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Cisgenesis: an important sub-invention for traditional plant breeding companies

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Abstract Modern plant breeding is highly dependent on new technologies to master future problems. More traits have to be combined, frequently originating from wild species. Traditional breeding is connected with linkage drag problems. The crop plant itself and its crossable species represent the traditional breeders gene pool. GM-breeding is a new way of improving existing varieties. Transgenes originate from non-crossable species and are representing a new gene pool. For release of GM-plants into the environment and onto the market in Europe Directive 2001/18/EC has been developed, primarily based on GM-technology and not on gene source. In society, opposition against GM crops is complicating the implementation of GM crops. In this paper, it is shown that not only transgenes, representing a new gene pool but also cisgenes and intragenes are available, representing the breeders gene pool. Cisgenes are natural genes and intragenes are composed of functional parts of natural genes from the crop plant itself or from crossable species. Cisgenesis is the combined use of only cisgenes with marker-free transformation, mimicking linkage drag free introgression breeding in one step. Therefore, cisgenesis is a new sub-invention in the traditional breeding field and indicates the need for reconsideration of GM Directives. Inventions are frequently containing not only hardware elements, but also software and orgware elements. For cisgenesis it is foreseen that the technical (hardware) and bioinformatic (software) elements will develop smoothly, but that implementation in society is highly dependent on acceptance and regulations (orgware). It could be made in a step by step approach by specific crop-gene derogations from the Directive, followed by adding cisgenesis to annex 1b of Directive 2001/18/EC for exemption. At present GM crops can only be introduced by large companies. An open innovation approach for cisgenesis by public private partnership including traditional SMEs has been discussed. Cisgenesis has been exemplified for resistance breeding of potato to *Phytophthtora infestans*.

Keywords Cisgenesis · GM-regulations · Linkage drag · Derogation · Exemption · Inventions

Introduction

Improvement of varieties is highly needed if we look to the new challenges of plant breeding such as global

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Table 1 Important ways of introducing useful genetic variation into crop plants

Way of breeding	Linkage drag	Solution
Intraspecific crosses		
Crosses within the crop species	A few genetically linked unfavorable alleles may be present	Repeated backcrosses
Interspecific crosses with wild species		
Introgression	Many alien alleles; some are unfavorable	Repeated backcrosses
Induced translocation	Many alien alleles; some are unfavorable	Repeated backcrosses and compensation breeding
Genetic modification		
Transgenesis	Transgenic selection markers, and backbone from vector	Marker free transformation, and selection for backbone free plants
Intragenesis	Backbone from vector	Selection for backbone free plants
Cisgenesis	Backbone from vector	Selection for backbone free plants

They all are with linkage drag problems at (multiple) gene or trait level in traditional as well as GM-plant breeding. However, solutions are different for cross breeding and GM-breeding

warming, population growth, (a)biotic stress, decreasing land resources and increasing demands for high food quality (Jacobsen and Karaba 2008). It means that many traits have to be combined in new varieties. The basis for that is traditional plant breeding consisting of searching for appropriate genetic variation and combining novel traits from wild germplasm with existing traits by crossing and selection. More often genetic variation is found not only within the own species but also in related species, and used by means of introgression or induced translocation breeding (Table 1). Genetic variation can also be induced by mutations (Chahal and Gosal 2002).

Many new tools for plant breeding have been found in plant biotechnology, such as in vitro techniques like embryo rescue, protoplast fusion, vegetative propagation, and transformation. Further genomics has been introduced, including genetic mapping, marker assisted selection, whole genome sequencing and gene isolation. All these new tools are very important for present and future plant breeding.

The combination of gene isolation and transformation is crucial for broadening genetic variation even outside the traditional breeders' gene pool. Nowadays, genetic modification (GM) is very important to improve existing varieties with single or a few new traits like herbicide and/or pest resistance. In 2007, 114 million hectares of GM crops were grown worldwide (Jacobsen and Schouten 2008). However, the seed of almost all these varieties have been produced and traded by a few companies. These companies have been positioned because of strict GMO regulations and the availability of patents, partly obtained by public money, on an exclusive base. In the USA, the Bayh Dole Act (Anonymus 1980) allowed universities to patent inventions including (exclusive) licensing. Meanwhile, the same kind of academic institutions are allowed to do so in many other countries of the world.

The invention of broadening genetic variation by genetic modification of organisms started 30 years ago and the researchers realized both the potential benefits and the potential risks of this new possibility. Since in the 1970s and 1980s of previous century in many countries GMO-regulations were designed, for contained use, introduction into the environment, and for market release of GMOs or products derived from GMOs. In this field, the Cartagena Biosafety Protocol (BSP) (Anonymus 2000) is the world wide multinational treaty and in Europe Directive 2001/18/EC (Anonymus 2001). Updating of this Directive is an ongoing process. For plants, GMOs are based on two factors: (1) the technology used, such as *Agrobacterium* or particle gun mediated gene transfer and protoplast fusion. These techniques are determining whether the product is GM or not and (2) the genetic source, which is determining whether a GMO will be treated according the Directive 2001/18/EC or BSP, or whether it is excepted or exempted from the regulation (Jacobsen and Schouten 2007).

In Europe, implementation of the Directive is, under pressure of NGOs, very strict and very expensive. It stimulates many repeated animal trials which are very costly but mostly superfluous and meaningless. Many of these animal experiments do not add anything to human safety (Schouten et al. 2006a, b).

In practice, this Directive and the strict implementation of it is frustrating GM plant breeding by the private sector, including SMEs in Europe and developing countries (Jacobsen and Schouten 2008).

In the meantime two new developments are observed, which have the potential to influence future GM regulation:

(1) Marker free transformation without linkage drag of antibiotic resistance (Table 1) is routine for many generatively propagated crops but also for vegetatively propagated crops.
(2) Modern genomics via advanced gene cloning techniques, whole genome sequencing and bioinformatics. Both are stimulating molecular isolation and insertion of many plant own genes, called cisgenes, allowing variety improvement with only natural alleles from the breeders gene pool (Schouten et al. 2006a, b).

Nowadays innovations in industry, including SMEs in plant breeding, are highly stimulated with public money by public private partnerships (PPP). In contrast to past developments in plant biotechnology, open innovations with new GMO sub-inventions, such as cisgenesis, is for the breeding industry, which is mainly consisting of SMEs, highly attractive. Most important bottle neck to be solved is whether this approach has to be classified at the same level as transgenesis within the present GMO regulations or not. In this paper it is proposed that cisgenesis with natural genes from the breeders gene pool should be exempted from GM regulations, starting with derogation for specific crop-gene combinations in a step-by-step approach. Above mentioned developments have been discussed and illustrated by sustainable resistance breeding of potato.

The role of inventions in agriculture

Inventions on products and production processes are defined as radical new ideas, perspectives and technologies that hold the potential to trigger a step change in production and development. They are ideas that may require further elaboration, further development or further analysis to assess their true potential before they can become the input for innovations. Because of their potential, inventions are expected to be implemented into different sub-innovations. Inventions are radically new, have no or limited history and hold the potential for a breakthrough and a step change in transition trajectories.

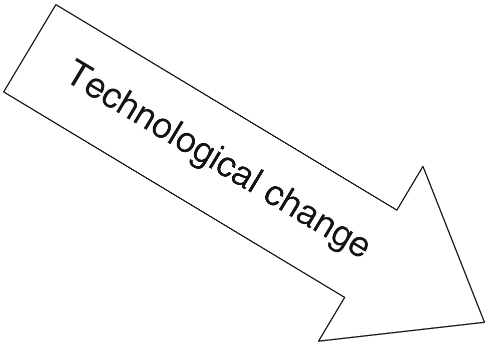
Nowadays most inventions do imply hardware, software and orgware elements for commercialization, diffusion and adoption (Table 2). It has to ensure that markets are reflecting societal preferences. Governmental regulations are needed to keep potential or theoretical risks of technological innovations at an acceptable level (MacGill 2007).

Worldwide, the importance of inventions is at a regular base discussed in all kind of settings. In 2003, for example, an international workshop on “Invention and innovation for sustainable development” was organized by the Lemelson-MIT program in London. In this workshop the importance of inventions as well as innovations in modern economies but also in less developed countries was clearly discussed and indicated (Anonymus 2003).

In the past, inventions have been tremendously important in the creation of the current agro-food knowledge infrastructure and will remain utmost important in the future when an altered agro-food knowledge infrastructure, more based on sustainability, will be developed. The difference is that more and more inventions and the implementation of it will not follow the old linear process of fundamental research (university), strategic research (research institute), applied research including extension (applied institutes), commercialization and diffusion but that today the processes are more often complex and non-linear (Leeuwis 2006; Anonymus 2003). It is more a complex interaction between human creativity, technology, entrepreneurship, society, regulations and the marketplace. In an ideal situation, iteration between these factors must happen before an invention has significant impact (Anonymus 2003). It is important to realize that in the past the prediction of Malthus (1798), that the world could not support an exponentially increasing population, has not become true yet. The main reason was the enormous impact of implemented new inventions and their related

Table 2 The place where inventions are created and the way they go by technological innovations in agriculture

Technological innovation	Technology		
	Hardware	Software	orgware
Fundamental research			
Basic inventions			
Strategic research (basic/sub) inventions			
Applied research			
Sub-inventions			
Commercialisation			
Diffusion/adoption			



In addition to technical hardware, orgware and software elements are increasingly of importance

innovations and transitions on a number of crucial economic sectors such as agriculture. It is, therefore, without doubt that new inventions will also be crucial to stimulate and enable sustainable development in modern agriculture, food production and agribusiness. Nowadays, inventions for sustainable development have to be seen in the complex interaction between human creativity, technology, sustainability and marketplace and not only technically.

Another issue related to inventions is intellectual property rights (IPR) by patentable inventions and their licensing to private companies. In the USA, the Bayh-Dole Act (Anonymus 1980), gave universities and institutes the possibility of ownership to discoveries in place of being freely available in the public domain (Zilberman et al. 2001). The possibility of licensing has greatly improved transfer of technology and application of it in, for example, the pharmaceutic and recently also in the agricultural sector, but there are also drawbacks. A major question is whether today all patentable inventions have to be treated in the free way as stimulated by Bayh and Dole or that there are also alternative ways to steer common interests more broadly, for example, by stimulating other ways of licensing, excluding exclusivity. With respect of stimulating sustainable agriculture with public money common interest should be used as main criterion for availability of inventions in this field.

It is important to recognize which inventions are serving, for example, sustainability but also which element of an invention (hardware, software or orgware, Table 2) needs specific input for a breakthrough. In the past (technical) inventions were worked out and implemented in isolation within sectors but without much input from society. Such societal input came in case of disasters often too late. This is the main reason that today sustainable agriculture is such a dominant issue.

Implementation of inventions, promoting the green revolution, are important examples from the past (Borlaug 2000). These inventions were not patented but they had a major impact at a global scale. The short straw wheat, rice and barley varieties enabled the development of modern agriculture in cereals. The altered allocation of carbohydrates among seeds and stem tripled the grain production since 1960 (Borlaug 2000). It stimulated applied research in countries like India to introduce short straw as a new trait in locally adapted varieties.

Another important invention in this field was the concept of hybrid varieties. This was firstly practiced in maize in the USA and later in other cereal crops such as pearl millet and rice (Chahal and Gosal 2002). In 2006, it was 50 years ago that the first hybrid variety was introduced into Europe starting in Hungary (Marton et al. 2003). Hybrid varieties increased yield to sixfold and in addition they stabilized yield. In this field an additional positive factor connected with the hybrid concept was the breeders' seed protection by natural breeders rights (Chahal and Gosal 2002). This aspect of natural breeders' rights protection in hybrid varieties has been the additional trigger of hybrid breeding and seed trading in many important crops such as maize, rice, tomato, and sweet pepper. During the years it resulted in a number of strong seed companies in vegetable and agricultural crops worldwide.

The most recent invention on hybrid variety breeding is reverse breeding by (re)synthesizing a particular hybrid plant with two selected non-GMO doubled haploids obtained from the same hybrid plant in which meiotic recombination was blocked by gene silencing (Dirks et al. 2003). For reverse breeding, the step in between with silencing of meiotic recombination can only be realised with GM-pants. However, the end product after crossing two selected doubled haploids is a non-GM hybrid variety. In this invention the orgware element on GMO interpretation has still to be solved. The interpretation by some lawyers using the GMO Directive 2001/18/EC is, that non-GM sexual offspring of a GM-plant, should still be considered as GM. Recently, a discussion group has started in the EU to solve this type of interpretation problems for a number of new techniques, including cisgenesis (unpublished).

Nowadays, important inventions in green biotechnology are based on in vitro techniques and omics. GM is an emerging field and can be indicated as next step in green revolution by gene revolution as well as gene evolution. The inventions in this field are more complex in understanding, protection and implementation, but they are crucial for stimulating sustainable development in modern agriculture, agribusiness and agro-ecology. In this field, the rate of inventions is at the moment much higher than the innovations getting realized. It is hypothesized that the main reason seems to be the fact that “natural and biological sciences have made larger gains in understanding natural and biological processes than scientists working in the social sciences have progressed in their efforts to produce knowledge about social behavior” (Norman 2005). It is important that more specific knowledge will be developed in order to know better how to improve implementation of inventions such as GM and what role IPR should play in the future in connection with plant breeders rights (Norman 2005). It is clear that coexistence problems between varieties with only breeders rights, including breeders exemption, and varieties with breeders and patent rights, without breeders exemption, have to be solved in order to safe the open innovation climate needed for breeding future improved varieties.

Gene evolution by cisgenesis in addition to gene revolution by transgenesis

The gene revolution in GM-plant breeding is focused on transfer of transgenes by genetic modification. For plant breeding, transgenes are representing gene revolution in a new gene pool (Table 3). Recent developments in gene isolation and the technique of genetic modification have changed the landscape in this field. Modern gene isolation techniques enables the isolation of more and more cisgenes (Table 3). These genes are representing natural gene evolution (Jacobsen and Schouten 2007).

Table 3 Description of transgenes, intragenes and cisgenes in plants

Type of gene	Definition	Plant with this gene regarded as modified organism according to	
		Cartagena Protocol	2001/18/EC
Transgene	A transgene is a (synthetic) gene with some or all regulatory sequences and coding sequences from donors other than crossable plants, including micro-organisms and animals. These genes belong to a new gene pool for plant breeding	Yes	Yes
Intragene	An intragene is a gene comprising of natural functional elements, such as coding part, promoter and terminator originating from different genes from the crop plant itself or from crossable species. All natural gene elements belong to the traditional breeders gene pool	Yes	Yes
Cisgene	A cisgene is an existing natural gene from the crop plant itself or from crossable species. It contains its native promoter and terminator. The gene belongs to the traditional breeders gene pool and is the already existing result of natural evolution.	No	Yes

Also it is indicated whether plants containing these genes are regarded as modified organism according to the Cartagena Protocol on Biosafety (Anonymus 2000) and according to European Directive 2001/18/EC (Anonymus 2001)

Table 4 The inventions on trans- and cis-genesis can be subdivided into hardware-, software- and orgware elements for commercialization and diffusion

Elements of inventions	Cisgenesis	Transgenesis
Hardware	Marker free transformation with cisgenes only	Transformation with transgenic marker genes and (foreign) target genes
Software	Bioinformatics and genomics of natural genes from own crop plant or crossable species	Bioinformatics and genomics of gene (elements) of all non-crossable organisms
Orgware	Exemption of directive 2001/18/EC starting with crop-gene specific derogation	Directive 2001/18/EC and its complicated implementation

The improved marker-free transformation techniques facilitate cisgenesis in many seed propagated and vegetatively propagated crops (Vetten et al. 2003; Schaart et al. 2004; Yu et al. 2006). Cisgenesis is mimicking introduction of single traits by traditional crosses and backcrosses within the species or between species by introgression or induced translocation breeding without the disadvantage of linkage drag (Table 1). It enables also stacking of more cisgenes in a simple way. In comparison to the traditional breeding methods it is a one step insertion approach without linkage drag instead of the multiple steps with linkage drag in traditional breeding. Another important aspect in comparison with traditional breeding is the fact that cisgenesis can be used for improving existing varieties. However, this is also true for transgenesis. Because of the gene source and the marker free gene transfer technology, cisgenesis is representing a new sub-invention in this field (Table 4). The invention of cisgenesis is the improvement of germplasm or existing varieties by one step insertion of natural genes or alleles only originating from the breeders gene pool. The big difference between cisgenesis and transgenesis is the gene source representing gene evolution and gene revolution, respectively.

Transgenesis, cisgenesis and intragenics

In addition to cisgenesis and transgenesis, intragenics is promoting another new gene type for GM breeding. In Table 3 simple definitions of these genes are given, including a possible interpretation of their present status of Living Modified Organism (LMO) in the Cartagena Biosafety Protocol (Anonymus 2000) and GMO status in the European Directive 2001/18/EC (Anonymus 2001).

The gene source for intragenes is the same as for cisgenes, i.e., the breeders' gene pool. Difference is that in the case of intragenes the smallest functional part is not the entire natural gene but smaller elements of it such as (parts of) coding sequences, promoters and terminators. Such genetic elements are combined into intragenes (Rommens et al. 2007). Intragenes are inserted in T-DNA containing so-called plant border sequences which are originating from the crop plant itself. It means that T-DNA in intragenics is only consisting of plant DNA. Intragenes like cisgenes are introduced into the plant with marker free transformation systems. Also in the case of intragenics it is proposed that GM-regulations should be changed. According to the existing regulations, the transfer system of intragenes makes also in this case a GMO, however, the gene source and even the source of all genetic elements is belonging to the breeders gene pool. Prominent difference between intragenes and cisgenes is the unit of functionality. In cisgenes, existing natural plant genes with its native promoter and terminator are the primary unit of functionality. This is comparable with the way traditional plant breeding is using existing genetic variation. In intragenes smaller functional genetic elements are fused in new combinations leading, for example, to altered gene expression patterns or gene silencing.

Parts of the biological effects that can be obtained with intragenes are also possible by natural or induced mutations (Heilersig et al. 2006; Rommens et al. 2006). In case of intragenic gene silencing using RNAi, the effect is comparable with loss of function mutations. Loss of function mutations in practice are restricted to one functional gene, and are inherited recessively. In allopolyploids like wheat, there are examples of loss of function mutations simultaneously in three homologous genes present in the three homoeologous genomes A, B and D, such as those coding for granule bound starch synthase (GBSS). In the past, they have been detected biochemically as single or double mutations in different Chinese wheat sources (Yamamori et al. 1994). Sexual combination of all three recessive GBSS alleles in one genotype resulted in the recessive waxy starch type in wheat kernels. RNAi is not only able to silence one functional gene but also several members of the same gene family. Another big difference is that gene silencing is inherited as a dominant trait. This is especially important for vegetatively propagated complex crops like apple, banana and potato. RNAi silencing can replace loss of function mutations very efficiently, even in existing varieties like amylose free potato (Heiligser et al. 2006). In our view, gene silencing is mimicking loss of function mutations and should be regulated less strictly in Directive 2001/18/EC (Anonymus 2001).

Another important new possibility of intragenes is alteration of gene expression, for example, in other organs or tissues by using promoters from other genes. The same effect may be obtained by induced mutations if they occur in promoter regions of the target gene. Changes as altered expression spectrum are expected to be inherited dominantly and will be the result of micro-mutations in or around the gene itself, of rearrangements in the genome (Chahal and Gosal 2002) or by altered feed back inhibition (Negrutiu et al. 1984). It means in practice that the biological effect of intragenes is comparable with loss of function mutations or altered expression pattern mutations. In case of cisgenesis, only complete genes are used, including their natural promoter and terminator. Therefore, it can be stated that cisgenesis is closer to classical plant breeding and existing natural variation within the sexual compatibility group, compared to intragenesis.

Cisgenesis and altering of GM-regulations

On regulations of GM-crops, two treaties have been mentioned earlier, i.e., worldwide the Cartagena Protocol on Biosafety and in Europe the Directive 2001/18/EC (Table 5). They implemented different definitions of (1) a living modified organism (LMO)

Table 5 Definitions of living modified organism (LMO) and genetically modified organism (GMO) in the Cartagena Protocol on Biosafety (Anonymus 2000) and in the Directive 2001/18/EC (Anonymus 2001), respectively, and their present applications

Cartagena protocol on biosafety

Definition of LMO: "Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology

"Modern biotechnology" means the application of:

a. *In vitro nucleic acid techniques*, including
    Recombinant deoxyribonucleic acid (DNA)
    Direct injection of nucleic acid into cells or organelles, or
b. *Fusion of cells* beyond the taxonomic family, that overcome natural physiological reproductive recombination barriers and that are not techniques used in traditional breeding and selection;

Directive 2001/18/EC

Definition of GMO: "genetically modified organism" means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination

Techniques not leading to a GM plant (annex 1a, Part 2):
    In vitro-fertilisation
    Polyploidy induction
    Our proposal is adding here in view of consistency with the Cartagena Protocol on Biosafety: Plants that contain no novel combination of genetic material that overcome natural, physiological reproductive recombination barriers.

Techniques leading to a GM plant, but these plants are exempted from the GMO legislation (annex 1b):
    Mutagenesis
    Fusion of cells of crossable plants
    In case annex 1a is not broadened, we propose to add to annex 1b: Plants that contain no novel combination of genetic material that overcome natural, physiological reproductive recombination barriers (e.g., Cisgenesis)

The table focuses on plants

in the Cartagena Protocol or (2) a genetically modified organism (GMO). In the Cartagena Protocol the definition of a LMO is: “living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (Article 3g of the Cartagena Protocol; http://www.cbd.int/biosafety/protocol.shtml). So there are two conditions for classifying a plant as an LMO: (1) the product—it must contain a novel combination of genetic material, and (2) the process—the plant must have been obtained through modern biotechnology. Only in case both conditions have been met, a plant should be considered as a LMO, according to the internationally agreed and signed Cartagena Protocol (Anonymous 2000). In case of cisgenic plants we are of the opinion that there is not a novel combination of genetic material, “that overcomes natural physiological reproductive or recombination barriers” (Article 3i of the Cartagena Protocol). Therefore, our conclusion is that a cisgenic plant is not a LMO. As a consequence, cisgenic plants are excluded from the Cartagena Protocol (oral presentation Piet van der Meer, 2 October 2008, Workshop in The Netherlands entitled “The New GMO Debate; a clash between legislations”).

In Directive 2001/18/EC (Anonymus 2001) only the process of genetic modification is determining whether a product is classified as GMO or not. Therefore, all genetic modification techniques like direct gene transfer but also induced mutations, protoplast fusions and polyploidisations are, according to this definition, resulting in GMOs. It means that in practice the definition of genetic modification is too broad. Therefore, the two annexes 1a and 1b have been introduced in order to overcome these problems (Table 5). It indicates that a phenomenon like polyploidisation, as shown in annex 1a, is not regarded as a technique of genetic modification, and that induced mutations and protoplast fusion between crossable plants are regarded as techniques of genetic modification, but plants developed by means of these techniques are exempted from the GMO regulation (annex 1b). Main reason for this is that in all mentioned cases genetic sources are not exceeding the gene pool of traditional plant breeding. It means that in practice GMO plants can be subdivided into three categories:

(1) *Annex 1a*: Not GMO because of natural occurrence of the phenomenon;
(2) *Annex 1b*: GMO, but exempted from the regulation because of the genetic sources used which are within the gene pool of traditional plant breeding;
(3) Full notification because of the technique applied and the genetic source used which is (partly) belonging to the new gene pool.

Although the European Community and different individual member states have signed the Cartagena Protocol (http://www.cbd.int/biosafety/signinglist.shtml) it is not fully implemented in the EU regulation. Table 5 proposes a solution for cisgenesis by broadening the annex 1b of the Directive. An alternative is broadening annex 1a.

The GMO regulations like Directive 2001/18/EC (Anonymus 2001) have been developed in a time frame that the availability of transgenes was dominating the discussion about GM crops. That made sense, because for gene transfer into the plant, selection genes were needed, which in practice were foreign genes, coding for antibiotic resistance and/or herbicide resistance. These genes usually have originated from microorganisms. Also foreign target genes were introduced, coding for herbicide resistance, insect resistance or virus resistance. Therefore, until now, all GM crop varieties on the market are transgenic.

Reconstructed logic

The dynamics of decision-making at the higher echelons of governments has a long-term history in which aspects like reconstructed logic are important (Yanarella 1975). Reconstructed logic is using reflective cycles. A nice recent example of the need of dynamics and reflective cycles in decision-making is GMO regulation. Time by time regulators are adapting the rules to new developments and insights in science, technology, practice and society. The developments regarding intragenes and cisgenes have shown that the existing gene pool of plant breeders is more and more available for the GM approach. In this paper, a suggestion has been made how to handle trans-, cis- and intra-genes in existing regulations like Directive 2001/18/EC. We have used two baselines being a natural gene or a hybrid gene. It is shown that transgenes can be both, but (part of) their origin is always outside the breeder’s gene pool. Therefore, full notification in the directive is needed. Until now, intragenes have been presented as chimeric genes consisting of functional elements of different genes of the breeder's gene pool. It means that a baseline with functional gene elements is appropriate. It is clear from the reasoning around intragenes that notification should be simplified or even exempted. For cisgenes there is only the baseline of natural functional genes within the conventional gene pool of the plant breeder. Because of the reasoning earlier discussed, exemption should be considered seriously. In genetical terms, cisgenesis is even more safe than several products of traditional breeding because (1) existing varieties with a history of safe use will be improved in which only the inserted cisgenes are new, rather than many more alleles in a new variety obtained by crossing, allele recombination and selection, and (2) only the target gene is introduced without linkage drag. If the gene source is a wild species, no additional checks for possible introduction of toxic compounds are needed which are practiced in the traditional breeding approach in crops like potato.

Proposed exemption of cisgenic plants in a step by step approach via derogation

How to come to updating of directives enabling new exemptions or how to proceed to obtain experience with new developments in the field with cisgenic crops? A frequently used possibility in EU directives is the phenomenon of derogation. In this case it could be the start of a step by step approach (Jacobsen and Schouten 2008) combined with a monitoring system in order to end up finally with exemption in Directive 2001/18/EC.

A derogation is the partial revocation of a law. It differs from dispensation in that it applies to the law and not to the person affected to the law. In terms of EU legislation, a derogation can also imply that (a) member state(s) has opted not to enforce or to recall a specific provision in a treaty, temporally or permanently, due to internal circumstances or new developments. Important examples are (1) Directive 2008/62/EC (Anonymous 2008) in which seed (potatoes) of conservation varieties are derogated for inclusion in the national catalogues of varieties of agricultural plant species as well as for the production and marketing of seed and seed potatoes of those varieties. (2) Derogation to use seed and vegetative propagating material in organic farming if, for example, not sufficient organically produced seed (of that particular variety or crop) is available. (3) The option to deviate from the EU Directive on nitrate for animal manure (Leeuwen et al. 2007). In line with these examples it must be possible also to define and accept derogations for cisgenesis at crop-gene level in order to investigate and monitor this possibility. Potato could be a very interesting first case because of its role in Europe with respect of economic and agricultural importance, absence of crossable relatives in nature, propagation by seed potatoes and not by botanical seed, its restricted level of out-crossing within the crop, the crop rotation cycle and the normal agricultural monitoring system preventing for volunteers and establishment of seed potatoes from seedlings. As GM crop, it has a favorable GM risk assessment profile also if co-existence aspects are included (Dijk 2004). In the coexistence recommendation for GM-potato in the Netherlands, a minimum isolation distance of 3 m with conventional potatoes has been recommended and of 10 m with organic potatoes.

Suggested steps for derogation and general exemption of cisgenes could be (Jacobsen and Schouten 2008):

(1) Application and approval of a crop-gene specific derogation, based on risk assessment.
(2) Phenotypic and molecular selection of backbone free (without vector-DNA from outside the TDNA borders), cisgenic plants in the glasshouse and gauzehouse. Specific monitoring and surveillance of selected plants in extending field experiments.
(3) Exemption of this crop-gene combination in annex 1b of the Directive as first step to general exemption of cisgenesis.
(4) Monitoring of more examples of crop-gene specific derogations, such as apple, followed after positive results by general exemption of these cisgenic plants.
(5) Finally the decision whether cisgenesis of plants in general can be added to annex 1b of the Directive or not.

Cisgenic resistance breeding in the potato-late blight interaction

Vegetatively propagated crops like potato are susceptible for aggressive diseases like *Phytophthora* *infestans*. Longterm traditional resistance breeding against this disease is without sustainable success until now. It means that other ways of breeding are needed to solve this problem. It turns out that for potato a sufficient number of *R*-genes is available in wild species but that stacking of them within one genotype by crossing is connected with major problems like linkage drag and mixing up of the alleles of many desired traits in the offspring plants (Table 1). Therefore, in traditional breeding, it takes too much time to come up with improved resistant varieties (Jacobsen and Schouten 2007). In the past, it has happened too often that the resistance in a new variety has been broken and, in potato, long-term breeding for field resistance stayed without significant success (Haverkort et al. 2008). Introgression breeding in heterozygous crops is complicated and needs a lot of pre-breeding efforts followed by selection for many traits united in one genotype. The most recent positive examples are the new resistant varieties cvs Toluca and Bionica (unpublished). Both varieties are the result of long-term introgression breeding with *S. bulbocastanum* using double bridge crosses with two other species *S. acaule* and *S. phureja* as first step to transfer one or a few strong *Rpi*-genes to cultivated potato (Hermsen and Ramanna 1973). There are strong indications that both varieties contain *Rpi-blb2* (unpublished) and that in between already a few broken late blight isolates have been found (Kessel, pers. Comm.). It indicates again that efficient stacking of *Rpi*-genes is highly needed. The whole procedure of introgression and variety breeding lasted almost 50 years.

At the other hand, we need more insight both into the resistance genes available and into the isolates of the pathogen. The improved cloning techniques such as, map based cloning, allele mining and all the possibilities in between bring cisgenic resistance breeding directly into practice by using not only stacked *Rpi* genes in plants but also the screening with *AVR*-genes (avirulence) from isolates of the pathogen. In potato, at the moment 15 *R*-genes (Table 6) and 5 *AVR*-genes (Vleeshouwers unpublished) have been isolated. All these genes belong to the NB-LRR class of *R*-genes and are members of different homologous clusters of varying sizes. A remarkable observation is that *Rpi* genes from different species can be highly homologous to each other with the same or nearly the same spectrum to a collection of *Phytphthora* isolates. Genes like *Rpi-blb1*, *Rpi-sto1* and *Rpi-pta1* belong to one class (Vleeshouwers et al. 2008) as well as *Rpi-blb3*, *R2*, *R2-like* and *Rpi-abpt* (Lokossou et al. 2009) and *Rpi-vnt1.1*, *Rpi-nrs1* and *Rpi-phu1* (Pel et al. 2009; Jones unpublished) and also *Rpi-3a* and *Rpi-3b* (Vossen unpublished). At this moment, *Rpi* genes belonging to seven different classes and clusters have been isolated. A major challenge that remains is the question how to predict durability of natural *Rpi* genes and how to select and introduce durable combinations of *Rpi* genes into existing and future varieties in the most efficient and sustainable way. We anticipate that more knowledge of effector diversity in relation to *Rpi* genes and *Rpi* alleles could be helpful. The recent discovery of a common RXLR motif in the oomycete AVR effector proteins promises to accelerate the discovery and functional profiling of late blight *Rpi*-alleles and -genes (Vleeshouwers et al. 2008) and of *P. infestans AVR*-alleles and -genes and thus the engineering of durable late

Table 6 Seven different clusters with 15 cloned *Rpi*-genes from *Solanum* species against *Phytophthora infestans*

R-genes	Chr.	Source	References
Rpi-R1	5	*S. demissum*	Ballvora et al. (2002)
Rpi-R2; *-R2-like*; *-abpt*; *-blb3*	4	*S. demissum, S. bulbocastanum,* unknown	Lokossou et al. (2009)
Rpi-R3a; *-R3b*	11	*S. demissum,*	Huang et al. (2005); Unpublished van der Vossen
RB/Rpi-blb1; *-sto1*; *-pta1*	8	*S. bulbocastanum, S.stoloniferum, S. papita*	Song et al. (2003); van der Vossen et al. (2003); Vleeshouwers et al. (2008)
Rpi-blb2	6	*S. bulbocastanum*	van der Vossen et al. (2005)
Rpi-vnt1.1; *-nrs1*	9	*S. venturii*	Pel et al. (2009)
Rpi-mcq1; *-phu*	9	*S. mochiquense*	Unpublished (Jones, TSL)

blight resistant potato varieties in the future. Cloned *Rpi*-genes and -alleles enable stacking of (1) multi-alleles of particular *Rpi*-genes with different specificities and/or (2) multi-genes from different Rpi-classes for sustainable resistance in combination with the use of existing varieties with long-term safe use. In the Netherlands, a proof of principle, called Durable Resistance to *Phytophthora* in potato (DUR-Ph; Haverkort et al. 2008), has been started to investigate: (1) whether sustainable resistance to *Phytophthora infestans* can be obtained by cloning sufficient *Rpi*-genes, and (2) to test cisgenic plants in mixed clones with different *Rpi* genes of the same variety or after stacking *Rpi*-genes in one clone of existing varieties (Haverkort et al. 2008). An important other new tool in this type of research is the availability of more and more *AVR*-genes which are cross reacting with cloned *Rpi* genes in order to estimate whether new *Rpi*-genes are potentially sustainable or not (Vleeshouwers et al. 2008). Testing of additive biological activity of stacked *Rpi* genes could be controlled in cisgenic plants effectively by the HR reaction if complementing *AVR*-genes have been isolated. It means that cisgenic breeding by stacking broad spectrum *Rpi* genes and their complementing *AVR*-genes are part of the new strategy to come to sustainable resistance to *Phytophthora infestans*.

Importance of open invention approaches

Development of GM-crops is at this moment almost the exclusive domain of multinational companies. They do have the infrastructure and financial power to develop improved varieties in large crops like maize, cotton and soybean, and to introduce these onto the market. Implementation problems of GM rules in practice are the main cause that new GM varieties are very expensive. Another obstacle is the weak IP position of small companies and developing countries and the combined protection of GM-varieties by breeders and patent rights. This is excluding GM-varieties as exempted breeding parents for future variety breeding which is normally common practice. Combination of these negative factors will decrease agro-biodiversity at variety level coming years. Worldwide, new GM-varieties will not be based on the variety of genetic backgrounds needed for safeguarding our food security. We still remember the discovery that maize varieties with Texas cytoplasm were cytoplasmic male sterile but also susceptible to toxin of *Helminthosporium maydis* race T (Levings 1990). It resulted in a disaster because of sudden susceptibility of all maize varieties with T-cytoplasm. Open invention approaches for sustainable resistance could partly help to solve these problems. It is important for small and medium sized enterprises and research institutions in public private partnerships (PPP) to share technology and knowledge regarding cisgenesis, for sustainable resistance breeding via stacking of resistance genes. Sharing of knowledge can be practiced at the three different phases of research, development and direct implementation of knowledge for creating improved cisgenic GM-varieties (Table 7). The risks taken in the different phases are entirely different and have to be taken into account when partners are entering such a collaboration. Exemption of cisgenesis could be the new fundament of the conservation of a broad level of agro-biodiversity within a crop like potato. As a first step, resistance of a number of frequently used free varieties can be improved through cisgenesis. Next step is exclusive use of *Rpi*-genes in protected

Table 7 Framework of open innovations in cisgenic breeding for SMEs and research institutes by Public Private Partnership in collaborative cloning and strategic use of cisgenes, technology and (tacit) knowledge

Phase	Activities	Economical risk
R	The cisgenesis concept and knowledge development by cloning specific *R* and *Avr*-genes and their use in GM plants	Very high
D	At world scale, development of location specific gene cassettes by investigating isolates using differential sets and effectors	High
I	Implementation of R and D knowledge with appropriate gene cassettes for improvement of varieties	Medium

Participation possible in R, D and I phase of knowledge development. The investment risks in the different phases have been indicated varieties owned by different member companies to assure a broad use of resistance genes in potato. Main prerequisite is a simplified regulation adapted to cisgenic plants.

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