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Debate on the Exploitation of Natural Plant Diversity to Create Late Blight Resistance in Potato

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Abstract This paper reports on a debate on intriguing propositions relating to the scientific, agronomic, societal and economic impact of the BIOEXPLOIT project, focusing on late blight resistance in potato. It discusses (i) whether identifying pathogen effectors will facilitate selecting durable *R* genes, (ii) whether breeding for durable late blight resistance requires deploying *Rpi* (for Resistance to *Phytophthora infestans*) genes, (iii) whether breeding strategies and cultural practices determine the durability of new resistance genes, (iv) whether marker-assisted breeding for *Phytophthora infestans* resistance is already in the stage of adoption, (v) to what extent genetically-modified organism technology can advance realizing late-blight resistant potato cultivars, and (vi) whether modifying *R* genes will result in novel broad spectrum resistance.

Keywords Breeding strategy · Cisgenesis · Durable resistance · Effector molecules · Genetic modification · Late blight · Marker-assisted breeding · *R* genes

Introduction

After the presentation of the progress within the BIOEXPLOIT project and an overview on the different strategies to combat late blight, the potato scientists present in the workshop in Brasov, Romania, 8 July 2008, on which this special issue reports, debated on the following six propositions:

1. Identification of pathogen effector molecules will facilitate the selection of durable *R* genes.

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2. Breeding for durable late blight resistance in potato requires deployment of *Rpi* (for Resistance to *P. infestans*) genes.
3. The durability of new resistance genes against late blight is determined by breeding strategy and cultivation practice.
4. How far is practical marker-assisted breeding for *Phytophthora infestans* resistance?
5. Things go much faster with genetically-modified organism (GMO) technology.
6. Modification of *R* genes will result in novel broad spectrum resistance.

This contribution highlights some of the current opinions on these issues without aiming at providing an accurate overview of all the views of the contributors to the debate. We focus on resistance against late blight in potato.

Identification of Pathogen Effector Molecules will Facilitate the Selection of Durable *R* Genes

Phytophthora infestans is a highly adaptable and rapidly evolving pathogen. Therefore resistance against this pathogen is easily overcome. There is a need for more durable resistance. A recent paradigm shift based on the effector molecules which the oomycete requires to become pathogenic might help in realizing that.

Effector molecules (or "effectors") bind to a protein and by doing so they change the activity of that protein. Birch et al. (2008) suggested that understanding the diversity in effector molecules in oomycete populations, in combination with understanding effector expression and function, can become essential tools in searching for sources of resistance that are more durable than the common ones. Their reasoning is that conserved and functionally non-redundant effectors that are required for the pathogen's pathogenicity could help to identify the weak spots of the pathogen. They further reason that deploying cognate *R* genes that are capable of recognising these effector molecules could result in a more durable form of resistance than can be achieved while deploying *R* genes that target non-conserved and functionally redundant effectors. In this reasoning, Birch et al. (2008) assume—as pointed out by Hein et al. (2009, this issue)—that "additional effectors from the pathogen do not interfere with the recognition event, or with processes downstream of the recognition event that lead, for example, to the hypersensitive response".

The availability of pathogen effector molecules will definitely facilitate the selection of durable resistance by allowing the recognition specificity of resistance (*R*) genes being determined at a molecular level. This requires research aiming at identifying and studying pathogen-derived effector molecules. This type of research occurs in several BIOEXPLOIT sub-projects. *P. infestans* effector molecules derived from these studies are now being increasingly incorporated into the characterisation of *R* gene specificity (see Hein et al. 2009, this issue). Expectations are that these effector molecules will also prove extremely useful in identifying *resistance mechanisms*, some of which might have the potential to be durable (Hein et al. 2009, this issue).

Breeding for Durable Late Blight Resistance in Potato Requires Deployment of *Rpi* Genes

Pyramiding major resistance genes has been discussed as an option to improve the durability of the resistance against late blight, but all major *R* genes have already been broken. Stacking minor resistance genes is not really an option in combating late blight as this will not result in a resistance level which is high enough.

It is claimed that the *Rpi-blb1* gene from *S. bulbocastanum* confers full resistance to complex isolates of *P. infestans*. Until recent no race specificity has been demonstrated for this gene. This suggests that this type of resistance is durable, although latest reports from laboratory experiments urge for caution. Proper management of this resistance gene is therefore essential, both in breeding programmes and in the practice of potato cultivation, and especially under organic conditions.

Within the BIOEXPLOIT project *Rpi-blb1* homologues have been found in *S. stoloniferum, S. papita, S. polytrichon* and *S. fendleri* (Hoekstra 2009, this issue). Several *Rpi* genes, notably *Rpi-mcd1* and *Rpi-dmsf1* (Hein et al. 2009, this issue), have been associated with field resistance to late blight. This type of quantitative resistance is essential as it is assumed to be race non-specific and polygenically inherited. However, the studies in BIOEXPLOIT until now have shown that one should not be overly optimistic about the availability of *Rpi* genes. The number of distinct functional *Rpi* genes that are available might be smaller than was suggested in the past and certainly than the number hoped for. Therefore, we need to employ all available taxonomic information when designing programmes to introgress *Rpi* genes into cultivated potato (Hein et al. 2009, this issue).

But still, there is a wide array of potato wild species available to breed for late-blight resistance, with most resistant clones present in the species *S. berthaultii, S. bulbocastanum, S. hougasii, S. brachistotrichum, S. trifidum* and *S. stoloniferum* (Hoekstra 2009, this issue). Specific clones from *S. tarijense* and the cultivated diploids *S. phureja* and *S. stenotomum* might also prove useful sources of resistance (Hoekstra 2009, this issue).

Durability of New Resistance Genes Against Late Blight is Determined by Breeding Strategy and Cultivation Practice

Haverkort et al. (2008; 2009, this issue) indicated that applying new scientific knowledge brings resistant potato varieties within reach. This involves a new paradigm of what durable resistance may imply (see also above) and how it can be used in combination with new ways to strategically manage resistance at the field, cropping system and landscape level. Many scientists claim that genetic modification of potato is a significant part of a breeding strategy aiming at the final solution of the late blight problem, preferably with cisgenes (Jacobsen and Schouten 2008), although there is significant resistance especially from organic agriculture where the late blight problem is most severe (Lammerts van Bueren et al. 2008). This is further elaborated below.

Haverkort et al. (2008) also indicated that intense monitoring of the current Dutch *P. infestans* population has shown that the virulence spectra for currently employed *R* genes are very complex. The current Dutch *P. infestans* population is also being monitored for presence and frequency of virulence against new resistance genes, to be introduced as transformation material, in order to optimize the breeding strategy and the resistance management.

Haverkort et al. (2008; 2009, this issue) also claim that transformation of a potato cultivar using different cassettes of resistance genes allows diversifying resistance within and between fields, without agronomical disadvantages. Such a diversification at different levels could also be instrumental in avoiding or at least delaying adaptation of the *P. infestans* population to this new resistance. This issue is currently investigated in the Netherlands under the DuRPh project by combining simulation studies and field experimentation to analyze different options for spatial and temporal diversification of resistance under various agronomic conditions (Haverkort et al. 2008). Strategies will be selected and these will subsequently be tested in the field during several years in order to assess whether these strategies are capable of reducing infection risk and slowing down the development of potato late blight epidemics (see also Skelsey et al. 2005). Also in the BIOEXPLOIT project a lot of attention is being paid to the proper management of resistance (see also Haverkort et al. 2009, this issue).

Employing diverse stands is also a strategy that might by useful in organic potato production (Finckh et al. 2007), especially since, in that niche market, genetic mixtures of tubers might be considered a positive asset in the eyes of buyers of fresh potatoes. Diverse potato stands may be created in the form of randomly mixed varieties, alternating rows or strips of varieties or strip intercropping of potato with other crop species (Finckh et al. 2007). Intimate mixing has by far the largest effect. Intimate mixing of potato varieties should be associated with the maximum diversification of race non-specific resistances and of major gene resistances.

How Far Advanced is Practical Marker-assisted Breeding for *Phytophtohora infestans* Resistance?

In general, DNA marker technologies can be used to identify and map genes, or specific alleles of those genes. In this way, it is possible to select for the desired genotype in a rapid and cost-effective way. Marker-assisted breeding can be a necessary instrument to achieve pyramiding of resistance genes in potato within a reasonable time. Marker-assisted breeding at the current stage of the technique and its economics has the potential to stack resistance genes but also to help to eliminate susceptible genotypes in an early stage of the breeding programme. It will also facilitate including resistance traits in varieties with a good performance with regard to other important traits including yield and quality traits.

In the BIOEXPLOIT project there is special attention to marker-assisted breeding for disease resistance in potato in sub-project 5 (see Carrasco et al. 2009, this issue). This sub-project has two work packages. In the first work package, it is tried to develop high-throughput marker technologies and molecular markers associated with disease resistance loci for commercial marker-assisted breeding and to validate these

technologies and markers. The sub-project tries to achieve this objective by designing and validating high-throughput marker technologies on the basis of converting existing markers into usable high-throughput applications (Carrasco et al. 2009, this issue). In the second work package, major *R* genes and/or quantitative trait loci (QTLs) will be stacked into elite materials applying marker-assisted breeding techniques (Carrasco et al. 2009, this issue). To realize this stacking, elite material and lines carrying *R* gene sources and QTLs have been crossed and individual plants in segregating populations have been screened using molecular marker techniques within the framework of sub-project 5 of BIOEXPLOIT. Part of the sub-project is also to carry out field tests under diverse conditions in order to assess the effectiveness of the various sources of resistance.

Genetically-modified Organism (GMO) Technology Greatly Advances Resistance Breeding

Many desirable traits in potato, including resistance against *Phytophthora infestans* and other pathogens, are obtained from wild *Solanum* species. The classical way to introduce such traits is through introgression breeding. Introgression breeding can be associated with a lot of linkage drag and in the case of the self-incompatible tetraploid potato the heterozygous nature of the varieties cause an additional bottleneck (Jacobsen and Schouten 2007). Many backcrosses are required to remove the linkage drag.

Genetic modification (GM) can be of help because specific new traits can be inserted into existing cultivars without linkage drag, albeit that the position of the new genes in the genome cannot be manipulated accurately and the expression level of the new gene inserts can vary manifold. Genetic modification can accelerate incorporating or stacking resistance genes from wild *Solanum* species into commonly grown cultivars compared with stacking through the classical way of introgression breeding using wild species containing *R* genes. Based on the source of the new genes genetic modification techniques are classified into transgenesis and cisgenesis. Transgenesis uses genes from viruses, bacteria or other non-crossable sources or even synthetic genes. In the case of potato, cisgenesis uses naturally occurring potato genes or genes from crossable species that are or can be used in current breeding programmes and with which potato can make natural crosses (Haverkort et al. 2008). Because backcrossing and selection is no longer necessary the major remaining technical bottlenecks are detecting, isolating and combining useful *R* genes, activities which are well under way in BIOEXPLOIT.

However, there are also societal bottlenecks. Many scientists have the opinion that cisgenesis is more likely to be acceptable than transgenesis (e.g., Jochemsen 2008). Some even argue that with the advent of cisgenesis a reconsideration of the rules for GMOs as described in Directive 2001/18/EC (Anonymous 2001) is necessary (Jacobsen and Schouten 2008) and that cisgenic plants should be exempted from the timely and costly procedures in testing GMOs, simply because there are no alien genes in the end product. At this stage the positions of the different groups of stakeholders are moving into the direction of accepting cisgenic approaches as part of traditional breeding.

The recent rapid progress in mapping and isolating newly discovered *P. infestans* resistance genes in the BIOEXPLOIT project will also be used more effectively when the cisgenesis route will be accepted as part of traditional breeding. This route greatly facilitates the rapid deployment of new resistances against *P. infestans*, either singly, in combinations or as 'mixtures', as indicated by Hein et al. (2009, this issue). Cisgenesis might give the potato breeding community the tool to finally combat *P. infestans* and stay ahead in the race against the oomycete. However, at this stage it is highly unlikely that cisgenesis will be accepted by the community of organic agriculture (Lammerts van Bueren et al. 2008).

Modification of *R* genes Will Result in Novel Broad Spectrum Resistance

In some parts of the world, wild *Solanum* species and *P. infestans* have co-existed for ages. The co-evolution of potato species and the oomycete resulted in a complex and "multi-layered defence system" in the potato (Slootweg et al. 2009, this issue). Some wild *Solanum* species in these regions show broad spectrum resistance against late blight. Genes conferring broad spectrum resistance may code for different molecular mechanisms to combat *P. infestans* and therefore may provide a durable and effective late blight resistance. It can be debated whether modifying *R* genes will result in novel broad spectrum resistance.

Some of the effector proteins which are used by pathogens to overcome the recognition systems of the plant can be recognized in resistant plants by so-called Resistance proteins (R proteins) (Slootweg et al. 2009, this issue). As soon as the plant has recognized the effector proteins, the plant cell will initiate certain defence responses, which usually cause local cell death, but in some cases can also stimulate a systemic acquired resistance response (Slootweg et al. 2009, this issue). There is scope for using these natural mechanisms of defence with the purpose of creating resistance against late blight. But in order to be able to do that we need much more knowledge and understanding of how these R proteins function and how they play their role in broader defence system of the plant (see Slootweg et al. 2009, this issue).

Modifying *R* genes may create the possibilities to change the resistance proteins and thereby may change the basic defence responses. Recently, it has been shown that in vitro induced subtle changes in the recognition domain of the Rx1 protein in potato not only result in a broader spectrum of disease resistance specificity to multiple strains of *Potato virus X* but also in broadening its resistance specificity to the distantly related *Poplar mosaic virus* (Farnham and Baulcombe 2006). This example demonstrates that it might be feasible to create novel types of broad spectrum resistance against late blight, a disease that takes so much of our resources and induces so many societal costs. It is time we find the tools to control it effectively.

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