Corn Borer. This pest bores through the stem and the ear of the plant causing it to topple over, or the ear to fall to the ground. On average, it destroys 4% of the world's annual crop and up to 20% in several infested regions. The European Corn Borer is traditionally controlled using chemical or biological insecticide sprays which are applied to the outside of the plant. However, these insecticides are only effective during the first three days in the corn borer's life cycle. The new varieties of maize contain a Bt gene that encodes a protein which kills the corn borer. The Bt gene variants are similar to those introduced into several American cotton plants as described earlier.

These new varieties of genetically-modified maize will be imported into Europe as seeds to be processed to starch and glucose syrups and for feed. The modified maize includes a marker gene for ampicillin resistance in bacteria, but this is inactive and not expressed in the maize. Ampicillin is an antibiotic used in medical treatment. Several countries have voiced concern over the presence of the ampicillin marker gene and the problems of labelling the products in which the maize has been used. The 0.6% of the 1996 US harvest which is from genetically-modified varieties has not been separately identified from the rest of the crop. Nevertheless the importation into the EU of *processed* forms of this maize for use in food products has been approved (January 1997).

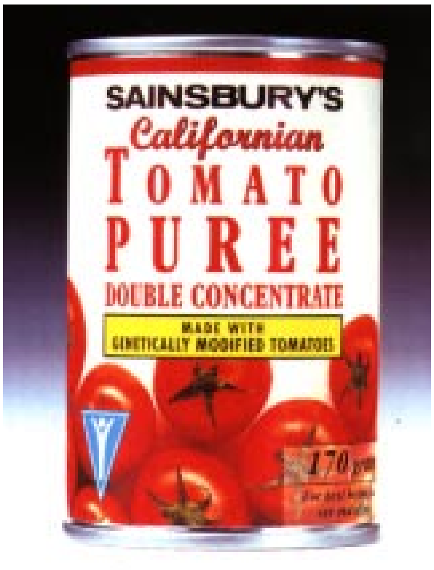
Tomatoes

In Europe, a purée from a tomato developed by *Zeneca Plant Sciences* in the United Kingdom with a shortened PG gene (see page 9) was the first transgenic food product to reach the consumer. The new purée has several advantages: less wastage during transport; reduction in energy requirements during processing and improved flavour due to lower processing temperatures. The *Zeneca* tomatoes are grown in Mexico and the USA, the paste is only available in cans in the United Kingdom. They are clearly labelled (Figure 8).

The *Zeneca* tomato also has a kanamycin resistance gene. The introduced genes are destroyed during the processing of the tomato. It was important, before it was approved for sale for the purée to be assessed for any nutritional changes or allergy-inducing potential (due to new proteins). All investigations so far have shown no problems of this sort.

It is actually possible with the polymerase

Figure 8: Purée from genetically modified tomatoes, as sold in the United Kingdom.



chain reaction (PCR) method (see EIBE Unit 2) to detect tiny amounts of the genetic material incorporated into the modified tomatoes in the processed tomato paste. DNA is an extremely stable molecule. These very few genes present in the paste cannot be considered harmful.

In the USA the *Flavr Savr®* tomato, produced using the antisense technique to slow ripening has been withdrawn from the market because of problems with its cultivation. The strain selected has been found to be susceptible to disease. During the growing season in 1995 it was sold throughout the USA.

Soya

The first herbicide (*Roundup®*) tolerant soya bean produced in the USA was released for marketing in the EU in April 1996. Three countries opposed its approval due to lack of regulations about labelling. They wanted the public to have the right to choose whether to buy food produced from genetically modified plants. *Roundup®* is considered an environmentally-acceptable herbicide due to its very quick breakdown in the soil. Nutritionally there is no difference between genetically modified and unmodified beans. The feelings aroused by the use of transgenic soya beans can be compared to those feelings that make people to choose organic food products instead of products from farms using more conventional management.

Therefore, when the ship *Hanjin Tampa* was on its way over the Atlantic, just before Christmas 1996, carrying 23 000 tons of soya beans to be used in a processed form or for cattle feed, it created headlines in the Danish media, and near chaos in the Danish parliament due to its cargo of a mixture of genetically-modified and 'normal' soya beans. In Denmark, according to a Parliamentary decision in 1994, genetically-modified foods have to be labelled. This decision will stand until the EU Novel Food Regulation comes into practice in 1997. The Novel Food Regulation will not require a company to label foods in situations where the food contains a manufactured product if there is not a mayor difference between the product made from transgenic plants and the original product, but companies may choose to label such foods.

While the *Hanjin Tampa* was approaching the Danish port of Århus, people in Denmark, and in the whole of Europe became aware of soya as a mayor compound in our manufactured food. More than 60% of the processed food contain soya or soya products. Very few consumers had previously been aware of this.

So far (February 1997) the soya in Århus has not been used in production of any food in Denmark, according to the normal users. The whole case has created a good deal of uncertainty which could have been avoided if the company had chosen another and more open and consumer-friendly policy. A parallel can be drawn to the tomato purée sold in the United Kingdom which *is* clearly labelled (Figure 8). The tomato paste has been reported to be a real success among the consumers. Consumers are not against the use of modern gene technology *per se*, if they can see the advantages and benefit from it. It is therefore very important to keep an open flow of information to avoid mistrust between producers and consumers.

Potentials and problems

★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★

There are many traditional areas of crop improvement currently being developed using transgenic techniques, these include improvements in nutritional content, resitance to a variety of pests, pathogens and weed control agents and improved survival during environmental stress. Transgenic plants also have potential for the production of new and improved raw materials for a wide range of industries: building and construction; textiles; dyes; packaging and medicine. For instance research is being carried out into production of new sorts of oils for both the food industry and in non-food applications, into biodegradable plastics and into the production of fuels by plants. In the medical area transgenic plants are being developed for the production of high value molecules such as antibodies, vaccines and anticoagulants.

Some problems to consider

What could be the impact of the development of resistance in crop pests?
Bt has been used as a spray for over thirty years without problems. It is one of the few methods of insect control that can be used by organic farmers. However, since the more widespread use of Bt in the 1990s, there are scattered reports pointing out the possibility of the emergence of resistant caterpillars in the cotton fields of Mississippi in the USA.

Will the use of transgenic plants reduce the levels of herbicide and pesticide use?
A Danish report gave some evidence that the presence of herbicide-tolerant plants could result in less use of herbicides, a sustained use or an increase in the use of herbicides in the future, according to the plant type.

The increasing concentrations of pesticides in ground water is cause for concern, particularly since it has been connected with a deterioration of human sperm quality and quantity. Will the development of pest-resistant crops reduce these levels?

Commercial benefits versus societal....?

There are also many questions of political, ecological, economic, social and ethical nature that need to be addressed, for example:

- Will rich countries develop and grow transgenic plants traditionally produced by the underdeveloped countries?
- Some companies developing the herbicide- and insect-resistant transgenic plants sell both the seeds and the herbicide. Powerful monopolies could develop.
- Should foods containing products from transgenic plants be labelled at point of sale? What other information does a comsumer require so that he or she can make a choice?
- What will be the effects on the nutritional content of both whole fruits and vegetables and on processed foods using new transgenic varieties?

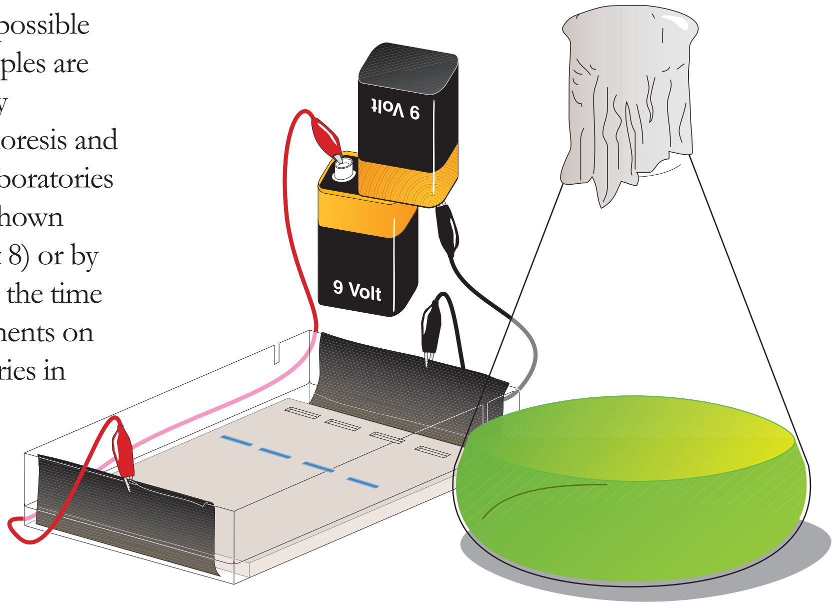
The Way Forward ...

Before permission is granted for the release of transgenic plants into the environment many factors must be taken into consideration:

- The plant should be safe for human beings and other animals;
- The plant should not cause ecological problems;
- The risks associated with resistance development should be assessed and strategies planned for its management;
- Traditional plant breeding should be developed along side transgenic plant development;
- There should be case-by-case study of every transgenic plant considered;
- Model systems should be studied to predict the effect of release into the environment;
- Interested individuals and organisations such as scientific, consumer, farming, environmental and other concerned groups should be kept informed and be allowed to participate in the debate.

Practical work in schools

Plants can be identified by looking at their protein composition. It is therefore possible to show whether different plant samples are from the same or different origins by separating their proteins by electophoresis and comparing the results. In research laboratories similarities or differences are often shown using an ELISA test (see EIBE Unit 8) or by doing a PCR (see EIBE Unit 2). For the time being it is not possible to do experiments on transgenic plants in school laboratories in any of the EU countries. Ideas have been put forward for safe practical experiments for schools and with the next revision of this Unit we hope to have some details.



Further reading

Risikovurdering ved gensplejsning, Munksgaard 1991

Gensplejsede planter - regulering og anvendelse,Teknologi rådet rapport 1996/1

Høring om gensplejsede planter, Teknologi rådet, høring 1/2 - 1996

The Prosamo Report: Testing the environmental impact of plant gene technology. David Fishlock. Published by the Laboratory of the Government Chemist, Queen's Road, Teddington, Middlesex, TW11 OLY, UK.

Roush, R. (1994), Managing Pests and Their Resistance to Bacillus thuringiensis: Can Transgenic Crops be Better than Sprays? *Biocontrol Science and Technology*, 4, 501-516.

Dale, P.J., J.A. Irwin and J.A. Scheffler, (1993) The Experimental and Commercial Release of Transgenic Crop Plants. *Plant Breeding* 111, 1-22.

A Public Voice on Biotechnology and Agriculture, Union of Concerned Scientists, Agricultural and Biotechnology Program, 1616 P Street, NW, Washington DC 20036 USA. *The Gene Exchange*. December 1996.

Calgene Fresh Inc. 1910 Fifth Street, Davis, CA 95616. USA.

Zeneca Plant Science, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, United Kingdom.

Holmes, B. (1995), Chips are down for killer potato. *New Scientist* 6th May, page 9.

Hoyle, R. (1995) EPA okays first pesticidal transgenic plants. *BioTechnology* 13, May, 434-435.

Estruch, Juan J. (1997) Transgenic plants: An emerging apporach to pest control. *Nature Biotechnology* vol. 15 no. 2

Winstanley, M. and Bowles, D. *Advances in Plant Biotechnology*. Biotechnology and Biological Sciences Research Council (BBSRC), Polaris House, Swindon SN2 1UH, United Kingdom.

Straughan, R. and Reiss, M.J. (1996) *Ethics, morality and crop biotechnology*. BBSRC. (See address above). ISBN: 0708405703.

The *New Scientist* and *Nature Biotechnology* have regular articles and comments on transgenic plants.

APPENDIX 1

Commission Decision of the EEC

This text is an extract of the decision of the Commission of the European Communities of November the 4th 1994, establishing simplified procedures concerning the deliberate release into the environment of genetically modified plants pursuant to Article 6 (5) of Council Directive 90/220/EEC.
(*Reference: 94/730/EC - OJ L 292/31, 12 November 1994*)

Article 1: The requests submitted by France and the United Kingdom pursuant to Article 6(5) of Directive 90/220/EEC and concerning the simplified procedures set out in the Annex are approved.

Article 2: This Decision is addressed to the Kingdom of Belgium, the Kingdom of Denmark, the Federal Republic of Germany, the Kingdom of Spain, the French Republic, Ireland, the Italian Republic, the Kingdom of the Netherlands, the Portuguese Republic and the United Kingdom of Great Britain and Northern Ireland.

Annex

1. The simplified procedure provides for a single notification dossier to be submitted pursuant to Part B of Directive 90/220/EEC, for more than one release of genetically modified plants which have resulted from the same recipient crop plant species but which may differ in any of the inserted/deleted sequences or have the same inserted/deleted sequence but differ in phenotypes.

2. A notifier can submit in a single notification information on several releases of genetically modified crop plants, to be released on several different sites, on the following conditions:

- the taxonomic status and biology of the recipient plants species is well known,
- information is available on the interactions of the recipient plant species in the ecosystems in which the experimental and/or agricultural releases are scheduled,
- scientific data is available on the safety to human health and the environment of experimental releases involving genetically modified plants of the recipient plant species,
- the inserted sequences and their expression products should be safe for human health and the environment under the conditions of the experimental release,
- the inserted sequences have been well characterised,
- all of the inserted sequences are integrated into the plant nuclear genome,
- all the releases are for an a priori specified programme of word,
- all the releases take place within an *a priori* specified time period.

....

5. In order to obtain one single consent covering several releases, all the necessary information for each release should be indicated in the single notification, including sufficient information on the different sites of the releases and on the experimental design, as well as indication of any conditions for risk management for each different release. Clear reference to each release to be covered should be made in the notification, and the appropriate information should be included to allow completion of the summary notification information format.

6. A notifier can also submit a single notification covering a whole, a priori specified, programme of development work with a single specific recipient plant species and a specified range of inserts/deletions over several years and on several different sites, and receive a single consent for the whole programme of work.

6.1. In such cases, detailed indications or descriptions of the different sites of the releases, subsequent intraspecific sexual crosses and/or the conditions of release need not to be given in the notification, as would be required under the conditions indicated in paragraph 5. However, the notification must contain sufficient information to enable overall an evaluation of risk, and a detailed risk assessment to be made for at least the first release in the programme of work.

....

8. When a single consent under simplified procedures is granted, conditions can be attached to each of the releases to which it refers. These conditions can subsequently be altered by the competent authority, as indicated in Article 6(6) of the Directive.

9. On completion of one or more of the releases approved within the simplified procedure, the notifier shall submit to the competent authority a report with the results of the release(s) at the time specified in the consent. Such reports may be submitted separately, or as a clearly identifiable section in support of a notification for subsequent releases.

10. The competent authority may alter the conditions of the original consent or intervene to alter the conditions of specific subsequent releases on the basis of the results indicated in the reports or on the basis of information obtained during inspections.

APPENDIX 2

Specimen Questionnaire

Concept of gene, plant and expression of genetic traits

Name: .... Age: .... Date: ....

Class: .... MALE FEMALE *(please circle)*

Please write your answers on the following questions in the space left under them.

1. Describe in your own way what you think genes are.

2. Where do genes occur?

3. Where do genes come from?

4. Where are genes located?

5. Do plants contain genes? Explain your answer.

6. It is now possible to transfer genes from elsewhere into plants? Which genes would you find interesting to transfer to plants and why?

7. Do you think there are some risks and some benefits connected to such transfer of genes?
   Risks YES NO Benefits YES NO *(please circle)*
   If so, which risks/benefits?

8. If you have personal opinion about gene technology, please make a statement here.