

Resistance to *Puccinia graminis* f. sp. *avenae* in Oat Genotypes and Challenges for Genetic Breeding in Argentina

Resistencia a *Puccinia graminis* f. sp. *avenae* en genotipos de avena y desafíos para el mejoramiento genético en Argentina

Juan Ignacio Dietz^{1,2,3*}, Laura Victoria Da Silva^{1,2,3}, María Rosa Simón^{1,2}, Pablo Campos³

Originals: Received: 23/06/2024 - Accepted: 26/05/2026

ABSTRACT

Oat stem rust caused by *Puccinia graminis* f. sp. *avenae* (*Pg*) significantly threatens oat cultivation globally and in some areas of Argentina, emphasizing the importance of studying local germplasm resistance. We evaluated 38 Argentine oat cultivars (16 old, 22 modern) and 20 breeding lines against prevalent *Pg* races (2005, 2021, 2022, and 2023) in both seedlings (greenhouse) and adult plants (field trials). We postulate several candidate genes (*Pg3*, *Pg9*, *Pg10*, *Pg15*, and *Pga*) in the tested germplasm. Seedling assays revealed a shift in cultivar resistance, with older cultivars becoming increasingly susceptible to recent *Pg* populations. Modern cultivars and lines exhibited limited seedling resistance, highlighting the vulnerability of current germplasm. Adult plant results showed variations in disease severity in some genotypes among years, suggesting shifts in *Pg* race prevalence and virulence. Resistance observed in cultivars such as Milagros, Payé, Aikén, and Yapa to specific races at the seedling stage may explain their favorable performance in certain seasons. Moreover, cultivars such as Máxima, Tucana, Polaris, U16, Maná, La Plata FA, and some lines showed severity values below 20% in the field. The findings provide useful insights into oat breeding programs and crop management strategies.

Keywords

oat stem rust • *Pg* genes • seedling resistance • *Avena sativa* • breeding for rust resistance

<https://doi.org/10.48162/rev.39.226>

1 Universidad Nacional de La Plata. Facultad de Ciencias Agrarias y Forestales. Cerealicultura. Av. 60 y 119. La Plata. Argentina.

2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Argentina.

3 Instituto Nacional de Tecnología Agropecuaria (INTA). EEA Bordenave CC44. C. P. 8187. Ruta 76 km 36. Bordenave. Buenos Aires. Argentina. * dietz.juan@inta.gob.ar



RESUMEN

La roya del tallo en avena, causada por *Puccinia graminis* f. sp. *avenae* (*Pg*) es una amenaza para el cultivo a nivel mundial, y recientemente ha afectado algunas áreas de Argentina, destacando la importancia de estudiar la resistencia del germoplasma local. En este trabajo, evaluamos 38 cultivares argentinos de avena (16 antiguos, 22 modernos) y 20 líneas contra razas prevalentes de *Pg* (2005, 2021, 2022 y 2023) en plántula (invernadero) y planta adulta (campo). Este trabajo permitió postular la presencia de varios genes (*Pg3*, *Pg9*, *Pg10*, *Pg15* y *Pga*) en el germoplasma testeado. Los resultados en plántula mostraron mayor susceptibilidad en los cultivares antiguos frente a las nuevas poblaciones *Pg*. Asimismo, cultivares modernos y líneas mostraron limitada resistencia, exhibiendo la vulnerabilidad del germoplasma actual. En planta adulta, la severidad varió entre genotipos y años, sugiriendo fluctuaciones en la prevalencia y virulencia de razas *Pg*. Nuestros resultados revelan que la resistencia en plántula de cultivares como Milagros, Payé, Aikén, y Yapa a razas específicas en invernadero, podría explicar su buen comportamiento a campo. Cultivares como Máxima, Tucana, Polaris, U16, Maná, La Plata FA y algunas líneas presentaron severidad menor al 20% en planta adulta. La información aquí obtenida es valiosa para los programas de mejoramiento de avena y manejo del cultivo.

Palabras claves

roya del tallo en avena • genes pg • resistencia en plántula • *Avena sativa* • mejoramiento de la resistencia a royas

INTRODUCTION

Oats (*Avena sativa* L.) are a widely cultivated cereal crop for both forage and grain. It is the sixth most important cereal in grain production, with approximately 23 million t growing around 10 million hectares (15). In Argentina, the crop covers 1.5 million hectares approximately, mainly for forage production (25). The crop is technically and economically viable, especially in livestock systems, due to its multiple uses: grain, forage, preserved forage (hay and silage), dual purpose (forage-grain), and use of stubble (9). Argentine oat genotypes have been extensively studied for key agronomic parameters like photoperiodic and vernalization responses, as well as the critical period for yield determination under optimal climatic conditions (11, 14). Furthermore, advances in understanding the nutritional quality of oat grain (20) and the health benefits of its consumption (17, 33) have revalued its use in human nutrition (2), leading to an increase in demand for oat-based products and driving oat cultivation for grain (1).

Oat stem rust, caused by *Puccinia graminis* f. sp. *avenae* Erikss and Henning (*Pg*), is globally recognized for its destructive potential under favorable conditions (27, 34). Van Niekerk *et al.* (2001) have reported yield losses of up to 85%, and a 45% decrease in test weight in susceptible materials after a stem rust epidemic, while Leyva *et al.* (2004) documented drops of up to 70% in grain yield. In Argentina, stem rust has been considered a minor disease, appearing sporadically toward the end of the crop cycle and affecting mostly grain production. Consequently, most research efforts in the country have focused on crown rust (*Puccinia coronata* f. sp. *avenae* (*Pc*)), particularly in resistance breeding and disease management (5, 9, 10, 12), while stem rust has received less attention. Although earlier reports (6, 39) describe occurrences of stem rust in certain seasons with low levels of disease among susceptible genotypes, more recent observation data (2021-2023) from field trials and farmers' fields across several locations in Argentina (Reconquista, La Plata, Barrow, and Bordenave) indicate increasing disease severity (Dietz, non-published data). For example, in Reconquista (Santa Fe province) during the 2023 growing season, many Argentine materials exhibited near 100% severity, stem breakage, and panicle retention. These observations suggest the possible emergence of new biotypes with broader virulence spectra.

The populations of *Pg*, as well as the populations of *Pc* demonstrate extensive race diversity and ongoing evolution (5, 12, 13, 26), which makes maintaining stable resistance in oat cultivars over time especially challenging (28). In this context, Campos & Giménez (2011) reported high variability among 83 *Pg* isolates collected between 2007 and 2009, demonstrating that most Argentine oat cultivars exhibit low seedling resistance. Wehrhahne & Storm (2014) observed that short-cycle cultivars (*e.g.*, Bonaerense INTA (B.I.) Maná, B.I. Calén, and B.I. Maja) suffer less diseases severity than long-cycle cultivars (Carlota INTA, Marita INTA, and Violeta INTA). However, additional studies are required to determine whether these differences are attributable to genetic associations between resistance and days to heading or if they result from environmental or epidemiological factors.

Genetic improvement is the most effective strategy for disease management (16, 21, 24, 38). However, only 16 stem rust resistance genes have been described worldwide (16), indicating that the genetic resources available for developing oat varieties resistant to this disease are limited. Most of these known genes are race-specific (major genes), which confer hypersensitive responses via incompatible host–pathogen interactions. They are generally effective from the seedling stage and confer resistance reactions even in adult plants, although only to certain races or pathotypes. Gene postulation in host genotypes using a set of differential races with distinct virulence/avirulence profiles (*i.e.*, pathotypes) is a standard phenotypic method for identifying resistance genes based on infection types (IT). In Argentina, several *Pc* resistance genes have been postulated in oat cultivars (5, 9), but to date, no *Pg* genes have been reported. Identifying and characterizing *Pg* genes in Argentine germplasm is essential to elucidate the genetic basis of resistance, to understand the performance of local cultivars, and to support targeted breeding programs.

The oat seed market in Argentina is much smaller than for wheat, reflected in fewer breeding programs and a limited catalog of commercial oat cultivars, often composed of older varieties. The long-term widespread use of a relatively small number of cultivars over many years may influence pathogen populations, such as those of *Puccinia coronata* (12). In fact, this practice has been suggested as one of the causes of shifts in race populations for *Pc* and *Pg*, and of increasing disease incidence in oat crops.

Given the diverse uses of oats in Argentina and the increasing disease pressure in many regions, this study aims to postulate *Pg* genes and evaluate resistance in advanced breeding lines and commercial varieties at both seedling and adult plant stages. Dissemination of these findings will enable oat breeders and producers to make informed decisions about germplasm deployment.

We hypothesize that the *Pg* population in Argentina has recently shifted, resulting in the emergence of races with expanded virulence spectra; and that most commercially available oat cultivars are vulnerable to these more virulent races.

MATERIAL AND METHODS

Plant Materials and Experimental Design

A total of 38 commercial Argentine oat cultivars (16 old and 22 modern) and 20 advanced breeding lines from different breeding programs (Universidad Nacional de La Plata, Instituto Nacional de Tecnología Agropecuaria (INTA) Bordenave, and INTA Barrow) were evaluated against different *Pg* races. Evaluations were conducted at the seedling stage (Experiment 1) and in field trials across two environments (Experiment 2, page 4).

Experiment 1: Greenhouse Studies of Seedling Resistance to Stem Rust

Seedling tests were carried out at the Rust laboratory of INTA Bordenave, Argentina (37°52' LS; 63°01' LW). A split-plot design was used, with each race as the main plot and genotypes as subplots, with two replications. All genotypes were tested against twelve races of *Pg* collected during the 2021, 2022, and 2023 growing seasons from different locations of Argentina [Supplementary Table S2]. These races were selected based on their high frequency in national surveys [*e.g.* *Pg* 21-7-(2), *Pg* 21-16-(1), *Pg* 22-1-(1), and *Pg* 23-10-(5)], and the rest representing different combinations in the virulence/avirulence patterns useful for discriminating resistance *Pg* genes in seedlings. Furthermore, this

study analysed a dataset from a trial conducted on the 16 old cultivars in 2006 using 10 predominant *Pg* races collected in 2005. This analysis considers potential changes in cultivar responses over time. The *Pg* races were previously characterized on a set of 11 differential oat lines (*Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg8*, *Pg9*, *Pg10*, *Pg13*, *Pg15*, *Pg16* and *Pga*), each line carrying a single race-specific *Pg* gene (19). This differential set is standard in Argentina for *Pg* population studies (6). Eight to ten seeds of each genotype were sown in 200 cm³ pots containing fertile loam soil. At twelve days after sowing, when seedlings had reached growth stage GS12 (two fully expanded leaves) (40)], plants were inoculated with freshly collected urediniospores of the selected races (Supplementary Table S1). For inoculation, a subsample of 2 mg of each *Pg* isolate was suspended in Soltrol® 170 mineral oil (diluted to 50 mg total volume) loaded into gelatin capsules (at 1/25 weight concentration), and sprayed onto plants using an air compressor with a spray nozzle. After inoculation, plants were placed in a dark chamber at 100% relative humidity and 20 +/-1°C for 20 h to allow spore germination and leaf penetration. They were then transferred to greenhouse benches maintained at 20 +/-1°C.

Disease reactions were scored 15 days post-inoculation using a 0-4 infection type (IT) scale, where 0 = no uredia or other macroscopic infection symptoms, 1 = small uredia surrounded by chlorosis or necrosis, 2 = small to medium size uredia surrounded by chlorosis, 3 = medium size uredia in a chlorotic area, and 4 = large uredia without chlorosis or necrosis (35). Seedlings showing IT of 0, 0+, 1, and 2 (including modifications such as 1+, 11+, 22+ T... etc.) were classified as resistant, and ITs of 3 or 4, as susceptible.

Because the experiment was observational and the *race* × *genotype* interaction is deterministic, no formal statistical analysis was carried out. The presence of *Pg* seedling resistance genes in the Argentine germplasm was postulated by comparing the IT responses of cultivars and breeding lines to those of the differential set.

Experiment 2: Field Studies of Adult Plant Resistance to Stem Rust

Adult plant resistance of the genotypes was evaluated under field conditions against natural infections of *Pg* at the J. Hirschhorn Experimental Station, Los Hornos (34°52' S; 57°58' W; Universidad Nacional de La Plata, Argentina), during the 2022 and 2023 growing seasons. The trials were conducted using a randomized complete block design, with environments (*years*) and genotypes as factors, and two replications. Sowing was performed in June under conventional tillage. Genotypes were arranged in 2 m rows, spaced 20 cm apart, at a target density of 200 plants.m⁻². Every five genotypes, two border rows of the susceptible cultivar "Boyera FA" were included to promote inoculum build-up. Disease severity (expressed as the percentage of diseased stem area, including the peduncle) was assessed using the modified Cobb scale (32) at GS75 [medium milky grain stage (40)], after grouping genotypes by phenological cycle. Data were analyzed by ANOVA using InfoGen software (3), and means were compared using the LSD test ($p=0.05$).

RESULTS

Seedling Resistance in Old Cultivars

The seedling responses of commercial oat cultivars released over 20 years to the ten races identified in 2005, and the twelve races of the 2021-2023 population are presented in table 1 (page 5) and table 2 (page 5-6). Against the 2005 *Pg* population, most cultivars exhibited resistance to the majority of races, except Millauquén INTA and Pilar INTA, which were entirely susceptible (table 1, page 5). Cultivars such as Rocío INTA and B.I. Payé were resistant to all ten races; Máxima INTA, INIA Polaris; Aurora INTA, INIA Tucana, B.I. Canaí, U16, Milagros INTA, and Graciela INTA exhibited resistance to nine races; whereas B.I. Maja, B.I. Calén, Cristal INTA, and Violeta INTA were resistant to six races. In contrast, when these cultivars were tested against the twelve races of the 2021-2023 *Pg* population, their resistance was substantially reduced. B.I. Maja and B.I. Calén showed resistance against three races; while Máxima INTA, Rocío INTA, and B.I. Payé resisted two races. Additionally, Aurora INTA, B.I. Canaí, INIA Tucana, INIA Polaris, U16, Milagros INTA, and Graciela INTA cultivars each resisted one race, while the remaining genotypes were completely susceptible.

Table 1. Seedling response of old Argentine cultivars against ten races of *Puccinia graminis* identified in Argentina in 2005.**Tabla 1.** Respuesta en plántula de antiguos cultivares argentinos frente a diez razas de *Puccinia graminis* identificadas en Argentina en 2005.

Cultivars	Postulated genes	<i>Pg</i> 05-70-1	<i>Pg</i> 05-5-1	<i>Pg</i> 05-15-1	<i>Pg</i> 05-16-3	<i>Pg</i> 05-7-2	<i>Pg</i> 05-9-1	<i>Pg</i> 05-6-2	<i>Pg</i> 05-7-1	<i>Pg</i> 05-14-1	<i>Pg</i> 05-6-1
Cristal INTA	<i>¿Pg13?</i>	3	3+	3	4	0	0	0	1	0	1
Millauquén INTA	<i>NO</i>	3+4	4	3	4	4	4	3	4	4	4
Pilar INTA	<i>NO</i>	4	3+	3+	4	4	3	3	4	4	4
Máxima INTA	<i>Pg10</i>	2+ 2++	3	2+ 2++	2	1	1	1+	1	1	1 1+
Aurora INTA	<i>Pga</i>	4	0	0	0;	0	0	;	0	0	0
Rocío INTA	<i>Pg10, Pga</i>	2+2++	0	;1	0;	0	0	;	0	0	0
B.I. Maja	<i>Pg9</i>	3	3	3	3	1	1	1	1	;1	1
B.I. Calén	<i>Pg9</i>	3 3+	3	3	3	0	;1	1	1 1+	0	1
B.I. Payé	<i>Pg10, Pga</i>	2+	0	0;	0;	0	0	0	0	0	0
B.I. Canaí	<i>Pga</i>	3 3+	2+ 2++	2+ 2++	0;	0	1	1	1	;1	1
INIA Tucana	<i>Pga</i>	3	1++	;1	1	1	1++	2	2 2+	1	2 2+
INIA Polaris	<i>Pg10</i>	2 2+	3+	2 2+	2 2+	;1	1	1	1	0;	1
U-16	<i>Pga</i>	3	1++	0	0;	1	;1	1	1	;1	1
Milagros INTA	<i>Pga</i>	3	1+	1 1+	1	2	1++	1	1 1++	1 1+	2
Violeta INTA	<i>¿Pg13?</i>	3	3	3	3	1	1	1+	1 1+	1	1
Graciela INTA	<i>Pga</i>	3 3+	0	0	0;	0	0	0	0	0	0

* *B.I.: Bonaerense INTA.

* Seedlings showing infection types (IT) of 0, 0+, 1, and 2 and their modifications (;1+, 11+, 22+ T... etc.) are classified as resistant, while those with ITs of 3 or 4 are considered susceptible. Cells highlighted in bold represent susceptible IT.

*Las plántulas que muestran tipos de infección (IT) 0, 0+, 1 y 2 y sus modificaciones (;1+, 11+, 22+ T... etc.) se clasifican como resistentes, mientras que aquellas con IT de 3 o 4 se consideran susceptibles. Las celdas resaltadas en negrita representan los tipos de infección susceptibles.

Table 2. Seedling response of old Argentine cultivars against twelve races of *Puccinia graminis* identified in 2021, 2022 and 2023 in Argentina.**Tabla 2.** Respuesta en plántula de antiguos cultivares argentinos frente a doce razas de *Puccinia graminis* identificadas en 2021, 2022 y 2023 en Argentina.

Cultivars	Postulated genes	<i>Pg</i> 21-7-2	<i>Pg</i> 21-10-2	<i>Pg</i> 21-6-2	<i>Pg</i> 21-4-3	<i>Pg</i> 21-16-1	<i>Pg</i> 22-4-2	<i>Pg</i> 22-2-1	<i>Pg</i> 22-1-1	<i>Pg</i> 23-1-1	<i>Pg</i> 23-7-1	<i>Pg</i> 23-2-3	<i>Pg</i> 23-10-5
Cristal INTA		3+	3	3	4	4	4	3	3+4	4	3+	4	4
Millauquén INTA	<i>NO</i>	3+ 4	4	3	4	4	3	4	4	4	3	4	4
Pilar INTA	<i>NO</i>	3 3+	3	3 3+	4	4	4	3 3+	4	4	3	4	4
Máxima INTA	<i>Pg10; Pg3</i>	3	3	2 2+	3	4	3+4	3+	4	3+4	1+	3+	3+
Aurora INTA	<i>Pga</i>	3	4	3	4	4	3 3+	4	4	1//2+	3	4	4
Rocío INTA	<i>Pg10, Pga</i>	3+	4	2 2+	4	3 3+	4	4	4	;n1	3	4	3
B.I. Maja	<i>Pg9</i>	3	3	3+ 4	3 3+	3+ 4	2 2-	2	4	3+ 4	3+	2 2-	3+
B.I. Calén	<i>Pg9</i>	3	4	3	3 3+	3+ 4	2 2-	2	4	3+	3	2	4
B.I. Payé	<i>Pg10, Pga</i>	3	3	2 2-	4	4	3 3+	4	4	0;	3	3	4
B.I. Canaí	<i>Pga</i>	4	4	3	4	4	3+4	4	4	1	3	3+	4

*B.I.: Bonaerense INTA.

*Seedlings showing infection types of 0, 0+, 1, and 2 and their modifications (;1+, 11+, 22+ T... etc.) are classified as resistant, while those with ITs of 3 or 4 are considered susceptible. Cells highlighted in bold represent susceptible IT.

*Las plántulas que muestran tipos de infección (IT) 0, 0+, 1 y 2 y sus modificaciones (;1+, 11+, 22+ T... etc.) se clasifican como resistentes, mientras que aquellas con IT de 3 o 4 se consideran susceptibles. Las celdas resaltadas en negrita representan los tipos de infección susceptibles.

INIA Tucana	<i>Pga</i>	3	3	3+ 4	3+ 4	4	4	4	3	1	3	3	3
INIA Polaris	<i>Pg10</i>	4	3+ 4	2+	3	4	3+4	3	3+4	3	3	3	4
U-16	<i>Pga</i>	3+	3	3	4	3	4	4	4	11+	3	3	3
Milagros INTA	<i>Pga</i>	3+ 4	3 3+	3 3+	4	3	4	4	4	1	3	3	3+
Violeta INTA		3	4	3	4	4	4	3	3+4	3+	3+	4	4
Graciela INTA	<i>Pga</i>	3	3 3+	3	3	3 3+	4	4	4	1	3+	4	4

Based on the 2005 race set, resistance gene postulation indicated the presence of *Pg10* + *Pga* in Rocío INTA and B.I. Payé; *Pga* in Aurora INTA, B.I. Canaí, U16, Milagros INTA, and Graciela INTA; *Pg10* in Máxima INTA and INIA Polaris; and *Pg9* in B.I. Calén and B.I. Maja. Some cultivars likely carry additional resistance genes, that could not be postulated due to the absence of corresponding virulence in the 2005 *Pg* population. Furthermore, the twelve races set from the 2021-2023 *Pg* population confirmed the presence of *Pg10*, *Pga*, and *Pg9* in these cultivars; *Pg3* in Máxima INTA, whereas *Pg2*, *Pg4*, *Pg8*, *Pg15*, and *Pg16* appear to be absent in the older cultivars.

Seedling Resistance in Modern Cultivars and Breeding Lines

The seedling resistance of modern oat cultivars and lines to twelve *Pg* races identified during 2021-2023 is detailed in table 3 (page 6-7) table 4 (page 7). Out of 264 cultivar × race interactions, 259 (≈98%) were susceptible, indicating that effective seedling resistance is largely absent in modern cultivars (table 3, page 7). Only a few cultivar-race combinations showed resistant reactions: Carlota INTA and B.I. Aikén were resistant to race *Pg23-1-1*, consistent with the presence of the *Pga* resistance complex, while Liliana INTA, María INTA, and B.I. Lola were resistant to *Pg21-16-2*, suggesting that they may carry *Pg10*. The remaining cultivars were susceptible to all races.

Among the tested lines, 221 of 240 interactions (≈92%) were susceptible (table 4, page 7). However, some lines exhibited race-specific resistance. The FA45 line displayed the broadest resistance, being effective against four of the twelve races, followed by Bv99-19, Bv131-19, FA19, Bw16-15, and Bw15-108, which each showed resistance to two races. Based on the resistance spectra and known differential reactions, we postulate that the genes *Pg10*, *Pga*, *Pg9*, and *Pg15* are present in certain breeding lines.

Table 3. Seedling response of modern Argentine cultivars against races of *Puccinia graminis* identified in 2021, 2022 and 2023 in Argentina.

Tabla 3. Respuesta en plántula de nuevos cultivares argentinos frente a doce razas de *Puccinia graminis* identificadas en 2021, 2022 y 2023 en Argentina.

Cultivars	Postulated genes	<i>Pg</i> 21-7-2	<i>Pg</i> 21-10-2	<i>Pg</i> 21-6-2	<i>Pg</i> 21-4-3	<i>Pg</i> 21-16-1	<i>Pg</i> 22-4-2	<i>Pg</i> 22-2-1	<i>Pg</i> 22-1-1	<i>Pg</i> 23-1-1	<i>Pg</i> 23-7-1	<i>Pg</i> 23-2-3	<i>Pg</i> 23-10-5
Lucía INTA		4	3	4	4	4	4	4	4	3 3+	3+	3+	3+
Marita INTA		3	3	3	4	4	3+4	4	4	3+4	3+	3+	3+
Florencia INTA		3+	3 3+	3	4	4	4	3	4	4	3+4	3+	4
Elizabeth INTA		3	3	3 3+	4	4	4	4	3	3 3+	3+	3+	4
Carlota INTA	<i>Pga</i>	3	3 3+	3	3	3	4	4	4	1+2	3	3	3
B.I. Maná		3	3	3	3	4	4	4	4	4	3	3	3
B.I. Sureña		3	4	3	3	4	4	4	4	3+	3	4	4
B.I. Aikén	<i>Pga</i>	3-	3+4	4	3+	3	3+	4	4	1	3	4	3 3+

*B.I.: Bonaerense INTA.

Seedlings showing infection types of 0, 0+, 1, and 2 and their modifications (;1+, 11+, 22+ T... etc.) are classified as resistant, while those with ITs of 3 or 4 are considered susceptible. Cells highlighted in bold represent susceptible IT.

*Las plántulas que muestran tipos de infección (IT) 0, 0+, 1 y 2 y sus modificaciones (;1+, 11+, 22+ T... etc.) se clasifican como resistentes, mientras que aquellas con IT de 3 o 4 se consideran susceptibles. Las celdas resaltadas en negrita representan los tipos de infección susceptibles.

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B.I. Yapa		3	3+	3 3+	3+ 4	4	4	4	4	3+	3	3	3 3+
Julieta INTA		3	3	3	3 3+	3	4	4	4	3+	3	3	3
La Plata FA		3 3+	4	4	4	3	4	4	4	3	3	3	3
Los Hornos FA		3+	3	3 3+	4	4	4	4	4	3 3+	3	3	3
Elena INTA		3 3+	3	3	3	3	4	3 3+	3	3	3 3+	3 3+	3 3+
Paloma INTA		3 3+	3	3	3+ 4	3	4	4	3+	4	3 3+	3 3+	3
Juana INTA		3	3+ 4	4	3 3+	3+ 4	4	3 3+	4	3+	3+	3+	3+
Sofía INTA		3	4	4	4	3+ 4	4	3	4	3+	3+	3+ 4	3+
Liliana INTA	<i>Pg10</i>	3+	4	2+	4	3	4	3 3+	3+ 4	4	4	4	3+
Pía INTA		3	3+ 4	4	3	3	4	4	4	4	4	3	3+
Susana INTA		3	4	4	3+	3	4	4	4	3+	4	4	3+
Maria INTA	<i>Pg10</i>	3	4	1	3	3	4	4	3 3+	4	3+	4	3+
B.I. Serena		3	4	3	4	3	4	4	3	4	3+	3+	3+
B.I. Lola	<i>Pg10</i>	3+	4	1	4	3	3+ 4	4	4	4	3	3+	3

Table 4. Seedling response of oat lines against races of *Puccinia graminis* identified in 2021, 2022 and 2023 in Argentina.

Tabla 4. Respuesta en plántula de líneas avanzadas de avena frente a doce razas de *Puccinia graminis* identificadas en 2021, 2022 y 2023 en Argentina.

Lines	Postulated genes	<i>Pg 21-7-2</i>	<i>Pg 21-10-2</i>	<i>Pg 21-6-2</i>	<i>Pg 21-4-3</i>	<i>Pg 21-16-1</i>	<i>Pg 22-4-2</i>	<i>Pg 22-2-1</i>	<i>Pg 22-1-1</i>	<i>Pg 23-1-1</i>	<i>Pg 23-7-1</i>	<i>Pg 23-2-3</i>	<i>Pg 23-10-5</i>
BV 50-16	<i>Pg15</i>	3	4	3	2+	3	4	4	4	3+	3	3	3
BV 374-19		3	4	3	3	3	4	4	4	4	3	3+	3
BV 108-19		3	4	3	3	4	4	4	3	4	3	3+	3
BV 21-16		3+	4	4	3	3+	4	4	3+	4	4	4	3
BV 449-19		3+	4	4	3	3+ 4	4	3	3+	4	4	4	3+
BV 99-19	<i>Pga; iPg4, Pg8?</i>	3	1	3+	3	4	4	4	4	1	4	3	3+ 4
BV 131-19	<i>Pg10; Pg15</i>	3+	3+	1	2+	4	4	4	4	4	4	3+	3+
FA 51	<i>Pg15</i>	3+	4	4	2+	3	4	4	4	3+ 4	4	4	4
FA 53	<i>Pg15</i>	3	4	4	1+	3	3 3+	4	4	3+	4	3+	4
FA 45	<i>Pg9; Pg10; Pg15</i>	4	4	2	2 2+	3	22-	22-	4	3	3+	4	4
FA 21	<i>Pg15</i>	3	4	4	2 2+	3	4	3	4	3	3	4	3+
FA 19	<i>Pg10; Pg15</i>	3+	3	1	1	3+	3	4	4	3+	3+	3+	3+
FA 39		3	3+	4	4	3	3	4	3+	3+ 4	3+	3+	3+
BW 14-10	<i>Pg10</i>	3	3	2	3	3	3 3+	4	3 3+	3+ 4	3+	3+	3+
BW 12-15	<i>iPg4, Pg8?</i>	3	0;	3	3	3	4	4	3	3+	3	3	3
BW 16-15	<i>Pg10; iPg4, Pg8?</i>	4	2	2	4	3	4	4	3+ 4	3+	3	3+	3+
BW 16-28	<i>Pg10</i>	3+	3	2+	4	3	4	4	4	3+ 4	3	3+	3+
BW 18-58	<i>Pg10</i>	3	3	2	4	4	4	4	4	3+	3+	3+	3+
BW 15-108	<i>Pg15; Pga</i>	3	3	4	1	3	4	3+ 4	3 3+	3+ 4	3	3+	3+
BW 15 113		3	3	4	4	4	4	3+ 4	3+ 4	3	3	3+	3+

* Seedlings showing infection types (IT) of 0, 0+, 1, and 2 and their modifications (;1+, 11+, 22+ T... etc.) are classified as resistant, while those with ITs of 3 or 4 are considered susceptible. Cells highlighted in bold represent susceptible IT.

*Las plántulas que muestran tipos de infección (IT) 0, 0+, 1 y 2 y sus modificaciones (;1+, 11+, 22+ T... etc.) se clasifican como resistentes, mientras que aquellas con IT de 3 o 4 se consideran susceptibles. Las celdas resaltadas en negrita representan los tipos de infección susceptibles.

Adult Plant Resistance

The ANOVA results revealed highly significant effects of *years* ($p<0.0001$) and *genotypes* ($p<0.0001$) treatments, and the *year x genotype* interaction ($p<0.0001$). The coefficient of variation was 10.2% (Supplementary Table S3). Mean disease severity was higher in 2023 (33.4%) than in 2022 (26.7%) (LSD= 0.78; ANOVA not shown), with severity values ranging from 2.5 to 95%, and 10 to 60% for 2023 and 2022, respectively (table 5 and table 6, page 9). The *year x genotype* interaction was explained by contrasting responses across genotypes. Some genotypes (*e.g.*, Milagros: 60% in 2022 vs. 15% in 2023; Payé: 50% vs. 15%; Aikén: 30% vs. 15%; Yapa: 20% vs. 5%; Lucía: 27.5% vs 20%), and lines such as Bv449-19 (60% vs. 32.5%), and FA51 (22.5% vs. 2.5%), exhibited lower severity in 2023 than in 2022. By contrast, other genotypes-Aurora (22.5% vs. 50%), Carlota (22.5% vs. 80%), Liliana (37.5% vs. 80%), Serena (35% vs 80%), and Lola (20% vs. 80%)-displayed significantly greater severity in 2023 compared to 2022. Additionally, some cultivars (*e.g.*, María: 60% in 2022 and 95% in 2023; Pía: 60% and 80%; Violeta: 60% and 80%) reached the highest severity levels in both years. In contrast, cultivars such as Máxima, Tucana, Polaris, U16, Maná, La Plata FA, and the lines Bv50-16 and Bv108-18 showed no significant differences between years and consistently maintained severity values below 20%. Some genotypes performed favorably only in a single year: Rocío (10%) and Juana (15%) in 2022, and Calén (20%) and Lucía (20%) in 2023. Moreover, Los Hornos FA and La Plata FA exhibited consistently low severity values (20-30%) across both years.

Table 5. Severity (%) in Argentine oat cultivars against natural *Puccinia graminis* infections in 2022 and 2023, at the J. Hirschhorn Experimental Station, Los Hornos, Argentina.

Tabla 5. Severidad (%) en cultivares argentinos de avena frente a infecciones naturales de *Puccinia graminis* en 2022 y 2023, en la Estación Experimental J. Hirschhorn, Los Hornos, Argentina.

Cultivars	Severity (%) 2022		Severity (%) 2023		Cultivars	Severity (%) 2022		Severity (%) 2023	
Lucía INTA	27.5	hij	20	efg	Marita INTA	35	klm	55	no
Cristal INTA	22.5	fgh	50	n	Carlota INTA	22.5	fgh	80	p
Millauquén INTA	25	ghi	32,5	jkl	B.I. Maná	15	cde	15	cde
Pilar INTA	22.5	fgh	32,5	jkl	B.I. Sureña	22.5	fgh	35	klm
Máxima INTA	10	bc	12,5	cd	B.I. Aikén	30	ijk	15	cde
Aurora INTA	22.5	fgh	50	n	B.I. Yapa	20	efg	5	ab
Rocío INTA	10	bc	30	ijk	Julieta INTA	37.5	lm	55	no
B.I. Maja	22.5	fgh	30	ijk	La Plata FA	20	efg	20	efg
B.I. Calén	22.5	fgh	20	efg	Los Hornos FA	20	efg	30	ijk
B.I. Payé	50	n	15	cde	Elena INTA	20	efg	75	p
B.I. Canaí	22.5	fgh	30	ijk	Paloma INTA	20	efg	30	ijk
Florencia INTA	22.5	fgh	30	ijk	Juana INTA	15	cde	30	ijk
Elizabeth INTA	22.5	fgh	30	ijk	Sofía INTA	20	efg	30	ijk
INIA Tucana	20	efg	15	cde	Liliana INTA	37.5	lm	80	p
INIA Polaris	12.5	cd	12,5	cd	Pía INTA	60	o	80	p
U-16	10	bc	15	cde	Susana INTA	35	klm	30	ijk
Milagros INTA	60	o	15	cde	María INTA	60	o	95	q
Violeta INTA	60	o	80	p	B.I. Serena	35	klm	80	p
Graciela INTA	35	klm	55	no	B.I. Lola	20	efg	80	p

*Different letters show significant differences in severity between the years and genotypes treatments, LSD $P<0.05$.

* Letras diferentes muestran diferencias significativas en severidad entre los años y genotipos de tratamiento, LSD $P<0,05$.

Table 6. Severity (%) in Argentine oat lines against natural *Puccinia graminis* infections in 2022 and 2023, at the J. Hirschhörn Experimental Station, Los Hornos, Argentina.**Tabla 6.** Severidad (%) en líneas argentinas de avena frente a infecciones naturales de *Puccinia graminis* en 2022 y 2023, en la Estación Experimental J. Hirschhörn, Los Hornos, Argentina.

Lines	Severity (%) 2022		Severity (%) 2023		Lines	Severity (%) 2022		Severity (%) 2023	
BV 50-16	10	bc	12,5	cd	FA 21	25	ghi	17,5	def
BV 374-19	17.5	def	17,5	def	FA 19	22,5	fgh	20	efg
BV 108-19	10	bc	15	cde	FA 39	22,5	fgh	32,5	jkl
BV 21-16	12.5	cd	32,5	jkl	BW 14-10	22,5	fgh	32,5	jkl
BV 449-19	60	o	32,5	jkl	BW 12-15	22,5	fgh	50	n
BV 99-19	40	m	32,5	jkl	BW 16-15	22,5	fgh	32,5	jkl
BV 131-19	60	o	17,5	def	BW 16-28	22,5	fgh	17,5	def
FA 51	22.5	fgh	2,5	a	BW 18-58	22,5	fgh	17,5	def
FA 53	22.5	fgh	15	cde	BW 15-108	22,5	fgh	15	cde
FA 45	22.5	fgh	15	cde	BW 15-113	22,5	fgh	15	cde

*Different letters show significant differences in severity between the years and genotypes treatments, LSD $P < 0.05$.

* Letras diferentes muestran diferencias significativas en severidad entre los años y genotipos de tratamiento, LSD $P < 0,05$.

DISCUSSION

Oat stem rust is a destructive disease that has recently emerged as a significant threat to oat cultivation in some areas of Argentina, making a comprehensive evaluation of local germplasm crucial for resistance breeding programs and effective disease management strategies. This study assessed 58 Argentine oat genotypes against the prevalent *Pg* races identified in Argentina during 2021, 2022, and 2023. Resistance gene postulation revealed the presence of *Pg10+Pga* in Rocío INTA and B.I. Payé, *Pg10+Pg3* in Máxima INTA; *Pga* in Aurora INTA, B.I. Canaí, U16, Milagros INTA, Graciela INTA, Carlota INTA and B.I. Aikén; *Pg10* in INIA Polaris, Liliana INTA, María INTA and B.I. Lola; and *Pg9* in B.I. Calén and B.I. Maja. Notably, *Pg2*, *Pg4*, *Pg8*, *Pg15*, and *Pg16* were not detected in any of the cultivars. Moreover, the use of races from the current *Pg* population confirmed the presence of *Pg10*, *Pga*, *Pg9*, and *Pg15* in breeding lines. Identifying and characterizing these resistance genes in Argentine genotypes is valuable for understanding the genetic resources within the national germplasm and for establishing optimal breeding strategies. By examining the genes present in breeding germplasm, breeders can identify sources of resistance with diverse genetic backgrounds, thereby enhancing genetic diversity and improving the resilience of oat cultivars against evolving *Pg* races. However, our findings suggest that certain genotypes harbor resistance genes that could not be fully characterized using the current set of *Pg* differential lines available in Argentina, underscoring the need for further research to determine whether these factors correspond to previously described genes or represent novel resistance mechanisms. In this context, given the proven effectiveness of *Pg6* in China (24) and its success in Brazil (18), future studies should evaluate its effectiveness against local *Pg* races in Argentina and its potential use in oat breeding programs. Furthermore, we evaluated the seedling resistance of commercial oat cultivars released over more than 20 years ago against *Pg* populations, and the results reveal a notable temporal shift in oat cultivar resistance. In 2005, most oat cultivars exhibited resistance to the majority of *Pg* races, with Rocío INTA and B.I. Payé resisting all ten races tested, and others resisting to between six and nine races. However, when tested against the 2021-2023 *Pg* population (twelve races selected based on their high frequency in Argentine surveys), the resistance of these older cultivars declined markedly. Only cultivars, such as B.I. Maja and B.I. Calén,

retained resistance to a limited number of races, while others increased susceptibility, with Violeta INTA and Cristal INTA becoming completely susceptible to all tested races. These findings confirm a clear shift in *Pg* population dynamics, whereby newly emergent races have overcome resistance genes (e.g. *Pg9*, *Pg10*, *Pga*) that were once effective, rendering older cultivars vulnerable. We suggest that reliance on a narrow set of cultivars or resistance genes in Argentine germplasm has promoted the continuous selection of *Pg* phenotypes virulent to those genes, a pattern also reported in *Puccinia coronata* populations (Dietz *et al.* 2023). Likewise, Meldrum and Oates (1997) documented that the release of oat cultivars carrying the *Pg12* gene (also known as *Pga*) in Australia during the early 1990s resulted in the emergence and increased frequency of isolates virulent to this gene. In South Africa, the deployment of cultivars with certain *Pg* genes increased the frequency of isolates virulent to *Pg9* and led to the first report of virulence to *Pg13* (4). In Argentina, the introduction and widespread adoption of genotypes carrying *Pg* genes, sourced from the Quaker Oat Nursery, either as cultivars or parental lines in germplasm, combined with large-scale cultivation, likely promoted cycles of selection and displacement of virulence phenotypes, consistent with Person's conceptual model (31). This phenomenon of directional selection occurs when pathogen isolates virulent to deployed resistance genes gain a selective advantage (survival, reproduction, spread), increasing their frequency in the population and ultimately leading to the breakdown of previously effective resistance genes (7, 8, 12).

The results of seedling resistance testing against the current *Pg* population indicate that modern oat cultivars possess limited resistance: in fact, 98% of host-pathogen interactions showed susceptible reactions, highlighting the vulnerability of current oat germplasm to stem rust. Similarly, resistance was generally low among the tested lines, except for line FA 45, which exhibited resistance to four *Pg* races.

Moreover, this study evaluated resistance under field conditions against natural *Pg* infections in 2022-2023. We observed differences in disease levels between those years, manifested in varying severity values. Environmental conditions play a critical role in disease development, with factors such as temperature, humidity, and rainfall influencing pathogen propagation and host susceptibility (30). Differences in weather patterns between 2022 and 2023 may have contributed to the higher disease pressure observed in 2023, thereby leading to greater severity levels. Despite higher precipitation in 2023, mean temperatures were substantially higher at the end of the growing cycle in 2022, which may explain why differences in mean severity between both years were modest.

The *year × genotype interaction* reflected differential performance of cultivars between 2022 and 2023. For example, Milagros INTA, B.I. Payé, B.I. Aikén, B.I. Yapa, and Lucía INTA showed higher severity in 2022, whereas Aurora INTA, Carlota INTA, Liliana INTA, B.I. Serena, and B.I. Lola exhibited significantly higher severity in 2023. These results suggest shifts in *Pg* race prevalence and virulence between the two years. It is plausible that certain oat cultivars may display higher susceptibility to the prevalent races each year leading to differences in disease severity. Additionally, the emergence of new pathogen races with distinct virulence profiles in 2023 could have further exacerbated disease severity for some genotypes, as they may lack effective resistance mechanisms against these novel races. Our findings also indicate that resistance observed in some genotypes at the seedling stage under greenhouse conditions may explain their favorable behavior in certain growing seasons.

Cultivars such as María (60 and 95% severity in 2022 and 2023), Pía INTA (60 and 80%), and Violeta INTA (60 and 80%) exhibited the highest severity levels across both years. In contrast, cultivars such as Máxima INTA, INIA Tucana, INIA Polaris, U16, B.I. Maná, La Plata FA, and lines Bv50-16 and Bv108-18 consistently showed severity levels below 20%, highlighting their potential value under Argentine agronomic conditions. Their demonstrated resistance suggests the presence of inherent genetic traits conferring durable protection against stem rust, consistent with the concept of non-race specific resistance or adult plant resistance (APR). Unlike race-specific resistance, APR offers a broader and more durable spectrum of protection against pathogens (22). Although the sources of stem rust include *Pg11* and *Pg17* (16), genotypes carrying *Pg17* have been reported to exhibit high disease severity levels (70-90%) in field trials conducted in Canada (36). Given the complex nature of disease resistance (shaped by genetic, environmental, and pathogen factors) continued multi-environment evaluations are recommended to assess the stability and performance of resistance traits under diverse conditions.

CONCLUSION

This study provides new insights into the response of oat genotypes under stem rust pressure in Argentina and identifies promising cultivars and lines. Such information is critical for breeding programs and integrated crop management strategies. Moreover, our findings underscore the need to expand efforts to discover new resistance genes and to deepen understanding of the APR genetic mechanisms underlying resistance to stem rust in oats.

SUPPLEMENTARY MATERIAL

<https://drive.google.com/file/d/1hXxUiB2ctCdVh7X8Vgkuj9axoxLutjDY/view?usp=sharing>

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ACKNOWLEDGEMENTS

We wish to thank Mauro Desch and Jesús Asencio, staff from the rust laboratory belonging to the National Institute for Agricultural Technology (INTA) in Bordenave, Argentina.

STATEMENTS AND DECLARATIONS

The authors declare that they do not have any actual or potential conflict of interest.

FUNDING

This study was funded by projects from ANPCyT (PICT-2021-I-A-00770), UNLP (A352-19 and A 393-25), and CICPBA (RESO 2022-517-GDEBA-CIC).