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Detection of homologous resistance genes to the late blight in wild potatoes

Detección de genes homólogos de resistencia al tizón tardío en patatas silvestres

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Summary: The identification of resistance genes to late blight (*Phytophthora infestans*) is essential in potato (*Solanum tuberosum*) breeding programs to advance in obtaining resistant cultivars. The objective was to detect sequences homologous to the resistance genes (R1, R2, R3a, R8, Sto-448, Rpi-blb1, Rpi-blb2, and Rpi-ber1) to *P. infestans* in 23 accessions of 14 wild potato species (*Solanum* spp.) with different resistance levels through the amplification of molecular markers. Eight primers sets were used to amplify the molecular markers. No amplifications of resistance markers were observed in highly susceptible plants in the field (accession 631200 of *S. chacoense*, accession 653801 of *S. berthaultii* and accession 631201 of *S. juzepzuckii*), whereas in plants immune to the field, such as *S. demissum* (653770), amplification of six molecular markers was observed. The R3a marker did not amplify for any accession. Therefore, no apparent association between the number of markers amplified and the resistance category was found by Chi-square test. These results highlight the complexity of genetic resistance in the studied potato species. Future studies could evaluate the functionality of the homologous genes and detect other molecular markers of the resistance genes.

Key words: Molecular markers, *Phytophthora infestans*, plant genetic resources, resistance genes.

Resumen: La identificación de genes de resistencia al tizón tardío (*Phytophthora infestans*) es fundamental en programas de mejoramiento de papa (*Solanum tuberosum*) para avanzar en la obtención de cultivares resistentes. El objetivo fue detectar secuencias homólogas a los genes de resistencia (R1, R2, R3a, R8, Sto-448, Rpi-blb1, Rpi-blb2 y Rpi-ber1) a *P. infestans* en 23 accesiones de 14 especies silvestres de papa (*Solanum* spp.) con diferentes niveles de resistencia mediante la amplificación de marcadores moleculares. En plantas altamente susceptibles en campo no se observaron amplificaciones de marcadores de resistencia (accesión 631200 de *S. chacoense*, accesión 653801 de *S. berthaultii* y accesión 631201 de *S. juzepzuckii*), mientras que, en plantas inmunes a campo como *S. demissum* (653770), se observó amplificación de seis marcadores moleculares. El marcador R3a no amplificó para ninguna accesión. Mediante análisis de Chi-cuadrado no se encontró asociación aparente entre el número de marcadores amplificados y la categoría de resistencia. Estos resultados resaltan la complejidad de la resistencia genética en las especies de papa estudiadas. Futuros estudios podrían evaluar la funcionalidad de los genes homólogos y detectar otros marcadores moleculares para genes de resistencia.

Palabras clave: Genes de resistencia, marcadores moleculares, *Phytophthora infestans,* recursos fitogenéticos.

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Introduction

The oomycete *Phytophthora infestans* (Mont.) De Bary causes the disease known as late blight, which results in significant losses in the worldwide production of potato tubers (*Solanum tuberosum* L.). This pathogen can reproduce sexually, producing more virulent offspring capable of overcoming genetic resistance in new cultivars (Enciso-Maldonado *et al.*, 2021). Since cultivated potatoes have a narrow genetic base and lack active resistance genes against *P. infestans*, the use of resistance genes from their wild relatives (*Solanum* spp.) is considered in potato breeding. These wild relatives have evolved in geographic regions with the most diverse populations of *P. infestans*, acquiring numerous functional R loci against late blight (Enciso-Maldonado *et al.*, 2021; Wang *et al.*, 2008). Therefore, the aim of this study was to detect the presence of homologous resistance genes to *P. infestans* in wild potato species (*Solanum* spp.) and associate the number of genes with observed resistance in the field.

Materials and Methods

Twenty-three accessions of 14 wild potato species (Table 1) exhibiting different resistance categories (Enciso-Maldonado *et al.*, 2022) were analyzed. Total genomic DNA was extracted from leaf samples using the CTAB technique (Doyle & Doyle, 1987). Dilutions were made from the extracted DNA to bring it to the working concentration (50 ng/µL). The 23 selected accessions were analyzed using 8 pairs of primers specific for late blight resistance genes, which are localized on different chromosomes as indicated in Table 1. These primers have been previously reported in the literature for other species of the *Solanum* genus (Table 1).

All PCR reactions were performed in a final volume of 25 µL, with a negative control included in each PCR run. The reagent concentrations were as follows: 1X Buffer (Green GoTaq Reaction Buffer), 0.5 U of Taq (GoTaq G2 DNA Polymerase, Promega), 0.2 mM of dNTPs (Promega), 0.2 µM of each primer, and 100 ng of template DNA. Regarding the cycling conditions, it started with an initial denaturation of 5 minutes at 94 °C, followed by 35 cycles that included denaturation for 45 s at 94 °C, annealing for 45 s at Tm, extension for 1 minute at 72 °C, and a final extension of 8 minutes at 72 °C. The specific Tm for each primer pair is listed in Table 2. Initially, calculated Tm for each primer pair were used; however, after not obtaining good results, Tm used by Tiwari *et al*. (2015) were tested, which allowed for the successful amplification of amplicons. The verification of the amplifications was done by electrophoresis (5V/cm) in 2% (w/v) agarose gels, using 0.5× TBE as the running buffer (Sambrook *et al*., 1989). The gels were stained with Diamond™ Nucleic Acid Dye (1:10,000), and the bands were visualized on a blue light transilluminator. The molecular weight marker used for reference was the 100 bp DNA Ladder (Invitrogen).

The presence/absence of homologous resistance genes in the potato accessions were recorded (Table 2). To explore the relationship between the number of molecular markers and the resistance category in wild potato species, a contingency table analysis was performed using the Chi-square test of independence. The provided data were loaded using *Python* with the pandas package for data manipulation, while the statistical test was conducted with *scipy.stats*. First, the Resistance category column was converted to a categorical type, and intervals were created for the number of molecular markers. Next, a contingency table was built using *pd.crosstab*, and the Chi-square test was applied with *chi2_contingency*. Additionally, the Contingency Coefficient (C) was calculated to determine the strength of the association between the studied categorical variables.

Results and Discussion

For the 23 accessions, different combinations of the eight markers analyzed were detected, varying between 0 and 6 in the same accession. The most present markers were Rpi-ber1 (19), Rpi-blb2 (18) and Rpi-blb1 (16) (Fig. 1). Markers R1 and R2 were only found in

Table 1. Molecular markers for detection of homologous resistance genes to late blight in wild potatoes.

Molecular marker	Chromosome	Length (pb)	Primers (5´ to 3´)	Tm (ºC)	Authors
Rpi-blb1	8	300, 500, 800, 1000	F: AACCTGTATGGCAGTGGCATG R: GTCAGAAAAGGGCACTCGTG	53	Wang *et al.* (2008)
R1	5	1205	F: CACTCGTGACATATCCTCACTA R: GTAGTACCTATCTTATTTCTGCAAGAAT	50	Sokolova *et al.* (2011)
Rpi-blb2	8	300, 500, 700, 976	F-GGACTGGGTAACGACAATCC R-ATTTATGGCTGCAGAGGACC	52	van der Vossen *et al.* (2005)
R2	4	1142	F: ATGGCTGATGCCTTTCTATCATTTGC R: TCACAACATATAATTCCGCTTC	50	Kim *et al.* (2012)
R3a	11	1380	F: TCCGACATGTATTGATCTCCCTG R: AGCCACTTCAGCTTCTTACAGTAGG	52	Sokolova *et al.* (2011)
R8	9	1276	F-AACAAGAGATGAATTAAGTCGGTAGC R-GCTGTAGGTGCAATGTTGAAGGA	50	Vossen *et al.* (2016)
Ssto-448	5	448	F - GTGGAACGCCGTCCATCCTTAG R - GCATAGGTGGTTAGATGTATGTTTGATTA	50	Sokolova *et al.* (2011)
Rpi-ber1	10	366	F: GAACGCGAAAGAGTGCTGATAG R: CCCGCTGCCTATGGAGAGT	50	Tan *et al.* (2010)

Table 2. Species, accession number (ACNO), resistance category and presence of the molecular marker in *Solanum* spp.

Species	Accession number	Resistance category*	Molecular marker							
			R1	*Rpi-ber1*	*R8*	*R2*	*Rpi-blb1*	*Rpi-blb2*	*Sto-448*	*R3a*
S. acaule	631194	HS		X			X	X		
S. acaule	653788	HS		X			X	X		
S. albicans	631198	HS		X			X	X		
S. albicans	631199	S		X	X		X	X		
S. berthaultii	653801	HS								
S. berthaultii	653829	R								
S. brevicaule	473378	HS		X	X		X	X		
S. brevicaule	631209	R		X						
S. chacoense	631200	HS								
S. chacoense	653781	HS		X	X		X	X		
S. demissum	653770	R	X	X	X		X	X	X	
S. demissum	225652	MR		X	X			X	X	
S. guerreroense	653828	R		X			X	X	X	
S. juzepzuckii	631201	HS								
S. microdontum	631212	S		X			X	X		
S. microdontum	631211	R		X	X		X	X		
S. michoacanum	653810	S		X			X	X		
S. pinnatisectum	275234	MR		X			X	X		
S. pinnatisectum	653808	HS		X			X	X		
S. stoloniferum	653771	HS		X	X	X	X	X	X	
S. stoloniferum	653823	R		X				X	X	
S. verrucosum	653784	HS		X			X	X	X	
S. vernei	473306	HS		X			X	X		

*HS: Highly susceptible; S: Susceptible; MR: Moderately resistant; R: Resistant (Enciso-Maldonado *et al*., 2022).

S. demissum (653770) and *S. stoloniferum* (653771), respectively. The R3a marker did not amplify in any accession.

As expected, no amplifications of resistance markers were observed in highly susceptible plants in the field (accession 631200 of *S. chacoense*, accession 653801 of *S. berthaultii* and accession 631201 of *S. juzepzuckii*), and plants immune to the field, such as *S. demissum* (653770), showed amplification of six markers (Table 2).

In contrast, the accession 653784 from *S. verrucosum*, 653781 from *S. chacoense*, and 473378 from *S. brevicaule*, were described as highly susceptible to late blight (Enciso-Maldonado *et al*., 2022); however, four markers of resistance were amplified; and in accession 631209 from *S. brevicaule* described as resistant (Enciso-Maldonado *et al*., 2022), only one marker of resistance was amplified.

The Chi-square statistic of 0.9583 and a p-value of 0.8113 indicated insufficient evidence to reject the null hypothesis of independence between the number of markers and resistance ($P < 0.05$). Additionally, the Contingency Coefficient ($C = 0.2$) suggests a weak relationship between the variables. As described for other analogous resistance genes, the markers may represent pseudogenes or have some loss-of-function mutation (Oosumi *et al.* 2009). This could explain the presence of resistance-related markers in susceptible accessions. In this way, future studies could focus on evaluating the functionality of the homologous genes mentioned in this work and identifying other potential resistance genes.

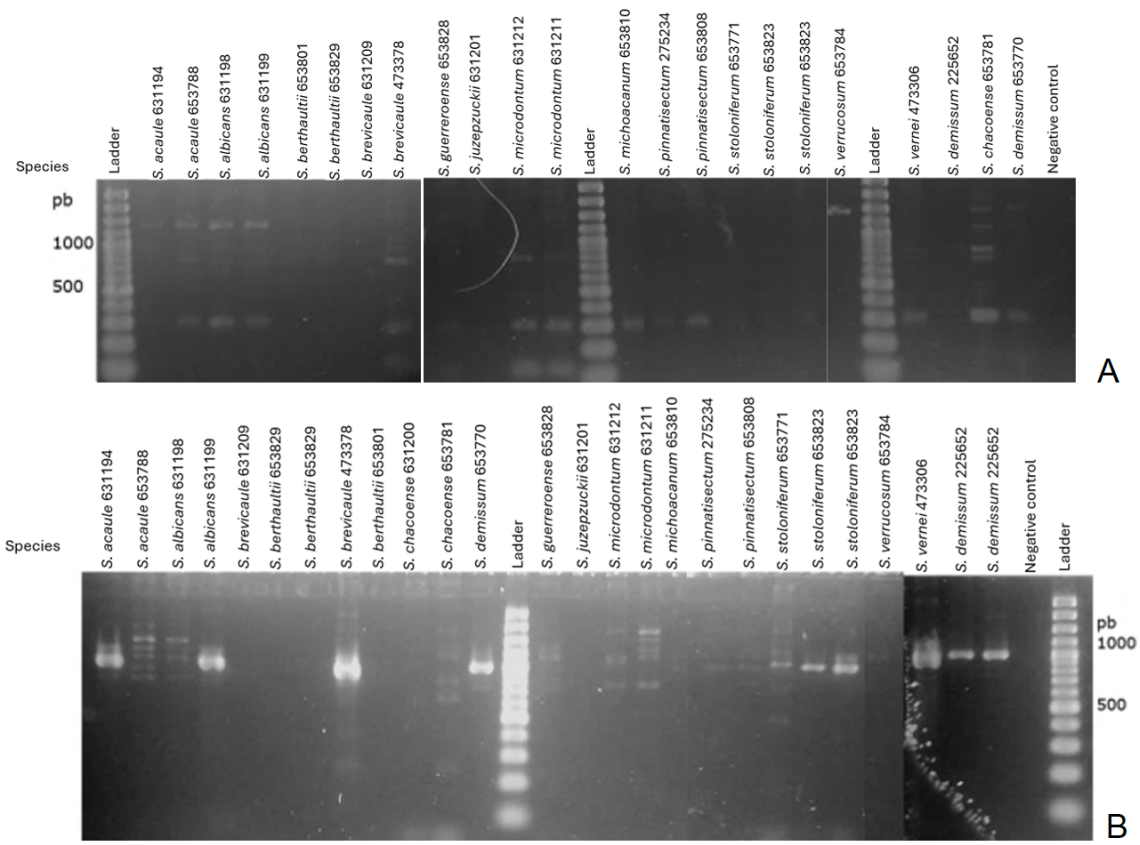

Fig. 1. Electrophoresis gel (2% agarose) analysis for the detection of homologous genes for late blight resistance in wild *Solanum* species using the molecular markers A. Rpi-blb1. B. Rpi-blb2.

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