

Molecular assisted assessment of late blight resistance in potato

Hannele Lindqvist-Kreuze, Matilde Orrillo, Willmer Perez, Leticia Portal, Manuel Gastelo, Juan Landeo, Merideth Bonierbale

International Potato Center (CIP), Apartado 1558, Lima 12, Peru
Email: h.kreuze@cgiar.org

Abstract

Late blight is one of the most important diseases of potato causing an estimated yearly economic loss of \$3.25 billion in potato-growing areas worldwide. We have identified molecular markers associated with late blight resistance in wild relatives of potato and advanced breeding materials available at CIP. The research focuses on wild species *S. paucissectum* that displays R-gene dependent and/or quantitative resistance, as well as on the advanced tetraploid breeding population B3, which displays high levels of quantitative resistance under high epidemic pressure in a wide range of tropical and subtropical agro ecologies. To understand the genetic components of resistance in these materials we have developed and characterized diploid experimental populations PCC1 (resistant *S. paucissectum*/ susceptible *S. chomatophilum*), and B3C1HP (resistant haploidized B3 clone/ susceptible *S. phureja*). Both of these populations express high levels of field resistance under high endemic late blight pressure, and quantitative trait loci (QTL) for late blight resistance have been located in chrXI in PCC1, and in chrIX in B3C1HP. We have identified molecular markers by 10K potato cDNA microarray and tested these and other markers for association with the resistance phenotype in our mapping populations. Our objective is to develop knowledge of the genetic base of the resistance and tools to facilitate its utilization or transfer across populations.

Keywords: Potato, late blight, molecular markers, resistance breeding.

Introduction

Late blight is one of the most devastating diseases of cultivated potato world wide. Despite a long history of breeding, durable resistance has not been achieved and the disease is mainly controlled by pesticide applications. In searches for durable resistance focus has been turned to the close wild relatives of potato and indeed, many wild *Solanum* species have proven to contain promising levels of late blight resistance (Perez et al., 2000). New R-genes (for example: Ewing et al., 2000; Foster et al., 2009; Song et al., 2003), as well as quantitative trait loci (for example: Villamon et al 2005; Bisognin et al., 2005) for late blight resistance have been discovered in several of these species. QTL for late blight resistance have been identified on almost every potato chromosome and in many cases the same region of the genome also contains clusters of R-genes (Gebhardt and Valkonen, 2001), thus R-genes are candidates for the QTL effect.

Screening plant material for late blight resistance is normally done by inoculating plants with different isolates or planting the tested material in field locations with endemic disease. However, this type of screening is laborious and its success dependent on environmental factors. Tracking the resistance phenotype by molecular assisted methods could cut back the costs considerably and allow for marker assisted selection of resistant plant material for breeding. Furthermore markers would be helpful in resolving the architecture of late blight resistance in and among available genetic resources.

In this study we are reporting the identification of genetic resistance components in an advanced tetraploid potato clone from the B3C1 population and in a wild species *S. paucissectum* with the help of molecular markers. We also demonstrate that the PCC1 progeny contains at least two new R-genes that locate in the major QTL in the chrXI.

Material and methods

Genotype 391011.17 from the tetraploid population B3C1, highly resistant to late blight, as challenged with *Phytophthora infestans*, and leaves were collected for RNA extraction. The cDNA synthesized was hybridized to

TIGR microarray consisting of approximately 10 000 probes originating from various potato cDNA libraries. Differentially regulated cDNA clones were selected by significant analysis of microarray (SAM) (Tusher et al., 2001) and placed in functional categories by MapMan (Thimm et al., 2004).

Marker CT182 is linked to the quantitative trait locus (QTL) in chr XI, in population PCC1 (*S. paucisectum* / *S. chomatophilum*) (Villamon et al., 2005). PCR based positional candidate markers were selected based on their location in proximity to this marker in published genetic maps of potato and tomato and tested for association with resistance phenotype in PCC1 population. We also generated AFLP (Amplified Fragment Length Polymorphism) and RGA (Resistance Gene Analog) markers for the same purpose. The significant ($P \leq 0.05$) effects of single marker alleles on AUDPC values were tested by Students t-test. A detached leaf assay was performed using the PCC1 progeny and *Phytophthora infestans* isolates PE84006, POX-067, PCO-093 and PCO-002 to test for presence of R-genes.

Results

Molecular markers in B3. Of the approximately 10000 probes present on the TIGR potato microarray 274 showed differential responses in B3 genotype due to late blight inoculation as revealed by SAM. The functional classification of these probes by MapMan indicated that 82 had no assigned function. The remaining probes belonged into various functional categories, of which the most interesting, stress related, is shown in Figure 1. According to the classification R-genes involved in recognition, R-gene dependent signaling genes as well as genes involved in hormone signaling, respiratory burst, proteolysis and several pathogenesis related (PR) genes were affected in the B3 genotype during late blight attack. Also secondary metabolism- and abiotic stress related genes and WRKY type transcription factors were affected. With the help of cDNA microarray we have identified a number of candidate genes responsible for the late blight resistance phenotype in B3 population. The location of eight of these candidate genes was determined in the B3C1HP (resistant haploidized B3 clone/ susceptible *S. phureja*) genetic map, and were found to tag chromosomes II, III, IV, VI, IX and XII.

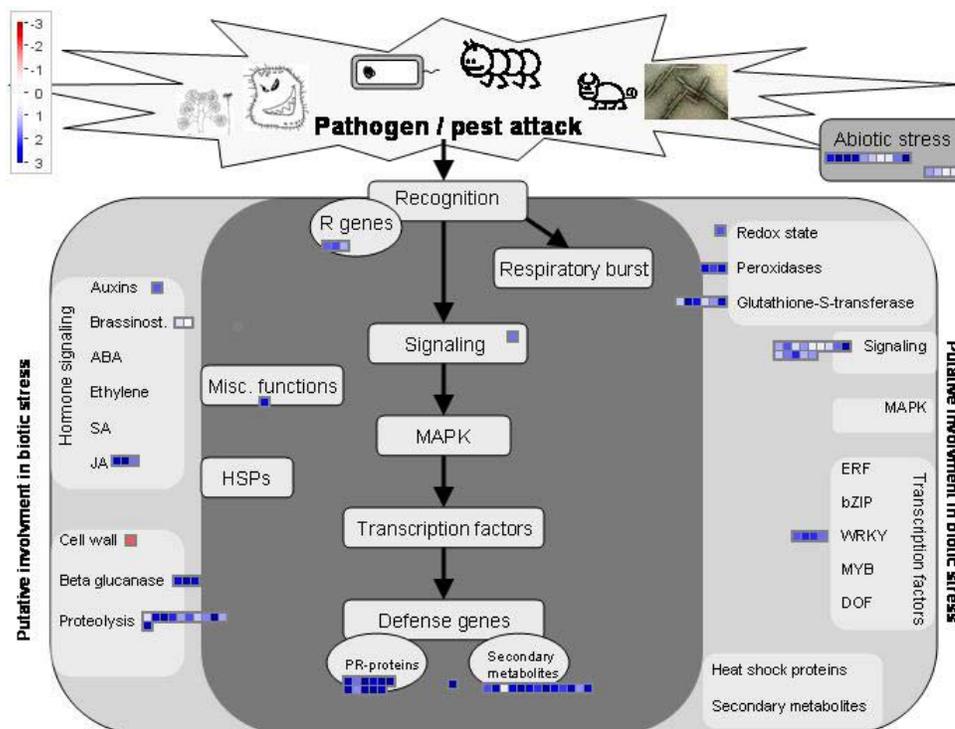


Figure 1. MapMan classified stress related genes differentially regulated in B3 during late blight attack. Each colored square represents a gene and the color of the square indicates its level of expression as compared to un-inoculated sample.

Molecular markers in PCC1. We used a range of techniques to identify molecular markers associated with the field resistance phenotype in PCC1 population. In total five informative positional candidate marker alleles were found. Four of these mapped into chromosome XI and were associated with the field resistance phenotype in both locations, while one located on chromosome I and was not significantly associated with field resistance. AFLP, NBS and SSR analysis yielded 44, 49 and 15 informative marker alleles, respectively. These marker alleles tagged all 12 chromosomes. In total six AFLP markers located in chromosomes I, II, V and XI, were associated with field resistance at least in one of the test locations (Table 1). Four NBS markers mapping into chromosomes IV and XI were associated with resistance. None of the SSR markers were associated with resistance.

Table 1. Marker type, number of loci scored and number of alleles associated with field resistance in PCC1 progeny in Comas 2002 and Oxapampa 2006

Marker type	loci	Associated with resistance	
		Comas	Oxapampa
AFLP	44	4	5
NBS	49	3	4
SSR	15	0	0
Cand gene	5	0	0
Position candidate	5	4	4
Resistance specificity	4	4	3
Total	122	15	16

R-genes in PCC1 progeny. There was a high frequency of incompatible interactions with the *P. infestans* isolates tested within the group of individuals that had low AUDPC values in the field experiment. The characteristic compatibility pattern in this group was incompatibility with all isolates (Table 2, pattern 3). The most susceptible groups of individuals had no incompatible interactions with any of the complex isolates (PCO93, POX67 and PCO002), but instead had a high number of incompatible reactions with isolate PE84006 (Table 2, pattern 2). The inability of the race 0 -isolate to infect a plant is considered indicative of presence of at least one of the known R-genes originating from *S. demissum* (Vleeshouwers et al 2000) or it may simply indicate that the isolates' avr factors match additional, previously unidentified R-genes. Although incompatibility to PE84006 was the most common reaction in the resistant progeny, there were a few individuals that displayed compatibility to PE84006 but were incompatible with the complex races suggesting the presence of a new R-gene (Table 2, pattern 1). This R-gene was absent from the susceptible progeny since all individuals with high AUDPC values showed incompatibility to PE84006 and compatibility with the complex races (Table 2, pattern 2). Therefore, it seems that the susceptible progeny has an R-gene with similar specificity as *S. demissum* R-genes other than R9, R5 or R8 originating from the *S. chomatophilum* parent, whereas the progeny with low AUDPC values contain at least two new R-genes originating from the *S. paucisectum* parent (Table 2, patterns 1 and 3). The association of resistance specificities against each *P. infestans* isolate with the field resistance phenotype were tested by T-test. All four resistance specificities were associated with the field resistance phenotype in test location Comas, and three in test location Oxapampa (Table 1).

Table 2. Compatibility patterns with four *P. infestans* isolates detected in DLAs of progenitors of PCC1 and 60 individuals of PCC1 progeny and the average AUDPC of the individuals having the indicated compatibility pattern

Pattern	<i>P. infestans</i> isolates				AUDPC Comas			Total	Progenitors of PCC1	
	race 0 PE84006	avr 5, 8, 9 PCO93	avr 8, 9 POX67	avr 5, 9 PCO002	60- 517	1100- 1760	2370- 3074		chm PI 310991-1	pcs PI 473489-1
1	C	I	I	I	6	1	0	7		
2	I	C	C	C	2	4	19	25	1	
3	I	I	I	I	15	4	0	19		1
4	C	C	C	C	1	7	1	9		
				Total	24	16	20	60		

Discussion

The TIGR 10K potato array was used to monitor global gene expression in an advanced tetraploid clone B3C1, under late blight attack. The inoculated samples were compared to healthy non-inoculated plants to filter out the genes expressed due to other reasons than the pathogen attack. cDNAs annotated as putative disease resistance proteins were found differentially expressed in B3, indicating the potential usefulness of identification of functional R-genes by transcriptome profiling. So far R-genes are likely candidates responsible for QTL effect only because of their co-localization on genetic maps. To date, we have preliminary indication of an expression of a major QTL in chr9 in a progeny of a cross where the resistance originates from one of the genotypes of the advanced B3 population, thus we may begin testing hypothesis of co-localization of some candidate R-genes in this genomic region. We have identified several new candidate genes for late blight resistance. We are interested in testing the effect of these genes in entire the B3 population to understand the genetic base of resistance in this population which shows good late blight resistance across a variety of tested tropical and subtropical environments.

Attempts to identify molecular markers associated with the field resistance phenotype by NBS profiling, AFLP and by utilizing positional candidates yielded markers linked with resistance but the best approach was by far the positional candidate method. As described previously by Villamon et al., (2005) a major QTL in the long arm of chr XI (designated QTLpcs11) explained the largest amount of the variation in Comas at 2002, and in a green house experiment. This map segment is a resistance hot spot containing several R-genes against viruses, wart disease and root knot nematode as well as QTL for resistance to other diseases including late blight (Leonards-Schippers et al. 1994, Ewing et al 2000). We identified several molecular markers in this QTL and associated with the field resistance phenotype. Molecular markers based on gene sequences previously mapped in this particular map segment either in potato or tomato were linked with field resistance phenotype. The utility of these markers for marker assisted selection (MAS) remains to be evaluated in *S. paucissectum* accessions.

The phenotypic resistance markers (incompatibilities to isolates POX-067, PCO-093, and PCO-002) indicative of presence of major genes show significant association with field resistance phenotype in both environments tested suggesting possible involvement in quantitative resistance. However, more research is needed to confirm this hypothesis.

References

- Bisognin, D.A.; Douches, D.S.; Buszka, L.; Bryan, G.; Wang, D. 2005. Mapping Late Blight Resistance in *Solanum microdontum* Bitter. *Crop Sci.* 45:340–345.
- Ewing, E., Simko, I., Smart, C.D., Bonierbale, M.W., Mizubuti, E.S.G., May, G.D. and Fry, W.E. (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. *Mol. Breed.* 6:25-36.
- Foster, S.J., Park, T.-H., Pel, M.A., Brigneti, G., Śliwka, J., Jagger, L., Van der Vossen, E.A.G. and Jones, J.D.G. (2009) Rpi-vnt1.1, a Tm-22 homolog from *Solanum venturii* confers resistance to potato late blight. *Mol. Plant-Microbe Interact.* 22:589-600.
- Gebhardt, C., and Valkonen, J.P.T. (2001) Organization of genes controlling disease resistance in the potato genome. *Annu. Rev. Phytopathol.* 39: 79-102.
- Leonards-Schippers, C.; Gieffers, W.; Schäfer-Pregl, R.; Ritter, E.; Knapp, S.J.; Salamini, F.; Gebhardt, C. 1994. Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. *Genetics* 137, 67–77.
- Pérez, W.; Salas, A.; Raymundo, R.; Huaman, Z.; Nelson, R.; Bonierbale, M. 2000. Evaluation of wild potato species for resistance to late blight. CIP Program Report 1999-2000, 49-62.
- Song, J.; Bradeen, J.M.; Naess, S.K.; Raasch, J.A.; Wielgus, S.M.; Haberlach, G.T.; Liu, J.; Kuang, H.; Austin-Phillips, S.; Buell, C.R.; Hegelson, J.P.; Jiang, J. 2003. Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *PNAS* 100, 9128-9133.
- Thimm; O.; Blaesing, O.; Gibon, Y.; Nagel, A.; Meyer, S.; Krüger, P.; Selbig, J.; Müller, L.A.; Rhee, S.Y.; Stitt, M. 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.* 37, 914-39.

- Tusher, V.G.; Tibshirani, R.; Chu, G. 2001. Significance analysis of microarrays applied to the ionizing radiation response. *PNAS* 98, 5116–5121.
- Villamon, F.; Spooner, D.; Orrillo, M.; Mihovilovich, E.; Perez, W.; Bonierbale, M. 2005. Late blight resistance linkages in a novel cross of the wild potato species *Solanum paucissectum* (series Piurana). *Theor. Appl. Genet.* 111,1201-1214.
- Vleeshouwers, V.; van Dooijeweert, W.; Govers, F.; Kamoun, S.; Colon, L. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* 210, 853-864.