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Cisgenesis, a New Tool for Traditional Plant Breeding, Should be Exempted from the Regulation on Genetically Modified Organisms in a Step by Step Approach

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Abstract Modern potato breeding requires over 100,000 seedlings per new variety. Main reasons are (1) the increasing number of traits that have to be combined in this tetraploid vegetatively propagated crop, and (2) an increasing number of traits (e.g., resistance to biotic stress) originates from wild species. Pre-breeding by introgression or induced translocation is an expensive way of transferring single traits (such as *R*-genes, coding for resistance to biotic stress) to the cultivated plant. The most important obstacle is simultaneous transfer of undesired neighbouring alien alleles as linkage drag. Stacking several genes from different wild sources is increasing this linkage drag problem tremendously. Biotechnology has enabled transformation of alien genes into the plant. Initially, transgenes were originating mainly from microorganisms, viruses or non-crossable plant species, or they were chimeric. Moreover, selection markers coding for antibiotic resistance or herbicide resistance were needed. Transgenes are a new gene source for plant breeding and, therefore, additional regulations like the EU Directive 2001/18/EC were developed. Because of a strong opposition against genetic modification of plants in Europe, the application of this Directive is strict, very expensive, hampering the introduction of genetically modified (GM) crops and the use of this technology by small and medium-sized enterprises (SMEs). Currently, GM crops are almost the exclusive domain of multinationals. Meanwhile, not only transgenes but also natural genes from the plant species itself or from crossable plant species, called cisgenes, are available and the alien selection genes can be avoided in the end product. This opens the way for cisgenic crops without alien genes. The existing EU directive for GM organisms is not designed for this new development. The cisgenes belong to the existing breeders' gene pool. The use of this classical gene pool has been regulated already in

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agreements regarding breeders' rights. We are proposing a step by step approach starting with a crop and gene specific derogation and monitoring towards a general exemption of cisgenic plants from the Directive. Two examples, i.e. development of cisgenic potato for resistance to *Phytophthora infestans* and cisgenic apple for resistance to *Venturia inaequalis* are discussed shortly for illustration of the importance of cisgenesis as a new tool for traditional plant breeding. Cisgenesis is simplifying introgression and induced translocation breeding tremendously and is highly recommended for SMEs and developing countries.

Keywords Apple breeding · Cisgenesis · Genetic modification · *Malus domestica* · *Phytophthora infestans* · Potato breeding · *Solanum tuberosum* · *Venturia inaequalis*

Introduction

Breeding of crops, including potato, is becoming increasingly complicated. The main reason for this is the increasing number of requirements for new varieties. Many required new traits are obtained from wild species (Jacobsen and Hutten 2006). For the introduction of such traits (for example resistance to scab in apple, late blight in potato, or black sigatoka in banana), introgression breeding is needed. In wheat and other allopolyploid crops, induced translocation breeding has to be applied (Friebe et al. 1996) which is even more complicated. Introgression breeding is relatively easy in self-pollinating crops such as tomato, barley and rice, but is more complicated in allopolyploid wheat (Chahal and Gosal 2002; Table 1). In these self-pollinating, generatively propagated crops, new traits from wild species can be incorporated into an existing variety by interspecific hybridization followed by back crossing. Main bottleneck in such an approach is linkage drag of hundreds of alien alleles neighbouring the gene of interest after introgression or translocation into the crop species. In case of self-incompatible, vegetatively propagated crops such as potato and apple, there is an additional bottleneck, i.e. the heterozygous nature of

Table 1 The different steps in introgression breeding, induced translocation breeding and cisgenesis

Step	Introgression breeding	Induced translocation breeding	Cisgenesis
1	Interspecific crossing with wild source containing the desired gene	Interspecific crossing with wild source	Isolation of the desired gene from wild source
2	Back cross procedure and removal of disturbing linkage drag	Back cross procedure resulting in monosomic addition line	Backbone free transformation and selection for cisgene expression
3	Crosses with breeding parent for variety selection	Irradiation and selection for useful translocations in offspring	Selection of cisgenic variety *without* linkage drag
4	Resistant variety *with* linkage drag	Crosses with induced translocation line for variety selection	
5		Resistant variety *with* linkage drag	

varieties (Jacobsen and Schouten 2007a). The unique composition of alleles that led to the final selection of the variety, is entirely and irreversibly destroyed in the progeny after making a cross. It means that a new combination of desired alleles has to be selected leading to a completely new variety, in addition to the complicated introgression process of the desired gene from the wild relative.

New possibilities in plant breeding have emerged through plant biotechnology enabling (1) a diversity of new in vitro techniques, such as micropropagation, plant regeneration, protoplast fusion and genetic modification, and (2) genomics with marker-assisted selection, sequencing of whole genomes and isolation of particular genes not only from non-crossable species, such as viruses and bacteria (transgenes), but also from the crop species itself or from crossable wild plant species (cisgenes; Schouten et al. 2006a).

Genetic modification (GM) of plants in plant breeding, leading to genetically modified organisms (GMOs) is accompanied with strict rules as described in Europe in EU Directive 2001/18/EC. Opposition of NGOs and organic farmers in Europe and other places has hampered introduction of new GM varieties considerably. It is important to realize that Directive 2001/18/EC (Anonymous 2001) has been based on the process of introducing transgenes, which are genes coming from viruses, bacteria or other non-crossable sources or synthetic genes. Until now also alien selection genes coding for antibiotic or herbicide resistance have been used for transformation, making every GM plant transgenic (Schouten et al. 2006a, b).

New developments in transformation technology that enable absence of alien selection genes in the end product, and utilization of cisgenes make a reconsideration of the rules for GMOs as described in Directive 2001/18/EC (Anonymous 2001) necessary.

Cisgenesis valorises the increasing wealth of information of plant genes in a safe way. Especially applications like stacking genes coding for resistance against *Phytophthora infestans* in potato may be the way for significant reduction of the tremendous quantities of fungicides applied in potato growing, both in conventional farming (synthetic fungicides) and in organic farming (copper). It may also prevent early flaming of potato crops in organic farming. Hampering or even abandoning cisgenesis for obtaining durable resistance in potato without a reliable alternative (Lammerts van Bueren et al. 2007) can be regarded as unethical.

In this contribution bottlenecks of traditional plant breeding, in particular of introgression breeding, are described and the potential solution of cisgenesis is discussed, including the reasoning why exemption of cisgenic plants from the Directive 2001/18/EC makes sense.

Traditional Plant Breeding

The gene pool for the traditional plant breeder consists of genes from the species itself, from crossable wild species, and genes altered by induced or spontaneous mutations (Chahal and Gosal 2002). Sexual compatibility in traditional plant breeding includes the application of embryo rescue, and crosses with bridging species (Anonymous 2001; Schouten et al. 2006a, b; Jacobsen and Schouten 2007a). All these techniques result into non-GM varieties without the need of applying additional rules such as GMO regulations. In potato, variety development is nowadays dependent on the selection of more than 100,000 seedlings per new variety (Jacobsen and Schouten 2007b). Main reason for that is, as indicated above, the growing number of traits that have to be combined into one genotype. Another reason is that the gene sources for important traits such as disease resistances or quality traits are frequently found in wild species. Introgression of such traits into a variety needs a lot of pre-breeding in which the gene of interest has to be "cleaned" from linkage drag caused by disturbing neighbouring wild alleles (Table 1). Stacking of several introgression traits from one or more wild species into a single variety worsens this problem (Jacobsen and Schouten 2007b). In cash crops such as tomato, but also in wheat, marker-assisted selection can help to speed up the process of combining several traits (William et al. 2007). In lettuce, an example has been described that marker-assisted selection can assist breeders in reducing linkage drag problems (Jansen 1997). However, marker-assisted selection is not easy. It still has to be further developed to allow for practical use in potato breeding and in breeding of many other crops. In potato, traditional introgression breeding has been successful in breeding for resistance to viruses and nematodes but it failed for sustainable resistance to *P. infestans*. The utilized sources of resistance against *P. infestans* were mainly tracing back to *Solanum demissum*. For this species 11 *R*-genes against *P. infestans* have been described (Jacobsen and Hutten 2006; Jacobsen 2007; Haverkort et al. 2008). All these resistance genes have been broken down and only four of them have been used extensively in varieties. Recently, in our laboratory over 1,000 accessions of 200 different species have been screened for resistance and new sources of resistance have been found (not published). The most important new source in traditional resistance breeding until now is *S. bulbocastanum* (Hermsen and Ramanna 1973). It contains several useful *R*-genes with broad spectrum resistance. Breeding with this source took more than 30 years before the first resistant varieties such as cv. Toluca was introduced into the market. Main reason for that was the need for multiple bridge crosses with *S. acaule* and *S. phureja* before the *R*-gene source came available into the potato background (Hermsen and Ramanna 1973).

Bridge cross hybrids with distantly related species like *S. bulbocastanum* have the additional disadvantage that meiotic recombination is decreased, hampering removal of disturbing linkage drag. Variety development takes even much more time if stacking of several *R*-genes from such far related species is needed. If such valuable *R*-gene sources are used one by one in new varieties, the arms race against the pathogen will remain very difficult. There is a serious danger of burning down important resistance genes in potato one by one, if they are introduced as single genes. For stacking several effective resistance genes, molecular information is required in the selection process. The EU Directive for organic agriculture states that organic products should be produced without genetically modified organisms or derived products. Unfortunately organic farming is persisting to go on with obstructing the use of molecular information such as marker-assisted breeding and cisgenesis in traditional breeding of new varieties (Lammerts van Bueren et al. 2007). This hampers the development of durably resistant cultivars tremendously.

New approaches are needed in plant breeding to solve problems like potato late blight, black sigatoka in banana and apple scab. The restrictions of introgression breeding described above are common in vegetatively propagated, heterozygous (polyploid) crops (Jacobsen and Schouten 2007a). Crosses disturb the combination of useful traits and require more and more seedlings to select new varieties with sufficient amounts of combined useful traits.

Another way of breeding is improving existing varieties. For vegetative, heterozygous crops, there are two ways of improvement of existing varieties with a long history of safe use, i.e. (induced) mutation breeding and genetic modification. Mutation breeding is still popular in vegetatively propagated ornamentals that are heterozygous like rose, *Alstroemeria*, *Chrysanthemum* but also in fruit trees like apple and peach (Ahloowalia et al. 2004). Main reason for improvement is not always the solution of problems but alteration of the phenotype with added value in the market. In potato, mutation breeding has been mainly restricted to altered traits like tuber skin colour and other tuber related traits.

The second possibility for improving existing varieties is genetic modification. With a restricted number of large crops like soybean, maize and cotton experience has been obtained. In the near future not only transgenes but also cisgenes and intragenes (Rommens et al. 2007) will become available. All these gene types can solve problems in the short run and they all need serious consideration as will be discussed below.

Transgenes, Intragenes and Cisgenes

Transgenes are the oldest type of molecularly isolated genes available for GM plant breeding. They originate mainly from viruses and bacteria, but also from other sources of non-crossable plant species. They are coding for selection markers for the transformation process such as antibiotic and herbicide resistance, and for agricultural traits such as resistance to herbicides, insects and viruses.

Intragenes (Rommens et al. 2007) are composed of genetic elements originating from the crop species itself or from crossable plant species. Genetic elements are, e.g., promoters, coding regions, and DNA sequences that are similar to T-DNA borders from *Agrobacterium tumefaciens*. These elements can originate from different genes and loci. For example, promoters can be chosen to alter the expression of a native gene. Intragenes are used in vectors from which the sequences of the L- and R-borders are originating from plant-DNA. Rommens et al. (2007) have proposed and argued that also this type of gene technology should be cleared through the regulatory process in a timely and cost-effective manner.

The source of a cisgene and an intragene is the same, i.e. the recipient species itself or a crossable species. The main difference between a cisgene and an intragene is the composition. A cisgene is an existing natural gene with its native promoter and terminator. For plant breeding, a cisgene belongs to the existing gene pool of traditional plant breeding. Breeding with this gene source is regulated in breeders' rights and annex regulations. An intragene is commonly a hybrid gene. Usually it is composed of the full or a partial coding part (RNAi) of a natural gene, frequently combined with another promoter and/or terminator from a gene of the same species or a crossable species. A well known special case is RNAi for silencing a gene or a number of related genes. It mimics loss of function mutations (Rommens et al. 2006; Heiligser et al. 2006). Intragenes are not new to the breeders' gene pool as far as functional parts of genes are concerned. However, it is new for the breeders' gene pool if one looks at the level of functional and natural genes. The reasoning in this paper is restricted to transgenes and cisgenes.

GM Breeding Using Transgenes

As indicated above, breeding with transgenes is the oldest way of GM breeding, and is using genes from new gene sources like viruses, bacteria and non-crossable plant species. It is also using synthetic or hybrid genes consisting of gene parts from crossable and non-crossable sources. For plant breeding, all these genes are new and GMO regulations have been developed to safeguard introduction of GM varieties into the environment and onto the market. The use of GM plants has been very successful for some major crops like maize, soybean and cotton in the USA and an increasing number of other places in the world. Main target genes have been restricted to Bt (*Bacillus thuringiensis*) coding for insect resistance, and herbicide tolerance. In 2007, commercial GM crops covered over 114 million hectares worldwide without significant biosafety problems due to the genetic modification itself (http://www.prweb.com/printer.php?prid=843044). However, in Europe, opposition against GM crops is persisting and strong. The main reason is the integration of the chemical industry with the seed industry. The public sector is not fond of this type of cross selling between varieties and agro-chemicals. The NGO lobby against the use of GM technology has been highly underestimated by the industry. However, this opposition has stimulated the development of very strict GMO regulations and a very strict application of this regulation. This makes approval of GM crops very expensive (Jacobsen and Schouten 2007b). In practice these requirements can only be fulfilled in large crops by multinationals. In this sense, it has to be said that the current regulations like Directive 2001/18/EC are only in favour of large companies with enough money while costs of development and approval are later compensated for in the growers' seed price. This means that the current strict regulation functions as a new protection for the large companies (Jacobsen and Schouten 2007b). For GM crops, the smaller companies are out of business or have shifted this type of work out of Europe.

The potato market is large but based on the use of many different varieties. Therefore, the present expensive approval of GM potatoes is hampering the development of GM potato varieties. The costs have to be paid back from a large acreage or the added value has to be significant. For potato it means that GM breeding with transgenes is usually not profitable. However, in starch potato, it is expected that varieties with amylose free starch can provide sufficient added value. Therefore, companies like BASF (http://www.corporate.basf.com/en/stories/loesungen/amflora/start.htm) are using RNAi for the development of amylose free varieties since a long time. Successful release of GM-varieties with altered starch is until now prohibited by the European Commission.

Cisgenesis and Food Safety

The use of cisgenes is a real new alternative for traditional and for GM- breeding, particularly in vegetative crops. Cisgenes belong to the gene pool of traditional breeding. With respect of marker-free transformation, cisgenesis is a realistic option, not only in generative crops but also in vegetative crops such as potato and apple (McKnight et al. 1987; Vetten et al. 2003; Schaart et al. 2004; Yu et al. 2006). In all these crops "clean" transformation is possible without leaving a selection marker gene behind, such as genes for antibiotic or herbicide resistance. For these crops agriculturally important cisgenes are becoming more and more available from specific research programmes but also from sequencing of whole genomes. In other words, the almost ideal way of "clean" introgression breeding with insertion of only the target gene is feasible now (Table 1). Instead of intensive pre-breeding for introgression of alien target genes and removal of many disturbing neighbouring alien alleles from the wild breeding parent, a targeted gene cloning strategy followed by transformation can be developed for isolating the useful alleles coding for, e.g., broad spectrum disease resistances or for quality traits such as yellow flesh in potato and red flesh in apple. The yellow flesh *Y* gene itself or the *Or* allele present in *S. phureja* for orange flesh colour are available for improving existing white fleshed varieties by metabolic engineering which is of importance in poor areas in order to improve vitamin A content, as earlier described for golden rice (van Eck 2007).

Currently, in the Netherlands two research programmes are ongoing in order to introduce cisgenesis into breeding practice.

1. The apple scab resistance project is isolating different resistance genes, and introduces these natural apple genes into existing elite varieties for obtaining durable polygenic resistance. In the past, introgression of scab resistance from *Malus floribunda* took 50 years before the first resistant variety with a good fruit quality was obtained. Main bottlenecks were the generation time of at least 6 years and linkage drag problems. However, after introduction of a scab resistant variety, this resistance is being overcome within several years in different orchards (Parisi et al. 1993; Belfanti et al. 2004). In this arms race another cycle of introgression breeding with new resistance genes has been needed. The new cisgenic approach does not need introgression breeding and stacking of resistance genes by expensive pre-breeding with wild material. However, resistance breeding using cisgenesis is the new pre-breeding approach, starting with isolation of new natural, broad spectrum, resistance genes, followed by direct, stacked, introduction via cisgenesis into existing elite varieties (Schouten et al. 2006b). Also the *MdMYB10* gene for red fleshed apples is being introduced into elite cultivars, enhancing the anti-oxidant capacity of the apples strongly (Espley et al. 2007).
2. Proof of principle in breeding for durable resistance of potato to *P. infestans*. In another contribution in this issue by Haverkort et al. (2008), the societal costs and the cisgenic approach have been described. It is based on the isolation of different classes of broad spectrum *R*-genes recognized by the use of isolated *Avr*-genes, present in the different isolates of this pathogen, and the combined application of the *R*-genes in existing and new varieties. This approach has to

replace the common breeding for polygenic resistance based on introgression breeding. One of the biggest outcomes of the approach with cloned *R*- and *Avr*-genes is the fact that homologous *R*-genes with (almost) the same resistance spectrum can be found in different wild species (E.A.G. van der Vossen, not published). This is saving traditional and cisgenic resistance breeding for the danger of working with the same *R*-gene, despite completely different resistance sources. The isolation of several broad spectrum *R*-genes with their cross reacting *Avr*-genes is enabling testing of their individual biological function after stacking of them by cisgenesis or even by introgression breeding. The disadvantage of disturbing linkage drag in multiple-step stacking of broad spectrum *R*-genes is not found in the one step approach of cisgenic resistance breeding (Jacobsen 2007). In cisgenesis, pre-breeding is altered into gene cloning. Stacking of broad spectrum *R*-genes by cisgenes in existing varieties with a history of safe use is an additional strong recommendation. These improved varieties also can be used as breeding parents for developing new varieties.

Generally, it can be said that cisgenesis is replacing more complicated introgression and induced translocation approaches and that it can be used for all dominantly inheriting traits.

Theoretical Drawbacks

Unknown insertion site A frequently mentioned drawback is the fact that cisgenes will be inserted at unknown places of the plant genome, which could bring unforeseen risks. The phenomenon of random insertion of pieces of alien DNA into the plant genome occurs also in traditional plant breeding. The most prominent examples are induced translocation breeding in wheat (Friebe et al. 1996). Wheat varieties from induced translocation breeding have been obtained during a long time without additional risks. There is a long history of safe use of these varieties. Wheat is an allopolyploid crop. Since the 1950s disease resistance genes from wild grasses are being introgressed into wheat. The interspecific cross between a diploid wild species and allopolyploid wheat is successful including the back cross procedure with the wheat plant itself. However, because of lack of homoeologous chromosome pairing, introgression of the resistance gene into the wheat genome by meiotic recombination is prohibited (Table 1). The end product is an addition line with an extra alien chromosome containing the resistance gene (Friebe et al. 1996). There is an extra step of induced translocation breeding using irradiation needed to introduce this resistance gene into the wheat genome. DNA breakages, induced by irradiation, are repaired. During this repair, at a very low frequency, small pieces of alien DNA with the resistance gene are inserted into the wheat genome. The result is a resistant wheat plant with an additional piece of alien chromosome randomly inserted into the wheat genome. The selected resistance gene is surrounded by many other alleles from the donor species, potentially causing linkage drag problems. This reduced agricultural fitness may be solved by compensation breeding because of lack of meiotic recombination in the inserted alien piece of DNA. The main message is that in comparison with cisgenesis insertion by induced translocation is not restricted to one or two genes but to a whole piece of alien DNA containing hundreds to thousands of alleles. This type of wheat varieties never led to additional risks.

Another example of gene insertion is the naturally occurring transposon activity in many plant species (Greco et al. 2001). The most well known crop plant with this phenomenon is maize. The active transposons are excised from certain places and inserted at other places in the genome. This randomly occurring phenomenon is even observed in existing varieties. Possibly occurring risks are dealt with during the existing breeding process. The most well known transposon based mutation used in practice is *waxy* starch in maize (Nelson 1968).

Mutation at insertion site Another theoretical drawback that is mentioned regarding cisgenesis is the mutation made at the insertion site of the cisgene and the unexpected accompanying phenotypic changes that might result from this. We just have seen that induced translocations and especially naturally occurring transposons can cause mutations in the crop genome, which even can be used. Usually, mutations are inherited recessively and represent loss of function. These mutations have never been accompanied with additional risks that were not covered by the breeding selection process. This is also true for the over 2,700 varieties which have been obtained in mutation breeding by using induced or spontaneously occurring mutations (Ahloowalia et al. 2004). Many of these varieties are the result of mutation induction by irradiation. The dose rate used in this method brings relatively high amounts of genetic damage which is not restricted to the target gene. However, the repair mechanisms seem to be efficient and the buffering capacity of the plant genome seems to be so high that at the end varieties obtained in this way are safe in use. At the moment, of more than 175 plant species, including rice, maize, wheat, barley, cotton, sunflower, apple, banana and many other crops, mutant varieties have been released and grown on millions of hectares worldwide (Ahloowalia et al. 2004). Many of these varieties are used at a large scale. Despite the large scale cultivation and consumption of products from varieties obtained from mutation breeding, it has never been accompanied with food safety problems or other safety issues. Plants selected in mutation breeding are always tested for undesired traits before varieties are market released. This selection process is also applied in cross breeding programmes or after genetic modification.

The presently known GM-varieties do show relatively few negative side effects although they may occur if they are marketed too quickly. The most prominent negative side effect expected is lower yield in comparison with the original variety (Heeres et al. 2002). However, sometimes higher yields (up to 5% increase) are observed (P. Heeres, pers. communication). Careful selection of the most optimal GM-plant can maintain yield at 100% or even higher. In the past, other problems occurred with the slow ripening FlavrSavr GM-tomato (Redenbaugh 1992). It is known that in this case not all selection steps needed for a new variety were covered well, so that not all traits in the whole chain until the market were tested well. These novice mistakes have been made by persons who were too closely connected to commercial interests and not connected with the selection process. These faults are quickly punished by the market itself. It is also known in mutation breeding that the improved variety sometimes is missing some other important trait or has too low yield. Such a variety will be replaced by another variety if available. This is the power of competition within a crop and between varieties of different companies. This highly appreciated competition is at this moment influenced negatively by the authorities because of a too stringent application of GM-directives which is only in favour of a very few, large companies.

Food safety An important advantage of cisgenesis is food safety. The use of wild *Solanum* species as source of genetic variation is bringing back different kinds of glycoalkaloids that have been removed during the breeding process in the past (van Gelder 1989). The use of wild species as new source of genetic variation is accompanied by the re-introduction of these compounds. These have to be removed again by means of back crosses during the introgression process of *R*-genes. Before release for food safety, new varieties have, therefore, always to be tested for the amount of glycoalkaloids that has to be below a certain threshold in the potato tuber. Existing varieties with a long history of safe use are the best source for the application of cisgenesis. It is expected that the cisgenic approach will not increase the existing glycoalkaloid content and that no new types of glycoalkaloids will appear. These expectations are important and can easily be tested in tubers of cisgenic potato plants.

Genetic Modification Regulation and Public Resistance

Gene transfer by means of cisgenesis is because of the process classified as genetic modification and amenable to the EU Directive 2001/11/EC (Anonymous 2001). However, cisgenic plants contain only genes that also can be introduced through traditional breeding procedures. In addition, cisgenic plants are as safe as or even safer than traditionally bred plants or plants obtained by means of induced mutation breeding. However, the approval for growth and market release of cisgenic varieties is expected to be as slow and expensive as with GM-plants containing transgenes. Main reason for that is the fact that the GM-regulation has been designed for transgenic plants and not for cisgenic plants (Schouten et al. 2006a). In this sense no classification is found in the present regulation for the cisgenic situation.

The regulation for GMOs has been based on the processes of recombinant DNA technology in living organisms such as bacteria, viruses, plants, animals and human beings and not primarily on the gene source. In the 1970s, the scientific world made a moratorium for these new techniques in order to prevent risks, specifically for individual researchers in the laboratory. Also labour unions were at that time actively involved in order to come to safety procedures. These laboratory rules have worked well. Until today, keeping these rules is the domain of the biological safety officer within a company or public institution. In the 1970s, the public debate about laboratory safety was intensive but relatively short. The same happened to the contained application of GM microorganisms to produce, for example, insulin, rennet or enzymes in washing powder. The contained use of GM microorganisms, which is normal in microbial production plants, is the main reason for application of microbial GM-products without a lot of public debate. In several countries, products obtained from GM-bacteria with self-cloned genes are free in contained use and in several countries products of it are free of labelling. In the Netherlands, there is a "not unless" rule for modification of animals, whereas application of recombinant-DNA in human is more and more shifting into the status of "a conditioned yes" as long as the technique is safe and the gene can not be inherited.

In the early 1980s with the first field experiments with GM crops there was a relatively low level of opposition. Small pressure groups were against and active but they did not have the sympathy of society. This image changed first in the UK where potential side effects in GM-potato expressing the *Galanthus nivalis* lectin (Ewen and Pusztai 1999) in animal trials were not treated well by industry. The opinion of society gradually became more negative when, in addition, it turned out that the first GM crop appeared to be herbicide tolerant soybean. The connection between seed business and chemical business activated many elements in society to be against GM crops. All kinds of problems were invented for which society proved receptive.

The power of NGOs was highly underestimated by industry and public authorities. The problems were even not taken seriously. GM plants were presented as a perfect product without disadvantages. Inquiries were avoided and answers were initially not given. Most inquiries were focused on the gene sources used. Genes from microorganisms which were not earlier present in the plant genome could enter and could spread into the environment in an uncontrolled way through seed and pollen grains. Another important problem was the use of antibiotic resistance genes that could lead to reduced effect of antibiotics in human healthcare. The third problem was the undesired link between chemical industry and the seed industry by the production of herbicide tolerant crop plants which is not decreasing the use of agro-chemicals but stimulating it. These three problems are removed in the cisgenic approach.

Definition of Genetic Modification

In the past, the different developments in plant biotechnology have brought a broad definition of genetic modification. The EU Directive 2001/18/EC (Anonymous 2001) defines a GMO as an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. This broad definition encloses all induced mutations, but also protoplast fusions. Because of common practice with certain common techniques, two Annexes 1A and 1B with exceptions have been introduced. In Annex 1B mutation induction and protoplast fusion between crossable plant species are, because of the techniques, indicated as GM plants but, because of the genetic content, exempted from the regulation. Although varieties from induced mutations are GM products, in practice they are not treated like that and not labelled because of the exemption. Such varieties are also commonly used in organic agriculture. If we consider the already accepted exceptions as exemplified in Annex 1A and 1B, it makes sense to add cisgenesis of plants also to Annex 1B. This would mean that because of the technique, the cisgenic plants obtained are genetically modified but because of the genetic content they are exempted as is the case with whole genomes in protoplast fusion between crossable species (Table 1). In cisgenesis only one or a few genes are involved instead of a whole genome of the breeder's gene pool. Exempting cisgenic plants from the regulation, would pave the path for cisgenesis in practical breeding. It is clear that cisgenesis is safer than traditional plant breeding, including mutation breeding.

Another major advantage of exemption of cisgenesis of the EU directive 2001/18/EC would be the new possibility of SMEs to come on board again as well as developing countries. A healthy competition within and between crops, which is not disturbed by monopolies anymore, is the best insurance for sufficient agro-biodiversity. It is also clear that cisgenic crops are stimulating development of improved varieties in developing countries, making them less dependent on the presently applied inefficient introgression- and translocation methods.

Derogation the First Step?

The process to come to exemption of cisgenesis could be made step by step (Table 2). A first proposed step could be to apply for derogation of cisgenic potato and/or apple or more precisely for a crop-gene combination.

Derogation is in the EU a common way of exempting rules for specific cases temporally or permanently. We advocate clearing the way for safe applications. Building up experience in this way could pave the path for exemption.

Conclusion

Cisgenesis adds no new risks in comparison with traditional plant breeding and mutation breeding. In the examples with apple-scab and potato-late blight interaction, it is indicated that safety, environment and durability of resistance are improved by cisgenesis both in the short and long run. Less stringent oversight on cisgenic crops, in view of their safety, would promote, in addition, valorisation of the growing knowledge on plant genes, not only by multinationals, but especially by small and medium-sized enterprises, research institutes and companies in developing countries. This would increase agro-biodiversity at the variety level and would break

Table 2 Proposed step by step approach via specific derogation to come to general exemption of cisgenesis in the EU Directive 2001/18/EC

Step
Step 1
Derogation: crop specific and gene type specific.
The genes belong to the breeders' gene pool.
Step 2
No disturbing observations on selected cisgenic plants during specific monitoring and general surveillance in the field.
Step 3
Exemption of cisgenes from the whole breeders' gene pool, by adding cisgenesis of plants to Annex 1B of the Directive

the present monopoly of multinationals in GM crops. We hope that cisgenesis will get a chance in the step by step approach via crop specific and/or gene specific derogation.

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