Identification of regions associated to late blight resistance and viruses in potato germplasm using molecular markers

Identificación de regiones asociadas a la resistencia del tizón tardío y virus en germoplasma de papa mediante marcadores moleculares

Identificação de regiões associadas à resistência à plaga tardia e vírus em germoplasma de batata usando marcadores moleculares

Ariadne Vegas García[1,2*]
Yanet Sandrea Rincón[2†]
Asia Zambrano[2†]
Lourdes González[3]
Martha Osorio[2]
Guillermo Trujillo[4]
Jorge Peralta Calle[5]

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[1]Instituto Nacional de Investigaciones Agropecuarias (INIAP). Estación Experimental Santo Domingo. Km 38 vía Santo Domingo – Quinindé. Cantón La Concordia. Santo Domingo de los Tsáchilas. Ecuador.
[2]Instituto Nacional de Investigaciones Agrícolas (INIA), Centro Nacional de Investigaciones Agropecuarias (CENIAP). Unidad de Biotecnología Vegetal. Apartado Postal 5653, Maracay 2101, Venezuela.
[3]Instituto Nacional de Investigaciones Agrícolas (INIA), Centro de Investigaciones Agropecuarias del Estado Mérida, Venezuela.
[4]Applied Biotecnology Laboratory. International Potato Center (CIP). P.O. Box 1558, Lima 12, Perú.
[5]Universidad Agraria del Ecuador, sede Guayaquil. Av. 25 de julio. Guayaquil 090104. Ecuador.

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Abstract

The combination of traits of economic interest in new potato cultivars, such as resistance to late blight, viral diseases, and culinary quality are important to achieve their adoption by farmers. In the present work, molecular markers were used to identify regions associated to late blight, the viruses PVY and PLRV resistance, in 50 materials belonging to the National Institute of Agricultural Research (INIA-Venezuela): commercial cultivars, differentials of blight, advanced clones from CIP and hybrids from the Fundación PROINPA of Bolivia. DNA extraction was carried out from vitroplants and known microsatellite, SCAR and CAPS molecular markers were used. Among 96 to 26% of the accessions amplified regions of the QTL *tbr* of chromosome XII, associated with resistance to blight. Only the differential R9 and crc2/P8 from PROINPA amplified the *R1* gene region. Between 18 and 68% of the genotypes presented the regions associated with the PVY and PLRV resistance genes (Ry_{adg} and *N* genes), respectively; only 10% amplified both regions; while in 24% these genes were not detected, among them are the commercial varieties Granola, Andinita and Cartayita. This study generated valuable information to support genebank curators and breeders in potato genetic improvement programs of this country.

*Corresponding author: ariadne.vegas@iniap.gob.ec

Resumen

La combinación de caracteres de interés económico en nuevos cultivares de papa, tales como la resistencia al tizón tardío, las enfermedades virales, y la calidad culinaria son importantes para lograr su adopción por los agricultores. En el presente trabajo se usaron marcadores moleculares para identificar regiones asociadas a la resistencia del tizón tardío y a los virus PVY y PLRV, en 50 materiales pertenecientes al Instituto Nacional de Investigaciones Agrícolas (INIA-Venezuela): cultivares comerciales, diferenciales del tizón, clones avanzados del CIP e híbridos de la Fundación PROINPA de Bolivia. La extracción del ADN se realizó a partir de vitroplantas y se utilizaron marcadores moleculares tipo microsatélites, SCAR y CAPS conocidos. Entre el 96 al 26 % de las accesiones amplificaron regiones del QTL *tbr* del cromosoma XII, asociado a la resistencia al tizón. Solo el diferencial R9 y crc2/P8 de PROINPA amplificaron la región del gen *R1*. Entre el 18 y 68 % de los genotipos presentaron las regiones asociadas con los genes de resistencia al PVY y PLRV (genes Ry_{adg} y *N*), respectivamente; solo el 10 % amplificaron ambas regiones; mientras que en 24 % no se detectaron estos genes, entre ellos se encuentran las variedades comerciales Granola, Andinita y Cartayita. Este estudio generó información valiosa de soporte a los curadores de los bancos de germoplasma y a los fitomejoradores en los programas de mejoramiento genético de papa del país.

Palabras clave: Oomicete, *Phytophthora infestans*, PLRV, PYV, *Solanum tuberosum*.

Resumo

A combinação de características de interesse econômico em novas cultivares de batata, como resistência à requeima, doenças virais e qualidade culinária, são importantes para sua adoção pelos produtores. No presente trabalho, marcadores moleculares foram utilizados para identificar regiões associadas à resistência à requeima e aos vírus PVY e PLRV, em 50 materiais pertencentes ao Instituto Nacional de Pesquisa Agropecuária (INIA-Venezuela): cultivares comerciais, diferenciais da requeima, clones avançados do CIP e híbridos da Fundación PROINPA da Bolívia. A extração de DNA foi realizada a partir de vitroplantes e foram utilizados microssatélites, marcadores moleculares SCAR e CAPS conhecidos. Entre 96 a 26 % dos acessos amplificaram regiões do QTL *tbr* do cromossomo XII, associadas à resistência à ferrugem. Apenas o diferencial R9 e crc2/P8 de PROINPA amplificou a região do gene *R1*. Entre 18 e 68 % dos genótipos apresentaram as regiões associadas aos genes de resistência PVY e PLRV (genes *N* e Ry_{adg}), respectivamente; apenas 10 % amplificou ambas as regiões; enquanto em 24 % esses genes não foram detectados, entre eles estão as variedades comerciais Granola, Andinita e Cartayita. Este estudo gerou informação valiosa para apoiar curadores de bancos de germoplasma e melhoristas de plantas nos programas de melhoramento genético da batata do país.

Palavras chave: Oomiceto, *Phytophthora infestans*, PLRV, PYV, *Solanum tuberosum.*

Introduction

In Venezuela, the potato (*Solanum tuberosum* L.) is considered a fundamental part of the diet, especially for inhabitants located in the Andean areas, states of Mérida, Táchira and Trujillo, due to its high content of carbohydrates, vitamins and minerals; constituting one of the main sources of economic income for small and medium farmers in these regions, where more than 80 % of the cultivated area is planted (González *et al*., 2017).

Globally, pests and diseases are the biggest problem facing potato growers, particularly small-scale growers in less developed countries, where certified seed and agrochemicals are not affordable. Among the most important diseases are late blight, bacterial wilt, and PVY (Potato Virus Y), PVX (Potato Virus X) and potato leafroll virus (PLRV) (Haverkort *et al*., 2016).

Late blight, commonly referred to as late candelilla or blight, is caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. The disease can destroy foliage and stems, and attack tubers, when conditions are favorable, at moderate temperatures, 16 to 22 °C, and high humidity (100 %). In prolonged humid conditions, all the tender and aerial organs of the plants wither and rot very quickly. It can be satisfactorily controlled using resistant cultivars, spraying with chemical compounds applied systematically, and cultural practices (Agrios, 2005). The oomycete comprises several pathotypes or physiological races, identified according to their virulence in differential potato genotypes. Pathotypes are the product of genetic variation due to mutations, sexual recombination; and possibly to changes in the ploidy of the pathogen or to hybridization with other *Phytophthora* species. New races with greater resistance to systemic fungicides, greater virulence and parasitic aptitude have been identified, as well as the existence of oospores resulting from the sexual reproduction of the pathogen in new agricultural regions (Alvarez-Morezuelas *et al*., 2021).

In Venezuela, the disease is present in almost all areas of the country where potatoes are grown, causing losses up to 100 %, when the infection occurs at critical times of crop development and before tuberization. Because the Granola cultivar, widely used in the country in the areas most affected by blight, has low resistance to candelilla, producers apply systemic fungicides without restriction (García and García, 2004). Isolates of *P. infestans* collected in various regions of the country have been characterized and are of the A1 compatibility type, with different susceptibilities to the metalaxyl fungicide (Rodríguez *et al*., 2008).

Two types of resistance to late blight have been identified in potato and related wild species, one based on major dominant genes (*R* genes), and the other polygenic, based on a Quantitative Trait Locus (QTL). The first type of resistance was introduced into cultivated potatoes through the introgression of *R* genes from the wild species *S. demissum*, of which 11 (*R1*-*R11*) have been identified. However, these genes were overtaken by newer races of the oomycete, and they were found not to provide durable resistance, either alone or in combination. For this reason, *R* genes present in other wild species, *S. berthaultii, S. bulbocastanum, S. guerreroense, S. neoantipoviczi* and *S. pinnatisectum*, among others, which have been incorporated in a pyramidal combination to *S. tuberosum*, were searched for (Ballesteros *et al*., 2010; Fadina *et al*., 2017; Zoteyeva *et al*., 2014). It has been argued that the best strategy to achieve longer-lasting resistance, possibly exerting less selection pressure on the pathogen, is the introgression of *R* genes that confer quantitative field resistance, as in the case of genes *R8*, *R9a*, *R10* and *Rpi-blb1* (Fadina *et al*., 2017; Jiang *et al*., 2018). Other breeders have chosen to select genotypes with quantitative field resistance, controlled by various non-race-specific genes, which has been described as horizontal, incomplete, and broad-spectrum (Fadina *et al*., 2017; Jiang *et al*., 2018). However, this type of resistance depends on external factors and has shown

a strong correlation with late maturity. Today it is known that, in general, QTLs correspond to groups of *R* genes (Jiang *et al*., 2018; Rubio *et al*., 2016).

Ballvora *et al*. (2002) cloned the first late blight resistance gene, called *R1*, located on chromosome V, in a dense region, where other resistance genes have also been found. To date, genes *R1*, *R2*, *R3*, and *R8* have been cloned (Jiang *et al*., 2018).

On the other hand, QTL or regions of the genome whose phenotypic effect is measurable on a continuous scale have been identified to identify potato genotypes resistant to late blight (Jiang *et al*., 2018; Trujillo, 2004). In this sense, Trujillo (2004) developed CAPS (Cleaved Amplified Polymorphic Sequences) and SCAR (Sequence Characterized Amplified Region) markers, which amplified regions of the QTL *tbr* of chromosome XII, from a segregating population of *S. phureja* (diploid, with resistance to late blight) x *S. tuberosum* (dihaploid, susceptible to the disease). This QTL of the parent *S. tuberosum* had a high contribution to field resistance to late blight, which also provided phenotypic variation of up to 43 % and was associated with markers linked to candidate genes involved in biochemical defense pathways, such as phenylalanine ammonia lyase (PAL) and chalcone isomerase (CHI) enzymes, WRKY transcription factors, osmotin, and cytochrome P450 induced by *P. infestans*.

Regarding viruses, in the Andean region of Venezuela, the potato crop is affected by a series of viruses, PLRV, PVY, PVX, Potato Virus S, Andean Mottled Virus (Potato Virus M, PVM) and Potato Virus A (PVA), indicated by García *et al*. (2005) and Pichardo *et al*. (2013).

Potato virus Y (PVY), from the Potivirus group, is one of the most important viruses in several Solanaceae species, including potato, tomato, tobacco and paprika (Scholthof *et al*., 2011; Thomas-Sharma *et al*., 2016). It is transmitted from infected seed potato tuber and by at least 20 species of aphids in a non-persistent manner and causes high yield reduction in most potato areas. Symptoms range from moderate to severe mottling to streaking or coalescing streaking of the leaves (Agrios, 2005). It has been reported that new strains of the virus have increased in incidence, which severely damage tubers (Kamangar *et al*., 2014). The most efficient protection to control potato viral diseases is achieved through the production of resistant cultivars. Several PVY resistance genes have been found in potato and its wild relatives. The *Ry* genes in *Solanum tuberosum* group Andigena (Ry_{adg}), *S. stoloniferum* (Ry_{sto}), *S. chacoense* (Ry_{chc}), and *S. tuberosum* group Phureja ($Ry\ (o)_{phu}$) are known to provide high levels of resistance to PVY, the first two located on chromosomes XI and XII, and the latter on chromosome IX (Herrera *et al*., 2018, Torrance *et al*., 2020). Kasai *et al*. (2000) developed the SCAR marker RYSC3 from a potato plant containing the Ry_{adg} gene, and a 320 bp fragment was amplified only in genotypes with this gene, becoming a powerful tool in marker-assisted selection in breeding programs.

Potato leaf curl, caused by PLRV, is one of the most important viral diseases and is distributed throughout the world, causing large production losses. It only affects this crop, causing prominent leaf curling and stunting of plant growth. In some varieties, the phloem necroses in the leaves and tubers. It is transmitted through infected seed tubers and in the field by 10 aphid species in a persistent manner, but is not mechanically transmitted (Agrios, 2005; Thomas-Sharma *et al*., 2016). It can cause between 20 and 60% yield reduction, and when it occurs in co-infection with PVY or PVX, its effects can be greater (Mesa *et al*., 2016, Pichardo *et al*., 2013). The spread of PLRV can be controlled with insecticide applications, however, the most economical and ecologically acceptable way is the use of resistant cultivars. There are two types of resistance to PLRV, one that works against infection by the viruliferous aphid and another that limits the multiplication and accumulation of the virus. Resistance to PLRV has been described as polygenically controlled, whereas resistance to PLRV accumulation appears to be under the control of a dominant gene (Barker and Solomon, 1990). The major QTL, *PLRV.1*, located on chromosome XI in the dense resistance region, contains several genes for qualitative and quantitative resistance to viruses and other potato pathogens. This QTL explains between 50 and 60 % of the phenotypic variance. The SCAR $NI27_{1164}$ marker was developed, which is closely linked to this region and allows to assist in the selection of cultivars with resistance to PLRV (Marczewski *et al*., 2001).

Molecular markers developed in potato, from known regions (microsatellites, genes, QTL) associated with resistance to pathogens are useful tools for the genetic study of germplasm collections, even when functional active genes cannot be distinguished from structurally homologous inactive ones, and allow the identification of promising genetic materials for genetic improvement, when they present the combination of various resistances, in addition to complementing field evaluations (Fadina *et al*., 2017; López, 2013).

The objective of this work was to identify regions associated with resistance to late blight (*Phytophthora infestans*) and to the PVY and PLRV viruses, in 50 promising commercial potato genetic materials for local use, belonging to the INIA – Mérida *in vitro* germplasm bank, using developed molecular markers (microsatellites, SCAR and CAPS).

Materials and methods

Plant Material, DNA Extraction and Amplification

The research was carried out in the Unidad de Biotecnología Vegetal, INIA-CENIAP. In the extraction of total genomic DNA, the methodology described by Zambrano *et al*. (2002) was used on *in vitro* plant samples of 50 potato genetic materials, including: 12 commercial cultivars (Andinita, Capiro, Caribay, Cartayita, Costanera, Fripapa INIA, Granola, Guadalupe, INIAFRIT, María Bonita, Monserrate, Única); 15 late blight differentials (R1, R2, R4, R5, R6, R7, R8, R9, R10, R11, R1R4, R2R3, R2R4, R3R4, R2R3R4), a population of 11 advanced CIP clones with high levels of horizontal resistance to late candelilla, high tuber yields and adaptation to different environments (391002.6, 391011.17, 391580.30, 392633.54, 393085.5, 393280.57, 393280.64, 393280.82, 393349.68, 393371.58) and I-1062; and 12 hybrids of Fundación PROINPA, Bolivia (Crc3/80, Crc3/84, Crc2/P8, Crc2/P9, UB6/69, 14okagran6, 14okagran7, 14okagran8, 14okagran11, 14okagran12, 14okagran17 y 14okagran20; which are part of the *in vitro* bank of germplasm from INIA-Mérida, Venezuela. The DNAs were resuspended in TE (pH 8). The integrity was determined by visualization in agarose gels Promega™ at 0.8 %, and the concentration and purity, with the use of a nanoDrop One®. Amplification was performed in a MJResearch-PC200 thermocycler, in a total volume of 25 µl containing 2.5 mM $MgCl_2$, Buffer B 1X, 200 µM DNTPs, 1 µM primers, 1U Taq Polymerase Promega™, 25 ng genomic DNA. The PCR conditions were in accordance with the protocols developed for microsatellite markers, SCAR and CAPS, according to Ballvora *et al* (2002), Kasai *et al* (2000), Marczewski *et al*. (2001), Milbourne *et al* (1998) and Trujillo (2004). To amplify the QTL *tbr* region of chromosome XII,

associated with resistance to late blight, six SCAR and CAPS type markers were used: e35m48.m, e32m61.w, e46m42.g, e44m42.j, e45m59.o and D15.16rr with specific sequences (Trujillo, 2004). For the CAPS marker (e46m42.g), the PCR products were digested with the enzyme *EcoRI* and incubated at 37 ºC overnight before electrophoresis. In all cases, 1 % agarose gels (Promega™) were used. Two other microsatellite markers STM0003 and STM0030, present on chromosome XII, associated with resistance to late blight were included (Milbourne *et al*., 1998). For the amplification of the *R1* gene, primers 76-2sf2 and 76-2SR, developed by Ballvora *et al*. (2002) were used.

The molecular markers associated with blight resistance used are known to be codominant, except for the markers e44m42.j, e45m59.o and *R1* (Ballvora *et al*., 2002; Kasai *et al*., 2000; Marczewski *et al*., 2001, Milbourne *et al*., 1998; Trujillo, 2004).

Two positive controls, donated by CIP, were used: DNA from two individuals of the diploid population (PD), from the cross between the female diploid parent (2n=2X=24) *Solanum tuberosum* Phureja (P) clone CHS-625 (2n) with resistance to late blight and the dihaploid male parent (n=2X=24) *Solanum tuberosum* Andigena (D) clone PS-3 susceptible to the disease; and DNA from two individuals of the tetraploid population B1B3, product of the cross between a genotype of the population B1 *Solanum tuberosum* Andigena, female, and a genotype of the population B3, male, which in turn comes from population A. The latter is characterized because it contains major genes introduced from *S. demisum*, and on the other hand, population B presents horizontal resistance but with the absence of *R* genes (Rodríguez *et al*., 2008).

To identify the Ry_{adg} gene, which confers resistance to PVY, DNAs were amplified with the primers SCAR 3.3.3s and ADG23R, associated with this resistance (Kasai *et al*., 2000). Potato cultivars Bintje, DTO-33 and Perrichioli, susceptible to PVY, were used as negative controls; and the cultivars TXY.11, DXY.7, and TXY.2, as positive controls, because they are resistant to the disease. The controls were donated by CIP. The PCR was programmed according to Kasai *et al*. (2000). To identify resistance to PLRV, a region of the *N* gene was amplified using primers NL27f and NL27r. The PCR was programmed according to Marczewski *et al*. (2001).

The amplification products were separated by horizontal electrophoresis in 1 % agarose gels (Promega™) and two repetitions of the amplifications were performed to corroborate the results. The 100 bp DNA ladder (Promega™) was used to estimate the size of the amplification products in the agarose gels. The presence or absence of amplification products associated with resistance to late blight and the PVY and PLRV viruses, in the 50 INIA potato genotypes, plus the positive and negative controls mentioned, were recorded, and analyzed using a Chemidoc® and the Quantity One® program.

Results and discussion

Molecular markers for resistance to *Phytophthora infestans*

The markers e35r48m, e32m61w, e45m59o and D1516rr were monomorphic in the agarose gels for the 50 genetic materials studied and the expected fragments of 339, 201, 123, 188 bp, respectively, were amplified (table 1). With the e44m42j marker, polymorphism was observed in agarose and the 155 bp fragment was taken as positive. In the results with the CAPS e46m42g marker, a single fragment of 172 bp product of the PCR could be observed, and two fragments after digestion with the restriction enzyme *EcoRI*. Positive controls PD (diploid population) and B1B3 (tetraploid population) gave the respective fragments mentioned (Data not shown). The markers e46m42g, e35m48.m and e45m59.o amplified in 96 to 92 % of the accessions, while the rest were present between 84 to 26 %, being the marker D15.16rr the least frequent (table 1). These results confirmed those obtained by Trujillo (2004) with respect to these markers associated with the QTL *tbr*.

On the other hand, the microsatellite markers STM0003 and STM0030 were highly polymorphic for the genetic materials studied, and the amplifications of the 141 and 147 bp fragments, respectively, were taken as positive, which were present in most of the materials, standing out STM0030 with 90 % (table 1).

The amplified fragment of 1.4 Kb, corresponding to the region of the *R1* gene for specific resistance to late blight, was only present in the differential R9, and in Crc 2/P8 of PROINPA (table 1) and it is confirmed that in population B from CIP, the *R1* gene is absent. This is explained because the accessions studied originally came from Population B developed at the CIP, without major genes (González *et al*., 2017; Jiang *et al*., 2018).

Under field conditions in 11 localities of the Mérida state, under natural infection of *P. infestans*, INIA accessions were evaluated during five consecutive years (2006-2010). The commercial varieties Granola, Capiro, Andinita and Montañita, showed the highest values of area under the progress curve of the candelilla disease, which indicates that they behaved as materials with low resistance, presenting the Granola variety, along with Fripapa INIA, Capiro and Única, the lowest yields, between 11.8 and 4.1 $t.ha^{-1}$. On the other hand, the advanced clones of CIP, among them, 301002.6, 391580.30, 393280.17, 393280.82, 392633.54, 393349.68, 393085.5, 393371.58 and 393080.57, stood out for their high tuber yields, between 49.6 and 14.9 $t.ha^{-1}$ and resistance to candelilla (González *et al*., 2011; González *et al*., 2019). Commercial cultivars released by INIA between 1987 and 2010 have been identified as tolerant to *P. infestans*, Cartayita, Fripapa INIA, INIAFRIT and Tibisay; moderately resistant cultivars Andinita and María Bonita; and Caribay is considered resistant. María Bonita has occupied an important place in the south of the Mérida State and localities of the Trujillo state. However, Granola continues to be the main cultivar in the Andean region, with high acceptance by farmers due to its precocity and post-harvest handling (González *et al*., 2017; González *et al*., 2019).

Molecular markers for resistance to PVY and PLRV viruses

The 321 bp amplified DNA fragment, which identifies the Ry_{adg} gene region, for PVY resistance was present in cultivars TXY.11, DXY.7 and TXY.2 (PVY resistant controls), but not in cultivars Bintje, DTO-33 and Perrichioli (controls susceptible to PVY) (Data not shown). This fragment was present in nine of the 50 samples: INIAFRIT, Capiro, Monserrate, Costanera, Caribay, 393280.57, R2, R4 and R5. On the other hand, in the Granola cultivar, which is highly susceptible to PVY, the fragment was absent in all the tests that were carried out. This cultivar could be used as susceptible material in the identification of the PVY resistance gene region. Other susceptible cultivars were FRIPAPA INIA, Guadalupe and María Bonita, several of the differentials, and PROINPA materials (table 1). These results are corroborated by Herrera *et al*. (2018), for positive and negative controls, and commercial materials, such as Costanera and Cartayita (identified by CIP as I-1039), which were positive and negative, respectively. However, there is controversy for the commercial variety Única, which did not present the fragment associated with the Ry_{adg} gene in our study.

Table 1. Amplified alleles associated with resistance to late blight and the PYV and PLRV viruses in 50 genetic materials from the INIA-Mérida potato germplasm bank.

Marker	Percentage (*)	Accessions that amplified with the molecular marker (**)
e35m48.m XII(***), 339 pb (****)	92	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27.28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46, 48, 49, 50
e32m61.w XII; 201 pb	84	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 44, 48, 49, 50
e46m42.g XII; 172 pb	96	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50
e44m42.j XII, 155 pb	78	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 20, 21, 25, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46, 47, 48, 49
e45m59.o XII, 123 pb	92	2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17,18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50
D15.16rr XII, 188 pb	26	1, 5, 6, 9, 10, 11, 28, 29, 32, 34, 39, 40, 43
STM0003 XII, 141 pb	44	7, 14, 16, 18, 19, 20, 21, 22, 24, 25, 26, 27, 34, 37, 39, 40, 41, 42, 45, 47, 48, 49
STM0030 XII, 147 pb	90	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 42, 43, 44, 46, 47, 48, 49
R1 V, 1.4 Kb	4	20, 41
Gen *Ryadg* XI, 321 pb	18	2, 3, 5, 9, 11, 14, 15, 16, 33
Gen *N* XI, 1.2 Kb	68	2, 6, 8, 9, 10, 12, 13, 14, 16, 17, 18, 20, 22, 24, 25, 26, 27, 28, 29, 31, 32, 33, 35, 36, 37, 38, 40, 41, 42, 43, 45, 46, 49, 50

(*) Percentage of accessions that amplified with the corresponding molecular marker; (**) Number corresponding to the accession: Andinita (1), Capiro (2), Caribay (3), Cartayita (4), Costanera (5), Fripapa INIA (6), Granola (7), Guadalupe (8), INIAFRIT (9), Maria Bonita (10), Monserrate (11), Única (12), R1 (13), R2 (14), R4 (15), R5 (16), R6 (17), R7 (18), R8 (19), R9 (20), R10 (21), R11 (22), R1R4 (23), R2R3 (24), R2R4 (25), R3R4 (26), R2R3R4 (27), 391002.6 (28), 391011.17 (29), 391580.30 (30), 392633.54 (31), 393085.5 (32), 393280.57 (33), 393280.64 (34), 393280.82 (35), 393349.68 (36), 393371.58 (37), I- 1062 (38), Crc3/80 (39), Crc3/84 (40), Crc2/P8 (41), Crc2/P9 (42), UB6/69 (43), 14okagran6 (44), 14okagran7 (45), 14okagran8 (46), 14okagran11 (47), 14okagran12 (37), 14okagran17 (49), and 14okagran20 (50); (***) Localization of molecular markers on chromosomes; (****) Molecular weights of the amplification products.

The 1.2 Kb fragment corresponding to the region of resistance to the PLRV virus (*N* gene) was present in 33 of the 50 materials evaluated, including: INIAFRIT, Capiro, Fripapa INIA, María Bonita, Guadalupe, 10 of the differentials of blight and materials from PROINPA Bolivia (table 1). Only in five genetic materials fragments were observed for both regions of resistance to the viruses evaluated: INIAFRIT, Capiro, 393280.57, R2 and R5. In Granola and Andinita the studied fragments were absent (table 1). In this research, amplification of the PLRV resistance region was observed in 68 % of the genetic materials evaluated, while only 18 % showed the PVY resistance region and 10 % amplified the two resistance regions for both viruses (table 1). These results are partially corroborated by Pichardo *et al*. (2013) who evaluated the presence of PVY, PLRV, PVM and PVS viruses *in vitro* plants and potato tubers, by the DAS-ELISA technique in Venezuela, finding that the Granola variety was the most affected material and presented mixed infections of the four viruses, followed by María Bonita which tested positive for the PVY and PVM viruses. Among the other varieties that were infected by only one of the viruses were: Andinita by PVM, INIAFRIT for PLRV, Capiro and Costanera for PVY.

Conclusions

The results show that the studied germplasm belonging to INIA Venezuela, composed of 50 potato genetic materials, including commercial cultivars, blight differentials, advanced CIP clones and hybrids from the Fundación PROINPA of Bolivia, have alleles associated with the resistance to late blight and to the PVY and PLRV viruses, located on chromosomes V, XI and XII.

In the case of markers developed for resistance to late blight, the accessions amplified regions located in the QTL *tbr* of chromosome XII, while only two materials did so for the *R1* gene. In the case of resistance to PLRV and PVY viruses, a higher percentage of the genetic materials amplified the region corresponding to the PLRV resistance gene, and a lower percentage amplified regions that corresponded to resistance to PVY or to both viruses. This study generated valuable information to support the curators of germplasm banks and plant potato breeders in Venezuela, however, the development and implementation of markers that manage to locate the greatest number of genes or QTLs associated with blight and virus resistance is necessary, with their respective validation through greenhouse and field evaluations to associate the specific allelic variants with the phenotypes.

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