Presentation

Fortuna et al.

Status and perspectives of GM approaches to fight late blight

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SUMMARY

The development of potatoes resistant to *Phytophthora infestans* via introduction of resistance (R) genes through genetic modification (GM) offers significant advantages over traditional breeding approaches. The different GM approaches of Wageningen University (the DuRPh project), of the Sainsbury Laboratory at the John Innes Centre in Norwich, and the development of Fortuna at BASF Plant Science Company, Limburgerhof, Germany are outlined.

The Phytophthora resistant potato Fortuna was developed by Agrobacterium-mediated transformation of the potato variety (var.) Fontane with two resistance (R) genes, R*pi-blb*1 and R*pi-blb*2, isolated from *Solanum bulbocastanum*. These genes encode BLB1 and BLB2 proteins, respectively, and impart plant resistance to late blight disease caused by *P. infestans*. Apart from the late blight resistance trait, Fortuna displays the same agronomic and tuber quality characteristics as its mother variety Fontane.

KEYWORDS

Phytophthora infestans, genetic modification, resistance genes, R-genes, *Solanum bulbocastanum*, Fortuna

INTRODUCTION

Potato late blight caused by the oomycete *Phytophthora infestans* is the most devastating potato disease. This fungus thrives best under wet and cold weather conditions and, if untreated, is able to destroy entire harvests. It is estimated to account for a destruction of 15% of the global potato production (International Potato Center, CIP). Most potato farmers in the European Union (EU) protect their crop against this disease through frequent (up to 16) fungicide applications per growing season (Haverkort *et al*., 2008). The protection against late blight causes by far the highest costs and requires the biggest efforts of the potato farmers as compared to the protection against other pests and diseases. Annual costs of potato late blight, i.e. of plant protection and damage in the EU are estimated to amount to about one billion Euro (Haverkort *et al*., 2008). Due to its huge genome with a lot of repetitive sequences, its short generation cycle and its ability to mate, *Phytophthora infestans* is evolving rapidly and has a strong ability to develop resistances to fungicides.

An alternate defence strategy via the development of late blight resistant cultivars therefore represents a major goal in potato breeding. The introgression of R-genes (resistance genes) from wild potato species resistant to late blight represents a major path since the 1930ies (Turner, 2008). However, for different reasons this approach so far has yielded only limited success:

(1) To cross *Solanum tuberosum* with wild potato species is often difficult and requires in some cases bridge crosses involving a third *Solanum* species.
(2) Wild potato species are typically small plantlets with low agronomic performance. In classical breeding some of these characteristics are typically co-segregating with the desired trait, rendering the progeny non-competitive based on their poor agronomical properties.
(3) Oftentimes race-specific R-genes have been introgressed that, in contrast to the broad-spectrum R-genes, proved not to confer durable resistance.
(4) Classical breeding methods typically yielded progeny containing single R-genes, which, especially when race-specific, have been overcome quickly by the evolving pathogen.

MATERIALS AND METHODS

Several test systems exist to test the resistance of potato cultivars against late blight either in the field (natural or artificial infection), the greenhouse, and or on isolated potato leafs in the detached leaf assay (Wang *et al.*, 2008, El-Kharbotly *et al.*, 1994). However, observations on effects made either via the detached leaf assay or on greenhouse grown plants need to be verified and validated in a field experiment, before valid conclusions can be drawn on the occurrence of resistance and its relevance for potato cultivation. For resistance conferred by the R*pi-blb*2 gene, resistance typically cannot be proven by the detached leaf assays beyond doubt and field evaluation is crucial (van der Vossen *et al.*, 2005).

RESULTS

Fortuna

The Phytophthora resistant potato Fortuna has been developed by introducing two R-genes, R*pi-blb*1 (van der Vossen, 2003) and R*pi-blb*2 (van der Vossen, 2005), from the wild potato species *Solanum bulbocastanum* into the broadly used European processing potato variety Fontane with the help of genetic modification.
To generate Fortuna, the R*pi-blb*1 and the R*pi-blb*2 genes were combined under control of their native regulatory elements. The resulting vector construct contained the genomic sequence of the R*pi-blb*1 gene under control of the native R*pi-blb*1 promoter and R*pi-blb*1 terminator, all derived from *S. bulbocastanum*, in combination with the genomic sequence of the R*pi-blb*2 gene under control of the native R*pi-blb*2 promoter and R*pi-blb*2 terminator all from *S. bulbocastanum*. The broadly used commercial potato variety Fontane was transformed with the R*pi-blb*1: R*pi-blb*2 double gene construct by using conventional Agrobacterium-mediated transformation using imidazoline resistance as selection marker.

The performance of the late blight resistance trait in Fortuna was tested in field trials at locations representative for potato cultivation, such as The Netherlands, Germany, United Kingdom, Czech Republic, and Sweden. In the absence of natural infection, and in order to further increase disease pressure, artificial infection using mixed local isolates of *Phytophthora infestans* were applied in the field. In over five years of testing *Phytophthora infestans* resistant potato lines in the field, late blight disease on potato lines expressing the combination of the R*pi-blb*1 and R*pi-blb*2 genes was never observed.

In addition, the resistance of potato lines expressing the combination of the R*pi-blb*1 and R*pi-blb*2 genes was tested in the greenhouse against a large collection of *Phytophthora infestans* isolates from all over the EU. Until today no isolate tested was able to overcome the resistance of Fortuna.

COMPARISON OF THE MAJOR GM APPROACHES TOWARD LATE BLIGHT RESISTANCE IN EUROPE

DuRPh project

The DuRPh (Durable resistance against Phytophthora) project is run by Wageningen University and Research Center, funded by the Dutch natural gas resources (national investment in innovation). The project focusses on cloning and analyzing from wild potatoes, testing R-genes in field trials, and developing resistance management strategies.

First field trials with Phytophthora resistant GM potatoes were performed in 2007, the trials are located at different sites in the Netherlands and at one site in Belgium. In 2011 the following R-genes were tested in the field trials: R3a, Sto1, Blb3, *Vnt*1 in single gene constructs and in double and triple stacks.

DuRPh employs cisgenic, marker-free genetic modification, and fosters the concept of dynamic cultivars to increase the sustainability of R gene mediated resistance.
In order to offer farmers access to DuRPh results requires the following steps: Variety development through breeders of the DuRPh consortium, the target varieties need to be decided by consortium members. Currently it is not clear by when such varieties could be launched, since the development of such varieties by the DuRPh consortium would require a change in the existing GM regulations, which would decrease the safety assessment requirements for cisgenic crops. As with all approaches, market acceptance will be required for a successful launch.

The Sainsbury Laboratory's approach

The project at the Sainsbury Laboratory is funded by the UK's Biotechnology & Biological Sciences Research Council (BBSRC). Its scope encompasses cloning and analyzing of R-genes from wild potato species and testing of these genes in potato field trials.
First field trials took place in 2010 at one site in the UK. In 2011 the *Vnt1* and *Mcq1* genes were tested in the field. The genes are introduced by standard transformation methods using an NPT2 resistance marker conferring kanamycin resistance to transformed cells in tissue culture.
The envisioned path to the farmer requires either the joint development of varieties in a public/private partnership or the outlicensing of R-genes to third parties. Goal in a public/private partnership would be to use the varieties Maris Piper and Desiree. The time of market launch can only be predicted once a commercial partner has been identified.

The BASF Plant Science approach

The project is funded by BASF SE. It builds upon existing potato fungicide knowledge and focusses on the in-licensing of R-genes, the development of Phytophthora resistant varieties, the conduction of regulatory safety studies, and the striving for regulatory approval and commercialization.
Field trials with Fortuna were performed since 2006 at over 20 sites in Germany, the Netherlands, Belgium, the United Kingdom, Czech Republic, and Sweden.
BASF Plant Science employs transgenic modification using an imidazoline herbicide resistance marker for selection of transformants. This marker does not have a field effect.
In order to bring Fortuna to the market a regulatory approval for cultivation in the EU is required as well as global import approvals.

On 31.10.2011 BASF Plant Science applied for EU approval for Fortuna.

On 16.01.2012 BASF Plant Science announced that it will stop all its GM crop developments targeted for the European market due to lack of acceptance for GM technology in Europe. This comprises also a stop of the Fortuna project. However, the company will further pursue the regulatory approval of Fortuna.

CONCLUSIONS

Genetic modification allows developing potato varieties which contain broad and durable resistance to *Phytophthora infestans* and possess all features of competitive cultivars. However, to create a solution which can be offered to the farmers some significant hurdles need to be taken, including a regulatory approval and acceptance in the market place.

REFERENCES

El-Kharbotly A, Leonards-Schippers C, Huigen DJ, Jacobsen E, Pereira A, Stiekema WJ, Salamini F, Gebhardt C. (1994) Segregation analysis and RFLP mapping of the R1 and R3 alleles conferring race-specific resistance to *Phytophthora infestans* in progeny of dihaploid potato parents. Mol Gen Genet. 1994 Mar;242(6):749-54

Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E. Lotz, L.A.P., Kessel, G.J.T., Visser, R.G.F., van der Vossen, E.A.G. (2008) Potato Res. 51: 47-57

Turner, R. S. (2008). Potato Agriculture, Late Blight Science, and the Molecularization of Plant Pathology. Historical Studies in the Natural Sciences, 38(2): 223-257.

Van der Vossen, E., Sikkema, A., Lintel Hekkert, B., Gros, J., Stevens, P., Muskens, M., Wouters, D., Pereira, A., Stiekema, W., Allefs, A. (2003). An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. The Plant Journal, 36:867-882

Van der Vossen, E. Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., Pereira, A., Allefs, A. (2005). The R*pi-blb*2 gene from *Solanum bulbocastanum* is a Mi-1 gene homolog conferring broad-spectrum late blight resistance in potato. The Plant Journal, 44: 208-222

Wang, M., Allefs, S., van der Berg, R. G., Vleeshouwer, V. G. A. A., van der Vossen, E., Vosman, B. (2008). Allele mining in *Solanum*: conserved homologues of R*pi-blb*1 are identified in *Solanum stoloniferum*. Theor. Appl. Genet., 116: 933-943.