Risks of the release of transgenic herbicide-resistant plants with respect to humans, animals, and the environment

Harry A. Kuiper*, Gijs A. Kleter, Maryvon Y. Noordam

RIKILT, Wageningen University and Research Centre, PO Box 230, NL-6700 AE, Wageningen, The Netherlands

Abstract

Cultivation of transgenic herbicide-resistant crops has become very attractive in the last 2–3 years. The potential risks and benefits on cultivation of such crops depend on the type of crop modification, herbicides to be used, but also of variable environmental conditions. Data on the use of herbicides on transgenic crops grown on a large scale are scarce. The safety of foods and animal feeding stuffs derived from transgenic crops is assessed through application of the concept of substantial equivalence. This process includes a thorough comparison of the composition of the modified foods with the proper traditionally grown product, a toxicological assessment of newly introduced gene products, and an evaluation of possibly unintended alterations. This information provides a solid basis for food and feed safety assessment. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Improvement of agricultural crops has primarily been focused on the identification and isolation of genes involved in pathways which control important agronomic traits of food plants. Food crops have been modified through the insertion of new traits or the inhibition of existing gene functions, resulting in plants with (i) improved tolerance for herbicides, (ii) improved disease resistance against plant viruses and fungi, and (iii) improved pest resistance against lepidoptera, beetles, and nematodes. These crop improvements may result in higher yields and decreased use of chemical pesticides, and are primarily of economic interest for plant breeders, farmers, food retailers, and processors. This paper deals with hazard identification and risk assessment of herbicide-resistant crops with respect to impact on the environment and on human and animal health.

2. Development of herbicide-resistant crops

Table 1 summarises the types of transgenic herbicide-resistant (tHR) crops approved for cultivation in the European Union, the United States, Canada, and/or Japan. Largest commercial application of herbicide resistance in crops is against the non-selective herbicides glufosinate ammonium and glyphosate, and to a smaller extent against bromoxynil and sulfonylurea herbicides. Herbicide-resistant crops covered 20.1 million hectares of agricultural land in 1998, which constituted 72% of the acreage of genetically modified crops worldwide. Herbicide-resistant soybean accounted for most of this, namely 14.5 million ha, while canola, corn, and cotton formed the remainder (James, 1998).

Other major crop plants modified with herbicide resistance are currently field tested, and may reach the market in the near future (Table 2).

Resistance to more than one herbicide would allow for the use of additional herbicides, increasing the flexibility of weed control measures. Modified target enzymes with single amino acid substitutions have been observed, for example, in the weed Kochia resistant to triazine, imidazolinone, and sulfonylurea (Foels et al., 1999). Multiple resistances can also be acquired by cloning genes coding for enzymes that detoxify herbicides. For example, cross resistance in weeds may be related to activities of glutathione transferase and cytochrome P450 (Cummins et al., 1999; Preston et al., 1996). A cytochrome P450 enzyme was recently cloned into potatoes, conferring resistance to atrazine and chlorotoluron (Inui et al., 1999). Native genes may be altered by chimaeraplasty, as described for the acetolactate

*Corresponding author. Tel.: +31-317-475422; fax: +31-317-417717.
E-mail address: h.a.kuiper@rikilt.wag-ur.nl (H.A. Kuiper).

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Table 1
Transgenic herbicide-resistant crops approved in EU, USA, Canada, and/or Japan

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Target</th>
<th>Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromoxynil</td>
<td>Photosystem II</td>
<td>Cotton, oilseed rape, tobacco</td>
</tr>
<tr>
<td>Glufosinate ammonium</td>
<td>Glutamine synthase</td>
<td>Maize, oilseed rape, rice, soy, sugar beet</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>5-Enolpyruvyl-shikimate-3-phosphate synthase (EPSPS)</td>
<td>Beet, cotton, maize, oilseed rape, soy</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>Acetolactate synthase</td>
<td>Cotton, flax</td>
</tr>
</tbody>
</table>

synthase gene in maize (Beetham et al., 1999), which may prevent the occurrence of unexpected effects in the organism due to introduction of new genes.

Risks of possible out-crossing of herbicide resistance traits between crops and weeds may be eliminated through genetic modification of chloroplast genomes rather than nuclear genomes. Gene flow of introduced genes through pollen dispersal can thereby be prevented. This method has the additional advantage that high levels of expression can be attained, due to the large number of chloroplasts within each plant cell. Recently, tobacco chloroplasts were successfully transformed with an EPSPS gene (Daniell et al., 1998).

3. Environmental issues

The main objective of weed control is prevention of crop losses and improvement of crop quality. Furthermore, harvest is easier if no weeds are present in crop fields and spread of pests and disease mediated by weeds may be kept in check. Herbicides are also used in rotation systems for the destruction of volunteer plants arising from the previous harvest. Several non-chemical techniques can be used by farmers to control weeds, like ploughing, hoeing, and other methods of soil cultivation, use of cover crops and residue burning. Since the introduction of synthetic herbicides in agriculture, however, most farmers in the more industrialised countries use herbicides to control weeds, especially in areas where tillage is not allowed or advisable due to risk of soil erosion. In the US, for example, 75% of the pesticides used in agriculture are herbicides (Duke, 1999). Herbicide-resistant crops expand the options a farmer has for weed control, but also may have some negative consequences. Some of the (perceived) potential environmental risks and benefits are summarised in Table 3.

The potential risks and benefits of cultivation of tHR crops depend among others upon the herbicide to be used, the crop involved, and the method of transformation. For instance, glyphosate and glufosinate ammonium are applied after weeds emerge and are relatively short acting and in forage maize, glufosinate ammonium may replace the herbicide atrazine, which is rather persistent. On the other hand, these chemicals are broad-spectrum herbicides, and spray drift of these herbicides may affect the vegetation around a crop field more than currently used single-spectrum herbicides. Glyphosate is considered as a relatively environmentally safe chemical, but a recent re-assessment of this chemical under the Directive 91/414/EEC indicates that glyphosate may cause harmful effects on non-target arthropods, like predatory mites (Pesticides Trust, 1999).

Data on use of herbicides on transgenic herbicide-tolerant crops grown on large scale like soybean and canola are scarce. Moreover, comparative data on use of agrochemicals on these crops before introduction of the transgenic varieties are not abundantly available either. Data on amounts of herbicides used can be misleading, because some herbicides are far more active than others thus requiring less kg per ha. Toxicity and persistence of herbicides also vary, which makes a comparison difficult if not impossible at this stage. In a study of the Economic Research Service (ERS) of the United States Department of Agriculture (USDA) it was shown that in 1997 the number of herbicide applications were significantly

Table 2
Transgenic herbicide-resistant crops approved for field testing in EU and/or Japan

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Target</th>
<th>Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asulam</td>
<td>Dihydropteroate synthase</td>
<td>Tobacco</td>
</tr>
<tr>
<td>Chlorsulfuron</td>
<td>Acetolactate synthase</td>
<td>Robusta coffee</td>
</tr>
<tr>
<td>Glufosinate ammonium</td>
<td>Glutamine synthase</td>
<td>Beet, broccoli, cauliflower, chicory, poplar, rice, robusta coffee, sugar beet, sunflower, wheat</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>5-Enolpyruvyl-shikimate-3-phosphate synthase</td>
<td>Aspen, beet, fodder beet, rice, sugar beet, tobacco</td>
</tr>
<tr>
<td>Isoxazole</td>
<td>$\mu$-Hydroxyphenylpyruvate dioxygenase</td>
<td>Maize, oilseed rape, soy, tobacco</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>Acetolactate synthase</td>
<td>Sugar beet</td>
</tr>
<tr>
<td>(Enzyme inhibitors)*</td>
<td>Imidazolesglycerol phosphate dehydratase</td>
<td>Sugar beet</td>
</tr>
</tbody>
</table>

*Specific compounds have not been defined by the available sources.
Table 3  
Potential benefits and risks of transgenic herbicide resistant crops*  

<table>
<thead>
<tr>
<th>Potential benefits</th>
<th>Potential risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simpler weed management based on fewer herbicides</td>
<td>Greater reliance on herbicides for weed control</td>
</tr>
<tr>
<td>Decrease in herbicide use</td>
<td>Increase in herbicide use</td>
</tr>
<tr>
<td>Less contamination of the ecosystem</td>
<td>More contamination of the water, soil and air and shift in exposure patterns</td>
</tr>
<tr>
<td>Use of environmentally more benign herbicides</td>
<td>Development of resistance in weed species by introgression of the transgenes</td>
</tr>
<tr>
<td>Reduction of the need for mechanical soil treatment</td>
<td>Shifts in population of weeds towards more tolerant species</td>
</tr>
<tr>
<td>Less crop injury</td>
<td>Increase in volunteer problems in agricultural rotation systems</td>
</tr>
<tr>
<td>Improved weed control</td>
<td>Negative effects of herbicides on non-target species</td>
</tr>
</tbody>
</table>


reduced in half of the areas where crops like herbicide-resistant corn, soybeans, and cotton were grown. In the other areas the number of herbicide applications were the same as in non-transgenic varieties (ERS, 1999).

The crop involved also determines the potential risks and benefits. The risk of out-crossing of resistance genes to weed species depends on the pollination properties of the crop (self- or cross-pollinator or both, and insect- or wind-pollinated) and on the presence of compatible weed species. For instance, it is known that Johnsonsgrass, a noxious weed, hybridises with cultivated sorghum varieties (Mitten, 1999). To make sorghum resistant to herbicide applications is therefore not advisable. In a similar fashion, oilseed rape is able to hybridise with related weed species, such as field mustard and wild radish, thereby conferring herbicide resistance to these weed species (Brown and Brown, 1996; Darmency et al., 1995). As noted above, transfer of resistance genes into the chloroplast rather than into the nuclear genome will reduce the chance for out-crossing via the pollen. Continuous use of the same herbicide may cause a shift in weed populations towards less sensitive species. In the long run, other herbicides will have to be used to control these species. In agricultural rotation systems the control of volunteer problems may become complex (Derksen et al., 1999). Crossing of canola with canola on nearby fields with resistance to different herbicides may seriously hamper control of volunteers if gene stacking occurs, e.g. canola varieties emerge with resistance to several herbicides. Experience with large-scale breeding of transgenic herbicide-tolerant crops has been gained up till now in the US, while in Europe cultivation of such crops is limited to relatively small field-plot experimentation. Further studies are needed with respect to the overall use of herbicides and potential changes in residue exposure patterns, to the development of resistance in weeds and changes in weed composition, and influences on farmland wildlife.

4. Food and feed safety assessment of tHR crops

Various regulatory bodies like the Organisation for Economic Cooperation and Development (OECD), the Food and Agriculture Organisation/World Health Organisation (FAO/WHO), and the European Union have designed strategies for the safety evaluation of genetically modified foods or food ingredients (EU, 1990, 1997a, b; FAO/WHO, 1991, 1996; OECD, 1993, 1996). Food and feed safety issues of tHR crops to be addressed are: (i) expression of new gene products, and their toxicological properties with respect to human or animal safety, (ii) formation/toxicity of new metabolites of herbicides on tHR crops, (iii) changes in patterns and levels of residues of herbicides on tHR crops, (iv) potential alterations in levels of nutrients or toxicants (unintended effects), (v) gene transfer between plants and the gut microflora of humans or animals, (vi) occurrence of residues in animal-derived edible products, and (vii) allergenicity.

These issues are examined on a case by case basis taking the type of food crop and the type of genetic modification into account (Noteborn et al., 1995). The safety of genetically modified food crops is assessed using a comparative approach, i.e. if a new food is found to be substantially equivalent to an existing traditional food, the new product can be considered to be as safe as the conventional food (OECD, 1993). This approach is not a safety assessment per se but a comparative analytical exercise, investigating agronomic, (bio)chemical, and nutritional parameters of the modified product relative to those of the existing traditional food.

It must be emphasised that foods are complex mixtures of nutrients and natural toxins which exert beneficial and adverse effects on human health. Conventional foods are considered to be safe on the basis of long-term experience and history of safe use, although a systematic safety assessment has generally not been conducted.
4.1. Detection and characterisation of unintended effects

A key issue in safety evaluation of genetically modified foods is the identification and characterisation of unexpected changes which may have taken place in the organism as result of the genetic modification process, which could affect the safety or nutritional status of the modified organism. In order to identify such effects, a systematic analytical comparison is made between the composition of the genetically modified organism and its parent grown under conditions that are as identical as possible. Compositional analysis is normally performed on single macro- and micro-nutrients and plant-specific known anti-nutrients or toxins. This approach has its limitations with respect to the detection of unknown anti-nutrients and natural toxins in less well-documented species. Animal experimentation with complex foods with the purpose to assess unintended effects, has its severe drawbacks like dietary deficiencies, sensitivity, and small margins of safety. Therefore, alternative methods are under development to identify unintended effects through DNA sequence and mRNA analysis, analysis of protein expression, or analysis of profiles of secondary metabolites (Kok et al., 1998; Noteborn et al., 1998; Kuiper et al., 1999).

4.2. Transfer of marker genes from genetically modified plants

One of the most debated safety issues with respect to the introduction of genetically modified organisms, is the possible transfer of newly introduced marker genes coding for antibiotic or herbicide resistance to gut microorganisms or cells of the gastrointestinal (GI) tract of humans or animals. Marker genes coding for aminoglycoside or β-lactam inactivating enzymes have been employed, and genes coding for resistance against glyphosate, glufosinate ammonium, bromoxynil and sulfonylureas. The likelihood of transfer of a gene from genetically modified plants to microorganisms is remote, but cannot be entirely ruled out. In case of the use of antibiotic-resistance marker genes, the human and animal use of the antibiotic and the prevalence of resistance to the same antibiotic in the GI microflora should be considered (FAO/WHO, 1996). In case of the use of herbicide-resistance coding genes, risks for humans and animals are low, given the specificity of enzymatic reactions involved and the non-prevalence of substrates in the GI tract of humans or animals (EU, 1998a).

4.3. Allergenicity

There is a clear need to assess the allergenic potential of novel foods produced through biotechnology. Genetic modification may increase the allergenic potential of a crop by introduction of a new allergenic protein, by raising endogenous levels of allergens or by modification of endogenous proteins. Internationally agreed approaches have been designed to identify and characterise introduced gene products with respect to potential allergenicity (Metcalfe et al., 1996). The proposed decision tree considers the source of the introduced gene, physicochemical properties of the protein and sequence homology with known allergenic proteins. For phosphinothricine acetyltransferase (PAT), for example, no relevant amino acid sequence similarity with known allergens could be found, and in addition PAT is degraded rapidly in a gastric model (Metcalfe et al., 1996).

However, much more must be understood of structural and functional parameters which determine the allergenic potential of a protein. Moreover, animal models to test for allergenicity are under development, but need further validation (Atkinson and Meredith, 1998; Knippeels et al., 1998). The available clinical and biochemical expertise yields a reasonable degree of certainty to identify new allergens before market introduction.

4.4. Residue assessment

After application of plant protective compounds to crops, residues and metabolites may occur on these plants, which may be used as animal feed and consequently appear in animal products destined for human consumption. For instance, glyphosate is slowly metabolised in tHR plants to amino-methylphosphonic acid (AMPA) as occurs in non-tolerant plants. In livestock animals, glyphosate and AMPA are not or to a minor extent metabolised and residue levels in animal-derived products are low or even below detection limits (EU, 1998b, c). Metabolism studies of glufosinate ammonium in transgenic crops indicate a rapid conversion of glufosinate to N-acetyl-glufosinate and depending on the plant variety and type of tissue, to 3-methylphosphinonic acid (MPP). Residue studies with ruminants and poultry indicated no detectable levels in animal-derived products (EU, 1998a,d).

5. Conclusions

Cultivation of tHR crops has become very attractive in the last 2–3 years, indicating that growers perceive strong benefits from growing such crops. The potential risks and benefits of cultivation of tHR crops depend on a large number of factors like the type of agronomic improvement of the crop involved, herbicides to be used, but also on variable environmental conditions. Data on the use of herbicides on tHR crops grown on a large scale are scarce.

The safety of foods and animal feeding stuffs derived from tHR crops is assessed through the application of the concept of substantial equivalence. A thorough
comparison of the composition of the modified foods with the properly traditionally grown product and a toxicological assessment of newly introduced gene products and of possibly unintended alterations provides a solid basis for food safety assessment.

References


