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Evolution of the *Puccinia coronata* population in Argentina and identification of resistance genes useful in oat breeding programs

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Abstract Crown rust caused by *Puccinia coronata* f. sp. *avenae* (*Pc*), is the most widespread and damaging disease in oat worldwide, causing significant losses in grain yield and quality. This research documents the evolution of the *Pc* population in Argentina and evaluates the potential of crown rust race-specific resistance genes for use in practical breeding in this region. For this, the virulence of 166 isolates of *Pc* collected in different locations of Argentina were characterized on a set of 9 oat differentials carrying *Pc* resistance genes. In total, 31 different races were found. Changes in the race population during the 2014–2021 period were observed with *Pc* populations in 2019 and 2021 being more complex and having a higher virulence frequency. This suggests that the use of a few genes (*Pc38, Pc39, Pc50*, and *Pc51*) as basis for resistance to crown rust in the Argentinian germplasm has resulted in the continuous selection of *Pc* phenotypes with virulence to these genes. In this sense, our results showed an increase in the frequency of virulence on *Pc*38 and *Pc*51 from 2014 to 2019–2021, reaching virulence frequencies greater than 80%. Likewise, *Pc39* and *Pc50* also exhibited frequencies higher than 80% in 2019, showing significantly higher values determined in previous work carried out with populations of *Pc* in Argentina. There were no races virulent on *Pc48*, *Pc52* and *Pc64* among the isolates collected. Furthermore, our data confirmed the effectiveness of these three genes in field trials. Data provided here may be helpful in making decisions on resistance breeding strategies, such as the deployment of major single genes or more complex gene pyramids.

Keywords Crown rust · *Pc* genes · Seedling resistance · *Avena sativa* · Physiologic specialization

Abbreviation
(*Pc*) *Puccinia coronata*

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Introduction

Crown rust caused by *Puccinia coronata* f. sp. *avenae* P. Syd & Syd *(Pc)*, is the most widespread and damaging disease in oat (*Avena sativa* L.) worldwide (Carson, 2008; Ohm & Shaner, 1992; Simons, 1985), causing significant losses in grain yield and quality (Doehlert et al., 2001; Holland & Munkvold, 2001; Gnanesh et al., 2015; Dietz, 2018; Dietz et al., 2019). In regions of South America, populations are highly complex and specialized, presenting high genetic variability for virulence (Campos et al., 2008; Dietz, 2018; Leonard & Martinelli, 2005; Vieira et al., 2007). Although there is no evidence that *Pc* have sexual reproduction in this area (Martinelli, 2000; Leonard & Martinelli, 2005), the genetic diversity in the populations is very high. Numerous works document its appearance in a large area of the Argentinian cereal zone (Campos et al., 2008; Dietz, 2018; Dietz et al., 2016, 2019; Wehrhahne & Storm, 2014;) being observed during the autumn, coinciding with the period of use as fodder and during the spring, affecting the oats destined for grain production. Argentina, Pérez & Molas (2000) report losses of up to 32% in biomass above ground and 26% in grain yield, while Wehrhahne (2008) mentions yield drops of more than 70% in susceptible genotypes in years of high disease incidence. Likewise, Dietz et al. (2016) showed that foliar diseases produced by foliar pathogens (mainly *Pc*) caused losses of 29.7% in biomass and 41% in yield, explained by reductions in the leaf area index (increases in severity due to foliar necrosis or accelerated death of tillers), in the growth rate of the crop and in the yield components. Furthermore, Dietz (2018) found a negative association between disease intensity (measured by % severity and area under the disease progress curve) and grain yield and quality parameters. Dietz et al. (2019) indicated that the magnitude of yield losses is determined by the resistance of the genotypes.

Host resistance is considered the most effective, economical, and environmentally friendly control method. Host–pathogen interaction in the oat-*Puccinia coronata* pathosystem is mainly based on a gene-for-gene relationship. However, the main limitation of this resistance is its low durability (Chong & Kolmer, 1993). In general, resistance determined by one or a few genes can be overcome by new pathogenic races that arise by mutation or migrate from other localities, and then increase in frequency due to strong selection pressure from the use of widespread genotypes containing these genes in large areas (Harder & Haber, 1992). New sources of resistance quickly become ineffective due to changes in pathogen virulence (Chong & Zegeye, 2004; Leonard, 2003). Despite this problem, interest in major gene resistance remains strong because crown rust control is very effective prior to the build-up of virulent individuals in the *Pc* population.

The oat growing regions of Argentina, Brazil, and Uruguay share a common epidemiological system (Campos et al., 2008; Leonard & Martinelli, 2005). The primary source of inoculum to infect oat in the fall comes from urediniospores produced on volunteer oat plants that survive the summer at the edges of fields of summer crops, in fence rows, and along roadsides. Prevailing wind patterns annually distribute urediniospores of *Pc* in a cyclical pattern throughout the whole growing region of these countries. Therefore, it might be expected that some races in the *Pc* population in Argentina be similar in their virulence pattern to races in Brazil and Uruguay. In this sense, Leonard & Martinelli (2005) found no evidence of distinct geographically separated populations of *Pc* within the states of southern Brazil and Uruguay. Previous works have provided valuable insights regarding the variability and the complexity of *Pc* populations in Brazil (Leonard & Martinelli, 2005; Vieira et al., 2007), Uruguay (Leonard & Martinelli, 2005) and Argentina (Campos et al., 2008), although most of them have been carried out with isolates collected more than 15 years ago. The characterization of the *Pc* population not only provides information about population size, diversity, and complexity but also allows for the identification of race specific genes potentially useful for breeding, which is essential to guide regional breeding programs and/or establish management guidelines that tend to reduce the losses caused by this disease. In this work we collected *Pc* isolates from a vast area of Argentina during 2014–2021, with the aims of i) examining the effectiveness of the *Pc* resistance genes that have been extensively used in the Argentinian germplasm and ii) studying the pathogen population evolution on these *Pc* genes.

Materials and methods

Seedling resistance response

Samples of oat leaves infected with *Pc* were collected during 2014, 2016, 2017, 2019, and 2021 (November) from experimental and farmer´s fields in different locations of Argentina [supplementary material; table S1)]. Each year after collection in November, samples were stored in a fridge at 4–6 °C and inoculated in May of the next season to carry out the virulence tests. The collection included 166 isolates, depicting for 25 in 2014, 54 in 2016, 31 in 2017, 18 in 2019, and 38 in 2021. A higher number of isolates was collected when the intensity of the disease was greater. For this reason, not all areas in Argentina were represented by isolates in each year.

Virulence tests were conducted at the Rust laboratory belonging to the National Institute for Agricultural Technology (INTA) in Bordenave, Argentina (37º 52' LS; 63º 01' LW), during the winter months. Urediniospores from each sample of rusted oat leaves were harvested using cotton swabs. They were immersed in lightweight mineral oil (Soltrol 170), and with an upward movement urediniospores were collected and then inoculated (using the same technique) on seedlings of the oat cultivar "Boyera", which is susceptible to all known races of *Pc* at seedling stage. The seedlings were left for 30 min to allow the oil to evaporate and then placed in a dew chamber where they were sprayed with a fine mist of sterile water and incubated at 18–22 °C for 20 h with a relative humidity close to 100%. When uredinia appeared, the Boyera seedlings were trimmed to leave one leaf with a single uredinium. Trimmed seedlings bearing single uredinia were then placed in polyethylene isolation cells in the greenhouse for several days to allow further sporulation in each isolated single uredinium. Urediniospores were then collected to establish one single-uredinial isolate per collection of rusted oat leaves. Each single-uredinial isolate was increased through one uredinial generation on Boyera oat seedlings in small plastic-covered isolation cages.

Each isolate was tested for virulence on a set of 9 *Pc* differentials (*Pc*35, *Pc*38, *Pc*39, *Pc*48, *Pc50, Pc51, Pc52, Pc64* and *Pc67*), each with a different single *Pc* gene for race specific crown rust resistance. The collection has been used in Argentina for a long time to characterize the *Pc* population and postulate genes in genotypes of interest (Campos et al., 2008). Several of these genes have already been documented in the Argentinean oat germplasm as conferring resistance to the *Pc* population (Dietz, 2018). For that reason, our aim was to evaluate if they were still effective due to possible changes in the *Pc* population.

Sowing of each *Pc* line was made in individual pots of 200 cm^3, with loam soil of good fertility. At 10–12 days after planting (when the seedlings had two expanded leaves; GS12, Zadoks et al., 1974), the seedlings in the differential set were inoculated with freshly collected urediniospores of a single-uredinial isolate. For inoculation a subsample of 2 mg of each *Pc* isolate was suspended in Soltrol 170 mineral oil (used as a vehicle) up to a total of 50 mg, which was placed in a gelatin capsule (1/25 of its weight concentration), mixed and sprayed using an air compressor and spray nozzle. Inoculated plants were placed in a 100% humidity dark chamber for 20 h at 18 to 22ºC for spore germination and penetration. After incubation, seedlings were placed on benches in the greenhouse at 18–22 °C and 60–80% relative humidity.

Disease reactions were scored on primary leaves at 12 to 15 days after inoculation according to a scale from 0 to 4, where 0 = absent 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. Responses 0, 1, and 2 were considered indicative of host resistance (low infection type) and responses 3 and 4 were considered indicative of host susceptibility (high infection type) (Murphy, 1935).

Data analysis

Races/isolates of *Pc* were determined taking into account the virulence/avirulence patterns on the 9 *Pc* differentials used in this study. For this, each isolate with a similar virulence/avirulence pattern, regardless of the year, received the same letter. Table 1 summarizes the isolates identified and the number of times they were found per year. Virulence parameters were described according to the Kosman approach (Kosman, 1996, 2003). The virulence within the pathogen population (virulence frequency) was calculated based on the average of the proportion of all *Pc* genes overcome by each isolate on the *Pc* set differential

Table 1 Virulence/avirulence pattern of the races identified and number of times found per year

Races	Virulence	Avirulence	Year					
			2014	2016	2017	2019	2021	Total
a	*Pc35-Pc38-Pc39-Pc50-Pc51-Pc67*	***Pc48-Pc52-Pc64***	2	5	2	13	11	**33**
b	*Pc35-Pc39-Pc50-Pc51-Pc67*	*Pc38-**Pc48-Pc52-Pc64***	6	14	16		4	**40**
c	*Pc35-Pc50-Pc51-Pc67*	*Pc38-Pc39-**Pc48-Pc52-Pc64***	2	3			3	**8**
d	*Pc35-Pc39-Pc50-Pc67*	*Pc38-**Pc48**-Pc51-**Pc52-Pc64***	1		4			**5**
e	*Pc35-Pc50-Pc67*	*Pc38-Pc39-**Pc48**-Pc51-**Pc52-Pc64***	9					**9**
f	*Pc35-Pc39-Pc67*	*Pc38-**Pc48**-Pc50-Pc51-**Pc52-Pc64***	2					**2**
g	*Pc35-Pc67*	*Pc38-Pc39-**Pc48**-Pc50-Pc51-**Pc52-Pc64***	1					**1**
h	*Pc35-Pc38-Pc39-Pc51-Pc67*	***Pc48**-Pc50-**Pc52-Pc64***	2	5	3		7	**17**
i	*Pc35-Pc38-Pc39-Pc51*	***Pc48**-Pc50-**Pc52-Pc64**-Pc67*		6	2			**8**
j	*Pc35-Pc39-Pc50-Pc67*	*Pc38-**Pc48**-Pc51-**Pc52-Pc64***		1				**1**
k	*Pc35-Pc50-Pc67*	*Pc38-Pc39-**Pc48**-Pc51-**Pc52-Pc64***		3				**3**
l	*Pc35-Pc39-*	*Pc38-**Pc48**-Pc51-**Pc52-Pc64**-Pc67*		1				**1**
m	*Pc35-Pc39-Pc50-Pc67*	*Pc38-**Pc48**-Pc51–**Pc52-Pc64***		1				**1**
n	*Pc35-Pc39-Pc51-Pc67*	*Pc38-**Pc48**-Pc50-**Pc52-Pc64***		5	2			**7**
ñ	*Pc35-Pc50-Pc51*	*Pc38-Pc39-**Pc48-Pc52-64-**Pc67*		2				**2**
o	*Pc35-Pc38-Pc51-Pc67*	*Pc39-**Pc48**-Pc50-**Pc52-Pc64***		1			1	**2**
p	*Pc35-Pc51*	*Pc38-Pc39-**Pc48**-Pc50-**Pc52-64-**Pc67*		1				**1**
q	*Pc51*	*Pc35-Pc38-Pc39-**Pc48**-Pc50-**Pc52-64-**Pc67*		1				**1**
r	*Pc35-Pc38-Pc67*	*Pc39-**Pc48**-Pc50-Pc51-**Pc52-Pc64***		1				**1**
s	*Pc35-Pc39-Pc50-Pc51*	*Pc38-**Pc48-Pc52-Pc64**-Pc67*		4		1		**5**
t	*Pc35-Pc38-Pc39-Pc51*	***Pc48**-Pc50-**Pc52-Pc64**-Pc67*			1	1		**2**
v	*Pc35-Pc38-Pc50-Pc67*	*Pc39-**Pc48-Pc52-Pc64***			1	1	7	**9**
w	*Pc35-Pc39-Pc51*	*Pc38-**Pc48**-Pc50-**Pc52-Pc64**-Pc67*				1		**1**
y	*Pc35-Pc38-Pc50-Pc67*	*Pc39-**Pc48**-Pc51-**Pc52-Pc64***				1		**1**
z	*Pc35-Pc38-Pc50-*	*Pc39-**Pc48**-Pc51-**Pc52-Pc64**-Pc67-*					1	**1**
za	*Pc35-Pc38-Pc39-Pc50-Pc51-*	***Pc48-Pc52-Pc64**-Pc67-*					1	**1**
zb	*Pc38-Pc50-Pc51-Pc67*	*Pc35-Pc39-**Pc48-Pc52-Pc64***					1	**1**
zc	*Pc38-Pc50-Pc51-Pc67*	*Pc35-Pc39-**Pc48-Pc52-Pc64***					1	**1**
zd	*Pc35-Pc50*	*Pc38-Pc39-**Pc48**-Pc51-**Pc52-Pc64**-Pc67-*					1	**1**
		Total	**25**	**54**	**31**	**18**	**38**	**166**

The Pc genes marked in bold were resistant (showed avirulence) against the races found in this work

(Supplementary Table S1). Results were subjected to the Kruskal–Wallis one way analysis of variance appropriate for data with distributions far from normal with the mean ranks post hoc comparison, using Infogen Software (Balzarini & Di Rienzo, 2016), to determine if there were significant differences in the virulence through the years. The virulence complexity was calculated as the mean number of virulence genes per isolate detected over the nine differential lines. In addition, frequency of virulence to each *Pc* differential was calculated as Frequency of virulence = (NVI/NTC)*100; where NVI is the number of times that a virulent reaction type was detected, and NTC is the number of all tested isolates collected each year.

Field evaluation for crown rust response

The resistance of the 9 *Pc* differentials was evaluated in the field against natural infections of *Pc* in different environments [1-INTA Bordenave in 2014, 2017 and 2021 (*Bv2014*, *Bv2017* and *Bv2021*); 2- J. Hirschhörn-Los Hornos Experimental Station, UNLP

(*Lp2021* and *Lp2022*)], which have been chosen for their contrasting conditions for the development of the disease. The experiment was sown using a split plot design, with the environments as the main plots and genotypes as the sub-plot, with three repetitions. The sowing was carried out in June under a conventional tillage system, where the genotypes were randomly sown in rows of two meters, spaced at 30 cm, with a sowing density of 200 pl.m^{-2}. A row with a susceptible check ("Boyera") was sown every 3 genotypes to help in the inoculum multiplication.

Disease severity was assessed by visual estimation (expressed as a percentage of diseased leaves over the total area) in GS82 (milky state, Zadoks et al., 1974), taking 7 to 10 plants at random from each plot. The flag leaf was used for the evaluation because it allowed for the most visible differences. Data were analyzed using the software Info gen (Balzarini & Di Rienzo, 2016) analysis of variance for split plots and the means were compared using the LSD test (P = 0.005).

Samples of oat leaves infected with *Pc* were collected in the field trials in Bordenave (Bv2014, Bv2017 and Bv2021) and La Plata (Lp2021 and Lp2022), and then assessed in the greenhouse (*similar to:2–1.Seedling resistance response*), for checking the predominant races at each location (Supplementary Table S2).

Results

Virulence/avirulence formula of isolates collected in Argentina in 2014, 2016, 2017, 2019 and 2021 are presented as supplementary material (Supplementary Table S1). In total, 31 different races were found among the 166 single-uredinial isolates collected during the 2014–2021 period (Table 1). In 2014, 8 races were identified from a total of 25 isolates (Supplementary Table S1). The race designated as "e" (*Pc35-Pc50-Pc67/Pc38-Pc39-Pc48-Pc51-Pc52-Pc64*) was the most frequent, while "d" and "g" were found only once (Table 1). In 2016, 16 races from 54 isolates were determined, with the race "b" being the most frequent *(Pc35-Pc39-Pc50-Pc51-Pc67/Pc38-Pc48-Pc52-Pc64)* collected 14 times, while other races (j, l, m, o, p, q, and r) were found only once. In 2017 from 31 isolates, 8 races were identified, with the race "b" being again the most frequent while "t" and "v" were found once. In 2019, 6 races were found between 18 isolates evaluated, and the race "a" *(Pc35-Pc 38-Pc 39-Pc50-Pc51-Pc67/ Pc48-Pc52-Pc64)* was the most frequent (13 times). Finally, in 2021 from 38 isolates, 11 races were identified, and the "a" race was also the most predominant (11 times).

Races "b", "a", and "h" were the most common phenotypes during the evaluated period (40, 33 and 17 times, respectively; Table 1). In the case of race "b", it was the most frequent in 2016 (14 times in 54 total isolates = 25%) and 2017 (16/31 = 51%), while race "a" predominated in 2019 (13/18 = 72%) and 2021 (11/38 = 29%). Likewise, analysing the dynamics of race "b", it showed a great change from 2016 (25%) to 2017 (51%), while in race "a" it was even greater, from 6.5% in 2017 to 72% in 2019.

The Kruskal–Wallis one-way variance analysis revealed statistically significant differences between the virulence of *Pc* populations collected in these years ($p < 0.001$). The mean ranks post hoc comparison revealed that isolates in 2014 were able to overcome an average of 44.5% of oat differential resistance lines (between 22 and 66%), which is similar to the 47% of the average in 2016 (between 11 to 66%), but statistically different from the rest of the years. The virulence frequency of the populations has increased in the last three years. In 2019, the average increased to 60.5%, a significant increase from 2017 (53.3%) but no difference from 2021 (55.3%). The level showed a range between 44 and 66% in these three years (2017, 2019, and 2021) (Fig. 1).

The Table 2 illustrates differences in the levels of virulence against reference genes. *Pc* isolates were able to overcome from 1 to 6 reference genes in all years. In 2014, most isolates (40%) were virulent on 3 *Pc* reference genes while only 8% were virulent on 6 *Pc*. In contrast, in 2019, most of isolates (72%) were able to overcome 6 *Pc* reference genes while 6% were only virulent on 3 *Pc* genes (Table 2).

There was no isolate that was virulent to *Pc*48, *Pc*52, or *Pc*64 (Table 3). Gen *Pc*38 was also very effective during 2014, 2016, and 2017 (16, 33 and 29% respectively), showing a severe increment in 2019 (83%), a similar response to *Pc*51 which increased from 48% in 2014 to 95% in 2019 and 2021. *Pc*39 and *Pc*50 also exhibited frequencies higher than 80% in 2019. The frequency of virulence on *Pc*35 and *Pc*67 was high and stable in all evaluated years

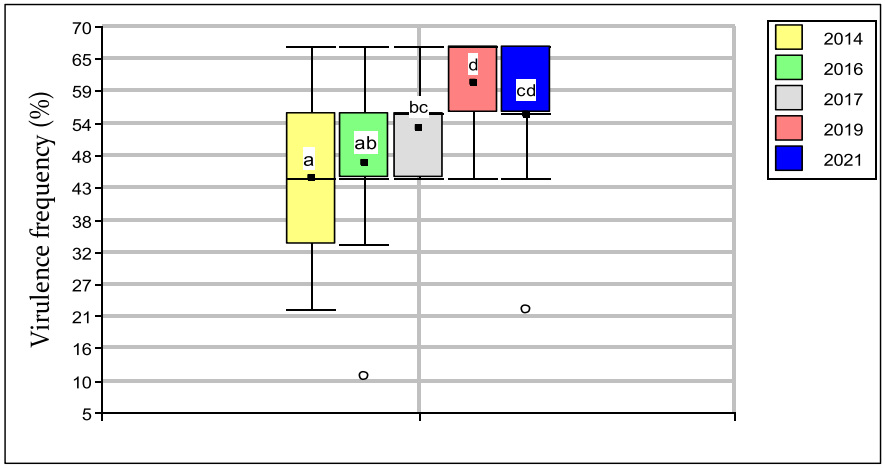


Fig.1 Box plot of virulence frequency of the *Puccinia coronata* isolates collected in Argentina during 2014, 2016, 2017, 2019 and 2021 on different *Pc* resistance genes.[Symbols in the figure represent; (—) Median; (▭) 25–75%, (○) Outliers]. Different letters show significant differences in the mean between years. LSD $p < 0.05$ (Kruskal Wallis, post hoc comparison)

Table 2 Percentage of isolates virulent on differential lines carrying *Pc* genes in *Puccinia coronata* populations collected in Argentina during 2014, 2016, 2017, 2019 and 2021

Number of *Pc* Genes	Percentage of isolates virulent per year (%)				
	2014	2016	2017	2019	2021
1 *Pc* gen	0	2	0	0	0
2 *Pc* genes	4	4	0	0	3
3 *Pc* genes	40	17	0	6	3
4 *Pc* genes	16	31	29	17	18
5 *Pc* genes	32	39	65	6	49
6 *Pc* genes	8	7	6	72	28
7 *Pc* genes	0	0	0	0	0
8 *Pc* genes	0	0	0	0	0
9 *Pc* genes	0	0	0	0	0
Total	100	100	100	100	100

(between 90–100%), with the exception of 2016 for *Pc*67 (72%) Table 3.

The field evaluation for crown rust response showed three environments with high disease levels [*Lp2021* (54%); *Bv2014* (49%) and *Bv2017* (32%)], while the remaining two [*Bv2021* and *Lp2022*] exposed low severity levels ($<=6\%$), not differentiating from each other (data not shown). The severity showed a wide range of values for *Bv2014* (86.7 to 0%); *Bv2017* (75 to 1%) and *Lp2021* (100 to 0%), allowing a clear differentiation of *Pc* genes resistant from those susceptible; and a narrow for *Bv2021* (16.7 to 0%) and *Lp2022* (12.7 to 0%). In those environments with a high level of disease, differential lines carrying *Pc*48, *Pc*52 and *Pc*64 were stood out for presenting % severity significantly lower than those of the susceptible control (Boyera) and the other *Pc* genes evaluated (Fig. 2). In this sense, *Pc*64 did not show signs of disease (0%) in *Bv2014*, while *Pc*48 and *Pc*52 showed a severity of 20%, significantly lower than the susceptible control, which reached 86.7%. In *Bv2017*, *Pc*52, *Pc*64 and *Pc*48 showed lower severity (1, 4 and 10% respectively) than *Pc*38 (75%). On the other hand, in *Lp2021* *Pc*64, *Pc*48 and *Pc*52 showed values of 10, 5 and 0% respectively, while Boyera reached 100% severity. The remaining *Pc* as well as Boyera reached high % severity, showing differences between genotypes and years. *Pc*35 presented a greater severity in *Bv2014* (53%) than in *Bv2017* and *Lp2021* (30%), while *Pc*39 showed the same trend, with a greater severity in *Bv2014* (80), followed by *Bv2017* (72%) and *Lp2021* (60%). *Pc*38 and *Pc*50 presented severity values greater than 50% in the three environments, while for *Pc*51 they were less than $<50\%$. For its part, *Pc*67 showed a low % severity (15) in *Bv2017*, compared to significantly higher values in *Bv2014* and *Lp2021* (55 and 90% respectively).

The collection of isolates in the field trials allows identifying the predominant races at each location (Supplementary Table S2). In this sense, races "b" and "d" were the most common in *Bv2014*, while in *Bv2017* and *Bv2021* races "a", "b" and "d" were identified. For its part, in *Lp21* races "a" and "h"

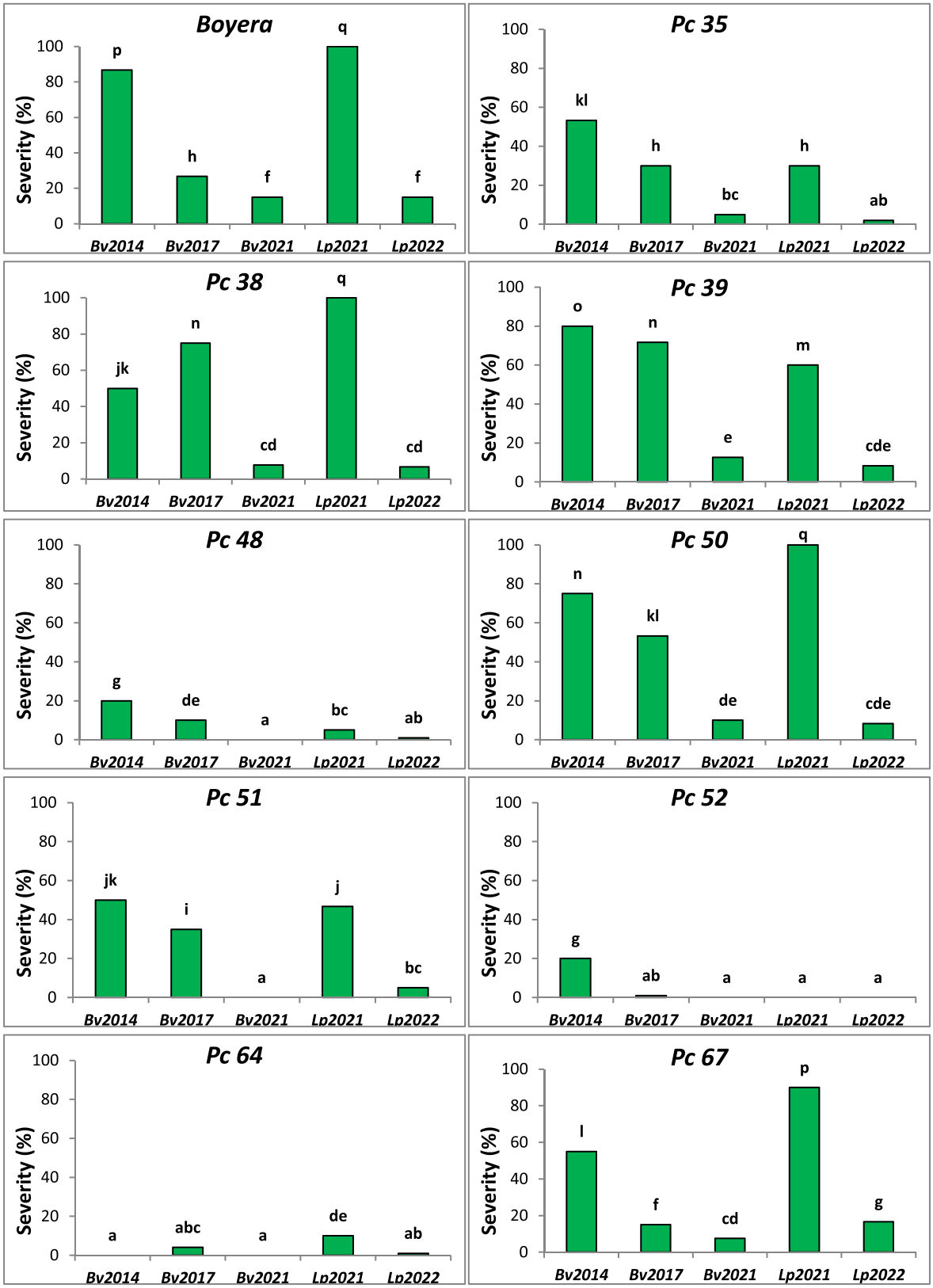
were found in equal proportion while in *Lp22* race "a" was the predominant.

Discussion

This research documents for the first time the structure and complexity of *Pc* populations based on 166 isolates collected in different sites in Argentina during 2014, 2016, 2017, 2019, and 2021. We found a great level of diversity in the *Pc* population as indicated by the 31 phenotypes (races) identified. The use of these 9 *Pc* differentials allows us a preliminary race designation, although some of the races identified could be different if we had used a wider *Pc* differential genotypes (cultivars, lines, etc.). In this sense, Dietz (2018) evaluating 32 *Pc* isolates collected in Argentina with these 9 *Pc* differentials found 16 races, while expanding the response on a set of cultivars allowed the differentiation of 29 races. The reasons for the high level of virulence polymorphism in *Pc* are not clear. Different authors (Martinelli, 2000; Leonard & Martinelli, 2005) have manifested that the genetic diversity in South American *Pc* populations is very high, although there is no evidence that *Pc* have sexual reproduction in this region. The explanation may be found in a high mutation rate, which can be conserved by having the same main host throughout the year. This can be attributed to the existence of a large region established of southern Brazil, Argentina and Uruguay, where oats are cultivated most of the year and during the interval between successive oat crops and also for the presence of volunteer oat plants (Leonard & Martinelli, 2005; Vieira et al., 2007). Additionally, the polymorphisms are maintained by some form of balancing selection or that genetic drift has little or no influence on the pathogen populations there (Leonard & Martinelli, 2005).

Likewise, changes in race dynamics during the 2014–2021 period were detected. In 2014, the race designated as "e" (virulent on *Pc35-Pc50-Pc67)* was the most frequent until race "b" (virulent on *Pc35, Pc39, Pc50, Pc51, Pc67*) became the most common in 2016 and 2017. In 2019, race "b" declined, and race "a" (virulent on *Pc35-Pc38-Pc39-Pc50-Pc51-Pc67*) developed the most frequent. Thus, race "a" showed a great change from 6.5% in 2017 to 72% in 2019. The fluctuations in the race dynamics population described here are evidence that the use of a few genes (*Pc38, Pc39, Pc50*, and *Pc51*) as a basis for resistance to crown rust in the Argentinian germplasm has resulted in the continuous selection of *Pc* phenotypes with virulence to these resistance genes, similar to what Kolmar mentioned (1991) for the wheat-*Puccinia triticina* pathosystem. The release of resistant cultivars in Argentina has created cycles of selection and displacement of specific virulences similar to Person`s idealized model (Person, 1966). Under directional selection, advantageous isolation increases as a consequence of differences in survival and reproduction between different phenotypes. Our results prove that the extensive commercial production of cultivars with a few major resistance genes generates great selection pressure, causing an increment in the proportion of races virulent on these genes and/or the appearance of new virulent races (Kolmer, 1991; Carson, 2011; Chong et al., 2011).

We found that the majority of the Argentinian *Pc* isolates were able to overcome from 3 to 6 reference genes in all years. The virulence complexity showed the highest level in 2019; where most of the isolates (72%) were able to overcome 6 *Pc* reference genes while 6% were virulent on only 3 *Pc* genes. Though, in 2014, most isolates (40%) were virulent on 3 *Pc* reference genes while only 8% were virulent on 6 *Pc*. Numerous studies carried out in Brazil have indicated the existence of virulent physiological races of *Pc* where many isolates have more than 16 virulence genes per genotype (Leonard & Martinelli, 2005; Vieira et al., 2007). Leonard & Martinelli (2005) detected that the virulence complexity of isolates from southern Brazil and Uruguay was greater than the complexity of isolates from a Russian population. Additionally, the Kruskal–Wallis one-way variance analysis revealed differences between the virulence frequencies of *Pc* populations collected during the period 2014–2021. The mean ranks post hoc comparison exposed that isolates from 2014 and 2016 were less virulent than those collected in 2017, 2019, and 2021. Moreover, the results showed an increment in the level of virulence within the pathogen population in each year from 2014 (44%) to 2019 (60.5%) and 2021(55.3%), indicating that the use of widespread genotypes containing a few genes in large areas of Argentina causes a strong selection pressure (Harder & Haber, 1992), and is the driving force for the increase of virulence in the Argentinian *Pc* population. Brun et al. (2014) suggest that long-term trends


Boyera
Severity (%)
100
80
60
40
20
0
p
h
f
q
f
Bv2014
Bv2017
Bv2021
Lp2021
Lp2022
Pc 35
kl
h
bc
h
ab
Pc 38
jk
n
cd
q
cd
Pc 39
o
n
e
m
cde
Pc 48
g
de
a
bc
ab
Pc 50
n
kl
de
q
cde
Pc 51
jk
i
a
j
bc
Pc 52
g
ab
a
a
a
Pc 64
a
abc
a
de
ab
Pc 67
l
f
cd
p
g

◂**Fig. 2** Means of severity (%) for each *Pc* differential and Boyera (susceptible check) in five environments [Bordenave (*Bv*) 2014, 2017, 2021; La Plata (*Lp*) 2021, 2022). Means followed by different letters show significant differences through all treatments [*Genotypes* × *environment* ($p < 0.05$)]

in virulence level revealed that the continued deployment of resistant host lines over wide regions of the United States has generated selection for an increased host genotype range. Likewise, the authors also indicated that increments in the virulence level would come with a fitness cost, an increase in the number of virulent genes per pathogen correlated with significant delays in the onset of reproduction and smaller pustule size. Nevertheless, the increment in virulence frequency as well as the complexity displayed in the Argentinian population would indicate a low fitness cost for carrying the *Pc* virulent.

Several of the *Pc* genes used in this work have already been documented in the Argentinean oat germplasm as conferring resistance to the *Pc* population (Dietz, 2018). The author postulated genotypes with two genes [Máxima and Milagros (*Pc38* + *Pc39*); Maná (*Pc38* + *Pc51*)] with single genes, and without either gene. For that reason, it is important to know if they are still effective taking into account possible changes in the *Pc* population. Despite the fact, the values mentioned here are not comparable to those of other similar works carried out with populations from South America (Leonard & Martinelli, 2005), North America (Carson, 2011) and Central Europe (Paczos-Grzeda & Sowa, 2019; Sowa & Paczos-Grzeda, 2020), which used a larger set of reference genes, the information gathered in this work is relevant to understanding the evolution of Argentinian *Pc* populations as it was carried out over a wide period. Our results showed an increase in the frequency of virulence on *Pc*38 and *Pc*51 from 2014 to 2019–2021, reaching virulence frequencies greater than 80%. *Pc38* was quite effective in 2014, 2016 and 2017 (16–33%), but the increase in the frequency of the race "a" from 2019 significantly decreased its effectiveness. Similarly, *Pc*39 and *Pc*50 also exhibited frequencies higher than 80% in 2019, showing values significantly greater than those reported by Campos et al. (2008) for *Pc* population in Argentina. Additionally, as result of the increment in the frequency on the *Pc*38, *Pc*39, *Pc*50 and *Pc*51 the *Pc* population in 2019 and 2021 were more complex and virulent. Moreover, the high and stable frequency of virulence on *Pc*35 and *Pc*67 in all the evaluated years (between 90–100%), indicates little apparent fitness cost to carrying this unnecessary *Pc* virulence in the pathogen population, because these genes are not known to be used in any oat cultivars in Argentina.

Another valuable result of this study is the determination of some potential *Pc* race-specific resistance genes for use in practical breeding in Argentinian conditions. There were no pathotypes virulent toward *Pc*48, *Pc*52, and *Pc*64 among the tested samples within the isolates collected at different locations over a wide period and in field essays. However, this result is not conclusive evidence of the nonexistence of virulent isolates to these genes in Argentina. It is worth noting that a survey of *Pc* carried out by Campos et al. (2008) found detectable virulence to *Pc64*, while Dietz (2018) reported that a few isolates collected in 2015 in Argentina (Los Hornos, Buenos Aires) showed virulence to *Pc48, Pc52,* and *Pc64.*

Furthermore, field trials were carried out to identify the predominant races in two locations (with possible distinct *Pc* populations) and check for possible breaks in resistance. Our data confirmed the effectiveness of *Pc*48, *Pc*52 and *Pc*64 genes under field trials. In those environments with a high level of disease, these genes stood out for not showing signs of disease (pustules) or for presenting a severity significantly lower than the susceptible check and the other *Pc* genes evaluated. It is necessary to mention that although these genes show a resistant infection type in the seedling stage (reactions with flecks or small pustules surrounded by chlorosis or necrosis), there are some races in which this kind of reaction could be associated with low severity percentages in adult field evaluations. Additionally, our field trial results showed that the severity of *Pc* susceptible (*Pc35, Pc38, Pc39, Pc50, Pc51, Pc67*) is not exclusively linked to the virulence/avirulence predominant isolates in these localities and years.

The data gathered in this work in greenhouses and field essays suggests that *Pc*48, *Pc*52 and *Pc*64 might be useful genes in oat breeding. As it was mentioned genes *Pc48*, P*c52*, *Pc64* are important resistance genes as the most frequent races considering years and locations, (a, b, h, d) carries avirulent genes for them. However, Leonard & Martinelli (2005) have documented virulence to *Pc*48, *Pc*52 and *Pc*64 in populations collected in Brazil and Uruguay,

Table 3 Frequency of virulence (%) on 9 *Pc* references genes of *Puccinia coronata* isolates collected in 2014, 2016, 2017, 2019 and 2021 in Argentina

Pc line	Frequency of virulence (%)				
	2014	2016	2017	2019	2021
Pc35	100	98	100	94	92
Pc38	16	33	29	83	79
Pc39	52	78	97	83	59
Pc48	0	0	0	0	0
Pc50	80	63	74	89	79
Pc51	48	85	87	94	95
Pc52	0	0	0	0	0
Pc64	0	0	0	0	0
Pc67	100	72	90	83	92

indicating a potential risk of loss of effectiveness if they are used individually in Argentinian oat cultivars. Therefore, the existence of a common epidemiological zone, the great genetic diversity and the high rate of mutations in South American populations will make it unlikely that long-lasting control of crown rust can be obtained with race-specific resistance. For these reasons, resistance management is fundamental in breeding programs to avoid resistance breaks and loss of gene effectiveness.

Our results confirmed changes in the race dynamics of the population as a consequence of the use of a few resistance genes, an increment in the virulence frequency and the existence of complex *Pc* races in Argentina. Taking into account that oats are a crop of great importance in the region, and crown rust disease presents a common epidemiological system; it would be interesting to carry out a comprehensive study that allows a broader and proper characterization of the populations of several South American countries. Likewise, as we have reported in this work, the resistance genes used by each of these countries have a great impact on the evolution of their populations, and therefore differences between populations are to be expected.

Successful implementation of durable resistance strategies relies on an understanding of the biology, genetic diversity and adaptability of the pathogen, and thus it is essential to maintain surveillance of the pathogen population. Data from this paper may be helpful in making decisions on resistance breeding strategies, such as the deployment of major single genes or more complex gene pyramids, based on the absence of races virulent toward these genes. Additionally, oat breeding programs should consider shifting their efforts toward race nonspecific, partial forms of crown rust resistance.

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Declarations

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

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