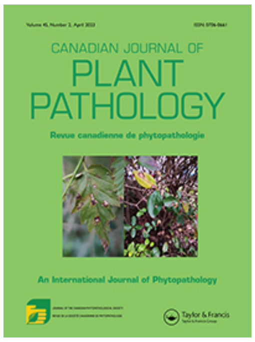

Canadian Journal of Plant Pathology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tcjp20

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To cite this article: Ahmet Cat, Mehmet Tekin, Kadir Akan, Taner Akar & Mursel Catal (2023) Virulence characterization of the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*, in Turkey from 2018 to 2020, Canadian Journal of Plant Pathology, 45:2, 158-167, DOI: 10.1080/07060661.2023.2166126

To link to this article: https://doi.org/10.1080/07060661.2023.2166126

Can. J. Plant Pathol., 2023
Vol. 45, No. 2, 158–167, https://doi.org/10.1080/07060661.2023.2166126

Genetics and Resistance / Génétique et Résistance

Virulence characterization of the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*, in Turkey from 2018 to 2020

AHMET CAT[1], MEHMET TEKIN[2], KADIR AKAN[3], TANER AKAR[2] AND MURSEL CATAL[4]

[1]*Department of Plant Protection, Faculty of Agriculture, Siirt University, Siirt, 56100, Turkey*
[2]*Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, 07059, Turkey*
[3]*Department of Plant Protection, Faculty of Agriculture, Kirsehir Ahi Evran University, Kirsehir, 40100, Turkey*
[4]*Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Antalya, 07059, Turkey*

(Accepted 3 January 2023)

Abstract: Stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is the most devastating disease of wheat in Turkey. Virulence characterization of the *Pst* population is critical to prevent large-scale epidemics, by developing wheat cultivars with durable resistance against the disease. In this study, 38 *Pst* races, including 25 races that were not previously reported, were identified from 140 isolates obtained from most regions of Turkey from 2018 to 2020 using a differential set containing 18 single *Yr* gene wheat lines (NILs) of 'Avocet'. Virulence to *Yr15* was not observed among any of the isolates. Virulence to the remaining 17-*Yr genes* was detected at various frequencies. The frequencies of virulence to *Yr6, Yr9, Yr7, Yr8, Yr43, YrExp2, Yr44, YrTr1*, and *Yr27* were high (57.1 to 100.0%), to *Yr1, Yr17, Yr32*, and *YrTye* were moderate (24.3 to 42.9%), and to *YrSP, Yr24*, and *Yr10* were low (9.3 to 17.1%). Only one race was virulent to *Yr5* (0.7%). Many of the races identified were common among regions, indicating that *Pst* races migrate throughout Turkey. However, the Black Sea (BS) and the Mediterranean (ME) regions were differentiated from other regions by unique races absent in other regions, and the *Yr5*-virulent race, respectively. To sum up, the virulences of the races identified and their distributions among regions provided an understanding of pathogen migration and may contribute to the development of resistant wheat cultivars against stripe rust.

Keywords: *Puccinia striiformis*, race, virulence diversity, wheat, yellow rust

Résumé: La rouille (jaune), causée par *Puccinia striiformis* f. sp. *tritici* (*Pst*), est la maladie du blé la plus dévastatrice en Turquie. La caractérisation de la virulence de la population de *Pst* est essentielle à la prévention des épidémies à grande échelle en développant des cultivars de blé offrant une résistance durable à la maladie. Dans cette étude, 38 races de *Pst*, y compris 25 races qui n'avaient jamais été rapportées auparavant, ont été identifiées à partir de 140 isolats obtenus de presque partout en Turquie, de 2018 à 2020, et ce, grâce à une série différentielle comprenant 18 lignées de blé portant le gène unique *Yr* du cultivar 'Avocet'. Aucun des isolats n'a affiché de virulence à l'égard de *Yr15*. La virulence à l'égard des 17 autres gènes *Yr* a été détectée à différentes fréquences. Les fréquences de la virulence à l'égard de *Yr6, Yr9, Yr7, Yr8, Yr43, YrExp2, Yr44, YrTr1* et *Yr27* étaient élevées (57,1 à 100,0%), à l'égard de *Yr1, Yr17, Yr32* et *YrTye*, modérées (24,3 à 42,9%), et à l'égard de *YrSP, Yr24* et *Yr10*, faibles (9,3 à 17,1%). Une seule race était virulente à l'égard de *Yr5* (0,7%). Plusieurs des races identifiées étaient communes à de nombreuses régions, ce qui indique que les races de *Pst* migrent dans toute la Turquie. Toutefois, les régions de la mer Noire et de la Méditerranée se distinguaient des autres régions par des races uniques trouvées nulle part ailleurs et la race virulente à l'égard de *Yr5*, respectivement. En résumé, la virulence des races identifiées et leur répartition permettent de comprendre la migration des agents pathogènes et peuvent contribuer au développement de cultivars de blé résistants à la rouille jaune.

Mots clés: blé, diversité de virulence, *Puccinia striiformis*, race, rouille jaune

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Correspondence to: Mehmet Tekin. E-mail: mehmettekin@akdeniz.edu.tr

Introduction

Turkey is one of the largest producers and consumers of wheat worldwide. Consumption of wheat in Turkey is approximately 170 kg per capita/year, which is one of the highest in the world (FAO 2022a). At the same time, Turkey is the world's largest flour exporter and the second largest macaroni exporter (FAO 2022b). Wheat (*Triticum* spp.) is grown on more than 6.6 million hectares annually in Turkey, and wheat production is approximately 20 million metric tons in the country (TUIK 2022). However, this production is under threat by many biotic and abiotic stress factors. Among biotic stresses, wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) can cause devastating epidemics resulting in extreme crop losses on susceptible wheat cultivars when disease-favourable weather conditions occur. The frequency, severity, and prevalence of wheat stripe rust vary based on many factors such as weather, phenology, and genetic background of the host.

Until now, wheat stripe rust has been reported in more than 60 countries (Chen 2020). It has been known that epidemics have routinely occurred every two years and affected over 25% of the wheat growing areas of most European, North African and Middle Eastern countries including Turkey (Chen 2020). Historically, epidemics have occurred at varying intensities throughout Turkey depending on weather conditions and cultivar resistance, and therefore crop losses caused by these epidemics have fluctuated. Although stripe rust affected wheat production generally in the inner regions from the 1930s to the early 1960s, epidemics occurred throughout Turkey in the 1960s to the early 1990s and caused serious yield losses of up to 80% in some growing areas (Akan 2019a, 2019b). From the 1990s to the 2010s, many regional and countrywide epidemics occurred, and these epidemics affected not only grain yield but also decreased grain quality dramatically (Mamluk et al. 1997; Cat et al. 2017).

Growing resistant cultivars is the most environmentally friendly and effective approach for controlling wheat stripe rust. Until now, over 80 *Yr* genes and many QTL loci associated with resistance have been identified (McIntosh et al. 2020). However, some of these genes have been ineffective due to the rapid evolution of *Pst*. Although the first report of wheat stripe rust in Turkey was in 1886 (Ozgen and Kinaci 1985), *Pst* races causing stripe rust were not identified until the 1990s. In the early 1990s, Louwers et al. (1992) reported *Pst* races 2E16, 6E16, 6E150 and 2E0 in the Central Anatolian region of Turkey. Between 1995 and 1998, while the resistance genes *Yr1, Yr3V, Yr9+, Yr7+, Yr8+, YrCV, YrSp, Yr2+*, and *Yr5* provided resistance to the races present at that time, *Yr2, Yr6, Yr7, Yr9, Gaby* and *A+* were ineffective (Mamluk et al. 1997; Cetin et al. 1999). Mert et al. (2012) determined adult-plant resistance (APR) under natural infection conditions in 12 different locations in Turkey from 2003 to 2011, and reported that the resistance genes *Yrl, Yr3V, Yr4+, Yr5, Yr10, Yrl5, YrSP* and *YrCV* were effective against the *Pst* races collected. In 2014, a new race was detected among isolates obtained from infected leaf samples of wheat growing fields in the Thrace region: This race was confirmed to be *Warrior (-)*, as defined by Hovmøller et al. (2016), with virulence formula *Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr25, Yr32*, and *YrSp* (Mert et al. 2016). However, the studies mentioned above were carried out with differentials that have different genetic backgrounds and carry one or more resistance genes. This differential set may not differentiate *Pst* races adequately due to its complexity.

Different race identification systems and many differentials for *Pst* have been used worldwide, but this complicates the comparison of races among countries. To overcome this complexity, Wellings et al. (2004) developed *Yr* near-isogenic lines (NILs) in the Avocet 'S' (AvS) background, and Cheng and Chen (2010) also developed several single-gene lines. Later, a new differential set, with 18 single *Yr* gene wheat lines, generated from a selection of these lines was first used in 2010 by Wan and Chen (2014). This differential set has a great advantage compared to other differential sets as it is suitable to evaluate a single *Yr* gene basis and gives more reliable data. In Turkey, Cat et al. (2021) first used this differential set and identified 25 *Pst* races from coastal areas of Turkey, and reported that the frequency of *Yr10*-virulent races was as much as 25%.

The objectives of this study were to: (i) detect *Pst* races throughout Turkey using a differential set of 18 single *Yr* gene wheat lines in the Avocet background and (ii) evaluate their frequencies and distributions.

Materials and methods

Field surveys

Field surveys for stripe rust were carried out by the authors and by collaborators throughout Turkey. Collection was done at the heading stage (Z45) (Zadoks et al. 1974) of wheat between mid-March and early June from 2018 to 2020 based on the phenology of each region. Infected leaf samples were collected from commercial fields and breeding nurseries in public research institutes in Turkey. One or more infected leaves were collected and put into a paper envelope. Samples were then sent to the lab as soon as possible so that the pathogen did not lose its viability. The samples were kept dry at 4°C in a desiccator until isolation of *Pst*.

Collection date, location, host species, cultivar, growth stage, infection type, disease severity and prevalence and other information were recorded for each isolate whenever possible. From seven geographic regions, 223 samples were collected, including 15 from the Aegean (AE) (six in 2018, two in 2019 and seven in 2020), 40 from the Black Sea (BS) (27 in 2018, 11 in 2019 and two in 2020), 77 from the Central Anatolia (CA) (41 in 2018, 15 in 2019 and 21 in 2020), four from the Eastern Anatolia (EA) in 2019, 31 from the Marmara (MA) (six in 2018, 12 in 2019 and 13 in 2020), 15 from the Mediterranean (ME) (six in 2018, two in 2019 and seven in 2020) and 41 from the Southeastern Anatolia (SA) (29 in 2019 and 12 in 2020).

Obtaining and maintaining *Pst* isolates

Urediniospores on dried leaf samples were collected into a gelatin capsule with a mini cyclone spore collector (Tallgrass solutions, USA). The bread wheat variety ‘Morocco’, which is susceptible to all *Pst* races at the seedling stage was used to obtain and maintain the *Pst* isolates. Twelve to 14-day-old seedlings of Morocco were inoculated with urediniospores collected from infected leaf samples to obtain single-pustule isolates. Urediniospores from each sample were suspended in NovecTM 7100 engineered fluid (3 M Company) at the ratio of 10-mg spores per 5-mL oil and then each suspension was sprayed onto the seedlings using an airbrush spray gun, as described by Sorensen et al. (2016). Inoculated seedlings were preincubated in a dew chamber at 10°C for 24 h in darkness. Later, they were transferred to a climate-controlled room programmed with a diurnal temperature cycle with a 16-h photoperiod, as described by Chen et al. (2002). Seedlings inoculated with each sample were isolated with separators to avoid cross-contamination. Urediniospores from a single-pustule were collected into gelatin capsules 15–19 days post inoculation (dpi) using the mini cyclone spore collector. This spore-multiplication process was repeated several times for each isolate until a sufficient spore quantity was obtained for virulence testing on differentials. Urediniospores in each gelatin capsule were dried in a desiccator at 4°C for five days before storing in foil bags at 4°C for short-term storage (1–2 months).

Virulence testing on differentials

Of the 223 *Pst* samples collected, 140 *Pst* isolates were recovered. In this manner, the isolates were obtained from all regions except for EA, and they were inoculated onto differentials (Avocet NILs) containing 18 single *Yr* gene lines (*Yr1, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr27, Yr32, Yr43, Yr44, YrSp, YrTr1, YrExp2* and *YrTye*) (Wan and Chen 2014). The inoculation process was carried out as described above. Infection types (ITs) were recorded at 19–21 dpi based on a 0 to 9 scale (Line and Qayoum 1992). The ITs 0 to 6 were considered avirulent and 7 to 9 virulent (Wan and Chen 2014). The tests were repeated twice and the virulence/avirulence pattern of each isolate was determined based on IT data. These confirmed patterns were used to determine *Pst* races, and then they were coded with the octal system described by Wan et al. (2016). After the identification of the races, the frequency, distribution and virulence frequency of each race was determined for both country and region wide.

Data analysis

To determine relationships among the races identified and among the race collections from six regions in Turkey, IT data were converted to virulence and avirulence as 1 and 0. A multivariate hierarchical clustering analysis using the unweighted pair-group method (UPGMA) based on Nei’s genetic distance (Nei 1978) was performed to reveal the similarities among both the races and the race collections using PAST v 3.0 (Hammer et al. 2001). Data were visualized in FigTree v. 1.4.4 (Rambaut 2018).

Results

Identification of *Puccinia striiformis* f. sp. *tritici* races

Of the 223 leaf samples collected from across of the country, 140 single pustule isolates were recovered. From the seven regions surveyed isolates were obtained from six; there was no data from EA since urediniospores on leaf samples collected from EA could not be recovered. Thirty-eight *Puccinia striiformis* f. sp. *tritici* (*Pst*) races, designated PSTr-4 to PSTr-52, were determined based on the reactions on the differentials to 140 *Pst* isolates (Table 1). Additionally, these races were coded using the octal system, and codes varied from 130 067 to 574 003. The number of virulences on differentials varied from 4 to 11. Race PSTr-31 (*Yr1, Yr6, Yr7, Yr9, Yr17, Yr27, Yr32, Yr43, Yr44, YrTr1* and *YrExp2*) had the highest number of virulences while PSTr-4 (*Yr6, Yr7, Yr9, Yr17*), PSTr-39 (*Yr6,* *Yr7, Yr8, Yr10*) and PSTr-43 (*Yr1, Yr6, Yr7, Yr9*) had the narrowest virulence spectrum. In addition, only 3 of them, PSTr-26 (PSTv-6), PSTr-27 (TRVR20-5) and PSTr-29 (PSTv-36), were identical to previously described *Pst* races (Cat et al. 2021).

Frequencies and distributions of PSTr races

The frequencies of each race varied from 0.7 to 22.7%. The predominant races were PSTr-29 (22.7%), PSTr-30 (17.0%), PSTr-31 (12.7%) and PSTr-28 (7.1%), and the remaining 34 races all had frequencies of <3% country-wide (Table 1). The distributions of the PSTr races in each region are given in Table 2. In general, the most prevalent race was PSTr-29 followed by PSTr-30 and PSTr-31, which were detected in four regions. Of the 38 races, 18 were identified from only one region.

The number of races in each region are shown in Table 2. From AE, nine races were characterized from 14 isolates, with PSTr-30 (28.6%) and PSTr-28

Table 1. Names, octal codes, virulence formulae, and frequencies of 38 races obtained from *Puccinia striiformis* f. sp. *tritici* collections in Turkey from 2018 to 2020.

No	Race name	Octal code	Virulence (bold)/avirulence formula (not bold)	*N*[a]	Frequency (%)	Region
1	PSTr-4[b]	151 000	**6,7,9,17**/1,5,8,10,15,27,32,43,44,SP,Tr1,Exp2,Tye	1	0.7	SA(1)
2	PSTr-6[b]	551 110	**1,6,7,9,17,32,SP**/5,8,10,15,24,27,43,44,Tr1,Exp2,Tye	2	1.4	CA(1), MA(1)
3	PSTr-7[b]	551 154	**1,6,7,9,17,32,43,SP,Tr1**/5,8,10,15,24,27,44,Exp2,Tye	2	1.4	CA(1), AE(1)
4	PSTr-8[b]	570 200	**1,6,7,8,9,27**/5,10,15,17,24,32,43,44,SP,Tr1,Exp2,Tye	4	2.8	MA(3), AE(1)
5	PSTr-12[b]	534 010	**1,6,8,9,10,SP**/5,7,15,17,24,27,32,43,44,Tr1,Exp2,Tye	3	2.8	CA(2), ME(1)
6	PSTr-13[b]	563 000	**1,6,7,8,10**/5,9,15,17,24,27,32,43,44,SP,Tr1,Exp2,Tye	2	1.4	AE(1), SA(1)
7	PSTr-14[b]	554 000	**1,6,7,9,10**/5,8,15,17,24,27,32,43,44,SP,Tr1,Exp2,Tye	1	0.7	AE(1)
8	PSTr-16[b]	130 250	**6,8,9,27,43,SP**/1,5,7,10,15,17,24,32,44,Tr1,Exp2,Tye	2	1.4	ME(1), MA(1)
9	PSTr-20[b]	544 440	**1,6,7,10,24,43**/5,8,9,15,17,27,32,44,SP,Tr1,Exp2,Tye	1	0.7	CA(1)
10	PSTr-22[b]	174 400	**6,7,8,9,17,24**/1,5,10,15,27,32,43,44,SP,Tr1,Exp2,Tye	1	0.7	CA(1)
11	PSTr-25[b]	141 630	**6,7,17,24,27,44,SP**/1,5,8,9,10,15,32,43,Tr1,Exp2,Tye	2	1.4	CA(1), SA(1)
12	PSTr-26[c]	570 250	**1,6,7,8,9,27,43,SP**/5,10,15,17,24,32,44,Tr1,Exp2,Tye	1	0.7	CA(1)
13	PSTr-27[c]	341 630	**5,6,7,17,24,27,44,SP**/1,8,9,10,15,32,43,Tr1,Exp2,Tye	1	0.7	ME(1)
14	PSTr-28	170 660	**6,7,8,9,24,27,43,44**/1,5,10,15,17,32,SP,Tr1,Exp2,Tye	10	7.1	MA(5), AE(3), CA(2)
15	PSTr-29[c]	170 266	**6,7,8,9,27,43,44,Tr1,Exp2**/1,5,10,15,17,24,32,SP,Tye	32	22.7	CA(15), SA(9), MA(3), BS(3), ME(1), AE(1)
16	PSTr-30	130 067	**6,8,9,43,44,Tr1,Exp2,Tye**/1,5,10,17,24,27,32	24	17.0	CA(10), ME(6), AE(4), SA(4)
17	PSTr-31	551 366	**1,6,7,9,17,27,32,43,44,Tr1,Exp2**,/5,8,10,15,24,Tye	18	12.7	CA(10), SA(4), ME(2), BS(2)
18	PSTr-32	570 046	**1,6,7,8,9,43,Tr1,Exp2**/5,10,15,17,24,27,32,44,SP,Tye	3	2.1	MA(3)
19	PSTr-33	570 106	**1,6,7,8,9,32,Tr1,Exp2**/5,10,15,17,24,27,43,44,SP,Tye	2	1.4	SA(2)
20	PSTr-34	164 607	**6,7,8,10,24,27,Tr1,Exp2,Tye**/1,5,9,15,17,32,43,44,SP	2	1.4	MA(2)
21	PSTr-35	551 143	**1,6,7,9,17,32,43,Exp2,Tye**/5,8,10,15,24,27,44,SP,Tr1	2	1.4	MA(2)
22	PSTr-36	551 146	**1,6,7,9,17,32,43,Tr1,Exp2**/5,8,10,15,24,27,44,SP,Tye	2	1.4	CA(2)
23	PSTr-37	551 170	**1,6,7,9,17,32,43,44,SP**/5,8,10,15,24,27,Tr1,Exp2,Tye	1	0.7	MA(1)
24	PSTr-38	551 440	**1,6,7,9,17,24,43**/5,8,10,15,27,32,44,SP,Tr1,Exp2,Tye	1	0.7	MA(1)
25	PSTr-39	164 000	**6,7,8,10**/1,5,9,15,17,24,27,32,43,44,SP,Tr1,Exp2,Tye	1	0.7	CA(1)
26	PSTr-40	175 110	**6,7,8,9,10,17,32,SP**/1,5,15,24,27,43,44,Tr1,Exp2,Tye	1	0.7	AE(1)
27	PSTr-41	171 100	**6,7,8,9,17,32/**1,5,10,15,24,27,43,44,SP,Tr1,Exp2,Tye	1	0.7	MA(1)
28	PSTr-42	570 000	**1,6,7,8,9**/5,10,15,17,24,27,32,43,44,SP,Tr1,Exp2,Tye	2	1.4	BS(2)
29	PSTr-43	550 000	**1,6,7,9/**5,8,10,15,17,24,27,32,43,44,SP,Tr1,Exp2,Tye	1	0.7	BS(1)
30	PSTr-44	574 002	**1,6,7,8,9,10,Exp2**/5,15,17,24,27,32,43,44,SP,Tr1,Tye	1	0.7	MA(1)
31	PSTr-45	574 003	**1,6,7,8,9,10,Exp2,Tye**/5,15,17,24,27,32,43,44,SP,Tr1	1	0.7	MA(1)
32	PSTr-46	171 200	**6,7,8,9,17,27**/1,5,10,15,24,32,43,44,SP,Tr1,Exp2,Tye	1	0.7	CA(1)
33	PSTr-47	171 310	**6,7,8,9,17,27,32,SP**/1,5,10,15,24,43,44,Tr1,Exp2,Tye	1	0.7	SA(1)
34	PSTr-48	570 043	**1,6,7,8,9,43,Exp2,Tye**/5,10,15,17,24,27,32,44,SP,Tr1	1	0.7	SA(1)
35	PSTr-49	571 310	**1,6,7,8,9,17,27,32,SP**/5,10,15,24,43,44,Tr1,Exp2,Tye	2	1.4	CA(2)
36	PSTr-50	571 303	**1,6,7,8,9,17,27,32,Exp2,Tye**/5,10,15,24,43,44,SP,Tr1	1	0.7	SA(1)
37	PSTr-51	571 011	**1,6,7,8,9,17,SP,Tye**/5,10,15,24,27,32,43,44,Tr1,Exp2	3	2.1	MA(2), AE(1)
38	PSTr-52	530 250	**1,6,8,9,27,43,SP/**5,7,10,15,17,24,32,44,Tr1,Exp2,Tye	3	2.1	BS(3)

[a] Number of isolates

[b] These identical races were first identified by Cat et al. (2021)

[c] PSTr-6 (PSTv-26) and PSTr-29 (PSTv-36) (Wan and Chen et al. 2014, Chen et al. 2021); PSTr-27 (TRVR20-5) (Tekin et al. 2021)

Table 2. Number of isolates, number of races identified, and their frequencies in six regions of Turkey from 2018 to 2020.

Region	Number of isolates	Number of races	PSTr race and frequency (%)
Aegean	14	9	7(7.1), 8(7.1), 13(7.1), 14(7.1), 28(21.4), 29(7.1), **30(28.6)**, 40(7.1), 51(7.1)
Black Sea	11	5	**29(27.3)**, 31(18.2), 42(18.2), 43(9.1), **52(27.3)**
Central Anatolia	51	15	6(2.0), 7(2.0), 12(3.9), 20(2.0), 22(2.0), 25(2.0), 26(2.0), 28(3.9), **29(29.4)**, 30(19.6), 31(19.6), 36(3.9), 39(2.0), 46(2.0), 49(3.9)
Marmara	27	14	6(3.7), 8(11.1), 16(3.7), **28(18.5)**, 29(11.1), 32(11.1), 34(7.4), 35(7.4), 37(3.7), 38(3.7), 41(3.7), 44(3.7), 45(3.7), 51(7.4)
Mediterranean	12	6	12(8.3), 16(8.3), 27(8.3), 29(8.3), **30(50.0)**, 31(16.7)
Southeastern Anatolia	25	10	4(4.0), 13(4.0), 25(4.0), **29(36.0)**, 30(16.0), 31(16.0), 33(8.0), 47(4.0), 48(4.0), 50(4.0)
Total	140	38	

(21.4%) as the most frequent races (Table 2). The BS region had only five races from 11 isolates, and PSTr-29 and PSTr-52 were the most prevalent with frequencies of 27.3%. In CA, there were 15 races, with PSTr-29 (29.4%), PSTr-30 (19.6%) and PSTr-31 (19.6%) the most frequent. In SA, there were 10 races, with PSTr-29 the most frequent. There were 14 races in MA, with PSTr-28 (18.5%), PSTr-29 (11.1%) and PSTr-32 (11.1%) the most frequent. There were only six races in ME, with PSTr-30 (50.0%) being the most frequent. In general, PSTr-29 in CA and SA, and PSTr-30 in AE and ME were the most frequent races. Additionally, PSTr-52 was observed only in BS.

Virulence frequencies and distributions

All 140 isolates, comprising the 38 races, were virulent to the *Yr6*-resistance gene. In addition, virulence to *Yr9, Yr7, Yr8, Yr43, YrExp2, Yr44, YrTr1*, and *Yr27* among the isolates was high (57.1 to 93.6%). Virulence to *Yr1, Yr17, Yr32*, and *YrTye* was moderate (24.3 to 42.9%) while virulence to *YrSP, Yr24*, and *Yr10* was low (9.3 to 17.1%) (Table 3). One isolate and therefore race was virulent to *Yr5*; however, there was no isolate virulent to *Yr15*.

The isolates had a wide range variation of virulence frequencies among the regions. While virulence to *Yr9* (100.0%), *Yr27* (72.7%), *Yr1* (72.3%), and *YrSP*

Table 3. Virulence frequencies of *Pst* isolates countrywide and in regions from 2018 to 2020.

	Turkey		Frequency (%) in regions					
Virulence	*N*[a]	Freq.[b](%)	Aegean	Blacksea	Central Anatolia	Marmara	Mediterranean	Southeastern Anatolia
Yr1	60	42.9	35.7	72.3	39.2	55.6	25.0	36.0
Yr5	1	0.7	0.0	0.0	0.0	0.0	100.0	0.0
Yr6	140	100	100.0	100.0	100.0	100.0	100.0	100.0
Yr7	107	76.4	83.3	72.3	76.5	96.3	33.3	84.0
Yr8	105	75.0	85.7	72.7	68.6	81.5	75.0	76.0
Yr9	131	93.6	92.9	100.0	94.1	92.6	91.7	92.0
Yr10	13	9.3	21.4	0.0	7.8	14.8	7.1	4.0
Yr15	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Yr17	43	30.7	21.4	18.2	35.3	29.6	25.0	32.0
Yr24	18	12.9	21.4	0.0	9.8	29.6	8.3	4.0
Yr27	80	57.1	35.7	72.7	62.7	51.9	41.7	64.0
Yr32	35	25.0	14.3	18.2	31.4	18.5	16.7	32.0
Yr43	102	72.9	64.3	72.7	82.3	59.2	83.3	72.0
Yr44	88	62.9	57.1	45.5	74.5	33.3	83.3	72.0
YrSP	24	17.1	21.4	27.3	15.7	18.5	25.0	4.0
YrTr1	85	60.7	42.8	45.5	74.5	29.6	75.0	76.0
YrExp2	97	69.3	35.7	45.5	72.5	44.4	75.0	84.0
YrTye	34	24.3	35.7	0.0	19.6	25.9	50.0	24.0

[a]N = number of isolates, [b]Freq. = frequency.

(27.3%) was higher in BS compared to other regions, virulence to *Yr8* (85.7%), and *Yr10* (21.4%) was higher in AE. Virulence to *Yr17* (35.3%) was higher in CA, and virulence to *YrExp2* (84.0%), *YrTr1* (76.0%) and *Yr32* (32.0%) in SA was higher than in other regions. Additionally, isolates from MA were more virulent to *Yr7* (96.3%) and *Yr24* (29.6%) than isolates from the remaining regions while virulence to *Yr43* (83.3%), *Yr44* (83.3%) and *YrTye* (50.0%) was higher in region ME (Table 3). Virulence to *Yr5* was only detected in ME. Virulence to all resistance genes was detected in all regions except for BS where virulence to *Yr10, Yr24* and *YrTye* was not found (Table 3).

Virulence relationships among either races or regions

Based on the avirulence/virulence reactions, the 38 races were classified into five virulence groups (VGs) (Fig. 1). VG1 consisted of two races including one (PSTr-27) from ME, and one (PSTr-25) from two regions. VG2 had four races including one (PSTr-34) from MA, and three (PSTr-28, PSTr-29 and PSTr-30) from three or more regions. VG3 had eight races, two (PSTr-20 and PSTr-36) from CA, three (PSTr-35, PSTr-37 and PSTr-38) from MA, three (PSTr-6, PSTr-7 and PSTr-31) from two or more regions. VG4 consisted of nine races including one (PSTr-40) from AE, one (PSTr-41) from MA, three (PSTr-22, PSTr-46 and PSTr-49) from CA, and

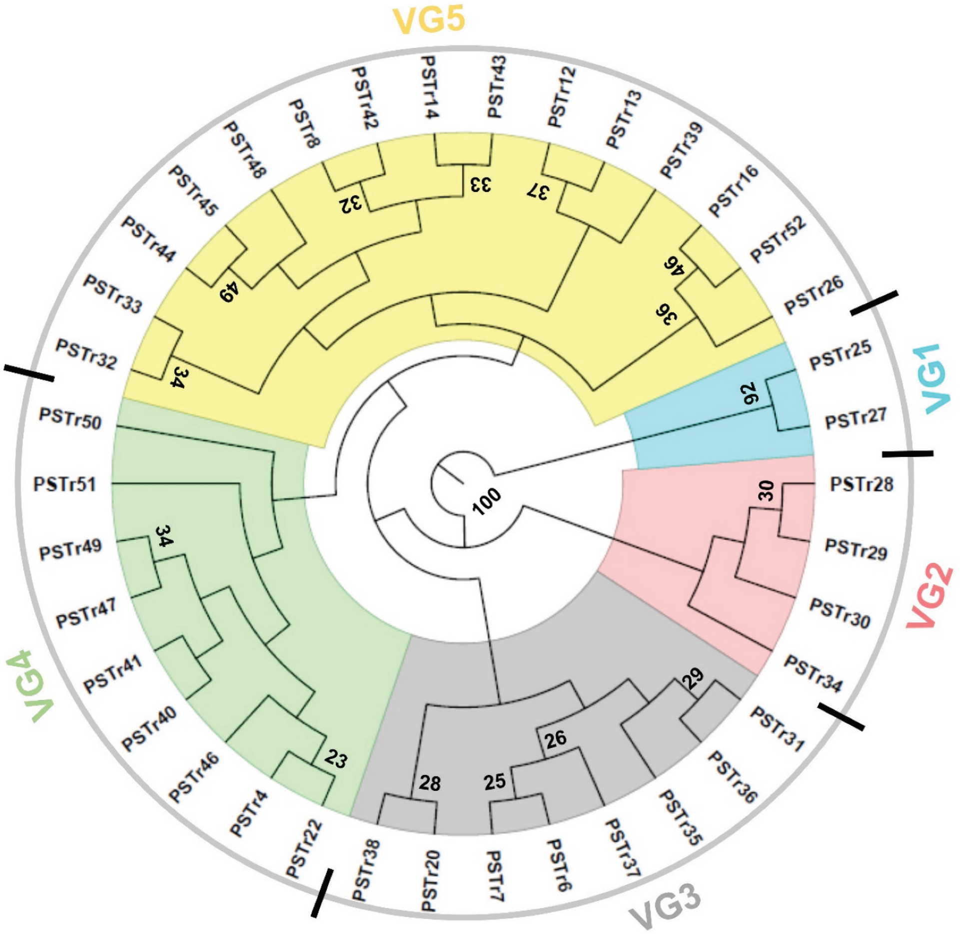

Fig. 1 Dendrogram constructed using the unweighted pair group method based on Nei's genetic distance showing relationships among 38 *Puccinia striiformis* f. sp. *tritici* races identified from six regions in Turkey.

three (PSTr-4, PSTr-47 and PSTr-50) from SA, and one (PSTr-51) from two regions. VG5 as the largest group had one (PSTr14) from AE, two (PSTr-26 and PSTr-39) from CA, two (PSTr-33 and PSTr-48) from SA, three (PSTr-42, PSTr-43 and PSTr-52) from BS, three (PSTr-32, PSTr-44 and PSTr-45) from MA, and four (PSTr-8, PSTr-12, PSTr-13 and PSTr-16) from two regions.

Considering the relationships of the races collected among the regions, the races from AE, CA and MA were similar to each other whereas those from SA and BS were more distant from each other. However, ME was clearly distant from the other regions (Fig. 2).

Discussion

Races of *Pst*, causing wheat stripe rust, have not been well characterized in Turkey because differentials with various genetic backgrounds carrying one or more resistance genes have been used in the past. However, a differential set of 18 single *Yr* gene wheat lines is now available to characterize *Pst* isolates. Sixteen single *Yr* gene NILs were used for the first time in Turkey by Cat et al. (2021) to identify 25 *Pst* races, eight of which were virulent to *Yr10*. They were identified in the coastal areas of Turkey where the frequency of *Yr10*-virulent races was up to 25%. In this study, 38 races from 140 *Pst* isolates were determined based on their reactions on an 18 NIL differential set, and these virulences on differentials varied from 4 to 11 (Table 1). Eleven of the 38 races identified were previously detected by Cat et al. (2021). The most prevalent races were PSTr-29, PSTr-30, PSTr-31 and PSTr-28. PSTr-29 was detected with a frequency of 22.7% in Turkey; it was previously detected in Mexico (Wang et al. 2018; Chen et al. 2021) and the USA (Wan and Chen 2014; Wan et al. 2016) and designated PSTv-36 (Wan and Chen 2014). Race PSTr-26 in this study was also identified previously as PSTv-6 in the USA (Wan and Chen 2014).

Long-distance wind dispersal and human activities can trigger movement of *Pst* race(s) from region to region. For instance, the first incursion of *Pst* into Australia in 1979 was probably the outcome of human facilitated movement from Europe (Wellings 2011). The race group 'Warrior' originated near the Himalayas and was probably moved into Europe by long-distance wind dispersal (Hovmøller et al. 2016). In this context, identical races identified in this study and other studies suggest intercontinental migration scenarios. However, 25 races identified in this study have not been previously reported.

In most regions of the world, the important *Yr* genes *Yr5, Yr10* and *Yr15* are still effective to control stripe rust. However, *Yr10*-virulent races have been previously reported in many countries such as Canada, Chile, China, Hungary, Italy, Kenya, Mexico, Nepal, Pakistan, Turkey, Uzbekistan, and the USA (Sharma-Poudyal et al. 2013; Cat et al. 2021; Chen et al. 2021; Ghanbarnia et al. 2021; Wang et al. 2022). In this study, 13 *Pst* isolates were virulent to *Yr10* with a frequency of 9.3% countrywide, and nine races identified as *Yr10*-virulent were detected in AE with a frequency of 21.4%.

Only one race (PSTr-27) was virulent to *Yr5*. This *Yr5*-virulent race, previously designated TRVR20-5, was reported in a disease note in 2021 (Tekin et al. 2021). Virulence to *Yr5* has been reported in India (Nagajaran 1986), Australia (Wellings and McIntosh 1990), China (Zhang et al. 2020), and Syria (Kharouf et al. 2021). Zhang et al. (2022) reported that the races TSA-6 and TSA-9, which are virulent to *Yr5* can be a serious threat to wheat production in China. On the other hand, Wellings et al. (2009) conducted field

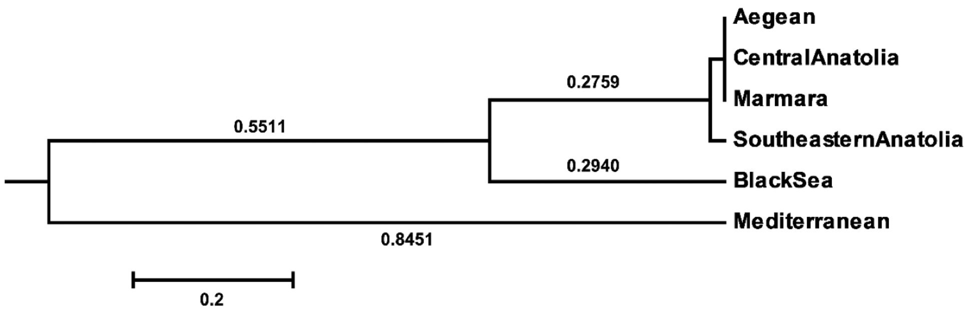

Fig. 2 Dendrogram constructed using the unweighted pair group method based on Nei's genetic distance showing the population relationships among *Puccinia striiformis* f. sp. *tritici* collections from six regions in Turkey.

studies with trap nurseries containing a NIL set in many countries from different continents during 1997–2001, and virulence to *Yr5* was recorded at the adult-plant stage in two locations in Turkey (Eskisehir in 1997 and Adana in 1998), and in single locations in both China and Tajikistan. However, it is not possible to determine whether a particular *Pst* race caused virulence to *Yr5* due to a lack of detailed race identification. Therefore, a connection could not be established between PSTr-27 identified in this study and virulence to *Yr5* reported by Wellings et al. (2009).

The region with the highest number of races was CA (15), followed by MA (14), SA (10), AE (9), ME (6), and BS (5) (Table 2). The most prevalent race in AE and ME was PSTr-30 while PSTr-29 was most prevalent in BS and CA. The most prevalent race was PSTr-28 in MA. Generally, many races were common in these regions, and identical races detected from multiple regions are a sign of *Pst* migration throughout Turkey. However, BS had unique races (PSTr-52, PSTr-42 and PSTr-43), which were absent in other regions (Table 2). It is known that *Berberis* spp. as an alternate host of *Pst* (Jin et al. 2010) are naturally distributed especially in BS of Turkey (Akci and Karakaya 2021). Therefore, it is considered that natural sexual or asexual reproduction on alternate hosts in this region can result in the emergence of new races or spread of current races.

Population relationships of race collections from six regions also support this hypothesis (Fig. 2). Virulence structure of *Pst* was similar in the AE, MA and CA, but races were more distant from each other in SA and BS. The ME region was clearly distant from other regions. The BS and the ME differed from other regions due to the absence of unique races in other regions, and due to the *Yr5*-virulent race, respectively.

In general, among the 18 *Yr* genes, virulence to 17 of these was detected at various frequencies (Table 3). All isolates were virulent to *Yr6*, and there was high virulence to *Yr9, Yr7, Yr8* and *Yr43* with frequency of >70%. Virulence to *Yr6, Yr7, Yr8* and *Yr9* was generally consistent with previous studies in Turkey (Mert et al. 2012; Mert et al. 2016). Unlike previous studies, races were avirulent to only *Yr15*. So far, a *Pst* race virulent to *Yr15* has not been reported from anywhere in the world (Chen et al. 2021; Wang et al. 2022). Moreover, the virulence patterns of the races on 18 *Yr*-gene lines were clustered into five virulence groups (VGs) (Fig. 1). VG5 was the largest group with 15 races, followed by VG4, VG3, VG2 and VG1. Each VG had a complex virulence structure with races from nearly every region. VG4 was differentiated from other VGs since it did not contain races identified in BS and ME (Fig. 1).

In this study, we identified 38 *Pst* races in Turkey. PSTr-29 was the most frequent and widely distributed race in the country from 2018 to 2020, followed by PSTr-30, PSTr-31, and PSTr-28. Race PSTr-29 is identical to PSTv-36, which has been identified in many countries on different continents (Wan et al. 2016; Chen et al. 2021; Wang et al. 2022). In addition, only one race, detected only in ME, was virulent to *Yr5*. This may be a sign of the incursion of new *Pst* races to Turkey. Therefore, breeding programmes should pay attention to more virulent, frequent, and widely distributed races.

Acknowledgements

The authors are grateful to Dr. Xianming Chen from Washington State University, USA for providing *Yr* single-gene line differentials. The authors also thank to many collaborators for collecting and sending infected leaf samples, especially in 2020.

Authors' contributions

TA, MC and MT designed the study; AC, MT and KA performed the virulence analyses; MT and AC analyzed the virulence data; MT wrote the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by The Scientific and Technological Research Council of Turkey (Grant number: 117O043; Türkiye Bilimsel ve Teknolojik Arastirma Kurumu)

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethics approval

There is no ethical issue in this study.

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