Potato Research (2009) 52:245–248
DOI 10.1007/s11540-009-9132-7

# Marker-assisted Breeding for Disease Resistance in Potato

**A. Carrasco · J. E. Chauvin · B. Trognitz · A. Pawlak · O. Rubio-Covarruvias · E. Zimnoch-Guzowska**

Received: 31 July 2008 / Accepted: 20 October 2008 /
Published online: 5 August 2009


**Abstract** Sub-project 5 of BIOEXPLOIT aims to design durable disease resistance through marker-assisted breeding by converting existing markers for high-throughput application, developing and validating high-throughput marker technologies and pyramiding major *R* genes and/or quantitative trait loci into elite material. Activities include (1) the fine mapping of the quantitative trait locus *PiXspg* which accounts for a large proportion of the variation in late blight resistance, (2) converting SNP-based markers and an AFLP marker to easy-to-use-markers, (3) testing of progenies with combined sources of late blight resistance for presence of *R* genes and agronomic features, (4) backcrossing new sources of resistance to *S. tuberosum* and molecular screening of breeding materials with marker GP94 linked with gene *Rpi-phu1*

A. Carrasco (✉)
APPACALE S.A., Valle de Mena 13, 09001 Burgos, Spain
e-mail: info@appacale.com

J. E. Chauvin
UMR ABV, Institut National de Recherche Agronomique (INRA), Domaine de Kéraïber,
29260 Ploudaniel, France

B. Trognitz
Biotechnology Department, ARC Seibersdorf Research GmbH, 2444 Seibersdorf, Austria

A. Pawlak
Zamarte Breeding Company Ltd., Zamarte 33, 89-430 Kamien Kraj, Poland

O. Rubio-Covarruvias
Programa de Papa, Instituto Nacional de Investigaciones Forestales y Agrícolas (INIFAP),
Conjunto SEDAGRO s/n, Metepec, Estado de México CP 52140, México

E. Zimnoch-Guzowska
Plant Breeding and Acclimatization Institute (IHAR), Research Centre Młochów, Platanowa 19,
05-831 Młochów, Poland

*Present Address:*
A. Carrasco
Newco S.L., Campus Agroalimentario de Arkaute, 01192 Arkaute, Spain
e-mail: acarrasco@newcosl.com

conferring late blight resistance, (5) evaluating potato clones with enhanced resistance against *Phytophthora infestans* under field conditions of Toluca (México), and (6) developing populations and marker-assisted breeding for disease resistance.

**Keywords** Durable resistance · Genotyping · High throughput marker technology · Late blight · Phenotyping · *R* genes · *Solanum tuberosum*

## Introduction

One strategic objective of BIOEXPLOIT is to accelerate the introduction of marker-assisted breeding and genetic engineering in the EU plant breeding industry. To achieve this target, the sub-project 5 aims to design durable disease resistance through marker-assisted breeding. This sub-project is divided into two work packages. The first work package concerns the development and validation of high-throughput (HTP) marker technologies and molecular markers associated with disease resistance loci for commercial marker-assisted breeding. To achieve this objective, existing markers will be converted for HTP application and HTP marker technologies will be developed and validated. The second work package has the objective of building pyramids of major *R* genes and/or quantitative trait loci (QTLs) by marker-assisted breeding into elite materials. Therefore, crosses between elite material and lines carrying *R* gene sources and QTLs were made and molecular marker screening of individual plants in segregating populations was carried out. Finally, field tests on effectiveness of the resistance sources were performed.

## Fine Mapping of QTL *PiXspg*

Participants from the Institut National de Recherche Agronomique (INRA) identified a QTL called *PiXspg* on Chromosome 10 which accounts for about 25% of the observed variation in late blight (*Phytophthora infestans*) resistance in the interspecific *Solanum* population 96D.32 (*S. tuberosum* Rosa H1 (2*x*) × *S. spegazzini*). Fine mapping was performed and ten successful crosses between six interspecific hybrids and a susceptible *S. tuberosum* dihaploid generated 1,700 seeds. These six interspecific hybrids from the 96D.32 population carried the marker linked to QTL *PiXspg* according to their resistance level evaluated in an outdoor assay with natural inoculum. Sowing and multiplication were carried out in 2007. Phenotyping in an outdoor assay with natural inoculum has been carried out in 2008 and will be carried out again in 2009. Genotyping of 1,107 plants with two PCR-markers has been performed in 2008. Comparison of the genotypic and phenotypic data will be carried out to test the efficiency of the markers identified in the 96D.32 progeny at the diploid level.

## Converting SNP Based Markers and an AFLP Marker to Easy-To-Use-Markers

Participants from ARC Seibersdorf Research are converting SNP based markers to easy-to-use-markers using primer modification by an LNA ("locked nucleic acid")

instead of a DNA base (Nakitandwe et al. 2007; BMC Plant Methods http://www.plantmethods.com/content/3/1/2). Allele-specific amplification using LNA primers was performed in a population of *S. caripense*. Moreover, high-throughput DNA isolation methods for application in breeding practice have been tested concluding that the method based on the Plant QuickExtract commercial kit (Epicenter) is faster and not more expensive. Finally, the AFLP Marker Pst1-108 from the *S. caripense* map was converted to a simple PCR marker. The marker is linked at less than 1 cM from the locus to be selected for.

## Testing of Progenies with Combined Sources of Late Blight Resistance for Presence of *R* Genes and Agronomic Features

Participants from the Zamarte Breeding Company in collaboration with those from the Plant Breeding and Acclimatization Institute (IHAR) harvested 930 individuals from 12 different crosses. Two of these populations were propagated in the field and tested for the presence of the marker GP94 by the PCR method. About 50% of the genotypes had the *R* gene *Rpi-phu1*. Moreover, clones from the second year of selection were tested in the laboratory in order to confirm the presence of the *R* gene by the detached leaflets assay method. All these clones descending from the second year showed resistance to the wart disease (*Synchytrium endobioticum)* and to the potato cyst nematode (*Globodera rostochiensis Ro1*). Field investigations were performed to evaluate yield and other agronomical traits. The Zamarte Breeding Company has got 12 clones with the *R* gene *Rpi-phu1*, with resistance to wart and nematode and with good suitability for consumption.

## Backcrossing New Sources of Resistance to *S. tuberosum* and Molecular Screening of Breeding Materials with Marker GP94 Linked with Gene *Rpi-phu1* Conferring Late Blight Resistance

Participants from the Plant Breeding and Acclimatization Institute (IHAR) made crosses between elite material and studied resistance sources both at 2*x* and 4*x* level. At the 2*x* level, nine dihaploids of early maturing Polish cultivars were used as seed parents. Three clones of *S. kurtzianum* (*ktz)* and two clones of *S. ruiz-ceballosii (rzc)* were pollen parents. In 19 combinations, 13,000 true seeds were obtained. Individuals with high level of resistance were only found in populations with *rzc* as pollen donor. In addition, molecular screening of breeding materials with marker GP94 linked with gene *Rpi-phu1* conferring late blight resistance was performed. Sixty-nine clones were analysed and six of them were recombinants.

## Evaluating Potato Clones with Enhanced Resistance Against *Phytophthora infestans* under Field Conditions of Toluca (México)

Potato clones obtained by participants from APPACALE were tested under field conditions in Toluca valley (México) by participants from the Programa de Papa,

Instituto Nacional de Investigaciones Forestales y Agrícolas (INIFAP). Results showed that there are promising potato cultivars with high late blight resistance that may be used in the breeding programmes. All the somatic hybrids with *S. pinnatisectum* presented lower infection than the other clones and resistance was decreasing with backcrossing.

## Developing Populations and Marker-assisted Breeding for Disease Resistance

Participants from APPACALE used *S. avilesi* (***avl***) PI 498091, *S. berthaultii* (***ber***) PI 265857 and *S. pinnatisectum* (***pnt***) PI 275236 (BGRC 8175) for interspecific crossings with susceptible lines of *S. tuberosum*. To obtain *tbr-pnt* populations, somatic hybrids with *S. pinnatisectum* were previously developed. Therefore, *tbr-ber* and *tbr-avl* progenies were diploid while *tbr-pnt* progenies were tetraploid. Two more populations with commercial varieties as parents are being studied for marker-assisted breeding following the results obtained by Bormann et al. (2004). Sixteen markers have been applied to different populations although more efforts are needed. However, the TPT marker is being studied in ***ber***-progenies. This marker is located on Chromosome 10 linked to a QTL for resistance in leaflets which accounted for 15.6% of the variance in resistance (Sliwka et al. 2007). Based on the results obtained from a *t*-test, the difference of the means is considered to be statistically significant. Other markers are being applied in the breeding programme of APPACALE such as markers for *Globodera rostochiensis, G. pallida*, *Potato Virus Y* and *Synchytrium endobioticum* resistance.

The most valuable markers are those which can identify a difficult trait because it would be cheaper and more reliable to apply marker-assisted selection (MAS) than performing the evaluation of the trait. In this case, 'difficult' markers could be applied. Moreover, other advantages of MAS are that good markers can avoid resistance tests and an early application of markers for agronomical traits would avoid performing many field assays. However, there are not many markers for important characteristics and another disadvantage is that they have to be checked for each parent.

## References

Bormann CA, Rickert AM, Castillo Ruiz RA, Paal J, Lübeck J, Strahwald J, Buhr K, Gebhardt C (2004) Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. Mol Plant Microbe Interact 17:1126–1138

Nakitandwe J, Trognitz F, Trognitz B (2007) Reliable allele detection using SNP-based PCR primers containing locked nucleic acid: application in genetic mapping. Plant Methods 3:2

Sliwka J, Jakuczun H, Lebecka R, Marczewski W, Gebhardt C, Zimnoch-Guzowska E (2007) Tagging QTLs for late blight resistance and plant maturity from diploid wild relatives in a cultivated potato (*Solanum tuberosum*) background. Theor Appl Genet 115:101–112