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Presentation

Monitoring the Dutch *Phytophthora infestans* population for virulence against new R-genes

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SUMMARY

New possibilities offered by marker assisted breeding and GMO breeding have sparked renewed international efforts to breed for durable potato late blight resistance. *Phytophthora infestans* is however known for its adaptability, a trait confirmed by recent discoveries on the structure of the *P. infestans* genome. One of the possibilities to enhance the durability of newly introduced host resistance is to monitor the pathogen population for virulence to new *R* genes, prior to - and after their introduction. The late blight control strategy should be adapted accordingly.

The Dutch *P. infestans* population was monitored during the growing seasons 2006 – 2008. *P. infestans* isolates were collected from blighted production fields and from bait fields in which *R* gene containing potato clones were grown without fungicide protection.

A selection of the *P. infestans* isolates collected were characterized for virulence to a range of new *R* genes using a detached leaf bio-assay. Virulence for all single *R* genes tested was found. When we focus on *R* genes *Rpi-blb1* and *Rpi-blb2*, no virulence was found in 2006. One *Rpi-blb1* virulent isolate was found in 2007. Another 2007 isolate was found to be virulent to *Rpi-blb2*. Depending on the genetic background in which *Rpi-blb1* was placed 13 or 21 isolates were virulent in 2008. Depending on the genetic background in which *Rpi-blb2* was placed 4 or 11 isolates were virulent in 2008. One isolate was found to infect the stacked *Rpi-blb1* and *Rpi-blb2* resistance genes in a detached leaf assay.

From these findings it is recommended that monitoring systems should be part of future potato late blight control strategies. The resulting information on the dynamics of virulence within the local *P. infestans* population can then be used to enhance the durability of newly introduced host resistance.

KEYWORDS

Solanum tuberosum; potato; late blight; *Solanum bulbocastanum*

INTRODUCTION

Phytophthora infestans, the causal organism of potato and tomato late blight, is one of the most important and destructive diseases of potatoes. Frequent fungicide applications are necessary to grow modern high yielding potato cultivars which are mostly susceptible to potato late blight. The resulting high fungicide input has a negative effect on the economic feasibility of the crop and on the environment. Development and application of cultivars with a high level of resistance to potato late blight is therefore highly desirable.

In the past *R* genes derived from *Solanum demissum* were used for breeding potato late blight resistant potato cultivars, but resistance proved not to be durable since *P. infestans* was able to adapt and break these *R* genes in rapid succession (e.g. Van der Plank 1971, Turkensteen 1993).

At present, many different *Solanum* spp. are used in classical and molecular breeding programs aiming to produce durably resistant potato cultivars. *S. berthaultii, S. bulbocastanum*, *S. stoloniferum*, and many other *Solanum* spp. are part of these efforts.

However, recent discoveries on the *P. infestans* genome (Haas & Kamoun *et al.* 2009) combined with the past experience with *S. demissum R* genes make it highly likely that *P. infestans* is also able to adapt and quickly overcome also these new *R* genes. Additional, durability enhancing, measures such as stacking *R* genes and low input chemical control strategies specifically designed for resistant cultivars are explored by e.g. the DuRPh project (www.DuRPh.wur.nl). Monitoring of the local *Phytophthora infestans* population for new virulences, by e.g. screening for effector variation, allows early detection of adaptation within the *P. infestans* population which gives the possibility to adapt the control strategy to the new situation.

In this paper we summarize the results of monitoring the Dutch *P. infestans* population for virulence to new *R* genes from 2006 till 2008.

MATERIALS AND METHODS

Isolate collection and storage

Over the period 2006-2008 *P. infestans* isolates were obtained from infected leaf samples collected from two different sources: infected growers fields, dumps and volunteer potatoes from all over The Netherlands and from bait fields established at three locations (Lelystad, Valthermond and Vredepeel) in the Netherlands. Bait fields contained 50-100 different *Solanum* genotypes (susceptible and resistant cultivars, Black's *S. demissum* R1-R11 differential set, advanced breeding lines and wild *Solanum* spp). Fungicides were not applied on bait field, thus genotypes were subjected to local infection pressure. Infected plant parts were collected weekly and used to obtain *P. infestans* pure cultures. Severely infected plants were removed entirely from the bait fields. The isolates were included in the Plant Research International *P. infestans* culture collection and were stored as sporangial suspensions in DMSO in liquid nitrogen.

Isolate characterization

P. infestans isolates were genotypically characterized using twelve microsatellite markers (SSR) as described by Li *et al.* (2010). SSR data were used to assign the isolates to SSR groups. From the most prominent SSR groups at least 1 isolate was selected for the detached leaf virulence assays. The number of isolates tested was 18, 38 and 31 for 2006, 2007 and 2008 respectively. Isolates were characterized for virulence against a set of *R* genes using *Solanum* accessions (wild *Solanum* spp.) with a known *R* gene content and *R* gene containing genetically modified *Nicotiana benthamiana* and *S. tuberosum* cv Desiree material as *R* gene differentials. The replicated differential experiments contained two leaflets per differential x isolate combination per petri dish and two petri dishes per replicate experiment. Leaflets were inoculated by spraying them with a sporangial suspension of

20.000 sporangia per ml of the appropriate *P. infestans* isolate. Petri dishes were incubated at 15 °C and a 16h/8h light/dark regime. Severity, as measured by the leaf area covered by necrotic lesions (0 – 100%), and sporulation (0, 1 or 2 for "no sporulation", "low level sporulation" and "high level sporulation" respectively) was assessed visually after 1 week incubation.

RESULTS

Virulence patterns
894 *P. infestans* isolates originating from infected field crops, waste piles, volunteer potatoes or bait fields were collected during the 2006-2008 period. Of these isolates, 562 were SSR genotyped. The virulence spectrum was determined for a selection of isolates after SSR genotyping. Overall, it was shown that virulence for all *R* genes tested was found using the detached leaf-assay although some virulences were rare in the Dutch *P. infestans* population so far.

Rpi-blb1 and Rpi-blb2
As an example we focus on two of the most commonly used *R* genes, *Rpi-blb1* and *Rpi-blb2*. Both genes originate from *S. bulbocastanum* and were crossed into *S. tuberosum*. Virulence for both *Rpi-blb1* and *Rpi-blb2* was first observed in 2007. One isolate (NL07377) was able to overcome *Rpi-blb1* and another isolate (NL07434) was able to overcome *Rpi-blb2*. Isolate NL07337 originated from a RHO3-424 clone. Isolate NL07434 originated from a naturally infected potato clone with an ABPT background. Both isolates were collected in bait fields at Valthermond in the North East (starch potato area) of the Netherlands.
In 2008 more virulent isolates were found for the two *S. bulbocastanum R* genes. In total 13-21 isolates were found with the virulence for *Rpi-blb1*, depending on the genetic background of the R-gene differential used. In total 4-11 isolates were found with virulence for *Rpi-blb2*, also depending on the background of the R-gene differential. Thus an increase in virulence for both *Rpi-blb1* and *Rpi-blb2* was found during the survey period. Furthermore, one isolate was virulent to both *Rpi-blb1* and *Rpi-blb2* separately and was also shown to infect the stacked *Rpi-blb1* and *Rpi-blb2* genes. This strain was isolated from a *S. venturii* clone at Vredepeel in the South East of The Netherlands in 2008.

DISCUSSION

P. infestans virulence pattern
P. infestans was isolated from bait fields and farmers fields. To establish the virulence patterns of a selected number of isolates, bio-assays were carried out on R-gene containing *Solanum* genotypes.
In general, virulence for almost all *R* genes tested was found in the bait fields and virulence for all R genes tested was found in the detached leaf bio-assays and thus in the Dutch *P. infestans* population although some virulences were rare. In our bait fields and bioassays virulence was also found for both *Rpi-blb1* and *Rpi-blb2*, both alone (bait fields and detached leaf assays) and in combination (detached leaf assays only). These two R genes belong to a small group of intensively used R-genes in current breeding programmes.
Commercial cultivars containing *Rpi-blb1* are not grown in The Netherlands during the survey period. Commercial cultivars containing *Rpi-blb2* are rarely grown in The Netherlands during the survey period. Yet virulent strains to both R genes were found, suggesting that these "new" virulences were already present in the Dutch *P. infestans* gene pool or they were the result of on site mutations, in response to a low level selection pressure.

R gene break through

Cultivars with new *R* genes are introduced at present. These new introductions are especially beneficial to organic farming since this is the primary market for resistant cultivars at this point in time. In the past *Phytophthora infestans* has shown to adapt very quickly to *R* genes originating from *Solanum demissum* (Turkensteen, 1992). It is now known that effector genes, the natural target for R-genes, are localized in highly dynamic and expanded regions of the *P. infestans* genome (Haas *et al.*, 2009). Taking both aspects into account it is thus highly likely that *P. infestans* is also able to adapt to new, non *S. demissum, R* genes which are used in modern breeding programmes.

If we assume that *P. infestans* generates natural variation within the effector gene family, an R gene break through could occur as follows:

Imagine two potato fields close to each other. A susceptible cultivar is grown in field 1 and is heavily blighted. An unprotected (not sprayed with fungicides) resistant cultivar is grown in field 2. Theoretically, up to $4x10^{12}$ *P. infestans* spores can be produced per ha of potato. These billions of spores are produced on the infected (susceptible) cultivar, transported through the atmosphere and deposited (in part) on the neighboring fields (Skelsey *et al.*, 2008), including field 2. Even with a very low mutation frequency it is likely that the natural variation of effector genes results in a few individuals (sporangia) that are virulent on the R-gene "planted" in field 2. If the spores produced in field 1 are deposited on the unprotected resistant cultivar in field 2, virulent genotypes are selected, with a late blight epidemic and a broken R gene as the result.

R-gene deployment strategies

As a logical consequence of the above, we should look into measures capable of enhancing the durability of host plant resistance. For future growing systems it is therefore recommendable to use a combination of multiple (stacked) host resistance genes with a low input protective spray strategy to protect the investment into the creation of host plant resistance and to benefit rentability of the crop and the environment. Also, effort must be put in preventing situations where massive amounts of *P. infestans* spores are allowed to be produced in order to minimize the number of undesired mutations.

CONCLUSIONS

Virulence for new *R* genes (*Rpi-blb1* and *Rpi-blb2*) was found in the Dutch *P. infestans* population. Multigenic resistance is more durable than resistance based on single genes but it must be assumed R-gene based resistance can be broken by *P. infestans.* Additional control measures are therefore needed to significantly enhance the durability of host plant resistance. These additional measures aim to prevent development of large *P. infestans* populations and aim to prevent selection of virulent *P. infestans* genotypes on resistant cultivars by applying (low input) fungicide applications during periods with high infection pressure. The combination of these additional control measures with already established control methods should be able to minimize the risk for R gene break through and minimize the consequences of R-gene break through once it has occurred thus enhancing the durability of newly introduced host plant resistance.

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