Genetic transformation of potato cultivars using *R* genes to increase resistance to late blight of potato caused by *Phytophthora infestans*

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Abstract

Several resistance (*R*) genes have recently been isolated and used in a transgenic approach. The *RB* gene of *Solanum bulbocastanum* confers partial resistance to many isolates of *Phytophthora infestans*, the causal agent of late blight (LB) disease in potato. We have selected three recipient varieties: (1) Desiree, which is easy to transform and has wide adaptability; (2) Granola which is the principal fresh market variety in Indonesia; and (3) Victoria (Asante) which is a well adopted variety in Uganda (and Kenya). Genetic transformation of Desiree was done with 2 batches of events. The first one resulted in 14 putatively transformed plants regenerated from wounded leaves and internodes. Out of these, 10 transgenic events have been selected as being independent events and 7 of them devoid of non-TDNA backbone vector sequences. These will be field trialed to determine their level of resistance to LB and other agronomical characteristics. The second batch resulted in 41 putative transformed plants. These plants are currently undergoing molecular characterization. With the variety Granola, 15 putative transformed events with the *RB* gene construct were produced. This variety displays much lower regeneration efficiency in addition to rapid oxidation of explants. Finally, the variety Victoria is being tested for regeneration efficiency and susceptibility to kanamycin and carbenicillin to select for transformed events while eliminating *Agrobacterium* cells. Two additional *R* genes, *Rpi-blb2* and *Rpi-vnt1.1* from *S. bulbocastanum* and *S. venturii* respectively, are now used in combination with *RB* gene in order to increase durability of resistance to late blight.

Keywords: Potato, late blight resistance, genetic engineering.

Introduction

Late blight caused by the oomycete *Phytophthora infestans* is the most devastating diseases of potato worldwide, causing an average production loss of 15% in developing countries which means approximately $2.75 billion loss as estimated by the International Potato Center (Anonymous, 1997). Since the middle of the 19th century when this pathogen turned out to be responsible of a major food crisis in Europe (the "Irish potato famine"), active efforts by plant breeders to control this disease have been carried out.

In the early days of breeding for LB resistance, eleven single resistance (*R*) genes from *Solanum demissum*, a wild potato species indigenous to México, were introgressed into modern potato varieties (Malcolmson and Black, 1966, Gebhardt and Valkonen, 2001). Even though *S. demissum* is highly resistant to late blight, their *R* genes only conferred race-specific resistance when transferred into potato cultivars. This resistance was rapidly overcome by new isolates (Wastie, 1991).

Other *R* genes were recently identified and isolated from *Solanum* species with resistance to a broad spectrum of isolates of *P. infestans*. Taking advantage of genetic engineering, a new strategy under development consists of transferring these *R* genes either as single events or (preferably) stacked into potato varieties and deploying these in such a way that the pathogen will not be able to overcome this multiple *R* gene mediated resistance. We report here our progress made in transferring the *RB, Rpi-blb2* (*Solanum bulbocastanum*) and *Rpi-vnt1.1* (*S. venturii*) in three potato varieties.

Materials and methods

Gene constructs

The plasmid pCLD04541 containing the 8,569 bp fragment corresponding to the *RB* gene was sent to the Applied Biotechnology Lab of CIP (ABL-CIP) from Dr. Jiang's lab at University of Wisconsin (Song et al., 2003). The *Rpi-vnt1.1* gene was obtained from the Sainsbury Lab (UK) but due to delays it was ultimately obtained from chemical synthesis, as well as the *Rpi-blb2* gene.

Plant material and genetic transformation

Solanum tuberosum varieties Desiree and Granola (CIP800048 and CIP800959, respectively) were obtained from CIP's genebank and propagated in vitro as published previously (Cuellar *et al.*, 2006). We used the strains EHA105 of *Agrobacterium tumefaciens* to transfer these R genes into the potato varieties using petioles and internodes as explants. Standard regeneration and transformation protocol were used (Cuellar *et al.*, 2006).

Analysis of transgenic plants

PCR analysis was done with genomic DNA of putative transformed plants using specific-primers to the *RB* and *npt*II genes for a rapid detection of transformants. The presence of the *RB* and the *nptII* genes were confirmed by PCR using specific primers. The extent of non-T-DNA sequence insertion was monitored by PCR using primer from three regions extending from the left border (LB) to the right border (RB) of the T-DNA. Southern blot hybridizations were performed using as probe a 597 bp DNA fragment of the *nptII* gene and genomic DNA digested with *Eco*RI.

Phytophthora infestans assays

Detached leaf and whole plants assays were performed with a complex isolate POX 067 which is virulent on the following *S. demissum* R genes: R1, R2, R3, R4, R5, R6, R7, R10, and R11). The isolate was inoculated on the transgenic events and six potato genotypes with known level of resistance using standard conditions developed at the CIP pathology lab.

Results and discusion

Development of R gene constructs

The *RB* gene from *S. bulbocastanum* is available in a single gene construct (pCIP68 and pCIP56 with and without the *nptII* kanamycin selectable marker gene, respectively). The *Rpi-vnt1.1 and Rpi-blb2* obtained through chemical synthesis were moved into a single R gene constructs (pCIP93 with *Rpi-vnt1.1* + *nptII*; pCIP95 with *Rpi-blb2* + *hptII* hygromycin selectable marker gene). We have also developed one double R gene construct (pCIP96 with *Rpi-blb2* + *Rpi-vnt1.1* + *nptII*). We plan to develop additional gene constructs (1) *Rpi-vnt1.1* + *Rpi-blb2* + *hptII* to stack them in *RB* events (kmR), using selection on hygromycin; a triple gene construct (*RB* + *Rpi-vnt1.1* + *Rpi-blb2* + *nptII*). A marker-free triple R gene construct will also be developed to adopt a cisgenic strategy when a good marker-free transformation protocol will be available at CIP.

Potato genetic transformation

Genetic transformation with the gene construct pCIP68 was carried out using 120 explants of the potato cultivar Desiree which produced 14 regenerants. Out of these, 11 plants have been characterized by PCR using primers for the TDNA (*RB* and *nptII* genes), and non-TDNA (backbone vector sequences, three pairs of primers) (Table 1). Two plants (50 and 62) were shown to have non-TDNA sequence and two others (47 and 83) were shown to be negative for all PCR primers (regenerants that are resistant to kanamycin but not transformed = "escapes"). Hence, 7.5% transformation efficiency was achieved with the gene construct pCIP68 in Desiree. Ten of the 11 putative transformed plants were characterised by Southern blotting (SB) using the *nptII* gene as probe (*RB* is homologous to many *R* genes present in the potato). One plant (67) has not yet been assessed by SB due to delays in plant propagation (in progress). The SB revealed that the 8 transgenic plants are independent events and that 4 events (9.1, 43, 53, 66) have one copy of the TDNA, 3 events (27, 50, 62) have two copies, and 1 event (28) has three copies.

Table 1. Molecular characterization of 11 independent events from the potato variety Desiree transformed with the RB gene

Event #	T-DNA	non-T-DNA	Copy number
Desiree [RB] 9.1	+	-	**1**
Desiree [RB] 27	+	-	**2**
Desiree [RB] 28	+	-	**3**
Desiree [RB] 43	+	-	**1**
Desiree [RB] 47	-	-	**none**
Desiree [RB] 50	+	+	**2**
Desiree [RB] 53	+	-	**1**
Desiree [RB] 62	+	+	**2**
Desiree [RB] 66	+	-	**1**
Desiree [RB] 67	+	-	**n.d.**
Desiree [RB] 83	-	-	**none**

Additional transformed events have been obtained. A second genetic transformation event with the pCIP68 gene construct was carried out in the variety Desiree. Three hundred and sixty explants (180 leaves with petioles and 180 internodes) were transformed which produced 41 regenerants from selection on kanamycin. For the variety Granola, we obtained 15 regenerants resistant to kanamycin from 630 explants from two events of transformation (210 leaves, 210 leaves with petioles and 210 internodes). More recently, the variety Victoria (Asante) has been tested for its ability to be genetically transformed through agroinfection. The plant propagation and regeneration *in vitro* appears to be very similar to Desiree. Preliminary result indicated that 50mg/l kanamycin and 250 mg/l carbenicillin are more appropriate to use in the first genetic transformation event.

Late blight resistance assay

A bio-assay using detached leaves to assess activity against *Phytophthora infestans* revealed a high level of resistance (hypersensitive reaction) for 2 of the 9 plants tested (7 transgenic events and 2 regenerants). The results were confirmed in whole plants assays (Table 2). Curiously, these 2 events (50 and 62) have non-TDNA sequence which makes them unsuitable for future product development. The other plants, including the untreated control Desiree, presented disease symptoms at 7 days post infection. The 9 transgenic events from Desiree will be characterized for transcriptional activity by real-time PCR under no infection and 5 days after inoculation and with the pathogen.

Table 2. Bio-assay using detached leaves of 9 transgenic events and 6 genotypes with known resistance levels against the isolate POX 067 of *Phytophthora infestans*

Event/Genotype	Severity (%)		
	Detached leaf		Whole plant
	Rep. 1	Rep.2	Rep. 1
Desiree[RB] 9.1	100	100	100
Desiree[RB] 27	100	100	100
Desiree[RB] 28	100	100	100
Desiree[RB] 43	100	100	100
Desiree[RB] 50	HR*	HR	HR
Desiree[RB] 53	100	100	100
Desiree[RB] 62	HR	HR	HR
Desiree[RB] 66	100	100	100
Desiree[RB] 67	100	100	100
Atzimba	100	100	100
Desiree	100	100	100
LBr 40	0	0	0
Monserrate	100	100	100
Pimpernell	100	100	100
Tomasa condemayta	100	100	100

*Hypersensitive reaction (rapid cell death

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