New Races with Wider Virulence Indicate Rapid Evolution of *Puccinia striiformis* f. sp. *tritici* in the Southern Cone of America

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Abstract

Wheat yellow (stripe) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases of wheat worldwide. *Pst* populations are composed of multiple genetic groups, each carrying one or more races characterized by different avirulence/virulence combinations. Since the severe epidemics in 2017, yellow rust has become the most economically important wheat foliar disease in Uruguay. A set of 124 *Pst* isolates collected from wheat fields in Uruguay between 2017 and 2021 were characterized phenotypically, and 27 of those isolates were subsequently investigated in-depth by additional molecular genotyping and race phenotyping analyses. Three genetic groups were identified, *PstS7*, *PstS10*, and *PstS13*, with the latter being the most prevalent. Two races previously reported in Europe, Warrior (*PstS7*) and Benchmark (*Pst*S10), were detected in four and two isolates, respectively. A third race, known as Triticale2015 (*PstS13*), that was first detected in Europe in 2015 and in Argentina in 2017 was detected at several locations. Additional virulence to *Yr3*, *Yr17*, *Yr25*, *Yr27*, or *Yr32* was detected in three new race variants within *PstS13*. The identification of these new races, which have not been reported outside South America, provides strong evidence of the local evolution of virulence in *Pst* during the recent epidemic years.

Keywords: genotypic characterization, race typing, wheat, yellow (stripe) rust

Wheat yellow (stripe) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases of wheat worldwide (Beddow et al. 2015; Chen et al. 2014; Stubbs 1985; Wellings 2011). *Pst* is favored by relatively low temperatures of 10 to 15°C and may cause very significant grain yield losses in susceptible cultivars (Carmona et al. 2019; Chen 2005; Roelfs et al. 1992). Historically, *Pst* has mainly been a problem in cool climates; however, since 2000, the pathogen has gained increased tolerance to higher temperatures and become an increasing problem in areas normally considered too warm for *Pst* establishment (Milus et al. 2009; Wellings 2007). Moreover, *Pst* epidemics originating from distant geographical areas have been reported, either as an incursion to new regions where it was previously absent or as a re-emergence of new races with increased aggressiveness (Bahri et al. 2009; Hovmøller et al. 2023b). As a consequence, *Pst* epidemics have been an increasing problem threatening global wheat production (Ali et al. 2014; Boshoff et al. 2002; Hovmøller et al. 2016).

Pst was detected for the first time in Uruguay and Argentina in 1929 (Rudorf and Job 1931). During 1929 and 1930, *Pst* caused widespread epidemics through most of the Southern Cone region, causing extremely high yield losses (Boerger 1934; Vallega 1938). From its first detection and until 2016, *Pst* occurred sporadically, rarely reaching epidemic levels in Uruguay (Germán et al. 2007, 2018; Germán and Caffarel 1999). Since 2017, *Pst* has caused generalized epidemics in Uruguay and Argentina (Carmona and Sautua 2018; Germán et al. 2018, 2021). *Pst* is currently the most economically important wheat foliar disease, requiring the highest number of fungicide applications relative to other prevalent diseases. This is probably due to the earlier onset of the disease during the growing season and because more than 50% of the wheat area has been deployed with susceptible or moderately susceptible cultivars (Silva et al. 2023). To control *Pst*, farmers typically spray two fungicide applications each growing season to obtain adequate disease control. The most susceptible cultivar to *Pst* included in the National Cultivar Evaluation trials in Uruguay had grain yield losses ranging between 71 and 82% in 2017 (Germán et al. 2018). Additionally, in Argentina, *Pst* races have overcome the resistance of most of the locally adapted cultivars (Carmona et al. 2019).

Mutations and subsequent selection are considered the main driving forces that generates new races with virulence to the deployed host resistance genes (de Vallavieille-Pope et al. 2012; Hovmøller and Justesen 2007). Sexual recombination is another mechanism that may generate variability in *Pst*, which has been reported under experimental conditions involving the alternate host *Berberis* spp. (Rodriguez-Algaba et al. 2014, 2020); however, it is rarely reported under natural conditions. As these secondary hosts are not reported in Uruguay, sexual recombination is thought to have no impact as a source of new variability locally, leading to the assumption of a strongly clonal *Pst* population in Uruguay as reported for other regions (Ding et al. 2021; Hovmøller et al. 2002).

Pst races are characterized phenotypically through their avirulence/virulence pattern to *Yr* resistance genes. Additionally, the *Pst* genetic diversity can be studied using microsatellite molecular markers to assign the different *Pst* isolates to different genetic groups (Ali et al. 2017; Bai et al. 2021; Sharma-Poudyal et al. 2020; Walter et al. 2016), allowing a better understanding of the evolutionary and dispersal dynamics of the pathogen (Ali et al. 2014; Ding et al. 2021; Thach et al. 2016).

Recent *Pst* epidemics worldwide and the spread of epidemics to new areas, where the disease was previously not relevant, make it urgent to understand the evolution of *Pst* races, their spread, and their establishment. Knowledge of the evolution of pathogen virulence is a key factor in determining the best strategy to breed locally adapted

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cultivars with effective and durable resistance to *Pst*. In the present study, we examined the population structure of *Pst* in Uruguay based on samples collected from wheat fields between 2017 and 2021. The isolates were race typed to study the diversity for virulence. Subsets of isolates were genotyped and assigned to internationally defined genetic groups, which allowed for interpretation of the results in a wider geographical and evolutionary context. The results may have strong implications for prevention and control of rust diseases in wheat.

Materials and Methods

A collection of 124 *Pst* isolates maintained at INIA-La Estanzuela (Colonia, Uruguay) was recovered from samples collected from spring bread wheat (*Triticum aestivum*) at different locations in the major wheat production area in Uruguay between 2017 and 2021. A set of 27 representative isolates were selected from the 124 *Pst* isolates based on the avirulence/virulence phenotypes observed at the preliminary race phenotyping (Table 1), sampled cultivars (when available), location, and year (Supplementary Table S1). Of the 27 samples, 4 were collected in 2017, 5 in 2018, 2 in 2019, 11 in 2020, and 5 in 2021 (Supplementary Table S1).

Sample recovery and race typing

The original samples, preserved at 5°C, were used to inoculate seedlings of susceptible wheat cultivar Morocco. A reduced differential set representing 13 R-genes (*Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Sp*, and *AvS*) was used for preliminary race phenotyping of the 38 Pst isolates collected between 2017 and 2020. An additional 86 samples collected in 2021 were race phenotyped using an extended differential set representing 17 R-genes (*Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *Sp*, *AvS*, and *Amb*). Based on these data (Table 1), 27 isolates (Supplementary Table S1) were selected for in-depth molecular genotyping and virulence phenotyping.

For the 27 selected isolates, infected leaves were collected separately and sent to the Global Rust Reference Center (GRRC) in Denmark following their standard practice recommendations for sample shipment. For isolate testing at the GRRC, the protocol described in Hovmøller et al. (2017) was used. Leaf segments were placed in Petri dishes with moist filter paper and incubated between 24 and 48 h to promote spore production. Seedlings of wheat cultivars Morocco and Morocco/*Lr*19 were used for multiplication of spores. Seedlings were grown in pots, with 12 to 15 plants per pot, and treated with 5 ml of 0.5% Antergon MH180 (Nordisk Alkali, Randers, Denmark) to regulate plant growth and enhance spore production. Leaves with newly emerged urediniospores were gently rubbed on the wheat seedlings. Inoculated seedlings were misted with water, incubated in a dew chamber at 10 to 12°C in darkness for 24 h, and transferred to spore-proof greenhouse cabins at 17°C during the day/12°C during the night with a 16-h photoperiod of natural light and supplemental sodium light (100 μmol/s/m) and 8 h of darkness, with a relative humidity of 70 to 80%. Pots were covered with cellophane bags (Helmut Schmidt Verpackungsfolien, Königswinter, Germany) prior to sporulation to prevent cross-contamination among isolates. Spores were harvested by shaking the plants inside the cellophane bag and transferred to cryovials. These were dried in a desiccator for approximately 3 days and preserved at −80°C until further use.

The 27 isolates were phenotyped using a set of 24 wheat differential lines (Table 2). A supplementary test was carried out including additional lines for further confirmation of specific race variants. Previously characterized reference isolates of the genetic groups detected for the Uruguayan isolates were included as controls. Ten to 12 seeds of each wheat genotype were sown in 7- × 7- × 8-cm pots with a 1:1 Pindstrup Substrate peat mix containing slow-release plant nutrients (Pindstrup Mose-brug A/S, Ryomgaard Denmark). Differential sets with one pot of each wheat genotype were placed in a tray, grown in spore-proof cabins in the greenhouse with a 17°C day/12°C night temperature regime, and inoculated approximately 12 days after sowing when the second leaf was half unfolded. Urediniospores were retrieved from −80°C and used for inoculation after heat shock treatment in a water bath at 40 to 42°C for 2 min. Approximately 25 mg of spores were suspended in 3 ml of engineered fluid 3M Novec 7100 (3M, St. Paul, MN, U.S.A.) and gently mixed. Wheat seedlings were spray inoculated using an airbrush spray gun (standard class, Revell, Bünde, Germany) in a laboratory fume hood. Seedlings were subsequently sprayed with mist water, incubated in a dew chamber, and transferred to the greenhouse

Table 1. Number of isolates with different virulence phenotypes for the 124 samples collected in Uruguay during 2017 to 2021 and number of isolates selected for in-depth phenotypic analysis[a]

Virulence phenotype	2017	2018	2019	2020	2021	Total	Selected for in-depth analysis
Yr1, *Yr2*, *Yr3*, *Yr4*, NA, *Yr6*, *Yr7*, –, *Yr9*, –, –, *Yr17*, NA, *Yr25*, –, *Yr32*, *Sp*, *AvS*, *Amb*					22	22	1
Yr1, NA, NA, NA, –, –, *Yr7*, –, *Yr9*, –, –, *Yr17*, –, NA, –, NA, *Sp*, *AvS*, NA		5		4		9	4
Yr1, *Yr2*, *Yr3*, *Yr4*, NA, *Yr6*, *Yr7*, –, *Yr9*, –, –, *Yr17*, NA, *Yr25*, –, *Yr32*, *Sp*, *AvS*, –					4	4	0
–, NA, NA, NA, –, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, –, –, NA, –, NA, –, *AvS*, NA	7					7	4
–, *Yr2*, –, –, NA, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, –, NA, –, –, –, –, *AvS*, –					3	3	1
–, NA, NA, NA, –, NA, *Yr7*, NA, *Yr9*, –, –, *Yr17*, –, –, –, –, –, *AvS*, NA		3	3			6	4
–, NA, NA, NA, –, NA, *Yr7*, NA, *Yr9*, –, –, *Yr17*, –, NA, –, NA, –, *AvS*, NA		2	2			4	2
–, NA, NA, NA, –, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *Yr17*, –, NA, –, *Yr32*, –, *AvS*, NA				12		12	7
–, *Yr2*, –, –, NA, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *Yr17*, NA, –, –, *Yr32*, –, *AvS*, –					10	10	1
–, *Yr2*, –, –, NA, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *Yr17*, NA, –, *Yr27*, *Yr32*, –, *AvS*, –					41	41	2
–, *Yr2*, *Yr3*, –, NA, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *Yr17*, NA, *Yr25*, –, *Yr32*, –, *AvS*, –					6	6	1

[a] Virulence phenotype numbers indicate virulence to yellow rust resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr25*, *Yr27*, *Yr32*, Spaldings Prolific (*Sp*), Avocet S (*AvS*), and Ambition (*Amb*), respectively. "–" indicates avirulence. "NA" indicates that the *Yr*-gene was not included in the differential set.

under the same conditions described above. Infection type (IT) was scored on individual plants/leaves after 15 to 17 days. The first and second leaves were scored separately using a 0 to 9 scale (McNeal et al. 1971), where scores between 7 and 9 indicated compatibility (virulence), and scores equal to or below 6 indicated incompatibility (avirulence).

Genotypic characterization of isolates

Genomic DNA was extracted from leaf-infected segments according to manufacturer instructions for the Sbeadex Mini Plant Kit (LGC Genomics, Germany) with an automated KingFisher Magnetic Particle Processor (Thermo Fisher Scientific, United States). The genotyping of the 27 isolates was based on 19 simple sequence repeat (SSR) markers according to Rodriguez-Algaba et al. (2017). PCR size fractionation was performed on Applied Biosystems 3,730 DNA analyzer (Thermo Fisher Scientific) using the service at KIGene, Karolinska University Hospital, Stockholm, Sweden. The amplicons were visualized in GeneMarker (Softgenetics), and allele sizes were manually scored using the GeneScan 600 Liz Size Standard (Thermo Fisher Scientific). This allowed a genetic grouping based on genotypic similarities, i.e., minor (or no) allele differentiation among individuals within a group and major differences between groups, following the principles of Ali et al. (2017). Allele sizes detected for individual isolates and associated genetic groups are presented in Supplementary Table S2.

Table 2. Wheat differential lines used for race typing of *Puccinia striiformis* f. sp. *tritici* isolates[a]

Differential line	Yellow rust resistance genes	GRRC standard set	Supplementary test
Chinese 166	*Yr1*	x	
Kalyansona	*Yr2*, +	x	x
Vilmorin 23	*Yr3*, +	x	x
Hybrid 46	*Yr4*, +	x	
Suwon	*Su*	x	
Heines Kolben	*Yr6*, +	x	
Avocet *Yr6*	*Yr6*, *AvS*	x	
Lee	*Yr7*, +	x	
Avocet *Yr8*	*Yr8*	x	
Avocet *Yr9*	*Yr9*, *AvS*	x	
Moro	*Yr10*	x	
Cortez	*Yr15*	x	
VPM1	*Yr17*, +	x	x
Avocet *Yr17*	*Yr17*, *AvS*	x	x
TP 981	*Yr25*, +	x	x
Spaldings Prolific	*Sp*, *Yr25*, +	x	x
Opata	*Yr27*, *Yr18*, +	x	x
Avocet *Yr32*	*Yr32*, *AvS*	x	x
Avocet S	*AvS*	x	x
Strubes Dickkopf	*Sd*, *Yr25*, +		x
Heines VII	*Yr2*, *Yr25*, +		x
Carstens V	*Yr32*, *Yr25*, +		x
Avocet *Yr27*	*Yr27*, *AvS*		x
Heines Peko	*Yr2*, *Yr6*, *Yr25*, +		x
Nord Desprez	*Yr3*		x
Ambition	*Amb*	x	
Benchmark	Unknown	x	
Kalmar	Unknown	x	
Nemo	Unknown	x	

[a] The + sign in the yellow rust resistance genes column denotes the presence of an additional *Yr* resistance gene not identified.

Results

Preliminary race typing

The preliminary race typing results for the 124 samples are presented in Table 1. This showed 11 distinct virulence phenotypes with different degrees of differentiation regarding avirulence/virulence towards 10 resistance genes. The first three virulence phenotypes shared virulence to *Yr1*, *Yr7*, *Yr9*, and *Yr17* but differed on their compatibility on cultivar Ambition. A second group (rows 4 to 11 in Table 1), shared avirulence to *Yr1* and virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, and *AvS* but differed on their avirulence/virulence to *Yr3*, *Yr17*, *Yr27*, *Yr25*, and *Yr32*. Based on these results, 27 isolates representing virulence diversity, host cultivars, locations, and years were selected for in-depth genotyping and additional virulence testing.

Pst genetic groups

The genetic profiles for the 27 samples analyzed with the 19 SSRs were compared with reference genetic profiles belonging to genetic groups reported worldwide (Ali et al. 2017; Hovmøller et al. 2016). The 27 isolates were assigned to one of the previously reported genetic groups if their genetic profiles for the set of 19 SSRs matched the reference profile. Three different *Pst* genetic groups were detected in Uruguay (Supplementary Table S2). *PstS13* was the most prevalent group with 78% of the isolates analyzed, followed by *PstS7* with 15% and *PstS10* with 8% (Table 3). The *PstS13* group was identified in samples collected from all sampling years, *PstS7* was found for the first time in 2018 and then in 2020 and 2021, and *PstS10* was only detected in 2020.

Race typing

The virulence phenotype of the 27 isolates and their corresponding genetic group are presented in Table 3. The primary results of the phenotyped isolates are presented in Supplementary Table S3. Four

Table 3. Number of isolates of different genetic groups and virulence phenotypes for samples collected in Uruguay during 2017 to 2021[a,b]

Genetic group	Race	Race variants in *PstS13*	Virulence phenotype	2017	2018	2019	2020	2021
PstS7	Warrior		*Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, –, *Yr9*, –, –, *Yr17*, *Yr25*, –, *Yr32*, *Sp*, *AvS*, *Amb*		1		2	1
PstS10	Benchmark		*Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, –, *Yr9*, –, –, *Yr17*, *Yr25*, –, *Yr32*, *Sp*, *AvS*, –				2	
PstS13	Triticale2015	a	–, *Yr2*, –, –, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, –, –, –, –, *AvS*, –	4	4			
		b	–, *Yr2*, –, –, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *17*, –, –, *32*, –, *AvS*, –			2	6	3
		c	–, *Yr2*, –, –, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *17*, –, *27*, *32*, –, *AvS*, –				1	
		d	–, *Yr2*, *Yr3*, –, *Yr6*, *Yr7*, *Yr8*, *Yr9*, –, –, *17*, *25*, –, *32*, –, *AvS*, –					1

[a] Virulence phenotype numbers indicate virulence to yellow rust resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, Spaldings Prolific (*Sp*), Avocet S (*AvS*), and Ambition (*Amb*), respectively. "–" indicates avirulence. Letters a, b, c, and d indicate different virulence phenotypes within genetic group *PstS13*.

[b] All isolates belonging to genetic group *PstS10* were virulent on differential line Benchmark.

isolates with virulence on *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, *Sp*, *AvS*, and *Amb* shared a virulence phenotype with a reference isolate of the 'Warrior' race (*PstS7*). Two isolates were assigned to the 'Benchmark' race (*PstS10*), which is characterized by avirulence to European cultivar Ambition and virulence to the European cultivar Benchmark. Four additional closely related virulence phenotypes were detected among the isolates belonging to *PstS13* (Table 4). Within *PstS13* group, a race virulent on *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, and *AvS* (Fig. 1A) was detected in 2017 and 2018, which corresponded to the original Triticale2015 race. The supplementary virulence test allowed the verification of three new race variants (Table 4). One variant, first detected in 2019, had gained virulence on *Yr17* and *Yr32*; a second variant, collected in 2020, had additional virulence on *Yr27*; and a third variant, collected in 2021, was virulent on *Yr3*, *Yr17*, *Yr25*, and *Yr32*, in addition to the virulence detected in the original Triticale2015 race.

Discussion

The Uruguayan collection of *Pst* isolates composed of 124 samples collected between 2017 and 2021 was preliminarily analyzed for virulence phenotype characterization. Based on the preliminary phenotypic results, a set of 27 isolates was analyzed in-depth both genetically and phenotypically. The set was considered representative of a local *Pst* population based on sampled cultivar, collection year, location, and preliminary race typing. The virulence and genotypic profiles allowed assignment to races within *PstS7*, *PstS10*, and *PstS13* genetic groups, some of which were previously reported as responsible for severe epidemics in the last decade (Ali et al. 2017; Beddow et al. 2015; Hovmøller et al. 2016; Sørensen et al. 2014). Races previously described in Europe within each genetic group were detected in this study, i.e., 'Warrior' (*PstS7*), 'Benchmark' (*PstS10*), and Triticale2015 (*PstS13*). Triticale2015, first detected in Europe in 2015 (Hovmøller et al. 2018) and in Argentina in 2017 (Carmona et al. 2019), was found in several of the *Pst* isolates. Additionally, three new race variants within the *PstS13* genetic group were also identified. These results provide strong evidence not only of migration and establishment of the disease in regions where *Pst* was not previously widespread but also reveal the emergence of new race variants with wider virulence, indicating the existence of regional evolution of *Pst.*

The appearance in the Southern Cone of America of *Pst* genetic groups previously reported in Europe may be associated with human activities (Ali et al. 2014; Brown and Hovmøller 2002; Stubbs 1985; Wellings 2007; Zadoks 1961) and/or long distance wind dispersal (Brown and Hovmøller 2002; Zadoks 1961). Argentina and Uruguay are located in the same rust epidemiological zone (Rajaram and Campos Vela 1974) where there are no geographical barriers for urediniospores dispersal, which likely explains the almost simultaneous development of severe epidemics in both countries (Carmona et al. 2019; Germán et al. 2018). More recently, *Pst* was detected in Paraguay for the first time (Fernández-Gamarra et al. 2023) and in Brazil. Before 2017, *Pst* did not cause severe epidemics in the epidemiological zone east of the Andes, as the pathogen survived during the summer distant from the wheat crop regions (Germán et al. 2007). Since 2017, a relevant epidemiological change associated with the presence of *Pst* in Uruguay is the likely increased oversummering capacity of the pathogen. In fact, oversummering of the pathogen has been observed in the Argentinian wheat crop area, which allows primary inoculum to infect crops earlier and thus cause severe epidemics not only in Argentina but also in Uruguay (Germán et al. 2018; Silva et al. 2023).

Among the three genetic groups detected in this study, *PstS7* and *PstS10* had the lowest prevalence. Since 2011 and 2012, both genetic groups have been associated with important epidemics with substantial economic losses in various parts of the world (Hovmøller et al. 2016; Hubbard et al. 2015). In Europe, new races from *PstS7* and *PstS10* genetic groups have been detected since 2011 and have replaced the previous pathogen population (Ali et al. 2017; Sørensen et al. 2014). So far, only one race has been described within the *PstS7* genetic group (Warrior race), which has spread from Europe to North Africa (Hovmøller et al. 2016) and South America (Hovmøller et al. 2023a). *PstS10* has been the most prevalent genetic group in Europe from 2013 to 2022. One race has been prevalent within this genetic group, but some variants virulent to widely grown cultivars have been described (Hovmøller et al. 2020). In 2018, it was also detected in Australia (Ding et al. 2021; Park et al. 2020). Up to now, it has not yet been possible to differentiate the new race variants within *PstS10* by molecular techniques nor by standard wheat differential lines (Hovmøller et al. 2022). In terms of virulence, the races within *PstS10* are similar to the Warrior race (*PstS7*), except for their avirulence/virulence to the European wheat cultivars Warrior and Ambition (Hovmøller et al. 2020).

In this study, *PstS13* was the most prevalent genetic group. *PstS13* was first reported in Europe in 2015 mainly affecting triticale and durum wheat (Hovmøller et al. 2018). A single race within this genetic group is still prevalent in Europe, although local variants have been observed (Hovmøller et al. 2018). The same genetic group caused severe epidemics on durum and bread wheat in Italy in 2017 (Hovmøller et al. 2018). A new *Yr10* virulent variant was detected in Poland in 2019 and in Germany in 2020 (Hovmøller et al. 2021). In Australia, *PstS13* was detected in 2018 and has become one of the most widespread genetic groups (Ding et al. 2021; Park et al. 2020). In South America, *PstS13* was reported as the prevalent genetic group in Argentina in 2017 and 2018 (Carmona et al. 2019; Hovmøller et al. 2018, 2019). Samples collected in Chile during 2018 were also assigned to this genetic group (Hovmøller et al. 2019). All samples from Argentina and Chile showed the same virulence phenotype as the original *PstS13* race reported in Denmark (Hovmøller et al. 2019, 2020). More recently, *PstS13* spread to Paraguay (Fernández-Gamarra et al. 2023). Since *PstS13* has been the most prevalent genetic group in South America accounting for severe *Pst* epidemics in the region, new virulence variants could be expected based on previously reported high mutation rates in yellow/stripe rust (Hovmøller and Justesen 2007).

Here, we report new race variants in South America with additional virulence within the *PstS13* genetic group. The emergence of new *Pst* races within the same genetic group is probably due to mutation and subsequent selection of races with virulence to the deployed host resistance genes as suggested by de Vallavieille-Pope et al. (2012) and Hovmøller and Justesen (2007). Through this mechanism, *Pst* isolates may acquire the ability to avoid recognition by resistance genes in host plants, resulting in step-wise mutations (Hovmøller et al. 2002). Similar patterns have been observed in Australia, where the sexual cycle of *Pst* does not occur, and new races usually differ from existing races by virulence on a single resistance gene (Ding et al. 2021; Park 2015; Wellings and McIntosh 1990). The detection of new races in higher frequencies may be related to the presence of the corresponding resistance genes in wheat cultivars used locally. The presence of new races with wider virulence was not associated to a dramatic change in field susceptibility of any of the widely grown cultivars. However, during 2017 to 2021, some cultivars changed their level of susceptibility from very low to intermediate (cultivars Génesis 6.87, Génesis 4.33, and SYN 211) or from intermediate to high (cultivar Algarrobo) (Castro et al. 2023). The observed change in the level of susceptibility is probably due to the presence of the newly detected *PstS13* race variants.

One possible pathway to explain the observed pathogen evolution in Uruguay starts with the original *PstS13* detected in samples from 2017 to 2018 (*PstS13*a, Table 3). *PstS13*b, detected in 2019, gained virulence on *Yr17* and *Yr32*. More recently, two new races emerged, *PstS13*c (in 2020), which gained virulence on *Yr27*, and *PstS13*d (in 2021), which gained virulence on *Yr3* and *Yr25* in addition to the virulence pattern observed for *PstS13*b. The new *PstS13*d variant, which is virulent on both *Yr3* and *Yr25*, could be the result of two subsequent single-set mutations. This hypothesis implies the existence of a race virulent on *Yr3* or *Yr25*, but it has not been detected in local surveys so far, which may be due to the small sample size or because it might not have been present in Uruguay. Another possible scenario of the appearance of *PstS13*d virulent on *Yr3* could be that

Table 4. Infection types on *Pst* differential lines of *Pst13* variants tested in the supplementary test[a]

Variety => *Yr*-genes =>	**AvocetS, *AvS***	**Strubes Dickkopf, *Sd*, *Yr25***	**Spaldings Prolific, *Sp*, *Yr25*, +**	**Kalyansona, *Yr2*, +**	**Heines Peko, *Yr2*, *Yr6*, *Yr25*, +**	**Heines VII, *2*, *Yr25*, +**	**Nord Desprez, *Yr3*, +**	**Vilmorin 23, *Yr3*, +**	**VPM1, *Yr17*, +**	**TP 981, *Yr25*, +**	**Avocet *Yr17*, *AvS*, *Yr17***	**Carstens V, *Yr25*, *Yr32*, +**	**Avocet *Yr32*, *AvS*, *Yr32***	**Avocet *Yr27*, *AvS*, *Yr27***	**Opata, *Yr18*, *Yr27*, +**	**Race**
DK69	7	1	5, 6, 7	7	2	1	2	1	0, 1	2	0	0, 1	0, 2, 3	0	2, 3, 4	Triticale 2015a
UY147	7	1	5, 6	7	2	1	2	1	1	2, 3, 4	0	0,1	2, 3	0	1, 2, 3	Triticale 2015a
UY150	7	1	5, 6, 7	7	2	1	2	1	1	2, 3, 4	0, 1, 2	0, 1, 2	2, 3	0, 1	0, 1, 2	Triticale 2015a
UY152	7	1	5, 6, 7	7	2	1	2	1	0, 1	2, 3, 4	0, 1, 2	0,1, 2	2, 3	0, 1	0, 1, 2	Triticale 2015a
UY158	7	1	5, 6, 7	7	2	1	2	1	0, 1	2	7	0, 1	7	3, 4	4, 5	Triticale 2015b
UY168	7	1	5, 6, 7	7	2	1	2	1	0, 1	2	6, 7	0, 1, 2	7	7	7	Triticale 2015c
UY623	7	2, 3	6, 7	7	7	5, 6	7	5, 6	2, 3	7	7	6, 7	7	4, 5, 6	4, 5	Triticale 2015d

[a] The first and the second leaves were scored using a 0 to 9 scale (McNeal et al. 1971); scores between 7 and 9 indicate compatibility (virulence); and scores equal to or below 6 indicate incompatibility (avirulence). Letters a, b, c, and d indicate different virulence phenotypes within genetic group *PstS13*.

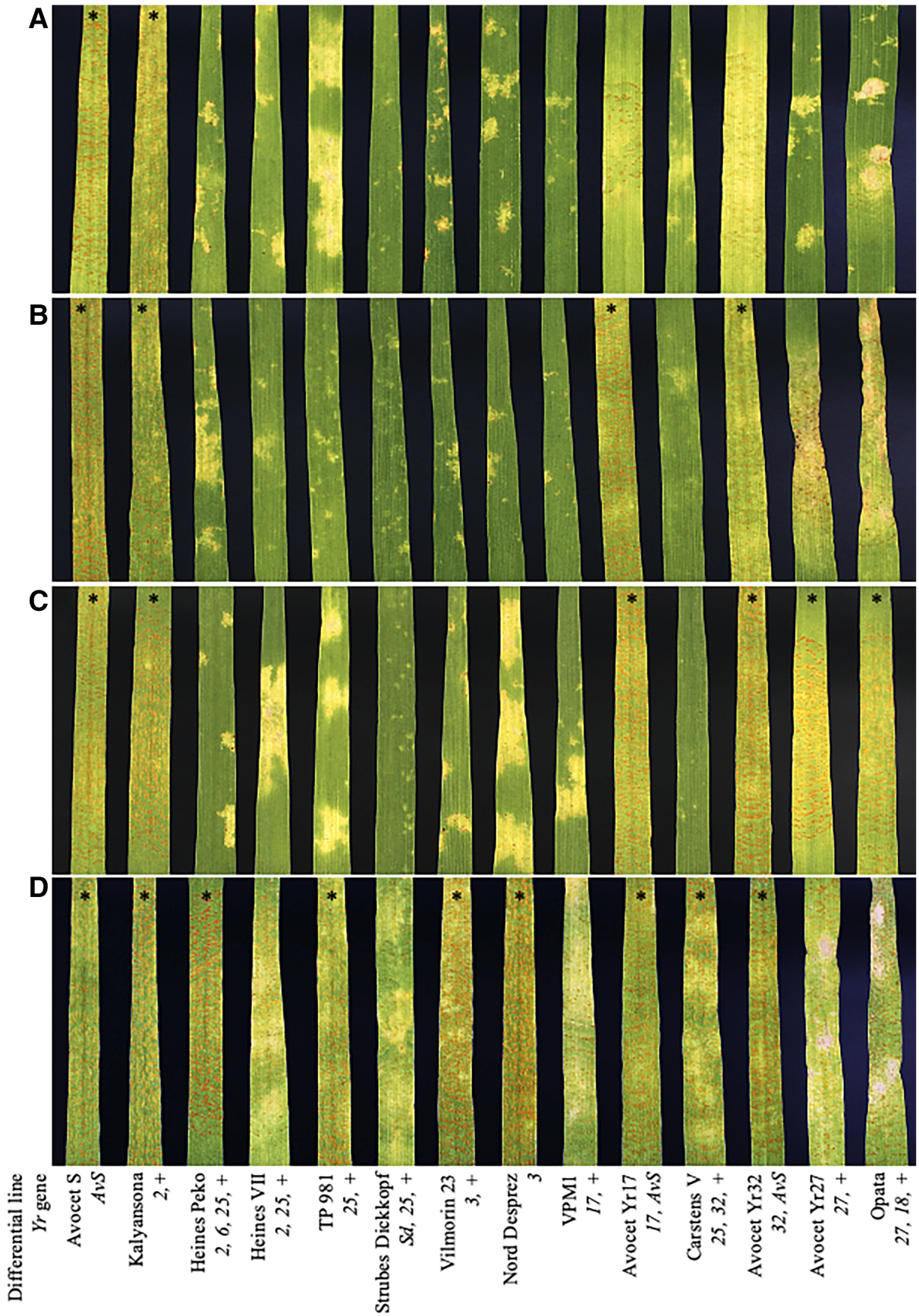

Fig. 1. *Puccinia striiformis* f. sp. *tritici* infections on differential lines for the four different races within the Triticale2015 race (*PstS13* genetic group): **A,** original Triticale2015 race; **B,** races with additional virulence to *Yr17* and *Yr27*; **C,** to *Yr17*, *Yr27*, and *Yr32*; and **D,** *Yr3*, *Yr17*, *Yr25*, and *Yr32*, respectively. * indicates a compatible interaction (infection type 7 to 9).

this race variant was already present in the *PstS13* races but was not detectable by the differential lines used up to date. The detection of *PstS13d* with virulence to *Yr3* and *Yr25* allows a better interpretation of the genes that might be present in some of the differential lines. Differential lines Vilmorin 23 and Nord Desprez, both carrying *Yr3* (Chen et al. 1996; Chen and Line 1993), resulted in a compatible interaction when tested with the *Yr25*-virulent race, which suggests that these differentials might also carry *Yr25*. The possible presence of *Yr25* in some differential lines, i.e., Carstens V, Spaldings Prolific, and Strubes Dickkopf, was previously suggested (Calonnec and Johnson 1998; Calonnec et al. 2002; Eriksen et al. 2004). Assuming that differential lines carrying *Yr3* may also carry *Yr25*, this suggests that *Yr3*, masked by the low IT conferred by the *Avr25*/*Yr25* phenotype, is probably ineffective in the previously reported *PstS13* race. If the two *Yr3* differentials also carry *Yr25*, we cannot securely confirm virulence/avirulence for *Yr3* using the current differential lines, except for mutant isolates with virulence on *Yr25*. Interpretation of virulence entirely depends on knowledge about R-genes (known or unknown) in the differential lines, and many of the differential lines currently used could also contain other uncharacterized R-genes (Johnson 1992). In this context, the use of multiple differential lines representing each *Yr*-gene could be recommended for a more precise race typing analysis.

In this study, we were able to detect three widely spread *Pst* genetic groups. However, additional *Pst* genetic groups could have been undetected. For example, *PstS14*, reported in Argentina in 2017 (Carmona et al. 2019), was not detected in Uruguay despite the geographical proximity of both countries. *PstS14* was first detected in Northern Africa and Europe in 2016, causing severe epidemics on bread wheat in Morocco in 2017 (Hovmøller et al. 2018). Future surveys, with a larger number of samples collected at a regional scale, could complement this work and provide further insights into the understanding of the current distribution of genetic groups in the Southern Cone of South America.

Conclusions

The hypothesis of initial migration of *Pst* into the Southern Cone of America from distant pathogen sources confirmed the current epidemic outbreaks observed in the region. The identification of *Pst* races in Uruguay that were not previously described provides strong evidence of recent evolution of virulence of *Pst* in Uruguay and possibly Argentina, where the most severe epidemics occurred. The emergence of new races of *Pst* with wider virulence has implications for the management of the disease and plant breeding for disease resistance in Uruguay and neighboring countries. In the short term, local management of ongoing epidemics on susceptible wheat varieties is limited to fungicide spraying. However, the deployment of resistant wheat cultivars appears as the most environmentally friendly strategy without additional cost for producers. The development of resistant cultivars implies permanent monitoring of the *Pst* races to generate cultivars with effective resistance to races present in a specific epidemiological zone. The occurrence of long-distance dispersal of races migrating from distant continents emphasizes the relevance of worldwide coordination of survey efforts. The new race variants reported in this work might migrate to other areas as well and potentially cause important yield losses in regions where cultivars that possess the overcome *Pst* resistance genes are deployed.

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