Scientific background report
Phytophthora-resistant potatoes

Phytophthora-resistant potatoes

Phytophtora infestans causes ‘late blight’. In regions of potato cultivation with a temperate climate, this is the single most dangerous disease. The disease costs farmers in Belgium about 55 million euros annually, and controlling it causes significant environmental pressure. However, in the last few years a number of resistant varieties based on conventional plant breeding techniques were introduced to the market, and work is being done on developing genetically modified *Phytophthora*-resistant lines. The conventional resistant varieties are the result of a complex plant breeding program lasting many years. Two of the available varieties – Bionica and Toluca – only contain a single resistance gene. The third – Sarpo Mira – a mixture of different genes providing adequate resistance. These conventional varieties are still used in small numbers; almost only in organic farming. Bionica and Toluca are only used in the fresh vegatable market. Sarpo Mira is used to make fries, amongst others.

Currently there are also genetically modified *Phytophthora*-resistant potatoes being developed. In many cases several resistance genes are introduced at once. And this is then done directly into potato varieties that have properties that are of interest to the industry. Such potatoes are still not available on the market, but are tested at various field test locations. In all probability it will take until 2013 before such potatoes are available on the market.

About this report

In this report the VIB ('Vlaams Instituut voor Biotechnologie') provides an overview of the scientific knowledge of potatoes that are resistant to *Phytophthora infestans*, also known as 'late blight'. VIB is a scientific institution with 1200 employees, with research groups at UGent, K.U. Leuven, Universiteit Antwerpen and Vrije Universiteit Brussel. The Flemish Government entrusted the VIB with disseminating evidence-based information on biotechnology.

About the references

Added to every paragraph you can find the references to the publications from which information was used. Sometimes you can fully download these publications; sometimes they are not freely available. In the latter case you can ask the author or look up the publication in the nearest university library.

Potatoes are globally cultivated and Belgium is no exception

Potato cultivation is important as a source of food and starch. Potatoes are cultivated in more than 100 countries. In 2006 the total global was 315 million tonnes of potatoes. China and India together produce more than 30% of all potatoes.

Originally, potatoes come from the South American Andes, a place where they have been cultivated for over 7000 years. Explorers brought them back to Europe in 1565, where they were kept in botanical gardens for a long period of time. After successive crop failures of traditional grains, they were introduced as food crop and began spreading over the whole world, starting from Europe. Potatoes grow best in temperate climates in which the frost-free period is sufficiently long. At temperatures under 10 ^{o}C and over 25 ^{o}C growth of the tuber is restrained.

Three types of growing potatoes can be distinguished: seed potatoes, consumption potatoes and starch potatoes. Growing seed potatoes is used for multiplying planting material. The Netherlands specialises in this field and exports planting material all over the world. Growing potatoes for human consumption is most important. It provides the potatoes we all eat, from potatoes for cooking to deep-frozen French fries, but also snacks like crisps. Starch potatoes produce starch for industrial applications: for adhesives, textile, paper, construction materials, etc. Starch potato varieties are specially selected for their suitability for producing starch for industrial applications. Starch potatoes can be eaten, but they are not very tasty.

In Belgium, the potato acreage has grown seriously in the last years to almost 81.000 hectares in 2010. This results in a growing annual potato production of more than 3 million tonnes. Flanders, the Dutch-speaking part of Belgium, produces most of this, 55 to 60% of the total. In Belgium, the vast majority of potato production consists of potatoes for consumption. The remainder consists of seed potatoes. In Belgium no starch potatoes are grown.

www.potato2008.org

A flourishing potato processing sector is present in Belgium

In Belgium, annually about 3 million tonnes of potatoes are processed in a flourishing and expanding potato processing sector. A large majority of companies in this sector are family businesses, which however have a strong international orientation.

The fact that the amount of potatoes produced in Belgium is equal to the amount that is processed in the country does not imply that all Belgium potatoes are processed within the country's borders. An intensive trade exists with the countries surrounding Belgium, as a result of which part of the potatoes produced in Belgium are processed abroad and part of the potatoes processed in Belgium are produced abroad.

Consumption potatoes are processed for a broad range of potato products in Belgium: from (deep frozen) French fries to mush, from cubes to wafers and from crisps to potato flakes. In contrast with the potato processing industry in some other countries, the potato processing companies in Belgium hardly have their own brands. Lutosa is one of the few exceptions. Most of the companies produce for other (house) brands.

www.belgapom.be

Phytophthora infestans – 'late blight' – is the largest threat to potato cultivation

Potato cultivation suffers from various diseases and pests, but 'late blight' forms the biggest threat in regions with a temperate climate. Other threats are nematodes *(Globodera pallida, Globodera rostochiensis* and *Meloidogyne spp)*, and some bacterial diseases such as brown rot (*Ralstonia solanacearum*), ring rot (*Clavibacter michiganensis*) and blackleg *(Dyckeya spp)*. Late blight is caused by the oomycete or water mould *Phytophthora infestans*, which is a fungus-like organism. Late blight is best known for the Great Famine it caused in Ireland around 1845. In a number of years the disease caused big potato crop failures in Ireland and proved to be a turning point in Irish history. About a million Irishmen died as a result of the famine and about the same number migrated, mostly to the U.S. to start a new life there. *Phytophthora infestans* emerged in Central America and crossed the Atlantic Ocean to Europe from there in 1843. The Great Famine took place only two years after.

The susceptibility of potatoes for *Phytophthora infestans* differs from variety to variety, but only very few varieties are characterized by complete resistance. The Bintje variety, which today still makes up between 40 to 50% of Flemish potato production, is highly susceptible to late blight. Potato farmers in Flanders spray their crops 10 to 15 times each year with agents that involve significant pressure to the environment. The number of times a crop is sprayed also strongly depends on weather conditions.

Phytophthora infestans affects both plant and tuber, and is highly contagious

Phytophthora infestans affects leaves, stems and tubers. When a plant is affected, lesions (spots on the leaves) occur on the leaves. How these spots look, depend on weather conditions. Usually one can see spots with a 1 to 2 cm diameter, which slowly turn dark brown. Initially, the brown spots are lined by a yellow ring. In damp weather conditions, the underside of the leaf will show a white fluffy mould. Under conditions that are favourable to the mould, affected leaves will ultimately shrivel up completely. The stems display large elongated lesions with a brown colour, reaching around the entire stem. The tubers are affected via buds or cracks in the skin; contamination can occur both during growth, harvesting and storage. Damage manifests itself as bluish spots becoming apparent from behind the skin, that turn rusty brown over time. Often, infestation of the tuber leads to dry rot or wet rot (bacterial infection) and the wet rot may then be passed on to unaffected tubers. In this way a limited number of tubers affected by *Phytophthora infestans* can eventually lead to significant losses during storage.

Photograph: A potato field with advanced Phytophthora infestation.

Phytophthora thrives best under temperate temperatures and under relatively high humidity. Quick penetration of the leave requires a sufficiently long wet leaf period. The disease especially spreads via asexual spores (zoospores) which are formed by the white fluffy mould. The disease is highly contagious. The spores are dispersed via wind or splashing rain drops.

Photograph: The result of Phytophthora in affected tubers.

Phytophthora infestans survives winter as hyphae in affected tubers. Potato waste heaps are notorious hideouts for the disease. Volunteer plants from tubers left in the field are also an important way in which the disease can survive winter. Since a number of years, however, the disease can also survive via sexual spores (oospores), which result from the joining of the so-called A1 and A2 mating types. Earlier, the A2 mating type did not occur in the Belgian area used for potato cultivation, so that survival via oospores is a relatively recent event. Oospores survive up to 1 to 3 years in the soil and may cause the disease to spread, especially when crop rotation turnover is too fast.

http://www.agris.be/nl/aardappelziekte/194003.asp#alg

Over the years, *Phytophthora infestans* turned more aggressive

Controlling the disease became more difficult over the years, as more aggressive isolates were naturally selected within the *Phytophthora infestans* population and diversity has increased due to recombination among various mating types previously not occurring within the same area. The increasing aggressiveness is expressed in a shorter penetration time and the disease's life cycle, more generations in a single growth season and a larger temperature tolerance.

Phytophthora infestans causes huge costs

Close to 2 million hectares of potatoes are grown in Europe, and the harvest represents a value of about 6 billion euros. According to estimates, late blight causes 1 billion euros of damage in Europe. This damage consists of costs for controlling the disease (costs for purchasing fungicides, plus costs for spray operations) and the costs that result from reductions in harvest and losses during storage. To Belgian potato farmers the late blight means an annual cost of roughly 55 million euros.

Haverkort et al., Societal Costs of Late Blight in Potato and Prospects of Durable Resistance Through Cisgenic Modification, Potato Research (2008) 51:47-57

Environmental pressure caused by controlling *Phytophthora infestans*

In Belgium, large quantities of fungicide are used annually for ensuring sufficient control of *Phytophthora*: over 1000 tons of active agent a year. In Flanders, an average of about 17 kg of active substance is applied per hectare per year for controlling *Phytophthora*. The three main active substances are (in order of decreasing importance): mancozeb, cymoxanil, and propamocarb.

Source: Flemish Government, Department of Agriculture and Food, Section Monitoring and Analysis – LMN, data 2008

Ways to keep the disease pressure of Phytophthora low

Important measures of reducing the disease pressure are controlling volunteer plants and destruction of potatoes in waste heaps. In some countries requirements exist to this purpose. Doing this prevents *Phytophthora* pockets from further spreading the disease, but it does not help in controlling sexual spores (oospores). Crop rotation that is sufficiently slow is effective against spreading the disease via oospores. A second way to reduce disease pressure is by carefully following the instructions of the monitoring services. A last resort is reducing potato cultivation itself – the less potato production, the lower the disease pressure, but of course that is hard to achieve in a country that is famous for its consumption of chips.

Growing resistant varieties is the best way to reduce disease pressure

In a country with intensive potato production the best way to reduce disease pressure is growing resistant varieties. The larger the area of cultivated resistant varieties, the lower the disease pressure. In doing this, it is important to insert a sufficient number of different resistance genes. If not, selection pressure will get too high or the limited amount of resistance genes will lead to a quick adaptation of the *Phytophthora* population.

The area of cultivated resistant varieties probably does not need to be 100% to sufficiently reduce the disease pressure. For comparison, look at the example of the virus resistant papaya on Hawaii. Eighty percent of the cultivated area of papaya on this island is planted with resistant crops, and that sufficiently reduces the disease pressure to be able to plant the remaining 20% of the cultivated area with non-resistant varieties without leading to problems with disease. Today, however, only a few potato varieties that are truly resistant are available: The varieties Bionica, Toluca and Sarpo Mira. More about these varieties later.

Acting fast is essential when disease occurs

Acting fast becomes essential when *Phytophthora* is found in a field. In practice, the disease is controlled by applying fungicides. Preventive action (treatment before penetration by the mould of the plant takes place) is needed to get adequate results. Only a limited number of agents have a sufficiently effective result.

Destruction of potato leafs and stems is another efficient method to prevent the disease from spreading outside the field. However, leaf and stem destruction at a time when the crop is not yet ready for the harvest has significant consequences. Certainly in late maturing varieties strong tuber growth occurs in the last weeks before harvesting. Prematurely destructing the potato leafs and stems would then result in lost yield potential. Instead of harvesting 45 to 60 tonnes per hectare perhaps only 35 tonnes are brought in, or even less. For this reason, destruction is usually done on leafs and stems that are already dying.

Improved resistance with conventional plant breeding techniques

Bionica and Toluca

The Dutch have started over 40 years ago to crossbreed natural resistance genes into commercially useful potato varieties. The resistance genes come from wild, tuber-forming *Solanaceae* from the Andes. The very first crossbreeding took place in 1959. It took until 2005 before some useful varieties were retrieved: Bionica and Toluca. Both varieties contain the same, single resistance gene *Rpi-blb-2* from *Solanum bulbocastanum*.

Crossing in resistance genes to the cultivated potato in a traditional way takes lots of work and patience. This is due to the fact that the potato can not always be crossbred directly with wild relatives in which the resistance genes are present. This necessitates application of a more complicated crossing diagram, using so-called 'bridge cross breeding'. It only allows crossing in one resistance gene at a time, unless you have the luck that the wild variant contains several resistance genes and that at the same time those resistance genes are very close together in the DNA of that wild variant. Furthermore, after crossbreeding with wild variants, many back-crossings are needed to arrive back at the desired cultivated potato properties. Below the crossing diagram is represented that led to the resistant variants Bionica and Toluca.

258 Potato Research (2009) 52:249–264

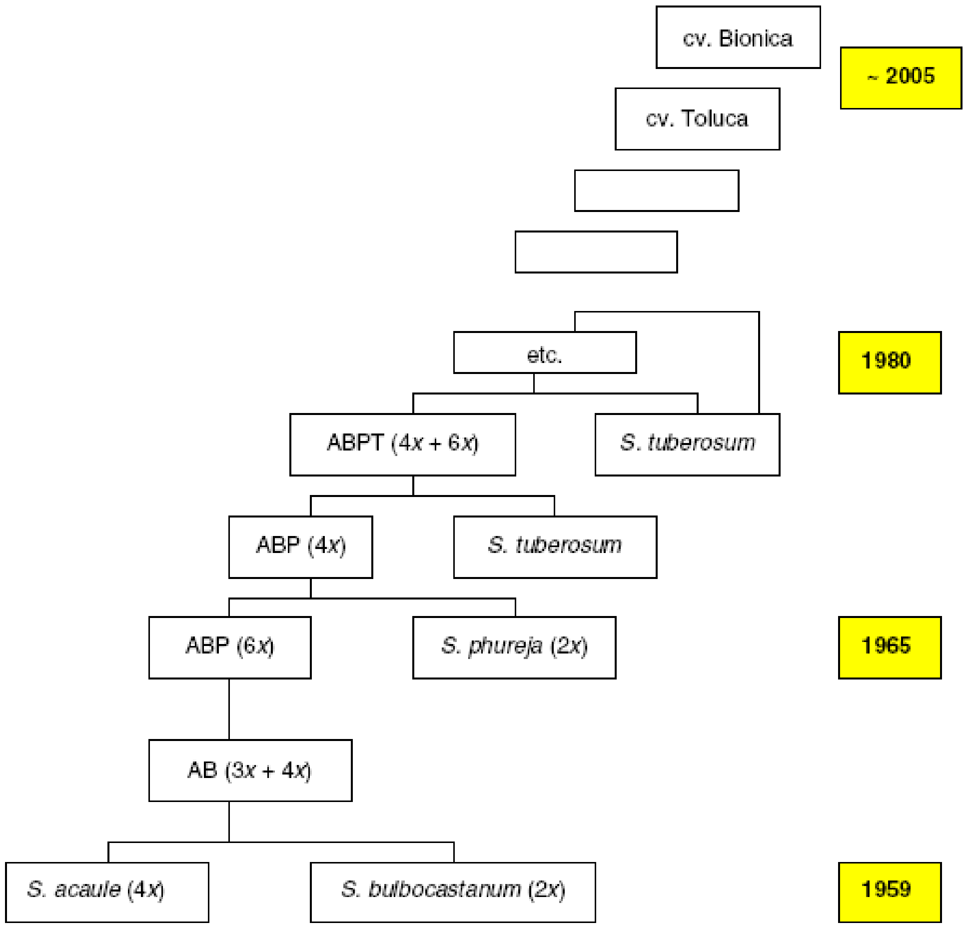

Fig. 3 Scheme of interspecific bridge cross breeding activities with late blight resistant *S. bulbocastanum* at Wageningen University and Research Centre and private breeding companies in the Netherlands. After 46 years the first resistant varieties Bionica and Toluca appeared, containing the single broad spectrum resistance gene *Rpi-blb2*. Note that stacking of *R* genes through this approach would even be more complicated and slow

www.meijer-potato.com

www.agrico.com

Sarpo Mira

Besides Bionca and Toluca there currently is a third potato variety on the market which features high resistance: the Sarpo Mira variety. The origin of this variety is not to be found in The Netherlands, but in Hungary. Like Bionica and Toluca, the variety has a long developmental history. According to legend, reaching its current level of resistance took 40 years. In achieving this, the following method was followed. As many different forms of resistance, tolerance and oversensitivity as possible were collected. On the basis of this gene pool, crossbreeding was done as much as possible. The plants that resulted from the generated seeds were subsequently sprayed with a mix of *Phytophthora infestans* that is as inclusive as possible. When 95% of the plans have died, the *Phytophthora* in the remaining plants was neutralized with a fungicide and these plants could reach maturity. The resulting tubers were subsequently used for further work. This lasted until the collapse of the communist regimes. The institute where the potatoes were kept was closed down. Thanks to the scientists involved, the best material was saved.

The material resurfaced a number of years later, when a total contamination with *Phytophthora* took place in Rumania. The Danish company Danespo then took over this material and provided the Eastern European scientists involved with a new basis for continuing their work. From the material that was subsequently obtained Sarpo Mira was marketed by Danespo.

www.danespo.com

Phytophthora easily overcomes single resistance genes

It is known that *Phytophthora* easily overcomes single resistances. The rich genetic diversity within *Phytophthora infestans* populations implies that genotypes are already present or emerge (the so-called mutants) and that these can circumvent the mechanism of the single resistance gene that is present. This allows these mutants to have a selective advantage vis-à-vis the wild types and subsequently quickly grow in numbers, dominating the population after a short while. When several resistance genes are present in a potato variety, it is much more difficult for the mould to adjust itself to both resistance genes at the same time. As stated earlier, the varieties Bionica and Toluca contain the single resistance gene *Rpi-blb2.* This resistance has not been overcome in the field for the moment. The varieties still offer adequate protection. The fact that the varieties are cultivated on a small scale only – mainly by organic and non-commercial vegetable gardeners – means that the selection pressure to overcome the resistance is still limited. However, it is known that isolates exist on which the *Rpi-blb2* gene has no effect. That is why in The Netherlands the growers of varieties Bionica and Toluca are explicitly asked to keep a close eye on their crop and immediately destruct the leafs and stems if they observe any sign indicating the presence of *Phytophthora*. If they would fail to do so, they could allow large-scale spreading of *Phytophthora* isolates that have overcome resistance. And that would render 40 years of plant breeding useless in a relatively short period of time.

Introducing resistance against Phytophthora infestans by means of genetic manipulation

Since a number of years resistance genes are also introduced into potatoes by means of genetic modification. This takes place in the U.S. (Cornell University), the U.K. (Sainsbury Laboratory, John Innes Centre), Peru (Centro Internacional de la Papa (CIP)), Germany (BASF) and The Netherlands (Wageningen UR), but not in Flanders. The advantage of the technology of genetic modification vis-à-vis classical plant breeding is that one can develop resistant varieties much faster and targeted and can also insert several resistance genes at the same time. Furthermore, this insertion via genetic modification can be done without losing properties of the variety.

Genetic modification for resistance against *Phytophthora infestans* step-wise

STEP 1: Identifying resistance genes

For making potatoes resistant to *Phytophthora*, the resistance genes should be obtained first. To do this, wild relatives of our cultivated potato are sown and tested for their resistance. This is initially done with a simple *in vitro* test. This way, thousands of different wild genotypes have been tested. Also, a so-called DNA fingerprint is made for each of those plants. Next, a leaf test with a number of specific *Phytophthora* isolates is performed on the plants that demonstrate resistance; this is to understand more about the interaction with the mould. The plants that demonstrate resistance in these tests as well are then subjected to field tests. In this way a smaller number of resistant genotypes remain.

By comparing the DNA of the resistant plants with the plants that are not resistant, the areas in that DNA can be located where the resistance is supposed to lay. Nowadays, the further quest for resistance genes is made easier by the fact that the potato genome at the base pair level is fully sequenced: the DNA order is known – every letter of it. Every year, several new resistance genes are discovered. Today, about two dozen different resistance genes are known and available. The isolated genes are also known as cisgenes because they come from varieties that can be crossbred, which can also be used for classical plant breeding.

STEP 2: Modification of potato lines

Once you have a resistance gene, it can be inserted into the potato. This procedure works as follows: as a first step, the gene is inserted into the DNA of soil bacterium *Agrobacterium tumefaciens*. It is built into a piece of the bacterium's DNA that is naturally transmitted to plant DNA if the bacterium infects a plant.

Small pieces of potato tissue are brought onto a Petri dish in which a growing medium is present in which the plant can grow. Next, the modified *Agrobacterium* is added to this potato tissue. The bacterium infects the tissue and transmits the resistance genes to the plant's DNA. Then, a new plant can be generated from the potato tissue. This can be done by adding various plant hormones to the growing medium, inducing the tissue to develop roots and shoots.

Not all cells are infected by *Agrobacterium*, so the desired DNA fragment is not present in all plant tissue cells. Therefore, plants should be selected that have actually incorporated the DNA. This can be done in two ways. The first way is to couple another gene to the resistance gene: a so-called selection marker. This can be an antibiotics resistance gene, for example, or a gene providing herbicide tolerance. By then adding the antibiotic or herbicide to the growing medium, only the plants survive that actually incorporated the DNA. This is a simple way of selection.

The selection markers are often obtained from bacteria or from plants with which the potato cannot naturally crossbreed. In other words, these are heterologous. Plants, in which heterologous genes are inserted via genetic modification, are also known as 'transgenic'.

In the second method of selection no selection marker is added to the DNA that is to be transmitted. Al regenerated plants are allowed to grow up, including the ones that did not incorporate DNA. Next, DNA is obtained from all developed plants. A genetic test based on PCR[1] determines whether a particular plant has incorporated the desired DNA. This selection method is a lot more complicated and time-consuming. Plants that have not incorporated the DNA are disposed of.

The resistant plants with selection markers, only contain DNA that is inserted via genetic modification from potatoes or plants with which the potato can naturally crossbreed. Such plants are also known as 'cisgenic'. Cisgenic plants to a high degree are comparable with plants which can be obtained via classical plant breeding. The only difference is the technology with which the DNA is introduced.

[1] PCR = Polymerase Chain Reaction

STEP 3: Testing the plants in a greenhouse

The first growth stages of the genetically modified potatoes usually take place in a plant nursery room. When the plants have become a bit bigger, they are brought to a greenhouse, where they can continue growth in a pot or in the soil. Growing them in the greenhouse has various purposes. Firstly, it is mainly about establishing whether the desired properties are present. For this, *Phytophthora* resistance is the dominant important property. Work only continues with plants that demonstrate resistance under a simple leaf test. Also, variety characteristics are checked. Are all relevant properties still there? Variety properties may be lost as a result of any of the next three phenomena: (1) ‘insertional mutagenesis’, (2) ‘pleiotrophy’, of (3) ‘somaclonal variation’.

Insertional mutagenesis indicates a mistake resulting from insertion of the additional piece of DNA, which ends up in an existing gene, disrupting the function of this particular gene. The potato is tetraploid, which means that it contains 4 copies of each chromosome (4 ‘homologous chromosomes’). The inserted DNA ends up in one of those four chromosomes, which means that even if the modification leads to a mutation, this will involve only one of the four genes present in the four homologous chromosomes. So the other three genes remain intact. Therefore, any mutation resulting from insertion of DNA will almost never lead to a noticeable change in potato properties.

Pleiotrophy is the phenomenon which involves unexpected and undesirable effects resulting from the fact that the additional piece of DNA ends up in an unexpected place of the plant's DNA. This might for example disrupt the functioning of nearby genes.

Somaclonal variation is the emergence of phenotypic (= observable) deviations from the potato from which it was derived, as a result of growing the plant *in vitro* and newly regenerating the potato. This latter phenomenon is not specific for the technology of genetic modification and also occurs during regular *in vitro* reproduction.

Therefore, work in the greenhouse requires strict selection, only selecting those plants that are true-to-type, in other words, which still have all relevant variety properties. The plants displaying resistance AND which are true-to-type are used for further work. With this material, leaf tests are performed with real *Phytophthora* isolates. And plants that also do well in these tests can in principle qualify for field tests.

Cultivation of conventional resistant varieties

Potato varieties differ in their susceptibility for *Phytophthora*. Varieties such as Bintje and Nicola are highly susceptible to the disease. Early varieties may potentially escape the biggest part of the *Phytophthora* pressure due to their short growth cycle. Only a small number of varieties such as Bionica, Toluca and Sarpo Mira contain true resistance based on one or several R genes. The resistance genes coming from *Solanum demissum* that are present in some varieties have often been overcome and therefore cannot be used anymore. Bionica, Toluca and Sarpo Mira are marketed by Dutch firms Meijer and Agrico and Danish firm Danespo, respectively. Bionica and Toluca are only cultivated for the market for fresh potatoes. Meijer purely offers the Bionica potato for the organic farming market. Agrico offers Toluca for both organic cultivation and conventional cultivation, but this potato as well is mainly used for organic farming. The potatoes are on the market since a number of years and in 2009 their field resistance still turned out to be good. In Belgium, Bionica is sold to non-commercial vegetable gardeners via AVEVE. In 2010 AVEVE sold about 10,000 kg of seed potatoes to non-commercial growers, translating to over 10 hectares of Bionica cultivation. Of course this is only a drop in the ocean, but non-commercial cultivation is traditionally seen as a potential source of spreading *Phytophthora* to commercial cultivation, because *Phytophthora* is hardly controlled in non-commercial cultivation. In this way, a very small number of Bionica can still have an impact on disease pressure.

Sarpo Mira still is not greatly appreciated by consumers due to its white/yellowish colour on the inside, the flowery cooking result and its not-so-smooth tuber face (faint red skin, irregular shape). The potato however turned out to be well suited for chips and flakes, granulates and mush. McCain exclusively uses Sarpo Mira for their organic chips.

www.avevewinkels.be

Sustainable resistance

Single resistances against *Phytophthora* are unsustainable. *Phytophthora* will overcome such a resistance sooner or later, and when that happens, the varieties with such single resistances lose a great deal of their value. Combination of two different resistance genes offers a higher degree of sustainability. This makes it not twice as difficult, but many times as difficult for *Phytophthora* to overcome the resistance. Triple resistance will provide sustainability that is still many times higher.

The most sustainable resistance is obtained if potato varieties with different multiple resistances are alternated in space and time. Scientists are convinced that in such a scenario –

supplemented with some spraying – problems with *Phytophthora* should be a thing of the past, as *Phytophthora* would not stand a chance of adapting itself.

BASF's Fortuna potato

BASF is most advanced in developing genetically modified, *Phytophthora*-resistant potatoes. They have created the 'Fortuna' potato. This potato is close to its market introduction. The potato contains two resistance genes, being *Rpi-blb1*, and *Rpi-blb2*. The combination of these two resistance genes implies that the resistance of this potato should be much more sustainable than that of the varieties Bionica and Toluca. The Fortuna potato also contains a selection marker in the form of a mutated AHAS gene. This mutated AHAS gene provides tolerance against sulfonyl-urea based herbicides, among others. BASF licensed the two resistance genes from a company having the original property rights to the use of these genes.

BASF does not make any official statements about this, but according to various sources, the Fortuna potato could have been derived from the Agri variety, a variety that is cultivated large-scale in many places in Europe, and is equipped with the right properties for processing to chips.

http://www.basf.com/group/corporate/en_GB/function/conversions:/publish/content/products-and-industries/biotechnology/images/Fortuna_VC.pdf

The DuRPh cisgenic project

The DuRPh project started in The Netherlands in 2006. DuRPh stands for '***Du***urzame ***R***esistentie tegen ***Ph***ytophthora' or Sustainable Resistance against *Phytophthora*. This is a 10-year project financed by the Dutch government which aims at identifying and characterising resistance genes, creating and testing genetically modified potato lines, and communicate this to a large target audience. The ultimate goal is to present a proof of principle with regard to sustainable resistance against this disease. The project was set up from a genuine sustainability approach and takes into account economic aspects, as well as ecological and social aspects. Wageningen UR took the initiative for the DuRPh project and carries it out.

In the DuRPh project, genetically modified potatoes are developed with a specific underlying idea. Of course the technology of genetic modification is used, and the end result is still subject to GMO legislation, but the plants that are created have the highest possible degree of similarity with plants that can also be obtained via conventional plant breeding. This is called cisgenesis, which implies that:

(1) The resistance genes come from *Solanaceae* with which the potato can naturally crossbreed.

(2) The resistance genes are not tampered with. They are inserted with their natural regulation signals.

(3) No selection markers will be available in the final lines, only cisgenes.

Work with constructs in which a marker is present is used in the first phases of development only, just because this is easier and takes less time, and a quicker insight is obtained in which genes (gene combinations) provide adequate and sustainable resistance.

Since 2006, Wageningen UR performs small-scale field tests with genetically modified Phytophthora-resistant potato lines on various locations in The Netherlands. In these field tests, the various lines that display adequate resistance during greenhouse testing are subjected to a first reality check. In the course of years, various constructs and lines are tested in this way. There are potatoes that include single resistance, double resistance, triple resistance, and lines with (transgenic) and without selection marker (cisgenic).

www.durph.nl

Cisgenesis is subject of regulatory discussions in the EU

Cisgenesis is genetic modification; there is no question about that. However, there is a discussion about the question whether cisgenesis should stay subject to GMO regulation or that it might be exempt from it. The existing legislation already provides a number of exceptions. Classical mutagenesis, for example, falls under the definition of genetic modification, but is exempt from European GMO legislation. A technical working group now considers the question whether there are any sound reasons to keep cisgenesis under GMO legislation or not. The working group does not only scrutinises cisgenesis, but also looks at a number of other modern ways to achieve DNA modifications, among which are reverse breeding and targeted DNA repair.

Genetically modified *Phytophthora*-resistant potatoes do not threaten the environment

Genetically modified plants should not have negative effects on the environment, such as pushing out existing species in nature or disturbing delicate natural balances. Genetically modified *Phytophthora*-resistant potatoes include resistance genes that naturally occur in wild, tuber-forming *Solanaceae.* The same or comparable genes are also present in *Phytophthora*-resistant potatoes that are obtained via conventional plant breeding. Therefore, the genes in the genetically modified potato lines are just as safe to the environment as the same genes in the varieties obtained via conventional plant breeding and those in wild relatives. Is that the end of the story? Not quite. It cannot be completely ruled out that genetic modification leads to unexpected phenomena having an effect on the survival of the potato plant in nature, or that other unexpected changes in plant properties emerge. For this reason, strict selection takes place during the quest for suitable genetically modified lines, in order to only select those lines that kept the right variant properties. In field tests, the phenotype and behaviour of the plants also undergo exhaustive scrutiny in order to locate any deviations, and to look whether deviations have undesirable consequences. Plants with undesirable properties will not be marketed.

In Europe, the potato is absent as a wild plant. Furthermore, the potato has no relatives with which it could crossbreed. Black nightshade is phylogenetically closest to the potato, but cannot be interbred with it. Furthermore, the cultivated potato is a highly domesticated plant that will not suddenly colonise nature. The plant lacks the power to successfully compete with wild plants. In practice, this means that neither the genetically modified potatoes, nor the genetically modified property itself will be able to colonise our environment. The potato will only be able to appear as volunteer plants in fields where it has previously grown, and in soil coming from such fields. These cases will, however, always be a temporary affair.

Food safety of genetically modified *Phytophthora*-resistant potatoes

Genetically modified *Phytophthora*-resistant potatoes contain the same or comparable resistance genes as traditional *Phytophthora*-resistant potatoes do. To provide a practical example: BASF's genetically modified *Phytophthora* potato and the potato varieties Bionica and Toluca that have been obtained via conventional plant breeding, all contain the same *Rpi-blb2* gene. For the genetically modified potatoes, the safety of the gene product that is synthesized by the resistance gene should be demonstrated. It should be demonstrated that the product is not toxic and does not cause allergic reactions. This is not a requirement in varieties like Bionica or Toluca which are the result of conventional plant breeding. These varieties can be marketed without further food safety tests. And all this despite the fact that the Rpi-blb2 gene in these plants is surrounded by hundreds of genes originating from the wild variety.

As indicated earlier, it cannot be ruled out that genetic modification can lead to unexpected side effects. Insertional mutagenesis, pleiotrophy and somaclonal variation were mentioned as possible causes of such side effects. For this reason, a genetically modified Phytophthora-resistant potato should undergo additional tests. The composition of the potato should be compared with that of conventional potatoes, for example. For this purpose the quantity of the main components of the potato is measured and compared. The OECD determined which substances should be included in the analysis. The potato naturally contains toxic glycoalkaloids. Their presence is the reason why one should peel potatoes before they can be safely eaten. Besides, the potato contains substances blocking our digestive enzymes. Eating raw potatoes leads to stomach-ache, so to prevent this we cook them before consumption. In the required analysis, the quantity of these kinds of substances is closely watched, because everything should be done to prevent marketing potatoes that carry too many hazardous substances. By the way, the amount of glycoalkaloids is checked for every new variety before market entry, whether it is genetically modified or not. In The Netherlands and Sweden an upper safety limit of a total glycoalkaloid level of 200 mg/kg applies. Other EU countries have not specified glycoalkaloid limits.

If analysis of the composition of the genetically modified potato shows an unexpected deviation in the level of certain substances, additional tests should be performed. Usually this takes the form of a 90-day food test on rats.

OECD, Consensus Document on the Biology of Solanum tuberosum subspecies tuberosum (potato)

Genetically modified *Phytophthora*-resistant potatoes not on the market for now

BASF's genetically modified *Phytophthora*-resistant Fortuna potato reached an advanced stage, but is still not on the market. BASF should first apply for market admission with the European Commission to achieve this. And once that file has been submitted, it will take some years more before the full procedure is completed. It is hard to look into the future, but it is expected to last until 2013 before a genetically modified *Phytophthora*-resistant potato is available on the market. All other lines that are being developed are less far advanced than the Fortuna potato and their market introduction will take a lot more time.