



Transformation of the potato variety Desiree with single or multiple resistance genes increases resistance to late blight under field conditions



Geert Haesaert^a, Jack H. Vossen^b, René Custers^c, Marc De Loose^d, Anton Haverkort^e, Betty Heremans^a, Ronald Hutten^b, Geert Kessel^e, Sofie Landschoot^a, Bart Van Droogenbroeck^d, Richard G.F. Visser^b, Godelieve Gheysen^{f,*}

^a Ghent University, Faculty of Bioscience Engineering, Department of Applied Biosciences, Valentin Vaerwyckweg 1, 9000 Gent, Belgium

^b Wageningen UR Plant Breeding, Wageningen University & Research Centre, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

^c VIB, Rijvisschestraat 120, 9052 Gent, Belgium

^d Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Science Unit, Product Quality and Innovation Research Group, Burg. Van Gansberghelaan 115, 9820 Merelbeke, Belgium

^e Plant Research International (PRI), Wageningen University & Research Centre, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

^f Ghent University, Faculty of Bioscience Engineering, Department of Molecular Biotechnology, Coupure Links 653, 9000 Gent, Belgium

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ABSTRACT

Late blight, caused by *Phytophthora infestans*, remains the most devastating disease in potato resulting in economic costs that sum up 5.2 billion euros, globally. The use of resistant varieties is a powerful, viable and environmentally friendly alternative or supplement for the current, commonly deployed chemical control strategies. In this study, we set out to improve the susceptible potato variety Desiree by transformation with single or multiple late blight (*R*) resistance genes. *Rpi-sto1*, *Rpi-vnt1.1* and a stack of *Rpi-sto1*:*Rpi-vnt1.1*:*Rpi-blb3*, were transformed and eight, eight, and ten independent transformants (events) respectively, were selected because of absence of vector backbone, low T-DNA copy number, responsiveness to the cognate Avr effectors, *P. infestans* resistance in detached leaf assays (DLAs) and preliminary field experiments. The performance of the selected events was studied under field conditions in The Netherlands and Belgium, after *P. infestans* inoculation and/or to natural late blight infection during two consecutive growing seasons. All selected events were more resistant than the non-transformed susceptible reference clone. The different individual *R* genes, however, contributed to different levels of resistance. The selected events were also compared to conventionally bred late blight resistant varieties with (partially) known *R* gene content. Generally, it was found that plants with single *R* genes showed a lower level of resistance than plants with *R* gene stacks. Only the events harbouring three late blight *R* genes remained unaffected until the end of the growing season, in both locations and in both growing seasons.

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1. Introduction

Late blight, caused by the oomycete *Phytophthora infestans*, is the most devastating disease of potato resulting in large global

yield losses. Costs of control and yield losses represent an annual cost of € 5.2 billion worldwide (Haverkort et al., 2009). *P. infestans* is a hemibiotrophic pathogen that initially requires living host cells; after a short biotrophic phase the pathogen causes extensive necrosis in leaves and stems which can completely destroy the photosynthetic capacity of the plant in a short period of time. Tubers will also be infected when spores from leaves are washed into the soil and enter the tuber through wounds, lenticels or eyes.

Currently, the vast majority of commercially grown potato varieties are susceptible to late blight (Netherlands catalogue of potato varieties, 2014). Potato late blight control, therefore, heavily depends on frequent applications of chemicals with a strong

* Corresponding author.

E-mail addresses: geert.haesaert@ugent.be (G. Haesaert), Jack.vossen@wur.nl (J.H. Vossen), rene.custers@vib.be (R. Custers), marc.deloose@ilvo.vlaanderen.be (M. De Loose), anton.haverkort@wur.nl (A. Haverkort), betty.heremans@ugent.be (B. Heremans), Ronald.hutten@wur.nl (R. Hutten), geert.kessel@wur.nl (G. Kessel), sofie.landschoot@ugent.be (S. Landschoot), bart.vandroogenbroeck@ilvo.vlaanderen.be (B. Van Droogenbroeck), richard.visser@wur.nl (R.G.F. Visser), godelieve.gheysen@ugent.be (G. Gheysen).

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oomycidal activity (referred to as fungicides in this manuscript). In countries with intensive potato cultivation and a humid moderate climate, like Belgium and The Netherlands, potato growers spray 12–15 times per growing season. In wet growing seasons the number of fungicide applications can go up to 20 or even more (Vanhaverbeke, 2012). The economic cost of chemical late blight control is estimated at € 100 and € 55 million per year for the Netherlands and Belgium, respectively (Haverkort et al., 2008). The high dependence on fungicides in combination with their frequent application leads to significant selection pressure on *P. infestans* populations. Despite active resistance management programs, this has resulted in wide spread resistance against the phenylamide metalaxyl and a reduced sensitivity to fluazinam in the Netherlands (Cooke et al., 2012; Schepers et al., 2013). Besides economic costs, frequent fungicide applications have undesirable environmental side effects whereas their mechanical application leads to soil compaction and additional CO₂ emission, thus negatively influencing the ecological footprint of potato production (Haverkort and Hillier, 2011).

The use of resistant varieties is a powerful, viable, environmentally friendly supplement to currently used integrated control strategies. Potato breeders have been selecting for late blight resistant varieties ever since the Potato Famines around 1845 (Goodrich, 1848). Well known are the *R1*, *R2*, *R3a*, *R3b*, *R4*, and *R10* genes from *Solanum demissum* which were introgressed into varieties like Craig's Snow White, Stirling and Pentland Ace (Bradshaw et al., 2004; Trognitz and Trognitz, 2007). Although initially promising, the *R* genes of *S. demissum* were rapidly overcome after field introduction because the action of *R* genes is based on a gene-for-gene interaction and one mutation in the pathogen can abolish recognition (Fry, 2008).

The structure of the *P. infestans* genome (Haas et al., 2009) in combination with the large number of progeny greatly contributes to its tremendous adaptive capability. Global population changes e.g. resulted from the second migration of *P. infestans* – including the A2 mating type – from Mexico to Europe in the latter part of the 20th century (Goodwin et al., 1998). The old, less aggressive, clonal population was quickly replaced, indicating that the new arrivals and the resulting new population were better adapted, more fit and more aggressive (Goodwin et al., 1992, 1998; Grunwald and Flier, 2005). Another example is the *P. infestans* genotype EU13-A2 that immediately became dominant after its emergence in Europe around 2005 (Cooke et al., 2012). These facts explain, at least partially, the increasing control problems of late blight during the last three decades.

Due to the increased genetic diversity and aggressiveness of *P. infestans* populations worldwide (Chmielarczyk et al., 2014; Goodwin et al., 1992, 1998), an upgraded, effective and sustainable control strategy for late blight is urgently needed. This future control strategy would build on the current integrated approach based on the most effective best agricultural practises available: reduction of primary inoculum, the use of resistant varieties and chemical control linked to a decision support system (McDonald and Linde, 2002; Schepers et al., 2009a,b). Host resistance is one of the most powerful tools available for a sustainable management of late blight (Mundt, 2014). Development of durably late blight resistant varieties would thus be key for the development of sustainable late blight control strategies (McDonald and Linde, 2002). Incorporation of host resistance into a preventive, zero tolerance, IPM control strategy for late blight is expected to reduce the necessary fungicide input to an absolute minimum.

A good strategy for breeding more durable resistance is to combine multiple late blight *R* genes in one potato genotype. These *R* genes must be chosen such that they recognise different AVR effectors (Vleeshouwers et al., 2011; Zhu et al., 2012, 2015) to achieve a complementary recognition spectrum and thus a broader

spectrum of resistance. In addition, with an increasing number of stacked *R* genes, it is increasingly less likely that *P. infestans* would overcome these multiple *R* genes, simultaneously, through random mutation. Obtaining broad-spectrum resistance through introgression of multiple *R* genes into potato genotypes by traditional breeding is slow due to the highly heterozygous and tetraploid character of commercial potato genotypes and linkage drag resulting from introgression using wild *Solanum* species.

Genetic transformation is a fast and efficient way to introduce different late blight *R* genes into an existing variety (Zhu et al., 2012; Jo et al., 2014), especially since in recent years novel sources of resistance have been identified and corresponding *R* genes have been cloned from crossable *Solanum* species, such as *Solanum bulbocastanum*, *Solanum venturii*, *Solanum stoloniferum* and *Solanum mochiuense* (Vleeshouwers et al., 2011). Until now, over 20 functional late blight *R* genes have been cloned (Jo, 2013). Since some of the *R* genes show a broader resistance spectrum than others, it is useful to study the biological function (including the effectors being recognised) of each *R* gene individually before combining them in transformation programs (Zhu et al., 2012). With genetic transformation two, three or even more genes can be introduced simultaneously (Lin et al., 2003; Zhu et al., 2012). *R* genes with a complementary effector interaction pattern can be combined for a broad spectrum and potentially durable resistance. In addition, the introduction of *R* genes by genetic engineering in an established variety is expected not to affect the agricultural and tuber characteristics of the variety. Nevertheless, several independent transformants (events) need to be screened as the *R* genes are integrated in different chromosomal locations which may influence the expression of the introduced resistance genes. Also the *in vitro* process may result in somaclonal variation. In this way the events that have gained late blight resistance but that retained the remaining variety characteristics are selected (True to Type events).

The overarching concept of this study is to contribute to the development of sustainable *P. infestans* resistance through a cisgenic approach. In this approach plants receive *R* genes only from crossable species and these *R* genes are under control of their natural regulatory elements. Furthermore, foreign genetic material such as selectable marker genes is absent (Jo et al., 2014). From a genetic point of view, cisgenic plants are highly similar to conventionally bred plants since only the way by which the genes are introduced is different. One argument in favour of this approach is that cisgenesis limits the introduction of genetic material into existing potato varieties to the absolute minimum required to achieve the desired trait. As a result, and from a societal point of view, cisgenic plants may be less controversial than transgenic plants (Eurobarometer, 2010; Gaskell et al., 2010). Unfortunately, according to the EFSA Panel on Genetically Modified Organisms (GMO) the same legislations and procedures could be applied towards transgenic, intragenic and cisgenic organisms (EFSA, 2012). It needs to be awaited if current regulatory discussions will consider that cisgenic plants are not subject to the requirements of the GMO legislation because the final gene combination could in principle also be obtained by breeding. A second argument is that the genetic engineering approach allows for the efficient transfer of multiple *R* genes in one single event.

Here, we describe the transformation of potato variety Desiree with individual or multiple *R* genes. *Rpi-sto1*, *Rpi-vnt1.1*, *Rpi-blb3*, originating from *S. stoloniferum*, *S. venturii*, and *S. bulbocastanum*, respectively, were used for this purpose. In this phase of the project, some of the transformations were performed using antibiotic selection to facilitate the selection of sufficient transformants, and therefore the obtained lines are by definition not cisgenic. Selected lines that carry either single or multiple late blight *R* genes were compared to conventional susceptible and resistant varieties

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