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CARACTERIZACIÓN FENOTÍPICA DE ***Solanum demissum*** Lindl.

TESIS

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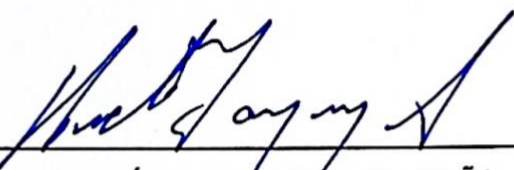
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CARACTERIZACIÓN FENOTÍPICA DE *Solanum demissum* Lindl.

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DEDICATORIA

A mis padres, con cariño, admiración y respeto

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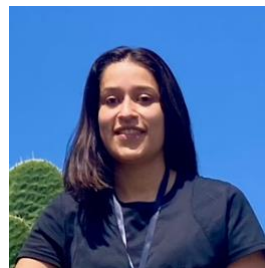
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RESUMEN GENERAL

CARACTERIZACIÓN FENOTÍPICA DE *Solanum demissum* Lindl.

Solanum demissum Lindl., una especie de papa silvestre originaria de México, ha sido una valiosa fuente de genes por su resistencia al tizón tardío (*Phytophthora infestans* (Mont.) de Bary.) y a heladas, convirtiéndola en una especie clave en el mejoramiento genético de la papa (*Solanum tuberosum* L.). La caracterización genotípica y fenotípica de especies silvestres proporciona información valiosa para la identificación, clasificación y selección de materiales en función de sus características. En este estudio se caracterizaron accesiones de *S. demissum* resistentes al tizón tardío y se aplicaron estrategias de fenotipado de alto rendimiento para analizar la diversidad morfométrica de las hojas. En el capítulo 3 se hace referencia al estado actual y perspectivas para el futuro en el estudio de la especie en donde se enfatiza la importancia previamente mencionada. En el capítulo 4 se evidencia la diversidad existente en los caracteres morfométricos de las hojas, mediante el procesamiento de imágenes, en algunas especies de papa silvestre incluida *S. demissum*, y en el capítulo 5 se confirma el grado de resistencia al tizón tardío de más de 100 accesiones de *S. demissum*, encontrándose que el 9 % y 30 % presentó síntomas en 2020 y 2021 respectivamente.

Palabras clave: papa silvestre, morfometría, tizón tardío, resistencia.

GENERAL ABSTRACT

PHENOTYPIC CHARACTERIZATION OF *Solanum demissum* Lindl.

Solanum demissum Lindl., a wild potato species native to Mexico, has been a valuable genetic resource due to its resistance to late blight (*Phytophthora infestans* (Mont.) de Bary.) and frost, making it a key species in potato breeding (*Solanum tuberosum* L.). Genotypic and phenotypic characterizations of wild species provide valuable information to identify, classify, and select materials based on their characteristics. In this study, accessions of late blight-resistant *S. demissum* were characterized and high-throughput phenotyping strategies were applied to analyze leaf morphometric diversity. Chapter 3 refers to the current status and future prospects in the study of the species, emphasizing the importance mentioned above. Chapter 4 highlights the existing diversity in the morphometric leaf traits through image processing in some wild potato species including *S. demissum*. In Chapter 5, the level of resistance to late blight is confirmed for over 100 accessions of *S. demissum*, with 9% and 30% showing symptoms in 2020 and 2021, respectively.

Keywords: wild potato, morphometrics, late blight, resistance.

CAPÍTULO I. INTRODUCCIÓN GENERAL

Las variedades comerciales de papa (*Solanum tuberosum* L.) presentan escasa variabilidad genética y una alta depresión endogámica, por lo tanto, las contribuciones que especies silvestres, conservadas regularmente en bancos de germoplasma, pueden aportar al incremento de la diversidad genética resulta fundamental en la mejora de este cultivo (Bamberg & Del Rio, 2007). Las especies de papa silvestre se han utilizado a lo largo de los años en programas de mejoramiento genético (Bradshaw et al., 2006), actualmente se conocen 151 especies de papas silvestres distribuidas en 16 países del continente americano (Spooner et al. 2019; <https://cipotato.org/potato/wild-potato-species>). Comúnmente, estas especies son utilizadas como fuente de resistencia a enfermedades (Ballesteros et al., 2010), plagas (Fürstenberg-Hägg et al., 2013) y estrés abiótico (Bradshaw et al., 2006).

La papa silvestre *Solanum demissum* Lindl. ha sido una valiosa fuente de resistencia al tizón tardío de la papa (*Phytophthora infestans* (Mont.) de Bary.) desde mediados del siglo XX (Ballesteros et al., 2010). y es considerada como una de las fuentes más importantes de resistencia a heladas (Vega & Bamberg, 1995). Aunado a esto, la actividad antioxidante (Friedman, 2006; Hale et al., 2008) y contenido de glicoalcaloides detectados establece una base sólida para futuras investigaciones en los campos agrícola y farmacéutico (Díaz-García et al., 2023; Distl & Wink, 2009; Friedman et al., 1997).

Por otro lado, para hacer un uso más eficiente de los recursos almacenados y conservados en los bancos de germoplasma, la caracterización genotípica y fenotípica de especies silvestres brinda información valiosa contribuyendo a la identificación, clasificación y selección de materiales (Singh et al., 2019). Particularmente, la caracterización fenotípica permite describir y establecer relaciones entre accesiones, además de identificar materiales prometedores para el mejoramiento y la conservación del germoplasma (Muli et al., 2021; Pereira-Dias et al., 2020; Plazas et al., 2014).

Los parientes silvestres de la papa han constituido una importante fuente de genes de resistencia en la generación de nuevas variedades de papa (Zoteyeva et al., 2012), sin embargo, la caracterización fenotípica de los mismos ha sido limitada debido a la baja precisión y capacidad de procesamiento del fenotipado tradicional, lo que ha dificultado el estudio de las grandes colecciones existentes en los bancos de germoplasma.

El fenotipado de alto rendimiento permite mayor procesamiento de información, disminución del error y facilita la cuantificación de caracteres complejos como el color o la forma (Díaz-García et al., 2016), abriendo un abanico de oportunidades

para el estudio y caracterización de materiales, brindando así información necesaria para selección en programas de fitomejoramiento, conservación y diversidad.

El objetivo de este trabajo ha sido la caracterización fenotípica de *Solanum demissum*, para la resistencia al tizón tardío y análisis morfométrico de las hojas. En el capítulo 3 se aborda una revisión sobre *S. demissum*, contribuciones y perspectivas, en el capítulo 4, análisis de la variabilidad morfométrica de hojas en distintas especies silvestres de papa, entre ellas *S. demissum*, y en el capítulo 5, la evaluación el grado de resistencia al tizón tardío de un panel de más 100 accesiones de *S. demissum* en el Valle de Toluca, el centro de diversidad más importante para *P. infestans*.

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CAPÍTULO II. REVISIÓN DE LITERATURA

2.1 *Solanum demissum*

Solanum demissum Lindl. es una papa silvestre perteneciente a la familia de las solanáceas (Spooner & Hijmans, 2001), de porte alto, flores de color violeta a morado, tubérculos blancos a bronceado, redondos a comprimidos (Hidalgo-Gómez, et al. 2022). Es una especie hexaploide autoincompatible ($2n = 6x = 72$, 4 EBN-Endosperm Balance Number) (Spooner & Hijmans, 2001) dando lugar a una gran variabilidad genotípica. Se encuentra distribuida en México y Guatemala en donde la mayor abundancia y diversidad se encuentra en territorio mexicano en el Eje Volcánico y la Sierra Madre del Sur, predominando el clima templado, sub-húmedo, con lluvias en verano de 800 a 900 mm anuales, alta humedad relativa y oscilación térmica, de 12° C a 20° C (Hijmans et al., 2002; Luna-Cavazos et al., 2012; Lozoya-Saldaña, 2005; USDA Germoplasm Resources Information Network (GRIN, <https://www.ars-grin.gov/>)).

2.1.1 Resistencia al tizón tardío

El interés en coleccionar y estudiar a las papas silvestres nació a partir de la hambruna irlandesa de la papa ocurrida en la década de 1840 a causa de “la enfermedad” actualmente conocida como tizón tardío de la papa, causada por el oomiceto *P. infestans*, provocando una gran crisis alimentaria que derivó en una grave escasez de alimento y en la pérdida de vidas humanas debido al hambre y desnutrición (Fry et al., 2015; Majeed et al., 2022). Posteriormente, a través de varias expediciones europeas se colectaron genotipos de papas silvestres para cruzarlas con la especie comestible con la finalidad de obtener variedades resistentes (Hawkes, 1941). *Solanum demissum* fue mencionada por primera vez por John Lindley en 1848 en el artículo Notes on the Wild Potato, publicado en The Journal of the Horticultural Society of London, en el cual el autor describe accesiones colectadas en el Valle de Toluca y Michoacán (México) como plantas que no presenta síntomas de “la enfermedad”. Donald Reddick (1951) de la

Universidad de Cornell, realizó una expedición de exploración de plantas a las montañas de México, cuya variabilidad e indicios de resistencia al tizón tardío detonaron un particular interés en estudiar a las papas silvestres mexicanas (Enciso-Maldonado et al., 2022; Zoteyeva et al., 2012). Actualmente, se considera que el centro de diversidad más importante de *P. infestans* se encuentra en el Valle de Toluca y se extiende hasta Michoacán y Tlaxcala (Wang et al., 2017), por lo tanto, las especies mexicanas han co-evolucionado con el patógeno y han adquirido una resistencia natural al mismo (Alfaro, 1995). Hasta la fecha, se han encontrado 14 genes de resistencia al tizón tardío funcionales en *S. demissum*: R1, R2, Rpi-demf1, R3a, R3b, R4a; R4MA, R5, R6, R7, R8, R9a, R10; R11 (Paluchowska et al., 2022).

2.1.2 Resistencia a otros factores

Se ha demostrado que *S. demissum* presenta resistencia a plagas como el escarabajo de la papa de Colorado (*Leptinotarsa decemlineata*) y la cigarrita de la papa (*Empoasca fabae*) (Fürstenberg-Hägg et al., 2013), a infección por nematodos causado por *Globodera pallida* y *G. rostochiensis* (Bachmann-Pfabe et al., 2019), a los Virus X de la Papa (PVX) y Virus Y de la Papa (PVY) (Zoteyeva et al., 2012) además de mostrar respuesta de hipersensibilidad contra el virus A de la papa (PVA) (Tiwari et al., 2022).

Por otra parte, *S. demissum* se ha categorizado dentro del grupo de parientes silvestres de papa como una de las especies más resistentes a heladas, soportando temperaturas de -2 °C (Vega & Bamberg, 1995), desencadenando, desde inicios del siglo XX, la obtención de híbridos a partir de cruza entre *S. demissum* y *S. tuberosum* resistentes a heladas (Del Rio & Bamberg, 2020).

2.1.3 Otras propiedades

Los glicoalcaloides son metabolitos secundarios encontrados en papa y sus parientes silvestres, los cuales han sido catalogados como compuestos tóxicos por lo que resultan útiles en la defensa ante el ataque de patógenos (Friedman, 2006; Kuc, 1992). Se han encontrado en *S. demissum* altas concentraciones de demissina, demissidina, tomatidina, chaconina y tomatidinol (Distl & Wink, 2009;

Friedman et al., 1997), compuestos que han mostrado efecto en el tratamiento de diferentes enfermedades ya que se han asociado a propiedades antiinflamatorias, antihiper glucémicas, antibióticas, anticolesterolemiantes y anticancerígenas (Friedman, 2006; Milner et al., 2011).

2.2 Fenotipado de alto rendimiento

El fenotipado de alto rendimiento (High-throughput phenotyping-HTP) o fenómica involucra una serie de técnicas de alto rendimiento para aumentar y automatizar la capacidad y precisión de la evaluación fenotípica, permitiendo mayor procesamiento de información, disminución del error de medición y facilitar la cuantificación de caracteres complejos como el color o la forma (Diaz-Garcia et al., 2016; Fritsche-Neto et al., 2014).

Algunas herramientas utilizadas en el fenotipado incluyen: imágenes bi y tridimensionales (permiten medir la forma, el tamaño, número de hojas, color, etc.), sistema de imágenes de infrarrojo lejano (para estudiar las diferencias de temperatura y sus efectos en la tolerancia a la salinidad, la eficiencia de la fotosíntesis, el uso de agua, etc.), sistema de imágenes de infrarrojo cercano (para medir el contenido de agua y sus patrones de movimiento en las hojas y el suelo), sistema de imágenes de fluorescencia (para análisis de la salud de las plantas), sistema de imágenes por resonancia magnética (para capturar imágenes geométricas en 3D de las raíces) y reflectancia espectral (para determinar la composición química de las plantas, como: niveles de clorofila y otros pigmentos en las hojas, carbohidratos solubles en agua y nitrógeno en hojas) (Roshni & Prajwala, 2019; Tao et al., 2022).

Los estudios de diversidad, el mapeo de genes y el mejoramiento genético (como el caso de selección genómica) requieren de la información obtenida gracias a HTP para obtener los resultados deseados (Sandhu et al., 2022).

2.2.1 Diversidad en plantas

Los estudios de diversidad son clave para la clasificación, conservación y selección de recursos fitogenéticos. La caracterización fenotípica es tan importante como la genotípica, ya que permite describir y establecer relaciones

entre grupos de cultivares y accesiones, además de identificar materiales potencialmente interesantes para el mejoramiento y la conservación del germoplasma (Furbank & Tester, 2011; Muli et al., 2021; Pereira-Dias et al., 2020; Plazas et al., 2014).

La evaluación de materiales es importante para descubrir fuentes útiles y predecir el potencial genético y valor de mejoramiento de los materiales existentes en los bancos de germoplasma. La evaluación fenotípica ha sido exitosa en la identificación de materiales adecuados para rasgos simples, pero los rasgos poligénicos con fuertes interacciones genotipo-ambiente son un desafío. Además, muchos rasgos importantes solo se expresan en etapas específicas de desarrollo, en entornos particulares o en respuesta a estreses bióticos o abióticos, y no son fácilmente capturados o incluidos en los esquemas de evaluación fenotípica. Predecir el comportamiento del material en diferentes condiciones ambientales es difícil, por lo que desarrollar colecciones caracterizadas es un desafío para una gran cantidad de características y entornos (Wang et al., 2017). La utilización de estas herramientas de fenotipado, junto con el genotipado, acelerará el uso de los recursos genéticos y el desarrollo de cultivos alimentarios más resistentes para el futuro (Nguyen & Norton, 2020).

2.2.2 Mapeo genético

El mapeo genético permite identificar la ubicación de los genes asociados o responsables de una característica fenotípica de interés en un organismo. Utiliza la información y variabilidad genética para establecer relaciones entre los marcadores genéticos y los rasgos fenotípicos, lo que permite la identificación de los loci específicos o regiones en el genoma responsables de la herencia de un carácter dado (Peña Malavera et al., 2014; Terwilliger & Göring, 2014).

La evaluación fenotípica es crucial en el mapeo de genes debido a que permite identificar y medir las características de los individuos que son determinadas por la combinación de sus genes y el ambiente en que se desarrollan. Para el caso particular del mapeo por asociación de genoma completo (GWAS), la identificación de una asociación entre un polimorfismo de nucleótido único (SNP) y un rasgo depende en su totalidad de la diversidad genotípica y fenotípica, de

las estrategias de fenotipado y genotipado y de la naturaleza del carácter a evaluar (Korte & Farlow, 2013), por lo que un fenotipado de alto rendimiento, preciso y eficiente, brinda mejores oportunidades para encontrar asociaciones positivas y confiables. En el caso de mapeo por QTL (locus de caracteres cuantitativos), incrementar el número de genotipos juega un papel importante en la detección de QTL positivos, el incremento de la precisión en el fenotipado con pocos genotipos no tiene efecto en la reducción de la probabilidad de detectar QTL falsos. HTP para el mapeo de QTL permite incrementar de forma significativa la cantidad de genotipos que es posible fenotipar (comparado con fenotipado tradicional) buscando reducir la probabilidad de falsos positivos (Lane & Murray, 2021).

2.2.3 Mejoramiento genético

El fenotipado es un proceso esencial en el mejoramiento genético de las especies cultivadas. Permite identificar y seleccionar individuos con características deseables para su uso en programas de mejoramiento, optimizando los procedimientos de selección y desarrollo de cultivares comerciales (Furbank & Tester, 2011; Morota et al., 2022). Al usar la selección fenómica en conjunto con adecuadas herramientas de fenotipado, la eficiencia de la selección genómica mejora (Krishnappa et al., 2021).

Un componente fundamental para acelerar el desarrollo de nuevas variedades de cultivo mejoradas es la evaluación fenotípica rápida y precisa de miles de líneas de mejoramiento en condiciones de campo. La falta de plataformas de fenotipado que puedan identificar de manera eficiente a los individuos de grandes poblaciones ha sido un obstáculo importante para la mejora de cultivos. Además, debido a que muchas características agrícolas de importancia están influenciadas por las interacciones genotipo-ambiente, se requiere el fenotipado a escala de campo. Por otra parte, la gran diversidad genética presente en colecciones de germoplasma, a menudo contienen parientes silvestres con características novedosas, que puede dar como resultado estudios de mapeo genético que identifiquen marcadores genéticos útiles para el mejoramiento

asistido, permitiendo así la generación de variedades de manera más rápida y eficiente (Migicovsky et al., 2017; Tanger et al., 2017; Watts et al., 2021).

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CAPÍTULO III. *Solanum demissum* Lindl. IN POTATO BREEDING

CAPÍTULO III. *Solanum demissum* Lindl. EN EL MEJORAMIENTO GENÉTICO DE LA PAPA

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Solanum demissum Lindl. in potato breeding

Solanum demissum Lindl. en el mejoramiento genético de la papa

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Abstract

Solanum demissum Lindl. is one of the most valued and used species for potato (*Solanum tuberosum* L.) breeding due to its richness in genes for resistance to various biotic and abiotic factors. Over the years, it has been a plant of interest, mainly for its resistance to potato late blight (caused by *Phytophthora infestans* [Mont.] de Bary.), which has led to crosses between *S. demissum* and *S. tuberosum* to obtain resistant cultivars. It has also been used for identifying physiological races of *P. infestans* and, recently, it has been associated with glycoalkaloids and antioxidant compounds, whose potential uses are of interest to various industries. Since there is great potential in the species *S. demissum*, not only as a source of resistance genes but also as a source of important bioactive compounds, this review summarizes the research conducted on the use of *Solanum demissum* in the breeding of cultivated potato, as well as its potential uses in other areas. In addition, areas of opportunity and limitations for breeding for resistance to late blight are highlighted to serve as a starting point for the development of new research or lines.

Keywords: wild potato, plant genetic resources, late blight, resistance, bioactive compounds.

Resumen

Solanum demissum Lindl. es una de las especies más valoradas y utilizadas para el mejoramiento genético de la papa (*Solanum tuberosum* L.) debido a su riqueza en genes de resistencia a diversos factores bióticos y abióticos. A lo largo de los años, ha sido una planta de interés, principalmente por ser resistente al tizón tardío de la papa (causado por *Phytophthora infestans* [Mont.] de Bary.), lo cual ha desencadenado cruces entre *S. demissum* y *S. tuberosum* para la obtención de cultivares resistentes. Asimismo, ha sido utilizada para la identificación de razas fisiológicas de *P. infestans* y, recientemente, se ha asociado a glicoalcaloides y compuestos antioxidantes, cuyos posibles usos son de interés para diversas industrias. Dado que existe un gran potencial en la especie *S. demissum*, no solo como fuente de genes de resistencia sino también como fuente de importantes compuestos bioactivos, esta revisión resume los aportes de la investigación de la especie *Solanum demissum* en el mejoramiento genético de la papa cultivada, así como sus usos potenciales en otras áreas. Además, se evidencian las áreas de oportunidad y limitaciones para el mejoramiento genético para la resistencia al tizón tardío, de tal manera que sirva como punto de partida para el desarrollo de nuevas investigaciones o líneas.

Palabras clave: papa silvestre, recursos fitogenéticos, tizón tardío, resistencia, compuestos bioactivos.

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Introduction

Crop wild relatives are plant species that are closely related to cultivated plants and constitute a huge reservoir of genetic diversity that can be used to breed new crop varieties resistant to diseases, pests and abiotic stresses, with the potential to contribute to food security in the face of climate change and population growth (Fonseka, Fonseka, & Abhyapala, 2020). In the specific case of cultivated potato (*Solanum tuberosum* L.), its wild relatives (*Solanum* spp.) are species of great interest, since they carry useful genes for the genetic improvement of this crop (Tiware et al., 2013). To date, 151 wild potato species distributed in 16 countries in the Americas are known (Spooner et al., 2019; <https://cipotato.org/potato/wild-potato-species>).

Among the wild relatives of potato, *Solanum demissum* Lindl. has been, for more than 100 years, one of the most valued and used species for potato breeding due to its richness in genes for resistance to potato late blight (*Phytophthora infestans* [Mont.] de Bary.). Fourteen vertical resistance genes have been identified, some introduced in commercial varieties, for which it is considered that about 50 % of the commercial varieties in the world have genes of this species (Enciso-Maldonado, Lozoya-Saldaña, Díaz-García, & López-Salazar, 2021; Lozoya-Saldaña et al., 2011; Paluchowska, Śliwka, & Yin, 2022; Rodríguez, 2015).

During the 19th and 20th centuries, different collections were made around the world, and from the 20th century, scientists focused on making crosses between *S. demissum* and *S. tuberosum* to obtain resistant cultivars (Turner, 2005); *S. demissum* was subsequently used to identify physiological races of *P. infestans* (Black, Mastenbroek, Mills, & Peterson, 1953; Malcolmson & Black, 1966), and it has demonstrated resistance to insects, viruses, bacteria, and nematodes (Bachmann-Pfabe, Hammann, Kruse, & Dehmer, 2019; del Rio & Bamberg, 2020; Eraso-Grisales, Mejía-España, & Hurtado-Benavides, 2019; Fürstenberg-Hägg, Zagrobelny, & Bak, 2013; Hidalgo-Gómez, Carrillo-Salazar, Rojas-Martínez, Rivera-Peña, & Ayala-Garay, 2022; Lambers, Chapin, & Pons, 2008; Tingey, 1984; Vega & Bamberg, 1995; Zoteyeva, Chrzanowska, Flis, & Zimnoch-Guzowska, 2012). In addition, high antioxidant activity has been detected (Friedman, 2006; Hale, Reddivari, Nzaramba, Bamberg, & Miller, 2008), as well as glycoalkaloids, compounds mainly found in *Solanum* spp., and which have been found to be useful in plant defense and against human diseases (Friedman, 2006; Kuc, 1992; Manrique-Carpintero, Tokuhisa, Ginzberg, Holliday, & Veilleux, 2013).

The objectives of this review were to summarize the research contributions on the use of the species

Introducción

Los parientes silvestres de los cultivos son especies de plantas que están estrechamente relacionadas con las plantas cultivadas, y constituyen un enorme reservorio de diversidad genética que se puede utilizar para producir nuevas variedades de cultivos resistentes a enfermedades, plagas y estrés abiótico, con el potencial de contribuir a la seguridad alimentaria frente al cambio climático y el crecimiento demográfico (Fonseka, Fonseka, & Abhyapala, 2020). En el caso específico de la papa cultivada (*Solanum tuberosum* L.), sus parientes silvestres (*Solanum* spp.) son especies de gran interés, ya que portan genes útiles para el mejoramiento genético de este cultivo (Tiware et al., 2013). Hasta la fecha, se conocen 151 especies de papas silvestres distribuidas en 16 países de América (Spooner et al., 2019; <https://cipotato.org/potato/wild-potato-species>).

Dentro de los parientes silvestres de la papa, *Solanum demissum* Lindl. ha sido, por más de 100 años, una de las especies más valoradas y utilizadas para el mejoramiento genético de la papa debido a su riqueza en genes de resistencia al tizón tardío (*Phytophthora infestans* [Mont.] de Bary.). Se han identificado 14 genes de resistencia vertical, algunos introducidos en variedades comerciales, por lo cual se considera que alrededor del 50 % de las variedades comerciales en el mundo cuentan con genes de esta especie (Enciso-Maldonado, Lozoya-Saldaña, Díaz-García, & López-Salazar, 2021; Lozoya-Saldaña et al., 2011; Paluchowska, Śliwka, & Yin, 2022; Rodríguez, 2015).

Durante los siglos XIX y XX se realizaron distintas colectas alrededor del mundo, y a partir del siglo XX los científicos se enfocaron en realizar cruces entre *S. demissum* y *S. tuberosum* para la obtención de cultivares resistentes (Turner, 2005); posteriormente, se utilizó para identificar razas fisiológicas de *P. infestans* (Black, Mastenbroek, Mills, & Peterson, 1953; Malcolmson & Black, 1966) y se demostró resistencia a insectos, virus, bacterias y nematodos (Bachmann-Pfabe, Hammann, Kruse, & Dehmer, 2019; del Rio & Bamberg, 2020; Eraso-Grisales, Mejía-España, & Hurtado-Benavides, 2019; Fürstenberg-Hägg, Zagrobelny, & Bak, 2013; Hidalgo-Gómez, Carrillo-Salazar, Rojas-Martínez, Rivera-Peña, & Ayala-Garay, 2022; Lambers, Chapin, & Pons, 2008; Tingey, 1984; Vega & Bamberg, 1995; Zoteyeva, Chrzanowska, Flis, & Zimnoch-Guzowska, 2012). Además, se ha detectado una alta actividad antioxidante (Friedman, 2006; Hale, Reddivari, Nzaramba, Bamberg, & Miller, 2008) y de glicoalcaloides, compuestos encontrados en *Solanum* spp., principalmente, y que han resultado ser útiles en la defensa de las plantas y ante enfermedades humanas (Friedman, 2006; Kuc, 1992; Manrique-Carpintero, Tokuhisa, Ginzberg, Holliday, & Veilleux, 2013).

S. demissum in the genetic improvement of the cultivated potato, as well as its potential uses in other areas, and to highlight the areas of opportunity and limitations of breeding for resistance to late blight so that the review can serve as a starting point for the development of new research or lines.

Methodology

A bibliographic search was conducted in specialized databases, both scientific and technical, including the Centro de Información Científica del CONACYT (<https://cicco.conacyt.gov.py/>), Google (<https://www.google.com/webhp?hl=es-419&sa=X&ved=0ahUKEwjU04Os9YfxAhVJJrkGHUOpCQcQPAgl>), Google Scholar (<https://scholar.google.es/schhp?hl=es>), Web of Science (<https://mjl.clarivate.com/search-results>), web pages of state institutions, and web pages of international entities related to potato plant genetic resources, among others.

First, keywords and connectors were combined to conduct the search in all fields: “*Solanum demissum*”, “late blight”, “*Phytophthora infestans*”, “abiotic stress”, “insect resistance”, “frost resistance”, “wild potatoes”, and “resistance genes”, with results being obtained from 1848 to 2022. Inclusion criteria such as year (period 1845-2022) and language (English, Spanish) were used. Subsequently, all technical and scientific materials available in these databases concerning the origin, distribution, characterization, resistance to biotic and abiotic factors, and chemical analyses related to *S. demissum* were selected.

Origin and distribution of *Solanum demissum*

Solanum demissum, a wild potato belonging to the family Solanaceae (section Petota of the genus *Solanum*) (Spooner & Hijmans, 2001), is a tall plant with a height of 60 cm, violet to purple flowers, white to tan tubers, round to compressed, and 5 cm in diameter (Figure 1) (Hidalgo-Gómez et al., 2022). It can multiply sexually through the seeds contained in the fruits, resulting in heterogeneous offspring, since it is a self-incompatible hexaploid species ($2n = 6x = 72$, with an endosperm balance number [EBN] of four) (Spooner & Hijmans, 2001), which gives rise to a complex and wide genotypic variability. This was first described by John Lindley (1848) in the article Notes on the Wild Potato, published in The Journal of the Horticultural Society of London. In this paper, the author describes accessions of *S. demissum*, collected in the Toluca Valley and Michoacán (Mexico), as plants that produce many runners and few tubers, and emphasizes that the species shows no symptoms of “the disease”.

The main center of wild potato distribution is in South America, in the Andean region between Peru,

Los objetivos de esta revisión fueron resumir los aportes de las investigaciones de la especie *S. demissum* en el mejoramiento genético de la papa cultivada, así como sus usos potenciales en otras áreas, y evidenciar las áreas de oportunidad y limitaciones del mejoramiento genético para resistencia al tizón tardío, de tal manera que la revisión sirva como punto de partida para el desarrollo de nuevas investigaciones o líneas.

Metodología

Se realizó una búsqueda bibliográfica en bases de datos especializadas, tanto científicas como técnicas, incluyendo el Centro de Información Científica del CONACYT (<https://cicco.conacyt.gov.py/>), Google (<https://www.google.com/webhp?hl=es-419&sa=X&ved=0ahUKEwjU04Os9YfxAhVJJrkGHUOpCQcQPAgl>), Google Académico (<https://scholar.google.es/schhp?hl=es>), Web of Science (<https://mjl.clarivate.com/search-results>), páginas web de instituciones estatales, páginas web de entidades internacionales relacionadas con los recursos fitogenéticos de papas, entre otras.

Primero, se combinaron las palabras clave y conectores para la búsqueda en todos los campos: “*Solanum demissum*”, “tizón tardío”, “*Phytophthora infestans*”, “estrés abiótico”, “resistencia a insectos”, “resistencia a heladas”, “papas silvestres”, “genes de resistencia”, donde se obtuvieron resultados de 1848 a 2022. Se utilizaron los criterios de inclusión, como el año (periodo 1845-2022) e idioma (inglés, español). Posteriormente, se seleccionaron todos los materiales técnicos y científicos disponibles en estas bases de datos referentes al origen, distribución, caracterización, resistencia a factores bióticos y abióticos, y análisis químicos relacionados con *S. demissum*.

Origen y distribución de *Solanum demissum*

Solanum demissum, papa silvestre perteneciente a la familia de las solanáceas (sección Petota del género *Solanum*) (Spooner & Hijmans, 2001), es una planta de porte alto con 60 cm de altura, flores de color violeta a morado, tubérculos blancos a bronceado, redondos a comprimidos, y de 5 cm de diámetro (Figura 1) (Hidalgo-Gómez et al., 2022). Se puede multiplicar sexualmente a través de las semillas contenidas en los frutos, lo que da como resultado una descendencia heterogénea, ya que es una especie hexaploide autoincompatible ($2n = 6x = 72$, con número de balance del endospermo [EBN, por sus siglas en inglés] de cuatro) (Spooner & Hijmans, 2001), lo cual da lugar a una compleja y amplia variabilidad genotípica. Esta fue descrita por primera vez por John Lindley (1848) en el artículo Notes on the Wild Potato, publicado en The Journal of the Horticultural Society of London. En dicho artículo, el autor describe accesiones de *S. demissum*, colectadas en el Valle de Toluca y Michoacán

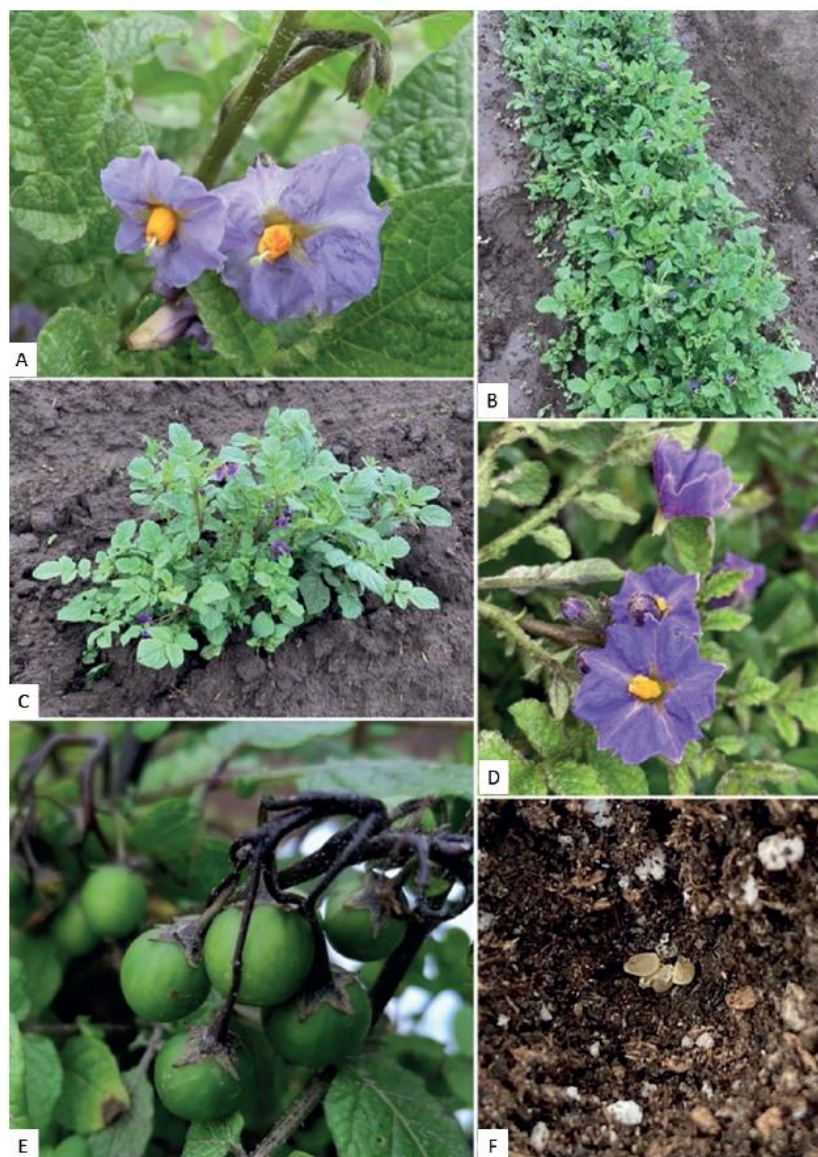


Figure 1. *Solanum demissum* cultivated in the Toluca Valley: A) flowers, B) plants, C) plant, D) flowers, E) fruits and F) seeds.
 Figura 1. *Solanum demissum* cultivada en el Valle de Toluca: A) flores, B) plantas, C) planta, D) flores, E) frutos y F) semillas.

Bolivia and Chile (Spooner et al., 2019), while Mexico is considered the second largest center of biodiversity for these species (Lozoya-Saldaña, 2005). *S. demissum* is distributed in Mexico and Guatemala (Figure 2). The greatest abundance and diversity is found in Mexico, in the Volcanic Axis and the Sierra Madre del Sur, between latitudes 19 and 21°, where the climate is temperate, sub-humid, and with summer rainfall of 800 to 900 mm annually, high relative humidity

(México), como plantas que producen muchos estolones y pocos tubérculos, y da énfasis en que la especie no presenta síntomas de “la enfermedad”.

El principal centro de distribución de papas silvestres se encuentra en Sudamérica, en la región Andina entre Perú, Bolivia y Chile (Spooner et al., 2019), mientras que México es considerado el segundo mayor centro de biodiversidad de estas especies (Lozoya-Saldaña,

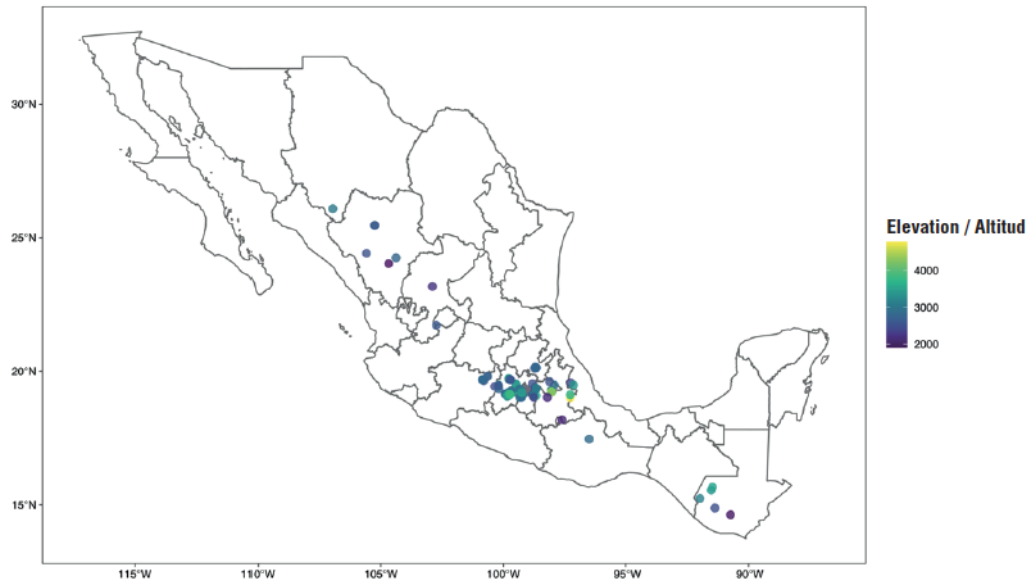


Figure 2. Geographic distribution of *Solanum demissum*. Map generated from the data of Hijmans et al. (2002) and USDA-GRIN (<https://www.ars-grin.gov/>).

Figura 2. Distribución geográfica de *Solanum demissum*. Mapa generado a partir de los datos de Hijmans et al. (2002) y USDA-GRIN (<https://www.ars-grin.gov/>).

and thermal oscillation from 12 to 20 °C (Hijmans, Spooner, Salas, Guarino, & de la Cruz, 2002; Lozoya-Saldaña, 2005; Luna-Cavazos, Romero-Manzanares, & García-Moya, 2012; USDA Germoplasm Resources Information Network [GRIN, <https://www.ars-grin.gov/>]). Spooner, Martinez, Hoekstra, and van den Berg (1997) indicate that *S. demissum* is a species that usually grows only in shady places under mature trees, among moss or pine litter.

Late blight resistance

Interest in collecting and studying wild potatoes grew out of the Irish potato famine, also known as the Great Famine, which occurred in the 1840s. In it, potato monocultures in Ireland were devastated by "the disease", now known as late blight, caused by the oomycete *P. infestans*, which caused a major food crisis resulting in severe food shortages and loss of human life due to starvation and malnutrition (Fry et al., 2015; Majeed, Siyar, & Sami, 2022; O'Neill, 2009; Schoina & Govers, 2014). After this event, through several European expeditions, genotypes of the potato's wild relatives were collected for crossing with the edible species in order to obtain resistant varieties (Hawkes, 1941). Edible potato varieties, at that time, were obtained through intraspecific crosses of a limited number of *S. tuberosum* genotypes, which generated a

2005). *S. demissum* se encuentra distribuida en México y Guatemala (Figura 2). La mayor abundancia y diversidad se encuentra en territorio mexicano, en el Eje Volcánico y la Sierra Madre del Sur, entre las latitudes 19 y 21°, donde el clima es templado, sub-húmedo, con lluvias en verano de 800 a 900 mm anuales, alta humedad relativa y oscilación térmica de 12 a 20 °C (Hijmans, Spooner, Salas, Guarino, & de la Cruz, 2002; Lozoya-Saldaña, 2005; Luna-Cavazos, Romero-Manzanares, & García-Moya, 2012; USDA Germoplasm Resources Information Network [GRIN, <https://www.ars-grin.gov/>]). Spooner, Martinez, Hoekstra, y van den Berg (1997) indican que *S. demissum* es una especie que, usualmente, crece sólo en lugares sombreados bajo árboles maduros, entre musgo u hojarasca de pino.

Resistencia al tizón tardío

El interés en coleccionar y estudiar las papas silvestres nació a partir de la hambruna irlandesa de la papa, también conocida como la Gran Hambruna, ocurrida en la década de 1840. En esta, los monocultivos de papa en Irlanda fueron devastados por "la enfermedad", actualmente conocida como tizón tardío, causada por el oomiceto *P. infestans*, la cual provocó una gran crisis alimentaria que derivó en una grave escasez de alimento, y en la pérdida de vidas humanas debido al hambre y la desnutrición (Fry et al., 2015; Majeed,

narrow genetic base that was easily overcome by the oomycete (Turner, 2005).

The oomycete *P. infestans* is one of the most studied pathogens in phytopathology; however, it remains a subject of research because it continues to cause major epidemics in potato and tomato crops worldwide (Fry et al., 2015). Until the mid-20th century, plant breeders believed that crosses between European varieties of *S. tuberosum* and their Mexican wild relatives would result in potato offspring resistant to late blight; however, Dr. Salaman at Cambridge would demonstrate the heritable nature of the resistance of a wild species (*S. endtense*) and initiated the first crosses between *S. tuberosum* and *S. demissum* (Black, 1954; Turner, 2005). Donald Reddick at Cornell University undertook a plant-exploring expedition to the mountains of Mexico (Reddick-Retires, 1951), where variability and indications of resistance to late blight sparked particular interest in studying Mexican wild potatoes (*S. bulbocastanum*, *S. pinnatisectum*, *S. hjertingii*, *S. papita*, *S. stoloniferum*, *S. polyadentum*, *S. verrucosum* and *S. demissum*, among others) (Enciso-Maldonado et al., 2022; Zoteyeva et al., 2012; Song et al., 2003).

Currently, the most important center of diversity of *P. infestans* is considered to be in the Toluca Valley, extending into Michoacán and Tlaxcala, where there is a metapopulation of the pathogen that could contribute to the repeated resurgence of *P. infestans* in the United States and elsewhere (Wang et al., 2017). In this regard, Mexican species are believed to have co-evolved with the pathogen over millions of years and have acquired a natural resistance to it (Alfaro, 1995), with *S. demissum* being one of the most studied species in the last 100 years (Figure 3) because it has vertical resistance genes (Enciso-Maldonado et al., 2021; Lozoya-Saldaña et al., 2011; Paluchowska et al., 2022).

One of the most important contributions that has been made with *S. demissum* is the identification of physiological races of *P. infestans*, since this oomycete can present different levels of virulence, with which it can overcome host resistance and cause the disease; furthermore, as mentioned above, *S. demissum* carries resistance genes. Genetic resistance, in the case of *P. infestans*-wild potato species interaction, can be explained by the gene-to-gene model, where for each host resistance gene there is a specific gene that determines the pathogenicity or virulence of the pathogen (Flor, 1959). Therefore, the plant's resistance gene is only effective if there is a corresponding avirulence gene in the pathogen (Alvarez-Morezuelas, Alor, Barandalla, Ritter, & de Galarreta, 2021; Serrano & Cadenas, 2008).

Black et al. (1953) and Malcolmson and Black (1966) inoculated different races of the pathogen in host

Siyar, & Sami, 2022; O'Neill, 2009; Schoina & Govers, 2014). Después de este acontecimiento, a través de varias expediciones europeas, se colectaron genotipos de parientes silvestres de papa para cruzarlos con la especie comestible con la finalidad de obtener variedades resistentes (Hawkes, 1941). Las variedades de papa comestibles, de ese entonces, se obtuvieron a través de cruces intraespecíficas de un limitado número de genotipos de *S. tuberosum*, lo cual generó una base genética estrecha, que fue superada fácilmente por el oomiceto (Turner, 2005).

El oomiceto *P. infestans* es uno de los patógenos más estudiados dentro de la fitopatología; sin embargo, sigue siendo tema de investigación debido a que continúa causando epidemias importantes en cultivos de papa y tomate en todo el mundo (Fry et al., 2015). Al principio, y hasta mediados del siglo XX, los fitomejoradores consideraban que la cruce entre variedades europeas de *S. tuberosum* con sus parientes silvestres mexicanos daría una descendencia de papas resistentes al tizón tardío; no obstante, el Dr. Salaman, en Cambridge, demostraría la naturaleza hereditaria de la resistencia de una especie silvestre (*S. endtense*), e inició las primeras cruces entre *S. tuberosum* y *S. demissum* (Black, 1954; Turner, 2005). Donald Reddick, de la Universidad de Cornell, realizó una expedición de exploración de plantas a las montañas de México (Reddick-Retires, 1951), donde la variabilidad y los indicios de resistencia al tizón tardío detonaron un particular interés en estudiar las papas silvestres mexicanas (*S. bulbocastanum*, *S. pinnatisectum*, *S. hjertingii*, *S. papita*, *S. stoloniferum*, *S. polyadentum*, *S. verrucosum* y *S. demissum*, entre otras) (Enciso-Maldonado et al., 2022; Zoteyeva et al., 2012; Song et al., 2003).

Actualmente, se considera que el centro de diversidad más importante de *P. infestans* se encuentra en el Valle de Toluca, y se extiende hasta Michoacán y Tlaxcala, donde existe una metapoblación del patógeno que podría contribuir al resurgimiento repetido de *P. infestans* en Estados Unidos y en otros lugares (Wang et al., 2017). En este sentido, se cree que las especies mexicanas han co-evolucionado con el patógeno durante millones de años y han adquirido una resistencia natural al mismo (Alfaro, 1995), siendo *S. demissum* una de las especies más estudiadas en los últimos 100 años (Figura 3) debido a que cuenta con genes de resistencia vertical (Enciso-Maldonado et al., 2021; Lozoya-Saldaña et al., 2011; Paluchowska et al., 2022).

Una de las contribuciones más importantes que se ha realizado con *S. demissum* es la identificación de razas fisiológicas de *P. infestans*, ya que este oomiceto puede presentar diferentes niveles de virulencia, con lo cual puede superar la resistencia del huésped y causar la enfermedad; además, como se mencionó anteriormente, *S. demissum* porta genes de resistencia.

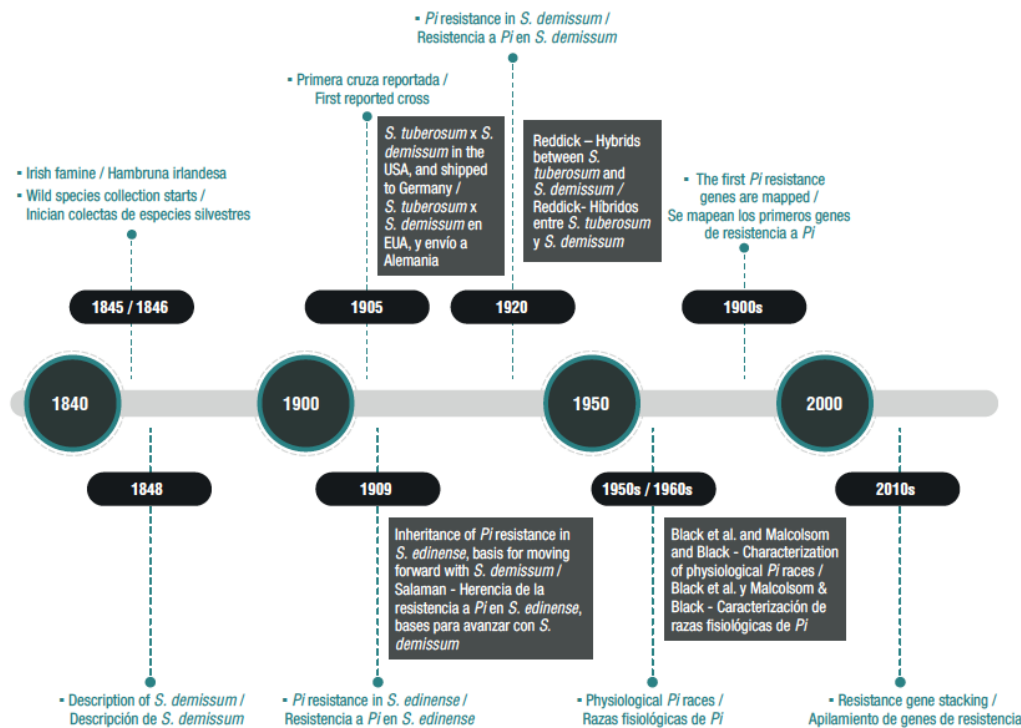


Figure 3. Chronology of the use of *Solanum demissum* in potato breeding for resistance to late blight (*Phytophthora infestans*). Source: author-made.

Figura 3. Cronología del uso de *Solanum demissum* en el mejoramiento de la papa para su resistencia al tizón tardío (*Phytophthora infestans*). Fuente: Elaboración propia.

plant differentials, with which they compared the immunity genes present in the host series and, from this, formulated a nomenclature system suitable for international application. In this way, they evaluated the virulence spectrum, which is the range of *Avr* genes expressed by the isolate when inoculated in the differential series of genotypes with *R* resistance genes. When isolates with the same virulence spectrum were observed, they were called physiological races, and the broader the virulence spectrum, the more complex was the race of the pathogen considered to be (Alvarez-Morezuélas et al., 2021). In this regard, Black et al. (1953) describe that race 1, 2 is capable of causing disease in genotypes carrying resistance genes *R1*, *R2* or *R1-R2*, but does not thrive in the presence of *R3* or *R4* genes. Therefore, susceptibility is only possible when the race has in its designation all the numerals present in the plant genotype. Consequently, the *R1-R2* genotype, which is the natural host for races 1, 2, is susceptible to races 1, 2; 1, 2, 3; 1, 2, 4 and 1, 2, 3, 4, and is only immune to the rest.

La resistencia genética, en el caso de la interacción *P. infestans*-especie silvestre de papa, se puede explicar mediante el modelo de gen a gen, donde por cada gen de resistencia del huésped existe un gen específico que determina la patogenicidad o virulencia del patógeno (Flor, 1959). Por lo tanto, el gen de resistencia de la planta solo es efectivo si hay un gen de avirulencia correspondiente en el patógeno (Alvarez-Morezuélas, Alor, Barandalla, Ritter, & de Galarreta, 2021; Serrano & Cárdenas, 2008).

Black et al. (1953) y Malcolmson y Black (1966) inocularon diferentes razas del patógeno en diferenciales de plantas hospedadoras, con lo cual compararon los genes de inmunidad presentes en la serie de hospederos y, a partir de ello, formularon un sistema de nomenclatura adecuado para la aplicación internacional. De esta manera, evaluaron el espectro de virulencia, que es el rango de genes *Avr* expresados por el aislado cuando es inoculado en la serie diferencial de genotipos con genes de resistencia *R*.

Black's differentials are used to this day to characterize *P. infestans* isolates into pathotypes or physiological races based on their virulence in plants with R genes from a differential group of genotypes (Alvarez-Morezuelas et al., 2021). Thanks to studies by Black et al. (1953) and Malcolmson and Black (1966), research was initiated to introduce the late blight resistance genes contained in *S. demissum* into potato cultivars, which were used by farmers for years.

Starting in the 2000s, the term effector began to be used and related to the term avirulence (Hogenhout, Van der Hoorn, Terauchi, & Kamoun, 2009). Effectors are defined as "all pathogen proteins and small molecules that alter host-cell structure and function", and whose alterations facilitate infection or trigger the plant's defense response (Hogenhout et al., 2009; Kamoun, 2007). The effectors present in *P. infestans* have been extensively studied and are broadly classified into apoplastic effectors and cytoplasmic effectors, although further classifications can be found in recent studies. The former are small cysteine-rich (SCR) proteins, such as PcF proteins (formerly called phytotoxins), a family of necrosis- and ethylene-inducing proteins (NLPs), and inhibitors of enzymes and extracellular proteases that degrade host structures, while cytoplasmic effectors are a family of RXLR proteins and CRN proteins (Fabro, 2022; Kamoun, 2006; Saraiva et al., 2022).

RXLR-type effector genes have been extracted from the *P. infestans* sequence and used in gene expression assays in wild potato species, with the aim of finding avirulence activity and accelerating the cloning of R genes (Lokossou, et al., 2009; Saunders et al., 2012; Schornack et al., 2009; Vleeshouwers et al., 2008). Several NLR genes (associated with effector-triggered immunity [ETI]), most of which belong to the CC-NLR family) have been identified, with R3a from *S. demissum* and Rpi-blb2 from *S. bulbocastanum* (recognizing the RXLR effectors AVR3a and AVRblb2, respectively) being of great interest, as they accentuate the importance and usefulness of effector studies in the search for and cloning of resistance genes (Majeed et al., 2022; Saraiva et al., 2022).

To date, 14 functional late blight resistance genes have been found in *S. demissum*: R1, R2, Rpt-demf1, R3a, R3b, R4^{al}, R4^{MA}, R5, R6, R7, R8, R9a, R10 and R11. Of these, the R1, R2, R3a, R3b, R8 and R9 genes have been cloned and classified within the family of genes encoding nucleotide-binding site and leucine-rich repeat domain-containing proteins (Paluchowska et al., 2022). Molecular cloning of genes has facilitated studies at the molecular level for the management of potato late blight resistance, as these can be used in genetic engineering to develop resistant cultivars (Rogozina, Beketova, Muratova, Kuznetsova, & Khavkin, 2021). However, these genes have not yet been widely

Cuando se observaron aislados con el mismo espectro de virulencia, se denominaron razas fisiológicas, y mientras más amplio fue el espectro de virulencia se consideró más compleja la raza del patógeno (Alvarez-Morezuelas et al., 2021). En este sentido, Black et al. (1953) describen que la raza 1, 2 es capaz de causar enfermedad en genotipos que carguen los genes de resistencia R1, R2 o R1-R2, pero no prospera en presencia de los genes R3 o R4. Por lo tanto, la susceptibilidad solo es posible cuando la raza tiene en su designación todos los numerales presentes en el genotipo de la planta. En consecuencia, el genotipo R1-R2, que es el huésped natural de las razas 1, 2, es susceptible a las razas 1, 2; 1, 2, 3; 1, 2, 4 y 1, 2, 3, 4, y solo es inmune al resto.

Los diferenciales de Black se utilizan hasta el día de hoy para caracterizar los aislados de *P. infestans* en patotipos o razas fisiológicas con base en su virulencia en plantas con genes R de un grupo diferencial de genotipos (Alvarez-Morezuelas et al., 2021). Gracias a los estudios de Black et al. (1953) y Malcolmson y Black (1966), se iniciaron investigaciones para introducir los genes de resistencia al tizón tardío contenidos en *S. demissum* en cultivares de papa, los cuales fueron utilizados por agricultores durante años.

A partir de la década de los 2000, se comenzó a emplear y relacionar el término effector con el término avirulencia (Hogenhout, Van der Hoorn, Terauchi, & Kamoun, 2009). Los efectores se definen como "todas las proteínas patógenas y moléculas pequeñas que alteran la estructura y función de la célula hospedante", y cuyas alteraciones facilitan la infección y desencadenan la respuesta de defensa de la planta (Hogenhout et al., 2009; Kamoun, 2007). Los efectores presentes en *P. infestans* han sido estudiados ampliamente y se clasifican, de forma general, en efectores apoplásticos y efectores citoplásmicos, aunque en estudios recientes se pueden encontrar más clasificaciones. Los primeros son proteínas pequeñas ricas en cisteína (SCR), como las proteínas PcF (antes llamadas fitotoxinas), una familia de proteínas inductoras de necrosis y etileno (NLP), e inhibidores de enzimas y proteasas extracelulares que degradan las estructuras del huésped. Mientras que los efectores citoplásmicos son una familia de proteínas RXLR y proteínas CRN (Fabro, 2022; Kamoun, 2006; Saraiva et al., 2022).

Genes efectores del tipo RXLR han sido extraídos de la secuencia de *P. infestans* y usados en ensayos de expresión de genes en especies silvestres de papa, esto con el objetivo de encontrar actividad de avirulencia y acelerar la clonación de los genes R (Lokossou, et al., 2009; Saunders et al., 2012; Schornack et al., 2009; Vleeshouwers et al., 2008). Varios genes NLR (asociados con la inmunidad desencadenada por efectores [ETI], por sus siglas en inglés), donde la mayoría pertenecen a la

introduced into potato cultivars, partly due to crossing difficulties arising from the difference in ploidy (Bethke, Halterman, & Jansky, 2017).

The ongoing coevolution of pathogen effectors and plant *R* genes represents the so-called arms race between plants and pathogens (Khavkin, 2015; Saraiva et al., 2022).

Insect resistance

Solanum demissum has been shown to be resistant to pests such as the Colorado potato beetle (*Leptinotarsa decemlineata*) and the potato leafhopper (*Empoasca fabae*) (Fürstenberg-Hägg et al., 2013). Resistance to the Colorado potato beetle is positively associated with the high content of the glycoalkaloids chaconine, demissine and leptines (Kuc, 1992), secondary metabolites known to act as a chemical defense against certain pathogens, although they are potentially harmful in humans (Eraso-Grisales et al., 2019; Lambers et al., 2008; Tingey, 1984). A limitation in obtaining insect-resistant genotypes of *S. tuberosum* is based on the fact that the offspring of resistant hybrids between *S. tuberosum* and *S. demissum* produce tubers with a high glycoalkaloid content.

Nematode resistance

Potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, make up an economically important group for potato cultivation. Their cysts can survive for more than 10 years in the soil, and persist even under unfavorable environmental conditions and the application of nematicides (Dandurand et al., 2019). Bachmann-Pfabe et al. (2019) identified genotypes resistant to nematode infection in 15 of 67 *S. demissum* accessions evaluated, and 45 of 67 with partial resistance. In addition, they indicate that the resistance of accessions originating in Mexico could be due to the presence of *G. mexicana* in Mexico, which is closely related genetically to *G. pallida*.

Resistance to other infectious agents

In the potato program of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), in Metepec, State of Mexico, *S. demissum* was found to present some resistance to the bacterium *Candidatus Liberibacter solanacearum*, so it could be included in breeding programs to mitigate the effects of this microorganism (Hidalgo-Gómez et al., 2022).

On the other hand, a screening of wild species carried out by Zoteyeva et al. (2012) showed that *S. demissum* exhibits resistance to potato virus X (PVX) and potato virus Y (PVY), which is part of the Bulgarian potato breeding program (Masheva, 2014). However,

familia CC-NLR, han sido identificados, siendo R3a de *S. demissum* y Rpi-blb2 de *S. bulbocastanum* (que reconocen los efectores RXLR AVR3a y AVRblb2, respectivamente) de gran interés, ya que acentúan la importancia y utilidad del estudio de efectores en la búsqueda y clonación de genes de resistencia (Majeed et al., 2022; Saraiva et al., 2022).

Hasta la fecha, se han encontrado 14 genes de resistencia al tizón tardío funcionales en *S. demissum*: R1, R2, Rpt-demf1, R3a, R3b, R4^d, R4^{Ma}, R5, R6, R7, R8, R9a, R10 y R11. De estos, los genes R1, R2, R3a, R3b, R8 y R9 han sido clonados y clasificados dentro de la familia de genes que codifican el sitio de unión de nucleótidos y las proteínas que contienen dominios repetidos ricos en leucina (Paluchowska et al., 2022). La clonación molecular de los genes ha facilitado la realización de estudios a nivel molecular para el manejo de la resistencia al tizón tardío de la papa, ya que estos se pueden utilizar en ingeniería genética para desarrollar cultivares resistentes (Rogozina, Beketova, Muratova, Kuznetsova, & Khavkin, 2021). Sin embargo, estos genes aún no se han introducido ampliamente en los cultivares de papa, en parte, debido a las dificultades de cruzamiento por la diferencia en ploidías (Bethke, Halterman, & Jansky, 2017).

La coevolución continua de los efectores de patógenos y los genes *R* de las plantas representa la llamada carrera armamentista entre plantas y patógenos (Khavkin, 2015; Saraiva et al., 2022).

Resistencia a insectos

Solanum demissum ha demostrado ser resistente a plagas, como el escarabajo de la papa de Colorado (*Leptinotarsa decemlineata*) y la cigarrita de la papa (*Empoasca fabae*) (Fürstenberg-Hägg et al., 2013). La resistencia al escarabajo de la papa de Colorado está asociada positivamente con el alto contenido de los glicoalcaloides chaconina, demissina y leptinas (Kuc, 1992), metabolitos secundarios conocidos por actuar como defensa química ante ciertos patógenos, aunque son potencialmente dañinos en humanos (Eraso-Grisales et al., 2019; Lambers et al., 2008; Tingey, 1984). Una limitante en la obtención de genotipos de *S. tuberosum* resistentes a insectos se basa en que la descendencia de híbridos resistentes entre *S. tuberosum* y *S. demissum* producen tubérculos con alto contenido glicoalcaloides.

Resistencia a nematodos

Los nematodos del quiste de la papa, *Globodera pallida* y *G. rostochiensis*, conforman un grupo económicamente importante para el cultivo de papa. Sus quistes pueden sobrevivir más de 10 años en el suelo, y persistir incluso en condiciones ambientales desfavorables y a la aplicación de nematicidas (Dandurand et al., 2019).

although Tiwari, Jeevalatha, Tuteja, and Khurana (2022) stress that the *S. demissum* *Ny_{ams}* gene confers a hypersensitive response (HR) against PVY and potato virus A (PVA), traditional breeding has not been entirely effective due to the rapid evolution of virulent strains (same case as late blight), making it important to use new technologies such as CRISPR-Cas (Doudna & Charpentier, 2014) for the generation of resistance by disruption of host factors or interference in the viral genome.

Frost resistance

Unlike pest and disease management, frost is not easily managed with cultivation practices or chemical treatments; therefore, the use of resistant varieties is recommended to reduce losses due to frost (Vega & Bamberg, 1995). *S. demissum* is classified, within the group of wild relatives of potato, as one of the most frost-resistant species, withstanding up to -2 °C (Vega & Bamberg, 1995). Since the beginning of the 20th century, this has led to the obtaining of frost-resistant hybrids from crosses between *S. demissum* and *S. tuberosum* (del Río & Bamberg, 2020).

Other properties

Glycoalkaloids, widely studied secondary metabolites, are commonly found in potato and its wild relatives, and have been classified as toxic compounds, making them useful in defending against pathogen attack and certain human diseases (Friedman, 2006; Friedman, McDonald, & Filadelfi-Keszi, 1997; Kuc, 1992; Manrique-Carpintero et al., 2013). High concentrations of α -tomatine, demissine, demissidine, tomatidine, chaconine, and tomatidenol have been found in *S. demissum* (Distl & Wink, 2009; Friedman et al., 1997); the last has been introgressed into *S. tuberosum* (McCue, 2009). These compounds have shown an effect in the treatment of different diseases, as they have been associated with anti-inflammatory, antihyperglycemic, antibiotic, anticholesterolemic and anticancer properties (Friedman, 2006; Milner et al., 2011). Tomatidine inhibits the growth of colon and liver cancer cells *in vitro* (Wölfling, 2007), α -chaconine and α -tomatine have a positive effect on the inactivation of herpes virus type 1 (Thorne, Clarke, & Skuce, 1985), and α -tomatine inhibits growth and induces apoptosis in myeloid leukemia cells (Huang et al., 2015).

In wild potato species, including *S. demissum*, high antioxidant activity has been reported, and phenolic compounds such as p-coumaric acid, chlorogenic acid and caffeic acid have been found. The last has antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory properties (Butiuk, Martos, Adachi, & Hours, 2016; Eroglu & Dogan, 2023; Friedman, 2006; Hale et al., 2008).

Bachmann-Pfabe et al. (2019) identificaron genotipos resistentes a la infección por nematodos en 15 de 67 accesiones de *S. demissum* evaluadas, y 45 de 67 con resistencia parcial. Además, indican que la resistencia de las accesiones originarias de México se podría deber a la presencia de *G. mexicana* en el territorio mexicano, que está estrechamente relacionada genéticamente con *G. pallida*.

Resistencia a otros agentes infecciosos

En el programa de papa del Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), en Metepec, Estado de México, se encontró que *S. demissum* presenta cierta resistencia a la bacteria *Candidatus Liberibacter solanacearum*, por lo cual se podría incluir en programas de mejoramiento genético para mitigar los efectos de este microorganismo (Hidalgo-Gómez et al., 2022).

Por otra parte, un cribado de especies silvestres realizado por Zoteyeva et al. (2012) demostró que *S. demissum* presenta resistencia a los virus X de la papa (PVX) y virus Y de la papa (PVY), lo cual forma parte del programa de mejoramiento de papa de Bulgaria (Masheva, 2014). Sin embargo, aunque Tiwari, Jeevalatha, Tuteja, y Khurana (2022) acentúan que el gen *Ny_{ams}* de *S. demissum* confiera una respuesta hipersensible (HR) contra el PVY y el virus A de la papa (PVA), el mejoramiento tradicional no ha resultado del todo efectivo debido a la rápida evolución de las cepas virulentas (mismo caso que el tizón tardío), por lo que resulta importante el uso de nuevas tecnologías como CRISPR-Cas (Doudna & Charpentier, 2014) para la generación de resistencia por medio de interrupción de factores del hospedero o la interferencia en el genoma viral.

Resistencia a heladas

A diferencia del manejo de plagas y enfermedades, las heladas no se manejan fácilmente con prácticas de cultivo o tratamientos químicos; por ello, se recomienda el uso de variedades resistentes para reducir las pérdidas por heladas (Vega & Bamberg, 1995). *S. demissum* se clasifica, dentro del grupo de parientes silvestres de la papa, como una de las especies más resistentes a heladas, al soportar hasta -2 °C (Vega & Bamberg, 1995). Desde inicios del siglo XX, esto ha desencadenado la obtención de híbridos a partir de cruces entre *S. demissum* y *S. tuberosum* resistentes a heladas (del Río & Bamberg, 2020).

Otras propiedades

Los glicoalcaloides, metabolitos secundarios estudiados ampliamente, se encuentran comúnmente en la papa y sus parientes silvestres, y han sido catalogados como compuestos tóxicos, por lo que resultan útiles en la

Discussion

Solanum demissum is an important phyto-genetic resource, valued for its resistance to pests and diseases, positioning itself on the map as one of the most exploited wild relatives of potato in breeding programs. However, despite the fact that genes for resistance to blight and other stress factors have been one of the most studied aspects in the last 100 years (by obtaining countless cultivars resistant to late blight), the results obtained from traditional breeding have not been entirely successful. *P. infestans* and other pathogens have managed to evade the genetic resistance of cultivars obtained through crosses with *S. demissum*, which has led to the development of new epidemics worldwide, such as in the United States, Canada, India, Mexico, Peru, the vast majority of European countries, Africa and Asia (Fry et al., 2013; Hwang et al., 2014; Dey et al., 2018; Romero-Montes, Lozoya-Saldaña, Mora-Aguilera, Fernández-Pavía, & Grünwald, 2012; Zoteyeva & Patrikeeva, 2010; Forbes, 2015).

As a result of the above, it is essential to continue researching, discovering and proposing improvements through the use of new technologies to make breeding programs more efficient. Currently, there are two aspects in the application of these tools: the search for and identification of candidate genes in *S. demissum*, and the introgression of the discovered genes into *S. tuberosum*.

For gene searching and identification, knowing the diversity, structure and relationships of the germplasm, genotyping by sequencing (GBS), genome-wide association studies (GWAS) and QTL's, to mention a few examples, are useful tools that can facilitate and accelerate the localization and selection of genes of interest, which will then be sought to be introduced or silenced in cultivated potato. Several studies have documented the feasibility of using these tools (Bastien, Boudhrioua, Fortin, & Belzile, 2018; Boudhrioua et al., 2017; Bradshaw, Hackett, Pande, Waugh, & Bryan, 2008; Hackett, McLean, & Bryan, 2013; Okada et al., 2019; Prodhomme et al., 2020; Rosyara, de Jong, Douches, & Endelman, 2016; Saidi & Hajibarat, 2020; Uitdewilligen et al., 2013; Zhang, Qu, Gu, Xu, & Xue, 2022). Some examples of genes that may be of interest are the late blight resistance genes (already mentioned above), the *GBSSI* gene (associated with amylose), the *Asn1*, *Asn2* and *Vlnv* genes (associated with acrylamide accumulation), the polyphenol oxidase gene (responsible for browning) and the beetle β -actin gene.

Regarding gene introgression into *S. tuberosum*, genetic transformation was the first strategy used, where the main methods are bio-ballistics, protoplast fusion and *Agrobacterium*-mediated transformation (Díaz-

defensa ante el ataque de patógenos y ante ciertas enfermedades humanas (Friedman, 2006; Friedman, McDonald, & Filadelfi-Keszi, 1997; Kuc, 1992; Manrique-Carpintero et al., 2013). En *S. demissum* se han encontrado altas concentraciones de α -tomatina, demissina, demissidina, tomatidina, chaconina y tomatidinol (Distl & Wink, 2009; Friedman et al., 1997); este último ha sido introgresado en *S. tuberosum* (McCue, 2009). Dichos compuestos han mostrado un efecto en el tratamiento de diferentes enfermedades, ya que se han asociado a propiedades antiinflamatorias, antihiper glucémicas, antibióticas, anticolesterolemiantes y anticancerígenas (Friedman, 2006; Milner et al., 2011). La tomatidina inhibe el crecimiento *in vitro* de células cancerosas de colon e hígado (Wölfling, 2007). La α -chaconina y la α -tomatina tienen efecto positivo en la inactivación del virus del herpes tipo 1 (Thorne, Clarke, & Skuce, 1985), mientras que α -tomatina inhibe el crecimiento e induce la apoptosis en células de leucemia mieloide (Huang et al., 2015).

En especies silvestres de papa, incluida *S. demissum*, se ha reportado alta actividad antioxidante, y se han encontrado compuestos fenólicos como ácido p-cumárico, ácido clorogénico y ácido cafeico. Estos últimos tienen propiedades antioxidantes, antibacterianas, antivirales, antifúngicas y antiinflamatorias (Butiuk, Martos, Adachi, & Hours, 2016; Eroglu & Dogan, 2023; Friedman, 2006; Hale et al., 2008).

Discusión

Solanum demissum es un recurso fitogenético importante, valorado por su resistencia a plagas y enfermedades, posicionándose en el mapa como uno de los parientes silvestres de la papa más aprovechados en los programas de mejoramiento genético. Sin embargo, a pesar de que los genes de resistencia al tizón y otros factores de estrés han sido uno de los aspectos más estudiados en los últimos 100 años (al lograr la obtención de innumerables cultivares resistentes al tizón tardío), los resultados obtenidos a partir del mejoramiento genético tradicional no han sido del todo exitosos. *P. infestans*, y otros patógenos, han logrado evadir la resistencia genética de los cultivares obtenidos a través de cruces con *S. demissum*, lo cual ha dado lugar al desarrollo de nuevas epidemias a nivel mundial, como en Estados Unidos, Canadá, India, México, Perú, la gran mayoría de los países europeos, África y Asia (Fry et al., 2013; Hwang et al., 2014; Dey et al., 2018; Romero-Montes, Lozoya-Saldaña, Mora-Aguilera, Fernández-Pavía, & Grünwald, 2012; Zoteyeva & Patrikeeva, 2010; Forbes, 2015).

Derivado de lo anterior, resulta indispensable continuar investigando, descubriendo y proponiendo mejoras mediante el uso de nuevas tecnologías para eficientizar

Granados & Chaparro-Giraldo, 2012). Bio-ballistics consists of the bombardment of microprojectiles covered with the DNA to be transferred, which carries, inside the cell, the genes of interest that will later be integrated into the plant genome. Protoplast fusion is a chemical-mediated transfer that most commonly uses polyethylene glycol to induce membrane permeability to allow the DNA to pass into the cell. The *Agrobacterium* system works because of the genus' ability to infect plant organisms and transfer DNA, and involves using the organism as a vector for gene transfer to the plant. This transformation can be trans-genesis or cis-genesis. The latter is used in the case of *S. demissum* and *S. tuberosum*, as they are closely related species (del Mar Martínez-Prada, Curtin, & Gutiérrez-González, 2021; Díaz-Granados & Chaparro-Giraldo, 2012; Nadakuduti, Buell, Voytas, Starker, & Douches, 2018; Nicolai, Fält, Hofvander, & Andersson, 2021; Toinga-Villafuerte, Vales, Awika, & Rathore, 2022; Van Eck, 2018; Zhang, Zhang, & Chen, 2020).

The second strategy used for introgression is gene editing. Zinc finger nucleases (ZFN), TALEN nucleases and the CRISPR-Cas9 complex are useful methods and another alternative for the use and introduction of genes of interest from *S. demissum* (Van Eck, 2018). Some reviews and trials have shown successful cases with the use of TALEN and CRISPR-Cas9 in *S. tuberosum*, such as the reduction of glycoalkaloids in tubers, resistance to herbicides, starch quality in tubers, self-incompatibility, and polyphenol oxidase (PPO) activity in tubers to reduce enzymatic browning.

The generation of genetically modified varieties produced and marketed since 1995 (NewLeaf™, NewLeaf™ Plus, NewLeaf™ Y, Innate™ 1.0 Innate™ 2.0, Amflora™, Starch potato, Elizaveta Plus, Lugovskoi Plus, SpuntaG2, Desiree, Ranger Russet, Taedong Valley, King Edward, Chicago, Atlantic, Kuras, Sayaka and Mayqueen) include cis-genesis, CRISPR-Cas9 and TALEN genetic transformation approaches, where acquired traits include incorporation of resistance to the Colorado potato beetle, PVY and late blight, reduction of acrylamide, glycoalkaloids, black spot and amylose, and an increase in TAG, carotenoids and vitamins (Abdallah, Hamwieh, Radwan, Fouad, & Prakash, 2021; del Mar Martínez-Prada et al., 2021; Hameed, Zaidi, Shakir, & Mansoor, 2018; Nadakuduti et al., 2018; Tiwari et al., 2022; Tussipkan & Manabayeva, 2021; Yasumoto et al., 2020; Wang et al., 2015).

Techniques such as gene silencing (RNAi) (used to confer resistance to viruses such as PVY, PVX, PVS, PLRV and late blight) (del Mar Martínez-Prada et al., 2021; Hameed et al., 2018; Sun et al., 2016) or gene blocking (gene knockout, suggested as an effective strategy to reduce acrylamide content) (del Mar

los programas de mejoramiento. Actualmente, se visualizan dos vertientes en la aplicación de estas herramientas: la búsqueda e identificación de genes candidatos en *S. demissum*, y la introgresión de los genes descubiertos a *S. tuberosum*.

Para la búsqueda e identificación de genes, conocer la diversidad, estructura y relaciones del germoplasma, el genotipado por secuenciación (GBS), los estudios de asociación de genoma completo (GWAS) y los QTL's, por mencionar algunos ejemplos, son herramientas útiles que pueden facilitar y acelerar la localización y selección de los genes de interés, que después se buscará introducir o silenciar en la papa cultivada. Diversos estudios documentan la factibilidad del uso de estas herramientas (Bastien, Boudhrioua, Fortin, & Belzile, 2018; Boudhrioua et al., 2017; Bradshaw, Hackett, Pande, Waugh, & Bryan, 2008; Hackett, McLean, & Bryan, 2013; Okada et al., 2019; Prodhomme et al., 2020; Rosyara, de Jong, Douches, & Endelman, 2016; Saidi & Hajibarat, 2020; Uitdewilligen et al., 2013; Zhang, Qu, Gu, Xu, & Xue, 2022). Algunos ejemplos de genes que pueden ser interesantes son los de resistencia a tizón tardío (ya mencionados anteriormente), el gen *GBSSI* (asociado con la amilosa), los genes *Asn1*, *Asn2* y *Vlnv* (asociados con la acumulación de acrilamida), el gen de la polifenoloxidasas (responsable del pardeamiento) y el gen de la β -actina del escarabajo.

Referente a la introgresión de genes a *S. tuberosum*, la transformación genética fue la primera estrategia utilizada, donde los métodos principales son la biobalística, la fusión de protoplastos y la transformación mediada por *Agrobacterium* (Díaz-Granados & Chaparro-Giraldo, 2012). La biobalística consiste en el bombardeo de microprojectiles cubiertos del ADN que se desea transferir, lo cual lleva, al interior de la célula, los genes de interés que después serán integrados al genoma de la planta. La fusión por protoplastos es una transferencia mediada por compuestos químicos que utiliza, más comúnmente, polietilenglicol para inducir permeabilidad en las membranas y así permitir el paso del ADN a la célula. El sistema *Agrobacterium* funciona gracias a la capacidad del género para infectar a organismos vegetales y transferir ADN, y consiste en utilizar al organismo como vector de transferencia del gen a la planta. Esta transformación puede ser trans-génesis o cis-génesis. Esta última es utilizada en el caso de *S. demissum* y *S. tuberosum*, ya que son especies estrechamente relacionadas (del Mar Martínez-Prada, Curtin, & Gutiérrez-González, 2021; Díaz-Granados & Chaparro-Giraldo, 2012; Nadakuduti, Buell, Voytas, Starker, & Douches, 2018; Nicolai, Fält, Hofvander, & Andersson, 2021; Toinga-Villafuerte, Vales, Awika, & Rathore, 2022; Van Eck, 2018; Zhang, Zhang, & Chen, 2020).

Martínez-Prada et al., 2021), used in conjunction with CRISPR-Cas9, have proven to be successful and have provided an interesting basis for further studies on gene functionality (Clasen et al., 2016; Kieu, Lenman, Wang, Petersen, & Andreasson, 2021; Toinga-Villafuerte et al., 2022; Wang et al., 2015).

From the wide possibilities for the application of transformation and gene editing tools, two major challenges have been visualized; on the one hand, there is the complexity represented by the variability of ploidy between species and, on the other, the government regulations imposed and in process for genetically modified organisms (Van Eck, 2018).

On the other hand, the antioxidant properties and the presence of glycoalkaloids in *S. demissum* set a starting point in agricultural and pharmaceutical research to take full advantage of these resources. Work has been carried out on the extraction of glycoalkaloids in cultivated potato (mainly α -chaconine and α -solanine), and different extraction methods have been proposed, such as aqueous extraction, obtaining acidified ethanolic extracts, obtaining extracts with ammonium hydroxide, and extraction with pressurized liquids (Eraso-Grisales et al., 2019; Sánchez-Maldonado, Mudge, Gänzle, & Schieber, 2014; Silva-Beltrán et al., 2015). In addition, the effectiveness of the extracts of these compounds against bacterial strains such as *E. coli*, *Salmonella typhimurium*, *Listeria ivanovi* and *Staphylococcus aureus* has been evaluated, as well as the biocidal effect against snails and the Colorado potato beetle (Kuc, 1992; Silva-Beltrán et al., 2015).

Regarding antioxidant activity, particularly for the extraction of p-coumaric acid, chlorogenic acid and caffeic acid (present in *S. demissum* and whose properties were described above), it may be feasible to use methodologies proven in other crops, such as artichoke or coffee, in which alcoholic, hydroalcoholic extracts and acid hydrolysis with hydrochloric acid are used (Eroglu & Dogan, 2023). Likewise, methodologies can be considered to determine the most suitable solvent for the extraction of the compounds (Aristizábal, Vargas, & Alvarado, 2019; Gani et al., 2006; Ky, Noiro, & Hamon, 1997).

Finally, it is worth emphasizing the importance that *S. demissum* has had over the years, being a milestone in the genetic improvement of potato, and contributing to the understanding of resistance and the identification of physiological races. *S. demissum* has been the focus of a large number of research projects, and today it has the known properties and elements to continue contributing to the improvement of crops and the production of useful compounds for humans.

La segunda estrategia utilizada para la introgresión es la edición genética. Las nucleasas de dedos de zinc (ZFN), las nucleasas TALEN y el complejo CRISPR-Cas9 son métodos útiles y otra alternativa para el uso e introducción de genes de interés procedentes de *S. demissum* (Van Eck, 2018). En algunas revisiones y ensayos se han demostrado casos de éxito con el uso de TALEN y CRISPR-Cas9 en *S. tuberosum*, como la reducción de glicoalcaloides en tubérculos, resistencia a herbicidas, calidad de almidón en tubérculo, autoincompatibilidad y actividad de polifenoloxidasas en tubérculo (PPO) para reducir el pardeamiento enzimático.

La generación de variedades modificadas genéticamente, producidas y comercializadas desde 1995 (NewLeafTM, NewLeafTM Plus, NewLeafTM Y, Innate[®] 1.0 Innate[®] 2.0, AmfloraTM, Starch potato, Elizaveta Plus, Lugovskoi Plus, SpuntaG2, Desiree, Ranger Russet, Taedong Valley, King Edward, Chicago, Atlantic, Kuras, Sayaka y Mayqueen), incluyen enfoques de transformación genética cis-génesis, CRISPR-Cas9 y TALEN, en donde las características adquiridas abarcan la incorporación de resistencia al escarabajo de la papa de colorado, al PVY y al tizón tardío, la reducción de acrilamida, glicoalcaloides, *black spot* y amilosa, y el incremento de TAG, carotenoides y vitaminas (Abdallah, Hamwiah, Radwan, Fouad, & Prakash, 2021; del Mar Martínez-Prada et al., 2021; Hameed, Zaidi, Shakir, & Mansoor, 2018; Nadakuduti et al., 2018; Tiwari et al., 2022; Tussipkan & Manabayeva, 2021; Yasumoto et al., 2020; Wang et al., 2015).

Técnicas como el silenciamiento de genes (ARNi) (utilizado para conferir resistencia a virus como el PVY, PVX, PVS, PLRV y el tizón tardío) (Del Mar Martínez-Prada et al., 2021; Hameed et al., 2018; Sun et al., 2016) o el bloqueo de genes (gene knockout, sugerido como una estrategia efectiva para reducir el contenido de acrilamida) (Del Mar Martínez-Prada et al., 2021), utilizadas en conjunto con CRISPR-Cas9, resultan ser exitosas y proveen una base interesante para otros estudios sobre la funcionalidad de los genes (Clasen et al., 2016; Kieu, Lenman, Wang, Petersen, & Andreasson, 2021; Toinga-Villafuerte et al., 2022; Wang et al., 2015).

A partir de las amplias posibilidades de aplicación de las herramientas de transformación y edición genética, se han logrado visualizar dos retos importantes. Por un lado, se tiene la complejidad que representa la variabilidad de ploidías entre especies y, por el otro, las regulaciones gubernamentales impuestas y en proceso para los organismos genéticamente modificados (Van Eck, 2018).

Por otro lado, las propiedades antioxidantes y la presencia de glicoalcaloides en *S. demissum* fijan

Conclusions

Solanum demissum is one of the species with great potential in different areas and for potato breeding. Traditional breeding has limitations that could be solved with the use of new molecular technologies, which, when used in an integral way, would result in an important advance in the field of improvement and use of plant species.

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- un punto de partida en la investigación agrícola y farmacéutica para aprovechar al máximo estos recursos. Se ha trabajado en la extracción de glicoalcaloides en papa cultivada (principalmente α -chaconina y α -solanina), y se han propuesto diferentes métodos de extracción, como la acuosa, la obtención de extractos etanólicos acidificados, la obtención de extractos con hidróxido de amonio y la extracción con líquidos presurizados (Eraso-Grisales et al., 2019; Sánchez-Maldonado, Mudge, Gänzle, & Schieber, 2014; Silva-Beltrán et al., 2015). Además, se ha evaluado la efectividad de extractos de estos compuestos ante cepas bacterianas como *E. coli*, *Salmonella typhimurium*, *Listeria ivanovi* y *Staphylococcus aureus*, así como el efecto biocida ante caracoles y el escarabajo de la papa de Colorado (Kuc, 1992; Silva-Beltrán et al., 2015).
- Respecto a la actividad antioxidante, particularmente para la extracción de ácido p-cumárico, ácido clorogénico y ácido cafeico (presentes en *S. demissum* y cuyas propiedades se han descrito anteriormente), puede ser factible el uso de metodologías probadas en otros cultivos, como la alcachofa o el café, en las que se utilizan extractos alcohólicos, hidroalcohólico y la hidrólisis ácida con ácido clorhídrico (Eroglu & Dogan, 2023). Asimismo, se pueden considerar metodologías para determinar el solvente más adecuado para la extracción de los compuestos (Aristizábal, Vargas, & Alvarado, 2019; Gani et al., 2006; Ky, Noirot, & Hamon, 1997).
- Finalmente, resulta valioso enfatizar la importancia que *S. demissum* ha tenido a lo largo de los años, al ser un parteaguas en el mejoramiento genético de la papa, y contribuir al entendimiento de las resistencias y a la identificación de razas fisiológicas. *S. demissum* ha sido protagonista en un gran número de investigaciones, y hoy en día cuenta con las propiedades y elementos para continuar contribuyendo en la mejora de cultivos y en la producción de compuestos útiles para el ser humano.

Conclusiones

Solanum demissum es una de las especies con gran potencial en diferentes áreas y para el mejoramiento genético de la papa. El mejoramiento tradicional presenta limitantes que podrían ser solventadas con el uso de nuevas tecnologías moleculares, ya que, al utilizarla de forma integral, resultarían en un importante avance en el campo de la mejora y aprovechamiento de especies vegetales.

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Fin de la versión en español

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CAPÍTULO IV. MORPHOMETRIC ANALYSIS OF WILD POTATO LEAVES

Como se envió a la revista *Genetic Resources and Crop Evolution*

MORPHOMETRIC ANALYSIS OF WILD POTATO LEAVES

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ABSTRACT

To catalog and promote the conservation and use of crop wild relatives, comprehensive phenotypic and genotypic information must be available. Plant genotyping has the power to resolve the phylogenetic relationships between crop wild relatives, quantify genetic diversity, and identify marker-trait associations for expedited molecular breeding. However, access to cost-effective genotyping strategies is often limited in underutilized crops and crop wild relatives. Potato landraces and wild species, distributed throughout Central and South America, exhibit remarkable phenotypic diversity and are an invaluable source of resistance to pests and diseases. Unfortunately, very limited information is available for these germplasm resources, particularly regarding phenotypic diversity and potential use as trait donors. In this work, more than 150 accessions corresponding to 12 species of wild and cultivated potatoes, collected from different sites across the American continent, were analyzed using computer vision and morphometric methods to evaluate leaf size and shape. In total, more than 1100 leaves and leaflets were processed and analyzed for nine traits related to size, shape, and color. The results produced in this study provided a visual depiction of the extensive variability among potato wild species and enabled a precise quantification of leaf phenotypic differences, including shape,

color, area, perimeter, length, width, aspect ratio, convexity, and circularity. Moreover, this study provided insights into the potential role of leaf size and shape variation in plant-insect interactions, productivity, and adaptation. Finally, we discussed the application and utility of inexpensive but comprehensive morphometric approaches to catalog and study the diversity of crop wild relatives.

keywords: wild potato, plant genetic resources, computer vision, crop wild relatives

ABBREVIATIONS

EFD - Elliptical Fourier Descriptor

ICAMEX - Agricultural Research and Training Institute, Mexico

INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) was domesticated from wild *Solanum* species native to the Peruvian Andes 8000-9000 years ago (Spooner et al., 2005). Through polyploidization (Watanabe and Peloquin, 1989), photoperiodism changes (Hosaka, 2003, 2004), and other diversification mechanisms (Hardigan et al., 2017), potato became adopted globally and it is now the third most important food crop for human consumption (Birch et al., 2012). Commercial potato cultivars exhibit great phenotypic diversity. For example, available germplasm varies in tuber shape, skin color, plant architecture, phenology, among others (<https://www.upov.int/edocs/tgdocs/es/tg023.pdf>). Despite this variability, most of the commercial cultivars are susceptible to late blight, a major disease caused by *Phytophthora infestans* (Mont.) de Bary and found worldwide. Late blight produces dark grey to brown water-soaked spots on leaf tissues, total necrosis of infected plants within 5 to 10 days, changes in tuber coloration, and rot (Kamoun et al., 2015; Dong and Zhou, 2022). Furthermore, late blight was responsible for the Irish Potato Famine in the mid-1800s, which led to widespread starvation, disease, and emigration, remaining as one of the most significant historical social crises caused by a plant pathogen (Majeed et al., 2022). Since then, plant breeders have looked for sources of resistance.

Potato landraces and wild species are widely distributed across Central and South America (Hardigan et al., 2015; Hijmans et al., 2002). These materials display remarkable

phenotypic variation, encompassing diverse tuber colors, sizes and shapes, canopy architectures, and growth habits (Spooner et al., 2005). More importantly, landraces and wild species are excellent sources of resistance to nematodes, viruses, insects, and late blight (Bachmann-Pfabe et al., 2019; Dandurand et al., 2019; del Rio and Bamberg, 2020; Fürstenberg-Hägg et al., 2013; Ruiz de Galarreta et al., 1998). The *Solanum* genus includes more than 151 wild potato species of varying ploidy levels (<https://cipotato.org/potato/wild-potato-species>), from diploid ($2n=2x=24$), to triploid ($2n=3x=36$), tetraploid ($2n=4x=48$), pentaploid ($2n=5x=60$), and hexaploids ($2n=6x=72$), and are distributed across most of the American continent (Hijmans et al., 2002). In recent years, much of the phylogenetic relationships across *Solanum* species have been revealed through the use of genomic information (Gagnon et al., 2022; Hardigan et al., 2015; Huang et al., 2019). Moreover, the integration of phenotypic and genotypic data has allowed researchers to identify marker-trait associations for tuber quality characteristics (Aversano et al., 2017; Sulli et al., 2017; Wolters et al., 2010), abiotic factors resistance (Esposito et al., 2017; Vega and Bamberg, 1995; Watanabe et al., 2011), and pest and disease resistance (Fulladolsa et al., 2015; Huang et al., 2023; Li et al., 2018c; Meade et al., 2020; Sudha et al., 2016; Tiwari et al., 2015; Yang et al., 2017). For instance, at least 14 loci have been identified for late blight resistance (Meade et al., 2020; Paluchowska et al., 2022; Tiwari et al., 2013), in addition to other resistance loci and tuber quality traits (Bhatia et al., 2023; Collins et al., 1999; Li et al., 2005). From a breeding perspective, potato wild relatives have become an important reservoir of novel allelic variation for trait introgression. For example, species like *Solanum acaule* Bitter, *Solanum bulbocastanum* Dunal, *Solanum chacoense* Bitter, *Solanum demissum* Lindl., and *Solanum stoloniferum* Schltdl. have been found to be highly resistant to light blight (Castañeda-Álvarez et al., 2015; Karki et al., 2020). Other wild species and landraces such as *Solanum brevicaule* Bitter, and *Solanum microdontum* Bitter are sources of resistance to pale cyst nematode (*Globodera pallida*) and abiotic stresses such frost and salinity (Azhar and Wani, 2021; Bachmann-Pfabe et al., 2019; Bashir et al., 2021; Nicolao et al., 2023). In this context, cataloging plant genetic resources is fundamental for predicting, assessing, and promoting the use of uncultivated or underutilized germplasm,

such as crop wild relatives, for plant improvement (Machida-Hirano, 2015; Spooner and Bamberg, 1994).

In recent decades, genebanks worldwide have accumulated extensive ex-situ collections of exotic germplasm, including crop wild relatives and wild species (Ellis et al., 2020). Advancements in genotyping technologies have revolutionized genetic characterization, enabling genome-wide analyses of numerous accessions and entire ex-situ collections within genebanks. High-throughput sequencing, in particular, has facilitated genome characterization (Tang et al., 2022) and the development of core collections, which aim to capture the majority of genetic diversity with a reduced number of accessions (Wambugu et al., 2018). However, the comprehensive characterization of phenotypic information and the exploration of environmental interactions, vital for harnessing the potential of exotic germplasm and identifying species and accessions suitable for climate resilience, still remains challenging. Moreover, the implementation of cost-effective genotyping strategies relies on the availability of high-quality genome assemblies and appropriate genotyping pipelines (Pavan et al., 2020), which is not always the case in minor crops or crop wild relatives.

Leaf morphometrics has been used to catalog genetic diversity in crop species such as grapevine (Demmings et al., 2019; Klein et al., 2017), tomato (Chitwood et al., 2013), cranberry (Diaz-Garcia et al., 2018), cotton (Andres et al., 2017) and apple (Migicovsky et al., 2017). Moreover, this approach has supported large-scale phenotyping efforts in orphan crops and crop wild relatives, including kura clover (Schlautman et al., 2020), *Solanum pennellii* (Li et al., 2018b), *Viburnum* (Spriggs et al., 2018), *Oxalis* (Morello et al., 2018), and *Passiflora* (Chitwood et al., 2018). Leaves serve as the primary photosynthetic organs in most plants, and in many cases, leaf-traits such as size and shape are highly informative and provide important insights into physiological and phenological states (Wang et al., 2021). In certain studies, leaf size and shape have been associated with distinct fruit characteristics. For example, leaf morphometry in oriental persimmon correlates with fruit shape (Maeda et al., 2018). In other species, like grape and apple, leaf color has been associated with berry flesh color (Migicovsky et al., 2017). While quantitative methods exist for measuring leaf size and color, the assessment of leaf

shapes often relies on qualitative measurements. This poses a challenge, particularly in plant species with multifoliate leaves, such as *Solanum* sp., where the absence of homologous landmark points complicates leaf shape comparisons (Li et al., 2017). In this study, more than 150 accessions corresponding to 12 species of wild and cultivated potato were analyzed using computer vision and advanced morphometric methods. The accessions analyzed here were collected from different sites across the American continent and represent a substantial portion of the existing diversity among wild potato species. More importantly, these species serve as a valuable gene reservoir of interest for plant breeding purposes. We used high-throughput phenotyping and morphometrics methods to evaluate leaf size and shape, which produced a dataset comprising 10 phenotypic variables and more than 7000 entries (i.e., leaves). The results produced in this study provided a visual depiction of the extensive variability among potato wild species, and enabled a precise quantification of phenotypic differences, including traits commonly considered to be qualitative, such as color or shape.

MATERIALS AND METHODS

Plant material

Seeds of 161 wild potato accessions spanning 12 *Solanum* species were obtained from the United States Potato Genebank (Peninsular Agricultural Research Station, University of Wisconsin-Madison) (Table S1). Accessions for these species were originally collected between 1940 and 2000 at different locations in Mexico, Bolivia, Peru, Argentina, Colombia, and Guatemala, at altitudes ranging from 1800 to 4100 masl (Figure 1). A Mexican *S. tuberosum* commercial cultivar ('Alpha') was also included in the study. Ploidy levels represented in the surveyed collection included diploids (n=23), triploids (n=1), tetraploids (n=27), and hexaploids (n=111; Figure 1).

The seeds were germinated in the greenhouse using 200-hole seedling trays and peat moss as media, for a period of six weeks and at 25°C. Once the plants developed roots and at least four leaves, they were transplanted to the field at ICAMEX, Estado de Mexico, Mexico (latitude 19.2435, longitude -99.59135, at 2606 masl). Plants were established in the field using a complete randomized design with two repetitions; each experiment unit

comprised two plants. For every plant in the experiment, two fully mature leaves collected from the middle of the canopy were manually sampled for imaging.

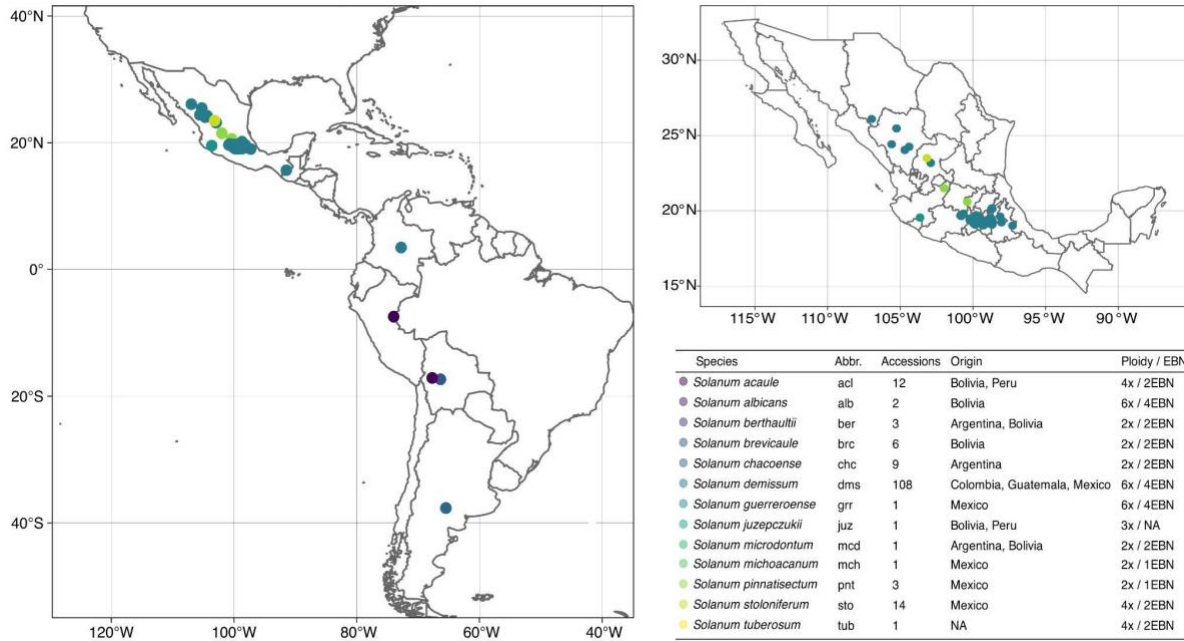


Fig. 1 The geographic distribution and ploidy of 13 *Solanum* accessions investigated in the present study. Upper-right panel zooms in into the collection sites for Mexican accessions. Different color points correspond to different accessions, as indicated in the lower-right table. EBN = Endosperm Balance Number

Image acquisition and morphometric analysis

Collected leaves were scanned using a flatbed scanner (Epson Perfection V39,) at 300 dpi. Two sets of images were obtained. In the first set (Figure 2a), the leaves were scanned with all their corresponding leaflets attached. In the second set of images (Figure 2b), the leaflets were detached and individually placed in the scanner for imaging, while recording the position of each leaflet within the leaf, from the apex to the base, and from left to right. Each image included a size reference and a QR code for image identification. Images were imported into R, thresholded, and converted into binary images using EBImage (Pau et al., 2010). Then, individual leaflets were selected and contours (outlines) were generated for downstream analysis with the R package Momocs (Bonhomme et al., 2014), as in (Chitwood et al., 2014). For each individual leaflet, several basic descriptors were computed, including area, perimeter, length, width, aspect ratio,

convexity, circularity, and color. Additionally, normalized elliptical Fourier descriptors based on 10 harmonics were calculated for each leaflet (Bonhomme et al., 2014). Because of the lack of common landmarks across accessions, the only variable computed for complete leaves (with attached leaflets, Figure 2a) was total leaf area.

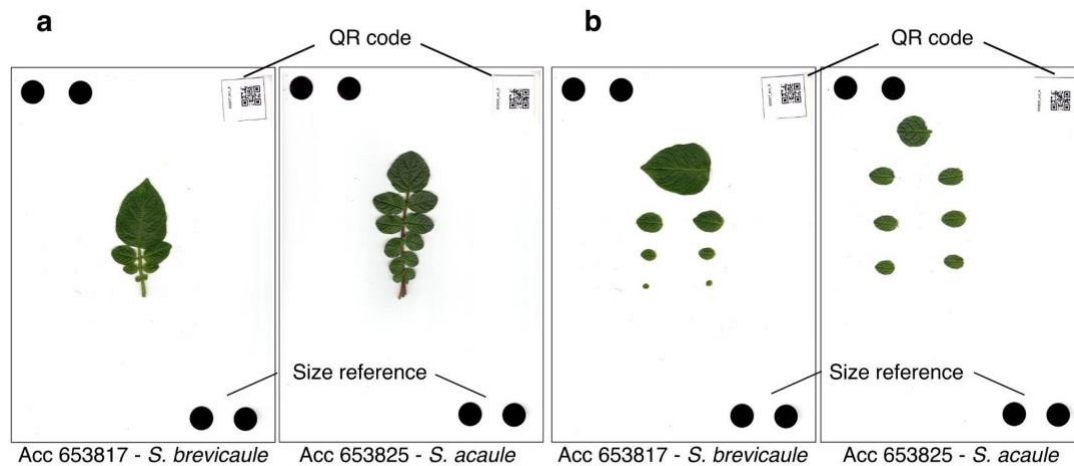


Fig. 2 Examples of the two sets of images produced in this study. (a) Complete leaves with attached leaflets. (b) Individual leaflets detached and imaged individually

Data visualization and statistical analysis

Leaflet contours were plotted using Momocs (Bonhomme et al., 2014) and visually inspected for errors. Incomplete leaves due to mechanical or insect damage, or errors during image segmentation were removed from the analysis. Correlations among leaf phenotypes were calculated using Pearson's correlations. All basic statistical functions were performed in R and visualized in the package ggplot2 (Wickham, 2009). Analysis of variance and Kruskal-Wallis tests were conducted for normally and non-normally distributed traits, respectively.

RESULTS

Leaf size and shape exhibit discernible patterns

More than 7500 leaflets sampled from 1129 leaves and 162 *Solanum* accessions were analyzed in this study. Leaf shape variation was surveyed in 13 *Solanum* species, more than in any other study before. This comprehensive study revealed large variation in leaf size and architecture, which arised from major differences in their corresponding leaflets.

For instance, leaflet size and shape varied as a function of its position within the leaf (Figure 3), which is further described below.

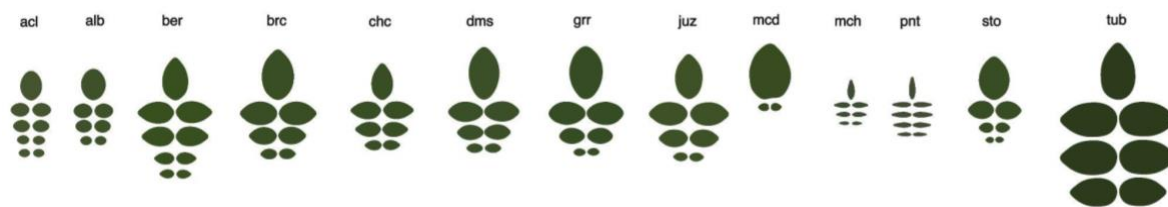


Fig. 3 Digital representation of leaf variation in 13 *Solanum* species. Each leaflet shape was generated by averaging leaflet shapes at similar leaflet positions within the leaf. Leaflets are colored according to the average color estimated from scanned images

Leaf size was determined using two different approaches. Firstly, leaf size was calculated from the images containing complete leaves (with their leaflets attached), thereby representing the true leaf area. Secondly, an alternative estimation was derived by aggregating the areas of individually scanned detached leaflets (Figure 4a). As expected, outcomes from both approaches exhibited a robust positive correlation ($r^2=0.95$, $p\text{-value}=2.2e-16$). Nevertheless, leaf area calculated from intact leaves, on average, surpassed the cumulative area of all leaflets by 11.1%. This difference can be attributed to the presence of small interjected leaflets located between the sampled leaflets and the petiole. Overall, discrepancies between leaf area (using complete leaves) and area derived from leaflet area summation ranged from 0.9% (no interjected leaflets, reduced petiole), to 18%. When comparing leaf sizes (using the cumulative leaflet areas) among different accessions, a twofold difference was observed. For example, alpha (*S. tuberosum*) exhibited the largest leaf size (221.08 cm²), followed by accessions 161367 (99.31 cm², *S. demissum*) and 161179 (98.16 cm², *S. demissum*). In contrast, the accessions 653799 (4.97 cm², *S. michoacanum*), 230489 (6.53 cm², *S. pinnatisectum*), and 275230 (7.97 cm², *S. pinnatisectum*) had relatively smaller leaf sizes.

The distribution of leaf area within the leaflets exhibited discernible patterns. Terminal leaflets consistently displayed larger sizes across all species, with accession 631212 (*S. microdontum*) having the largest terminal leaflet size (96.11 cm²), and accession 653799 (*S. michoacanum*), with a 1.15 cm² terminal leaf. Additionally, leaflet size decreased

gradually as a function of their position along the petiole, which was expected as apical leaflets emerge and develop first.

By discerning the specific position of each leaflet along the petiole, other descriptive variables were calculated. For example, the ratio of terminal leaflet size to non-terminal leaflet size was highly variable across accessions (Figure 4b). Species like *S. tuberosum* and *S. pinnatisectum* had higher ratios (0.84-0.73), indicating that lateral leaflets were approximately three quarters the size of the terminal leaflet. On the other hand, species like *S. microdontum* and *S. albicans* had a ratio closer to 0, indicating significantly larger terminal leaflets compared to lateral leaflets. Overall, these findings collectively underscore the influence of leaflet position on size distribution, with the apical locations predominantly exhibiting larger leaflets.

Leaf shape variation

Leaflet shape also exhibited variation depending on leaflet position. Two distinct patterns were observed based on the aspect ratio of the leaflets (Figure 4c). In certain species like *S. acaule*, *S. albicans*, *S. berthaultii*, and *S. tuberosum*, an asymptotic-like relationship was observed between aspect ratio and leaflet position. These species displayed larger aspect ratios (indicating more elongated leaflets) in their second and third leaflet pairs, while the aspect ratios were smaller for the first and fourth leaflet pairs, indicating more rounded leaflets. Conversely, species such as *S. michoacanum* and *S. pinnatisectum* consistently showed an increase in aspect ratio as the leaflet position progressed from basal to apical positions. Unlike the species with the asymptotic relationship, *S. michoacanum* and *S. pinnatisectum* exhibited considerably larger aspect ratios ranging between two and four. The circularity of the leaflets followed a similar pattern, as it was highly correlated with the aspect ratio ($r^2=0.91$).

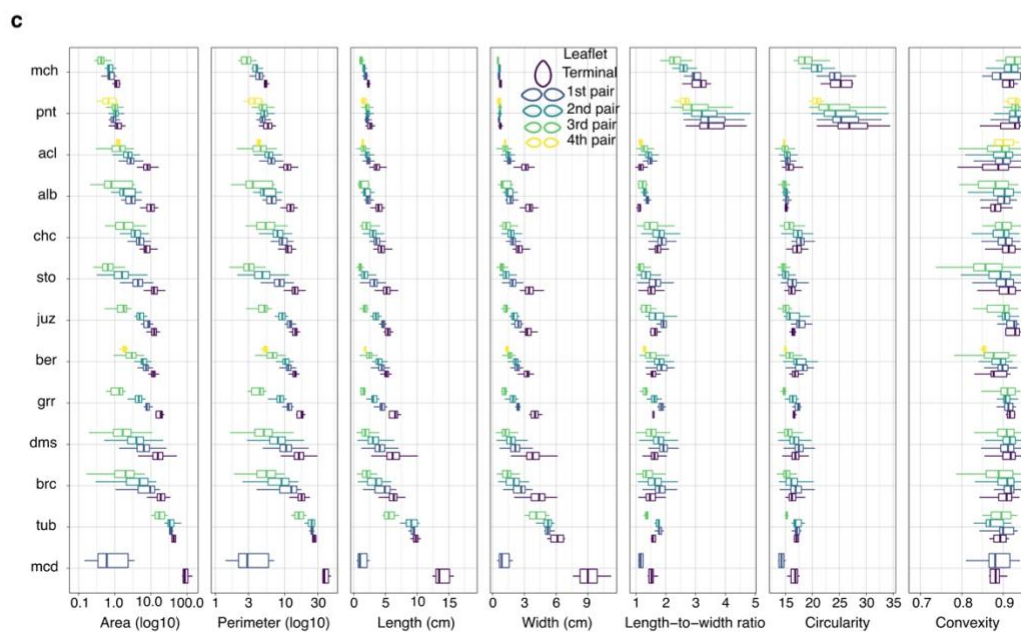
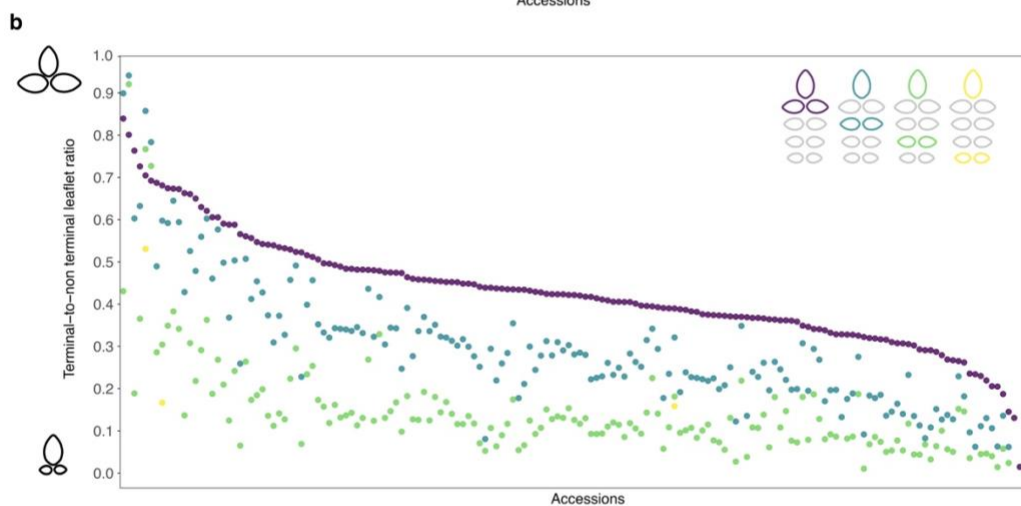
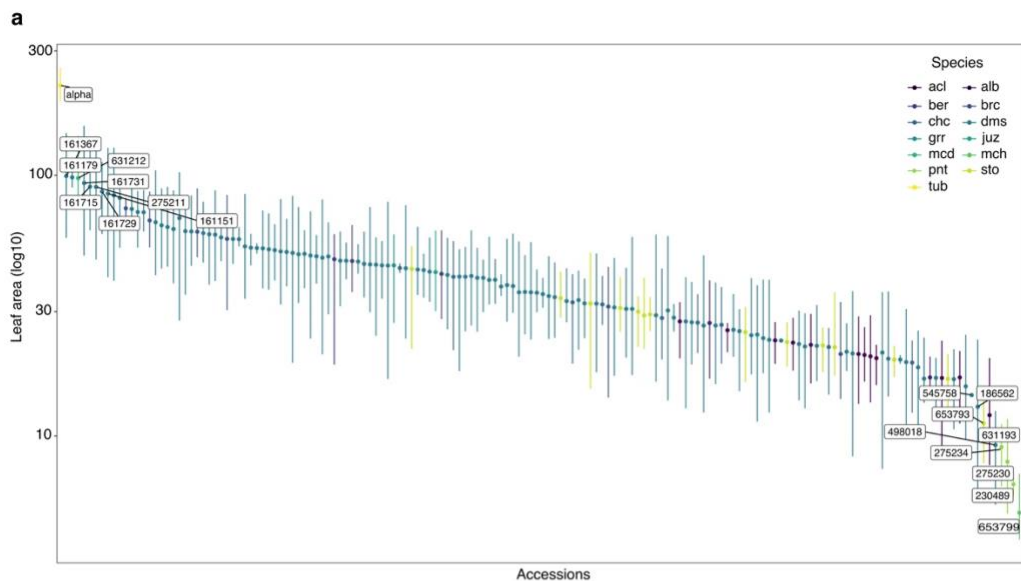


Fig. 4 Leaflet size and shape shows great variability across wild potato accessions. Variation in (a) leaf area and (b) the ratio of terminal leaflet to non-terminal leaflet. (c) Species variability in leaflet area, perimeter, length, width, aspect ratio, convexity and circularity

Correlation among traits

Size-related descriptors including area, perimeter, length, and width exhibited a strong positive correlation ($r^2=0.92$ to 0.99). Among the three basic shape descriptors, circularity and aspect ratio showed a high positive correlation, while convexity exhibited no significant correlation with any of the traits. In general, no correlation was observed between leaflet size and shape (Figure 5a). However, upon conducting a more in-depth exploration of trait correlations across various species, distinct patterns of species-specific significant correlations came to light (Figure 5b). For instance, although leaflet circularity and leaflet area showed poor correlation when considering all species together, they exhibited a medium to high negative correlation ($r^2=0.80$ to 0.18) specifically within the *S. microdontum* species. Similarly, while convexity showed little correlation with other traits overall, it displayed a medium negative correlation ($r^2=-0.32$ to -0.36) with traits such as width, aspect ratio, perimeter, and length specifically in the *S. tuberosum* species. Conversely, the opposite trend was also observed, with high correlations among all species except for specific ones. For example, width and length exhibited a medium correlation ($r^2=0.50, 0.60$) in the *S. pinnatisectum* and *S. tuberosum* species, while for the rest of the species, the correlation between these traits was larger than 0.8 (average $r^2=0.93$). These findings reveal the complexity and high variability of *Solanum* leaf size and shape across different species, with leaflet properties playing a major role in determining these characteristics.

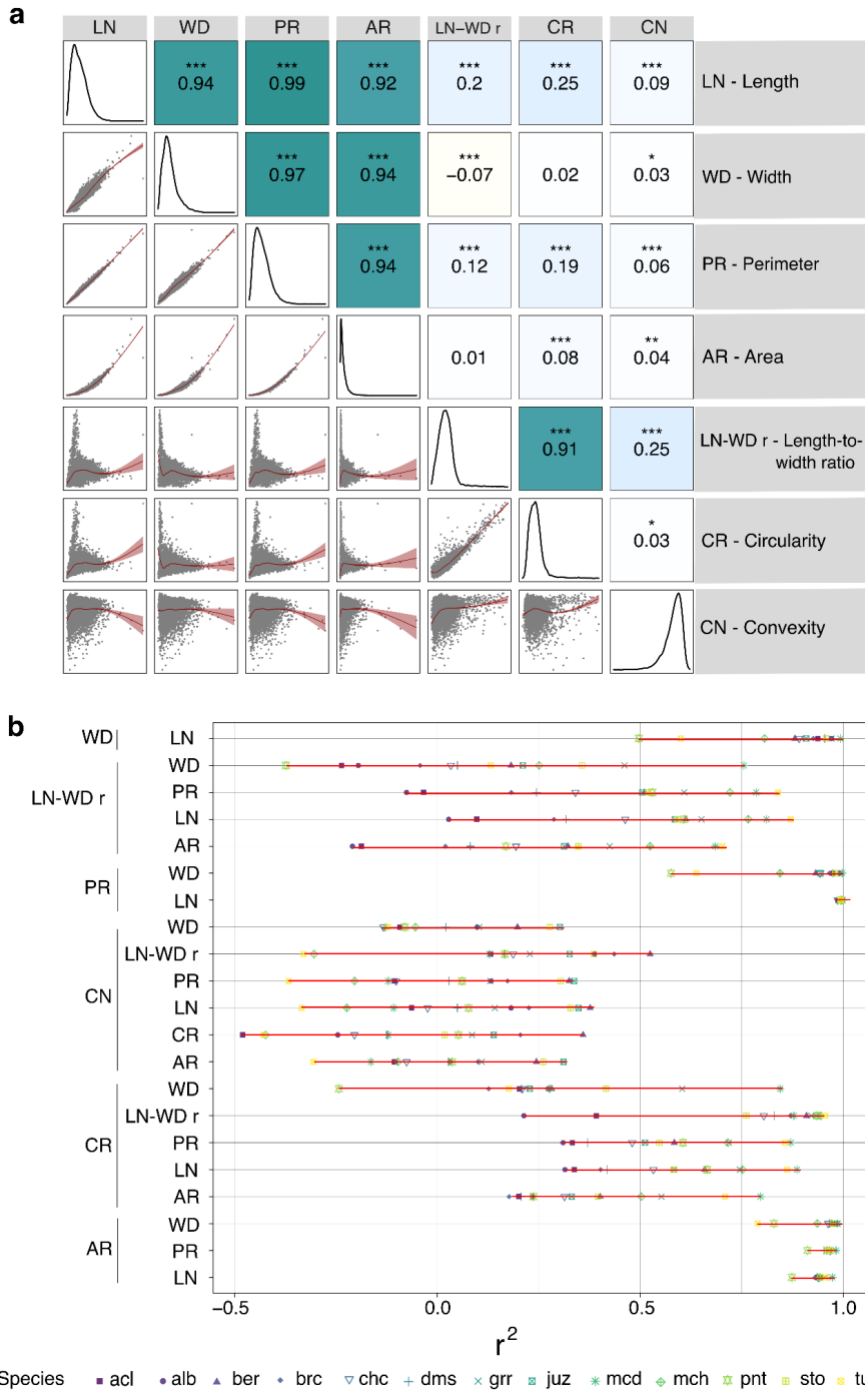


Fig. 5 Correlation among shape and size traits. (a) Pearson's correlation using non-averaged leaflet values. (b) Species-specific variation in trait correlations. Different marker symbols/colors correspond to different species; red lines highlight the extend of the variation in the correlation coefficients for each pair of traits

Elliptical Fourier descriptors

Compared with basic shape descriptors such as aspect ratio, circularity, and convexity, the Fourier analysis allows the description of global and local features to quantify variance among different shapes (Chitwood et al., 2013, 2014). This approach extracts the information embedded in the object outlines (leaflets) into a weighted sum of wave functions with varying frequencies. Then, the Fourier coefficients determine the contribution of each waveform to the shape under inspection. For instance, lower-order harmonics provide insights into the overall shape, while higher-order harmonics capture local variations in the outlines (Iwata et al., 1998). One notable advantage of the EFD-based methods is its ability to separate and independently analyze symmetric and asymmetric variances in shape, which is a common feature in biological systems

Leaflet shape variation was analyzed using symmetrical and asymmetrical principal components, which allowed for a better understanding of the results from the elliptical Fourier descriptor analysis (Figure 6). The first three principal components (PC) collectively accounted for 96.2% of the symmetric variance in leaflet variation, while the first three asymmetric components explained only 89.9%. Particularly, PC1 accounted for 90% of the symmetric variance, but only 82.3% of the asymmetric variance.

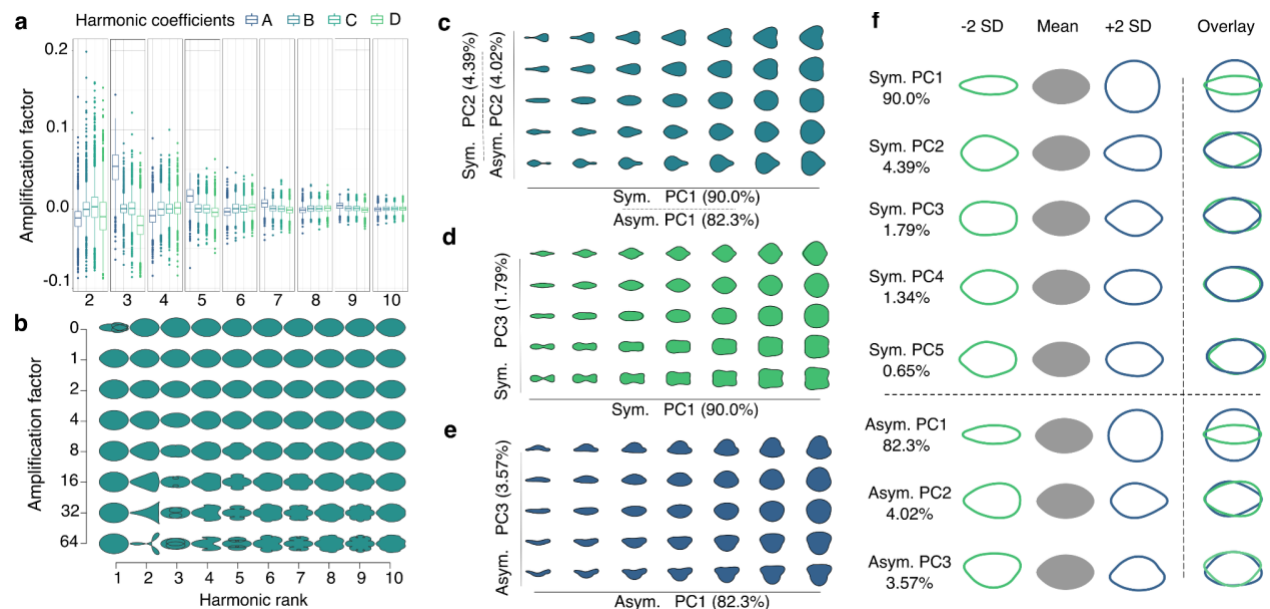


Fig. 6 Elliptical Fourier Descriptors. (a) Variation of harmonic coefficients. (b) Harmonic contributions to shape resulting from EFD analysis. Principal components (c) PC1 and PC2, (d, e) PC1 and PC3. (f) PCs explaining variance in leaflet shape

Analysis of full leaves

Significant variation in both leaflet size and shape was observed among the accessions examined in this study. Leaflet position and the allocation of leaf area among different leaflet positions (Figure 4) were identified as major sources of variation. However, complete leaves, as a whole, also exhibited significant variation, not only in size as discussed above, but in structure (i.e. number of leaflets) and shape. For instance, different species and accessions displayed varying numbers of leaflets. Some had only one pair of lateral leaflets (*S. microdontum*), while others had three (*S. albicans*, *S. brevicaule*, *S. chacoense*, *S. demissum*, *S. guerreroense*, *S. juzepczukii*, *S. michoacanum*, *S. stoloniferum*, *S. tuberosum*), or the maximum observed, four (*S. acaule*, *S. berthaultii*, *S. pinnatisectum*). Interjected leaflets at different leaflet positions were also observed in certain accessions. Additionally, petiole size varied across species, and in some cases (e.g., *S. tuberosum*), accounted for a significant portion of the total leaf area.

Comparative analysis of morphometric phenotypes in multifoliate leaves is challenging because of the absence of clearly defined homologous points (e.g., landmarks) across different leaf structures (Li et al., 2018a). Therefore, techniques like persistent homology have been used to quantitatively account for shape variation in complex traits, providing a promising avenue for analysis (Diaz-Garcia et al., 2018; Li et al., 2019; Schlautman et al., 2020). In this study, the set of images containing complete leaves (with attached leaflets, Figure 2a) was subjected to persistent homology analysis. A shape barcode consisting of 800 values was generated for each leaf, and principal component analysis was performed on the entire dataset, considering the barcodes of all the leaves. The first three principal components derived from persistent homology explained 44, 22, and 6% of the variance in leaf shape, respectively. Seventeen principal components from persistent homology explained 90% of the shape variance, indicating the significance of local and accession-specific shape features. Two analyses were conducted with the persistent homology data. First, a correlation analysis was performed between the principal components derived from persistent homology (using complete leaves) and those obtained from the elliptical Fourier descriptor analysis (using leaflets). Interestingly, a strong correlation was observed between the first principal components of persistent homology and EFD, likely suggesting that persistent homology applied to complete leaves

is sensitive to shape variation in individual leaflets. Second, by plotting principal components 1 and 2 of persistent homology, the accessions were partially grouped according to their assigned species. For example, all three *S. pinnatisectum* accessions formed a distinct group along principal component 1, while the *S. berthaultii* accessions also formed a well-defined group. However, species with a larger number of sampled accessions, such as *S. demissum* and *S. acaule*, showed more scattered grouping, indicating pronounced intraspecies leaf shape variation.

DISCUSSION

The leaf holds vital importance within plants, orchestrating essential physiological processes like photosynthesis, respiration, and transpiration. Both size and shape wield a profound impact on photosynthetic efficiency, intimately intertwined with agronomic productivity (e.g. yield) and tolerance to biotic and abiotic stressors (Nicotra et al., 2011; Wang et al., 2021; Zhang et al., 2021). Moreover, leaves exhibit many heritable characteristics, including size and shape (Karamat et al., 2021), color (Sarker and Oba, 2020), anatomy (Feldman et al., 2017; Radenović et al., 2019), and spectral profile (Radenović et al., 2019), among others. In this sense, leaves are highly informative organs with great potential for taxonomic classification and germplasm curation. Characterizing leaf morphometry in wild potato species not only reveals the existing variability among them but also offers a comprehensive overview of the adaptability and functionality each species can exhibit within specific environments, and enhances our understanding of how plants interact with their surroundings. Comprehensive leaf morphometrics also holds significant implications for the conservation and classification of genetic resources, prebreeding, and sustainable agriculture. Finally, by understanding how leaf size and shape influence a plant's capacity to carry out essential processes, it becomes possible to make more informed decisions regarding crop management and optimization.

Size and shape in adaptation

The role of leaf size in plant adaptation to their environment and growth conditions has been extensively documented. It directly influences physiological functions, including light capture, thermoregulation, water absorption, and transpiration, while also interacting with

surrounding organisms (Niinemets et al., 2006; Pickup et al., 2005; Sarlikioti et al., 2011; Westoby et al., 2002). This feature is crucial for survival in diverse habitats and for addressing specific challenges. Concerning leaf boundary layer thickness, particularly in terms of heat exchange, heat transfer diminishes with increasing leaf size and dissection. Larger leaves develop thicker boundary layers, making them more susceptible to overheating compared to smaller leaves, resulting in higher respiratory and transpiration costs (Sattler, 1978; Wright et al., 2006). Smaller leaves, as seen in species like *S. michoacanum* and *S. pinnatisectum*, prove advantageous in warm, dry habitats with high solar radiation. Conversely, larger leaves, such as those in species like *S. berthaultii*, *S. demissum*, *S. guerreroense*, *S. juzepczukii*, and *S. tuberosum*, excel in environments with lower irradiation, cooler temperatures, and higher humidity (Ackerly et al., 2002; Falster and Westoby, 2003; Givnish and Vermeij, 1976; Gates, 1965; Niinemets et al., 2006). Additionally, a positive relationship between leaf thickness and leaf biomass is observed with increasing altitude, as evidenced in *Fritillaria przewalskii* (Ma et al., 2020). The influence of leaf shape and size on plant development and biomass can also impact plant fitness and stress responses as well (Karamat et al., 2021). When plants are exposed to concurrent biotic and abiotic stressors, such as drought, there is a pronounced inhibition of aboveground biomass, disruption of metabolic processes, nutrient assimilation imbalance, and delayed root growth (Nawaz et al., 2023).

The variation in leaf size and shape is often considered a product of physiological and biomechanical demands imposed by different habitats. However, biotic stresses, for example, herbivore insects, have also played a significant role in the evolution of leaf size and shape, particularly in the context of physical barriers that prevent herbivory (Bright and Rausher, 2008; Brown and Lawton, 1991; Ferris, 2019). Deeply divided compound leaves, as seen in species like *S. michoacanum* and *S. pinnatisectum*, can reduce insect feeding efficiency, leading herbivores to prefer plants with simple or less divided compound leaves, such as *S. tuberosum* and *S. microdontum* (Bright and Rausher, 2008; Brown and Lawton, 1991; Ferris, 2019). At the same time, leaf size and shape impact plant choice by insects, revealing sensitivity to leaf morphology in many cases. Both psyllids (Diptera) and butterflies (Lepidoptera) are attracted to leaves with specific shapes (Prokopy and Owens, 1983). For instance, adult *Pegomya nigritarsis* flies (Diptera) adjust

egg quantities based on host leaf size, although they might rely more on chemical signals than visual cues (Brown and Lawton, 1991). Aphids (Homoptera) also respond to size; adult females of *Pemphigus betae* show a preference for larger leaves (Brown and Lawton, 1991; Dell'Aglio et al, 2016). These interactions between leaf morphology and insect choice underscore the importance of leaf characteristics in plant ecology and evolution. On the other hand, it has been observed that leaf size and shape also affect the individual performance of insects. The movement, mating, oviposition, feeding, and shelter of herbivores and their adversaries can be influenced by leaf design (Brown and Lawton, 1991). Surface properties of the leaf, such as roughness, wax presence, and pubescence, affect insects' ability to cling, move, and feed (Strong et al., 1984). For example, female *Lithocolletis quercus* moths (Lepidoptera) tend to lay eggs on larger leaves. In contrast, females of *Perga affinis* (Hymenoptera: Symphyta) cannot deposit eggs on hosts with broad leaves, as they need to grip the leaf edges during oviposition. Lepidopteran caterpillars may encounter difficulties maneuvering and feeding on the small and often scarce leaflets of African acacias' pinnate leaves (Brown and Lawton, 1991). Moreover, larvae of various moth species can discern differences between basal, lateral, and terminal leaves of their compound leaf food plants. Basal leaflets are less palatable than lateral or terminal leaflets, and this relative aversion to basal leaflets may serve as a defense against phytophagous insects in compound-leaved plants (Gall, 1987).

Comprehensive phenotyping of wild species

Germplasm banks play an essential role in the conservation and characterization of plant biodiversity, particularly when dealing with wild species (Smale and Jamora, 2020). These institutions are dedicated to the collection and preservation of plant materials with the aim of maintaining the genetic diversity of plants (Singh et al., 2019). The conservation of wild species in germplasm banks allows for their study in crop improvement, as these species may carry genes resistant to diseases, tolerance to extreme environmental conditions, and other desirable traits that can be crucial in creating more resilient and sustainable crops (Smale and Jamora, 2020; Wang et al., 2017).

Genotypic and phenotypic characterization of the materials stored in germplasm banks provides valuable information that not only allows for more efficient resource utilization but also contributes to the identification, classification, and selection of materials based on their characteristics (Smale and Jamora, 2020; Thormann et al., 2012). Genotyping enables the identification of specific genetic variants within the species. However, it is not always sufficient to understand the phenotypic adaptations of these plants to different environments, and it does not provide direct information on how these genes manifest in visible traits, which can be measured through phenotyping. In this regard, phenotypic characterization is as important as genotypic characterization, as it allows for the description and establishment of relationships between cultivar groups and accessions, as well as the identification of promising materials for germplasm improvement and conservation (Muli et al., 2021; Pereira-Dias et al., 2020; Plazas et al., 2014).

Plant phenotyping is essential to understand how wild species adapt to their environment. While traditional phenotyping relies on manual observation and measurement, high-throughput phenotyping uses advanced technologies such as image analysis and morphometrics to efficiently measure multiple traits on a large scale, enabling comprehensive characterization of wild species, revealing complex traits and precise adaptations (Diaz-Garcia et al., 2016; Wang et al., 2017). This is especially valuable when searching for specific traits crucial for crop adaptation. Moreover, these high-throughput techniques are fundamental in creating collections that more accurately and efficiently represent genetic diversity (Schlautman et al., 2020).

Historically, wild relatives of potatoes have been a significant source of genes in cultivated potato breeding programs (Zoteyeva et al., 2012). However, the study of the large collections in germplasm banks has been limited due to the constraints of traditional phenotyping. Implementing high-throughput phenotyping in these collections would provide greater opportunities for characterization (Wang et al., 2017).

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AUTHOR CONTRIBUTIONS

GDG and LDG contributed to the conception and design of the study; JB provided the plant material; GDG and HLS set up the field material and collected the data. Data analysis, validation, and visualization were carried out by GDG and LDG. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTAL MATERIAL

Table S1. List of accessions used in this study. PI Number: US National Plant Germoplasm System "plant introduction number".

Accession	PI	
Number	Collection	Species
631192	VIR 9786	<i>Solanum acaule</i> Bitter
631193	VIR 17901	<i>Solanum acaule</i> Bitter
631195	VIR 18004	<i>Solanum acaule</i> Bitter
631218	VIR 4114	<i>Solanum acaule</i> Bitter
653786	VIR 04250	<i>Solanum acaule</i> Bitter
653787	VIR 04253	<i>Solanum acaule</i> Bitter
653788	VIR 04263	<i>Solanum acaule</i> Bitter
653812	VIR 09784	<i>Solanum acaule</i> Bitter
653825	VIR 18010	<i>Solanum acaule</i> Bitter
653826	VIR 18014	<i>Solanum acaule</i> Bitter
653827	VIR 18021	<i>Solanum acaule</i> Bitter
658586	VIR 18522	<i>Solanum acaule</i> Bitter
631198	VIR 9814	<i>Solanum albicans</i> Ochoa
631199	VIR 9813	<i>Solanum albicans</i> Ochoa

653801	VIR 07635	<i>Solanum berthaultii</i> Hawkes
653802	VIR 07642	<i>Solanum berthaultii</i> Hawkes
653829	VIR 23047	<i>Solanum berthaultii</i> Hawkes
473378	HHCH 4498	<i>Solanum brevicaule</i> Bitter
631209	VIR 7370	<i>Solanum brevicaule</i> Bitter
631210	VIR 7381	<i>Solanum brevicaule</i> Bitter
653800	VIR 05764	<i>Solanum brevicaule</i> Bitter
653817	VIR 09798	<i>Solanum brevicaule</i> Bitter
653818	VIR 09808	<i>Solanum brevicaule</i> Bitter
631200	VIR 2733	<i>Solanum chacoense</i> Bitter
631202	VIR 7610	<i>Solanum chacoense</i> Bitter
631203	VIR 7613	<i>Solanum chacoense</i> Bitter
631207	VIR 3701	<i>Solanum chacoense</i> Bitter
653782	VIR 03678	<i>Solanum chacoense</i> Bitter
653783	VIR 03702	<i>Solanum chacoense</i> Bitter
653785	VIR 04236	<i>Solanum chacoense</i> Bitter
653792	VIR 05257	<i>Solanum chacoense</i> Bitter
653822	VIR 15994	<i>Solanum chacoense</i> Bitter
160220	COR 14199	<i>Solanum demissum</i> Lindl.
160221	COR 14200	<i>Solanum demissum</i> Lindl.
160227	COR 14215	<i>Solanum demissum</i> Lindl.
160230	COR 14220	<i>Solanum demissum</i> Lindl.
161149	COR 14203	<i>Solanum demissum</i> Lindl.
161151	COR 14209B	<i>Solanum demissum</i> Lindl.
161164	COR 14237	<i>Solanum demissum</i> Lindl.
161165	COR 14238	<i>Solanum demissum</i> Lindl.
161166	COR 14239	<i>Solanum demissum</i> Lindl.
161167	COR 14240	<i>Solanum demissum</i> Lindl.
161168	COR 14243	<i>Solanum demissum</i> Lindl.
161169	COR 14244	<i>Solanum demissum</i> Lindl.
161175	COR 14259	<i>Solanum demissum</i> Lindl.

161176	COR 14260	<i>Solanum demissum</i> Lindl.
161179	COR 14265	<i>Solanum demissum</i> Lindl.
161180	COR 14266	<i>Solanum demissum</i> Lindl.
161181	COR 14267	<i>Solanum demissum</i> Lindl.
161365	COR 14274	<i>Solanum demissum</i> Lindl.
161366	COR 14283	<i>Solanum demissum</i> Lindl.
161367	COR 14286	<i>Solanum demissum</i> Lindl.
161686	COR 14435	<i>Solanum demissum</i> Lindl.
161693	COR 14319	<i>Solanum demissum</i> Lindl.
161715	COR 14379	<i>Solanum demissum</i> Lindl.
161719	COR 14413	<i>Solanum demissum</i> Lindl.
161729	COR 14378	<i>Solanum demissum</i> Lindl.
161731	COR 14411	<i>Solanum demissum</i> Lindl.
175403	EBS 17/1	<i>Solanum demissum</i> Lindl.
175404	EBS 17/28	<i>Solanum demissum</i> Lindl.
175408	EBS 46/33	<i>Solanum demissum</i> Lindl.
175409	EBS 46/34	<i>Solanum demissum</i> Lindl.
175411	EBS 14/144	<i>Solanum demissum</i> Lindl.
175423	EBS 48B/36	<i>Solanum demissum</i> Lindl.
186551	HAW 1077	<i>Solanum demissum</i> Lindl.
186552	HAW 1079	<i>Solanum demissum</i> Lindl.
186561	HAW 1117	<i>Solanum demissum</i> Lindl.
186562	HAW 1118	<i>Solanum demissum</i> Lindl.
195165	CPC 43.2	<i>Solanum demissum</i> Lindl.
201853	BEL DEM 52	<i>Solanum demissum</i> Lindl.
201854	BEL DEM 55	<i>Solanum demissum</i> Lindl.
205515	CPC 14.2	<i>Solanum demissum</i> Lindl.
205516	CPC 14.3	<i>Solanum demissum</i> Lindl.
205518	CPC 1363.1	<i>Solanum demissum</i> Lindl.
205519	CPC 1364.3	<i>Solanum demissum</i> Lindl.
205625	CPC 2127	<i>Solanum demissum</i> Lindl.

218047	BB s.n.	<i>Solanum demissum</i> Lindl.
225652	CCC 606	<i>Solanum demissum</i> Lindl.
225653	CCC 620A	<i>Solanum demissum</i> Lindl.
230558	EBS 18	<i>Solanum demissum</i> Lindl.
230559	EBS 44	<i>Solanum demissum</i> Lindl.
230578	SPB s.n.	<i>Solanum demissum</i> Lindl.
230579	SPB s.n.	<i>Solanum demissum</i> Lindl.
230589	RDD 178	<i>Solanum demissum</i> Lindl.
230591	RDD 696	<i>Solanum demissum</i> Lindl.
230592	RDD 886	<i>Solanum demissum</i> Lindl.
275206	HAW 1295	<i>Solanum demissum</i> Lindl.
275208	HAW 1601	<i>Solanum demissum</i> Lindl.
275209	HAW 1657	<i>Solanum demissum</i> Lindl.
275210	HAW 1608	<i>Solanum demissum</i> Lindl.
275211	HAW 1782	<i>Solanum demissum</i> Lindl.
310961	UGN 1514	<i>Solanum demissum</i> Lindl.
310962	UGN 2448	<i>Solanum demissum</i> Lindl.
338618	ROW 1	<i>Solanum demissum</i> Lindl.
338619	ROW 2	<i>Solanum demissum</i> Lindl.
347760	TRN 64	<i>Solanum demissum</i> Lindl.
347761	TRN 88	<i>Solanum demissum</i> Lindl.
347762	TRN 142C	<i>Solanum demissum</i> Lindl.
365381	TRN 7A	<i>Solanum demissum</i> Lindl.
365387	TRN 15D	<i>Solanum demissum</i> Lindl.
473520	EBS 2624	<i>Solanum demissum</i> Lindl.
498012	TRHRG 123	<i>Solanum demissum</i> Lindl.
498013	TRHRG 150	<i>Solanum demissum</i> Lindl.
498014	TRHRG 167	<i>Solanum demissum</i> Lindl.
498015	TRHRG 186	<i>Solanum demissum</i> Lindl.
498016	TRHRG 192	<i>Solanum demissum</i> Lindl.
498017	TRHRG 194	<i>Solanum demissum</i> Lindl.

498018	TRHRG 196	<i>Solanum demissum</i> Lindl.
498229	OCH 14154	<i>Solanum demissum</i> Lindl.
498230	OCH 14155	<i>Solanum demissum</i> Lindl.
498231	OCH 14156	<i>Solanum demissum</i> Lindl.
498233	OCH 14217	<i>Solanum demissum</i> Lindl.
498299	HAW 1134	<i>Solanum demissum</i> Lindl.
545754	TRHRG 198	<i>Solanum demissum</i> Lindl.
545756	TRHRG 203	<i>Solanum demissum</i> Lindl.
545758	TRHRG 204C	<i>Solanum demissum</i> Lindl.
545765	TRHRG 294	<i>Solanum demissum</i> Lindl.
545767	TRHRG 304	<i>Solanum demissum</i> Lindl.
558051	HJT 7259	<i>Solanum demissum</i> Lindl.
558389	SHGRF 4261	<i>Solanum demissum</i> Lindl.
558390	SHGRF 4045	<i>Solanum demissum</i> Lindl.
558391	SHGRF 4062	<i>Solanum demissum</i> Lindl.
558392	SHGRF 4064	<i>Solanum demissum</i> Lindl.
558464	SHGRF 4048	<i>Solanum demissum</i> Lindl.
558482	SHGRF 4019	<i>Solanum demissum</i> Lindl.
607841	RSSV 920	<i>Solanum demissum</i> Lindl.
607849	RSSV 940	<i>Solanum demissum</i> Lindl.
607869	RSSV 975	<i>Solanum demissum</i> Lindl.
607873	RSSV 984	<i>Solanum demissum</i> Lindl.
653762	VIR 02353	<i>Solanum demissum</i> Lindl.
653770	VIR 03287	<i>Solanum demissum</i> Lindl.
653772	VIR 03341	<i>Solanum demissum</i> Lindl.
653773	VIR 03342	<i>Solanum demissum</i> Lindl.
653774	VIR 03355	<i>Solanum demissum</i> Lindl.
653776	VIR 03362	<i>Solanum demissum</i> Lindl.
653779	VIR 03540	<i>Solanum demissum</i> Lindl.
653789	VIR 04445	<i>Solanum demissum</i> Lindl.
653803	VIR 08446	<i>Solanum demissum</i> Lindl.

653804	VIR 08462	<i>Solanum demissum</i> Lindl.
653805	VIR 08466	<i>Solanum demissum</i> Lindl.
653828	VIR 18407	<i>Solanum guerreroense</i> Correll
631201	VIR 9742	<i>Solanum juzepczukii</i> Bukasov
		<i>Solanum michoacanum</i> (Bitter)
653799	VIR 05763	Rydb.
631212	VIR 5684	<i>Solanum microdontum</i> Bitter
230489	ROC S-44	<i>Solanum pinnatisectum</i> Dunal
275230	HAW 1424	<i>Solanum pinnatisectum</i> Dunal
275234	HAW 1456	<i>Solanum pinnatisectum</i> Dunal
631206	VIR 8505	<i>Solanum stoloniferum</i> Schltdl.
631213	VIR 8416	<i>Solanum stoloniferum</i> Schltdl.
631214	VIR 8475	<i>Solanum stoloniferum</i> Schltdl.
631215	VIR 2492	<i>Solanum stoloniferum</i> Schltdl.
631216	VIR 4226	<i>Solanum stoloniferum</i> Schltdl.
653763	VIR 02534	<i>Solanum stoloniferum</i> Schltdl.
653764	VIR 02536	<i>Solanum stoloniferum</i> Schltdl.
653771	VIR 03326	<i>Solanum stoloniferum</i> Schltdl.
653777	VIR 03527	<i>Solanum stoloniferum</i> Schltdl.
653778	VIR 03533	<i>Solanum stoloniferum</i> Schltdl.
653780	VIR 03554	<i>Solanum stoloniferum</i> Schltdl.
653793	VIR 05347	<i>Solanum stoloniferum</i> Schltdl.
653794	VIR 05431	<i>Solanum stoloniferum</i> Schltdl.
653823	VIR 16888	<i>Solanum stoloniferum</i> Schltdl.
	commercial	
'Alfa'	cultivar	<i>Solanum tuberosum</i> L.

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**CAPÍTULO V. FIELD SCREENING OF *Solanum demissum*
CONFIRMS ITS LATE BLIGHT RESISTANCE IN THE
TOLUCA VALLEY, MEXICO**

Como se envió a la revista *American Journal of Potato Research*

FIELD SCREENING OF *Solanum demissum* CONFIRMS ITS LATE BLIGHT RESISTANCE IN THE TOLUCA VALLEY, MEXICO

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ABSTRACT

Late blight is a major threat to potato crops worldwide. Despite the host's genetic improvements, continuous evolution of the pathogen and climate change makes it necessary reconfirmation of genetic resistance sources. *Solanum demissum* has long been identified as a valuable source of late blight resistance genes. The aim of this study was to reevaluate *S. demissum* accessions for late blight resistance under natural infection in the Toluca Valley, México. Most of the accessions exhibited resistance, with minor variations between two consecutive growing seasons. Eighty three out of 94 accessions that were evaluated in both years were resistant, thirty-three accessions that were exposed to the pathogen for only one season were also resistant. However, a response variation was observed in some accessions. This research characterizes and reconfirms the resistance of *S. demissum*, providing information for breeding programs and optimizing the use of these genetic resources.

INTRODUCTION

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, remains one of the main threats to potato (*Solanum tuberosum* L.) crops worldwide (Akino et al., 2014). Despite advances in genetic improvement, the continuous evolution of pathogen strains and changes in environmental conditions have highlighted the need to continue searching for sources of genetic resistance. In this context, wild potato species have proven to be a valuable source of late blight resistance genes (Enciso-Maldonado et al., 2021).

In a recent review, Blossei et al. (2022) emphasize that there are accessions from 85 wild potato species, distributed from the southwestern United States to central Argentina and Chile, compiled from 74 studies published over the last four decades, with the potential to provide genetic resources for enhancing late blight resistance in potatoes. To date, more than 70 *Rpi* genes have been discovered and mapped in 32 *Solanum* species. Most of these genes come from tuber-bearing species, the most notable being those of Mexican origin (*S. bulbocastanum*, *S. demissum*, *S. edinense*, *S. hjertingii*, *S. michoacanum*, *S. pinnatisectum*, *S. schenckii*, *S. stoloniferum*, *S. verrucosum*), Bolivian

(*S. avilesii*, *S. berthaultii*, *S. capsicibaccatum*, *S. circaeifolium* ssp. *quimense*, *S. ruiz-ceballosii*, *S. tarijense*), Peruvian (*S. huancabambense*, *S. mochiquirense*, *S. paucissectum*, *S. piurae*), Argentinian (*S. microdontum*, *S. neorossii*, *S. venturii*), Paraguayan (*S. chacoense*), from USA (*S. jamesii*) and a species commonly found in the Andes region (*S. phureja*) (Paluchowska et al., 2022). However, not all species have the same ease of use for breeding, as wild potatoes exhibit different levels of ploidy, with the majority being diploids, and to a lesser extent, tetraploids and hexaploids (Haverkort et al., 2016). In some cases, there are species with triploid, pentaploid, or multiple ploidy levels. Furthermore, incompatibility between species is usually attributed to insufficient development of the endosperm, causing the death of the embryo within the seed. For the endosperm to develop properly, the specific proportion must be 2:1 of maternal: paternal factors (Johnston et al., 1980).

Solanum demissum Lindl. (section Petota, $2n = 6x = 72$) (Spooner and Hijmans, 2001) is a wild species harboring valuable late blight resistance genes (Paluchowska et al., 2022), and has been shown to be resistant to pest insects such as the Colorado potato beetle (*Leptinotarsa decemlineata*), the potato leafhopper (*Empoasca fabae*), potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, the bacterium *Candidatus Liberibacter solanacearum*, potato X virus (PVX), and potato Y virus (PVY), as well as abiotic factors such as frost (Díaz-García et al., 2023). It is widely distributed in Mexico and Guatemala, and its greatest abundance and diversity is found in Mexican territory in the Volcanic Axis and the Sierra Madre del Sur, mostly between latitudes 19° and 21° (Fig. 1), where the climate is temperate, sub-humid, with summer rains, from 800 to 900 mm per year, high relative humidity and thermal oscillation, from 12° C to 20° C (Hijmans et al., 2002; Luna-Cavazos et al., 2012; Díaz-García et al., 2023). The greatest genetic diversity of *P. infestans* is known to be found in Mexico, therefore, Mexican wild potatoes and the pathogen have co-evolved for millions of years in central Mexico and beyond. This coevolution has allowed *S. demissum* to develop mechanisms of resistance to late blight and, to date, it is known to carry 14 resistance genes to *P. infestans* (Rpi) (Paluchowska et al., 2022; Lozoya-Saldaña, 2005; Alfaro, 1995).

S. demissum is a species of great importance because for over a century it has been used in potato breeding programs worldwide to combat late blight (Turner, 2005). Being recognized as a naturally resistant species to late blight, it has been collected in various regions of Mexico and Guatemala (Rodríguez, 2015) and stored in germplasm banks worldwide (Ramsay and Bryan, 2011). Intensive research on potato late blight has led to the discovery of dominant resistance genes against *P. infestans* (*Rpi* genes), which have been introduced into potato cultivars (Paluchowska et al., 2022). However, due to the high genetic variability of *P. infestans*, these introduced *Rpi* genes from *S. demissum* were quickly overtaken by new virulent strains of the pathogen (Jo et al. 2014). The resistance of *S. demissum* to late blight can be either vertical or horizontal. Vertical resistance shows a high degree of resistance but to a few strains of the pathogen, mediated by one or a few R genes that are race-specific, exhibit Mendelian inheritance, and are easily incorporated to generate new resistant genotypes. In contrast, horizontal resistance is polygenic and nonspecific, providing protection to the plant against various strains of the pathogen (Enciso-Maldonado et al., 2021).

The importance of characterizing resistance to this disease lies in its potential to optimize the use of these phylogenetic resources and provide valuable information for future breeding programs (Smale and Jamora 2020). Previously, in 2015, DS Douches conducted a field evaluation of a collection of 158 accessions of *S. demissum* to determine their resistance category to late blight (data available on the USDA Germplasm Resources Information Network [GRIN] website: <https://www.ars-grin.gov/>). Subsequently, Enciso-Maldonado et al. (2022) assessed *S. demissum* accessions to identify late blight resistance under field conditions in the Toluca Valley. Also, del Rio and Bamberg (2020) characterized 149 accessions of *S. demissum* of diverse geographical origin with 1403 AFLP markers and found that all accessions were possible to distinguish individually with these markers, suggesting that the samples are not genetically diverse.

The Toluca Valley in central Mexico is known to be the primary center of diversity for *P. infestans*. In this location, the pathogen can undergo sexual reproduction due to the presence of both mating types, A1 and A2, in a 1:1 ratio, allowing for a high and complex genetic diversity of the pathogen. Additionally, a factor contributing to this complexity is

the presence of wild species of the *Solanum* genus. Outside the Toluca Valley, *P. infestans* spreads clonally to worldwide production regions, making it less challenging than in a location where it continuously recombines into new strains. Therefore, the Toluca Valley is a favorable site for studying the resistance of potato cultivars and wild relatives to late blight, as well as assessing the effectiveness of fungicide control. It is likely that potato genotypes resistant to late blight evaluated in this location will maintain their resistance in other regions (Flier et al., 2003; Grünwald and Flier, 2005; Lozoya-Saldaña, 2005; Wang et al., 2017; Enciso-Maldonado et al., 2022).

Since RN Salaman first discovered late blight resistance in *S. edinense* (a natural hybrid of *S. demissum* x *S. tuberosum*) (Akino et al., 2014) and crosses between *S. demissum* and *S. tuberosum* were initiated, expeditions to Mexico were launched in search of materials exhibiting resistance to late blight, the central trait of interest in this species (Reddick-Retires, 1951). Given that *S. demissum* has been the subject of study for the last 100 years, it is essential to determine whether there is variability in this property between different materials collected in various parts of the world. This approach is critical to maximizing the effectiveness of breeding efforts and ensuring that the potential of this species is fully realized in the fight against one of the most devastating potato diseases. The present study focuses on running field screenings of *S. demissum* accessions to evaluate their resistance to late blight in the Toluca Valley, Mexico.

METHODS

During two seasons, the resistance of *S. demissum* accessions from the US Potato Genebank (USPG) (Sturgeon Bay, Wisconsin, United States) was evaluated at the ICAMEX-SEDAGRO Experimental Station, located in the Toluca Valley, Mexico. One hundred and nineteen accessions were transplanted in 2020 and 108 in 2021; 94 accessions were evaluated in both seasons (Table 1). Some accessions were evaluated in only one year. The seeds were sown in 200-hole trays with a seedbed substrate based on peat moss C1 R8089 (Kekkilä Professional) and perlite in a 1:1 ratio on May 9, 2020, and May 1, 2021. The transplants were established under rainfed conditions and natural infection by *P. infestans*.

The experiment followed a completely randomized block design with three replicates for both years. Each experimental unit consisted of five seedlings. A susceptible control, Potato cv. Agata (*S. tuberosum*), was included. No fungicides or irrigation were applied throughout the experiment. Late blight was visually evaluated once a week using a late blight severity scale (Henfling 1987) based on the percentage of foliar area infected. To quantify disease progression, the area under disease progress curve (AUDPC) was calculated following the method of Shanner and Finney (1977). Since AUDPC values differed in terms of time, they were standardized (sAUDPC) by dividing each value by the time elapsed between the first and last disease observations. Plants were categorized as highly susceptible (HS), susceptible (S), moderately resistant (MR), or resistant (R) based on their Maximum Disease (Dmax) values, falling within the ranges of 85.1 to 100, 35.1 to 85, 15.1 to 35, and 0 to 15, respectively, as described by Deahl et al. (1974). Due the variables did not exhibit a normal distribution, they underwent non-parametric test and rank comparison tests using the *Agricolae* package in the R software (de Mendiburu, 2023), using a probability of error of 5 % ($\alpha = 0.05$).

RESULTS

The climatic conditions in both seasons were typical and ideal for the development of both the host plants and the pathogen in the field (Enciso-Maldonado et al., 2022; Lozoya-Saldaña 2005). In 2020, the average minimum and maximum temperatures were 6.0 and 29.0 °C, respectively, and precipitation was 638.7 mm, while in 2021, the average minimum and maximum temperatures were 0 and 28 °C, respectively, and precipitation was 761.9 mm (Fig. S2).

Accession/Year had a significant effect on maximum disease at the end (Dmax) and sAUDPC (Table 1). Infection initially occurred in the control cultivar on August 10, 2020 (39 DAP) and August 11, 2021 (40 DAP). In both years, severity ranged from 0 to 100%, depending on the genotype. The average maximum disease severity for all accessions was 6.6 % and 6.0 % in 2020 and 2021, respectively. The percentages of plants categorized in the different levels of resistance were similar in both seasons. In 2020, 92.4% of accessions were R; 1.7% were MR; 2.5% were S; and 3.4% were HS.

Meanwhile in 2021, 92.6 % of accessions were R; 3.7% were MR; 2.8% were S; and 0.9% were HS. The virulence of *P. infestans* was reflected in the cv. Agatha, which was HS in both years.

Most of the *S. demissum* accessions were categorized as R. Of the 94 accessions that were evaluated in both seasons, 83 accessions were resistant in both years. On the other hand, 33 accessions evaluated one year were also R. However, a response variation was observed in accessions 161719, 160220, 161367 and 161181, which were categorized as R in 2020 and as MR, S and HS in 2020, 2021, respectively. Whereas accessions 161168 and 275206 categorized as S in 2020 and R in 2021 and accessions 225653 and 607841 as HS in 2020 and R and MR in 2021, respectively. Accession 653789 categorized as MR in both years. Finally, accessions 160208, 161169, 225711, 545764 and 558051 were evaluated only in one year and resistance categories S, MR, HS, MR and HS, respectively.

DISCUSSION

Prior to this assessment, Douches conducted an evaluation with most of these accessions in a greenhouse, where he found 147 resistant accessions out of a total of 158 accessions studied (data published in GRIN website: <https://www.ars-grin.gov/>). However, we found some discrepancies with the results obtained by Douches. He reports accessions 160208, 161769, and 545761 as moderately resistant, while we found that these accessions were categorized as S, R, and R, respectively. Similarly, accessions 225653, 230591, 275211, and 545764 were categorized by Douches as S, while we found that 225653 was R in 2020 and S in 2021, 230591 and 275211 were R in both years, and 545764 was categorized as MR. For the other hand, this is the first time that a collection of *S. demissum* of this magnitude is being evaluated at this site to identify sources of resistance to late blight in Toluca Valley. Before this work, Enciso-Maldonado et al., (2022) evaluated 97 accessions from 14 potato species in the Toluca Valley for two seasons, where variability in intra- and interspecific resistance response to late blight was observed. However, these authors included only 11 accessions of *S. demissum*, all of which exhibited complete resistance in both seasons, except for accession 653789, which

changed to MR in the second season. This study recorded a similar behavior, where some accessions were classified as resistant in one season and then changed their category, and vice versa (Table 1). This could be attributed to changes in *P. infestans* populations in the environment, leading to different interactions between the plant and pathogen due to the involvement of different effectors that influence the host in various ways (Martynov and Chizhik, 2020). The change in *P. infestans* populations is attributed to the existence of the A1 and A2 mating types of the pathogen in a 1:1 ratio in the field, promoting genetic diversity through sexual reproduction of *P. infestans*. This phenomenon is widely prevalent in the central valleys of Mexico (Enciso-Maldonado et al., 2022; Shakya et al., 2018; Belmar-Díaz and Lozoya-Saldaña, 2013; Flier et al., 2003). On the other hand, the variation in response among accessions of *S. demissum* can be attributed to the fact that, being an allopolyploid species, it exhibits significantly greater heterozygosity than other Mexican diploid species. This reflects allelic diversity among its different wild subgenomes (Hardigan et al., 2015).

The results of this quantification allowed the identification of *S. demissum* accessions that exhibit complete resistance to late blight, as in other studies (Enciso-Maldonado et al., 2022; Zoteyeva et al. 2012). These accessions can be valuable for potato breeding programs, as their resistance genes can be transferred to cultivated potato varieties (*S. tuberosum*) to develop new cultivars resistant to this disease (Rogozina et al., 2021). The prospects include the development of more robust commercial varieties that are less dependent on chemical pesticides.

The characterization reveals the genetic diversity present in the *S. demissum* accessions, which can provide a solid foundation for further diversifying the genetics of cultivated potato varieties. Our work complements previous research that aims to classify the materials in germplasm banks (del Rio and Bamberg, 2020). In addition to characterizing the accessions based on their resistance levels, further efforts have been directed towards reclassifying them in germplasm banks. By adding information to the germoplasm bank database, researchers and breeders can streamline their efforts towards working with plant materials that possess the desired traits. This process contributes to the optimization of breeding programs, ensuring that only the most

promising and resistant varieties are cultivated and developed for future agricultural practices (Singh et al., 2019).

Genetic diversification is essential for increasing crop resilience and adapting them to future challenges, such as climate change and new pathogen strains (Kissoudis et al., 2016). The data collected in the characterization can be used for genome-wide association studies (Lindqvist-Kreuze et al., 2014). These studies can help identify specific genes responsible for resistance and provide a better understanding of the molecular mechanisms involved (Angmo et al., 2023; Wang et al., 2021; Mosquera et al., 2016).

The results of the characterization can also open new areas of scientific research in plant genetics. Scientists can study genes and metabolic pathways related to late blight resistance and other stress factors in *S. demissum*, contributing to the overall knowledge of plant biology and plant-pathogen interactions (Diaz-Garcia et al., 2023).

The information obtained through this research will be included in GRIN, making it available to all researchers interested in using these materials. This allows for broader collaboration for the global benefit of agriculture and food security.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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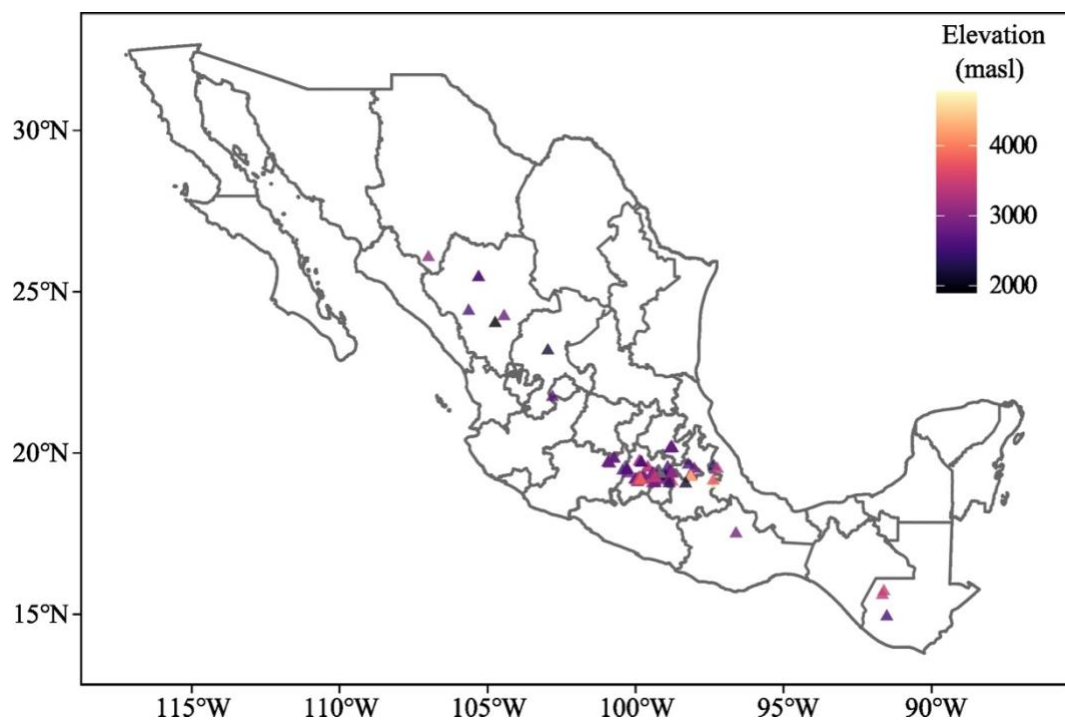


Fig 1. Geographic distribution of *Solanum demissum*. Map generated from the data of USDA- GRIN (<https://www.ars-grin.gov/>) and Hijmans et al. (2002).

Table 1. Maximum disease (Dmax), standardized Area Under Disease Progress Curve (sAUDPC), resistance rating.

Accession	ID collection	2020					2021				
		Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v	Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v
160208	COR						85.0 ± 0.0	329.0 a	34.8 ± 0.0	329.0 ab	S
160220	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	43.3 ± 5.8	323.5 ab	22.7 ± 0.0	326.0 ab	S
160221	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	5.0 ± 0.0	255.5 g	3.6 ± 1.3	277.5 efghi	R
160222	COR						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
160227	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	5.0 ± 5.0	219.7 h	0.6 ± 0.5	206.7 j	R
160229	COR						11.7 ± 2.9	289.5 cdef	3.6 ± 1.3	275.3 fghi	R
160230	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	10.0 ± 0.0	282.5 defg	4.5 ± 0.8	287.0 defghi	R
161149	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	10.0 ± 0.0	282.5 defg	4.9 ± 0.0	291.5 cdefghi	R
161151	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161153	COR						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161154	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161155	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	10.0 ± 0.0	282.5 defg	3.6 ± 1.3	277.5 efghi	R
161164	COR	5.0 ± 0.0	341.0 b	3.1 ± 0.0	341.0 c	R	5.0 ± 0.0	255.5 g	2.2 ± 1.4	263.3 hi	R
161165	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161166	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161167	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	5.0 ± 0.0	255.5 g	1.8 ± 0.8	258.3 i	R
161168	COR	65.0 ± 0.0	353.0 ab	39.5 ± 0.0	358.0 abc	S	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161169	COR	20.0 ± 0.0	347.0 ab	10.9 ± 1.1	345.7 bc	MR					
161175	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
161176	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161179	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161180	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161181	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	100.0 ± 0.0	333.5 a	51.3 ± 3.9	332.7 a	HS
161365	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
161366	COR	15.0 ± 0.0	344.0 b	10.3 ± 2.2	345.3 bc	0					
161367	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	50.0 ± 0.0	325.5 ab	8.2 ± 0.8	308.3 abcdefg	S

Table 1. (continued)

Accession	ID collection	2020					2021				
		Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v	Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v
161686	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
161693	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	15.0 ± 0.0	303.5 abcde	8.9 ± 2.4	309.0 abcdef	0
161715	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161719	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	30.0 ± 0.0	319.5 abc	14.9 ± 1.1	323.0 abc	MR
161729	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	15.0 ± 0.0	303.5 abcde	9.6 ± 2.4	310.7 abcdef	R
161731	COR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161732	COR						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
161769	COR						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
175403	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
175404	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
175405	EBS						8.3 ± 2.9	273.5 efg	4.9 ± 1.3	290.8 cdefghi	R
175408	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
175409	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
175411	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
175423	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
186551	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
186552	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
186561	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
186562	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
195165	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
201850	BEL						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
201851	BEL	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
201853	BEL	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
201854	BEL	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
205514	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
205515	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					

Table 1. (continued)

Accession	ID collection	2020					2021				
		Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v	Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v
205516	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
205518	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
205519	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
205625	CPC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
218047	BB	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
225652	CCC	85.0 ± 0.0	357.5 ab	39.3 ± 0.0	355.0 abc	S	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
225653	CCC	100.0 ± 0.0	368.0 a	52.3 ± 2.4	370.3 ab	HS	6.7 ± 2.9	264.5 fg	3.1 ± 0.8	273.0 ghi	R
225711	CCC	100.0 ± 0.0	368.0 a	48.1 ± 1.6	364.3 abc	HS					
230448	ROC	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
230558	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
230559	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
230578	SPB	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
230579	SPB	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
230589	RDD	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
230591	RDD	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	3.3 ± 2.9	210.7 hi	0.6 ± 0.0	206.7 j	R
230592	RDD	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
275206	HAW	85.0 ± 0.0	357.5 ab	40.8 ± 5.5	355.3 abc	S	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
275208	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
275209	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
275210	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
275211	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	5.0 ± 0.0	255.5 g	2.3 ± 0.0	263.0 hi	R
310961	UGN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
310962	UGN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	10.0 ± 0.0	282.5 defg	3.1 ± 0.8	273.0 ghi	R
338618	ROW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	3.3 ± 2.9	210.7 hi	1.0 ± 1.1	211.3 j	R
338619	ROW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
347760	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R

Table 1. (continued)

Accession	ID collection	2020					2021				
		Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v	Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v
347761	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
347762	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
365380	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
365381	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
365386	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
365387	TRN	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
473520	EBS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498012	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498013	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	15.0 ± 0.0	303.5 abcde	6.2 ± 0.0	300.0 abcdefg	R
498014	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498015	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498016	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498017	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498018	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498229	OCH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498230	OCH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498231	OCH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498232	OCH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498233	OCH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
498299	HAW	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545754	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545756	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545757	TRH						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545758	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545759	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545760	TRH						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R

Table 1. (continued)

Accession	ID collection	2020					2021				
		Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v	Dmax (%) ^z	Dmax (rank) ^y	sAUDPC ^x	sAUDPC (rank) ^w	Resistance Rating ^v
545761	TRH						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545763	TRH						10.0 ± 0.0	282.5 defg	4.5 ± 0.8	287.0 defghi	R
545764	TRH						16.7 ± 2.9	306.3 abcd	10.6 ± 0.8	312.7 abcde	MR
545765	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	11.7 ± 2.9	289.5 cdef	5.3 ± 0.7	294.3 bcdefgh	R
545766	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
545767	TRH	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
558051	HJT	100.0 ± 0.0	368.0 a	46.6 ± 1.2	363.0 abc	HS					
558388	SHG						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
558389	SHG	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
558390	SHG	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
558391	SHG	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
558392	SHG	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
558464	SHG	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
558482	SHG	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
607841	RSS	100.0 ± 0.0	368.0 a	50.0 ± 0.0	368.0 ab	HS	26.7 ± 2.9	316.5 abc	12.3 ± 0.0	319.0 abcd	MR
607849	RSS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R					
607869	RSS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
607873	RSS	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
607877	RSS						0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
653762	VIR	0.0 ± 0.0	170.0 d	0.7 ± 1.2	226.0 e	R	15.0 ± 0.0	303.5 abcde	2.8 ± 4.8	184.3 jk	0
653770	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
653772	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	5.0 ± 0.0	255.5 g	1.8 ± 0.8	258.3 i	R
653773	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R
653774	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	2.5 ± 2.7	188.3 ij	0.4 ± 0.5	185.5 jk	R
653776	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	1.7 ± 2.6	165.8 j	0.8 ± 1.2	169.0 k	R
653779	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R	0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R

Table 1. (continued)

Accession	ID collection	2020							2021						
		Dmax (%) ^z		Dmax (rank) ^y	sAUDPC ^x		sAUDPC (rank) ^w	Resistance Rating ^v	Dmax (%) ^z		Dmax (rank) ^y	sAUDPC ^x		sAUDPC (rank) ^w	Resistance Rating ^v
653789	VIR	17.5 ± 19.2	260.0 c	12.8 ± 14.1	259.8 d	MR		17.5 ± 8.2	298.8 bcde	6.7 ± 4.5	292.7 cdefgh	MR			
653803	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R		0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R			
653804	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R		0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R			
653805	VIR	0.0 ± 0.0	170.0 d	0.0 ± 0.0	169.5 f	R		0.0 ± 0.0	121.0 k	0.0 ± 0.0	122.0 l	R			
Control S. tuberosum	NA	100.0 ± 0.0	368.0 a	58.9 ± 1.5	374.0 a	HS		100.0 ± 0.0	333.5 a	52.9 ± 2.7	334.3 a	HS			
X ²			358.2	315.7				319.3		352.3					

^z Maximum disease severity reached at the end of the epidemic. Means ± standard deviation for three replications.

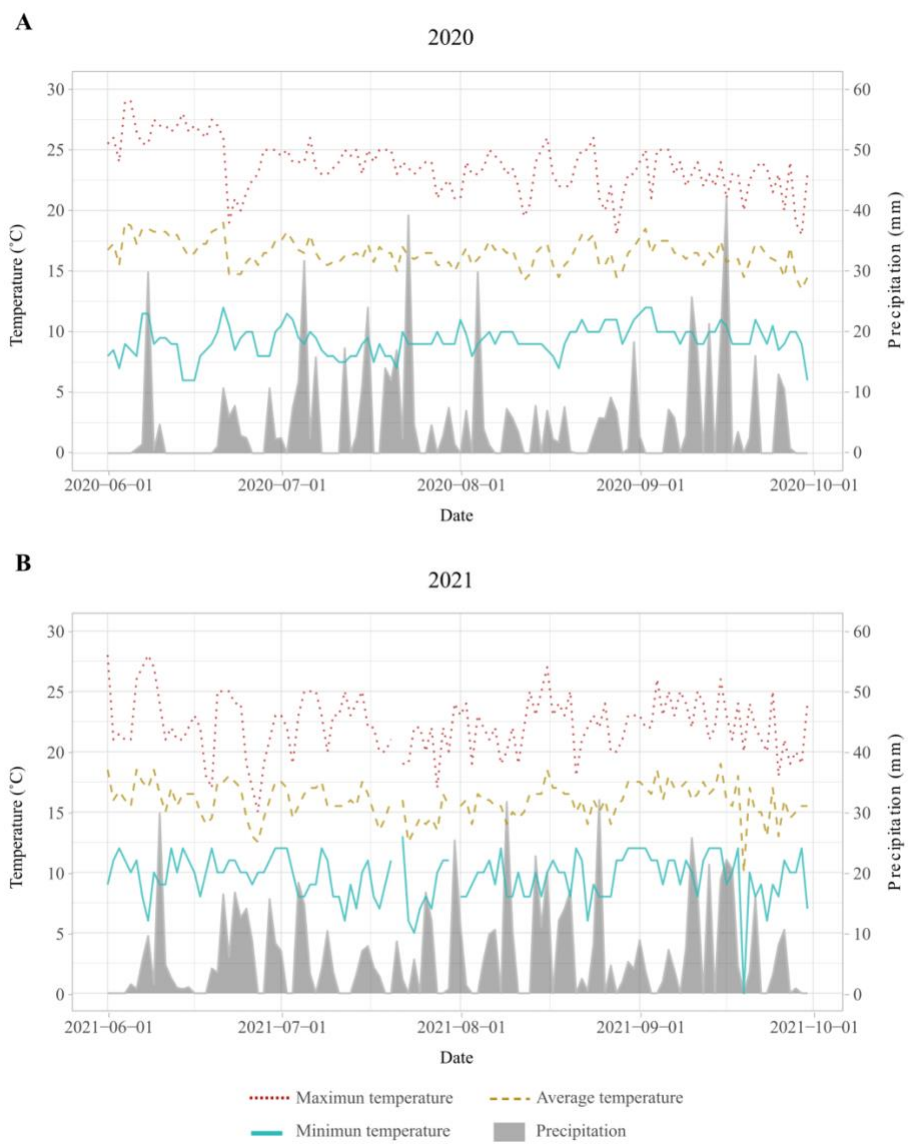
^y Maximum disease severity rank of the statistical analysis.

^x Standardized Area Under Disease Progress Curve, obtained dividing AUDPC value by the time lapse between the first and last disease rating for each accession. Means ± standard deviation for three replications.

^w Standardized Area Under Disease Progress Curve rank of the statistical analysis.

^v Plants between 85.1 to 100 % severity were considered Highly Susceptible (HS), 35.1 to 85 % Susceptible (S), 15.1 to 35 % Moderately Resistant (MR) and plants with values between 0 and 15 % were considered Resistant (R).

SUPPLEMENTAL MATERIAL



FigS2. Meteorological conditions along the growing cycles: A, 2020; B, 2021.