



The first DuRPh field trials are encouraging



Not long ago, the results of the first field trials from the DuRPh research programme were made known. The programme involves tests whereby Wageningen scientists are looking at the possibilities of genetic modification in the fight against Phytophthora. Even though the first results are only the start of the programme, Anton Haverkort, the researcher and coordinator of the project is very hopeful about its continuation.

After starting the Durable Resistance programme against Phytophthora (DuRPh) with the appropriate laboratory tests in 2006, we were able to carry out the first field trials in Wageningen and in De Krim last year. We selected three varieties for these trials: Aveka, Désirée and Première. We had used only one single gene against Phytophthora for these three varieties. We could not use more than one because first we had to gain experience in cloning, transformation and selection. In

addition to the aforementioned field trial – in which we infect potato plants with Phytophthora – we also started a trial in which chemicals are used against Phytophthora. This is to check whether the new variant of the variety without Phytophthora remains exactly the same as the variety itself when fully grown. Moreover, we have also multiplied the material in jars in a gauze screen house, which will serve as seed next year.



Plant breeder Ronald Hutten (left) during an excursion in July 2008 shows healthy new R-gene containing plants and infected plants of the original variety.



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R-gene: difference between life and death

The trial in Wageningen was planted during April 2008 and infected with *Phytophthora* spores on July 1. Earlier infection is forbidden by the Hoofd Productschap Akkerbouw (HPA) (Main Commodity Board for Arable farming) on account of the significant risk of contamination in nearby potato fields. All trial plots were infected with a mixture of widely known *Phytophthora* strains. Via overhead irrigation, the foliage was being kept wet as much as possible, and after a month, the untreated early varieties were virtually completely dead, only the late maturing Aveka variety was able to hold on just a bit

longer. There were big differences in the degree of infection in the fields. The plants with a resistant gene (R-gene) from a wild species were still beautifully green, whilst the plants of the non-adjusted original variety, or the wild type, were affected.

Significant reduction in pesticides

It is clear that the approach, inserting an R-gene, works effectively and can lead to a significant reduction in the use of pesticides. What we also see is that such varieties can be of real value. However, it is necessary that resistance is lasting and

What is DuRPh and what is the objective

In 2005, the Dutch government decided to stimulate potato breeding and genetic modification in particular. From the Economic Structure Enhancing Fund – FES (natural gas revenues), 10 million euros were made available to Wageningen University to research a *Phytophthora*-resistant potato. The terms of reference for this research were to explore the possibilities that genetic modification offers, because the Dutch government feared that, in the long run, the seed potato sector would lose the competitive battle. Especially if companies in other countries (America, China), where consumers and environmental organisations have fewer objections to GMO crops, were to continue on the path of genetic modification. At this moment – in 2009 – there are already more than 130 million hectares of genetically modified crops worldwide (that is more than 30 x the surface area of the Netherlands). These crops are predominantly maize, soya and cotton, which have been made resistant to insects or pesticides such as glyphosate or glufosinate: transgenic crops with genes from other varieties than the plant variety itself (from the *Bacillus thuringiensis* bacteria or Bt, for example). Consumers in Europe have objections to these transgenes in particular. At all events, our chickens, pigs and cows are eating plenty of genetical-

ly modified feed from modified soya and maize from which oil and starch have been extracted for human consumption.

Stimulus for sustainable resistance

The government subsidy enabled us to give our aim for sustainable resistance a generous stimulus, as sustainable resistance had not yet been successful. This is due to the fact that the single gene introduction from the wild variety *Solanum demissum* was broken down by *Phytophthora* after a few years. This fear of broken down resistance also exists for prospective new varieties treated with a single gene from the wild *Solanum bulbocastanum* species. It is, however, not easy to combine both *Phytophthora* resistance and other characteristics such as colour of flesh and baking quality, as a result of which many varieties are many decades to more than a hundred years old. The programme is called DuRPh: Durable Resistance against *Phytophthora* (in dutch this means 'courage'), and is based on a number of principles.

The principles of DuRPh

DuRPh uses modern laboratory techniques, which accelerate the breeding process enormously, in this case genetic modification. In doing so, we only work with cisgeneses. This is a method whereby, when transferring genes, we



only use genes that are derived from wild potato species, the same genes as those that can also be used for normal crossing. The final product is therefore pure potato without genes from other crops. In doing so, we keep the varieties we have with all their historically-proven culinary characteristics. We want to avoid the risk whereby inserting a sin-



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will not be abruptly broken down. This means that gene stacking, resistance management in place and time, and a few other preconditions are needed. In the latter case, it is quite conceivable to consider patenting the R-genes, agreeing with breeders on the use of such genes, the way in which we have to register such new "old" varieties and how to regulate their seed potato supply. A seed potato company such as Averis has already started to use R-genes in a non-food starch variety. As from today, DuRPh still has seven years to go and if the trials of the basic principles outlined above are successful, we will definitely also find answers for the preconditions.

There remains the question of how the consumer and the legislator will deal with the acceptance of this form of genetic modification (cisgenesis). Is the final product only going to be judged on the process of its development, genetic modification, or will it be judged on its characteristics: only the potato. We will need to make considerable progress with DuRPh in the coming seven years on this point also. ●

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ective?



from a variety at different times and places in order to outsmart *Phytophthora* permanently. Furthermore, we develop marker-free plants, which do not contain any herbicide or antibiotics resistance at all. This is possible by making many transformants and by establishing whether they are resistant against all *Phytophthora* strains that we have available (that is, in fact, the "marker"). Moreover, we check in the laboratory with PCR whether the gene can, indeed, be found.

Ten years and five subsidiary projects

The DuRPh Project has a length of 10 years (2006-2015) and a budget of one million euros a year. There are five subsidiary projects. The first part involves the identification of R-genes in wild potato species. To do this, we cross with a susceptible variety and check whether the offspring roughly consists of very susceptible and very resistant genotypes. In the laboratory, the researchers "cut" this R-gene and multiply (clone) it with the *E. coli* bacterium. The second part involves the transfer (transformation) of the cloned genes to existing varieties. For our research, we use *Première*, *Désirée* and *Aveka*, three varieties with increasing lateness. In part three, we select the plants that were made in part two and have "rege-

nerated" or grown from a single cell, because they can show individual difference – although they are genetically identical. In this part, we select plants that contain the gene, are resistant (tests) and look exactly the same as the original variety. The latter is of importance so that the variety can be made flexible at a later stage. It is at this stage that permission to plant transformed plants in the field is requested at the Ministry of Environment and implementation of the field trials takes place. Part four involves resistance management or the management of the battle between the resistance mechanism of the plant and the attack strategy of the disease. Here we check how many genes need to be stacked and which different cassettes need to be inserted and removed, and used again later. We also research how the cassettes of genes can be inserted into, or how they should be divided among fields to minimise the chance of a break down. We also look at the *Phytophthora* populations in the Netherlands and in Europe to know which resistant genes are likely to be the most successful. The last part of DuRPh is what we are also doing now: communicate with and inform every interested party and stakeholder, so that they can form an opinion about our method of operation. ●

gle gene resistance is quickly broken down again, so we stack 3 to 5 genes from various wild varieties to make a cassette, which is subsequently inserted into a variety. To prevent this stacking from failing to last long enough, we introduce the concept of "dynamic variety", whereby we temporarily insert or remove sets of resistance genes in or