AFPP-6TH CONFERENCE ON ALTERNATIVE METHODS OF PROTECTION FOR AN INTEGRATED PRODUCTION LILLE-21st, 22nd AND 23rd OF MARCH 2017

BREEDING FOR LATE BLIGHT RESISTANCE IN POTATO

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ABSTRACT

Late blight, caused by the oomycete *Phytophthora infestans*, is one of the major pathogens of potato. The predominant method to control this disease is the frequent use of fungicides. Already in the beginning of the 20th century breeding for resistance was started, but soon after introduction of the first varieties, resistance was defeated and breeding shifted to obtaining partial resistance, which was supposed to be more durable. However, during the last decennia, several new resistance genes have been identified and cloned. Breeding for resistance has now moved to obtaining an optimal combination of different R-genes that result in a more durable level of resistance. The challenge for the breeder is to determine the optimal combination of R-genes in combination with all the other traits that are essential for modern potato production. The presentation highlights this development from the perspective of Desmazières, part of Agrico, a (Dutch) cooperative of seed potato growers.

Keywords: late blight, *Phytophthora infestans*, potato, resistance breeding, sustainability.

Introduction

Late blight is caused by the oomycete *Phytophthora infestans*. It is the most destructive disease of potato (*Solanum tuberosum*) worldwide (Fry and Goodwin, 1997). This pathogen can destroy a crop in a couple of weeks' time. It infects the foliage, stems and can also infect the tubers and fruit if not controlled by chemicals. This devastating disease became established in the 19th century in the US and Europe, when it caused the Irish potato famine (1845). It caused severe crop loss during several years and due to starvation and emigration the Irish population was halved in a couple of years (Fry, 2008).

Potato is the fourth most important food crop in the world, after rice, wheat and maize (Gebhardt, 2012). The crop is grown worldwide, with the exception of countries near the equator that lack cold agro-ecological zones in the highlands (Haverkort *et al*., 2009). At the same time, late blight is endemic in most potato-cropping areas (Pilet *et al.*, 2005). Currently, late blight is mainly controlled by chemical fungicides that need repeated application. During a growing season up to 16 rounds of spraying need to be applied, depending on the season and the earliness of the variety that is grown. As a consequence late blight control contributes to 10-20% of total production cost, based on the costs of the chemicals and their application (machinery, labor and fuel). In areas where growers do not have access to chemicals, yields tend to be 25% of those in Northern Europe or USA (Haverkort *et al.*, 2009). Despite the availability of chemicals, the spraying cycle can be disturbed by weather conditions, leaving the crop temporarily unprotected. Together with heavy disease pressure, late blight can still result in crop loss, either by shortening the growing season or by decreasing storability. Overall global yield losses due to late blight are estimated to be 16% (Haverkort *et al*., 2009).

History of resistance breeding

A good alternative for chemical protection is provided by resistance to the pathogen. The *Solanum* gene pool consists of about 150 tuber bearing species, of which a significant number of species contain late blight resistance (Vleeshouwers *et al*., 2011) . In the first half of the 20th century resistance breeding was started, mainly using the species *Solanum demissum*. This highly resistant Mexican species was supposed to contain 11 different R-genes, named R1 till R11 (Black *et al*., 1653). Later, new genes from other species were coded Rpi-xyzn, where *xyz* is the triletter code of the species involved and *n* is the number indicating the sequence of discovery (Van der Vossen *et al*., 2003). Unfortunately, soon after introduction or even during the breeding phase of the first resistant varieties, the resistance was defeated. Due to this disappointing result breeders stopped utilizing these R-genes, with the result that from *S. demissum* only R1, R3a/b and possibly R10, R11 and R2 were utilized in commercial varieties. By the 1970's, breaking strains for every known R-gene were present. A similar situation was found with the Ph genes from tomato (Malcolmson, 1969; Wastie, 1991).

Subsequently, until the 1980's chemicals were used as the sole measure to control late blight. Concerns about the environmental impact of fungicides triggered renewed interest in breeding. As a consequence of the knowledge of the initial failure of the use of R-genes, breeders were concentrating on quantitative or partial resistance. This type of resistance was believed to be more durable because of its supposed polygenic nature (Andrivon *et al*., 2003). It was searched for in wild species, but also in relatively old varieties, that were late maturing. Furthermore, breeding clones from the International potato centre (CIP)-program were used. However, this breeding strategy was rather unsuccessful, as resistance appeared to be strongly correlated with late maturity.

From 2000 on, a renewed interest in R-genes was established due to the discovery and introduction of Rpi-blb2 from *S. bulbocastanum* in the so-called ABPT material (Hermsen and Ramanna, 1973). In order to find new R-genes, extensive screening programs of potato gene banks for new sources of resistance have been conducted. This has resulted in the SolRgene database (Vleeshouwers *et al*.,

2011) . Late blight resistance was found in more than 20 different species, that originate from both Central and South-America. Among them were the sources that for a long time were known for their resistance under the high natural disease pressure in Mexico: *S. demissum*, *S. bulbocastanum*, *S. pinatisectum*, *S. stoloniferum*, but also relatively new genes originating from South-America like *S. berthaultii*, *S. microdontum*, *S. paucissectum* and *S. venturi*.

Disease assay and R-gene mapping

The screening for late blight resistance can be done using detached leaf assays (DLA's) or whole plant test by field screening. A detached leaf assay is a relatively quick lab-test, in which detached leaves of greenhouse grown plants are put in florist foam. Each leaf is inoculated with two drops of inoculum on the abaxial side and incubated under optimal conditions, i.e. with high humidity, sufficient light and optimal temperature (17˚C). After 6 days the leaves either show a small necrotic spot (HR) or are covered with mycelium (Figure 1). With this assay, a lot of R-genes were traceable. However, a few genes were only expressing resistance under field conditions. Good examples of such monogenic resistance genes that are only detectable under field conditions are Rpi-blb2, R8 and R9 and it has become clear that this class of R-genes should not be missed as they are indeed very valuable.

Figure 1. Example of a DLA. Individual leaves are inoculated with droplet of inoculum (left) and after 6 days incubation under optimal conditions, leaves either show HR or mycelium (right).

As soon as resistance is observed, segregating populations need to be developed by crossing a

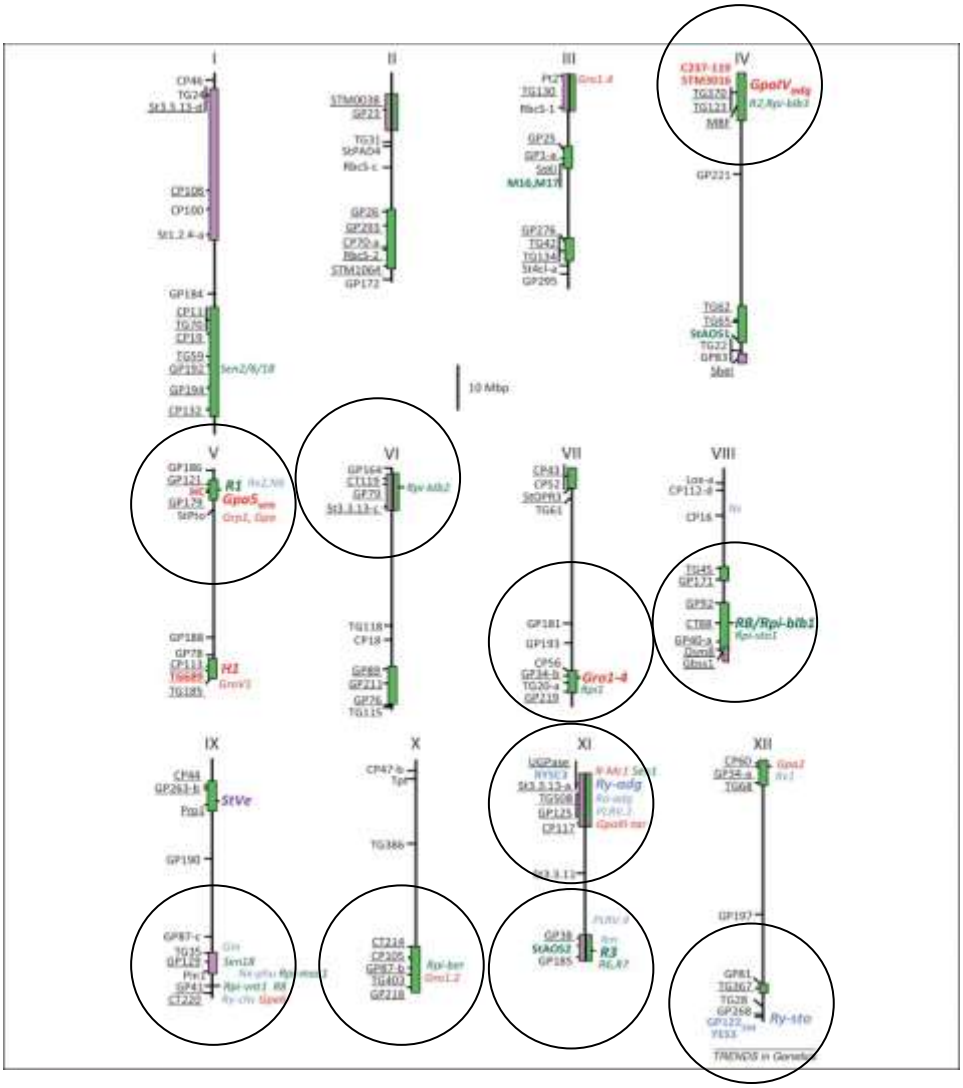
resistant genotype with a susceptible genotype. When the progeny shows clear segregation in a susceptible (S) and resistant (R) group, the population is suitable for determination of the position of the R-gene on the genetic potato map. This has led to the localization of several Rpi genes from wild *Solanum* species. Currently, all identified late blight R-genes seem to map to approximately 10 known R-gene clusters on the potato map (figure 2; Gebhardt, 2013). At the same time the potato genome sequence became available revealing the physical position of the R-gene clusters, indicated by the dense regions which overlap with the late blight loci (Jupe *et al*., 2012). There appears to be a couple of more positions where R-genes are located, but, so far, no late blight resistance genes were mapped in those clusters.

Naturally occurring resistance (examples from nature)

Up to now, intensive screening of the *Solanum* gene pool has shown that there is a big resource of natural Rpi genes available. Breeders question how to apply these genes in a durable and sustainable way, keeping in mind the negative results of application of single R-genes in the previous century. One way to deal with this is to consider the strategy of the different resistant *Solanum* species coping with late blight in their natural habitat. Thanks to intensive mapping work, it has become clear that resistance in wild species is based on the presence of two to at least six R-genes (Lokossou, 2010; Huang *et al*., 2004). The first and most curious example is *Solanum demissum*. This species is growing in an area known to contain a high natural infection pressure in the Toluca valley of Mexico. Although initially 11 R-genes were identified based on the differentials of Black (Black *et al*., 1953; Malcolmson and Black, 1966), up to 6 really different R-genes have been mapped so far, i.e. R1, R2,

R3(ab), R4, R8 and R9 (Ballvora *et al*., 2002; Li *et al*., 1998; Huang, 2005; van Poppel, 2010; Jo *et al*., 2011; Jo, 2013). The R3a gene cluster is complex and consists of several allelic versions of R3a, i.e. R3b, R6, R7, R10 and R11 (Huang, 2005; Bradshaw *et al*., 2006). Although previously mapped to the R3 cluster (Huang, 2005) differential R5 appears to be a stack of at least three other *S. demissum* genes R1, R2, R3b and possibly R8 (unpublished results) and it is therefore feasible that R5 as an individual R-gene does not exist. The mapped *S. demissum* R-genes are located in five different
R-gene clusters. Six of these *S. demissum* R genes (R1, R2, R3, R4, R7 and R8) have been found to occur simultaneously in a single genotype from a *S. demissum* accession (Huang *et al*., 2004). Another example of a resistant *Solanum* species is *S. x edinense,* which is a natural hybrid of *S. demissum* and *S. andigena,* and also native to Mexico. This species seems to handle *P. infestans* with at least three genes, i.e. Rpi-edn1, Rpi-edn2 and Rpi-edn3 (Verzaux, 2010). Based on mapping and effector studies it was concluded that Rpi-edn1 is an R2-homologue and Rpi-edn3 is identical to $R4^{Ma}$. The third gene, Rpi-edn2 also maps in a region where *S. demissum* genes reside, i.e. R8 and R9 on chr. 9 (Verzaux, 2010; Jo, 2013). Furthermore, these 3 Rpi-edn genes were found as a natural stack in a single genotype (Verzaux, 2010).

Figure 2. Genomic positions of R genes in potato. Circles indicate the R-gene clusters. Copied from Gebhardt, 2013. *Trends in Genetics* 29, 251.



The third example is an unrelated species *S. bulbocastanum*, also native to Mexico. Again in this species, three different R-genes were identified, Rpi-blb1, Rpi-blb2 and Rpi-blb3 (Van der Vossen *et al.*, 2003; Van der Vossen *et al.*, 2005; Park *et al*., 2005). Of these three genes, Rpi-blb3 is similar to R2, but the other two genes are completely different to the previous species both in gene and

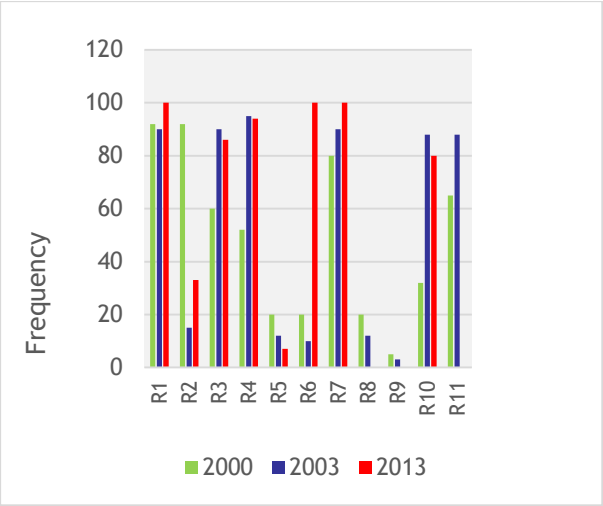
genome position. Although three R-genes are available, it was shown that natural stacking of two of these three genes in a single *S. bulbocastanum* genotype occurs with relative high frequency (Lokossou, 2010).

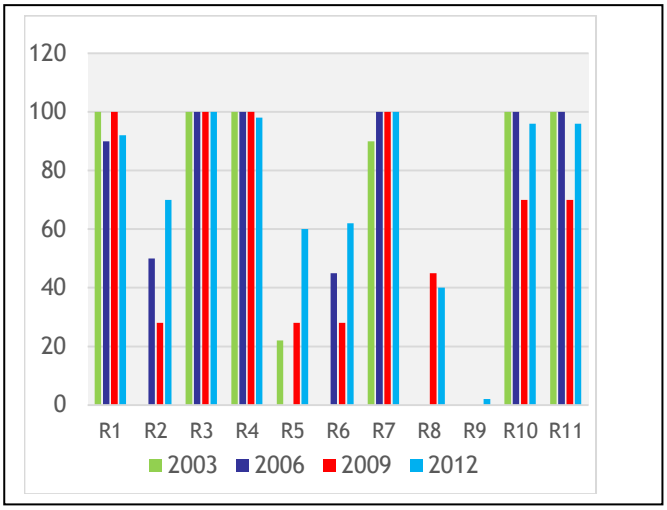
Based on the few examples that were just given, in combination with what is further known in literature, it can be concluded that species use at least 2 to 3 R-genes to combat late blight. These combinations can be strongly overlapping (*S. x edinense* and *S. demissum*), partly overlapping (Rpi-blb3 and R2) or completely different (Rpi-blb1 and Rpi-blb2). Besides the described three species R-genes from other species have been described or even cloned, like for instance Rpi-vnt1 on chr.9 from *S. venturii* (Foster *et al*., 2009), Rpi-ber1 on chr. 10 in *S. berthaultii* (Ewing *et al*., 2000; Rauscher *et al*., 2006)

Defeated R-genes

Based on experimental and practical research it has become clear that some of the *S. demissum* derived R-genes, like R1, R3a, R3b and R4 have no agricultural value when applied as a single R-gene in varieties (Fry, 2008). This is in agreement with several studies in which field collected *P. infestans* isolates were characterised. The majority of collected isolates contain virulence for these four genes (Andrivon, 1994; Lebreton *et al*., 1998). However, there are also examples of single R-genes, such as R2, that do seem to have a practical value when applied individually. A Dutch field study performed by our company showed that a little less than half of the isolates is avirulent for R2, indicating that R2 can still have a function depending on year and location. Similar results were obtained by a long-term field study called Eucablight (eucablight.org). In literature, it is often stated that defeated or "broken" R-genes have no practical value and that continued production of varieties that contain such genes in large areas will ultimately cause a fixation of virulence in the pathogen. While this might be the case for *S. demissum* genes R1, R3, R4, R7, R10 and R11 (Eucablight, 2003), the R2 gene is a nice example of a defeated R-gene that is grown in large areas and that still has some practical value. The R2 gene is present in several varieties, like for instance Innovator and Kuras. In 2015 the seed potato area of several R2 containing varieties grown in the EU exceeded 6000 ha. When looking at ware potato area this amount can roughly be multiplied by 10 to a total of 60.000 ha. Although this production area will predominantly be protected by chemicals, the population of *P. infestans* is exposed to a large area of R2-expressing hosts. A good example was the recent growing season of 2016, when a lot of infection could be observed in fungicide sprayed crops, either due to an extremely high infection pressure in combination with a fast growing crop or due to a disturbed interval of spraying as a consequence of bad weather conditions. Still, only half of the isolates seem to contain virulence for R2 (unpublished results). This is visualized in figure 3. Within the Eucablight project and within our company, monitoring of virulence for *S. demissum* R-genes has been performed over several years in the Netherlands (AR) and across Europe (Eucablight). It is obvious that certain virulences, as mentioned before, are present in almost all collected isolates, for example R1, R3, R4 and R7. However, there are also examples that show that it can fluctuate per year. For instance in the Netherlands, in 2000 most of the collected isolates appeared to be virulent for R2, while this was reduced to less than 20 percent 3 years later. Similar data were obtained in Belgium. This implies that although virulence is broken, certain R-genes can still have a function. Furthermore, avirulence for R2 still exists in the *P. infestans* population even in the presence of large areas of R2-containing varieties.

Figure 3. Monitoring virulence in the field in The Netherlands (left) and Belgium (right) over several years (data obtained from Eucablight database and unpublished results of Agrico Research).





Breeding for resistance at Agrico Research

At Agrico Research (AR), breeding for late blight resistance started round 1980 but was shifted towards the exploitation of R-genes at the end of the 20th century. Around 2000, efforts were remarkably increased to find and deploy new R-gene sources. While pre-breeding was in full progress, it was closely followed by attempts to map the corresponding R-genes. As a result AR played a substantial role in the cloning of Rpi-blb1 (van der Vossen *et al*, 2003), Rpi-blb2 (Van der Vossen *et al*., 2005) and to a lesser extend also to Rpi-blb3 (Park *et al*., 2005).

At the same time, markers were developed that could be applied in the introgression of these species. As a result of this intensive resistance breeding program, Agrico currently has six different varieties with late blight resistance on the market. At this moment, the resistance is merely based on single major R-genes, possibly supplemented with unknown minor R-genes. Of these six varieties, Toluca is already taken from the market as a big disadvantage was its lack of tuber resistance, which appeared to be a problem during organic cultivation. Furthermore, the consumers were not very fond of the rather white flesh color. Variety Athlete is only grown in the UK, where it is grown in a conventional way but with low input (with only few times spraying). However, the expectation is that its commercial production will diminish and disappear in a couple of years. Carolus is a very promising variety that is actually also grown in a conventional way. The variety is suitable for both making French fries and fresh consumption, and at the moment very popular in Sweden in the King Edward segment. The red eye of Carolus is a disadvantage for the processing industry and distrusted by Dutch consumers, while it has sufficient processing quality. Alouette is a very promising variety as well. At this moment it is mainly grown for the organic market, but when seed area increases, there are possibilities in the conventional segment as very promising results were obtained from trials in Central America and North Africa, where it performs well during short day conditions. The cultivars Twinner and Twister are new varieties that are not on the variety list yet. The expectation is that Twinner will be mainly grown organic and in private allotments, as the yield is too low for conventional growers. Twister has more potential for conventional growth and is suitable for the retail segment.

Long-term strategy

The long-term strategy of AR is to produce resistant varieties for all segments that can compete with existing varieties in the conventional market. While the current varieties are mainly based on single major R genes, current efforts are aimed to imitate nature. Either by combining major genes from different species (like for instance combining the R-genes from Toluca and Athlete) or by combining major and minor genes resembling naturally occurring combinations, (like they are present in

S. demissum or *S. bulbocastanum)*. A good example is the variety Sarpo Mira (Developed by Danespo recognized for a very good late blight resistance) for is that is known to contain at least five different R-genes of which at least four are derived from *S. demissum* (Rietman *et al.,* 2012). Sarpo Mira may be considered as a true durable potato variety.

Sustainability label

In order to describe the true nature of resistance of new and future varieties, in the future we could work with sustainability labels that resemble the so-called energy labels. For instance, a variety like Toluca that only has a single R-gene, that is functional but rather vulnerable could obtain label E, while a variety with 5 genes or more ultimately could obtain label A. Up to now, a good example of this last class is variety Sarpo Mira. Breeding however is not only about resistance. The obtained variety should be able to compete with the already existing varieties. Thus, next to the challenge of obtaining durable resistance based on stacking the optimal combination of R-genes, the variety should also perform well on all other traits that are essential for modern potato production.

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