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REVIEW

A transgene-centred approach to the biosafety of transgenic plants: overview of selection and reporter genes

P. L. J. METZ and J. P. NAP*

Department of Molecular Biology, CPRO-DLO, PO Box 16, 6700 AA Wageningen, The Netherlands

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INTRODUCTION

Recombinant DNA techniques have given plant breeding a new dimension. No longer hampered by crossing barriers, it is now possible to isolate genetic information from any organism and transfer it to virtually any plant of interest. Plants in which new pieces of DNA are introduced by procedures other than sexual crossing are generally referred to as genetically modified, or transgenic, plants. From the first possibilities to create such transgenic plants, however, discussions began. There is general concern with respect to the relative safety and admissibility of the transgenic plants involved. The novelty of the transgenic approach as well as the apparent concern ask for careful evaluation of the acquired characteristics of any transgenic plant (Dale *et al.* 1993).

Research has been and is devoted to the consequences of the introduction of transgenic plants in the environment. Studies of pollen dispersal and gene transfer from transgenic crops to related species (Kerlan *et al.* 1992; Scheffler *et al.* 1993; Dale 1994; Eijlander & Stiekema 1994; Mikkelsen *et al.* 1996; Timmons *et al.* 1996; Metz *et al.* 1997, this issue) can be considered 'transgene independent', yielding information irrespective

*Correspondence author.

of the transgene applied. Many such 'transgene independent' studies conclude that specific transgenic plants should be evaluated on a case-by-case basis. This call for a case-by-case evaluation is the motivation for the alternative, or 'transgene-centred' approach (Nap *et al.* 1995) we propose here. Concentrating on the presence of gene *xxx* and gene product XXX allows definite questions to be evaluated and may identify open issues more readily than general considerations. Such a transgene-centred approach to the evaluation of biosafety should provide a useful body of knowledge reflecting the current state of affairs of gene transfer technology for regulatory authorities. That may contribute to discussions and aid in prudent policy-making regarding the transgene under scrutiny. Before embarking on such a transgene-centred approach to the biosafety of transgenic plants, it is important to determine what issues should be considered for each (trans)gene and what gene should be evaluated first.

BIOSAFETY ISSUES

The regulatory framework for the biosafety of transgenic plants in the European Union consists of the 1990 Directives for Contained Use (90/219) and Deliberate Release (90/220). These directives require the evaluation of the risks of each proposed release and to take appropriate measures to avoid adverse effects on human health and the environment. Unfortunately, neither the risks concerned, nor the extent of issues considered relevant, are clarified and defined. The directives differ considerably from the guidelines that are operational in the United States (Butler, 1995; Koehler, 1995).

For the 'transgene-centred approach to biosafety' we propose a distinction between 'biosafety in the narrow sense' and 'biosafety in the broad sense'. Biosafety in the narrow sense involves the characteristics, ecology and toxicology of the transgene and the transgene product, whereas biosafety in the broad sense covers everything else that is or could be considered relevant. In the last section of this review we will address some of the issues belonging to the 'biosafety in the broad sense'. A proper starting point for the assessment of the biosafety in the narrow sense of a transgene could be: given current agricultural practice, what would be the consequences of agronomic crops being transgenic?

Concerns with respect to the biosafety in the narrow sense of transgenes in plants should involve the ecology and toxicology (see, for example, Glandorf *et al.* 1997, this issue; Van Raamsdonk & Schouten 1997, this issue) of both release and use of transgenic crop plants. The ecological concerns focus on weediness and can be summarized as follows: the transgenic trait may transform the crop into a weed beyond control; the transgene may spread vertically to wild relatives that as a result become a weed; the transgene may spread horizontally to other organisms that as a result become problematic; the transgene may disturb the ecological relations of the crop or any wild relative in another unknown way. The toxicological concerns focus on food safety and consumption: the transgenic trait may render the plant unsuitable for consumption or processing; the transgene may affect the toxicological characteristics of the crop or any product derived from it in another unknown way. In Fig. 1 a flow diagram is given that depicts the hypothetical spread of a transgene and its transgene product into the environment and in consumers (after Nap *et al.* 1992).

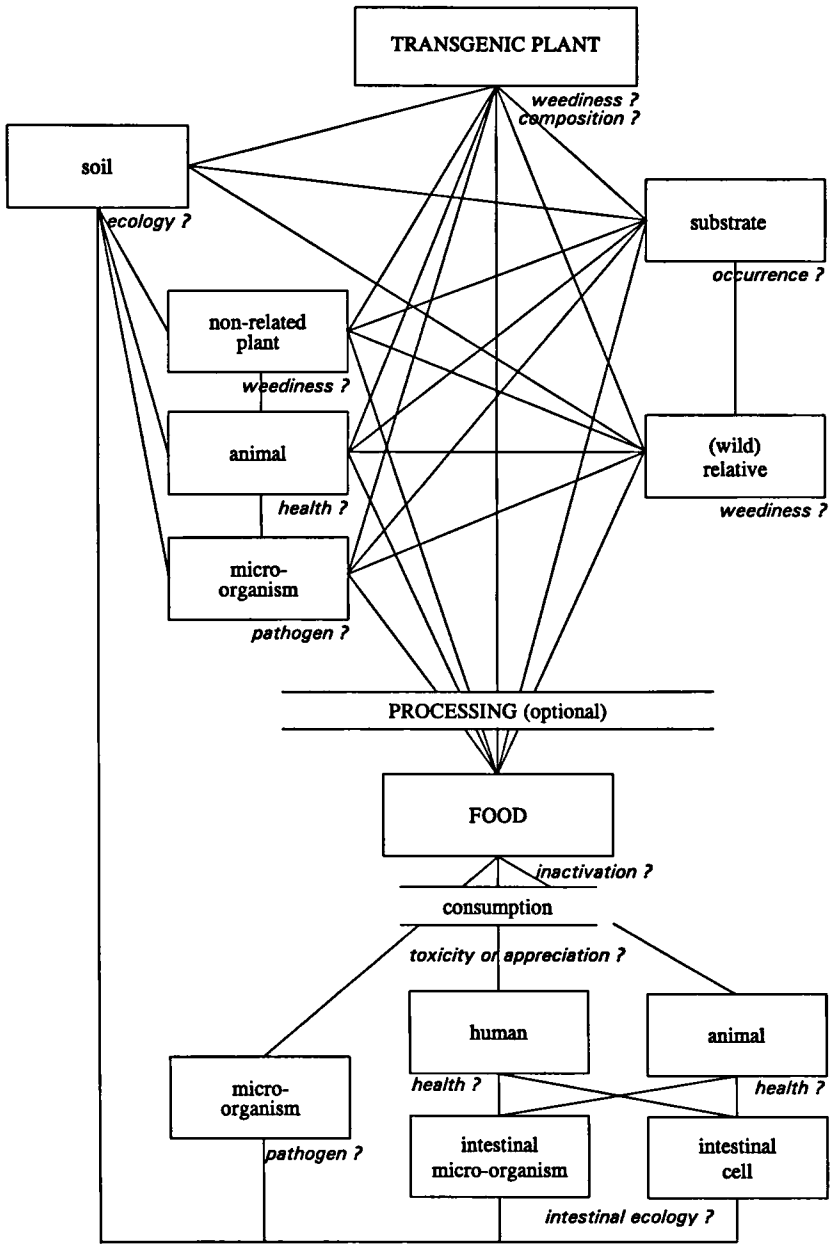

Fig. 1. Hypothetical spread of a transgene and its gene product into the environment. For the sake of clarity, not all possible relationships are shown in the flow diagram. The main concern of each possibility (see text) is given in italics.

SELECTION AND REPORTER GENES

Gene classes

The transgenes most appropriate to evaluate first would seem to be the transgenes most frequently used. These are the selection and reporter genes. There are several methods

Table 1. Selection and reporter genes used in plants

Gene	Enzyme	Selective agent or substrate
I. Selection genes: antibiotic resistance		
—*aphA1*	aminoglycoside-3′-phosphotransferase,	kanamycin and other
aphA2	APH(3′)I,II, and III, NPTI,II,	aminoglycosides
aphA3	and III	
—*aacC1*	gentamicin-acetyltransferase,	gentamicin and other
aacC3	AAC(3′)I,III, and IV, AAC(6′)	aminoglycosides
aacC4		
aacA		
—*hpt*	hygromycin phosphotransferase,	hygromycin B
(*aphIV*)	HPT	
—*spt*	streptomycin phosphotransferase,	streptomycin
*spt**	SPT, SPT*	
—*aadA*	aminoglycoside-3″-adenyltransferase,	streptomycin
	AAD(3″)	spectinomycin
—*ble*	drug binding protein (no enzyme)	bleomycin
shble		phleomycin
—*dhfr*	dihydrofolate reductase, DHFR	methotrexate, anti-folate drugs
—*bsr*	blasticidin S deaminase, BSR	blasticidin S
—*sulI*	dihydropteroate synthase, DHPS	sulfonamides
II. Selection genes: herbicide tolerance		
—*bar*	phosphinothricin-N-acetyltransferase,	glufosinate, bialaphos,
pat	PAT	phosphinothricin (Basta, Finale)
—*epsps*	5-enolpyruvylshikimate-3-phosphate	glyphosate (Roundup)
epsps5	synthase, EPSPS, plant origin	
—*aroA*	5-enolpyruvylshikimate-3-phosphate	glyphosate (Roundup)
sm1	synthase, EPSPS, microbial origin,	
cp4	mutated forms.	
—*gox*	glyphosate oxidoreductase, GOX	glyphosate (Roundup)
bxn	bromoxynil nitrilase, BXN	bromoxynil
—*tfdA*	2,4-dichlorophenoxyacetate	2,4-D
	monooxygenase	
—*als*	acetolactate synthase, ALS	sulfonylureas
(*ahas*)	acetohydroxy acid synthase, AHAS	imidazolines
csr1-1,-2		triazolopyrimidines
suRB-S4-hra		pyrimidylbenzoates

Continued on next page

of introducing new DNA into the hereditary material of plant cells. These methods, however, are relatively inefficient (Fraley 1989). To improve the efficiency of obtaining the desired transgenic cell or plant, generally an extra gene is co-introduced with the gene-of-interest. This extra gene allows positive selection of transformation events, because it confers to cells the ability to proliferate in the presence of a selective agent. Table 1 gives an overview of the selection genes currently available.

Three classes of selection genes can be distinguished. The first class confers resistance to antibiotics, such as kanamycin or gentamicin, mostly by inactivating the antibiotic through modifying enzymes. The second class confers tolerance to herbicides that generally have a broad spectrum. Herbicide tolerance is based on detoxifying the herbicide or on reducing the efficacy of the herbicide. Generally the herbicide

Table 1. *Continued*

Gene	Enzyme	Selective agent or substrate
III. Selection genes: metabolism		
tdc	tryptophan decarboxylase, TDC	4-methyltryptophan
dhps	dihydrodipicolinate synthase, DHPS,	S-aminoethyl-L-cysteine (AEC)
dhps-r1	DHDPS	
ak	aspartate kinase, AK	threonin and lysin
badh	betaine aldehyde dehydrogenase, BADH	betaine aldehyde
manA	6-phosphomannose isomerase, PMI	mannose
aprt	adenine phosphoribosyltransferase, APRT	azaserine, alanosine and adenine
IV. Reporter genes		
uidA (*gus*)	β-glucuronidase, GUS	β-glucuronides
luc	firefly luciferase, LUC	luciferin, ATP and oxygen
luxA *luxB*	bacterial luciferase, LUC	decanal, $FMNH_2$ and oxygen
lacZ	β-galactosidase, β-GAL	galactosides
cat	chloramphenicol acetyltransferase, CAT	chloramphenicol
gfp	green fluorescent protein, GFP (no enzyme)	oxygen required

tolerance serves a dual purpose. The tolerance allows the selection during the transformation phase, and also changes the agronomic properties of the resulting transgenic plant. The last class of selection genes involves genes concerned with various metabolic pathways of plants. Field trial applications in The Netherlands (courtesy R. van de Graaf) as well as international (OECD 1993c) indicate that the kanamycin resistance gene *aphA2* is the selection gene by far the most frequently used. The herbicide tolerance genes *bar* and *pat* are runners-up. The detailed evaluations of the most frequently used *aphA2* transgene and transgene product (Calgene 1990; Flavell *et al.* 1992; Nap *et al.* 1992; Fuchs *et al.* 1993a,b; Redenbaugh *et al.* 1994; Wood *et al.* 1995) can be considered to set the stage for evaluations of subsequent genes and gene products.

To monitor the successful transfer of the transgene to the recipient genome, and/or to study its expression, very often a so-called reporter gene is included. A good reporter gene should result in (often enzymatic) activity that is not present in the recipient host, and can be assayed easily, reproducibly and quantitatively. The application of reporter genes is generally confined to the laboratory or small-scale field trials and is not primarily directed towards full-scale commercial use. More recently it has become clear that, especially in case of plant species that are difficult to transform, the presence of a reporter gene can be essential for obtaining the desired transgenic plants (Jefferson & Wilson 1991). Transformation of chrysanthemum, for example, relies on the early detection of GUS activity (de Jong *et al.* 1994). We predict, therefore, that in the longer run, ornamental and food crops will be considered for marketing while still carrying

reporter genes. Table 1 gives an overview of the reporter genes currently in use. The most frequently used reporter gene is the *E. coli* β-glucuronidase gene *uidA*.

Alternatives

Apart from herbicide tolerance genes, selection and reporter genes are not aimed at any change or improvement of the agronomic characteristics of the crop involved. Attempting to obtain a transgenic crop without the agronomically useless gene(s) is therefore an obvious strategy (Bryant & Leather 1992). It would obviate the need for biosafety assessments of such genes and may ease part of the public concerns about transgenic plants. Several approaches to obtain transgenic plants not carrying selection genes have been suggested.

No selection or reporter gene. In most cases, the overall efficiency of gene transfer is so low, that genetic modification without any selection seems no realistic alternative. Direct DNA transfer via microinjection of DNA into cells of microspore-derived embryoids may improve the efficiency to acceptable values. Neuhaus *et al.* (1987) and Neuhaus & Spangenberg (1990) found 27–51% of the regenerated plants carrying stably integrated copies of the injected DNA. Cell finder systems in which a computer-controlled microscope allows easy positioning and relocation of cultured cells and protoplasts (Hall *et al.* 1995), in combination with improved gene delivery techniques (Blackhall *et al.* 1995), may also develop into a system that allows the identification and isolation of transgenic plants only carrying the gene-of-interest.

Only a reporter gene. Especially the selection genes encoding antibiotic resistance and herbicide tolerance are issues for debate. Using only a reporter gene to monitor transformation is another attempt to do without these genes. The reporter genes now most frequently in use, such as the GUS gene (Jefferson *et al.* 1987), require assays that destruct the transgenic plant material. The more recently used reporter genes encoding firefly luciferase (Ow *et al.* 1986) and the green fluorescent protein (Haseloff & Amos, 1995) can be assayed non-destructively. This opens the possibility of monitoring transformation during the development of the transgenic shoot, and select the transgenic shoot in a very early stage.

Inactivation of the selection gene. The major issues of biosafety are related to the transgene-encoded protein. Limiting the expression of the selection gene to the stages at which selection for transformation is applied will result in transgenic plants in which the transgene is present, but not the transgene-encoded protein. It has been demonstrated that the wound-inducible promoter AoPR1 isolated from asparagus (*Asparagus officinalis*) when fused to the kanamycin resistance selection gene allows selection during transformation, but results in very low levels of the transgene product in the mature plant (Özcan *et al.* 1993a,b).

Removal of the selection gene. Especially when using *Agrobacterium tumefaciens* T-DNA transfer as means of transformation, the selection gene, being on the same T-DNA as the gene-of-interest, is in genetic terms absolutely linked to the gene-of-interest. When unlinked, one can subsequently segregate the gene-of-interest away from the selection gene and plants carrying only the gene-of-interest can be identified with routine procedures. Currently there are three approaches proposed to unlink the selection gene

from the gene-of-interest. These three approaches are co-transformation, excision by site-specific recombination and transposon-mediated unlinking.

(a) *Co-transformation.* In some cases it is demonstrated that two separate *Agrobacterium* T-DNA molecules have a tendency of ending up in the same cell, without being linked (McKnight *et al.* 1987). It is possible, therefore, to place the gene-of-interest on one T-DNA and the selection gene on another. De Block & Debrouwer (1991) found 21% of two such separate T-DNAs to be genetically unlinked and therefore in principle be separable. Biolistics with independent DNA molecules may also result in unlinked co-transformation (Carrer & Maliga, 1995).

(b) *Excision by site-specific recombination.* A recombinase that is able to recombine the DNA present between specific recombination sites could be used to remove the selection gene from the recipient genome. The Cre-*lox* system was the first example of a single-polypeptide recombinase used for this purpose (Odell *et al.* 1990; Dale & Ow, 1991). Two other recombination systems, FLP-*frt* and R-*rs*, are also being evaluated in plants (Ow 1996).

(c) *Transposon-mediated unlinking.* A transformation system utilizing the *Ac/Ds* maize transposable element was described that included either a gene-of-interest or the selection gene between non-autonomous *Ds* elements (Goldsbrough *et al.* 1993; Yoder & Goldsbrough 1994). With the help of transposase, the selection gene jumps away from the gene-of-interest, or vice versa, to become unlinked from the gene-of-interest.

Prospects

Most if not all strategies to obtain transgenic plants without a selection gene have been successful in some, typically 'model' cases. However, these approaches are far from routine or from being generally applicable. The techniques could be further optimized for more routine transgenic plant production. Approaches employing protoplasts are likely to suffer from disadvantages compared to *Agrobacterium*-mediated plant transformation, due to somaclonal variation. Approaches that are based on segregating away the unlinked selection gene require crops that are relatively homozygous and that can be easily back-crossed. For an agriculturally important crop such as potato, for example, these approaches will be essentially impossible. Possibly gene silencing (Maessen 1997, this issue) may be developed into a method to obtain plants without selection gene activity. Nevertheless, agricultural biotechnology will continue to face the presence of selection and reporter genes in transgenic plants aimed for release on the market. Biosafety assessments of these genes are therefore useful. In Table 2 we present a 'passport' description of each selection and reporter gene currently used in or proposed for use in plants. Such a gene passport could serve as a starting point for gene-centred evaluations.

The (theoretical) possibility of removal or inactivation of a selection or reporter gene from specific crops poses special challenges for regulatory authorities. It will have to be decided whether regulations should be on a gene-alone basis (i.e. gene *xxx* in any plant), or on a gene–plant basis (gene *xxx* in plant P). Ideally, the admissibility of a transgene in crops should be based upon the characteristics of the gene (product) and not upon the feasibility of its removal or inactivation. For example, allowing a transgene in potato while requiring the removal of the very same transgene from tomato only because it is possible to remove it from tomato seems undesirable.

Table 2. Gene passport of selection and reporter genes currently used in or proposed for plants

I. Selection genes: antibiotic resistance	
Gene	: *aphA1* (*nptI*) and *aphA2* (*nptII*)
Origin	: *Escherichia coli* transposon Tn*903*, *E. coli* transposon Tn5, respectively
Gene product	: aminoglycoside-3′-phosphotransferase I and II (APH(3′)I or NPTI; APH(3′)II or NPTII)
Protein action	: ATP dependent phosphorylation of the 3′-hydroxyl moiety
Substrate	: kanamycin, neomycin, geneticin (G418), paromomycin, APH(3′)III also amikacin (aminoglycoside antibiotics)
Substrate action	: impaired chloroplast protein synthesis
Reference	: Bevan *et al.* (1983); Pietrzak *et al.* (1986)
Remarks	: *aphA2* is most frequently used selection gene. Characteristics extensively investigated and reviewed (Calgene 1990; Nap *et al.* 1992; Fuchs *et al.* 1993ab)
Gene	: *aphA3* (*nptIII*)
Origin	: *Streptococcus faecalis* R plasmid
Gene product	: aminoglycoside-3′-phosphotransferase III (APH(3′)III or NPTIII)
Protein action	: ATP dependent phosphorylation of the 3′-hydroxyl moiety
Substrate	: kanamycin, neomycin, geneticin (G418), paromomycin, amikacin (aminoglycoside antibiotics)
Substrate action	: impaired chloroplast protein synthesis
Reference	: Pietrzak *et al.* (1986)
Gene	: *aac*C1
Origin	: *E. coli* transposon Tn*21*
Gene product	: aminoglycoside-3′-acetyltransferase I (gentamicin-3′-acetyltransferase I; AAC(3′)I)
Protein action	: acetylation of the free 3′-amino moiety
Substrate	: gentamicin (aminoglycoside antibiotics)
Substrate action	: impaired chloroplast protein synthesis
Reference	: Carrer *et al.* (1991)
Remark	: AAC(3′)I has a comparatively narrow substrate range
Gene	: *aacC3* and *aacC4*
Origin	: R plasmids from *Pseudomonas aeruginosa* and *Salmonella* ssp., respectively
Gene product	: aminoglycoside-3′-acetyltransferase III and IV (AAC(3′)III and IV) or gentamicin-3′-acetyltransferase III and IV, respectively
Protein action	: acetylation of the free 3′-amino moiety
Substrate	: gentamicin, kanamycin, tobramycin, neomycin, paromomycin; AAC(3′)IV also apramycin and G418 (aminoglycoside antibiotics)
Substrate action	: impaired chloroplast protein synthesis
Reference	: Hayford *et al.* (1988)
Gene	: *aacA* (*6′gat*)
Origin	: *Shigella* sp.
Gene product	: aminoglycoside-6′-acetyltransferase (AAC(6′))
Protein action	: acetylation of the free 6′-amino moiety
Substrate	: gentamicin, kanamycin, tobramycin, neomycin, paromomycin, amikacin, netilmicin (aminoglycoside antibiotics)
Substrate action	: impaired chloroplast protein synthesis
Reference	: Gosselé *et al.* (1994)
Remarks	: AAC(6′) is a large group of enzymes with a wide distribution. Which gene exactly is used is unclear

Table 2. *Continued*

Gene	: *hpt* (*aphIV*)
Origin	: *E. coli*
Gene product	: hygromycin phosphotransferase (HPT)
Protein action	: phosphorylation of hydroxyl group
Substrate	: hygromycin B (aminoglycoside antibiotic)
Substrate action	: inhibits translocation resulting in mistranslation
Reference	: Waldron *et al.* (1985), Van den Elzen *et al.* (1985)
Gene	: *spt*, *spt**
Origin	: Tn5 of *Klebsiella*/*E. coli*
Gene product	: streptomycin phosphotransferase (SPT) and mutated form (SPT*)
Protein action	: phosphorylation of hydroxyl group
Substrate	: streptomycin (aminoglycoside antibiotic)
Substrate action	: inhibits chloroplast protein synthesis
Reference	: Jones *et al.* (1987); Maliga *et al.* (1988)
Remarks	: spt selection is based on colour differentiation rather than toxicity spt* is a mutated form that allows more stringent selection
Gene	: *aadA*
Origin	: *Shigella flexneri* plasmid
Gene product	: aminoglycoside-3″-adenyltransferase (AAD(3″))
Protein action	: transfer of adenyl moiety
Substrate	: streptomycin, spectinomycin (aminoglycoside antibiotics)
Substrate action	: blocks chloroplast protein synthesis
Reference	: Svab *et al.* 1990
Remarks	: selection is based on colour differentiation or toxicity
Gene	: *ble*, *shble*
Origin	: Tn5 of *Klebsiella*/*E. coli*, *Streptoalloteichus hindustanus*
Gene product	: drug binding protein
Protein action	: no enzymatic activity; drug binding impairs DNA cleavage
Substrate	: bleomycin, phleomycin (glycopeptide antibiotics)
Substrate action	: DNA breakage
Reference	: Hille *et al.* (1986); Perez *et al.* (1989)
Remarks	: resistant plant cells may suffer from DNA lesions
Gene	: *dhfr*
Origin	: *E. coli* R plasmid R67; mouse
Gene product	: mutated dihydrofolate reductase (DHFR)
Protein action	: NADPH-dependent reduction of dihydrofolate to tetrahydrofolate; mutated enzyme is insensitive to the action of the antibiotics
Substrate	: methotrexate, trimethoprim, pyrimethamine (anti-folate drugs)
Substrate action	: inhibition of DHFR, resulting in a lack of tetrahydrofolate, and therefore in a blocked nucleotide biosynthesis
Reference	: Herrera-Estrella *et al.* (1983); Eichholtz *et al.* (1987); Pua *et al.* (1987)
Gene	: *bsr*
Origin	: *Bacillus cereus* K55–S1
Gene product	: blasticidin S deaminase
Protein action	: inactivates blasticidin S by deamination
Substrate	: blasticidin S (antimicrobial; fungicide)
Substrate action	: inhibits protein synthesis
Reference	: Kamakura *et al.* 1990
Remarks	: high phytotoxicity of fungicide may limit efficiency

Table 2. *Continued*

Gene	: *sulI*
Origin	: R plasmid R46
Gene product	: dihydropteroate synthase (DHPS)
Protein action	: bacterial DHPS is not susceptible to sulfonamides. Enzyme acts in folic acid biosynthesis
Substrate	: asulam, suldadiazine (sulfonamide antibiotics)
Substrate action	: inhibits DHPS
Reference	: Guerineau *et al.* (1990)
II. Selection genes: herbicide tolerance	
Gene	: *bar* and *pat*
Origin	: *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*
Gene product	: phosphinothricin-N-acetyltransferase (PAT)
Protein action	: acetylation of the free NH_2 group using acetyl coenzyme A as cofactor
Substrate	: glufosinate, L-phosphinothricin (PPT), bialaphos
Substrate action	: inhibition of the enzyme glutamine synthetase (GS) resulting in an accumulation of ammonia and eventually cell death
Herbicide	: Basta, Finale, Radicale, Herbiace
Reference	: De Block *et al.* (1987); Wohlleben *et al.* (1988)
Remarks	: most popular selection gene for monocots
Gene	: *epsps*
Origin	: *Petunia hybrida*, *Arabidopsis thaliana*
Gene product	: 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), endogenous gene product is overproduced with strong promoter
Protein action	: formation of 5-enolpyruvylshikimate-3-phosphate (EPSP) from shikimate-3-phosphate and phospho-enolpyruvate
Substrate	: glyphosate, N-phosphonomethylglycin
Substrate action	: inhibition of EPSPS. This prevents synthesis of aromatic amino acids and secondary metabolites and results in an accumulation of shikimate.
Herbicide	: Roundup
Reference	: Shah *et al.* (1986); Klee *et al.* (1987)
Remarks	: targeting EPSPS to the chloroplast improves glyphosate tolerance, but is not essential. Significant reduction in plant growth observed due to glyphosate accumulation (Kishore & Shah 1988)
Gene	: *aroA*, *aroA(sm-1)* and *aroA(cp4)*
Origin	: *Salmonella typhimurium*, *E. coli* B, and *Agrobacterium* CP4, respectively
Gene product	: mutated bacterial EPSPS with a reduced affinity to glyphosate
Protein action	: formation of 5-enolpyruvylshikimate-3-phosphate (EPSP) from shikimate-3-phosphate and phospho-enolypyruvate
Substrate	: glyphosate, N-phosphonomethylglycin
Substrate action	: inhibition of EPSPS. This prevents synthesis of aromatic amino acids and secondary metabolites and results in an accumulation of shikimate
Herbicide	: Roundup
Reference	: Comai *et al.* (1985); Della-Cioppa *et al.* (1987); Padgette *et al.* (1989); Barry *et al.* (1992); Zhou *et al.* (1995)
Remarks	: targeting to chloroplast improves tolerance
Gene	: *epsps*; *epsps5*
Origin	: *Petunia hybrida*
Gene product	: mutated plant EPSPS with a reduced affinity to glyphosate
Protein action	: formation of 5-enolpyruvylshikimate-3-phosphate (EPSP) from shikimate-3-phosphate and phospho-enolpyruvate

Table 2. *Continued*

Substrate	: glyphosate, N-phosphonomethylglycin
Substrate action	: inhibition of EPSPS. This prevents synthesis of aromatic amino acids and secondary metabolites and results in an accumulation of shikimate
Herbicide	: Roundup
Reference	: Hinchee *et al.* (1988); Padgette *et al.* (1989)
Gene	: *gox*
Origin	: *Achromobacter* sp. strain LBAA
Gene product	: glyphosate oxidoreductase
Protein action	: metabolization of glyphosate to aminomethylphosphonate using FAD as co-factor
Substrate	: glyphosate, N-phosphonomethylglycin
Substrate action	: inhibition of EPSPS. This prevents synthesis of aromatic amino acids and secondary metabolites and results in an accumulation of shikimate
Herbicide	: Roundup
Reference	: Kishore & Barry (1992); Barry *et al.* (1992); Zhou *et al.* (1995)
Remarks	: targeting to chloroplast; used in combination with mutated EPSPS
Gene	: *bxn*
Origin	: *Klebsiella ozaenae*
Gene product	: a bromoxynil-specific nitrilase
Protein action	: degradation of bromoxynil to 3,5-dibromo-4-hydroxy benzoic acid
Substrate	: 3,5-dibromo-4-hydroxybenzonitrile
Substrate action	: inhibition electron transfer in photosynthesis by binding to a component of Photosystem II
Herbicide	: Bromoxynil
Reference	: Stalker *et al.* (1988)
Remarks	: used specially in monocots
Gene	: *tfdA*
Origin	: *Alcaligenes eutrophus* JMP134
Gene product	: 2,4-dichlorophenoxyacetate monooxygenase (DPAM)
Protein action	: degradation of 2,4-dichlorophenoxy-acetic acid (2,4-D) to 2,4-dichlorophenol
Substrate	: 2,4-D
Substrate action	: competition with indole-3-acetic acid (IAA), by occupying binding sites of endogenous auxin receptors
Herbicide	: various commercial formulations
Reference	: Streber & Willmitzer (1989)
Remarks	: monocot plants are tolerant for high concentrations of 2,4-D
Gene	: *als* (*ahas*), or *csr1-1*, *csr1-2*, *suRB-S4-hra* and *ahas3*
Origin	: *Arabidopsis thaliana*, *Nicotiana tabacum* and *Brassica napus*
Gene product	: mutated acetolactate synthase (ALS) (other name: acetohydroxy acid synthase, AHAS), with reduced affinity for herbicide
Protein action	: FAD-requiring formation of 2-hydroxybutyrate (acetolactate) from α-ketobutyrate
Substrate	: sulfonylureas, imidazolinones, triazolopyrimidines and pyrimidylbenzoates
Substrate action	: inactivation of ALS. Exact mechanism depends on herbicide. ALS is a key enzyme in branched-chain acid synthesis. Subject to complex feedback inhibition mechanisms. Inactivation ultimately results in cell death
Herbicides	: many different commercial formulations available, among many others Chlorsulfuron, Sulfometuron methyl, Chlorimuron ethyl, Imazapyr, Imazaquin, Triazolopyrimidine

Table 2. *Continued*

Reference	: Lee *et al.* (1988); Haughn *et al.* (1988); Sathasivan *et al.* (1991); Hattori *et al.* (1995)
Remarks	: several resistant agronomic lines (corn, canola) obtained without genetic modification
III. Selection genes: metabolism	
Gene	: *tdc*
Origin	: *Catharanthus roseus*
Gene product	: tryptophan decarboxylase (TDC)
Protein action	: conversion of tryptophanes to tryptamines
Substrate	: L-tryptophan, 4-methyltryptophan, fluorotryptophan
Substrate action	: 4-methyltryptophan is toxic to plant cells
Reference	: Goddijn *et al.* (1993)
Remarks	: requires plants without endogenous TDC activity
Gene	: *dhps, dhdps-r1*
Origin	: *E. coli, Nicotiana sylvestris*
Gene product	: dihydrodipicolinate synthase (DHPS, DHDPS)
Protein action	: bacterial DHPS or mutated plant DHPS is less sensitive for lysine feedback inhibition than endogenous plant DHPS. The increased DHPS activity results in lysine overproduction
Substrate	: S-aminoethyl-L-cysteine (AEC)
Substrate action	: AEC is a toxic lysine analog that inhibits endogenous DHPS
Reference	: Perl *et al.* (1993); Ghislain *et al.* (1995)
Remarks	: protein is targeted to chloroplasts
Gene	: *ak*
Origin	: *E. coli*
Gene product	: aspartate kinase (AK)
Protein action	: bacterial AK is less sensitive to feedback inhibition by a lysine/threonine combination. The increased AK activity results in threonine overproduction.
Substrate	: millimolar concentrations of threonin and lysine
Substrate action	: inhibition of endogenous AK results in methionine starvation
Reference	: Perl *et al.* (1993)
Remarks	: protein is targeted to chloroplasts
Gene	: *badh*
Origin	: spinach and sugar beet
Gene product	: betaine aldehyde dehydrogenase (BADH)
Protein action	: conversion of betaine aldehyde (BA) in glycine betaine
Substrate	: BA
Substrate action	: BA is toxic to plant cells
Reference	: Rathinasabapathi *et al.* (1994)
Remarks	: targeting to chloroplasts; requires plants with low or without glycine betaine pathway; growth reduction observed due to BA toxicity
Gene	: *manA*
Origin	: *E. coli*
Gene product	: 6-phosphomannose isomerase (PMI)
Protein action	: interconversion of mannose-6-phosphate and fructose-6-phosphate
Substrate	: mannose
Substrate action	: PMI enables mannose 6-phosphate to be catabolized via the glycolytic pathway
Reference	: Miles & Guest (1984); Bojsen *et al.* (1994)

Table 2. *Continued*

Gene	: *aprt*
Origin	: *Arabidopsis thaliana*
Gene product	: adenine phosphoribosyltransferase
Protein action	: generates adenosine-monophosphate (AMP)
Substrate	: combination of azaserine, alanosine and adenine
Substrate action	: blocks *de novo* AMP synthesis, making APRT essential
Reference	: Schaff (1994)
Remarks	: requires inhibition of endogenous APRT
IV. Reporter genes	
Gene	: *uidA* (*gus*)
Origin	: *E. coli*
Gene product	: β-glucuronidase
Protein action	: hydrolysis of glucuron(os)ide ester
Substrate	: wide range of β-glucuronides, also some β-galacturonides
Reference	: Jefferson *et al.* (1987)
Remarks	: Most popular reporter gene. Many substrates available
Gene	: *luc*
Origin	: *Photinus pyralis* (firefly)
Gene product	: firefly luciferase
Protein action	: ATP-dependent oxidative decarboxylation of luciferin results in light
Substrates	: luciferin, ATP, O_2
Reference	: Ow *et al.* (1986)
Remarks	: allows non-destructive assays; likely to become much more popular
Gene	: *luxA* and *luxB*
Origin	: *Vibrio harveyi*
Gene product	: bacterial luciferase
Protein action	: oxidation of fatty acid generates light
Substrates	: decanal (long chain fatty aldehydes), $FMNH_2$, O_2
Reference	: Koncz *et al.* (1987)
Remarks	: enzyme is heterodimer
Gene	: *lacZ*
Origin	: *E. coli*
Gene product	: β-galactosidase
Protein action	: hydrolysis of galactoside ester
Substrate	: wide range of galactosides
Reference	: Teeri *et al.* (1989)
Remarks	: of relatively little use in plants due to too high background of endogenous galactosidase levels
Gene	: *cat*
Origin	: *E. coli* Tn9
Gene product	: chloramphenicol acetyltransferase (CAT)
Protein action	: acetylation of chloramphenicol
Substrate	: chloramphenicol
Substrate action	: inhibits prokaryotic protein synthesis; too leaky to become a selection gene
Reference	: Herrera-Estrella *et al.* (1983)
Remarks	: many different assay possibilities
Gene	: *gfp*
Origin	: *Aequorea victoria, Renilla reniformis* (jellyfish)
Gene product	: green fluorescent protein
Protein action	: GFP absorbs blue or UV light and emits green fluorescent light
Substrate	: oxygen required
Reference	: Haseloff & Amos (1995)
Remarks	: Both fluorescence and protein are very stable. *Aequorea* GFP wavelength mutants available. High levels of GFP may interfere with regeneration

TRANSGENE-CENTRED ECOLOGY

Weediness

The concerns with respect to enhanced weediness are that the transgenic trait enables the crop to become a weed in agricultural systems, or establishes itself in ecosystems outside agricultural fields, or both. A weed can be broadly defined as a plant at the wrong place and/or the wrong time. As such, weeds constitute a serious limitation to crop productivity in agricultural systems all over the world. The weediness of a plant largely depends on the interplay between the intrinsic characters of the plant, in combination with its specific habitat (Keeler 1989; Williamson *et al.* 1990).

Central in transgene-centred ecological considerations will be the influence of the transgene product on the fitness of the crop. The scenario most relevant to biosafety is an enhancement of fitness due to the transgene product. Will the transgene product result in drastic or minor changes in habitat performance? Will there be any selective advantages of the transgenic crop over the parent non-transgenic crop and if so, in what circumstances? Is there a possibility for a change in the ecological range of the crop? More generally, is there an analogy between the problems with introductions of exotic plant species into new environments and the introduction of transgenic plants into environments (NRC 1989)?

The expression of selection genes gives an almost absolute advantage to transgenic plants in the laboratory. Generally, no selective conditions are expected outside the laboratory, with the exception of the cases of herbicide tolerance genes. Herbicide tolerance genes are aimed at giving a selective advantage in the field upon spraying. An important biosafety issue is, however, the selective advantage of the transgene in the absence of selection, either within or outside agricultural fields.

Considerable research attention has been given to the ecological consequences of transgenic tolerance against the herbicide Basta in relation to selection. Crawley *et al.* (1993) studied the demographic parameters of transgenic phosphinothricin-tolerant oilseed rape (*Brassica napus*) and non-transgenic oilseed rape in a variety of habitats and under a range of climatic conditions. They observed no indication for any increase in invasive potential conveyed by the phosphinothricin tolerance. Transgenic lines tended to be less invasive and less persistent than their non-transgenic counterparts. Fredshavn *et al.* (1995) investigated the competitiveness of transgenic phosphinothricin-tolerant oilseed rape. In their trials, the overall variation was high and actual differences between transgenic and non-transgenic lines could have been hidden by the overall variation. It proved important to include a species in the experimental design for which significant differences in competitiveness could be established. In the trials, the more aggressive crucifer white mustard (*Sinapis alba*) was included as calibration for the extent of competitiveness. The results allowed the conclusion that any change in competitiveness would not exceed the level of the more competitive white mustard (Fredshavn *et al.* 1995).

The research by Crawley *et al.* (1993) has been called a 'landmark paper in ecology' (Kareiva 1993), but also resulted in many discussions among ecologists and others about its scientific merits (Crawley 1993, 1994; Miller *et al.* 1993, 1994; Miller 1994). The same seems true for most other ecological data concerning transgenic plants. Experimental designs, as well as the validity and generality of the conclusions drawn, are controversial (Williamson 1992; Kareiva 1993; Crawley 1993, 1994; Miller *et al.* 1993, 1994; Miller 1994; Mellon & Rissler, 1995). These controversies may indicate that the science of

ecology is in the process of developing the methods required for routine assessment of ecological parameters. Transgenic herbicide-tolerant crops are likely to prove very useful for the further development of ecological science, especially in combination with the application of molecular techniques (Williamson 1992). Such a 'molecular ecology' will yield valuable insights to the dynamics and plasticity of ecosystems. The experimental data available to date seem sufficient to allow the conclusion that also in the case of transgenic herbicide tolerance an increased weediness is highly unlikely in the absence of selective conditions.

Spread of the transgene

Another major concern of transgenic crops is gene flow from the transgenic plant to wild relatives via cross pollination, resulting in a wild relative that is more weedy than its parent. The spread of any transgene to wild relatives depends on a myriad of ecological situations, genetic factors and stochastic events (Tiedje *et al.* 1989; Keeler & Turner 1991). In view of the global interests of current agriculture, it is prudent to assume that the transgene may spread by cross-pollination in some conditions and at some locations with a certain probability, that if desired can be estimated. More important would be the foreseeable effect on putative weediness of the spread of the transgene. As result of the transgene, a wild relative may impair agronomic practice or go out of control outside agricultural fields. The concerns about weediness are generally the same as those for the transgenic crop, although the wild relative by itself may be better adjusted to non-agricultural environments. A weedy wild relative will only be able to go out of control in case of selective conditions. Such conditions, except perhaps occasionally in verges adjacent to production fields, are unlikely to occur in natural ecosystems, but may pose problems in agricultural ecosystems. Outcrossing to a weedy wild relative next to a field may result in a weed that moves back into the field and poses problems. This scenario is particularly relevant for transgenic herbicide tolerance. The introduction of transgenic herbicide tolerance may result in a higher likelihood of the occurrence of acquired herbicide tolerance in weeds. This could ultimately lead to the 'loss' of the herbicide for agriculture. Having accepted a herbicide as relatively benign, any impairment of its current use due to gene flow from transgenic plants should be considered a negative development.

Horizontal, or non-sexual, transfer of the transgene to any other organism may also have undesired consequences. Horizontal gene transfer between organisms requires a chain of events, each step having a little likelihood (Schlüter *et al.* 1995). Any final outcome, irrespective of the time it will take to happen, will be an organism that normally would have been destroyed or affected, but is now able to cope with the substrate for the product of the selection gene. The consequence will, again, be dependent on the presence of selective conditions. These are unlikely to occur in natural ecosystems.

Familiarity

An important concept in the biosafety evaluations of the ecology of transgenic plants is the concept of familiarity (OECD 1993b). Assessment of familiarity implies the evaluation of how novel the transgenic phenotype is for the ecosystem under scrutiny. In some cases, such as the *aphA2* kanamycin resistance selection gene (Nap *et al.* 1992) and the β-glucuronidase reporter gene *uidA* (Gilissen *et al.* 1996), both (trans)gene and (trans)gene product are clearly familiar to ecosystems. In other cases, however, the

transgene-encoded properties due to selection and reporter genes will generally be unfamiliar for ecosystems. In this case, the concept of familiarity seems of no help. However, the familiarity concept could be applied to the result of the transgenic phenotype, rather than to the specific trait itself. 'Herbicide tolerance' as such is familiar to most agricultural ecosystems, only the herbicide used to obtain the tolerant phenotype is different in case of transgenic herbicide-tolerant crops. This could be taken as a form of familiarity.

TRANSGENE-CENTRED TOXICOLOGY

The introduction of a transgene in crops implies that two additional types of molecules can be present: the transgene DNA and its metabolites and the transgene product and its metabolites. In case of transgenic herbicide tolerance, the transgenic plants are likely to be challenged with the herbicide during the growing season. This is a situation different from a herbicide treatment of the non-transgenic parent crops. Therefore, the plants might contain a third type of molecules: the herbicide itself or its metabolites. Each of these molecules and metabolites may give rise to undesirable effects upon consumption. The safety of consumption needs to be evaluated in relation to the natural background of identical, similar or related compounds (IFBC 1990; OECD 1993a).

Transgene DNA

The large amount of DNA that passes the digestive tract daily indicates that DNA in itself is not intrinsically toxic to human beings. Most DNA is efficiently degraded and no functional genes are assumed to remain present (Berkowitz 1990). In this respect, transgene DNA will not differ from any other DNA and will not pose any (additional) threats. Possibly intestinal cells or micro-organisms can be transformed by the passing of transgenic DNA. The absence of selective pressure in the digestive tract of consumers will preclude any conceivable harm.

Transgene-encoded enzyme activity

Undesirable effects due to presence of the transgene-encoded protein can result from enzymatic activity of the protein in either transgenic plant or digestive tract, from the presence of the protein itself and/or from metabolites of the protein. Situations may build up during growth and development of the plant, during harvesting and storage, or may occur at actual consumption. In the transgenic plant, the transgene-encoded enzyme could generate secondary metabolites that are undesirable for consumption. This will depend on the substrate specificity of the enzyme. Assessment of the substrate specificity of the transgene-encoded protein will therefore be an important aspect of all transgene-centered evaluations. Are there endogenous look-alike enzymes? Where do these occur? Are there endogenous substrates? If so, can these substrates meet the transgene-encoded enzyme? What is the role of such putative endogenous substrates? What would be the consequence of transgene-encoded catalytic activity towards such substrates? Especially in the case of reporter genes, these questions would seem to be major issues. Metabolites generated in the human digestive tract will depend on the availability of substrates as well as on the likelihood of enzyme activity under the

conditions in the digestive tract. In general, the gastric conditions in the human digestive tract are not such that proteins encoded by selection or reporter genes are thought to have any catalytic activity.

Protein and substrate residues

Without enzymatic activity, the protein molecule itself, or its degradation products, could prove toxic or allergenic. Generally, proteins are non-toxic (Jones & Maryanski 1991). Recently, the OECD has summarized the criteria which may suggest an allergenicity of a protein (FDA/EPA/USDA 1994). In addition to the relative abundance, important criteria are resistance to proteolytic degradation or heat denaturation, as well as the occurrence or likelihood of glycosylation. These three criteria indicate that no allergenicity of selection or reporter gene-encoded proteins or degradation products are to be expected. In general, the proteins are not likely to exceed 0·1% of the total soluble protein content of the transgenic plant material. Glycosylation requires transport of the protein over membranes. Generally, selection and reporter gene products are not transported over membranes, excluding their glycosylation.

In particular, transgenic herbicide tolerance in crops may result in a situation different from the common use of the herbicide. Most herbicides used to obtain transgenic herbicide tolerance against are non-selective herbicides, already in use for general, pre-emergence weed control or control at the seedling stage. These are not used during maturation of the crop. In transgenic, herbicide-tolerant crops, the herbicide or its metabolites could be present upon harvesting. Studies concerning the toxicology and residues of the herbicide have generally been performed on herbicide sensitive material. The situation in herbicide-tolerant material may be different. The relevant molecules to evaluate will involve (1) the herbicide itself; (2) the 'normal' metabolites, i.e. the metabolites also formed in the untransformed plants upon spraying with the herbicide; (3) the modified, inactivated herbicide molecule resulting from the product of the transgene-encoded enzyme; as well as (4) the metabolites of the inactivated herbicide molecule. This will require detailed biochemical analyses of the transgenic material after herbicide application.

Substantial equivalence

A concept proposed for the safety evaluations of transgenic food is the concept of 'substantial equivalence' (OECD 1993a). When a new or modified food is found substantially equivalent to an existing food, further safety or nutritional concerns are expected to be insignificant. Such foods could be treated as their analogous conventional counterparts, for which the 'generally recognized as safe' (GRAS) concept is applicable (IFBC 1990; Jones & Maryanski 1991). In the case of transgenic food, however, the concept of 'substantial equivalence' may prove to be too poorly defined to be operationally useful. Even the reintroduction of a plant gene by genetic modification may, theoretically, change metabolism beyond substantial equivalence. More information about the boundary limits of natural variation in gene activities and metabolite spectra would seem to be a prerequisite to establish 'substantial equivalence'.

PLEIOTROPIC EFFECTS

The presence of the transgene or the transgene-encoded product, or any one of its metabolites, may in some way alter any of the manifold ecological relationships of the

crop itself, any wild relative derived from outcrossing, or any organism derived from horizontal gene transfer. For example, the wild relative may be more (or less) appreciated as food by some predators. An example of an ecological pleiotropic effect from classical breeding is the relation between male sterility and susceptibility against a leaf blight fungus in corn (Levings 1990). Similarly, the presence of the transgene or the transgene product, or any one of its metabolites, may in some unexpected way alter any of the manifold toxicological characteristics of the crop or any product derived from it.

Unfortunately, there are little data available with respect to transgenic plants containing a selection or reporter gene. There are no methods yet to approach these issues in an undisputed way. There is some evidence of pleiotropic variation from field trials with transgenic potatoes containing GUS and NPTII (Dale & McPartlan, 1992). The presence of GUS was reported to have a negative effect on plant growth and development. It cannot be excluded, however, that the observed pleiotropic variation is not due to the presence of GUS activity *per se* but to somaclonal variation introduced during the tissue culture steps of the genetic modification. Putatively pleiotropic effects due to specific genes are, therefore, very difficult issues for biosafety assessments. Can or must the prediction of such effects be required or requested? To only a certain and limited extent it can (and should) be attempted to predict undesired side effects of the introduced transgene. For example, the more central a target of a transgene product is in plant growth and development, the more relevant seems prediction and assessment of such effects. Also in this case, a transgene-centred approach will be useful.

At the moment, for both the ecology and the toxicology of transgenic crops it is unclear whether unpredictable pleiotropic effects due to the presence of a specific transgene do occur to the extent that any effects can be measured in a meaningful way. If they can be measured, it is unclear whether these effects have any relevance for ecological or toxicological relationships; if such an effect has any relevance for such relationships, the outcome does not necessarily need to be an adverse effect. Attention for putatively pleiotropic effects in ecological relationships could perhaps be part of post-marketing monitoring. The use of classical semi-chronic animal tests in the decision trees for the evaluation of transgenic plant products (Voedingsraad 1993) has resulted in debates concerning the suitability of such tests for toxicological research on complex plant products. The science of toxicology requires new and better methods to evaluate transgenic food. To answer questions concerning the safety of transgenic novel foods, proper analytical and toxicological tools are being developed (Kok 1993; Kok *et al.* 1994). In several (inter)national cooperative projects it is investigated how DNA analyses and RNA and metabolite profiling can contribute to the evaluation of food safety. These investigations are aimed at a better assessment of the toxicological characteristics of complex plant products. The fairly precisely defined change brought about by a transgene in a crop offers excellent material for analysis and development of improved methods for analysis.

In general, however, it could be pointed out that agriculture implies a constant flux in ecological systems. The dynamics and self-regulatory properties of ecosystems, in combination with the natural background of mutations and changes, such as the existence of transposons, would seem to create sufficient 'ecological noise'. Similarly, the dynamics and self-regulatory properties of man as a consumer, in combination with the natural background of foodstuffs and the relatively minor and well documented changes brought about by a transgene product, are likely to create sufficient 'toxicological noise'. It will be a difficult, but interesting and valuable, scientific exercise to

determine metabolite variability in food and feed to see of the variability within transgenic food stuffs is sufficiently low to assess any pleiotropic influence of a transgene in such food. For the issue of biosafety and biosafety regulations, however, the general considerations would seem to allow the conclusion that putatively pleiotropic effects, either ecologically or toxicologically, will be of no or only minor importance. It is important to stress, however, that these notions are controversial among ecologists (Regal 1994) as well as toxicologists (Kok, pers. comm.).

BIOSAFETY BEYOND THE TRANSGENE

The concerns and issues with respect to the biosafety in the broad sense of transgenes in plants reflect social, ethical and/or economic views with respect to current agriculture and to the role of life science research. We will mention a few examples in random order of arguments that have been put forward and should ideally be included in assessments of 'biosafety in the broad sense'. Genetic modification that overcomes species barriers is seen as tampering with the natural order of life. Evolutionary 'boundaries' should be considered as provisional warning signs of danger (Suzuki & Knudtson 1989). Transgenic crops could threaten the centers of crop diversity (Rissler & Mellon 1993). Resources used for genetic modification are thought to be better spent on more important issues. Research into transgenic herbicide-tolerant crops, for example, could distract from research into alternatives such as mechanical weed control (Reijnders 1993). The combination transgene/transgene-containing crop/transgene substrate is generally owned by the 'agro–industrial complex'. This may limit the options of farmers, may impair development of agriculture in third-world countries and generally will result in too high profits for too few (Lucassen *et al.* 1990; Rissler & Mellon 1993). Biotechnology becoming a high school topic, requiring not much more than a computer and some inexpensive laboratory equipment, may also appeal to 'bioterrorists' (Woodhouse & Hamlett 1992). The differences in regulation between transgenic plants and the products of modern plant breeding are confusing. An ethical reevaluation of traditional breeding practices and the regulation thereof has been proposed (Kockelkoren 1993).

Companies, on the other hand, point out that the investments made into agricultural biotechnology are high. Preferably early in product development a product should promise to be cost-effective. Cost-effectiveness will depend on patent and license fees, alternatives, environmental impact, environmental policies and taxes (Bijman 1994). Such concerns play also a role in the social and public acceptance of the transgenic crops. Related topics are the communication of risks and benefits to the lay audience; the developments with respect to property rights; as well as the necessity for and methods of labelling foods derived from modern biotechnology. Each of these topics is currently generating a respectable bibliography (e.g. Scholten *et al.* 1991; Durant 1992; Bryant & Leather 1992; Dunwoody 1992; Van Wijk *et al.* 1993; Barefoot *et al.* 1994). Full clearance and/or clarity for particular transgenes in as early a stage as possible would be advantageous for especially small and medium-sized enterprises.

Moreover, the 'biosafety of genetically modified organisms' is developing into a scientific discipline by itself, with its own conferences, pecking order and terminology (e.g. Stone 1994; Jones 1994). This is paralleled by a fair growth of the civil service involved in various aspects of regulation and control of regulation. As pointed out by

management theories covering the public sector (Lawton & Rose 1991), there is some risk that such a body of science and civil service will generate its own justification for its own sake. Such a body could usurp on budgets to an extent that is out of proportion, especially considering the drastic science budget cuts all over the world.

Unfortunately, between (and within) EU member states there are clear differences in the conceptualization of 'risk' and disagreements with respect to the environmental impacts that should be taken into consideration. For example, potential effects that could result from agricultural applications of a herbicide-tolerant transgenic crop are that upon outcrossing, farmers may loose the option of using the herbicide, whereas the herbicide itself is considered to be more environmentally friendly than alternatives. The latter effects, the potential loss of the herbicide and the environmental impact of the herbicide, are clearly secondary or indirect effects, or, in the terminology we propose, issues of biosafety in the broad sense. Regulatory authorities in EU member states such as the United Kingdom and The Netherlands tend to consider only the narrow sense ecological effects of the transgenic plants a biosafety issue. Broad sense effects are not seen as an issue of biosafety. Such effects are considered to be the competence of other committees and/or are covered by different laws and jurisdiction. Other member states, such as Austria, Denmark and Sweden, however, indicate that also broad sense effects should be included in assessments of transgenic plants. Their national legislation links biotechnology with broader criteria, such as sustainability, socioeconomics and ethics. Commandeur *et al.* (1996) give a recent overview of the situation in various EU countries.

In such a complex and politically sensitive context, full assessments of all aspects of the 'biosafety in the broad sense' are very demanding and interdisciplinary tasks. More consensus about 'need-to-know' issues versus 'nice-to-know' issues would seem to deserve the utmost priority (CCRO 1995; Miller 1996). Precise definitions of what exactly is discussed could help and contribute to more consensus among participants. Consensus on the 'narrow sense' issues of individual transgenes may contribute to more easy 'broad sense' assessments. It is very possible that a transgene is evaluated to be biosafe in the narrow sense, but poses undesirable characteristics with respect to its biosafety in the broad sense. An example would be a particular transgene conferring herbicide tolerance to a herbicide with an adverse environmental impact. The presence of the transgene and the transgene product in plants could be fully biosafe, but the associated increased use of the environmentally adverse herbicide would imply a negative biosafety in the broad sense. A transgene-centred evaluation of genes used in plants as outlined in this review will hopefully contribute to streamlining such discussions.

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