

Genetic engineering of crops as potential source of genetic hazard in the human diet

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Abstract

The benefits of genetic engineering of crop plants to improve the reliability and quality of the world food supply have been contrasted with public concerns raised about the food safety of the resulting products. Debates have concentrated on the possible unforeseen risks associated with the accumulation of new metabolites in crop plants that may contribute to toxins, allergens and genetic hazards in the human diet. This review examines the various molecular and biochemical mechanisms by which new hazards may appear in foods as a direct consequence of genetic engineering in crop plants. Such hazards may arise from the expression products of the inserted genes, secondary or pleiotropic effects of transgene expression, and random insertional mutagenic effects resulting from transgene integration into plant genomes. However, when traditional plant breeding is evaluated in the same context, these mechanisms are no different from those that have been widely accepted from the past use of new cultivars in agriculture. The risks associated with the introduction of new genes via genetic engineering must be considered alongside the common breeding practice of introgressing large fragments of chromatin from related wild species into crop cultivars. The large proportion of such introgressed DNA involves genes of unknown function linked to the trait of interest such as pest or disease resistance. In this context, the potential risks of introducing new food hazards from the applications of genetic engineering are no different from the risks that might be anticipated from genetic manipulation of crops via traditional breeding. In many respects, the precise manner in which genetic engineering can control the nature and expression of the transferred DNA offers greater confidence for producing the desired outcome compared with traditional breeding. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Plant breeding has been successfully used for many years to genetically improve crop plants. It

began with unconscious selection by early hunter-gatherer communities at the dawn of agriculture, followed by the deliberate saving of seed from preferred plant types. Over many hundreds of years, such ancient practices resulted in the gradual domestication of the majority of crop plants known today. Although plant breeding was a well established activity before the genetic basis of inheritance was elucidated by Mendel, it became recognised as a

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more scientifically directed endeavour as the understanding of classical genetics improved in the early decades of the 1900s. Over the past 50 years the combined efforts of plant breeders to successfully develop and release new crop cultivars have provided the basis for the consistent supply of food in a changing global environment and ever-changing pest and disease populations. This has been a major contributing factor toward the alleviation of world hunger and suffering, and the consequent maintenance of political stability.

Improvements in yield performance, specific quality attributes, resistance to specific diseases and pests, and environmental adaptation are constantly demanded from plant breeders. Over the recent history of modern plant breeding, 'new technologies' have been used regularly to facilitate the development of new gene combinations during the breeding of new cultivars. These technologies have included:

- manipulating ploidy levels by generating haploid plants and chemical induction of chromosome doubling;
- chromosome manipulation through the development of specific addition and substitution lines;
- chemical and radiation treatments to induce mutations and chromosome rearrangements;
- cell and tissue culture approaches such as embryo rescue, *in vitro* fertilisation and protoplast fusion to allow the recovery of interspecific and intergeneric hybrids, as well as the recovery of plants with spontaneous genome rearrangements.

The genetic gains made possible by integrating these technologies into mainstream plant breeding have culminated in substantial improvements in the performance of the resulting cultivars. Over the past four to five decades these approaches have become a routine component of the genetic improvement of crops and have quietly revolutionised plant breeding. They have been usually utilised by plant geneticists involved in a 'pre-breeding' phase associated with the development of novel germplasm. This material has been subsequently used as a resource from which plant breeders have developed new cultivars. Consequently, the contribution that these technologies have made to the final production of new cultivars is often overlooked.

Recent advances in molecular biology have resulted in the development of two technologies that

offer the basis for the next level of genetic gain in crop cultivars.

- The construction of genetic maps saturated with DNA markers, and the subsequent design of relatively simple PCR-based assays to facilitate the selection of desired alleles at closely linked loci and the resulting development of plant lines with desired combinations of traits (marker-assisted selection);
- The cloning and DNA sequencing of specific genes, the reassembly of specific DNA fragments into functional chimeric genes, and the transfer of such genes to single plant cells from which complete plants can be regenerated via cell and tissue culture.

The first of these two approaches to crop improvement involves the molecular diagnosis of the existing genetic material in crop plants. The latter approach involves genetic engineering of plants and offers immense opportunities for the incorporation of new genes into crop cultivars. Most genetically engineered crop plants currently on the market or under development involve the transfer of genes conferring resistance to pests, diseases, herbicides, and environmental stress, as well as quality traits such as improved post-harvest storage, flavour, nutrition, and colour. The resulting novel germplasm is predicted to allow plant breeders to respond much more quickly to the market need for new and improved cultivars, and satisfy the increasing consumer demand for a consistent supply of high quality, blemish-free grains, fruits and vegetables with reduced pesticide residues.

The potential benefits of this new technology for improving the reliability and quality of the world food supply have been contrasted with public concerns raised about the food safety of the resulting products. In particular, debates have focused on the possible unforeseen risks associated with the accumulation of new metabolites in crop plants that may contribute to toxins, allergens and genetic hazards in the human diet. In this review, we examine the potential for new hazards to appear in foods as a direct consequence of genetic engineering in crop plants. No attempt is made to address specific hazards that may result from the transfer of individual genes to particular crops. Instead, we discuss the various mechanisms by which such hazards may arise during genetic engineering. Furthermore, we assess whether these mechanisms are fundamentally different from those that may arise from the well

established and accepted practice of traditional plant breeding and the existing use of new cultivars in agriculture.

2. Characteristics of genetic engineering in crop plants

In order to assess the various mechanisms which may give rise to food hazards during genetic engineering of crop plants, it is important to first summarise the basic features of plant transformation, especially the nature of transgenes and the manner in which they are expressed. This provides an appropriate basis for identifying the mechanisms that can give rise to hazards.

2.1. Gene transfer approaches

The preferred method for genetic engineering of crops involves the use of the natural gene transfer mechanisms of *Agrobacterium*, a common soil bacterium [1]. A modified transfer-DNA (T-DNA) vector is constructed in which the desired DNA fragment is inserted between the T-DNA border regions (specific 25 bp direct repeat regions) of *Agrobacterium*. This vector is transferred into *Agrobacterium* and the virulence gene products of *Agrobacterium* actively recognise, excise, transport, and integrate the T-DNA region into host plant genomes. However, the natural host range of *Agrobacterium* limits this approach in some crops, especially the cereals and other monocotyledonous species. For such crops, alternative approaches such as direct uptake of naked DNA into protoplasts or tissues using electroporation or particle gun bombardment are available [1]. The co-transfer of a selectable marker gene along with the gene of interest allows the preferential growth of the transformed cells in cell culture. Successive manipulations of the chemical composition of the culture medium, especially the plant hormones, allows the regeneration of complete plants. The ease with which genetically engineered plants can be developed varies markedly between crops, and even between different genotypes within crop species. Despite this, genetically engineered plants have been recovered in virtually all crop plants [1].

2.2. Nature of transferred DNA

The amount of DNA transferred is usually less than 10 kb, which is minuscule compared to the genome size of crop plants (262 million base pairs/1C nucleus in peaches to nearly 16,000 million base pairs/1C nucleus in onions and wheat; [2]). The complete DNA sequence of the DNA region to be transferred is either known or can be deduced from the component DNA fragments. In most cases, when the technology has reached the point of transferring genes for genetic improvement of crops, the nature of all encoded functions is well understood. Furthermore, the manner in which the transgenes are intended to be expressed in the resulting plant can be controlled by the use of appropriate cis-regulatory sequences. In particular, promoters can be selected that either allow constitutive gene expression or limit gene expression to only specific cell types or in response to specific environmental stimuli. The translational fusion of specific signal sequences to the peptide coding region can target expression to particular subcellular or extracellular locations.

2.3. Transgene integration into plant genomes

Transformation events are genetically equivalent to small addition-deletion mutations and the transgenic plants initially regenerated are hemizygous for the inserted DNA and therefore segregate upon self-pollination or outcrossing [3]. The DNA fragment intended for transfer may become integrated as a single discrete copy or as repeated regions, and insertion may occur at one or more integration sites, e.g., [4–7]. At any site of incorporation into plant chromosomes, the transferred genes may integrate in a complete, a truncated, or a rearranged manner. In some instances, *Agrobacterium*-mediated transformation can result in complex integration patterns which may include vector sequences outside the T-DNA region [8]. The preferred integration event is clearly the insertion of one complete intact DNA fragment at a single locus. This is a relatively common event for the T-DNA transfer events associated with *Agrobacterium*-mediated transformation, whereas direct DNA transfer approaches often result in long concatamers of the transferred DNA [9,10].

Although the intact nature of the transferred DNA cannot be guaranteed prior to any transformation event, the nature of transgene integration can be accurately determined by Southern blot analysis after the genetically engineered plant is recovered. Complex integration patterns can also be effectively analysed by fluorescence in situ hybridisation on extended DNA fibres [8]. Furthermore, the site of integration into the plant chromosomes can be determined if this was important. Genetic mapping studies have established that gene integration occurs throughout the genome along the length of all chromosomes in plants. This has been well documented for several plants species, e.g., potatoes [11,12], tomato [13], *Arabidopsis* [14] and petunia [15].

2.4. Variability in transgene expression

Plant genetic engineering is also highly unpredictable with respect to the expression of the transferred genes, and the stability of their performance [3]. The magnitude of transgene expression, and sometimes the developmental pattern of expression, can vary markedly among a population of plants independently transformed with the same transgene. This general phenomenon is referred to as the 'position effect', and is believed to be a consequence of each transformation event involving the random integration of the transgene into different sites of the plant chromatin [16–18]. These position effects can be mitigated by flanking transgenes with specific matrix-associated regions which are considered to insulate transcriptional regulation from the effects of surrounding chromatin [19,20].

Even when a transgenic line with high expression of the transgene is identified, a range of gene silencing phenomena can occur subsequent to the initial transformation event. The continuity and stability of expression can be occasionally influenced by a variety of allelic and non-allelic interactions [21]. Homology-dependent gene silencing may occur in response to homozygosity of the transgene, or in conjunction with DNA sequence homology with another transgenic locus or an endogenous gene. Transgenic loci with such epistatic dominant silencing action usually have complex DNA integration patterns and are often associated with inverted repeats [22–25]. The precise nature of the silencing agent is unknown,

but is thought to involve a transgene-derived, putatively aberrant RNA [26].

When transgenes are inserted as one intact DNA fragment at a single locus, their expression generally behaves in a highly consistent manner. Such transgenic loci exhibit the expected additive gene action both within loci (hemizygous versus homozygous status) and between loci (dihybrids between homozygous transgenic individuals) [24,27,28]. Loss of transgene function is rare in such transgenic lines (one in 10^4), which is consistent with the performance of many endogenous plant genes [29].

2.5. Advantages of genetic engineering technology

Many of the characters targeted for improvement by genetic engineering are similar to those achieved by gene transfer from wild species via traditional breeding [30]. Does genetic engineering technology offer any benefit over traditional breeding for crop improvement? Several key advantages can be identified.

1. The germplasm base is extended from related wild species to any source of DNA such as other plants, microorganisms and animals, or even artificially synthesised genes. This therefore provides new opportunities to complement the existing genes in the declining gene pools of traditional breeding. This is especially important for pest and disease resistance, due to the constant selection pressure on the target pests and diseases to overcome the resistance genes already transferred via traditional breeding.

2. Genetic engineering allows the repeated transfer of new genes directly into existing cultivars or elite lines in plant breeding programmes. This contributes to the more efficient development of new cultivars without many generations of hybridisation and selection to recover agronomically useful lines. In the case of gene transfer from wild species, this process may require up to 20 generations of additional plant breeding cycles.

3. The transfer of single discrete genes can be readily achieved via genetic engineering. This overcomes the problem of linkage drag in traditional plant breeding which is associated with the transfer of many unwanted and undefined closely linked genes. The negative effects of such linkage drag are more pronounced when chromosome fragments are

introgressed from more distantly related germplasm sources.

4. New gene formulations can be specifically designed and constructed using the tools of molecular biology. This may involve the use of promoter regions that target gene expression at a desired magnitude, time, or location in plants. Alternatively, it is possible to target the specific ‘knock out’ of discrete gene functions through antisense or gene silencing approaches.

3. Mechanisms by which food hazards may arise during crop genetic engineering

Risks associated with the appearance of toxins, allergens or genetic hazards in foods derived from genetically engineered crops may arise as a consequence of the biosynthesis of specific chemical constituents in the portion of the crop that is eaten. Alternatively, hazards may arise from the elimination of metabolites that play important roles in reducing health risks (e.g., antioxidants). In general terms, the mechanisms by which food hazards may arise from genetic engineering of crops fall into three categories [31]:

- inserted genes and their expression products,
- secondary and pleiotropic effects of gene expression,
- insertional mutagenesis resulting from gene integration.

3.1. Inserted transgenes and their expression products

The DNA of genes and their RNA expression products are composed of nucleic acids. Since the chemical components of nucleic acids are identical in all living organisms, the physical presence of the transferred genes and their RNA expression products do not cause any new health risks over existing foods. When the transferred genes are expressed in plant cells, it is the effects of the protein expression products that need to be considered. The direct protein expression products of transgenes transferred via genetic engineering are generally known and sensitive assays for their presence are usually available. Therefore, the amount and stability of these proteins

in edible components of plants, before and following harvest, storage and food processing, can be determined. Furthermore, since the protein expression products of transgenes are known, any potential toxic, immunological, allergenic or genetic hazards can be evaluated if health concerns are associated with the presence of the specific proteins in food sources.

3.2. Secondary and pleiotropic effects of gene expression

Many transgenes transferred by genetic engineering encode the production of enzymes that catalyse biochemical reactions. These enzymes are often expressed at a high level, which may lead to altered metabolic flow-through in biochemical pathways. In turn, this may give rise to unanticipated increases or decreases in certain other biochemicals. The biosynthesis of enzymes from transgene expression may result in the depletion of the enzymatic substrate and the concurrent accumulation of the enzymatic product. An example of such effects in transgenic crops involves the transfer and expression of a nopaline synthase gene in asparagus. The nopaline synthase enzyme catalyses a condensation reaction between arginine and α -keto glutarate to produce nopaline. In both callus tissue and shoot cultures of transgenic asparagus, the accumulation of the product (nopaline) is accompanied by a decrease in the content of arginine (the substrate) [32].

The potential for altering flow-through of metabolites in biochemical pathways is well illustrated by the over-expression of a yeast ornithine decarboxylase gene in tobacco [33]. Some of the derived transgenic lines had a 10–20 fold increase in ornithine decarboxylase activity with no change in the activity of other downstream enzymes in the pathway to nicotine. A two-fold increase in the accumulation of both putrescine and nicotine was observed, with no change in the content of polyamines or other intermediate substrates.

The expression of a new enzymatic activity in plant cells may also result in the diversion of metabolites from one secondary metabolic pathway to another. To promote longer shelf life of tomatoes, ethylene biosynthesis was substantially reduced by diverting metabolism of the aminocyclopropane carboxylic acid intermediate away from ethylene

biosynthesis by the transfer and expression of a bacterial aminocyclopropane carboxylic acid deaminase gene [34]. Similarly, the transfer and expression of tryptophan decarboxylase from the medicinal plant *Catharanthus roseus* to canola plants, resulted in an accumulation of tryptamine and a corresponding lower content of tryptophan-derived indole glucosinolates considered to be antinutritional factors in crucifer crops [35]. The development of artificial metabolic sinks to redirect metabolites away from a specific secondary pathway is usually the desired metabolic effect being targeted. However, this may result in unpredictable effects in other intermediary metabolites and the diversion of metabolic flow to other secondary pathways.

Dramatic pleiotropic effects of transgene expression can result from the expression of specific transacting factors which may result in the up-regulation of a whole biochemical pathway. For example, the transfer and expression of the *R* and *C1* regulatory genes from maize to several dicotyledonous species resulted in some tissues being highly anthocyanin-pigmented due to the activation of specific genes in the anthocyanin biosynthetic pathway [36,37]. The *R* gene also induced the development of trichomes (hairs) in *Arabidopsis* [36].

The possibility of secondary effects of transgene expression will depend on the key regulatory points and rate limiting steps in biochemical pathways. In general terms these are poorly understood in plants, and can be expected to vary between crops as well as between cultivars and breeding lines within the same crop species [31]. In some instances the RNA or protein expression products of the transferred genes can influence the expression of existing genes in plants. Secondary effects of gene expression may occur in plant tissues beyond those in which the transgene is expressed. It is conceivable that metabolites accumulating as a consequence of transgene-derived biochemical activity in one tissue are translocated within the plant to other tissues and organs such as the harvested food.

3.3. Insertional mutagenesis resulting from transgene integration

A possible consequence of the random integration of transgenes into the genomes of plants is that

insertional mutagenesis may disrupt or modify the expression of existing genes in the recipient plant. Experiments using promoter-less reporter genes in a range of plant species have recovered a high frequency (30–70%) of transgenic plants with reporter gene activity [38–43]. This suggests integration into transcriptionally active DNA, downstream of endogenous sequences with promoter activity. The analysis of genome sequences flanking transgene insertion sites in plant genomes suggests that about half of all insertions involve low/single copy DNA [44], which further substantiates the possibility of insertional mutagenesis being a relatively common event.

The most common insertional mutagenic event is expected to be inactivation of endogenous genes from the physical disruption associated with integration of an exogenous DNA fragment. When such insertions occur into existing genes this can result in the appearance of mutant forms, which will become apparent in later generations when the transferred gene (and corresponding insertional mutant) occur in the homozygous state [45,46]. Another consequence of random insertion is the potential for the production of fusion proteins resulting from the read-through of the genetic code from flanking plant DNA into the inserted DNA (or vice versa) [31]. Such translational fusions have been detected at low frequencies between endogenous genes and a β -glucuronidase (GUS) reporter gene which can tolerate large amino-terminal additions while retaining enzymatic activity. Using a vector with a promoter-less GUS gene which was also devoid of its ATG initiation site, GUS expression was detected in 1.6% of the regenerated plants arising from independent transformation events [39]. In most instances, such fusion proteins will result in the biosynthesis of nonsense expression products of no biological significance. In the production of transgenic crops, such events will be recognised and eliminated by the transgene expression products having an incorrect size, biophysical property or function.

Theoretically, insertional mutagenesis can also result in gene activation of otherwise silent genes in plants [31]. This may arise via read-through from highly expressed promoter regions of transgenes into coding regions of the flanking plant DNA, especially if 'fortuitous' truncations of transgene occurred at

some integration sites. The marked influence of transcriptional read-through on gene expression of downstream genes was observed following transformation of plants with hybrid genes specifically designed to test this possibility [47]. In this context, the possibility of activating genes that encode enzymes in biochemical pathways toward the production of toxic secondary compounds raises the most concern for food safety. The wild relatives of many crop plants are known to contain various secondary compounds with varying levels of toxicity. During the early phases of crop domestication, such compounds have been substantially reduced in the edible portion of crop plants [48]. For example, although potato tubers have a greatly reduced glycoalkaloid content, high levels of the glycoalkaloids, α -solanine and α -chaconine, are retained in the foliage [49]. Similarly, the various tissues of vegetable brassicas markedly differ in their content of glucosinolates, well known to be goitrogenic [50–52]. The reductions of toxic constituents in the edible portion of crops may have resulted from mutations in the DNA regions that regulate where the genes are expressed. The activation of such silenced genes by the chance insertion of a transgene at a neighbouring site is therefore plausible, although an exceedingly remote possibility.

4. Assessment of risks relative to traditional crop breeding

As outlined above, several distinct mechanisms can potentially give rise to food hazards during the genetic engineering of crops. Are these mechanisms unique to genetic engineering? Can identical situations be identified within the bounds of traditional plant breeding?

4.1. Inserted genes and their expression products

Genetic engineering allows the nature of the DNA intended for transfer to be controlled in a very precise manner and limited to the exact minimal segment of DNA capable of conferring the desired trait. This is in marked contrast to traditional breeding where undefined genes are routinely transferred between breeding lines, species and even genera. The parental lines used in traditional plant breeding

are often assumed to have large regions of uncharacterised DNA transferred from wild species. Only a small proportion of this transferred DNA is expected to carry the desired character such as resistance to pests and diseases, and the vast majority of the transferred DNA has been presumed to involve linked genes of unknown function. Theoretical analyses of 'near-isogenic' lines after six generations of backcrossing have calculated that the average size of introgressed regions ranges from 24–38 cM for chromosomes ranging in size from 50–200 cM [53]. Two well studied examples involve the introgression of resistance genes from *Lycopersicon peruvianum* to tomato (*Lycopersicon esculentum*). Genetic mapping studies involving molecular markers have determined that the introgressed chromosomal region around the Tm-2 locus, conferring resistance to tobacco mosaic virus, varied from 4 to 51 cM over a wide range of cultivars [54]. Likewise, the *Mi* locus conferring resistance to the nematode *Meloidogyne incognita*, which was transferred to cultivated tomato over 50 years ago, has been located to an introgressed chromosomal region of < 2 cM to 8 cM in a series of tomato cultivars [55]. Since the genome size of tomato is approximately 950 million base pairs/1C nucleus [2] and the total map length is about 1300 cM [56], an average of 730 kb per cM can be calculated for the tomato genome. The chromosomal regions derived from *L. peruvianum* can therefore be estimated to range from 1500–40,000 kb, which is up to several orders of magnitude larger than the < 5 kb usually associated with cloned and sequenced resistance genes, e.g., [57,58].

The recently developed techniques of GISH (genomic in situ hybridisation) and FISH (fluorescence in situ hybridisation) offer powerful tools for analysing the extent to which plant breeders have introgressed 'foreign' DNA into crop plants. These technologies enable large chromosome segments originating from wild species to be clearly visualised as distinct regions on condensed chromosome preparations in a wide range of crops, including: wheat [59], barley [60] and onions [61]. One remarkable example in wheat involves the identification of introgressed chromosomal segments conferring resistance to five important diseases following hybridisations with plants from three other genera: *Secale* (rye), *Thinopyrum*, and *Avena* (oat) [62].

4.2. Secondary effects of gene expression

The concerns about secondary effects of gene expression are not confined to genetic engineering. They can also unwittingly occur in traditional breeding programmes by the complementation of genes from different parental lines. Complementary genes may specify enzymes with different activities along biochemical pathways and provide opportunities for unintended increases in secondary compounds, especially when the parental lines contain wild species in their pedigrees. A classic illustration of secondary effects in traditional breeding programmes involves the development of novel fruit colours in tomato following introgression of genes from some accessions of wild *Lycopersicon* species [63,64]. One specific example involves a substantial increase in the intensity of the red pigmentation of tomato fruit, attributed to the concentration of lycopene, following gene transfer from *Lycopersicon hirsutum*. This was unanticipated considering the fruit of *L. hirsutum* remain green, even when fully ripe, due to the lack of an active enzyme for the final step in the pathway to lycopene [65]. The wild tomato presumably contributed a gene that enhances earlier steps in the biosynthetic pathway toward lycopene, which results in greater metabolic flow-through to higher pigment production. In a similar context, advanced potato breeding lines with novel, toxic glycoalkaloids in their tubers have been produced when wild species exist in their pedigrees [66].

The risk of secondary effects of gene expression products during the development of genetically engineered crops are clearly not new, since cultivars with genes transferred from wild species have been commercialised by plant breeders for many years. When a transgene is expressed in a crop plant, the biochemistry underlying the new character is better understood than for most genes utilised in traditional breeding programmes. This increases the opportunity to predict possible secondary effects, which can be investigated if hazardous situations are envisaged.

4.3. Insertional mutagenesis resulting from gene integration

The implications of random insertion events are also not unique to genetic engineering, and do not present a new health risk beyond the risks that can

be anticipated from traditional plant breeding. Naturally occurring phenomena involving factors such as chromosomal rearrangements [67], activity of transposable elements in plant genomes [68,69], as well as various forms of genetic recombination, can all give rise to identical ‘random’ effects in plant genomes. Chemical and radiation treatments to induce mutations and chromosome rearrangements have also played an important role in traditional plant breeding of most crop plants [48,70].

Gene introgression from wild species into crop plants, especially for genes conferring disease resistance, often involves homoeologous recombination or induced translocations between chromosomes. Such gene transfers have a well documented history in cereal breeding. In the case of wheat, homoeologous pairing and recombination can be induced in specific genetic stocks involving the *ph* mutant gene and deleted chromosome 5B [71]. Disease resistance genes have also been introgressed by radiation-induced random translocations of undefined chromosome regions [72,73] or cell culture-induced breakage and fusion [74].

Even without the use of transgenics, other aspects of biotechnology are constantly pushing forward the introgression of DNA between species. Considerable progress has been made toward the introgression of tomato chromosomal DNA into the potato genome following somatic fusion of protoplasts and subsequent backcrossing of the regenerated plants [75,76]. More remarkable is the recent use of Southern hybridisation analysis to confirm the transfer of genomic DNA between monocotyledonous and dicotyledonous plants following somatic hybridisation. This has been achieved for carrots following the regeneration of plants from the fusion products of carrot protoplasts with barley protoplasts [77] and irradiated rice protoplasts [78].

5. Perspective on risks associated with genetic modifications

5.1. Plant breeding

Despite the apparent risks associated with traditional plant breeding, the food products from new cultivars have been readily accepted as part of the human diet for many years. Similarly, new crops

species have been ‘domesticated’ without any food safety assessment. A classic example is triticale, an artificial crop species developed from the intergeneric hybridisation of durum wheat and rye [79]. Combining the complete genomes of these two species established the potential for many new unpredictable interactions involving epistasis and complementation between genetic elements from each parental species. A recent example of a new species launched on the world market is a yellow-fleshed kiwifruit, *Actinidia chinensis*, which is becoming widely accepted for its novelty value, but has never been evaluated for food safety considerations.

Over the history of crop improvement, breeders have been especially successful at introgressing chromosomal regions from wild species. For example, in potato breeding the development of new cultivars has been greatly assisted by the transfer of genes from over 20 related *Solanum* species, which have contributed resistance to pests, diseases, and frost, as well as some quality traits such as content of low reducing sugars, starch and protein [30]. More than half of all current potato cultivars have genes introgressed from *Solanum demissum*, mainly for resistance to *Phytophthora infestans* (potato blight), with some cultivars containing introgressions from several wild *Solanum* species [80].

The genes introgressed from wild species are usually associated with pest and disease resistance traits. In most instances the biochemical basis for the transferred character is undetermined. As the biochemical knowledge of plants improves, such resistance traits are subsequently associated with the accumulation of proteins such as lectins, ribosome inactivating proteins, proteinase inhibitors, antifungal hydrolases and thionins, as well as a wide range of secondary compounds [81]. Many of these traits are now being transferred between plants by transgenic means. It is incongruous that the agricultural use of transgenic plants containing these genes raises immediate concern, whereas the use of such genes in traditional breeding has been widely accepted for many decades.

5.2. Genetic engineering of crop plants

Many of the potential hazards that may arise in crop plants as a consequence of genetic engineering

would only be expected to arise at very low frequencies. Even if such events did occur, they would not pose any new risks beyond the risks that might be expected from existing food sources and genetic manipulation of crops via traditional breeding. Compared to the knowledge base about genes transferred via traditional breeding, genes transferred via genetic engineering are very well understood at the molecular level. Consequently, very sensitive assays are available to monitor the presence and expression of transgenes in crop plants. Therefore, genetic manipulation using the power and precision of molecular biology offers greater confidence of achieving the desired outcome than traditional breeding.

The focus of food safety assessment of transgenic crops should be on the protein expression products of the introduced transgene. Since the identity of such products is known, any potential concerns about the toxic, immunological, allergenic or genetic hazards that may arise from the introduced gene can be readily recognised and assessed if health concerns exist. Such issues become more important when the proteins expressed from transgenes result in new components in the organ harvested from a crop, so that the resulting food is no longer substantially equivalent to previous products. Secondary and pleiotropic effects of the inserted genes, as well as random mutational effects associated with gene insertion, can raise health concerns. However, similar events have been readily accepted with traditional plant breeding. In most instances the possibility of secondary biochemical effects of gene transfer are less predictable for traditional breeding than they are for genetic engineering, since less is known about the nature of the genes being transferred.

5.3. Assessing the products of genetic modification

The possibility of inadvertent biochemical changes occurring during genetic modification by either traditional or transgenic approaches, highlights the importance of routinely testing new cultivars for their content of natural compounds that may pose toxic, allergenic, or genetic hazards in the human diet. What analyses should be performed on new cultivars before they are marketed as a food source? It is clearly important that the potential toxins in specific crops are not increased. The toxins that may accumu-

late in response to rare biosynthetic activation events are those found in the non-harvested organs of the crop plant or in closely related species. For example, it would be important to monitor new cultivars of vegetable brassicas for glucosinolates and new cultivars of potatoes for glycoalkaloids. It is also important that the essential nutrients or metabolites, that specific crops contribute to the human diet, are maintained following genetic modification. Such analyses should take into account the standard processing and cooking methods for food derived from new cultivars of crop plants.

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References

- [1] R.G. Birch, Plant transformation: problems and strategies for practical application, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48 (1997) 297–326.
- [2] K. Arumuganathan, E.D. Earle, Nuclear DNA content of some important plant species, *Plant Mol. Biol. Rep.* 9 (1991) 208–218.
- [3] A.J. Conner, M.C. Christey, Plant breeding and seed marketing options for the introduction of transgenic insect-resistant crops, *Biocontrol Sci. Technol.* 4 (1994) 463–473.
- [4] J.D.G. Jones, D.E. Gilbert, K.L. Grady, R.A. Jorgensen, T-DNA structure and gene expression in petunia plants transformed by *Agrobacterium tumefaciens* C58 derivatives, *Mol. Gen. Genet.* 207 (1987) 478–485.
- [5] R. Jorgensen, C. Snyder, J.D.G. Jones, T-DNA is organized predominantly in inverted repeat structures in plants transformed with *Agrobacterium tumefaciens* C58 derivatives, *Mol. Gen. Genet.* 207 (1987) 471–477.
- [6] S.C. Deroles, R.C. Gardner, Analysis of T-DNA structure in a large number of transgenic petunias generated by *Agrobacterium*-mediated transformation, *Plant Mol. Biol.* 11 (1988) 365–377.
- [7] M.C. Christey, B.K. Sinclair, Regeneration of transgenic kale (*Brassica oleracea* var. *acephala*), rape (*B. napus*) and turnip (*B. campestris* var. *rapifera*) plants via *Agrobacterium rhizogenes*-mediated transformation, *Plant Science* 87 (1992) 161–169.
- [8] A.M.A. Wolters, L.M. Trindade, E. Jacobsen, R.G.F. Visser, Fluorescence in situ hybridization on extended DNA fibres as a tool to analyse complex T-DNA loci in potato, *Plant J.* 13 (1998) 837–847.
- [9] A.P. Czernilofsky, R. Hain, L. Herrera-Estrella, H. Lörz, E. Goyvaerts, B.J. Baker, J. Schell, Fate of selectable marker DNA integrated into the genome of *Nicotiana tabacum*, *DNA* 5 (1986) 101–113.
- [10] A.P. Czernilofsky, R. Hain, B. Baker, U. Wirtz, Studies of the structure and functional organization of foreign DNA integrated into the genome of *Nicotiana tabacum*, *DNA* 5 (1986) 473–482.
- [11] J.M.E. Jacobs, H.J. van Eck, P. Arens, B. Verkerk-Bakker, B. te Lintel Hekkert, H.J.M. Bastiaanssen, A. El-Kharbotly, A. Pereira, E. Jacobsen, W.J. Stiekema, A genetic map of potato (*S. tuberosum*) integrating molecular markers, including transposons, and classical markers, *Theor. Appl. Genet.* 91 (1995) 289–300.
- [12] A. El-Kharbotly, J.M.E. Jacobs, B. te Lintel Hekkert, E. Jacobsen, M.S. Ramanna, W.J. Stiekema, A. Pereira, Localization of Ds-transposon containing T-DNA inserts in the diploid transgenic potato: linkage to the R1 resistance gene against *Phytophthora infestans* (Mont.) De Bary, *Genome* 39 (1996) 249–257.
- [13] C.M. Thomas, D.A. Jones, J.J. English, B.J. Carroll, J.L. Bennetzen, K. Harrison, A. Burbidge, G.J. Bishop, J.D.G. Jones, Analysis of the chromosomal distribution of transposon-carrying T-DNAs in tomato using the inverse polymerase chain reaction, *Mol. Gen. Genet.* 242 (1994) 573–585.
- [14] Y.G. Liu, N. Mitsukawa, T. Oosumi, R.F. Whittier, Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR, *Plant J.* 8 (1995) 457–463.
- [15] T.P. Robbins, A.G.M. Gerats, H. Fiske, R.A. Jorgensen, Suppression of recombination in wide hybrids of *Petunia hybrida* as revealed by genetic mapping of marker transgenes, *Theor. Appl. Genet.* 90 (1995) 957–968.
- [16] C. Dean, J. Jones, M. Favreau, P. Dunsmuir, J. Bedbrook, Influence of flanking sequences on variability in expression levels of an introduced gene in transgenic tobacco plants, *Nucleic Acids Res.* 16 (1988) 9267–9283.
- [17] K.S. Blundy, M.A.C. Blundy, D. Carter, F. Wilson, W.D. Park, M.M. Burrell, The expression of class I patatin gene fusions in transgenic potato varies with both gene and cultivar, *Plant Mol. Biol.* 16 (1991) 153–160.
- [18] C. Peach, J. Velten, Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters, *Plant Mol. Biol.* 17 (1991) 49–61.
- [19] L. Mlynárová, A. Loonen, J. Heldens, R.C. Jansen, P. Keizer, W.J. Stiekema, J.P. Nap, Reduced position effect in mature transgenic plants conferred by the chicken lysozyme matrix-associated region, *Plant Cell* 6 (1994) 417–426.
- [20] L. Mlynárová, R.C. Jansen, A.J. Conner, W.J. Stiekema, J.P. Nap, The MAR-mediated reduction in position effect can be uncoupled from copy number-dependent gene expression in transgenic plants, *Plant Cell* 7 (1995) 599–609.

- [21] M.A. Matzke, A.J.M. Matzke, How and why do plants inactivate homologous (trans)genes?, *Plant Physiol.* 107 (1995) 679–685.
- [22] F.F. Assaad, K.L. Tucker, E.R. Signer, Epigenetic repeat-induced gene silencing (RIGS) in *Arabidopsis*, *Plant Mol. Biol.* 22 (1993) 1067–1085.
- [23] S.L.A. Hobbs, T.D. Warkentin, C.M.O. DeLong, Transgene copy number can be positively or negatively associated with transgene expression, *Plant Mol. Biol.* 21 (1993) 17–26.
- [24] J.P. Nap, A.J. Conner, L. Mlynárová, W.J. Stiekema, R.C. Jansen, Dissection of a synthesized quantitative trait to characterize transgene interactions, *Genetics* 147 (1997) 315–320.
- [25] M. Stam, R. de Bruin, S. Kenter, R.A.L. van der Hoorn, R. van Blokland, J.N.M. Mol, J.M. Kooter, Post-transcriptional silencing of chalcone synthase genes in *Petunia* by inverted transgene repeats, *Plant J.* 12 (1997) 63–82.
- [26] D.C. Baulcombe, RNA as a target and an initiator of post-transcriptional gene silencing in transgenic plants, *Plant Mol. Biol.* 32 (1996) 79–88.
- [27] S.L.A. Hobbs, P. Kpodar, C.M.O. DeLong, The effect of T-DNA copy number, position and methylation on reporter gene expression in tobacco transformants, *Plant Mol. Biol.* 15 (1990) 851–864.
- [28] A.J. Conner, L.C.P. Keizer, L. Mlynárová, W.J. Stiekema, J.-P. Nap, Evaluation of diallel analysis using β -glucuronidase activity from transgenes in *Nicotiana tabacum*, *Euphytica* 102 (1998) 161–168.
- [29] A.J. Conner, L. Mlynárová, W.J. Stiekema, J.-P. Nap, Meiotic stability of transgene expression is unaffected by flanking matrix-associated regions, *Mol. Breed.* 4 (1997) 47–58.
- [30] A.J. Conner, J.M.E. Jacobs, R.A. Genet, Transgenic versus ‘traditional’ potatoes: what’s the difference?, in: G.D. McLean, P.M. Waterhouse, G. Evans, M.J. Gibbs (Eds.), *Commercialisation of transgenic crops: risk, benefit and trade considerations*, Cooperative Research Centre for Plant Science and Bureau of Resource Sciences, Canberra, 1997, pp. 23–36.
- [31] A.J. Conner, Food safety issues relating to genetic engineering of crop plants, *Agricultural Science* 6 (1993) 36–41.
- [32] A.J. Conner, H.J. Bezar, J.W. Ashby, Genetic engineering of plants for weed, disease and pest control: Science vs. politics?, *Proceedings of the New Zealand Weed and Pest Control Conference*, 43 (1990) 200–208.
- [33] J.D. Hamill, R.J. Robins, A.J. Parr, D.M. Evans, J.M. Furze, M.J.C. Rhodes, Over-expressing a yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* can lead to enhanced nicotine accumulation, *Plant Mol. Biol.* 15 (1990) 27–38.
- [34] H.J. Klee, M.B. Hayford, K.A. Kretzmer, G.F. Barry, G.M. Kishore, Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants, *Plant Cell* 3 (1991) 1187–1193.
- [35] S. Chadavej, N. Brisson, J.N. McNeil, V. De Luca, Redirection of tryptophan leads to production of low indole glucosinolate canola, *Proc. Natl. Acad. Sci. U.S.A.* 91 (1994) 2166–2170.
- [36] A.M. Lloyd, V. Walbot, R.W. Davis, *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators *R* and *C1*, *Science* 258 (1992) 1773–1775.
- [37] F. Quattrocchio, J.F. Wing, H.T.C. Leppen, J.N.M. Mol, R.E. Koes, Regulatory genes controlling anthocyanin pigmentation are functionally conserved among plant species and have distinct sets of target genes, *Plant Cell* 5 (1993) 1497–1512.
- [38] C. Koncz, N. Martini, R. Mayerhofer, Z. Koncz-Kalman, H. Körber, G.P. Redei, J. Schell, High frequency T-DNA-mediated gene tagging in plants, *Proc. Natl. Acad. Sci. U.S.A.* 86 (1989) 8467–8471.
- [39] S. Kertbundit, H. De Greve, F. DeBoeck, M. Van Montagu, J.P. Hernalsteens, In vivo random β -glucuronidase gene fusions in *Arabidopsis thaliana*, *Proc. Natl. Acad. Sci. U.S.A.* 88 (1991) 5212–5216.
- [40] J.F. Topping, W. Wei, K. Lindsey, Functional tagging of regulatory elements in the plant genome, *Development* 112 (1991) 1009–1019.
- [41] K. Lindsey, W. Wei, M.C. Clarke, H.F. McArdle, L.M. Rooke, J.F. Topping, Tagging genomic sequences that direct transgene expression by activation of a promoter trap in plants, *Transgenic Research* 2 (1993) 33–47.
- [42] A.J. Conner, T.S. Shum, J.L. Tynan, M.K. Williams, M.C. Christey, Frequency of transcriptional gene fusion during *Agrobacterium*-mediated transformation in potato, *Abstracts of the Third International Symposium on the Molecular Biology of the Potato*, Santa Cruz, CA, 1993, p. 78.
- [43] M.C. Christey, A.J. Conner, B.K. Sinclair, Frequency of transcriptional gene fusion during *Agrobacterium*-mediated transformation of Brassica, *Abstracts of the 8th Crucifer Genetics Workshop*, 21–24 July, Saskatoon, Canada, 1993, p. 91.
- [44] J.M.E. Jacobs, B. te Lintel Hekkert, A. El-Kharbotly, E. Jacobsen, W.J. Stiekema, A. Pereira, *Ac-Ds* transposons mapped near disease resistance loci for targeted tagging in potato, in: W.R. Belknap, M.E. Vayda, W.D. Park (Eds.), *The Molecular and Cellular Biology of the Potato*, 2nd edn., CAB International, Wallingford, 1994, pp. 21–30.
- [45] K.A. Feldmann, M.D. Marks, M.L. Christianson, R.S. Quatrano, A dwarf mutant of *Arabidopsis* generated by T-DNA insertion mutagenesis, *Science* 243 (1989) 1351–1354.
- [46] K.A. Feldmann, T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum, *Plant J.* 1 (1991) 71–82.
- [47] I. Ingelbrecht, P. Breyne, K. Vancompernelle, A. Jacobs, M. Van Montagu, A. Depicker, Transcriptional interference in transgenic plants, *Gene* 109 (1991) 239–242.
- [48] N.W. Simmonds, *Principles of crop improvement*. Longman, London, 1979, 408 pp.
- [49] W.M.J. Van Gelder, Steroidal glycoalkaloids in *Solanum*: consequences for potato breeding and for food safety, in: R.F. Keller, A.T. Tu (Eds.), *Handbook of Natural Toxins*, Vol. 6, *Toxicology of Plant and Fungal Compounds*, Marcel Dekker, New York, 1991, pp. 101–134.
- [50] D.G. Carlson, M.E. Daxenbichler, C.H. VanEtten, H.L. Tookey, P.H. Williams, Glucosinolates in crucifer vegetables: turnips and rutabagas, *J. Agric. Food Chem.* 29 (1981) 1235–1239.

- [51] D.G. Carlson, M.E. Daxenbichler, C.H. VanEtten, C.B. Hill, P.H. Williams, Glucosinolates in radish cultivars, *J. Am. Soc. Hortic. Sci.* 110 (1985) 634–638.
- [52] C.H. VanEtten, M.E. Daxenbichler, W.F. Kwolek, P.H. Williams, Distribution of glucosinolates in the pith, cambial cortex, and leaves of the head in cabbage, *Brassica oleracea*, *J. Agric. Food Chem.* 27 (1979) 648–650.
- [53] P. Stam, A.C. Zeven, The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing, *Euphytica* 30 (1981) 227–238.
- [54] N.D. Young, S.D. Tanksley, RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding, *Theor. Appl. Genet.* 77 (1989) 353–359.
- [55] R. Messeguer, M. Ganal, M.C. de Vicente, N.D. Young, H. Bolkan, S.D. Tanksley, High resolution RFLP map around the root knot nematode resistance gene (*Mi*) in tomato, *Theor. Appl. Genet.* 82 (1991) 529–536.
- [56] S.D. Tanksley, M.W. Ganal, J.P. Prince, M.C. de Vicente, M.W. Bonierbale, P. Broun, T.M. Fulton, J.J. Giovannoni, S. Grandillo, G.B. Martin, R. Messeguer, J.C. Miller, L. Miller, A.H. Paterson, O. Pineda, M.S. Röder, R.A. Wing, W. Wu, N.D. Young, High density molecular linkage maps of the tomato and potato genomes, *Genetics* 132 (1992) 1141–1160.
- [57] D.A. Jones, C.M. Thomas, K.E. Hammond-Kosack, P.J. Balint-Kurti, J.D.G. Jones, Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging, *Science* 266 (1994) 789–793.
- [58] W.Y. Song, G.L. Wang, L.L. Chen, H.S. Kim, L.Y. Pi, T. Holsten, B. Wang, W.X. Zhai, L.H. Zhu, C. Fauquet, P. Ronald, A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*, *Science* 270 (1995) 1804–1806.
- [59] T. Schwarzacher, K. Anamthawat-Jonsson, G.E. Harrison, A.K.M.R. Ilam, J.Z. Jia, I.P. King, A.R. Leitch, T.E. Miller, S.M. Reader, W.J. Rogers, M. Shi, J.S. Heslop-Harrison, Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat, *Theor. Appl. Genet.* 84 (1992) 778–786.
- [60] R.A. Pickering, A.M. Hill, R.G. Kynast, Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic substitution plant derived from *Hordeum vulgare* L. × *Hordeum bulbosum* L. crosses, *Genome* 40 (1997) 195–200.
- [61] L.I. Khrustaleva, C. Kik, Cytogenetical studies in the bridge cross *Allium cepa* × (*A. fistulosum* × *A. roylei*), *Theor. Appl. Genet.* 96 (1998) 8–14.
- [62] S. Tang, J. Zhuang, Y. Wen, S.A. Ai, H. Li, J. Xu, Identification of introgressed segments conferring disease resistance in a tetrageneric hybrid of *Triticum*, *Secale*, *Thinopyrum*, and *Avena*, *Genome* 40 (1997) 99–103.
- [63] G. Mackinney, C.M. Rick, J.A. Jenkins, Carotenoid differences in *Lycopersicon* hybrids of an unusual race of *L. pimpinellifolium*, *Proc. Natl. Acad. Sci. U.S.A.* 8 (1954) 695–699.
- [64] C.M. Rick, Fruit and pedicel characters derived from Galápagos tomatoes, *Economic Botany* 21 (1967) 171–184.
- [65] S.D. Tanksley, S.R. McCouch, Seed banks and molecular maps: unlocking genetic potential from the wild, *Science* 277 (1997) 1063–1066.
- [66] W.M.J. Van Gelder, J.J.C. Scheffer, Transmission of steroidal glycoalkaloids from *Solanum vernei* to the cultivated potato, *Phytochemistry* 30 (1991) 165–168.
- [67] G.S. Khush, Cytogenetics of aneuploids, Academic Press, New York, London, 1973.
- [68] N.V. Fedoroff, About maize transposable elements and development, *Cell* 56 (1989) 181–191.
- [69] T. Oosumi, B. Garlick, W.R. Belknap, Identification and characterization of putative transposable DNA elements in Solanaceous plants and *Caenorhabditis elegans*, *Proc. Natl. Acad. Sci. U.S.A.* 92 (1995) 8886–8890.
- [70] A.M. van Harten, Mutation breeding: theory and practical applications, Cambridge Univ. Press, 1998.
- [71] R. Riley, Cytogenetics of chromosome pairing in wheat, *Genetics* 78 (1974) 193–203.
- [72] E.R. Sears, The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat, *Brookhaven Symposia in Biology* 9 (1956) 1–22.
- [73] B. Friebe, J. Jiang, B.S. Gill, P.L. Dyck, Radiation-induced nonhomoeologous wheat-*Agropyron intermedium* chromosomal translocations conferring resistance to leaf rust, *Theor. Appl. Genet.* 86 (1993) 141–149.
- [74] P.M. Banks, P.J. Larkin, H.S. Bariana, E.S. Lagudah, R. Appels, P.M. Waterhouse, R.I.S. Brettell, X. Chen, H.J. Xu, Z.Y. Xin, Y.T. Qian, X.M. Zhou, Z.M. Cheng, G.H. Zhou, The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat, *Genome* 38 (1995) 395–405.
- [75] H.J. de Jong, A.M.A. Wolters, J.M. Kok, H. Verhaar, J. van Eden, Chromosome pairing and potential for intergeneric recombination in some hypotetraploid somatic hybrids of *Lycopersicon esculentum* (+) *Solanum tuberosum*, *Genome* 36 (1993) 1032–1041.
- [76] F. Garriga-Calderé, D.J. Huigen, A. Angrisano, E. Jacobsen, M.S. Ramanna, Transmission of alien tomato chromosomes from BC1 to BC2 progenies derived from backcrossing potato (+) tomato fusion hybrids to potato—the selection of single additions for seven different tomato chromosomes, *Theor. Appl. Genet.* 96 (1998) 155–163.
- [77] H. Kisaka, M. Kisaka, A. Kanno, T. Kameya, Production and analysis of plants that are somatic hybrids of barley (*Hordeum vulgare* L.) and carrot (*Daucus carota* L.), *Theor. Appl. Genet.* 94 (1997) 221–226.
- [78] H. Kisaka, H. Lee, M. Kisaka, A. Kanno, K. Kang, T. Kameya, Production and analysis of asymmetric hybrid plants between monocotyledon (*Oryza sativa* L.) and dicotyledon (*Daucus carota* L.), *Theor. Appl. Genet.* 89 (1994) 365–371.
- [79] E.N. Larter, Triticale, in: N.W. Simmonds (Ed.), *Evolution of crop plants*, Longman Group, London, 1976, pp. 117–120.
- [80] H. Ross, Potato breeding—problems and perspectives, *J. Plant Breeding Suppl* 13 (1986) .
- [81] S.L. Franck-Oberaspach, B. Keller, Consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity, *Plant Breeding* 116 (1997) 1–17.