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Societal Costs of Late Blight in Potato and Prospects of Durable Resistance Through Cisgenic Modification

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Abstract In the European Union almost 6 Mha of potatoes are grown representing a value of close to €6,000,000,000. Late blight caused by *Phytophthora infestans* causes annual losses (costs of control and damage) estimated at more than €1,000,000,000. Chemical control is under pressure as late blight becomes increasingly aggressive and there is societal resistance against the use of environmentally unfriendly chemicals. Breeding programmes have not been able to markedly increase the level of resistance of current potato varieties. New scientific approaches may yield genetically modified marker-free potato varieties (either trans- and/or cisgenic, the latter signifying the use of indigenous resistance genes) as improved variants of currently used varieties showing far greater levels of resistance. There are strong scientific investments needed to develop such improved varieties but these varieties will have great economic and environmental impact. Here we present an approach, based on (cisgenic) resistance genes that will enhance the impact. It consists of five themes: the detection of *R*-genes in the wild potato gene pool and their function related to the various aspects in the infection route and reproduction of the late blight causing pathogen; cloning of natural *R*-genes and transforming cassettes of single or multiple (cisgenic) *R*-genes into existing varieties with proven adaptation to improve their value for consumers; selection of true to the wild type and resistant genotypes with similar qualities as the original variety; spatial and temporal resistance management research of late blight of the cisgenic genetically modified (GM) varieties that contain different cassettes of *R*-genes to avoid breaking of resistance and reduce build-up of epidemics; communication and interaction with all relevant stakeholders in society and transparency in what research is doing. One of the main challenges is to explain

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the different nature and possible biological improvement and legislative repercussions of cisgenic GM-crops in comparison with transgenic GM-crops. It is important to realize that the present EU Directive 2001/18/EC on GM crops does not make a difference between trans- and cisgenes. These rules were developed when only transgenic GM plants were around. We present a case arguing for an updating and refinement of these rules in order to place cisgenic GM-crops in another class of GM-plants as has been done in the past with (induced) mutation breeding and the use of protoplast fusion between crossable species.

Keywords Cisgenesis · Cloning · Communication · Late blight · *Phytophthora infestans* · Potato · Resistance management · Selection · Transformation

Introduction

The most important disease in potato - the second most important arable crop (after wheat) in Europe - is late blight caused by a fungus-like microorganism (oomycete) with the scientific name *Phytophthora infestans*. The potato originates from the Andes in South America and in the beginning was grown in Europe in absence of this disease. When finally the disease struck in Europe, it led to famine, especially in Ireland. Initially the yields of potato decreased drastically but towards the end of the 19th century the disease was controlled with "Bordeaux mixture", an environmentally very unfriendly crop protection agent consisting of copper sulfate and calcium hydroxide. Since mid-20th century chemical products based on manganese and tin became available and later also systemic products were developed. To prevent damage, growers in North and West Europe have to apply these chemical substances almost weekly. Besides the high costs incurred, the chemicals pollute the environment and give potato a bad image. In central and eastern Europe and in developing countries the crops are treated chemically much less frequently but here yields are much lower. A potato late blight resistant potato in terms of the triple P concept and principles would considerably reduce costs in regions with intensive potato production and would increase yields in areas with more extensive cultivation (Profit), would make production more environmentally friendly (Planet) and would reduce exposure to chemicals, would improve the image of arable farming and would enhance food security in developing countries (People).

Applying new scientific knowledge brings resistant potato varieties within reach. The concept of durable resistance combined with strategic resistance management in an integrated cropping system will have a major global impact. Wageningen Plant Sciences carries out a major research effort aimed at obtaining genetically modified potatoes to accelerate the solution of the late blight problem either using transgenes, but more preferably using innovative genetic modification with cisgenes (Jacobsen and Schouten 2007). Cisgenes are defined as natural indigenous potato genes or those from crossable species that are or can be used in current breeding programmes with which potato can make natural crosses. We aim at a proof of principle of an integrated resistance approach for the benefit of the industry (breeding firms and farmers) and the environment (much less spraying of plant protection chemicals). Acceleration occurs through a Genetic Modification (GM) approach by incorporation of only cisgenes from wild species (stacking them in currently grown varieties) rather than stacking during classical introgression breeding with *R*-gene containing wild species. In this GM-approach at least 15 years are saved as backcrossing and selection programmes to get rid of the linkage drag (introduction of unwanted properties) are not required. The major bottle neck at the moment for this approach is the detection and isolation of useful *R*-genes.

The proof of principle of the development of a *Phytophthora* resistant cisgenic potato variety may be a convincing way to breach the public ban on GM-crops and possibly also to contribute to the next version of the Directive 2001/18/EC which is streamlining the introduction into the environment of GM-plants and the release of GM-varieties. The approach involves in potato only the use of cisgenic *R*-genes in the absence of antibiotic resistance genes as selection marker.

The objectives of a European wide cooperative research and development project are to show the perspectives of a GM technique which distinguishes itself from the current transgenic one to arrive at a durable resistance strategy that will save the environment, reduce costs of production, increase yields and have a strong economic, ecological and scientific impact as desired in the Lisbon agenda (Lisbon Strategy 2004). There is a need not only for Europe but also for developing countries to make GM affordable and worthwhile in crops other than maize and soya bean, and to search how to arrive at a special classification of such cisgenic GM-crops.

The aim of this paper is to show the relevance of combating a major disease in arable farming in Europe, to highlight the scientific perspectives of innovative research that may reduce that problem significantly by the cisgenic resistance approach and to indicate its impact on the people, planet and profit aspects.

Economic Importance of Potato and Late Blight

Data for the calculations below were taken from databases of the Netherlands Potato Organization (NAO 2007), EUROstat (n.d.), FAOstat (n.d.) and KWIN (De Wolf and van der Klooster 2006). The latter contains all costs of operations in arable farming in the Netherlands. The most important commodity in the EU-25 by far is cereals (52 Mha grown per year) of which slightly less than half consists of wheat. Other cereals are barley, oats, rye and rice. Potatoes are grown on almost 2 Mha, comparable to sugar beet (2.2 Mha). Average sugarbeet yields are almost twice that of potato. So the total production of sugar beet is also double. Yet the value of the two crops is about equal as farmers receive roughly €50 per Mg for sugar beet against €100 per Mg for potato. This means that the total value of the 60 million Mg potato crop in the EU is about M€6,000, comparable to the sugar beet crop and about 1/5th of the €31 billion value of the wheat crop (at €250 per Mg).

Major potato producing countries with more than 1.5 million Mg of potatoes harvested are Belgium, Germany, Spain France, Italy, the Netherlands, Poland and the United Kingdom. The importance of the crop can vary considerably per country as e.g. Malta, with a small population, grows less than 1000 ha but a major proportion is exported as valuable early potato to northern Europe. To estimate the economic importance of the disease the Netherlands are taken as a case and some extrapolation will be derived from those figures.

Table 1 Potato production data in the Netherlands in rounded figures (NAO 2007)

Type	Area (ha)	Yield (Mg/ha)	Production Million Mg	Price (€/Mg)	Value (M€)
Ware	75 000	50	3.75	100	375
Seed	40 000	38	1.52	200	304
Starch	50 000	43	2.15	50	108
Total	165 000	45	7.42	-	787

The total area of 165 000 ha with a yield of 45 Mg/ha yields an amount of potatoes of 7.9 million Mg that represents an average value of about M€790 (Table 1). Applying fungicides is associated with costs of the chemical and the costs of applying them (machines, labour, and energy). The number of sprays varies between 10 and 16 per season. Seed potatoes receive fewer sprays as they are harvested prematurely but the chemicals used there are more costly per kg. Growers tend to alternate systemic fungicides with contact fungicides to avoid build up of resistance of the disease against the chemicals. Tables 2 and 3 show the most frequently used chemicals and their costs.

The cost of the 1,424 Mg chemicals applied on 165 000 ha in the Netherlands this way are calculated at M€61.1 per year. The costs of applying on average 15 times per season (machinery, labour and fuel) are calculated (KWIN, De Wolf and van der Klooster 2006) at €330 per hectare (M€54.4 national). This means that costs of control (chemical + application) amount to M€115.5 for the country per year.

Potato late blight not only leads to costs of control but also to costs due to losses. Incidental premature harvest and immediate delivery to the market due to bad storability in ware potato are estimated (once in 5 years on 10% of the area leading to 5% loss of value) to represent a value of M€1.4. Spraying machines leave tracks in the fields and locally damage the crop reducing yields by 3%. As crops are partly also sprayed because of other pests and diseases (especially seed potato crops) 1% of losses in the ware crop and starch crops and 0% of losses in the seed crops is attributed to the tracks representing a value of M€4.8.

In the Netherlands organic production of seed potatoes takes place on 350 ha and production of ware potatoes on 1050 ha with average yields of 27 and 29 Mg/ha, respectively whereas conventional yields are 38 and 50 Mg/ha. In total, organic production on 1400 ha yields 25,900 Mg less than it would have in a conventional situation. Assuming that half of the yield loss is due to late blight and assuming a farm gate price of €250/Mg leads to a loss of M€3.2. Adding up all losses incurred from late blight in the Netherlands leads to a figure of M€9.4. Added to the M€115.5 for control totals the costs of late blight in the Netherlands at M€124.9, which is 15.8% of total farm gate price (Table 1).

Conservatively applying 15% costs to the EU level, losses in the EU are estimated at M€900 (15% of M€6,000). In some countries in central Europe the costs of

Table 2 Most frequently used chemicals to control late blight and their costs per unit and per hectare (De Wolf and van der Klooster 2006)

Active ingredient	Trademark	Price in €
cymoxanil + mancozeb	Curzate	10.80 per kg
cymoxanil + famoxadone	Tanos	43.20 per kg
fluazinam	Shirlan	67.10 per l

Table 3 Amount and costs of late blight control chemicals per ha (De Wolf and van der Klooster 2006)

	Amount (kg/ha)	Costs (€/ha)
Ware potato	9	350
Seed potato	8	450
Starch potato	9	300
Average	9	370

application may be less as growers do not control or only a few times but here losses due to the occurrence of the disease are considerable. This is one of the main reasons of the low yields per ha in countries like Lithuania, Latvia, Poland and Slovakia that are about one third of yields in countries such as Denmark, Germany and the Netherlands. Globally 20 Mha of potato are grown with an average yield of 16 Mg/ha with a value of about M€32,000. A percentage of 15 of this represents a value of M €4800 as a very conservative loss annually caused by late blight. A late blight free potato would increase income and food security - with special impact on people in developing countries - where losses would decrease strongly and where the fourth commodity (after wheat, rice and maize) would increase even more rapidly than it does at present.

Environmental Aspects

Energy wise it costs about 25 GJ to grow one ha of potato. The application of 1 kg of fungicide per ha costs 40 MJ (energy content) and 2 l of diesel (×55 MJ per l indirect costs such as the costs of making the machinery included) makes it 150 MJ per spray. If crops are sprayed 15 times total energy costs are 2.25 GJ which represents 9% of the total energy costs of potato production. (All data from KWIN, De Wolf and van der Klooster 2006).

Other environmental costs are Environmental Pressure Points as, e.g., calculated by the Netherlands Centre for Agriculture and Environment (CLM). The Netherlands Committee for the Admission of Crop Protection Agents (CTB) uses these norms for groundwater and surface water in their admission procedures. There is some environmental pressure from fungicides used in controlling late blight, especially the effect of mancozeb on groundwater, but this is only a fraction of pressure from a treatment with herbicides against weeds or insecticides against pests. The effect of fungicides on human health is also much less than that of herbicides and especially insecticides according to the “Safety Information Sheets” that chemical companies have to establish following EU directives (taken from the websites of companies such as DuPont (mancozeb) and Syngenta (fluazinam)). The active ingredients can cause some damage (irritation of skin or eyes) but are not very toxic with LD_{50} values between 2000 and 5000 mg/kg whereas, e.g., insecticides are much more toxic with LD_{50} values below 100 mg/kg.

Employment

In the Netherlands potatoes are grown by 2,200 seed potato growers, 7,000 ware crop growers and 2,500 farmers who produce starch potatoes (NAO 2007). The whole potato sector - from farm gate onwards - adds value: there are over 50 export and breeding companies, over a dozen large processing factories that process 3.2 million Mg of potato into deep frozen French fries, crisps and chilled products and into starch for food and non-food uses. The total consumer value of fresh and processed potato in the Netherlands is estimated at over three billion Euros: four times the farm gate price. In the rest of Europe this is somewhat less (less seed and processing) but the 6 billion Euro farm gate price at the consumer level will represent a value of over 15 billion Euros.

Durable Resistance Against Phytophthora (DuRPh)

Breeding for resistance against late blight proved not very successful over the last 100 years. The most important source of resistance was introgressed early to mid 20th century from the wild potato species *Solanum demissum* by interspecific crosses and successive backcrosses with cultivated potato and simultaneous selection for resistance. However, all 11 major resistance genes (*R*) in due course were overcome by virulence of the disease. Mainly four of these *R*-genes have been used in varieties as single resistance gene or in combination. To combat late blight is to get involved in the struggle between the virulence genes of *Phytophthora* and the defence genes of *Solanum*. The oomycete contains avirulence genes that produce a protein that when recognised by a cell in the resistant plant triggers it to kill itself. The result is a hypersensitivity reaction that makes it impossible for late blight to infect the plant. Late blight, however, always introduced pathotypes with absence or mutated avirulence genes so that the variety did not recognise the germinating spore at cellular level so that it became susceptible.

Given the economic, social and environmental implication of late blight and the fact that conventional breeding is not able to enhance resistance, Wageningen University and Research Centre in the Netherlands started an ambitious programme in 2005 that will run for 10 years. The research programme aims at considerably reducing the costs of late blight and involves some principles that may influence positively the application of GM-food crops: continue the use of existing successful varieties but improved by cisgenic *R*-gene modification:

- only ‘free’ (without breeders rights) potato varieties are used that have proven to be safe and are adapted to growing conditions in Europe and elsewhere and that are known by consumers and processors;
- the cisgenic modifications are only carried out with single or low numbers of natural indigenous *R*-genes directly isolated from wild potato species that can be crossed with *Solanum tuberosum* following conventional techniques in potato breeding;
- the transformation will only be carried out with cisgenes without selection markers such as resistance against herbicides or antibiotics.

The initiative aims at showing the proof of principle of this approach, not at the creation and exploitation of commercial varieties. Once the principle is proven commercial companies will develop varieties for their benefit.

Therefore, in the programme but also in other, matching, projects, *R*-genes are identified, isolated from the genome of wild species and transferred (single or combined in sets of varying composition) to some 'free' varieties. The resulting genotypes are screened for resistance to late blight, their agricultural, processing and consumer characteristics are tested and they are screened for (desired) resemblance to the original variety. We expect that resistance management needs continuous attention, not only during the research phase but also once new resistant varieties become operational. To learn of factors that affect public acceptance of a cisgenic late blight resistant potato variety, resources (funding and scientists' time) are allocated to listening and communication with the general public.

The five themes needed to achieve the aims are briefly outlined below.

Theme "Cloning"

Before the start of the programme, a limited set of *R*-genes was cloned (van der Vossen et al. 2003, 2005), which were freely available for research but not for commercial application. It is planned that in this programme 4-6 additional *R*-genes from other wild species, e.g., *S. berthaultii, S. pinnatisectum* and *S. chacoense* will be isolated (2nd generation genes). In this way, together with other matching projects, a set of 11-13 *R*-genes will eventually become available for the design of novel *R*-gene cassettes which will be transferred to several different potato varieties (see theme "Transformation"). These GM varieties will subsequently be used in the subsequent themes. The additional set of *R*-genes will be cloned through candidate gene and map-based cloning approaches which include the following steps:

- Creation of segregating populations for late blight resistance to detect major genes;
- Determination of the resistance spectrum and genetic basis of late blight resistance in *Solanum* genotypes previously identified as harbouring late blight resistance;
- Development and identification of molecular markers genetically highly linked to the *R*-loci of interest;
- Generation of physical maps of the *R*-loci using large genomic DNA insert libraries (BAC libraries);
- Functional characterization of *R*-gene candidates present on BAC clones that span the *R*-loci through complementation analysis.

In the meantime (mid 2007), at least 6 *R*-genes from different wild species have been isolated in other projects which are not only based on map based cloning, but also on allele mining. New developments in the field of effector research of *Phytophthora* are influencing the quick isolation of *R*-genes by allele mining. It means that based on this research interspecific homologues of *R*-genes can be recognized and isolated more easily and that the *R*-genes have to be divided into different classes. Members of the same class, with a similar resistance spectrum, can be found in different species. This throws also another light on the classical resistance breeding with *R*-genes. For a durable resistance the cloned *R*-genes, preferably belonging to different classes, are used for cisgenic transformation of potato varieties in the proof of principle experiments.

Theme "Transformation"

The goal of this theme is to transform a set of potato varieties with *R*-gene cassettes carrying varying numbers and different combinations of cloned *R*-genes. These

GM-varieties are ultimately used in the theme "Resistance Management" to study the effect of *R*-gene pyramiding and mixed cultivation on the epidemiology of *Phytophthora* outbreaks in the field. For the development of the cisgenesis concept, various *R*-gene cassettes are transferred to the selected potato varieties using the cloned indigenous *R*-genes combined with marker free transformation technology. The marker free technology is based on an efficient transformation protocol so that a sufficient number of transformed regenerants can be selected without using antibiotic or herbicide resistance selection markers in the vector (de Vetten et al. 2003; Heeres et al. 1997, 2002).

The following steps are of importance:

- Analysis and optimization of transformation efficiencies of a selected set of potato varieties;
- Development of single and multiple *R*-gene cassettes using different sets and 'generations' of *R*-genes;
- Classic and marker free transformation of three different potato varieties with *R*-gene cassettes carrying different combinations of *R*-genes by using optimized *Agrobacterium tumefaciens* mediated transformation protocols;
- About 50-100 independent transformants per transformation event are grown from callus on inoculated explants and subjected to a functional, phenotypic and molecular analysis before being handed over to research within the theme 'Selection".

Theme "Selection"

The activities in this theme are aimed at the selection of potato transformants that express the new late blight resistance gene(s) correctly and have no discrepancies from the wild type due to the transformation event or to somaclonal variation arising from the callus stage in the transformation procedure (Dale and McPartlan 1992; Kaniewski and Thomas 1999). Plant selection on resistance and morphology starts in vitro in the growth chamber, and is continued in the greenhouse, the gauze house and in the field in the years thereafter. A number of selected plants in the first field experiment will be characterized molecularly in order to level up the field experiment into a next stage for larger experiments. For stacking of *R*-genes it is important to investigate possibilities for testing their biological activity one by one against *Phytophthora*. For stacking of genes without available virulent isolates, the availability of *Avr*-genes with their HR reaction is a great help. It is important to know before-hand whether the *R*-genes of interest do have the potential to be more durable. Investigations with diverse isolates of *Phytophthora* will therefore be crucial.

One of the aims in this theme is also the creation of flexible varieties. We assume that even when a number of (new) *R*-genes are stacked in current varieties, the risk remains that late blight may overcome this barrier. Whenever this will occur we should have GM-lines available with a new set of potentially more durable resistance genes to replace the first ones. Temporal and spatial variation in the composition of the set is part of the approach. The selected plant material is used in the theme "Resistance management".

Theme "Resistance Management"

Transformation of a potato cultivar using different cassettes of resistance genes allows diversification of resistance within and between fields, without agronomical disadvantages, to avoid or delay adaptation of the *P. infestans* population to this new resistance. A combination of simulation and field experiments is used to select the most effective options for spatial and temporal diversification of resistance. Model tools, available from adjacent research within the Netherlands Umbrella Plan Phytophthora, allow simulation of *P. infestans* epidemics in space and time under a wide array of scenarios including variation in spatial scale, level of resistance, maturity type, climate and late blight management strategy. Models are used as a selection tool to identify potentially efficient options for diversification of resistance in space and time. Selected strategies will then be evaluated in multiple year field experiments for their capability to reduce infection risk and epidemic development of potato late blight (Skelsey et al. 2005).

Additionally, monitoring of the current Dutch *P. infestans* population has revealed very complex virulence spectra for currently employed *R*-genes. The current Dutch *P. infestans* population is also being monitored for virulence for new resistance genes, to be introduced as transformation material. We annually monitor the *P. infestans* population for presence and frequency of virulence against new resistance genes.

Theme "Communication and Interaction"

Communication and interaction with all relevant stakeholders in the Netherlands, transparency about what research is doing and explanation of the nature of the GM crops (cisgenic and marker free) aimed at acceptance of genetically modified cisgenic potatoes by the majority of consumers are needed. The ultimate goal is that stakeholders receive all relevant information about pros and cons of the development of a cisgenic *Phytophthora* resistant potato, in such a way that they are able to develop their own opinion about this innovation. Similar approaches have been developed with respect to stakeholders' attitude in the Netherlands concerning transgenic herbicide resistant crops (Lotz et al. 1999) and the way Dutch stakeholders have agreed on measures to prevent inadvertent admixture of genetically modified organisms with products from conventional and organic farming (Van de Wiel and Lotz 2006).

First we identify relevant stakeholder groups. With these groups and in interaction with the European Commission and researchers, themes are selected that are worked out in, e.g., workshops. A first set of proposed themes consists of the present societal costs of *Phytophthora* (in terms of people, planet, profit), the development of varieties with cisgenes, marker-free constructs, compared to varieties from traditional breeding, field experiments, preconditions and results, implementation into crop protection strategies, non-food applications versus food application. Stakeholders include breeding companies (field trials, acceptability), representatives of growers (resistance and cropping strategies), representatives of organic growers (acceptability of approach), processing industry regarding use of GM potatoes, consumer organizations, water authorities (costs of cleaning impurities), NGOs and policymakers (regulation and legislation).

Prospects

Flexible Varieties

Many potato varieties were bred dozens of years ago and some are almost one hundred years old, e.g., varieties Bintje in the Netherlands and Russet Burbank in the USA. Apparently it is very difficult to improve taste and processing quality of ware potatoes. The approach chosen will allow the insertion of genes in existing varieties thereby maintaining the desired quality but adding an important trait: sustainable resistance to late blight. The technique allows breeders to insert different combinations of genes in different varieties and even to diversify in space and time. Theoretically different farms could have a different set of resistance genes or a field could have a mixture of plants with different sets of genes. This should considerably reduce the chance of breaking resistance and if it does it would strongly reduce the spread of the disease because of a slower production of spores and of a reduced rate of infection. To further reduce the chance of resistance breaking, every few years newly discovered *R*-genes could be inserted replacing the old ones, thus continually staying one step ahead of the pathogen. The cost of such a breeding programme with cisgenic insertions would only be a fraction of the total costs associated with late blight exceeding 100 M€ per annum in the Netherlands alone. Aspects that need attention are the protection of intellectual property, registration of cisgenic improved varieties resulting in, e.g., new ‘Désirées’ and ‘Spuntas’ with enhanced resistance but for the rest indistinguishable from the original ‘wildype’.

Acceptance, Legislation and Deregulation

National legislation regarding GM varies from country to country. There is a request and a positive attitude within several factions important in the potato chain of the European Union to warrant less stringent oversight for cisgenic plants, and that legislators differentiate cisgenic from transgenic plants. Transgenic plants contain genes from plants or other species that could not be introduced through introgression (crossing). Cisgenic plants contain indigenous genes from crossable species. The GM-directive 2001/18/EC in the European Union has not made a difference between the two types of GMOs discussed here. Cisgenes with their native promoters introduced into a potato variety do not introduce new phenotypic traits into a species as they were there already. They do not introduce new fitness traits so putatively will not influence the environment nor the risks in food or feed in another way than with traditional breeding. Cisgenesis may even be safer than conventional breeding because it prevents introduction of genes via linkage drag which could lead to all kinds of unwanted traits (e.g., increase glycoalkaloid content to a higher level than allowed in the regulations for breeder’s rights). Results of research projects such as DuRPh on durable resistance through gene stacking should help national and EU legislators in establishing rules and regulations that are properly targeted at societal needs.

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