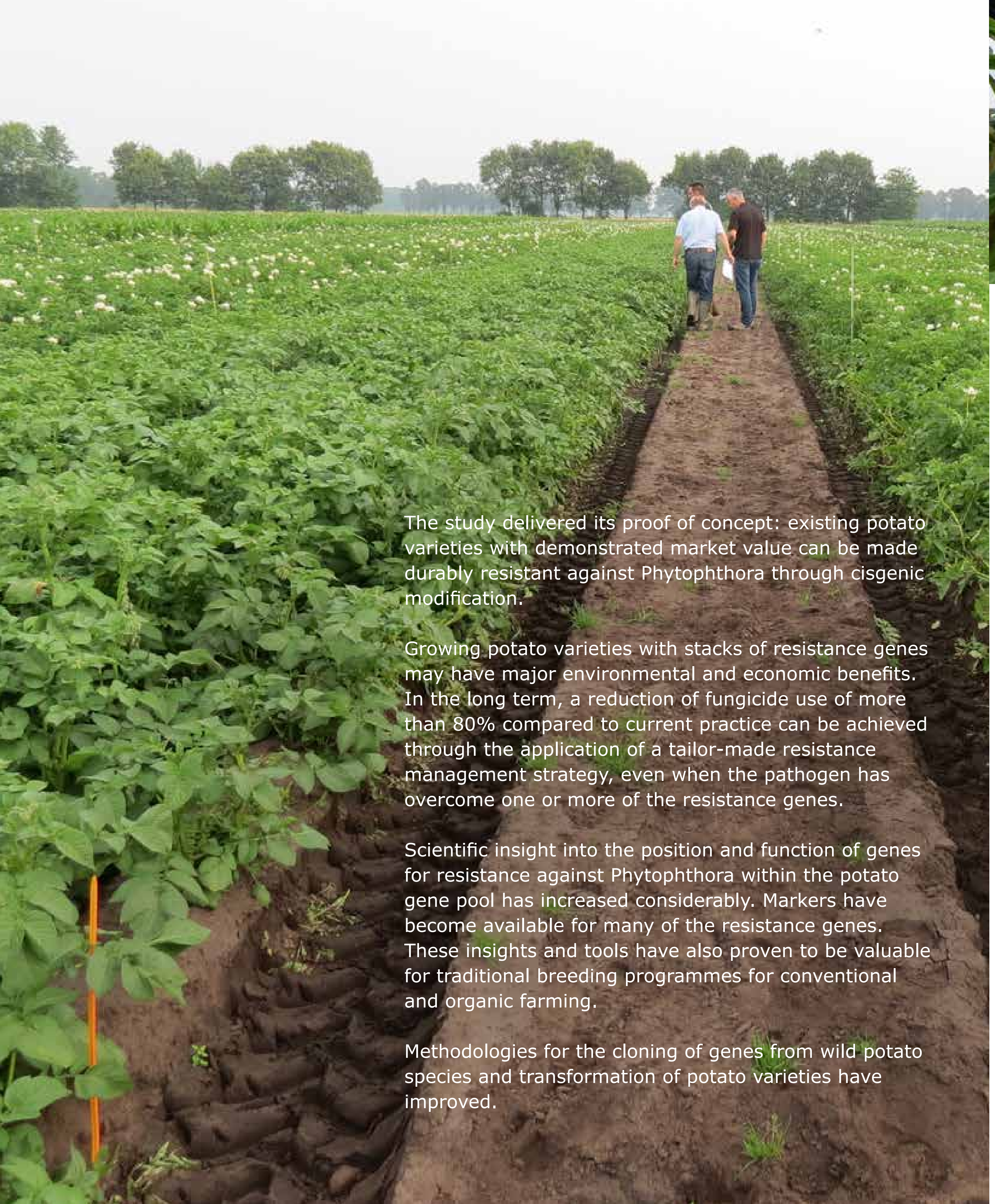
Main results of DuRPh



The study delivered its proof of concept: existing potato varieties with demonstrated market value can be made durably resistant against Phytophthora through cisgenic modification.

Growing potato varieties with stacks of resistance genes may have major environmental and economic benefits. In the long term, a reduction of fungicide use of more than 80% compared to current practice can be achieved through the application of a tailor-made resistance management strategy, even when the pathogen has overcome one or more of the resistance genes.

Scientific insight into the position and function of genes for resistance against Phytophthora within the potato gene pool has increased considerably. Markers have become available for many of the resistance genes. These insights and tools have also proven to be valuable for traditional breeding programmes for conventional and organic farming.

Methodologies for the cloning of genes from wild potato species and transformation of potato varieties have improved.

DuRPh and stakeholders

The DuRPh project has enhanced international scientific collaboration on making potato production more sustainable by means of cisgenic modification.
DuRPh plants have been tested:

- in the EU-projects *GMO risk assessment and communication of Evidence* (GRACE) and *Assessing and monitoring the impacts of genetically modified plants on agro-ecosystems* (AMIGA), e.g. with field trials performed by TEAGASC, Ireland
- in several other collaborative projects, e.g. with a Belgian research consortium centred on the University of Ghent, and with the Swiss institute Agroscope.

During the open days of DuRPh, Dutch breeders indicated that they are able to commercially exploit the DuRPh results by themselves, only if varieties which originate from cisgenesis do not have to undergo the expensive and time-consuming European approval procedure which currently applies to transgenic plants (Directive 2001/18/EC).

DuRPh scientists regularly met with farmers and other operators in the conventional potato chain, both in their own and DuRPh meetings. These meetings showed that there is a willingness to grow, process and market Phytophthora-resistant potatoes created via cisgenesis as soon as there is a viable market.

Representatives of organic farming often expressed appreciation for the developed knowledge and methods that are specifically valuable for programmes related to the cross breeding of potatoes.

Environmental groups reacted in various ways, ranging from positive criticism – mostly referring to the beneficial effects on the environment – to various types of negative feedback.
A number of groups indicated that the DuRPh programme produced a well-documented case which is suitable as input for a constructive discussion on the use of cisgenesis in potato breeding.

The DuRPh programme was funded by the Netherlands Ministry for Economic Affairs and conducted by Wageningen UR (University & Research centre).

Ministry of Economic Affairs

The following scientists were involved: Piet Boonekamp, Anton Haverkort, Ronald Hutten, Evert Jacobsen, Geert Kessel, Bert Lotz, Richard Visser and Jack Vossen.

www.durph.nl

Contact
Wageningen UR
Erik Toussaint
erik.toussaint@wur.nl

Durable resistance against Phytophthora through cisgenic modification (DuRPh)

An overview of results and responses from the wider society

WAGENINGEN UR
For quality of life

A publicly funded research programme entitled *Durable Resistance against Phytophthora (potato late blight) by cisgenic modification* – with the acronym DuRPh – was carried out between 2006 and 2015. Central to the DuRPh research was the deployment of multiple resistance genes from wild potato species in well-established potato varieties via genetic modification, and a low-input resistance management strategy based on Phytophthora population monitoring to ensure the durability of the acquired resistance. The aim of the programme was to establish proof of concept that existing potato varieties could be made durably resistant with this approach. The project showed that stacking multiple genes, combined with smart resistance management, allows for an 80% reduction in fungicide use.

This folder describes the approach and summarises the results of the DuRPh programme as well as responses from the scientific community and society at large.

The DuRPh approach: cisgenesis and resistance management

Genes for resistance to late blight from crossable wild potato species were cloned and inserted into existing potato varieties through marker-free transformation. This type of marker-free genetic modification, using natural genes from crossable plant species only, is called cisgenesis. The resulting potato plants were subsequently tested for resistance to late blight and similarity to the original variety in lab, greenhouse and field trials. Resistance management studies were conducted to assure maximum resistance longevity following the introduction of resistance genes.

Farmers apply fungicides up to 15 times during a growing season to protect their crop against Phytophthora.

Potato crops suffer from late blight, a potato disease caused by the oomycete *Phytophthora infestans*. Farmers in developed countries apply fungicides up to 15 times during a given growing season to protect their crop against the disease. Organic growers depend largely on resistant varieties and early haulm killing when symptoms appear. So far Phytophthora has shown itself able to easily break single resistance genes in potato. Creating Phytophthora resistant varieties using conventional breeding tools currently requires large multi-annual breeding programmes. The multitude of characteristics which breeders have to consider in a commercial setting makes the insertion of multiple resistance genes in a single variety difficult and time consuming, and even practically impossible for some combinations.

Potato leaves infected by *Phytophthora infestans*. Spores allow the pathogen to spread easily.

Cloning and transformation of resistance genes

Overall, 30 resistance genes from wild potato species are now known and mapped on different chromosomal positions in the genome. More than 20 resistance genes have been isolated and are available for transformation (i.e. genetic alteration by insertion of DNA in a plant cell). This ultimately resulted in four potato varieties (Première, Désirée, Aveka, Atlantic) equipped with one to three resistance genes.

All resistance genes identified so far encode for the same group of proteins (so called NBS-LRR proteins). These host proteins allow the potato plant to recognise a Phytophthora infection and induce a resistance response to stop the infection in its tracks.

Potato stem explants have undergone the transformation procedure for the insertion of resistance genes. Cells in which the transformation was successful grow into a new plant.

Lab, greenhouse and field tests

Similarly to the selection process in conventional breeding, the DuRPh programme also started with a selection step – but instead of in a greenhouse, here the first selection step took place in the lab, where only those plants were selected in which all inserted resistance genes were expressed.

Subsequently, resistance to late blight and variety characteristics were quantified in the greenhouse and in field tests to result in potato plants that were resistant but otherwise indistinguishable from the original varieties.

This clearly showed that inserted resistance genes are capable of turning a susceptible variety into a resistant one, in lab, greenhouse and field. The resistance genes used varied in broadness of resistance, indicating that some genes resulted in plants that are resistant to a broad spectrum of Phytophthora genotypes, while others conferred resistance only to a smaller group. Thus, different combinations of resistance genes (so called stacks) were tested and this resulted – for a number of stacks – in complete protection against all Phytophthora genotypes tested.

Potato plants were grown in a greenhouse for molecular studies to test whether the resistance genes were successfully inserted, and to establish that they do not deviate from the original variety.

Plants with up to three inserted resistance genes are able to defend themselves against Phytophthora.

Resistance management

Phytophthora is known for rapidly breaking down any resistance that is encoded by a single resistance gene. The origin of this extreme adaptive capability resides in its genome and its high reproductive capacity. Experiments and simulation studies have shown that resistance based on a stack of genes is much more difficult for Phytophthora to break down. Despite the robust design of the latter, however, it is assumed that the pathogen can also eventually overcome stacks of resistance genes if given the time and opportunity. To prevent or delay this, a resistance management strategy was developed on paper and tested in experiments, deploying resistance genes judiciously in time and space and using low doses of fungicides when necessary.

Stakeholders were kept regularly informed about the development of successful resistance management.

The DuRPh resistance management strategy builds on the preventive and integrative principles of Integrated Pest Management (IPM): robust host resistance, pathogen population monitoring and sanitation, combined with minimal use of fungicides as a last resort. Annual monitoring is used to detect and track adaptation in the pathogen population. The crop is not sprayed as long as two or more resistance genes are still effective. When only one effective resistance gene is left, a preventive low-input spray strategy is applied to protect the (still functional) resistance gene stack while a new effective stack is introduced. Reduced dose rates of protectant fungicides (e.g. 25% of the label-recommended dose rate) were proven to be sufficient to prevent the resistance gene stack from being overcome and the crop from being infected.

Communication and interaction with society at large

Research was monitored, discussed and adapted by an advisory board consisting of representatives from breeding companies, the Netherlands Farmers Organisation and the Netherlands Ministry of Economic Affairs. There were plenty of communications with other stakeholders through annual field demonstrations, articles published in newspapers and professional magazines, presentations on radio and television, at conferences and webinars, and, of course, in scientific publications. In this way, DuRPh scientists supplied relevant reports to the public to allow it to form an educated view of the role of cisgenesis as a tool for making potato farming more sustainable.

DuRPh scientists actively followed the communications around the project via activities such as monitoring DuRPh items in the news media. The press appeared to have a great deal of interest in the project developments. Although the fact that DuRPh uses genetic modification initially attracted lots of attention, the press eventually focused more on the aims and results of the DuRPh research.

DuRPh scientists demonstrated their research progress to the press and public every year.