

Identification of virus and nematode resistance genes in the Chilota Potato Genebank of the Universidad Austral de Chile

Marlon López¹, Ricardo Riegel², Carolina Lizana², and Anita Behn^{2*}

Potato Genebank of the Universidad Austral de Chile (UACH) is an important gene bank in Chile. The accessions collected all over the country possess high genetic diversity, present interesting agronomic and cooking traits, and show resistance to biotic and abiotic stress. A particularly interesting subgroup of the gene bank includes the accessions collected in the South of Chile, the Chilota Potato Genebank. The focus of this study is the identification of virus and nematode resistant genes in potatoes (*Solanum tuberosum* L.), using the RYSC3 and YES3-3B molecular markers. The *Potato virus Y* (PVY) resistance genes *Ry_{adg}* and *Ry_{sto}* were identified. Furthermore, the CP60 marker was used to assess the *Rx* resistance gene that confers resistance to *Potato virus X* (PVX). In addition, the HC and GRO1-4 markers were utilized to identify the *GpaV_{vrn}-QTL* and *Gro1-4*, resistance genes of *Globodera pallida* and *Globodera rostochiensis*, respectively. Both *G. pallida* and *G. rostochiensis* are Potato Cyst Nematodes (PCN). The plant material used in this study included leaves from 271 accessions of the gene bank. These samples were collected in the field where natural pathogen pressure of potential viruses and diseases exists. ELISA assays were run for field detection of PVY and PVX. However, there have been no previous reports of nematode presence in the plant material. The results herein presented indicate presence of virus and nematode resistance genes in accessions of the Chilota Potato Genebank. In terms of virus resistance, 99 accessions out of the 271 tested possess the *Ry_{adg}* resistance gene and 17 accessions of these 271 tested have the *Ry_{sto}* resistance gene. Also, 10 accessions showed positive amplification of the *Rx1* resistant gene marker. As to nematode resistance, 99 accessions have possible resistance to *G. pallida* and 54 accessions show potential resistance to *G. rostochiensis* as detected using the available molecular markers.

Key words: *Globodera*, molecular marker, *Solanum tuberosum*, PVY, PVX.

INTRODUCTION

Pests and diseases are the major threat to potato (*Solanum tuberosum* L.) cultivation worldwide. Specifically, these threats are most catastrophic on smallholder farms in less developed countries where certified seed potatoes and chemical protection are not generally accessible (Gebhardt et al., 2006). It is known that 30% of the world potato yield is lost due to pests and diseases (Jellis and Boulton, 1984). Worldwide, there are 50 viruses that could infect *Solanum* species and at least 60 nematode species affecting the potato crop (Salazar, 1995). Classical breeding for pest resistant crops involves the identification of resistant sources, which are often found in wild and unadapted germplasm. Once these resistant sources have been found, introgression of the resistance factors into cultivars

is facilitated by repeated backcrossing to different *S. tuberosum* L. subsp. *tuberosum* clones. Following this, resistant progeny are phenotypically selected (Ross, 1986). Some of the known resistance factors have been located on molecular linkage maps using DNA-based markers. These resistance factors were mapped either as major genes (R genes) or as quantitative trait loci (QTL) (Gebhardt y Valkonen, 2001). Molecular markers are useful for constructing linkage and transcriptome maps (Ritter et al., 2008). Molecular markers can also be used to locate monogenic and polygenic traits to allow for the efficient introgression and selection of individuals with specific characteristics already in the seedling stage (Ritter et al., 2009). The use of DNA markers and marker-assisted selection (MAS) could increase the accuracy and efficiency of potato breeding programs (Bertrand et al., 2008).

Potato virus Y (PVY, genus *Potyvirus*) is one of the most important viral pathogens of the cultivated potato, and it also affects other *Solanaceae* species such as tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), and pepper (*Capsicum annum* L.) (Brunet et al., 1996). PVY, which can cause crop losses up to 80%, spreads through the use of infected tuber-seeds, mechanical contact, and through more than 25

¹Fundación Hondureña de Investigación Agrícola (FHIA), Apartado Postal 2067, San Pedro Sula, Cortés, Honduras.

²Universidad Austral de Chile, Facultad de Ciencias Agrarias, campus Isla Teja s/n, Valdivia, Chile.

*Corresponding author (anita.behn@uach.cl).

Received: 7 August 2014.

Accepted: 16 April 2015.

doi:10.4067/S0718-58392015000400008

aphid species in a non-persistent way. Different defense mechanisms of PVY have been described including extreme resistance based on major genes, tolerance, and hypersensitive responses (Bendahmane et al., 1999). Resistance to PVY has been reported in native potatoes (Valkonen et al., 1996). Several major genes such as *Ry* in *S. tuberosum* L. subsp. *andigena* (Juz. & Bukasov) Hawkes (*Ry_{adg}*), *Ry_{hou}* in *S. hougassii* Correll and *Ry_{sto}* in *S. stoloniferum* Schldtl. & Bouché (Barker 1997) are known to confer resistance. Kasai et al. (2000) developed the RYSC3 polymerase chain reaction (PCR)-based marker that can be used for the detection of both the *Ry_{adg}* gene and the *Na_{adg}* gene that function in the hypersensitive response to *Potato virus A* (PVA). Some years later, Song et al. (2005) mapped the *Ry_{sto}* gene on chromosome XII that co-segregates with the molecular marker STM0003. Song and Schwarzfischer (2008) developed the STS molecular markers YES3-A and YES3-B to co-localize the *Ry_{sto}* gene.

Potato virus X (PVX) is one of the main viruses infecting potatoes worldwide; it can cause yield losses ranging from 5-20%, depending up on the virus strain, the potato genotype, and if the infection occurs simultaneously with other viruses like PVY and PVA (Adams et al., 2004). The two dominant genes *Rx1* and *Rx2*, located on the top arm of chromosome XII and the upper arm of chromosome V, respectively, confer extreme resistance to PVX in potatoes (Bendahmane et al., 1997; Ritter et al., 1991).

There are several nematode species that affect potato crops. The *Globodera* genus is highly specific to *Solanaceous* species, and two *Globodera* species *G. pallida* and *G. rostochiensis* are found abundantly on potatoes. These are commonly known as potato cyst nematodes (PCN) and reduce tuber yields due to a reduction in the tuber size (Mugniéry and Phillips, 2007). Both species of PCN can be divided into pathogenic variants or pathotypes. An effective and long-lasting way to control PCN is the use of resistant potato cultivars. The dominant gene *Gro1* resistant to *G. rostochiensis* most likely originated from *S. spegazzinii* Bitter as this gene was mapped to potato chromosome VII (Barone et al., 1990). At the molecular level, the *Gro1* locus consists of a clustered family of NB-LRR type genes. One member of this family, *Gro1-4*, was shown to confer resistance to *G. rostochiensis* pathotype *Ro1* (Paal et al., 2004). Schwarzfischer et al. (2009) showed that the *Gro1-4* gene is informative for identifying varieties that possess full pathotype resistance to *Ro1-5* of *G. rostochiensis*. The most prominent and reproducible QTL for *G. pallida* resistance has been mapped to chromosome V. The diagnostic value of using the linked DNA marker SPUD1636 to identify sources of resistance was demonstrated for some accessions of *Solanum vernei* Bitter & Wittm (Bryan et al., 2002). Following this, Sattarzadeh et al. (2006) reported that the PCR marker HC has a higher diagnostic value for identifying

resistance in *G. pallida*. Furthermore, Schwarzfischer et al. (2009) showed that HC is an appropriated diagnostic tool to identify resistance in a wide spectrum of German potato varieties.

The genetic pool of wild tuber-bearing species of *Solanum* has been and will still be used as a source of resistance to viruses and nematodes in potato breeding. The range of genes available from wild species will allow potato breeders to combat pests and diseases in the future (Contreras and Tapia, 1983). The Chiloe Province in southern Chile, is considered to be a sub-center of the origin of potato species. The Potato Genebank at the Universidad Austral de Chile has a large number of accessions collected from the Chiloe Province, and therefore this repository most likely houses important genetic resources, including specific genes and genomic regions that can be used to look for pest and disease resistance. The objective of this study was to determine the existence of resistance alleles to *Potato virus Y*, *Potato virus X*, and potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*. Here conventional molecular markers and accessions of the Chilota Potato Genebank exposed to the natural pressure of viruses and nematodes were used.

MATERIAL AND METHODS

Plant material

Two hundred and seventy one accessions of the working collection of the Chilota Potato Genebank of the UACH maintained on the Southern Agricultural Experiment Station in Valdivia, Chile, were tested. The Potato Genebank was founded in 1958 at the university (UACH) when the Chilean Ministry of Agriculture donated potato accessions. Since then, the collection has been cultivated yearly at the Southern Agricultural Experiment Station of the UACH. The collection grew further due to many nationwide excursions conducted by Prof. Andrés Contreras and others. The colored tetraploid potatoes collected in the Chiloe Province were utilized for this study. Most of these potatoes are well described in recompilations of Contreras and Castro (2008).

The samples used in this study were collected from the experimental fields of the UACH and were not inoculated with viruses or nematodes so that the natural pressure of potential viruses and other diseases could be considered.

Serological procedure

Serological ELISA tests were used to determine the presence of PVY and PVX. The sap of fresh leaves from the 271 samples was extracted by mechanically (reductor machine R52 DT71K4/2, SEW EURODRIVE, Bruchsal, Germany) crushing five leaves of each accession while adding an extraction buffer. The procedure followed the protocol of BIOREBA for virus identification though some modifications were made. The BIOREBA (Reinach,

Switzerland) PVY polyclonal and PVX monoclonal reagent sets were used.

DNA extraction and quantification

Plant DNA was obtained from fresh leaves of five different plants of each accession collected from the field. Leaves were processed with the protocol described by Doyle and Doyle (1990) with some modifications. The quantification of the genomic DNA was performed using a 16-well Infinite F200/M200 NanoQuant spectrophotometer (TECAN, Männedorf, Switzerland) with absorption wavelengths of 260 and 280 nm. The DNA concentrations were diluted to 50 ng μL^{-1} DNA per sample.

Diagnostic PCR marker assays

PCRs were performed in a thermal cycler (MULTIGENE, International Labnet Inc., Edison, New Jersey, USA). The primers used in each assay are shown in Table 1. The reactions for amplifying the RYSC3 SCAR marker were performed in a final volume of 13 μL following the protocol of Kasai et al. (2000). The reaction contained 1X buffer (INVITROGEN, Carlsbad, California, USA), 100 μM of each dNTP, 2 mM MgCl_2 , 0.2 μM of each forward and reverse primer, 1 U of Taq polymerase (Invitrogen) and 50 ng of genomic DNA. The PCR program consisted of an initial denaturation step at 93 $^{\circ}\text{C}$ for 9 min, followed by 35 cycles of denaturation at 95 $^{\circ}\text{C}$ for 45 s, primer annealing at 52 $^{\circ}\text{C}$ for 45 s and primer extension at 72 $^{\circ}\text{C}$ for 1 min, followed by a final extension at 72 $^{\circ}\text{C}$ for 10 min. The negative control used was 'Desiree' (susceptible to PVY) and the positive control used was 'Eva' that possesses the *Ry^{adg}* gene and is immune to PVY, as described in Sagredo et al. (2009).

According to the protocol described by Song and Schwarzfischer (2008), the *Ry^{sto}* gene was detected using the YES3-3B STS marker. The optimum PCR band produced using this marker is 286 bp in size. The reaction was performed in a final volume of 13 μL containing 1X buffer (INVITROGEN), 100 μM of each dNTP, 2 mM MgCl_2 , 0.2 μM of each forward and reverse primer, 1 U Taq polymerase (Invitrogen) and 50 ng of genomic DNA. The PCR program consisted of an initial denaturation of 94 $^{\circ}\text{C}$ for 2 min, followed by 10 cycles of denaturation at 94 $^{\circ}\text{C}$ for 40 s, primer annealing at 60 $^{\circ}\text{C}$ for 40 s and primer extension at 72 $^{\circ}\text{C}$ for 1 min, followed by 30 cycles of denaturation at 94 $^{\circ}\text{C}$ for 40 s, primer annealing at 55 $^{\circ}\text{C}$ for 40 s, and primer extension at 72 $^{\circ}\text{C}$ for 1 min, followed for a final extension of 72 $^{\circ}\text{C}$ for 10 min.

The negative control used was 'Desiree' (susceptible to PVY) and the positive control used was 'Amado', which is immune to PVY (DNA of 'Amado' was kindly provided by Dr. A. Schwarzfischer, Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, 2012).

The *Rx1* gen was identified using the CP60 CAPS marker after restriction digestion with enzyme *DdeI* according to Bendahmane et al. (1997). A 350 bp *DdeI* fragment was indicative of positive *Rx1* presence in the accessions with resistance to PVX. The PCR reaction was performed in a final volume of 40 μL containing 1X buffer, 100 μM of each dNTP, 2 mM MgCl_2 , 0.2 μM of each forward and reverse primer, 1 U Taq polymerase and 50 ng genomic DNA. The PCR program consisted of an initial denaturation step of 94 $^{\circ}\text{C}$ for 4 min, followed by 35 cycles of denaturation at 94 $^{\circ}\text{C}$ for 45 s, primer annealing at 60 $^{\circ}\text{C}$ for 45 s and primer extension at 72 $^{\circ}\text{C}$ for 45 s, followed by a final elongation at 72 $^{\circ}\text{C}$ for 10 min. The negative control used was 'Desiree' (susceptible to PVX) and the positive control used was 'Atlantic'.

The *Gro1-4* gen was identified using the Gro1-4 SCAR marker described by Paal et al. (2004), which generates an amplification product of 602 bp and is associated with resistance to *G. rostochiensis*. The PCR reaction was performed in a final volume of 13 μL containing 1X buffer, 100 μM of each dNTP, 2 mM MgCl_2 , 0.2 μM of each forward and reverse primer, 1 U Taq polymerase and 50 ng of genomic DNA. The PCR program consisted of an initial denaturation of 94 $^{\circ}\text{C}$ for 3 min, followed for 35 cycles of denaturation at 92 $^{\circ}\text{C}$ for 45 s, primer annealing at 52 $^{\circ}\text{C}$ for 45 s, and primer extension at 72 $^{\circ}\text{C}$ for 1 min, and a final step at 72 $^{\circ}\text{C}$ for 10 min. The positive and negative controls were the same as those used for the HC marker.

The HC primers described by Sattarzadeh et al. (2006) generate a specific amplification product of 276 bp for the QRL-HC locus associated with resistance to *G. pallida*. The PCR reaction was performed in a final volume of 13 μL containing 1X buffer, 100 μM each dNTP, 2 mM MgCl_2 , 0.2 μM of each forward and reverse primer, 1 U Taq polymerase and 50 ng of genomic DNA. The PCR program consisted of an initial denaturation step of 94 $^{\circ}\text{C}$ for 4 min, followed by 35 cycles of denaturation at 94 $^{\circ}\text{C}$ for 45 s, primer annealing at 60 $^{\circ}\text{C}$ for 45 s and primer extension at 72 $^{\circ}\text{C}$ for 45 s, followed by a final elongation at 72 $^{\circ}\text{C}$ for 10 min. The negative control used was 'Desiree', susceptible to *G. pallida* and *G. rosotochiensis*.

Table 1. Molecular markers used and their primer sequences.

Marker	Type/ restriction	Gene	Size (bp)	Primers 5'-3'	Reference
RYSC3	SCAR	<i>Ry^{adg}</i>	321	ATACACTCATCTAAATTTGTATGG AGGATATACGGCATCATTTTTCGG	Kasai et al. (2000)
YES3-3B	STS	<i>Ry^{sto}</i>	290	TAACTCAAGCGGAATAACCC CATGAGATTGCCTTTGGTTA	Song and Schwarzfischer (2008)
CP60	CAPS/ <i>DdeI</i>	<i>Rx1</i>	350	CAGCCTACCGCGAAAGTGCCTTCG GCCAACCCACGAGTTTCTCACTGAC	Bendahmane et al. (1997)
Gro1-4	SCAR	<i>Gro1-4</i>	602	TCITTTGGAGATACTGATCTCTCA GCACCTAAAATGAAAAGCATCT	Paal et al. (2004)
HC	SNP	<i>GpaV_{vm-QTL}</i>	276	ACACCACCTGTTTGATAAAAACCT GCCTTACTTCCTGCTGAAG	Sattarzadeh et al. (2006)

'Brisant' was used as a positive control as it is immune to *G. pallida* and *G. rostochiensis* (DNA of 'Brisant' was kindly provided by Dr. A. Schwarzfischer, Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding, 2012).

RESULTS

Resistance to PVY, PVX, and *Globodera* spp.

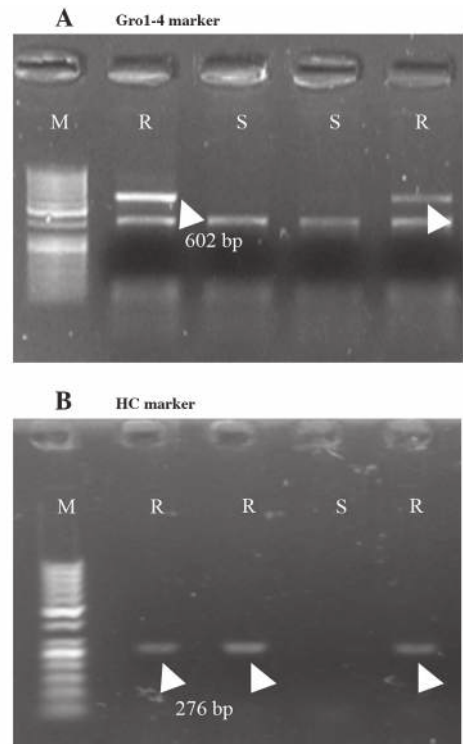
To determine the presence of the Ry_{adg} and Ry_{sto} PVY resistance genes, the RYSC3 SCAR marker and the YES3-3B STS marker were used, respectively. Ninety nine accessions out of the 271 showed amplification of the RYSC3 marker associated with resistance to PVY in *S. tuberosum* subsp. *andigena* source (Figure 1). Seventeen accessions showed amplification of the YES3-3B marker associated with resistance to PVY in *S. stoloniferum* (Figure 1). Finally, there were seven accessions that had amplification for both markers associated with PVY-resistance.

In resistance to PVX, the CP60 CAPS marker was used to locate the *Rx1* gene, which confers resistance to PVX. Ten accessions showed successful amplification of a 350 bp product (Figure 1). Due to difficulties in the amplification of the PCR product only 57% of the accessions of the gene bank were assessed.

In resistance to *Globodera* spp., the Gro1-4 SCAR marker and the HC SNP marker were used to locate the *Gro1-4* gene and the *GpaV_{vm}*-QTL, which are resistance genes to *G. rostochiensis* and *G. pallida*, respectively (Figure 2). Positive amplification of the *Gro1-4* gene associated with resistance to *G. rostochiensis* was possible in 54 accessions and in 99 accessions the HC marker related to resistance to *G. pallida* was amplified.

Combined resistance

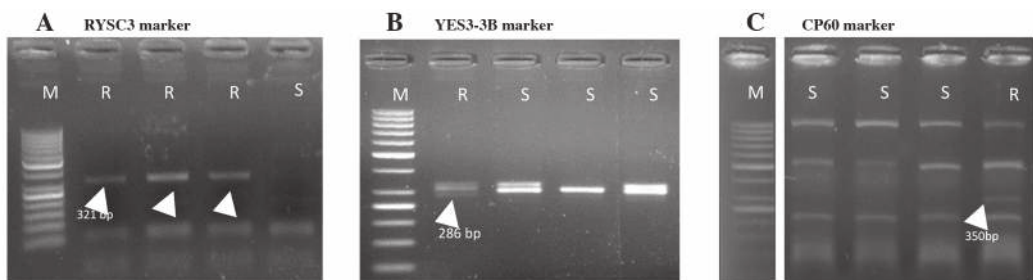
As gene pyramiding is an important tool to enhance the development of new potato varieties, the marker combinations used in this study should be considered in further breeding experiments. In the present analysis, one accession was found to possess both markers for



Thicker bands represent 250 and 500 bp; R = resistant; S = susceptible.

Figure 2. Examples of amplification products and diagnostic bands (with arrow) in potato accessions with markers associated with nematode resistance A) Gro1-4 marker associated with resistance to *Globodera rostochiensis* (Paal et al., 2004); HC marker for *G. pallida* resistance (Sattarzadeh et al., 2006). M = 50 bp DNA ladder (Thermo Scientific).

PVY and for nematode resistance. Amplification using the diagnostic markers RYSC3, YES3-3B, GRO1-4, and HC was possible for accession 97-AU-1296. Furthermore, two other accessions showed positive amplification products for the YES3-3B, Gro1-4, and HC markers. In addition, amplification of three molecular markers (GRO1-4, HC, and RYSC3) was possible for eight accessions. Finally, the RYSC3 and HC molecular markers produced positive amplification in 34 accessions.



Thicker bands represent 250 and 500 bp; R = resistant; S = susceptible.

Figure 1. Examples of amplification products and diagnostic bands (with arrow) in potato accessions with markers associated with virus resistance: A) RYSC3 marker for *Potato virus Y* (PVY) resistance (Ry_{adg}) (Kasai et al., 2000); B) YES3-3B marker for PVY resistance (Ry_{sto}) (Song and Schwarzfischer, 2008); C) CP60 marker associated with *Potato virus X* (PVX) resistance (Bendahmane et al., 1997). M = 50 bp DNA ladder (Thermo Scientific).

These two markers are associated with resistance to PVY and *G. pallida*, respectively. The GRO1-4 marker related to *G. rostochiensis* resistance and the RYSC3 marker for PVY resistance were used for successful amplifications in ten accessions. Specifically, one accession showed amplification for the markers RYSC3, CP60, and HC, one for CP60, GRO1-4, HC. Two accessions showed positive amplification for the CP60 and HC markers, and

amplification using the RYSC3 and CP60 markers was possible for one accession (Table 2).

Serological and molecular results

The ELISA tests were additional to the molecular results. Figure 3 shows all accessions evaluated, including the accessions without marker amplification. From the 271 evaluated accessions, 247 showed negative ELISA results

Table 2. Accessions with a combination of two or more amplified molecular markers.

Accession	RYSC3	YES3-3B	CP60	GRO1-4	HC	Resistance genes
280-CON-755	+	+	-	(+)	-	<i>Ry_{adg}</i> , <i>Ry_{sto}</i> , <i>Gro1-4</i>
366-UA-1186A	+	+	-	-	-	<i>Ry_{adg}</i> , <i>Ry_{sto}</i>
521-UA-1625B	+	+	-	-	-	<i>Ry_{adg}</i> , <i>Ry_{sto}</i>
97-UA-1296	+	+	-	+	+	<i>Ry_{adg}</i> , <i>Ry_{sto}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
233-UA-1593	-	+	-	+	+	<i>Ry_{sto}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
530-CON-1114	-	+	-	+	+	<i>Ry_{sto}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
254-CON-902	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
219-UA-1534	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
4-CON-1154	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
247-CON-1100	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
NG-117	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
NG-82	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
INIA-S/N-P1	+	-	-	+	+	<i>Ry_{adg}</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
373-UA-1587	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
NN-1	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
93-COCHRANE-14	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
244-CON-1266	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
344-CON-1148	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
418-UA-1243	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
298-UA-1362	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
229-CON-1039	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
205-UA-1014	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
227-CON-824	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
166-CON-1089	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
181-UA-1022	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
161-CON-1093	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
487-UA-1191	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
93-COCHRANE-13	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
336-CON-784	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
184-UA-1356	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
481-CON-1099	+	+	-	-	+	<i>Ry_{adg}</i> , <i>Ry_{sto}</i> , <i>GpaV_{vrn-QTL}</i>
NG-94	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
488-UA-1354	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
156-CON-1171	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
512-UA-1079	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
NG-88	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
586-CON-1153	+	+	-	-	+	<i>Ry_{adg}</i> , <i>Ry_{sto}</i> , <i>GpaV_{vrn-QTL}</i>
93-COCHRANE-6	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
195-CON-1296	+	-	+	-	+	<i>Ry_{adg}</i> , <i>Rx</i> , <i>GpaV_{vrn-QTL}</i>
133-CON-1051	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
185-UA-1560	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
NG-120	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
311-UA-1093	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
421-CON-921	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
422-UA-1023	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
563-UA-1157	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
CARAHUE-CON-1365	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
LOS LAGOS	+	-	-	-	+	<i>Ry_{adg}</i> , <i>GpaV_{vrn-QTL}</i>
58-CON-1041	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
260-CON-917	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
169-UA-1517	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
NG-92	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
NG-80	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
585-UA-1633	+	+	-	+	-	<i>Ry_{adg}</i> , <i>Ry_{sto}</i> , <i>Gro1-4</i>
541-UA-1042	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
562-UA-1397	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
547-UA-1053	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
NG-140	+	-	-	+	-	<i>Ry_{adg}</i> , <i>Gro1-4</i>
426-CON-926	-	-	+	+	+	<i>Rx</i> , <i>Gro1-4</i> , <i>GpaV_{vrn-QTL}</i>
464-UA-1360	-	-	+	-	+	<i>Rx</i> , <i>GpaV_{vrn-QTL}</i>
195-CON-1296	-	-	+	-	+	<i>Rx</i> , <i>GpaV_{vrn-QTL}</i>
42-UA-1258	+	-	+	-	-	<i>Ry_{adg}</i> , <i>Rx</i>

+ = Marker presence - = Marker absence.

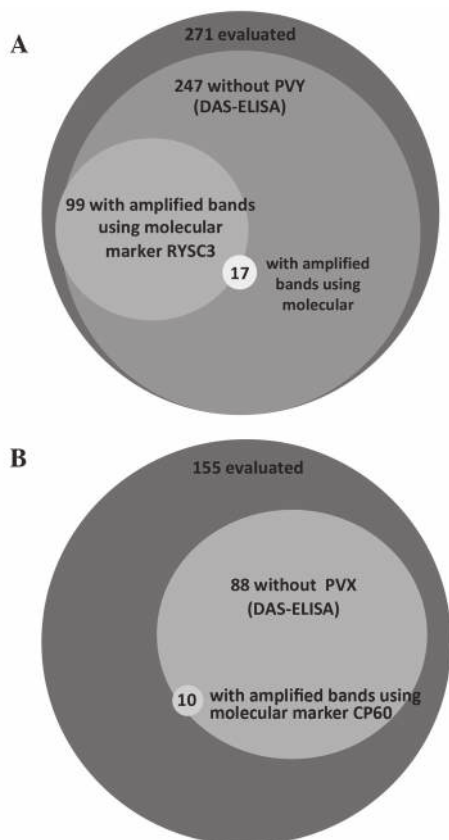


Figure 3. A) Number of accessions evaluated using markers RYSC3 and YES3-3B with and without virus infection according to the DAS-ELISA results as well as presence and absence of both markers. B) Number of accessions analyzed with the CP60 marker with and without virus infection according to the DAS-ELISA results as well as presence and absence of the CP60 band.

for PVY presence, which means that 91% of the accessions from the field showed no PVY infestation. Ninety-nine accessions of the 247 accessions mentioned above showed positive amplification bands when the RYSC3 marker was used. In addition, 17 accessions showed positive amplification when the YES3-3B marker was used. Seven accessions showed negative ELISA results for PVY and negative PCR products for both of the PVY-resistance markers (RYSC3 and YES3-3B). The remaining 148 accessions (60% of the accessions without PVY presence) showed no virus infection and no amplification was possible for any of the molecular markers.

Out of the 155 accessions evaluated for PVX presence, 67 showed PVX positive and 88 showed PVX negative ELISA results. Nine out of the 10 accessions that presented band amplifications using the CP60 marker had negative ELISA results.

DISCUSSION

For many years, the characteristics of the accessions of the UACH Chilota Potato Genebank have been evaluated.

Traits such as yield, disease resistance, potato flavor, and taxonomic classification (part of this information is available in the catalogue of Contreras and Castro (2008) and at www.potatogenebank.cl), have been investigated. However the present study is the first molecular marker evaluation of virus and nematode resistance in Chilota potatoes.

The utilization of the RYSC3 marker has been validated by many authors such as Dalla Rizza et al. (2006), Sagredo et al. (2009), Veramendi et al. (2011), Ortega and Lopez-Vizcon (2012), and Lopez-Pardo et al. (2013). In the evaluation of the 271 *Solanum tuberosum* subsp. *tuberosum* accessions, amplification using the RYSC3 marker was possible in 99 accessions. That means that 37% of the measured accessions possess the resistance marker. There are clear differences between *S. tuberosum* subsp. *tuberosum* and *S. tuberosum* subsp. *andigena* (Hawkes, 1990). However, the source of origin of these two subspecies has been delimited geographically and both subspecies seem to have co-evolved together and genes resistant to viruses and nematodes are present in both subspecies.

The *S. stoloniferum* (*Ry_{sto}*) PVY resistance was first reported by Cockerham (1943). This gene confers resistance to all strains of PVY and PVA (Ross, 1986; Barker, 1997). The YES3-3B molecular marker has been used by breeders in Europe. Lindner et al. (2011) used YES3-3A and YES3-3B for evaluation of PVY in German potato varieties showing that 21 of the 31 varieties evaluated possess *Ry_{sto}*, the gene for resistance. By evaluating tetraploid crosses of *S. phureja*, Schwarzfischer et al. (2009) found 10 varieties of potato with extreme resistance to PVY. In the present study, 17 accessions were found to have an amplified gene product using the YES3-3B marker. Although the geographical origin of *S. stoloniferum* and *S. tuberosum* ssp. *tuberosum* seems to be different, some *S. tuberosum* ssp. *tuberosum* accessions of the Potato Genebank possess the same loci for resistance as *S. stoloniferum*. This situation might be explained by ancestral history.

The marker RYSC3 produced positive amplification for the following accessions: 525-UA-1244, 236-UA-1031, 502-UA-1071, 512-UA-1079, and 181-UA-1022. These accessions were reported to have absence of PVY according to the DAS-ELISA. In various publications (Contreras and Banse, 1982; Contreras and Tapia, 1983), these accessions have been shown to lack PVY, therefore it seems to be accessions with a PVY resistance, lasting through the time. Out of the 99 accessions the amplified for the marker RYSC3, 95 showed absence of PVY according to DAS-ELISA. These represent a 95% correlation between molecular and serological results. Both methods are usually applied as a tool for virus resistances evaluations; like Gebhardt et al. (2006), Sagredo et al. (2009), and Ortega and Lopez-Vizcon (2012). The remaining 148 accessions showed no virus

infection and no molecular marker amplification, task that might be explained by unknown PVY resistance sources, since that plant has been exposed to natural pest presence on the field.

An important number of wild *Solanum* species have been used as resistance source for PVX. The *Rx_{adg}* (*Rx1*), *Rx_{ibr}*, *Rx_{acl}* (*Rx2*) genes conferring extreme resistance to PVX have been reported in *S. tuberosum* subsp. *andigena*, *S. tuberosum* L. subsp. *chiloense* (A. DC.) L.I. Kostina and *S. acaule* (Cockerham, 1970). Bendahmane et al. (1997) localized the *Rx1* gene on chromosome XII, using the GP34 and CP60 molecular markers. The CP60 marker has been used by other authors such as Gebhardt et al. (2006) and Veramendi et al. (2011). Comparing the same accessions used in this investigation that showed amplification using the CP60 marker, with previous evaluations of the Potato Genebank, Contreras and Banse (1982) reported that accessions 548-UA-1328, 464-UA-1360, and 186-UA-1045 are not resistant to PVX as determined by serological evaluation.

The genes resistant to PCN have been reported in *S. spegazzinii* and *S. vernei* (Ross, 1986; Phillips, 1994). The HC and Gro1-4 molecular markers associated with resistance to PCN were used by other authors: Gebhardt et al. (2006), Veramendi et al. (2011), Milczarek et al. (2011), Ortega and Lopez-Vizcon (2012), Lopez-Pardo et al. (2013), Dalton et al. (2013). In this study 99 accessions (36% of the 271 accessions) showed amplification bands using the HC marker. In addition, 54 accessions (20% of 217 accessions) showed positive amplification products for marker Gro1-4. This result is promising considering that fewer accessions were expected to amplify for these markers, because of previous information about the accessions and the impossibility of co-evolution of the disease with the potato crop due to the absence of *G. rostochiensis* in the South of Chile. The PCN is considered a quarantine pest in European and Mediterranean Plant Protection Organization (EPPO) member countries and in seed potato growing areas of Chile (SAG, 2008). Therefore, nematode resistance genes for *G. pallida* and *G. rostochiensis* have a great relevance in the potato breeding programs all over the world.

Milla and Krausz (2004) found three accessions with extreme PCN resistance, three accessions with partial resistance, and eight accessions susceptible in a preview evaluation of 14 accessions of the Potato Genebank. From the accessions with extreme resistance, two accessions (280-CON-755 and 254-CON-902) amplified for the Gro1-4 marker in this study. Further studies will test other molecular markers for PCN resistances as well as inoculation tests of resistant accessions in the lab.

CONCLUSIONS

Molecular markers linked to resistance genes for the PVY, PVX and Potato Cyst Nematode (PCN) were found

within the Chilota Potato Genebank of the Universidad Austral de Chile. As all of the used markers are known to be linked to major genes, they are a real tool to be used for gene pyramiding in further potato breeding programs. The PVY resistance genes of *Solanum tuberosum* subsp. *andigena* (*Ry_{adg}*) were found in a higher proportion than those of *Solanum stoloniferum* (*Ry_{sto}*). The genes resistant to PCN were found in a greater proportion for *Globodera pallida* than for *G. rostochiensis*. However, there were also accessions found that possess both resistance genes. The tested accessions are highly recommended for potato crop breeding as they possess resistance to diseases and pathogens relevant at national and global scales.

ACKNOWLEDGEMENTS

This work was funded by the DID 2012-03 project from the Universidad Austral de Chile.

LITERATURE CITED

- Adams, M.J., J.F. Antoniwi, M. Bar-Joseph, A.A. Brunt, T. Candresse, G.D. Foster, et al. 2004. The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Archives of Virology* 149:1045-1060.
- Barker, H. 1997. Extreme resistance to potato virus V in clones of *Solanum tuberosum* that are also resistant to potato viruses Y and A: evidence for a locus conferring broad-spectrum potyvirus resistance. *Theoretical and Applied Genetics* 95:1258-1262.
- Barone, A., E. Ritter, U. Schachtschabel, T. Debener, F. Salamini, and C. Gebhardt. 1990. Localization by restriction fragment length polymorphism mapping in potato of a major dominant gene conferring resistance to the potato cyst nematode *Globodera rostochiensis*. *Molecular Genetics and Genomics* 224:177-182.
- Bendahmane, A., K. Kanyuka, and D.C. Baulcombe. 1997. High resolution genetical and physical mapping of the *Rx* gene for extreme resistance to potato virus X in tetraploid potato. *Theoretical and Applied Genetics* 95:153-162.
- Bendahmane, M., M. Koo, E. Karrer, and R.N. Beachy. 1999. Display of epitopes on the surface of tobacco mosaic virus: impact of charge and isoelectric point of the epitope on virus-host interactions. *Journal of Molecular Biology* 290:9-20.
- Bertrand, C., Y. Collard, and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363:557-572.
- Brunt, A.A., K. Crabtree, M.J. Dallwitz, A.J. Gibbs, and L. Watson. 1996. *Viruses of plants*. 1484 p. CAB International, Wallingford, UK.
- Bryan, G.J., K. McLean, J.E. Bradshaw, W.S. De Jong, M. Phillips, L. Castelli, et al. 2002. Mapping QTLs for resistance to the cyst nematode *Globodera pallida* derived from the wild potato species *Solanum vernei*. *Theoretical and Applied Genetics* 105:68-77.
- Cockerham, G. 1943. Potato breeding for virus resistance. *Annals of Applied Biology* 30:105-108.
- Cockerham, G. 1970. Genetical studies on resistance to potato viruses X and Y. *Heredity* 25:309-348.
- Contreras, A., y J. Banse. 1982. Determinación de virus en el germoplasma chileno de papas (*Solanum* sp.) *Agro Sur* 10(2):84-89.
- Contreras, A., y I. Castro. 2008. Catálogo de variedades de papas nativas de Chile. 236 p. Universidad Austral de Chile, Valdivia, Chile.
- Contreras, A., y V. Tapia. 1983. Resistencia a virus en variedades antiguas de papa chilena (*Solanum tuberosum* L.) *Agro Sur* 11(2):69-73.

- Dalla Rizza, M., F. Vilaro, D.G. Torres, and D.C. Maeso. 2006. Detection of PVY extreme resistance genes in potato germplasm from the Uruguayan breeding program. *American Journal of Potato Research* 83:297-304.
- Dalton, E., D. Griffin, T. Gallagher, N. De Vetten, and D. Milbourne. 2013. The effect of pyramiding two potato cyst nematode resistance loci to *Globodera pallida* Pa2/3 in potato. *Molecular Breeding* 31:921-930.
- Doyle, J.J., and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.
- Gebhardt, C., D. Bellin, H. Henselewski, W. Lehmann, J. Schwarzfischer, and J.P. Valkonen. 2006. Marker-assisted combination of major genes for pathogen resistance in potato. *Theoretical and Applied Genetics* 112:1458-1464.
- Gebhardt, C., and J.P.T. Valkonen. 2001. Organization of genes controlling disease resistance in the potato genome. *Annual Review of Phytopathology* 39:79-102.
- Hawkes, J. 1990. *The Potato, evolution, biodiversity and genetic resources*. Belhaven Press, London, UK.
- Jellis, G.J., and R.E. Boulton. 1984. Damage and loss caused by potato diseases p. 255-266, 327. In Wood, R.K.S., and G.J. Jellis (eds.) *Plant diseases: Infection, damage and loss*. Blackwell Scientific Publications, Oxford, UK.
- Kasai, K., Y. Morikawa, V.A. Sorri, J.P.T. Valkonen, C. Gebhardt, and K.N. Watanabe. 2000. Development of SCAR markers to the PVY resistance gene *Ry_{adg}* based on a common feature of plant disease resistance genes. *Genome* 43:1-8.
- Lindner, K., A. Behn, A. Schwarzfischer, and Y. Song. 2011. Extreme Y-Resistenz im aktuellen deutschen Kartoffelsortiment. *Journal für Kulturpflanzen* 63(4):97-103.
- Lopez-Pardo, R., L. Barandalla, E. Ritter, and J. Ruiz De Galarreta. 2013. Validation of molecular markers for pathogen resistance in potato. *Plant Breeding* 132:246-251.
- Milczarek, D., B. Flis, and A. Przetakiewicz. 2011. Suitability of molecular markers for selection of potatoes resistant to *Globodera* spp. *American Journal of Potato Research* 88:245-255.
- Milla, A., y C. Krausz. 2004. Detección de resistencia al nematodo dorado (*Globodera rostochiensis* Woll) en accesiones pertenecientes al germoplasma chileno de papa (*Solanum tuberosum* L.). *Agro Sur* 32(1):28-34.
- Mugniéry, D., and M.S. Phillips. 2007. The nematode parasites of potato. p. 569-594. In Vreugdenhil, D., J. Bradshaw, C. Gebhardt, F. Govers, M. Taylor, D. MacKerron, H. Ross (eds.) *Potato biology and biotechnology. Advances and perspectives*. Elsevier, Wageningen, The Netherlands.
- Ortega, F., and C. Lopez-Vizcon. 2012. Application of molecular Marker-Assisted Selection (MAS) for disease resistance in a Practical Potato Breeding Programme. *Potato Research* 55:1-13.
- Paal, J., H. Henselewsk, J. Muth, K. Meksem, C.M. Menéndez, F. Salamini, et al. 2004. Molecular cloning of the potato *Gro1-4* gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. *Plant Journal* 38:285-297.
- Phillips, M.S. 1994. Inheritance of resistance to nematodes. p. 319-337. In Bradshaw, J.E., and G.R. Mackay (eds.) *Potato genetics*. CAB International, Wallingford, UK.
- Ritter, E., T. Debener, A. Barone, F. Salamini, and C. Gebhardt. 1991. RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). *Molecular Genetics and Genomics* 227:81-85.
- Ritter, E., J.I. Ruiz de Galarreta, M. Hernandez, G. Plata, L. Barandalla, R. Lopez, et al. 2009. Utilization of SSR and cDNA markers for screening know QTLs for late blight (*Phytophthora infestans*) resistance in potato. *Euphytica* 170:77-86.
- Ritter, E., J.I. Ruiz de Galarreta, H.J. van Eck, and I. Sanchez. 2008. Construction of a potato transcriptome map based on the cDNA-AFLP technique. *Theoretical and Applied Genetics* 116:1003-1013.
- Ross, H. 1986. *Potato breeding: Problems and perspectives. Advances in Plant Breeding. Fortschritte der Pflanzenzüchtung (Suppl 13)*. Paul Parey, Berlin und Hamburg, Germany.
- SAG. 2008. Declara área libre de *Globodera rostochiensis*, *Globodera pallida*, *Thecaphora solani* (*Angiosorus solani*) y *Ralstonia solanacearum* (raza 3, biovar 2), al área comprendida por la Provincia de Arauco, VIII Región y la IX, X y XI Regiones. Modificada por Res. n° 890 de 2006 y n° 2.238 de 2008. Servicio Agrícola y Ganadero (SAG), Santiago, Chile. Available at http://www.sag.cl/sites/default/files/RES_2104_2003.pdf (accessed 08.04.2015).
- Sagredo, B., M. Mathias, C. Barrientos, I. Acuña, J. Kalazich, and J. Rojas. 2009. Evaluation of a SCAR RYSC3 marker of the *Ry_{adg}* gene to select resistant genotypes to *Potato virus Y* (PVY) in the INIA Potato Breeding Program. *Chilean Journal of Agricultural Research* 69:305-315.
- Salazar, L.F. 1995. *Los virus de la papa y su control*. Centro Internacional de la Papa, Lima, Perú.
- Sattarzadeh, A., U. Achenbach, J. Lübeck, J. Strahwald, E. Tacke, H.R. Hofferbert, et al. 2006. Single nucleotide polymorphism (SNP) genotyping as basis for developing a PCR-based marker highly diagnostic for potato varieties with high resistance to *Globodera pallida* pathotype Pa2/3. *Molecular Breeding* 18:301-318.
- Schwarzfischer A., A. Behn, J. Groth, M. Reichmann, A. Kellermann, and Y. Song. 2009. Marker-assisted selection in practical potato breeding - experience and outlook. *Tagung Züchtung und Genressourcen gegen abiotische Stressfaktoren. Markergestützte Selektion in der Praxis*. 24-26 November 2009. 81-85. Raumberg-Gumpenstein, Germany.
- Song, Y.S., L. Hepting, G. Schweizer, L. Hartl, G. Wenzel, and A. Schwarzfischer. 2005. Mapping of extreme resistance to PVY (*Ry_{sto}*) on chromosome XII using anther-culture-derived primary dihaploid potato lines. *Theoretical and Applied Genetics* 111:879-887.
- Song, Y., and A. Schwarzfischer. 2008. Development of STS markers for selection of extreme resistance (*Ry_{sto}*) to PVY and maternal pedigree analysis of extremely resistant cultivars. *American Journal of Potato Research* 85:159-170.
- Valkonen, J.P.T., M.M. Kyle, and S.A. Slack. 1996. Comparison of resistance to potyviruses within *Solanaceae*: infection of potatoes with tobacco etch potyvirus and peppers with potato A and Y potyviruses. *Annals of Applied Biology* 129:25-38.
- Veramendi, S., M. Baldeomar, A. Teran, y J. Gabriel. 2011. Marcadores moleculares asociados a genes/QTLs de resistencia para factores bióticos en nuevas variedades de papa (*Solanum tuberosum* L.) de Bolivia. *Revista Latinoamericana de la Papa* 16:209-232.