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Special Issue: Rust Diseases of Field Crops and Forest Trees

Races of *Puccinia striiformis* f. sp. *tritici* identified from the coastal areas of Turkey

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Abstract: Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a devastating disease of wheat in Turkey and worldwide. This pathogen can overcome known resistance genes and negatively affect the wheat production. The objectives of this study were to determine the virulence patterns of wheat stripe rust isolates collected from the coastal areas (Aegean and Mediterranean regions) of Turkey, and to examine the genetic diversity of the pathogen populations by simple sequence repeat (SSR) marker analysis. The majority of the isolates collected were virulent at various frequencies on the resistance genes *Yr1* (50%), *Yr6* (100%), *Yr7* (78%), *Yr8* (50%), *Yr9* (84%), *Yr10* (25%), *Yr17* (38%), *Yr24* (22%), *Yr27* (31%), *Yr32* (22%), *Yr43* (47%), *Yr44* (6%), *YrSp* (41%), *YrTr1* (6%), and avirulent on *Yr5* and *Yr15*. Based on the analysis of virulence, all isolates were determined as 25 races and clustered into six virulence groups (VGs). In contrast, the isolates clustered into four molecular groups (MGs) based on genotypic data. All four MGs were found in Aegean region of Turkey, while only three MGs (MG1, MG2 and MG3) were found in the Mediterranean region of the country. Additionally, analysis of molecular variance indicated that 80.9% of genetic variation was found within regions and 19.1% was found between the regions. The results suggest that interregional migration of the pathogen was high. The data can be helpful for the management of stripe rust and understanding the population structure and migration of *Pst* in Turkey.

Keywords: molecular characterization, population structure, race dynamics, virulence, yellow rust

Résumé: La rouille jaune, causée par *Puccinia striiformis* f. sp. *tritici* (*Pst*), est une maladie dévastatrice du blé en Turquie et partout dans le monde. Cet agent pathogène peut contourner les gènes de résistance connus et nuire à la production de blé. Les buts de cette étude étaient de déterminer les schémas de virulence d'isolats de rouille jaune collectés dans les régions côtières de Turquie (régions égéenne et méditerranéenne) et d'examiner la diversité génétique des populations d'agents pathogènes par l'analyse des marqueurs des répétitions de séquences simples (SSR). La majorité des isolats collectés était virulente à différents degrés à l'égard des gènes de résistance *Yr1* (50%), *Yr6* (100%), *Yr7* (78%), *Yr8* (50%), *Yr9* (84%), *Yr10* (25%), *Yr17* (38%), *Yr24* (22%), *Yr27* (31%), *Yr32* (22%), *Yr43* (47%), *Yr44* (6%), *YrSp* (41%) et *YrTr1* (6%), et avirulent à l'égard de *Yr5* et *Yr15*. En se basant sur l'analyse de virulence, tous les isolats constituaient 25 races qui formaient 6 groupes de virulence (GV). En revanche, en se basant sur les données génotypiques, les isolats se sont regroupés en quatre groupes moléculaires (GM). Les quatre GM ont été trouvés dans la région égéenne de la Turquie, tandis que trois seulement (MG1, MG2 et MG3) l'ont été dans la région méditerranéenne du pays. De plus, l'analyse de la variance moléculaire a indiqué que 80,9% de la variation génétique se trouvait dans les régions et 19,1%, entre les régions. Les résultats suggèrent que le taux de migration interrégionale était élevé. Les données peuvent servir à gérer la rouille jaune et à comprendre la structure et la migration des populations de *Pst* en Turquie.

Mots clés: Caractérisation moléculaire, structure de population, dynamique des races, virulence, rouille jaune

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Introduction

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most devastating foliar diseases of wheat, causing up to 100% crop losses on susceptible cultivars under favourable climatic conditions (Chen et al. 2014). Therefore, surveillance for the disease in areas with frequent epidemics is of great importance to identify new virulent *Pst* races and identify new genetic resources with efficient resistance genes to prevent devastating epidemics. It has been reported that approximately 88% of the wheat growing areas in more than 60 countries are under threat of wheat stripe rust (Schwessinger 2017). The urediniospores of the pathogen can be carried by wind (Zeybek and Yigit 2004) from one geographically distant region to another, from America to Australia (Wellings 2007), from Europe to West Asia, and from the Middle East to South Africa (Boshoff et al. 2002). The frequency of the occurrence of stripe rust and damage vary with climatic conditions and plant growth stage for each country. At a global level, many large-scale epidemics of *Pst* have occurred and caused economically important losses. Stripe rust causes frequent and devastating epidemics with 5–10% crop losses in some regions, including the United States of America, Australia, India, Pakistan, Ethiopia, Kenya and the UK. Epidemics have also occurred every 2 of 5 years with average 1–5% crop losses in over 25% of the wheat-growing regions of other countries, including Turkey (Chen 2020). In Turkey, wheat stripe rust epidemics caused crop losses of 26.5% in 1992 and the monetary losses were estimated to be $568 million, $53 million in 2000 and $10 million in 2010 (Solh et al. 2013).

The deployment of resistant cultivars and cultural practices are environmentally friendly and cost-effective approaches to prevent large crop losses. Approximately 83 designated and 67 provisional resistance genes and more than 300 quantitative trait loci (QTLs) have been characterized in the wheat genome against *Pst* races so far (McIntosh et al. 2020). However, new virulent *Pst* races are able to overcome resistance genes and negatively affect wheat production. For instance, *Yr9*-virulent races were first identified in the Soviet Union in 1973 (Stubbs et al. 1977) and migrated worldwide (Wang et al. 1986; Saari 1996). Serious large-scale epidemics occurred in many countries such as Iran, Pakistan, India and Turkey between 1992 and 1996 due to *Yr9*-virulent races (Dusunceli et al. 1996; Saari 1996; Nazari and Torabi 2000). Similarly, *Yr27*-virulent races, initially detected in Australia in 2007, subsequently caused devastating epidemics and crop losses of up to 70% in Africa, the Middle East and western Asia, including Turkey and Syria in 2009 and 2010 (Chen 2017), because the races were actually virulent on the resistance genes *Yr10* and *Yr24* in addition to *Yr27* (Hovmøller 2012).

The race dynamics and population structure of *Pst* have been studied intensively using virulence and molecular characterization given the appearance of new races that overcome resistance genes in a target environment and their dissemination to new areas where *Pst* may have been absent or scarce (Rioux et al. 2015; Boshoff et al. 2020). In particular, codominant simple sequence repeat (SSR) markers have been effectively used to distinguish homokaryotic from heterokaryotic isolates since *Pst* urediniospores are dikaryotic (Mboup et al. 2009; Zhan et al. 2012; Sharma-Poudyal et al. 2020). Moreover, a high-level of heterozygosity in *Pst* has been reported in previous studies (Zheng et al. 2013; Xia et al. 2018). Generating virulence and molecular groups based on virulence phenotype and molecular data aid in evaluating the diversity among isolates of *Pst* in a target environment.

Sharma-Poudyal et al. (2013) studied an international *Pst* collection including 53 isolates from Turkey and determined that most of the isolates from Turkey were virulent on at least 11 resistance genes with very high frequency (>70%) and avirulent on *Yr5, Yr10* and *Yr15*. Hovmøller et al. (2020) reported that a race belonging to the PstS11 lineage first detected in Afghanistan in 2012 was found in six African countries and the Middle East including Ethiopia, Kenya, Rwanda, Tanzania, Uganda and Turkey, and had the virulence formula: *Yr2, Yr4, Yr6, Yr7, Yr8, Yr17, Yr27, Yr32, AvS*. In addition, the race 'Triticale2015′ (virulence formula: *Yr2, Yr6, Yr7, Yr8, Yr9, AvS*) and a race belonging to the PstS14 lineage (virulence formula: *Yr2, Yr3, Yr6, Yr7, Yr8, Yr9, Yr17, Yr25, Yr32, YrSp* and *AvS*) were recorded in Turkey in 2019 (Hovmøller et al. 2020). In contrast, Akan (2019) reported that three *Pst* populations (I, II and III) predominated in Turkey, and the populations caused local epidemics during the 2006–2010, 2011–2014 and 2014–2015 growing seasons. He also noted that the resistance genes *Yr5, Yr10* and *Yr15* were effective against *Pst* races present in Turkey. While there are some studies related to the characterization of the wheat stripe rust isolates in Turkey, these are based mainly on virulence tests at the phenotypic level without any race identification at the molecular level. Therefore, the objectives of this study were to characterize *Pst* isolates collected from the southwest coastal areas of Turkey, the Aegean and Mediterranean regions, in

which two severe stripe rust epidemics occurred during the past two decades. Characterization was based on virulence and molecular analyses and the variability and distribution of the races collected in the 2018 and 2019 growing seasons was also determined.

Materials and methods

Field surveys, isolate collection and maintenance of Pst *isolates*

Field surveys were carried out across the wheat-growing areas in the Aegean and Mediterranean regions of Turkey. The regions cover 20% of the wheat production areas in Turkey and are situated between the Middle East and Europe. Wheat crops were sampled at the adult-plant stage (mid-March to early June) during the 2018 and 2019 growing seasons. Infected leaves of wheat and its relatives, preferably with single pustules, were collected from farmer fields during the surveys. The collection site and date, collector, infection type and severity, infected host species, cultivar and other relevant information were recorded for each isolate. A total of 28 isolates, 15 from the Mediterranean region and 13 from the Aegean region, were collected (Table 1). These isolates were immediately placed in paper envelopes and brought to the lab at Akdeniz University, Turkey, for multiplication and analyses.

The infected leaves were dried in a desiccator for one week and then spores on the leaves for each isolate were collected into a gelatin capsule with a mini cyclone spore collector (Tallgrass Solutions, USA). The susceptible bread wheat variety 'Morocco' was used for multiplication of spores of the isolates. The seeds of 'Morocco' were sown in plastic trays with 49 pots (7 x 7 × 10 cm) containing peat-based soil and grown in a climate-controlled room at 12–17°C with a relative humidity of 70–80%. The 12–14day-old seedlings were inoculated with urediniospores suspended in NovecTM 7100 engineered fluid (3 M Company) in a glass vial at a ratio of 10 mg spores per 5 mL oil, and the suspension was then sprayed onto the seedlings using an airbrush spray gun

Table 1. Virulence pattern and prevalence of the *Pst* races.

Race	Isolate	Virulence pattern	Number of virulences	Host species	Collection site	Region
PSTr-1	TRYR17	*-,-,6,7,-,9,10,-,-,-,-,-,43,-,-,-,AvS*	5	*T. aestivum*	Camliyayla/Mersin	Mediterranean
PSTr-2	TRYR23	*-,-,6,7,8,9,-,-,-,24,-,-,-,-,-,-,AvS*	5	*X Triticosecale*	Aliaga/Izmir	Agean
PSTr-2	TRYR28	*-,-,6,7,8,9,-,-,-,24,-,-,-,-,-,-,AvS*	5	*T. durum*	Merkez/Kutahya	Agean
PSTr-3	TRYR16	*1,-,6,7,-,9,-,-,17,-,27,-,-,-,-,-,AvS*	6	*T. aestivum*	Dörtyol/Hatay	Mediterranean
PSTr-4	TRYR24	*-,-,6,7,-,9,-,-,17,-,-,-,-,-,-,-,AvS*	4	*T. aestivum*	Sarıgöl/Manisa	Agean
PSTr-5	TRYR26	*1,-,6,7,8,9,-,-,17,-,27,-,-,-,-,AvS*	7	*T. durum*	Merkez/Uşak	Agean
PSTr-6	TRYR35	*1,-,6,7,-,9,-,-,17,-,32,-,-,-,Sp,-,AvS*	7	*T. aestivum*	Merkez/Edirne	Thrace
PSTr-7	TRYR13	*1,-,6,7,-,9,-,-,17,-,-,32,43,-, Sp,Tr1,AvS*	9	*T. aestivum*	Acıpayam/Denizli	Agean
PSTr-8	TRYR22	*1,-,6,7,8,9,-,-,-,-,27,-,-,-,-,-,AvS*	6	*T. aestivum*	Torbalı/İzmir	Agean
PSTr-9	TRYR56	*-,-,6,7,8,9,-,-,-,-,-,-,-,-,-,-,AvS*	4	*T. aestivum*	Haymana/Ankara	Central Anatolia
PSTr-10	TRYR43	*-,-,6,7,8,9,-,-,-,-,27,-,-,-,-,-,AvS*	5	*T. aestivum*	Ceylanpınar/Sanliurfa	Southeastern Anatolia
PSTr-10	TRYR44	*-,-,6,7,8,9,-,-,-,-,27,-,-,-,-,-,AvS*	5	*T. aestivum*	Akcakale/Sanliurfa	Southeastern Anatolia
PSTr-11	TRYR1	*1,-,6,-,-,9,10,-,17,-,-,-,-,-,-,-,-,-,AvS*	5	*T. aestivum*	Sarıcam/Adana	Mediterranean
PSTr-12	TRYR6	*1-,6,-,8,9,10,-,-,-,-,-,-,Sp,-,AvS*	6	*T. aestivum*	Onikisubat/Kahramanmaras	Mediterranean
PSTr-13	TRYR25	*1,-,6,7,8,-,10,-,-,-,-,-,-,-,-,AvS*	5	*X Triticosecale*	Fethiye/Mugla	Agean
PSTr-14	TRYR27	*1,-,6,7,-,9,10,-,-,-,-,-,-,-, -,-,AvS*	5	*T. aestivum*	Dinar/Afyonkarahisar	Agean
PSTr-15	TRYR3	*-,-,6,-,8,9,-,-,-,-,-,43,-,Sp,-,AvS*	5	*X Triticosecale*	Serik/Antalya	Mediterranean
PSTr-15	TRYR4	*-,-,6,-,8,9,-,-,-,-,-,43,-,Sp,-,AvS*	5	*X Triticosecale*	Manavgat/Antalya	Mediterranean
PSTr-15	TRYR5	*-,-,6,-,8,9,-,-,-,-,-,43,-,Sp,-,AvS*	5	*T. aestivum*	Döşemealtı/Antalya	Mediterranean
PSTr-15	TRYR7	*-,-,6,-,8,9,-,-,-,-,-,43,-,Sp,-,AvS*	5	*T. aestivum*	Konyaaltı/Antalya	Mediterranean
PSTr-16	TRYR8	*-,-,6,-,8,9,-,-,-,-,27,-,43,-,Sp,-,AvS*	6	*T. durum*	Kemer/Antalya	Mediterranean
PSTr-17	TRYR2	*-,-,6,7,-,9,-,-,-,-,27,32,43,44,-,-,AvS*	7	*T. aestivum*	Tarsus/Mersin	Mediterranean
PSTr-18	TRYR18	*1,-,6,7,-,-,-,-,-,-,27,-,43,-,-,-,AvS*	5	*T. aestivum*	Mentese/Mugla	Agean
PSTr-19	TRYR19	*1,-,6,7,-,-,10,-,-,24,27,-,43,-,-,-,AvS*	7	*T. durum*	Pamukkale/Denizli	Agean
PSTr-20	TRYR20	*1,-,6,7,-,-,10,-,-,24,-,-,43,-,-,-,AvS*	6	*T. aestivum*	Salihli/Manisa	Agean
PSTr-21	TRYR12	*1,-,6,7,-,9,-,-,17,-,-,32,43,-,-,-,AvS*	8	*T. aestivum*	Merkez/Afyonkarahisar	Agean
PSTr-22	TRYR14	*-,-,6,7,8,9,-,-,17,24,-,-,-,-,-,-,AvS*	6	*T. aestivum*	Merkez/Burdur	Mediterranean
PSTr-23	TRYR15	*-,-,6,7,8,9,10,-,-,24,-,-,-,-,-,-,AvS*	6	*T. aestivum*	Yalvac/Isparta	Mediterranean
PSTr-24	TRYR21	*1,-,6,7,-,9,-,-,17,-,-,32,43,-,Sp,-,AvS*	8	*T. aestivum*	Merkez/Aydın	Agean
PSTr-24	TRYR11	*1,-,6,7,-,9,-,-,17,-,-,32,43,-,Sp,-,AvS*	8	*T. durum*	Elmalı/Antalya	Mediterranean
PSTr-24	TRYR10	*1,-,6,7,-,9,-,-,17,-,-,32,43,-,Sp,-,AvS*	8	*T. durum*	Bucak/Burdur	Mediterranean
PSTr-25	TRYR9	*-,-,6,7,-,-,-,-,17,24,27,-,-,,44,Sp,-,AvS*	7	*T. durum*	Merkez/Isparta	Mediterranean

connected to a vacuum pump from a distance of 10 cm to 15 cm by rotating the tray (Hovmøller et al. 2017). Inoculated seedlings were kept in a dew chamber for 24 h in darkness at 10°C and then transferred to a climate-controlled room where the temperature was set to 18–12°C (day-night) and the relative humidity to 70–80%. The pathogen was re-isolated from plants inoculated with each isolate, and isolated with separators to avoid cross-contamination. Urediniospores of each isolate from a fresh single-uredinium on 'Morocco' were collected in gelatin capsules 15–19 days after inoculation using the mini cyclone spore collector. Each gelatin capsule was placed in a desiccator at 4°C for a short time until testing on the differential set. This reproduction process was repeated several times for each isolate until a sufficient spore quantity was obtained for virulence testing on differentials.

Virulence testing on differentials

Twenty-eight *Pst* isolates (Table 1), of which 17 were from bread wheat (*Triticum aestivum* L.), seven from durum wheat (*Triticum turgidum* spp. *durum*) and four from triticale (x *Triticosecale*), collected in 2018 and 2019 from the Aegean and Mediterranean regions of Turkey were used for virulence characterization on a differential set of wheat genotypes containing 16 *Yr* near-isogenic lines (NILs) of 'Avocet', each differentiating for a single-resistance gene (*Yr1, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr27, Yr32, Yr43, Yr44, YrSp* and *YrTr1*) (Wellings et al. 2009). In addition, four isolates (TRYR35, TRYR43, TRYR44 and TRYR56) that caused regional epidemics during the 2008, 2010, 2011 and 2015 growing seasons in different regions of Turkey were included in the collection as controls to compare with the other isolates (Table 1). Infection types (ITs) were recorded at 19 days after inoculation according to a 0-to-9 scale described by Line and Qayoum (1992). ITs of 0 to 6 were considered avirulent, and 7 to 9 virulent (Wan and Chen 2014).

The virulence/avirulence pattern of each isolate was determined based on IT data from the 'Avocet' NILs. If the isolates produced the same virulence/avirulence pattern, they were classified within a common virulence group (VG), but if the pattern was different, they were identified in another VG.

DNA extraction and PCR amplification

Genomic DNA was extracted directly from about 20-mg dried urediniospores for each isolate. Approximately 150 mg sterile sea sand (Merck, Germany) and 200 µL lysis buffer [2% Cetyl trimethylammonium bromide (CTAB), 1.4 M NaCI, 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 1% β-mercaptoethanol] were first added to a 2.0 mL microcentrifuge tube containing about 20 mg urediniospores for each isolate, and the mixture was homogenized using a micro pistil and vortexed regularly. CTAB solution (200 µL) was added to each microcentrifuge tube and homogenization continued. After adding 10 µL *RNase A* to the microcentrifuge tubes, the homogenized samples were incubated at 65°C for 1 h in a thermo-shaker (Biosan, Latvia). Following incubation, 25:24:1 (v/v) phenol/chloroform/isoamyl alcohol was added to the tubes, mixed gently and centrifuged for 20 min at 13 000 rpm. Next, approximately 700 µL of clean lysate (supernatant) was transferred to another microcentrifuge tube, and 500 µL of cold isopropyl alcohol was added. The contents of the tube were mixed gently and centrifuged for 20 min at 13 000 rpm. Later, with 700 µL of 70% ethanol (−20°C) added into the tubes before centrifuging again at 13 000 rpm. After centrifugation, the ethanol was discarded and the tubes were air dried at room temperature for 1 h. Genomic DNA was dissolved in 100 µL TE (10 mM Tris-HCL and 1 mM EDTA, pH 8.0) buffer and kept for 1 h at room temperature. The quality and quantity of the DNA samples were checked on an agarose gel, and visualized using a gel imaging system. Stock DNA was stored at −20°C until use.

The genomic DNA of the *Pst* isolates was genotyped using 21 polymorphic *Pst*-specific SSR markers (Enjalbert et al. 2002; Chen et al. 2009; Cheng et al. 2012). Marker information is given in Supplementary Table 1. The total volume of the reaction mixture was 12 µL containing 100 ng genomic DNA, 1X PCR buffer (Thermo Fisher Scientific, USA), 1.5 mM $MgCI_2$ (Thermo Fisher Scientific), 0.2 mM of each dNTP (Thermo Fisher Scientific), 1 µM forward primer, 1 µM reverse primer, and 1 U *Taq* DNA polymerase (Thermo Fisher Scientific). Amplifications were performed in a thermocycler (BioRad, USA) under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 41–58°C (Supplementary Table 1) for 30 s, and extension at 72°C for 1 min, and a final extension of 10 min at 72°C. The PCR products were resolved in 2% agarose gel sand visualized under UV light with a gel-imaging system (UVP UVsolo touch, Analytik Jena, Germany) after staining with ethidium bromide.

Structural analyses of Pst *isolates based on virulence and molecular data*

A multivariate hierarchical clustering analysis based on the unweighted pair-group method (UPGMA) was

performed to evaluate the similarities among all isolates based on virulence patterns using PAST v 3.0 (Hammer et al. 2001) and a phylogenetic tree was modified in FigTree v. 1.4.4. (Rambaut 2018). Genetic variation parameters such as observed alleles (*Na*), effective alleles (*Ne*), Shannon's information index (*I*), observed heterozygosity (*Ho*) and expected heterozygosity (*He*), and Nei's genetic distance (GD) were calculated to reveal mean genetic variations based on the SSR loci using Popgene v. 1.31 (Yeh et al. 1997) for all isolates. In addition, the population structure and fixation index (*Fst*) among all isolates were analysed by the analysis of molecular variance (AMOVA) in Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010). A phylogenetic tree based on genetic distances was produced in FigTree v. 1.4.4. (Rambaut 2018).

Results

Virulence characterization

The collection sites and virulence patterns of the isolates on the 16 *Yr* single-gene differentials are given in Table 1. All of the isolates were virulent on *Yr6*. The isolates were also most frequently virulent on the resistance genes (≥70%) on *Yr7* (78%) and *Yr9* (84%), and virulent at moderate frequencies (between 20% and 70%) on *Yr1* (50%), *Yr8* (50%), *Yr10* (25%), *Yr17* (38%), *Yr24* (22%), *Yr27* (31%), *Yr32* (22%), *Yr43* (47%) and *YrSp* (41%). Isolates (TRYR2 and TRYR9) were least frequently virulent on *Yr44* (6%) and *YrTr1* (6%). All isolates were avirulent on *Yr5* and *Yr15*.

The isolates were virulent on four to nine of the differentials, and classified as 25 *Pst* races (Table 1). Race PSTr-7 was virulent on most resistance genes (*Yr1, Yr6, Yr7, Yr9, Yr17, Yr32, Yr43, YrSp* and *YrTr1*). The races PSTr-4 (*Yr6, Yr7, Yr9* and *Yr17*), while PSTr-9 was virulent on the fewest (*Yr6, Yr7, Yr8* and *Yr9*). Eight *Yr10*-virulent races (PSTr-1, PSTr-11, PSTr-12, PSTr-13, PSTr-14, PSTr-19, PSTr-20 and PSTr-23) were identified (Table 1).

In the hierarchical clustering analysis, the isolates were classified into six virulence groups (VGs) based on their virulence patterns (Fig. 1*a*), with VG1 including the most isolates (12), followed by VG3 (5), VG4 (5), VG5 (5), VG2 (4) and VG6 (1). The control isolates with previously known virulence formulas grouped into VG1.

In VG1, all isolates were virulent on *Yr9* and avirulent on *Yr44*. In VG2, all isolates were virulent on *Yr1* and *Yr10* and avirulent on *Yr24, Yr27, Yr32, Yr43, Yr44* and *YrTr1*. In contrast, all isolates were virulent on *Yr8, Yr9, Yr43* and *YrSp*, and avirulent on *Yr1, Yr7, Yr10, Yr17, Yr24, Yr44* and *YrTr1* in VG3, whereas only *YrTr1* and

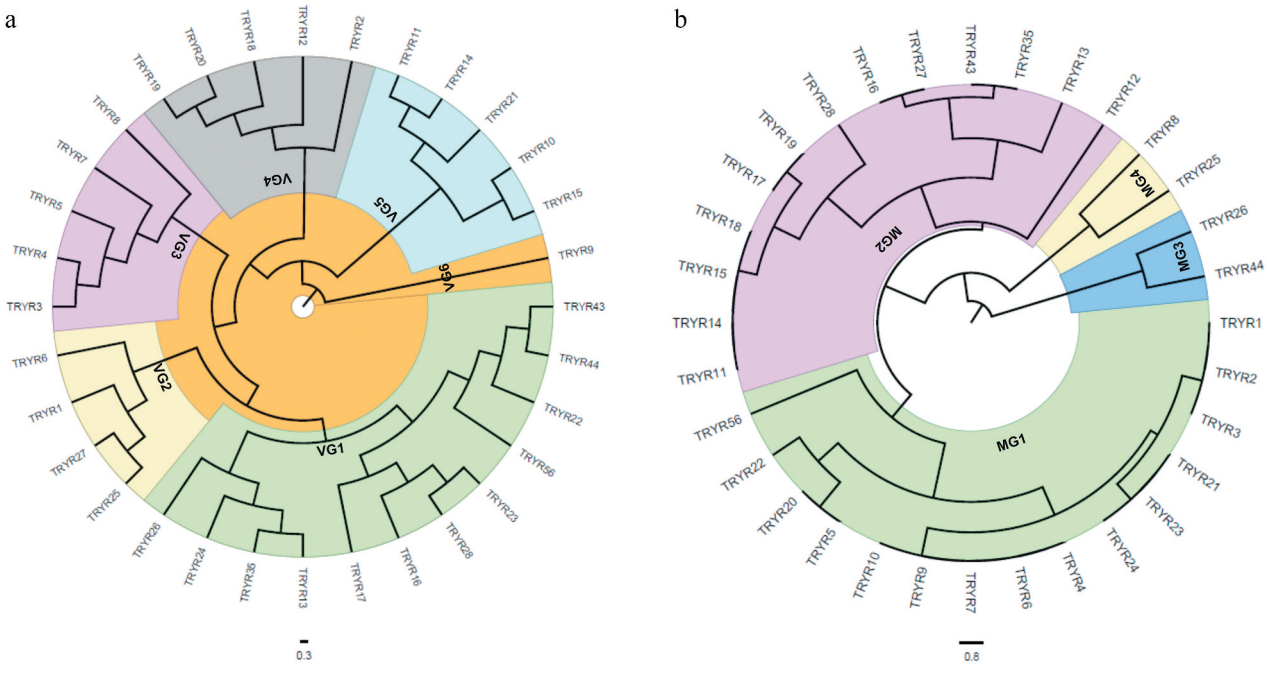

Fig. 1 Dendrogram of virulence **(a)** and molecular groups **(b)** of the *Pst* isolates.

Yr44 were effective in VG4 and VG5, respectively. VG6 contained only one isolate (TRYR9) with virulence on *Yr6, Yr7, Yr17, Yr24, Yr27, Yr44* and *YrSp* (Fig. 1*a*).

Population diversity and heterozygosity based on molecular data

Twenty-one polymorphic SSR primer pairs were used in this study. Information on the SSR loci, allele sizes and the number of alleles is given in Supplementary Table 1. The number of alleles per locus ranged from one to three. Ten of 21 SSR loci were monomorphic among the isolates, illustrating that there was no difference between the isolates collected from the Aegean and Mediterranean regions for at least 10 SSR loci. The diversity of the *Pst* isolates was estimated using several genetic variation parameters (Table 2). The mean observed alleles ranged from 1.095 in TRYR43, from the southeastern Mediterranean region, to 1.476 in the Aegean region. Furthermore, effective alleles in the Aegean region were higher (1.338) in comparison with the Mediterranean region (1.271), TRYR44 (1.238), TRYR56 (1.190) and TRYR43-TRYR35 (1.095). While the highest Shannon's information index was also observed in the Aegean region (0.264), it was the lowest in TRYR43 (0.066). Moreover, the highest expected heterozygosity values were calculated in the Aegean (0.175) and Mediterranean (0.145) regions. Unlike these, TRYR56 (0.238) and TRYR44 (0.238) had the highest observed heterozygosity.

The highest frequency of polymorphic loci was found in the Aegean region (42.9%) compared with the Mediterranean region (33.3%) and the controls. The isolate TRYR3 from the Mediterranean, and the isolates TRYR19 and TRYR27 from the Aegean, and a control isolate, TRYR44, had the most specific alleles (Table 2).

The phylogenetic tree based on Nei's genetic distance clustered the 32 isolates, including 28 isolates from the Aegean and Mediterranean regions and four control isolates, into four molecular groups (MGs) (Fig. 1*b*). MG1 consisted of 15 isolates (46.8%), mostly from the Mediterranean region, and MG2 of 13 isolates (40.6%) from both regions. MG3 and MG4 were the smallest groups with only two isolates in each. While MG3 was more distant from the other MGs, MG1 and MG2 were more closely related to each other, and MG4 had the same distance to MG1 and MG2. The distribution of MGs in the Aegean and Mediterranean regions is illustrated in Supplementary Fig. 1. While all MGs were detected in the Aegean region, the Mediterranean region had three MGs (MG1, MG2 and MG3). In addition, MG2 was predominant in both regions, followed by MG1. The presence of the same MGs in both regions suggests migration of *Pst* isolates between regions. Additionally, the relationship between the regions is illustrated in Fig. S2.

The analysis of molecular variance (AMOVA) showed that genetic variation among isolates occurred mostly within regions accounting for 80.9% ($p < 0.01$). Genetic variation among regions was not statistically important, with only 19.1% of the variation explained. The genetic distance, number of effective migrants and the fixation index between the *Pst* isolates of both regions were calculated based on molecular data (Table 3). While the most distinct relationship was Mediterranean-TRYR44 (0.145) based on genetic distance, the Mediterranean and Aegean regions (0.011) were very close to each other. The number of effective migrants varied from 0.161 (between Mediterranean and TRYR44) to 5.069 (between Mediterranean and Aegean) in parallel to genetic distance.

The fixation index values between regions also changed at similar rates as genetic distance, and both

Table 2. Genetic variation parameters of the *Pst* isolates collected from different geographical regions.

Origin of isolate	Observed alleles (N_a)	Effective alleles (N_e)	Shannon information index (I)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Polymorphic loci (%)
Mediterranean region	1.429 ± 0.148	1.271 ± 0.092	0.222 ± 0.072	0.167 ± 0.065	0.145 ± 0.047	33.33
Aegean region	1.476 ± 0.131	1.338 ± 0.107	0.264 ± 0.074	0.167 ± 0.062	0.175 ± 0.050	42.86
TRYR56*	1.190 ± 0.112	1.190 ± 0.112	0.165 ± 0.066	0.238 ± 0.095	0.119 ± 0.048	23.81
TRYR43**	1.095 ± 0.066	1.095 ± 0.066	0.066 ± 0.045	0.095 ± 0.066	0.048 ± 0.033	9.52
TRYR44**	1.238 ± 0.095	1.238 ± 0.095	0.165 ± 0.066	0.238 ± 0.095	0.119 ± 0.048	23.81
TRYR35***	1.095 ± 0.095	1.095 ± 0.095	0.099 ± 0.054	0.143 ± 0.078	0.071 ± 0.039	14.29

*TRYR56 (2015) is originated from central Anatolia region of Turkey,
**TRYR43 (2010) and TRYR44 (2011) are originated from southeastern region of Turkey,
***TRYR35 (2008) is originated from Thrace region of Turkey.

Table 3. Nei's genetic distance (GD), number of effective migrants (N_m) and fixation index (F_{ST}) values of the regions.

Origin of isolate	GD	N_m	F_{ST}
Mediterranean-Aegean	0.011	5.069	0.047
Mediterranean-TRYR56	0.117	0.359	0.410
Mediterranean-TRYR43	0.058	0.940	0.210
Mediterranean-TRYR44	0.145	0.161	0.609
Mediterranean-TRYR35	0.109	0.359	0.410
Aegean-TRYR56	0.071	2.086	0.108
Aegean-TRYR43	0.080	1.439	0.149
Aegean-TRYR44	0.107	0.304	0.451
Aegean-TRYR35	0.067	2.086	0.108

parameters were positively correlated (r= 0.99, P< 0.01). The Mediterranean and Aegean regions had little differentiation (0< F_{ST}≤0.05) and three region relations (Aegean-TRYR43, Aegean-TRYR56 and Aegean-TRYR35) had moderate differentiation (0.05< F_{ST} ≤0.15). In addition, while Mediterranean and TRYR43 had high differentiation (0.15< F_{ST}≤0.25), three region relations (Mediterranean-TRYR56, Mediterranean-TRYR35, Aegean-TRYR44 and Mediterranean-TRYR44) had very high differentiation (0.25< F_{ST}) (Table 3). The combination of VGs and MGs of the isolates are given in Supplementary Table 2; MG1 contained all VGs followed, by MG2, MG4 and MG3.

Discussion

Turkey has had several stripe rust epidemics in both the highland and coastal areas including the Aegean and Mediterranean regions during the last 20 years. However, few studies of the virulence of *Pst* isolates and field observations with trap nurseries have been conducted. In one of these studies, Mert et al. (2012) reported that *Yr1, Yr3V, Yr4+, Yr5, Yr10, Yr15, YrSp*, and *YrCV* genes were resistant to the *Pst* population in each location by using trap nurseries at 12 different locations in Turkey. It was also reported that a new race, virulent on the *Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr25, Yr32* and *YrSp* genes, was detected in the Thrace and Marmara regions of Turkey during the 2014 and 2015 growing seasons (Mert et al. 2016). This new race was identical to the 'Warrior' race, based on a seedling test conducted under greenhouse conditions (Hovmøller et al. 2016). In these studies, the resistance genes *Yr5, Yr10* and *Yr15* were found to be effective against all *Pst* populations in Turkey (Anonymous 2004; Mert et al. 2012, 2016). These results were similar to the findings of this study, except that the isolates collected from the Aegean and Mediterranean regions in 2018 and 2019 in the current report were virulent on the *Yr10* gene with moderate frequency (25%). Zeybek and Yigit (2004) also characterized the virulence of 491 *Pst* isolates collected from the southern Aegean and western Mediterranean regions of Turkey during 2000 and 2001. They determined that most isolates were virulent on the *Yr2, Yr3a, Yr9, Yr17* and *Sd* resistance genes at high frequency, while *Yr6, Yr7, Yr8* and *Yr10* were resistant to all isolates. Although their study was conducted in similar regions to this study, the resistance genes found by Zeybek and Yigit (2004) were mostly ineffective (*Yr6* and *Yr7*) or partially ineffective (*Yr10*) to all *Pst* isolates in contrast with our study. This may reflect changing population dynamics of the pathogen over the past 18 years. Until this study, there have not been comprehensive studies to characterize *Pst* isolates in Turkey.

The *Pst* isolates collected from the two regions in this study together with the four control isolates collected previously were determined as 25 races (Table 1), and all isolates clustered into six virulence groups (VGs) based on virulence analyses (Fig. 1*a*). In particular, VG1 had the most complex virulence structure, supporting the hypothesis of interregional migration in Turkey. Other VGs were more stable and generally contained isolates from the same region. There have also been international studies, apart from studies conducted by the Turkish groups, based on virulence characterization or field observations with differential sets, which also included *Pst* isolates from Turkey (Wellings et al. 2009; Sharma-Poudyal et al. 2013). Wellings et al. (2009) reported that the resistance genes *Yr7, Yr8* and *Yr9* were ineffective against *Pst* isolates in the Adana, Izmir, Ankara and Haymana locations of Turkey in 1998, but virulence on *Yr5* was recorded in Adana in 1998 and in Eskisehir in 1999. Sharma-Poudyal et al. (2013) studied 235 international *Pst* isolates, including 53 isolates from Turkey, and reported that most isolates from Turkey were virulent on *YrA, Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr28, Yr31* and *YrExp2* at very high frequencies (>70%), moderately virulent on *Yr1* and *Yr25* at frequencies of 20% and 70%, respectively, and virulent on *Yr24, Yr32* and *YrSp* at very low frequencies (7%). The genes *Yr5, Yr10* and *Yr15* were resistant to all isolates collected from Turkey based on virulence characterization. In this study, all the isolates were virulent on *Yr6* (Table 1). Additionally, 28 *Pst* isolates were also

virulent on other resistance genes at various frequencies and our results were mostly compatible with those of Sharma-Poudyal et al. (2013) except for *Yr10*.

In the molecular portion of this study, the results indicated that there was high-genetic diversity among the *Pst* isolates. The 32 *Pst* isolates, including 28 isolates from the Aegean and Mediterranean regions and four control isolates, were allocated into four molecular groups (MGs) (Fig. 1*b*). While all four MGs were found in the Aegean region, three were found in the Mediterranean region (MG1, MG2 and MG3). In addition, MG2 was predominant in both regions, followed by MG1. The presence of the same MGs in both regions is a sign of migration of *Pst* isolates between the regions. Walter et al. (2016) studied 566 *Pst* isolates selected worldwide, including more than 5000 isolates available at BIOGER-CPP, INRA and the Global Rust Reference Center (GRRC). They determined that 15 of 18 *Pst* isolates collected from Turkey were included in the genetic lineage PstS2 and the remaining three isolates in the other genetic lineage. The races in PstS2 had different virulence formulas by sampling year. The virulence formula was *Yr2, Yr6, Yr7, Yr8, Yr9, Yr10, Yr24, Yr25, AvS* in the sampling year 2004, 2005 and 2009, and *Yr1, Yr2, Yr6, Yr7, Yr8, Yr9, Yr25, AvS* in 2009. In this study, MG1 and MG2 were mostly similar to the genetic lineage PstS2 presented by Walter et al. (2016). Moreover, Hovmøller et al. (2020) reported that the races belonging to the lineages PstS11, PstS13, PstS14 and ME2018 were detected in Turkey in 2019. In our study, MG3 was similar to the genetic lineage PstS13 including the race Triticale2015, identified by Hovmøller et al. (2020) and Walter et al. (2016). The AMOVA also showed that genetic variation was determined within regions. However, genetic variation among regions was not statistically significant, owing to the fact that identical isolates were found between neighbouring collection sites in both regions.

The fixation index and number of effective migrants are widely used to show genetic differentiation among isolates in studies focusing on population structure (Mboup et al. 2009; Zhan et al. 2012; Sharma-Poudyal et al. 2020). In particular, the low *Fst* ($0< F_{ST}\leq 0.05$) and high number of effective migrants (5.069) between the Aegean and Mediterranean regions of Turkey also illustrated that there was no difference between the regions (Table 3). It has been known that there is a resident clonal population like the lineage PstS2 in the western part of Turkey, and the migration route of this population was estimated to be from the Middle East to South Africa by Ali et al. (2014). Ali et al. (2014) also demonstrated that *Pst* populations from the Mediterranean, the Middle East and central Asia were similar in spite of the fact that this is a large geographic area. Bahri et al. (2009) previously confirmed that in the clonal population, 83% of the isolates collected from Mediterranean regions belonged to the lineage PstS2.

This study has provided new information regarding the population structure and migration of the *Pst* population in western Turkey. We report, for the first time, *Yr10*-virulent races detected with moderate frequency (25%) in the coastal areas of Turkey. Continued monitoring populations of the stripe rust pathogen will be critical for the early detection of new virulent races with the potential to cause devastating epidemics.

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Supplementary material

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References

Akan K. 2019. Evaluation of some lines selected from bread wheat landraces for their reaction to yellow (stripe) rust. Fresen Environ Bull. 28 (8):6339–6351.

Ali S, Gladieux P, Leconte M, Gautier A, Justesen AF, Hovmoller MS, Enjalbert J, De Vallavieille-pope C. 2014. Origin, migration routes and worldwide population genetic structure of the wheat yellow rust *Puccinia striiformis* f. sp. *tritici*. Plos Pathog. 10 (1):e1003903. doi:10.1371/journal.ppat.1003903

Anonymous. 2004. National cool season cereals project, resistance studies for cereal diseases. Turkey: Field Crops Central Research Institute.

Bahri B, Leconte M, Ouffroukh A, De Vallavieille-Pope C, Enjalbert J. 2009. Geographic limits of a clonal population of wheat yellow rust in the Mediterranean region. Mol Ecol. 18(20):4165–4179. doi:10.1111/j.1365-294X.2009.04267.x

Boshoff WHP, Pretorius ZA, van Niekerk BD. 2002. Establishment, distribution, and pathogenicity of *Puccinia striiformis* f. sp *tritici* in South Africa. Plant Dis. 86(5):485–492. doi:10.1094/PDIS.2002.86.5.485

Boshoff WHP, Visser B, Lewis CM, Adams TM, Saunders DGO, Terefe T, Soko T, Chiuraise N, Pretorius ZA. 2020. First report of

Puccinia striiformis f. sp. tritici, causing stripe Rust of Wheat, in Zimbabwe. Plant Dis. 104(1):290. doi:10.1094/PDIS-07-19-1395-PDN

Chen CQ, Zheng WM, Buchenauer H, Huang LL, Lu NH, Kang ZS. 2009. Isolation of microsatellite loci from expressed sequence tag library of *Puccinia striiformis* f. sp. *tritici*. Mol Ecol Resour. 9:236–238. doi:10.1111/j.1755-0998.2008.02423.x.

Chen WQ, Wellings C, Chen XM, Kang ZS, Liu TG. 2014. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Mol Plant Pathol. 15(5):433–446. doi:10.1111/mpp.12116

Chen X. 2017. Stripe rust epidemiology. In: Chen X, Kang Z, editors. Stripe rust. Dordrecht: Springer Nature; p. 283–352.

Chen XM. 2020. Pathogens which threaten food security: *Puccinia striiformis*, the wheat stripe rust pathogen. Food Secur. 12(2):239–251. doi:10.1007/s12571-020-01016-z

Cheng P, Chen XM, Xu LS, See DR. 2012. Development and characterization of expressed sequence tag-derived microsatellite markers for the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici*. Mol Ecol Resour. 12:779–781.

Dusunceli F, Cetin L, Albustan S, Beniwal S. 1996. Occurrence and impact of wheat stripe rust (Puccinia striiformis) in Turkey in 1994/95 crop season. *The European and Mediterranean Cereal Rusts Powdery Mildew Conference*; 1996; Lunteren, Netherlands.

Enjalbert J, Duan X, Giraud T, Vautrin D, De Vallavieille-Pope C, Solignac M. 2002. Isolation of twelve microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Puccinia striiformis* f.sp. *tritici*. Mol Ecol Notes. 2:563–565. doi:10.1046/j.1471-8286.2002.00322.x.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 10(3):564–567. doi:10.1111/j.1755-0998.2010.02847.x

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron. 4(1):9.

Hovmøller MS. 2012. Report on *Puccinia striiformis* race analyses 2011. Flakkebjerg: Global Rust Reference Center (GRRC), Aarhus University.

Hovmøller MS, Rodriguez-Algaba J, Thach T, Sørensen CK. 2017. Race typing of *Puccinia striiformis* on wheat. In: Periyannan S, editor. Wheat rust diseases methods and protocols. New York: Humana Press; p. 29–47.

Hovmøller MS, Patpour M, Rodriguez-Algaba J, Thach T, Justesen AF, Hansen JG. 2020. GRRC annual report 2019: stem- and yellow rust genotyping and race analyses. Aarhus: Global Rust Reference Center.

Hovmøller MS, Walter S, Bayles RA, Hubbard A, Flath K, Sommerfeldt N, Leconte M, Czembor P, Rodriguez-Algaba J, Thach T, et al. 2016. Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. Plant Pathol. 65(3):402–411. doi:10.1111/ppa.12433

Line RF, Qayoum A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-87. Washington: US Department of Agriculture.

Mboup M, Leconte M, Gautier A, Wan AM, Chen W, De Vallavieille-pope C, Enjalbert J. 2009. Evidence of genetic recombination in wheat yellow rust populations of a Chinese oversummering area. Fungal Genet Biol. 46(4):299–307. doi:10.1016/j.fgb.2008.12.007

McIntosh RA, Dubcovsky J, Rogers WJ, Xia XC, Raupp WJ. 2020. Catalogue of gene symbols for wheat: 2020 supplement. Annual Wheat Newsletter. 66:109–128.

Mert Z, Dusunceli F, Akan K, Cetin L, Yazar S, Bolat N, Yorgancilar A, Unsal R, Ercan B, Ozseven I. 2012. An overview of the network for important cereal diseases management research in Turkey between 2003 and 2011. 3th International Cereal Rusts and Powdery Mildews Conference; Beijing, China.

Mert Z, Nazari K, Karagoz E, Akan K, Ozturk I, Tulek A. 2016. First incursion of the warrior race of wheat stripe rust (*Puccinia striiformis* f. sp *tritici*) to Turkey in 2014. Plant Dis. 100(2):528. doi:10.1094/PDIS-07-15-0827-PDN

Nazari K, Torabi M. 2000. Distribution of yellow rust (*Yr*) resistance genes in Iran. Acta Phytopathol. Entomol. Hung. 35:121–131.

Occurrence and impact of wheat stripe rust (*Puccinia striiformis*) in Turkey in 1994/95 crop season. The European and Mediterranean Cereal Rusts Powdery Mildew Conference; 1996; Lunteren, Netherlands.

Rambaut A. 2018. FigTree v.1.4.4. [accessed 2020 Oct 25]. http://tree.bio.ed.ac.uk/software/figtree/.

Rioux S, Mimee B, Gagnon AE, Hambleton S. 2015. First report of stripe rust (*Puccinia striiformis* f. sp *tritici*) on wheat in Quebec, Canada. Phytoprotection. 95(1):7–9. doi:10.7202/1028400ar

Saari EE. 1996. The Yr9 virulence factor in Puccinia striiformis and South Asia: coevolution or migration from a distant place. The European and Mediterranean Cereal Rusts & Powdery Mildews Conference; 1996; Lunteren, Netherlands.

Schwessinger B. 2017. Fundamental wheat stripe rust research in the 21 st century. New Phytol. 213(4):1625–1631. doi:10.1111/nph.14159

Sharma-Poudyal D, Bai Q, Wan A, Wang M, See D, Chen X. 2020. Molecular characterization of international collections of the wheat stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* reveals high diversity and intercontinental migration. Phytopathology. 110(4):933–942. doi:10.1094/PHYTO-09-19-0355-R

Sharma-Poudyal D, Chen XM, Wan AM, Zhan GM, Kang ZS, Cao SQ, Jin SL, Morgounov A, Akin B, Mert Z, et al. 2013. Virulence characterization of international collections of the Wheat Stripe Rust Pathogen, Puccinia striiformis f. sp. tritici. Plant Dis. 97 (3):379–386. doi:10.1094/PDIS-01-12-0078-RE

Solh M, Burak M, Nazari K, Keser M, Karaman Y, Baum M, Mert Z. 2013. Stripe rust and the Turkey-ICARDA regional cereal rust research center at Izmir, Turkey. Izmir: BGRI; [accessed 2020 May 13]. https://globalrust.org/sites/default/files/2013-BGRI-session-3-1-Solh.pdf.

Stubbs RW, Slovencikova V, Bartos P. 1977. Yellow rust resistance for some European wheat cultivars derived from rye. Cereal Rusts Bulletin. 5:45–47.

Walter S, Ali S, Kemen E, Nazari K, Bahri BA, Enjalbert J, Hansen JG, Brown JKM, Sicheritz-Pontén T, Jones J, et al. 2016. Molecular markers for tracking the origin and worldwide distribution of invasive strains of *Puccinia striiformis*. Ecol Evol. 6 (9):2790–2804. doi:10.1002/ece3.2069

Wan AM, Chen XM. 2014. Virulence characterization of *Puccinia striiformis* f. sp *tritici* using a new set of *Yr* single-gene line differentials in the United States in 2010. Plant Dis. 98(11):1534–1542. doi:10.1094/PDIS-01-14-0071-RE

Wang KN, Hong XW, Wu LR, Xie SX, Meng QY, Chen SM. 1986. The analysis of the resistance of varieties in the wheat stripe rust nurseries in 1951–1983. Acta Phytophylacica Sin. 13:112–123.

Wellings CR. 2007. Puccinia striiformis in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979-2006. Aust J Agr Res. 58(6):567–575. doi:10.1071/AR07130

Wellings CR, Singh RP, Yahyaoui AH, Nazari K, McIntosh RA. 2009. The development and application of near-isogenic lines for monitoring cereal rust pathogens. The Borlaug Global Rust Initiative Technical Workshop; Obregon, Mexico.

Xia CJ, Wang MN, Yin CT, Cornejo OE, Hulbert SH, Chen XM. 2018. Genome sequence resources for the wheat stripe rust pathogen (*Puccinia striiformis* f. sp *tritici*) and the barley stripe rust pathogen (*Puccinia striiformis* f. sp *hordei*). Mol Plant Microbe In. 31 (11):1117–1120. doi:10.1094/MPMI-04-18-0107-A

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. 1997. POPGENE, the user-friendly shareware for population genetic analysis. Canada: Molecular Biology and Biotechnology Centre, University of Alberta; [accessed 2020 Oct 25]. https://sites.ualberta.ca/~fyeh/popgene.html.

Zeybek A, Yigit F. 2004. Determination of virulence genes frequencies in wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) populations during natural epidemics in the regions of southern Aegean and western Mediterranean in Turkey. Pakistan J Biol Sci. 7(11):1967–1971. doi:10.3923/pjbs.2004.1967.1971

Zhan GM, Chen XM, Kang ZS, Huang LL, Wang MN, Wan AM, Cheng P, Cao S, Jin S. 2012. Comparative virulence phenotypes and molecular genotypes of *Puccinia striiformis* f. sp *tritici*, the wheat stripe rust pathogen in China and the United States. Fungal Biol-Uk. 116 (6):643–653. doi:10.1016/j.funbio.2012.03.004

Zheng WM, Huang LL, Huang JQ, Wang XJ, Chen XM, Zhao J, Guo J, Zhuang H, Qiu C, Liu J, et al. 2013. High genome heterozygosity and endemic genetic recombination in the wheat stripe rust fungus. Nat Commun. 4(1):2673. doi:10.1038/ncomms3673