Canadian Journal of Plant Pathology

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

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To cite this article: Xianming Chen, Meinan Wang, Anmin Wan, Qing Bai, Mingju Li, Pedro Figueroa López, Marco Maccaferri, Anna Maria Mastrangelo, Charles W. Barnes, Diego Fabricio Campaña Cruz, Albert U. Tenuta, Samar M. Esmail & Abdelrazek S. Abdelrhim (2021) Virulence characterization of *Puccinia striiformis* f. sp. *tritici* collections from six countries in 2013 to 2020, Canadian Journal of Plant Pathology, 43:sup2, S308-S322, DOI: 10.1080/07060661.2021.1958259

To link to this article: https://doi.org/10.1080/07060661.2021.1958259

Can. J. Plant Pathol., 2021
Vol. 43, No. 2, S308–S322, https://doi.org/10.1080/07060661.2021.1958259

Special Issue: Rust Diseases of Field Crops and Forest Trees

Virulence characterization of *Puccinia striiformis* f. sp. *tritici* collections from six countries in 2013 to 2020

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(Accepted 16 July 2021)

Abstract: *Puccinia striiformis* f. sp. *tritici* (*Pst*) causes wheat stripe rust (also called yellow rust, *Yr*), one of the most important diseases worldwide. Characterization of virulence in *Pst* populations is essential for developing wheat cultivars with effective and durable resistance to control the disease. A total of 138 *Pst* races, including 120 races that were not previously reported, were identified from stripe rust collections made from Canada, China, Ecuador, Egypt, Italy and Mexico in 2013–2020 using a set of 18 *Yr* single-gene differentials. Virulence of the resistance gene *Yr5* or *Yr15* was not found in isolates from any of the countries, indicating their effectiveness against the *Pst* populations. Virulence to 16 *Yr* genes was detected, but the frequencies varied greatly among countries. On average, the frequencies of virulence to *Yr6, Yr7, Yr9, Yr43, Yr44* and *YrExp2* were high (81.7–90.6%), those to *Yr1, Yr8, Yr17, Yr27, YrSP* and *Yr76* were moderate (34.0–56.8%), and those to *Yr10, Yr24, Yr32* and *YrTr1* were low or very low (0.4–18.5%). The same races detected in different countries and different races from different countries clustered into the same virulence groups, indicating *Pst* migration among different countries, especially between eastern Asia and the Mediterranean region. These results should be useful for breeding wheat cultivars with effective resistance to stripe rust in these countries as well as globally.

Keywords: *Puccinia striiformis*, races, stripe rust, virulence, wheat, yellow rust

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Published online 27 Sep 2021

Résumé: *Puccinia striiformis* f. sp. *tritici* (*Pst*) cause la rouille jaune du blé (aussi appelée « rouille jaune », *Yr*), une des plus graves maladies à l'échelle de la planète. La caractérisation de la virulence dans les populations de *Pst* est essentielle au développement de cultivars possédant une résistance efficace et durable permettant de lutter contre la maladie. En tout, 138 races de *Pst*, y compris 120 races qui n'avaient pas été préalablement rapportées, ont été identifiées à partir de collections de rouille jaune du blé créées, de 2013 à 2020, à partir du Canada, de la Chine, de l'Équateur, de l'Égypte, de l'Italie et du Mexique avec un ensemble de 18 différentiels d'*Yr* à gène unique. La virulence à l'égard du gène de résistance *Yr5* ou *Yr15* n'a été détectée dans aucun des isolats provenant de ces pays, ce qui indique leur efficacité contre les populations de *Pst*. La virulence à l'égard du gène *Yr16* a été détectée, mais les fréquences variaient grandement d'un pays à l'autre. En moyenne, les fréquences de virulence à l'égard de *Yr6, Yr7, Yr9, Yr43, Yr44* et *YrExp2* étaient élevées (81,7 à 90,6%), celles à l'égard de *Yr1, Yr8, Yr17, Yr27, YrSP* et *Yr76* étaient modérées (34,0 à 56,8%) et celles à l'égard de *Yr10, Yr24, Yr32* et *YrTr1* étaient faibles ou très faibles (0,4 à 18,5%). Les mêmes races détectées dans différents pays et différentes races provenant de différents pays se regroupaient dans les mêmes groupes de virulence, ce qui met en évidence la migration de *Pst* d'un pays à l'autre, particulièrement entre ceux de l'Asie de l'Est et de la région méditerranéenne. Ces résultats devraient servir à sélectionner des cultivars de blé possédant une résistance efficace contre la rouille jaune dans ces pays de même que mondialement.

Mots clés: blé, *Puccinia striiformis*, races,, rouille jaune, rouille jaune du blé, virulence

Introduction

Stripe rust also called yellow rust (Yr), of wheat, barley and grasses is caused by different *formae speciales* of *Puccinia striiformis*, of which those attacking wheat and barley are economically important. Wheat stripe rust, caused by *P. striiformis* f. sp. *tritici* (*Pst*), is one of the most important diseases threatening global wheat production. The disease has been reported in more than 60 countries and occurs on all continents except Antarctica, where wheat is not grown (Stubbs 1985; Chen 2005, 2020; Wellings 2011; Chen and Kang 2017a). Widespread stripe rust epidemics occur frequently in more than a dozen countries including China, Ecuador, Mexico and the United States, and although less frequent, the disease can cause significant yield loss in many other countries including Canada, Egypt, Italy and Morocco (Wellings 2011). In a recent survey, stripe rust of wheat was among the most important diseases causing annual global yield loss of more than 1% (Savary et al. 2019), and the annual cost of wheat stripe rust damage was estimated to be more than one billion US dollars (Chen 2020). Stripe rust can cause 100% yield loss in highly susceptible cultivars when the disease is extremely severe (Chen 2005, 2014).

Stripe rust can be controlled by growing resistant cultivars, fungicide applications and to some extent cultural practices (Chen and Kang 2017b). Among these approaches, developing and growing resistant cultivars is considered the most effective, easy-to-use, and economically and environmentally friendly (Chen 2013). Different types of resistance have been used in breeding programmes to develop resistant cultivars. Among these, all-stage resistance (ASR), which is expressed throughout all growth stages and adult plant resistance (APR), which is expressed mostly in the late growth stages, have been characterized and used in breeding programmes. ASR, which usually provides a high-level of resistance, is controlled by single genes and is therefore easier to incorporate into new cultivars. However, ASR is race-specific; widespread use of single ASR genes exerts selection pressure on the pathogen, resulting in the development of virulent races in the pathogen population. In contrast, APR is generally non-race specific and durable. However, APR is not usually effective at the seedling stage, and therefore does not provide adequate protection in areas where stripe rust often occurs at the seedling stage. APR is almost always affected by temperatures and is more effective under high temperatures. Because APR is influenced by temperature and plant growth stage, it is also called high-temperature adult-plant (HTAP) resistance (Line 2002; Chen 2005, 2013). It is important to recognize the effects of temperature and growth stage on stripe rust resistance as cultivars with ASR genes can change from resistant to susceptible by pathogen virulence, whereas HTAP resistance may be ineffective at low temperatures and in early plant growth stages. To take advantage of both ASR and HTAP resistance and overcome the disadvantages, combining ASR and HTAP resistance by pyramiding effective ARS genes and HTAP resistance genes is the best approach to develop cultivars with highly effective and durable resistance to stripe rust (Chen 2013). Virulence characterization of *Pst* populations and the identification of races are needed to provide information on the effectiveness of resistance genes and races for screening germplasm and breeding material for both ASR and HTAP resistance.

The stripe rust fungus can rapidly become virulent through various genetic mechanisms, such as mutation, somatic recombination, and sexual recombination (Zhao et al. 2016; Lei et al. 2017; Yuan et al. 2018; Li et al. 2019, 2020; Xia et al. 2020). Virulence genes are

characterized phenotypically by testing *Pst* isolates on a set of cultivars or single resistance gene lines that are used
to differentiate races within each *forma specialis*. In the United States, *Pst* races were characterized using a set of 20 wheat cultivar differentials (Line and Qayoum 1992; Chen et al. 2002, 2010; Wan and Chen 2012) until 2009. Since 2010, a new set of 18 wheat lines carrying single *Yr* genes has been established and used to differentiate races of *Pst* (Wan and Chen 2014; Wan et al. 2016). Using this set of *Yr* single-gene line differentials, more than 300 *Pst* races have been identified from historical and recent collections from the United States and other countries (Wan and Chen 2014; Wan et al. 2016; Liu et al. 2017; Wan et al. 2017; httpp://striperust.wsu.edu).

As *Pst* is a biotrophic fungus and prefers relatively low temperatures, controlled conditions are required to conduct testing to identify races and to store isolates. Research on *Pst* race identification has been conducted in only a few countries with established programmes. In a previous study, we identified 129 races from 235 isolates collected from 13 countries (Algeria, Australia, Canada, Chile, China, Hungary, Kenya, Nepal, Pakistan, Russia, Spain, Turkey and Uzbekistan) from 2006 to 2010 (Sharma-Poudyal et al. 2013). In a later study, we identified 18 *Pst* races from 97 isolates collected from Ethiopia in 2013–2014 (Wan et al. 2017). These studies provide useful information on the virulence of *Pst* in these individual countries and shed light on the dissemination of pathogen races or genotypes among different countries and continents (Sharma-Poudyal et al. 2013, 2020; Wan et al. 2017).

The objectives of the present study were to identify races from *Pst* collections from six countries (Canada, China, Ecuador, Egypt, Italy and Mexico) from 2013 to 2020 on the 18 *Yr* single-gene differentials, determine virulence frequencies in these countries, and evaluate the relationships of the *Pst* populations among these countries. Races and virulence factors of *Pst* in these countries will be discussed in comparison with those in the United States. The results should be useful to understand the global distribution, migration and evolution of *Pst* and to breed cultivars resistant to stripe rust.

Materials and methods

Stripe rust collection and spore production

A total of 491 *Pst* isolates were obtained from six countries, including 16 from Canada (one in 2013, two in 2016 and 13 in 2017), 139 from China in 2016, 45 from Ecuador (33 in 2015 and 12 in 2016), two from Egypt in 2018, 167 from Italy (25 in 2014, 108 in 2016, 22 in 2017, three in 2018 and nine in 2020) and 122 from Mexico (13 in 2015 and 109 in 2016). Leaf samples with stripe rust uredinia were stored at 4°C when received, and urediniospores from the samples were revived and multiplied as soon as possible.

To revive urediniospores, leaf samples were cut into 3 cm pieces and kept on moist-blotting paper in Petri dishes for about 24 h under a diurnal temperature cycle from 20°C at 2:00 PM to 4°C at 2:00 AM and back to 20°C at 2:00 PM (Chen and Line 1992). Fresh urediniospores produced on the leaf pieces were transferred with a clean fine paint brush onto the leaves of the *Pst*-susceptible winter wheat cultivar 'Nugaines' at the two-leaf stage. Inoculation and growth conditions before and after inoculation follow the standard procedure and conditions used in our programme (Chen and Line 1992). The inoculated plants were kept in a dark dew chamber for 18–24 h at 10°C, and then grown in a growth chamber programmed with a diurnal cycle with the temperature gradually changing from 4°C at 2:00 AM to 20°C at 2:00 PM and with 8 h dark from 10 PM to 6 AM and 16 h light from 6 AM to 10 PM. Pots with inoculated seedlings were isolated from each other with transparent plastic cylinders to prevent cross-contamination. Urediniospores were collected 16 days after inoculation and once every 2 days until 26 days after inoculation. Multiplication was repeated on 'Nugaines' for isolates that did not produce adequate spores during the first multiplication. Urediniospores were dried in a desiccator at 4°C for 5 days before being stored at 4°C for up to 2 months or in liquid nitrogen for years. Fresh urediniospores, or those stored at 4°C for less than 2 months, were used for testing on the host differentials.

Virulence testing and race identification

A set of 18 wheat *Yr* single-gene lines listed in Supplementary Table 1 (Wan and Chen 2014; Wan et al. 2016) were used to determine the races of the *Pst* isolates. Seedlings at the two-leaf stage were dust-inoculated with urediniospores mixed with talc at a 1:20 ratio, incubated, and grown under the conditions described above. Infection type (IT) was recorded 20–22 days after inoculation on a 0 to 9 scale (0 = no visible signs or symptoms; 1 = necrotic and/or chlorotic flecks, no sporulation; 2 = necrotic and/or chlorotic blotches, no sporulation; 3 = necrotic and/or chlorotic blotches, trace sporulation; 4 = necrotic and/or chlorotic blotches, light sporulation; 5 = necrotic and/or chlorotic blotches

intermediate sporulation; 6 = necrotic and/or chlorotic blotches, moderate sporulation; 7 = necrotic and/or chlorotic blotches, abundant sporulation; 8 = chlorosis behind sporulating area, abundant sporulation; and 9 = no necrosis or chlorosis, abundant sporulation) as described by Line and Qayoum (1992). Infection types 0 to 6 were considered avirulent and 7–9 virulent (Wan and Chen 2014). If an original isolate produced an avirulence/virulence pattern different from previously identified races, two to three single uredinial isolates were obtained from individual uredinia on the differential(s) showing the different reaction(s) and tested on the whole set of differentials to confirm the pattern. These confirmed patterns were used to determine and name races for the original and single-uredinial isolates. *Pst* races with octal codes were named in sequential order following the system described by Wan et al. (2016).

Distributions and frequencies of virulence factors and races

The ITs of each isolate on the differentials were assigned with binary codes of 0 for virulence (ITs 0–6) and 1 for avirulence (ITs 7–9). Frequencies were calculated for all virulence factors and races in the collection from each country and compared among countries. The virulence and race frequencies in these countries were compared with those in the United States for the same period (http://striperust.wsu.edu).

Relationships of Pst races and collections from different countries

Virulence relationships among the races and among *Pst* collections from different countries were determined using IT data converted to avirulence and virulence. A dendrogram was generated to show the relationships of all *Pst* races identified using the 'aboot' function of the R package 'poppr' and bootstrap probability was calculated with 1000 bootstrap repeats based on the Nei genetic distance values (Nei 1978) calculated from the virulence data between races to generate a dendrogram using the unweighted pair group method with arithmetic mean (UPGMA) (Kamvar et al. 2015). The same software was used to construct a neighbour-joining tree to show the relationships among the *Pst* collections from different countries.

Results

Recovery of stripe rust samples

From 1550 wheat stripe rust samples received from six countries (Canada, China, Ecuador, Egypt, Italy and Mexico) from 2013 to 2020, 491 isolates (31.7%) were recovered, with each isolate recovered from a different sample (Table 1). Recovery rates varied greatly from country to country. The highest recovery rate (48.8%) was obtained from the Chinese collection of 285 samples, followed by the collections of Italy (43.4% of 385 samples), Canada (34.8% of 46 samples), and Mexico (30.0% of 406 samples). The Ecuadorian collection had a recovery rate of 18.1% from 248 samples. The Egyptian collection had the lowest recovery rate (1.1%), with only 2 isolates obtained from 180 samples. Information on the origin of the country, province/region, nearest city, year collected, IT on the 18 *Yr* single-gene differentials, virulence formula, and race for each of the 491 isolates is provided in Supplementary Table 2.

Pst races identified from six countries

Of the 491 isolates, 138 races were identified based on their virulence formula on a set of 18 *Yr* single-gene differentials (Table 2). These races were named according to the system in our programme for races identified in the United States, which uses a sequential number with the prefix 'PSTv'. Each race was associated with

Table 1. The number of samples received, isolates recovered, and races of *Puccinia striiformis* f. sp. *tritici* identified from six countries in 2013–2020.

Country	Year	No. of samples	No. of isolates	Recovery rate (%)	No. of races	Race/isolate ratio
Canada	2013, 2016, 2017	46	16	34.8	5	1/3.2
China	2016	285	139	48.8	59	1/2.4
Ecuador	2015, 2016	248	45	18.1	21	1/2.1
Egypt	2018	180	2	1.1	2	1/1.0
Italy	2014, 2016–2018, 2020	385	167	43.4	41	1/4.1
Mexico	2015, 2016	406	122	30.0	29	1/4.2
Total (mean)	2013–2020	1,550	491	(31.7)	138	(1/3.6)

Table 2. Races of *Puccinia striiformis* f. sp. *tritici* identified from collections of six countries from 2013 to 2020.

Races	Octal code[1]	Virulence to *Yr* genes	No. of virulence factors	No. of isolates	Freq. (%) (n = 491)	Country distribution (No. of isolates)[2]
PSTv-4	511 211	1,6,9,17,27,SP,76	7	2	0.41	EC(1), MX(1)
PSTv-11	571 263	1,6,7,8,9,17,27,43,44,Exp2,76	11	1	0.20	EC(1)
PSTv-14	571 267	1,6,7,8,9,17,27,43,44,Tr1, Exp2,76	12	1	0.20	CA(1)
PSTv-15	551 273	1,6,7,9,17,27,43,44,SP,Exp2,76	11	2	0.41	CN(1), EG(1)
PSTv-17	571 273	1,6,7,8,9,17,27,43,44,SP,Exp2,76	12	1	0.20	CN(1)
PSTv-18	000000	/	0	6	1.22	EC(1), IT(2), MX(3)
PSTv-20	001000	17	1	2	0.41	EC(2)
PSTv-28	151 022	6,7,9,17,44,Exp2	6	1	0.20	MX(1)
PSTv-30	170 026	6,7,8,9,44,Tr1,Exp2	7	1	0.20	MX(1)
PSTv-36	170 266	6,7,8,9,27,43,44,Tr1,Exp2	9	5	1.02	MX(5)
PSTv-37	171 266	6,7,8,9,17,27,43,44,Tr1,Exp2	10	61	12.42	CA(10), EC(1), MX(50),
PSTv-42	471 221	1,7,8,9,17,27,44,76	8	1	0.20	EC(1)
PSTv-47	571 266	1,6,7,8,9,17,27,43,44,Tr1,Exp2	11	2	0.41	CA(1), MX(1)
PSTv-52	171 262	6,7,8,9,17,27,43,44,Exp2	9	25	5.09	CA(3), EC(1), MX(21)
PSTv-53	510 011	1,6,9,SP,76	5	6	1.22	CN(1), MX(5)
PSTv-71	550 263	1,6,7,9,27,43,44,Exp2,76	9	1	0.20	CN(1)
PSTv-78	160 262	6,7,8,27,43,44,Exp2	7	2	0.41	MX(2)
PSTv-91	161 762	6,7,8,17,24,27,32,43,44,Exp2	10	1	0.20	IT(1)
PSTv-97	561 062	1,6,7,8,17,43,44,Exp2	8	1	0.20	CN(1)
PSTv-102	160 266	6,7,8,27,43,44,Tr1,Exp2	8	1	0.20	MX(1)
PSTv-106	551 262	1,6,7,9,17,27,43,44,Exp2	9	2	0.41	EC(2)
PSTv-109	174 266	6,7,8,9,10,27,43,44,Tr1,Exp2	10	1	0.20	MX(1)
PSTv-120	510 211	1,6,9,27,SP,76	6	1	0.20	EG(1)
PSTv-121	450 031	1,7,9,44,SP,76	6	1	0.20	IT(1)
PSTv-124	560 170	1,6,7,8,32,43,44,SP	8	1	0.20	IT(1)
PSTv-125	550 073	1,6,7,9,43,44,SP,Exp2,76	9	23	4.68	CN(14), IT(9)
PSTv-127	551 072	1,6,7,9,17,43,44,SP,Exp2	9	67	13.65	IT(67)
PSTv-128	000002	Exp2	1	2	0.41	IT(2)
PSTv-129	411 022	1,9,17,44,Exp2	5	1	0.20	IT(1)
PSTv-130	000022	44,Exp2	2	1	0.20	IT(1)
PSTv-131	160 062	6,7,8,43,44,Exp2	6	1	0.20	IT(1)
PSTv-132	061462	7,8,17,24,43,44,Exp2	7	1	0.20	IT(1)
PSTv-133	061062	7,8,17,43,44,Exp2	6	3	0.61	IT(3)
PSTv-134	060062	7,8,43,44,Exp2	5	1	0.20	IT(1)
PSTv-135	021022	8,17,44,Exp2	4	1	0.20	IT(1)
PSTv-136	020062	8,43,44,Exp2	4	2	0.41	IT(2)
PSTv-137	020022	8,44,Exp2	3	7	1.43	IT(7)
PSTv-138	030033	8,9,44,SP,Exp2,76	6	1	0.20	IT(1)
PSTv-140	450 021	1,7,9,44,76	5	2	0.41	CN(1), IT(1)
PSTv-144	571 272	1,6,7,8,9,17,27,43,44,SP,Exp2	11	1	0.20	IT(1)
PSTv-157	000023	44,Exp2,76	3	1	0.20	IT(1)
PSTv-175	551 263	1,6,7,9,17,27,43,44,Exp2,76	10	1	0.20	IT(1)
PSTv-183	140 022	6,7,44,Exp2	4	1	0.20	MX(1)
PSTv-192	550 277	1,6,7,9,27,43,44,SP,Tr1,Exp2,76	11	2	0.41	CN(1), IT(1)
PSTv-198	170 262	6,7,8,9,27,43,44,Exp2	8	13	2.65	CA(1), MX(12)
PSTv-201	020000	8	1	1	0.20	MX(1)
PSTv-203	551 271	1,6,7,9,17,43,44,SP,76	9	2	0.41	IT(2)
PSTv-205	551 032	1,6,7,9,17,44,SP,Exp2	8	1	0.20	IT(1)
PSTv-212	511 231	1,6,9,17,27,44,SP,76	8	1	0.20	IT(1)
PSTv-220	571 277	1,6,7,8,9,17,27,43,44,SP,Tr1, Exp2,76	13	2	0.41	MX(2)
PSTv-221	140 262	6,7,27,43,44,Exp2	6	3	0.61	EC(3)
PSTv-225	550 273	1,6,7,9,27,43,44,SP,Exp2,76	10	43	8.76	CN(43)
PSTv-226	540 273	1,6,7,27,43,44,SP,Exp2,76	9	4	0.81	CN(4)
PSTv-227	550 272	1,6,7,9,27,43,44,SP,Exp2	9	4	0.81	CN(4)
PSTv-228	550 233	1,6,7,9,27,44,SP,Exp2,76	9	5	1.02	CN(5)
PSTv-229	570 073	1,6,7,8,9,43,44,SP,Exp2,76	10	3	0.61	CN(3)

(*Continued*)

Table 2. (Continued.)

Races	Octal code[1]	Virulence to *Yr* genes	No. of virulence factors	No. of isolates	Freq. (%) (n = 491)	Country distribution (No. of isolates)[2]
PSTv-230	550 271	1,6,7,9,43,44,SP,76	8	2	0.41	CN(2)
PSTv-231	550 033	1,6,7,9,44,SP,76	7	3	0.61	CN(3)
PSTv-232	550 072	1,6,7,9,43,44,SP,Exp2	8	12	2.44	CN(3), IT(9)
PSTv-233	561 262	1,6,7,8,17,27,43,44,Exp2	9	3	0.61	CN(3)
PSTv-234	550 003	1,6,7,9,Exp2,76	6	1	0.20	CN(1)
PSTv-235	050073	7,9,43,44,SP,Exp2,76	7	1	0.20	CN(1)
PSTv-236	050233	7,9,27,44,SP,Exp2,76	7	1	0.20	CN(1)
PSTv-238	410 220	1,9,27,44	4	1	0.20	CN(1)
PSTv-240	530 003	1,6,7,Exp2,76	5	1	0.20	CN(1)
PSTv-241	040040	7,43	2	1	0.20	CN(1)
PSTv-242	556 272	1,6,7,9,17,27,43,44,SP,Exp2	10	2	0.41	IT(2)
PSTv-243	470 270	1,7,8,9,27,43,44,SP	8	1	0.20	CN(1)
PSTv-244	561 766	1,6,7,8,17,43,44,Tr1,Exp2	9	2	0.41	CN(2)
PSTv-246	110 071	6,9,43,44,SP,76	6	1	0.20	CN(1)
PSTv-248	050040	7,9,43	3	1	0.20	CN(1)
PSTv-250	000230	27,44,SP	3	2	0.41	CN(1), IT(1)
PSTv-251	010060	9,43,44	3	1	0.20	CN(1)
PSTv-252	511 030	1,6,9,17,44,SP	6	1	0.20	MX(1)
PSTv-253	540 043	1,6,7,43,Exp2,76	6	1	0.20	CN(1)
PSTv-254	541 273	1,6,7,17,27,43,44,SP,Exp2,76	10	1	0.20	CN(1)
PSTv-255	100 025	6,44,Tr1,76	4	1	0.20	CN(1)
PSTv-256	050070	7,9,43,44,SP	5	1	0.20	CN(1)
PSTv-257	150 051	6,7,9,43,76	5	1	0.20	CN(1)
PSTv-258	000001	76	1	2	0.41	CN(1), IT(1)
PSTv-259	170 072	6,7,8,9,43,44,SP,Exp2	8	11	2.24	CN(1), IT(10)
PSTv-260	150 071	6,7,9,27,43,44,SP,76	8	1	0.20	CN(1)
PSTv-261	150 061	6,7,9,43,44,76	6	1	0.20	CN(1)
PSTv-262	071264	7,8,9,17,27,43,44,Tr1	8	1	0.20	MX(1)
PSTv-263	400 040	1,43	2	1	0.20	CN(1)
PSTv-264	450 231	1,7,9,27,44,SP,76	7	1	0.20	CN(1)
PSTv-265	450 033	1,7,9,44,SP,Exp2,76	7	1	0.20	CN(1)
PSTv-266	540 073	1,6,7,17,43,44,SP,Exp2,76	9	1	0.20	CN(1)
PSTv-267	550 035	1,6,7,9,44,SP,Tr1,76	8	3	0.61	CN(3)
PSTv-268	551 001	1,6,7,9,17,76	6	1	0.20	CN(1)
PSTv-269	550 021	1,6,7,9,44,Exp2,76	7	2	0.41	CN(2)
PSTv-270	550 053	1,6,7,9,44,SP,Exp2,76	8	2	0.41	CN(2)
PSTv-271	150 023	6,7,9,44,Exp2,76	6	1	0.20	CN(1)
PSTv-272	170 022	6,7,8,9,44,Exp2	6	1	0.20	MX(1)
PSTv-274	551 233	1,6,7,9,17,27,44,SP,76	9	1	0.20	CN(1)
PSTv-275	570 471	1,6,7,8,9,24,43,44,SP,76	10	1	0.20	CN(1)
PSTv-276	170 544	6,7,8,9,24,32,43,Tr1	8	1	0.20	MX(1)
PSTv-277	570 273	1,6,7,8,9,27,43,44,SP,Exp2,76	11	1	0.20	CN(1)
PSTv-278	551 073	1,6,7,9,17,43,44,SP,Exp2,76	10	12	2.44	CN(2), IT(10)
PSTv-279	040003	7,Exp2,76	3	1	0.20	CN(1)
PSTv-280	150 073	6,7,9,43,44,SP,Exp2,76	8	1	0.20	CN(1)
PSTv-281	510 033	1,6,9,44,SP,Exp2,76	7	1	0.20	CN(1)
PSTv-282	100 072	6,43,44,SP,Exp2	5	1	0.20	CN(1)
PSTv-283	171 264	6,7,8,9,17,27,43,44,Tr1	9	2	0.41	MX(2)
PSTv-285	470 220	1,7,8,9,27,44	6	1	0.20	EC(1)
PSTv-286	140 062	6,7,43,44,Exp2	5	5	1.02	EC(5)
PSTv-287	141 072	6,7,17,43,44,SP,Exp2	7	1	0.20	EC(1)
PSTv-289	140 272	6,7,27,43,44,SP,Exp2	7	1	0.20	EC(1)
PSTv-290	001024	17,44,Tr1	3	1	0.20	EC(1)
PSTv-291	001054	17,43,SP,Tr1	4	1	0.20	EC(1)
PSTv-292	501 231	1,6,17,27,44,SP,76	7	1	0.20	MX(1)
PSTv-294	541 262	1,6,7,17,27,43,44,Exp2	8	2	0.41	EC(2)
PSTv-295	574 270	1,6,7,8,9,10,27,43,44,SP	10	1	0.20	IT(1)
PSTv-296	571 223	1,6,7,8,9,17,27,44,Exp2,76	10	1	0.20	MX(1)

(*Continued*)

Table 2. (Continued.)

Races	Octal code[1]	Virulence to *Yr* genes	No. of virulence factors	No. of isolates	Freq. (%) (n = 491)	Country distribution (No. of isolates)[2]
PSTv-297	570 262	1,6,7,8,9,27,43,44,Exp2	9	1	0.20	MX(1)
PSTv-298	551 267	1,6,7,9,17,27,43,44,Tr1,Exp2,76	11	1	0.20	EC(1)
PSTv-299	551 070	1,6,7,9,17,43,44,SP	8	6	1.22	IT(6)
PSTv-300	551 076	1,6,7,9,17,43,44,SP,Tr1,Exp2	10	1	0.20	IT(1)
PSTv-301	551 052	1,6,7,9,17,43,SP,Exp2	8	4	0.81	IT(4)
PSTv-302	530 211	1,6,8,9,27,SP,76	7	1	0.20	MX(1)
PSTv-303	041040	17,43	2	1	0.20	EC(1)
PSTv-304	000040	43	1	1	0.20	MX(1)
PSTv-305	141 262	6,7,17,27,43,44,Exp2	7	9	1.83	EC(9)
PSTv-306	141 062	6,7,17,43,44,Exp2	6	1	0.20	EC(1)
PSTv-307	140 242	6,7,27,43,Exp2	5	1	0.20	MX(1)
PSTv-309	150 002	6,7,9,Exp2	4	1	0.20	MX(1)
PSTv-310	130 000	6,8,9	3	1	0.20	MX(1)
PSTv-311	064060	7,8,43,44	4	2	0.41	IT(2)
PSTv-312	540 023	1,6,7,44,Exp2,76	6	1	0.20	CN(1)
PSTv-313	550 271	1,6,7,9,27,43,44,SP,76	9	1	0.20	CN(1)
PSTv-314	550 013	1,6,7,9,SP,Exp2,76	7	1	0.20	CN(1)
PSTv-315	001011	17,SP,76	3	1	0.20	CN(1)
PSTv-316	571 231	1,6,7,8,9,17,27,44,SP,76	10	3	0.61	IT(3)
PSTv-317	171 022	6,7,8,9,17,43,44,Exp2	8	3	0.61	IT(3)
PSTv-318	571 221	1,6,7,8,9,17,27,44,76	9	1	0.20	IT(1)
PSTv-319	171 076	6,7,8,9,17,43,44,SP,Tr1,Exp2	10	1	0.20	IT(1)
PSTv-320	151 262	6,7,9,17,27,43,44,SP,Exp2	9	1	0.20	IT(1)
PSTv-327	550 262	1,6,7,9,27,43,44,Exp2	8	8	1.63	EC(8)

[1]Octal codes were determined using the scheme presented in Wan et al. (2016).
[2]CA = Canada, CN = China, EC = Ecuador, EG = Egypt, IT = Italy and MX = Mexico.

an octal code. For example, race PSTv-4 with octal code 511 211 is virulent to *Yr1, Yr6, Yr9, Yr17, Yr27, YrSP* and *Yr76*; but avirulent to *Yr5, Yr7, Yr8, Yr10, Yr15, Yr24, Yr32, Yr43, Yr44, YrTr1* and *YrExp2*.

Virulence spectra and frequencies of races

The 138 races were virulent to different numbers of resistance genes among the 18 *Yr* genes in the differentials, ranging from 0 to 13 (Table 2), with a mean of 6.9. Only one race (PSTv-18) was avirulent to all genes, and one race (PSTv-220) was virulent to 13 of the 18 *Yr* genes. Five races were virulent to 1, 4 races to 2, 9 races to 3, 8 races to 4, 10 races to 5, 18 races to 6, 17 races to 7, 22 races to 8, 19 races to 9, 15 races to 10, 7 races to 11 and 2 races to 12 of the 18 *Yr* genes. The distribution of races with various numbers of virulence factors is shown in Fig. 1A. Virulence to 8 *Yr* genes had the highest number (22) of races, followed by 9 *Yr* genes (19 races) and 6 *Yr* genes (18 races). The majority (101) of the races were virulent to 5–10 of the *Yr* genes. No races had virulence to 14 or more of the 18 *Yr* genes. The highest number of isolates (151 isolates or 30.8%) were virulent to 9 *Yr* genes, followed by virulence to 10 *Yr* genes (78 isolates; 15.9%) and virulence to 8 *Yr* genes (77 isolates; 15.7%) (Fig. 1B). Neither virulence to very few (e.g., 0–2) nor to very many (e.g., 11–13) *Yr* genes were common. These results indicated that races with virulence to between 8 and 10 resistance genes tended to be predominant in most countries.

Among the 138 races, PSTv-127 (octal code 551 072) with virulence to *Yr1, Yr6, Yr7, Yr9, Yr17, Yr43, Yr44, YrSP* and *YrExp2* was the most frequent (13.7%) although only in Italy (Table 2). The second most frequent race was PSTv-37 (12.4%), detected in Canada, Ecuador and Mexico. The third most frequent race was PSTv-225 (8.8%), detected only in China. PSTv-52 ranked fourth (5.1%) and PSTv-125 fifth (4.7%); the former was detected in Canada, Ecuador, and Mexico, and the latter in China and Italy. Forty-six races were detected from two (0.4%) to 13 isolates (2.7%) from 1 to 3 countries, while 87 races each were detected from only one isolate. Of the 138 races, 16 were detected in two or three countries, while 132 were detected from only one country. Among the 16 races detected in two or three countries, in addition to the four most common races mentioned above, seven races (PSTv-140, PSTv-192, PSTv-232, PSTv-250, PSTv-258, PSTv-259 and PSTv-278) were detected in both China and Italy,

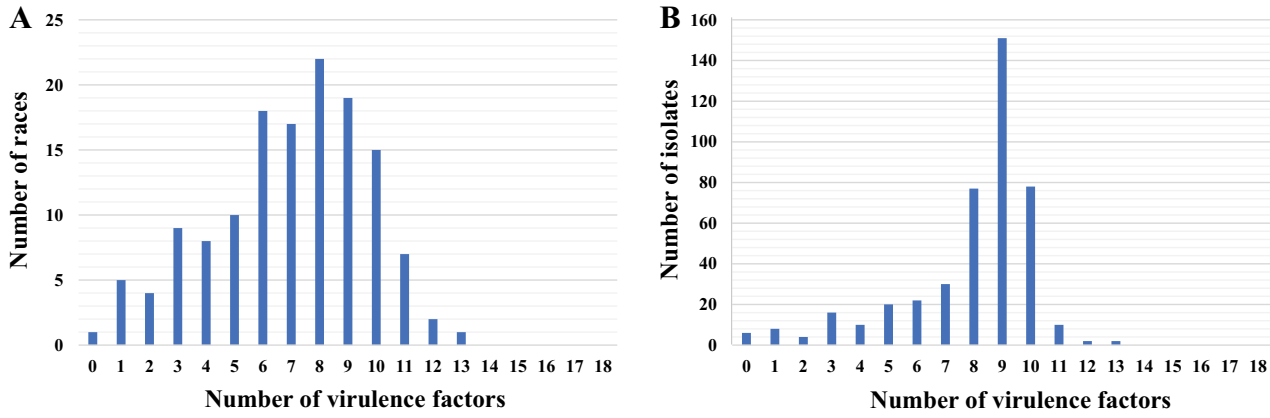

Fig. 1 Distributions of races (**A**) and isolates (**B**) with different numbers of virulence factors.

two races (PSTv-47 and PSTv-198) in both Canada and Mexico, one race (PSTv-15) in China and Egypt, one race (PSTv-53) in China and Mexico, one race (PSTv-4) in Ecuador and Mexico, and one race (PSTv-18) in Ecuador, Italy and Mexico.

Virulence frequencies and distributions

Among the 18 *Yr* genes, virulence to 16 genes was detected at various frequencies (Table 3). No virulence to either *Yr5* or *Yr15* was detected, indicating that these genes were effective against the *Pst* populations in the six countries. High frequencies were found for virulence to *Yr9* (81.7%), *Yr43* (82.1%), *YrExp2* (83.3%), *Yr6* (88.0%), *Yr7* (88.8%) and *Yr44* (90.6%). Moderate frequencies were observed for virulence to *Yr76* (34.0%), *Yr8* (37.1%), *Yr27* (49.3%), *Yr17* (51.1%), *YrSP* (53.8%) and *Yr1* (56.8%). Low frequencies were found for virulence to *YrTr1* (18.5%), and very low frequencies were observed for *Yr10* (0.4%), *Yr32* (0.6%) and *Yr24* (1.0%).

Virulence frequencies varied among countries. In Canada, virulence to *Yr6, Yr7, Yr8, Yr9, Yr27, Yr43, Yr44* and *YrExp2* was 100%, indicating that all isolates were virulent to these genes. Most isolates were virulent

Table 3. Frequencies (%) and distributions of virulence to *Yr* genes in the *Puccinia striiformis* f. sp. *tritici* isolates collected from six countries in 2013 to 2020.

Yr gene	No. of isolates	Freq. (n = 491)	Canada (n = 16)		China (n = 139)		Ecuador (n = 45)		Egypt (n = 2)		Italy (n = 167)		Mexico (n = 122)	
			No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
Yr1	279	56.8	2	12.5	120	86.3	17	37.8	2	100.0	124	74.3	14	11.5
Yr5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Yr6	432	88.0	16	100.0	123	88.5	37	82.2	2	100.0	138	82.6	116	95.1
Yr7	436	88.8	16	100.0	128	92.1	38	84.4	1	50.0	146	87.4	107	87.7
Yr8	182	37.1	16	100.0	14	10.1	5	11.1	0	0.0	41	24.6	106	86.9
Yr9	401	81.7	16	100.0	116	83.5	17	37.8	2	100.0	139	83.2	111	91.0
Yr10	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0	1	0.6	1	0.8
Yr15	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Yr17	251	51.1	15	93.8	15	10.8	26	57.8	1	50.0	112	67.1	82	67.2
Yr24	5	1.0	0	0.0	1	0.7	0	0.0	0	0.0	3	1.8	1	0.8
Yr27	242	49.3	16	100.0	74	53.2	32	71.1	2	100.0	14	8.4	104	85.3
Yr32	3	0.6	0	0.0	0	0.0	0	0.0	0	0.0	2	1.2	1	0.8
Yr43	403	82.1	16	100.0	105	75.5	38	84.4	1	50.0	141	84.4	102	83.6
Yr44	445	90.6	16	100.0	126	90.7	39	86.7	1	50.0	157	94.0	106	86.9
YrSP	264	53.8	0	0.0	113	81.3	4	8.9	2	100.0	134	80.2	11	9.0
YrTr1	91	18.5	12	75.0	7	5.0	4	8.9	0	0.0	3	1.8	65	53.3
YrExp2	409	83.3	16	100.0	109	78.4	36	80.0	1	50.0	144	86.2	103	84.4
Yr76[1]	167	34.0	1	6.3	116	83.5	4	8.9	2	100.0	33	19.8	11	9.0

[1] *Yr76* = *YrTye* (Wan et al. 2016; Xiang et al. 2016).

to *Yr17* (93.8%) and *YrTr1* (75.0%), while only 12.5% of that isolates were virulent to *Yr1* and 6.3% to *Yr76*. In China, most isolates were virulent to *Yr7* (92.9%), *Yr44* (90.1%), *Yr6* (88.5%), *Yr1* (86.3%), *Yr9* (83.5%), *Yr76* (83.5%), *YrSP* (81.3%) and *Yr43* (75.5%). Moderate frequencies were found for virulence to *Yr27* (53.2%), low or very low frequencies were observed for virulence to *Yr24* (0.7%), *YrTr1* (5.0%) and *Yr8* (10.1%), and no isolates were virulent to *Yr10* or *Yr32* in addition to *Yr5* and *Yr15*. In Ecuador, high frequencies were found for virulence to *Yr44* (86.7%), *Yr7* and *Yr43* (84.4%), *Yr6* (82.2%), *YrExp2* (80.0%) and *Yr27* (71.1%); moderate frequencies to *Yr17* (57.8%), *Yr1* and *Yr9* (37.8%); and low frequencies of virulence to *YrSP, YrTr1* and *Yr76* (8.9%) and *Yr8* (11.1%). No virulence was found to *Yr10, Yr24* and *Yr32*, in addition to *Yr5* and *Yr15*. Only two isolates that were identified as two races were from Egypt. Both isolates were virulent to *Yr1, Yr6, Yr9, Yr27, YrSP* and *Yr76*, while only one of that isolates was virulent to *Yr7, Yr17, Yr43, Yr44* and *YrExp2*. In Italy, high frequencies of virulence were found to *Yr44* (94.0%), *Yr7* (87.4%), *YrExp2* (86.4%), *Yr43* (84.4%), *Yr9* (83.2%), *Yr6* (82.6%), *YrSP* (80.2%), *Yr1* (74.3%) and *Yr17* (67.1%), while low frequencies of virulence were observed to *Yr8* (24.6%) and *Yr76* (19.8%), and very low frequencies to *Yr10* (0.6%), *Yr32* (1.2%), *Yr24* and *YrTr1* (1.8%), and *Yr27* (8.4%). In Mexico, high frequencies of virulence were found to *Yr6* (95.1%), *Yr9* (91.0%), *Yr7* (87.7%), *Yr8* and *Yr44* (86.9%), *Yr27* (85.3%), *YrExp2* (84.4%) and *Yr43* (83.6%); moderate frequencies of virulence to *Yr17* (67.2%) and *YrTr1* (53.3%); low frequencies to *Yr17* and *Yr76* (9.0%); and very low frequencies to *Yr10, Yr24* and *Yr32* (0.8%). The differences in virulence frequency among the six countries are illustrated in Fig. 2. Great variation in frequency among these countries was observed for virulence to *Yr1, Yr8, Yr17, Yr27, YrSP, YrTr1* and *Yr76*.

Virulence relationships among races and among collections from different countries

Based on the avirulence/virulence reactions, the 138 races identified were clustered into nine virulence groups (VGs) (Fig. 3). VG1 consisted of 17 races including six (PSTv-241, PSTv-248, PSTv-255, PSTv-263, PSTv-279 and PSTv-315) from China, four (PSTv-20, PSTv-303, PSTv-290 and PSTv-291) from Ecuador, three (PSTv-128, PSTv-130 and PSTv-157) from Italy, two (PSTv-201 and PSTv-304) from Mexico, and two (PSTv-18 and PSTv-258) from two or more countries (Ecuador, Italy and Mexico for PSTv-18; and China and Italy for PSTv-258).

VG2 had five races including PSTv-129 and PSTv-238 from China, PSTv-285 from Ecuador, PSTv-252 from Mexico, and PSTv-250 from two or more countries (China and Italy).

VG3 was the largest group with 40 races. This group can be further separated into three subgroups. SubVG3-1 had six races (PSTv-28, PSTv-30, PSTv-183, PSTv-272, PSTv-309 and PSTv-310) all from

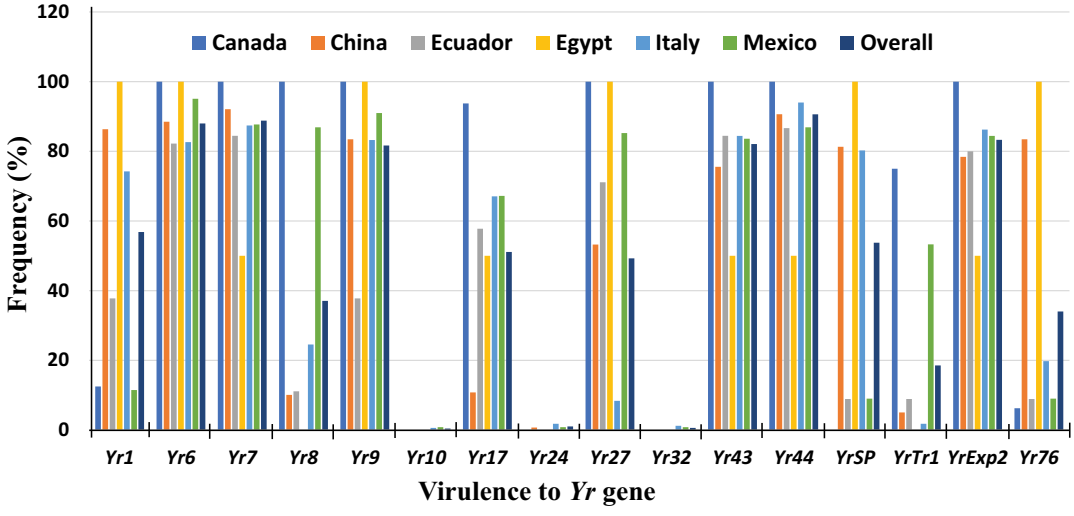

Fig. 2 Distributions and frequencies of virulence of *Puccinia striiformis* f. sp. *tritici* to 16 *Yr* genes in six countries.

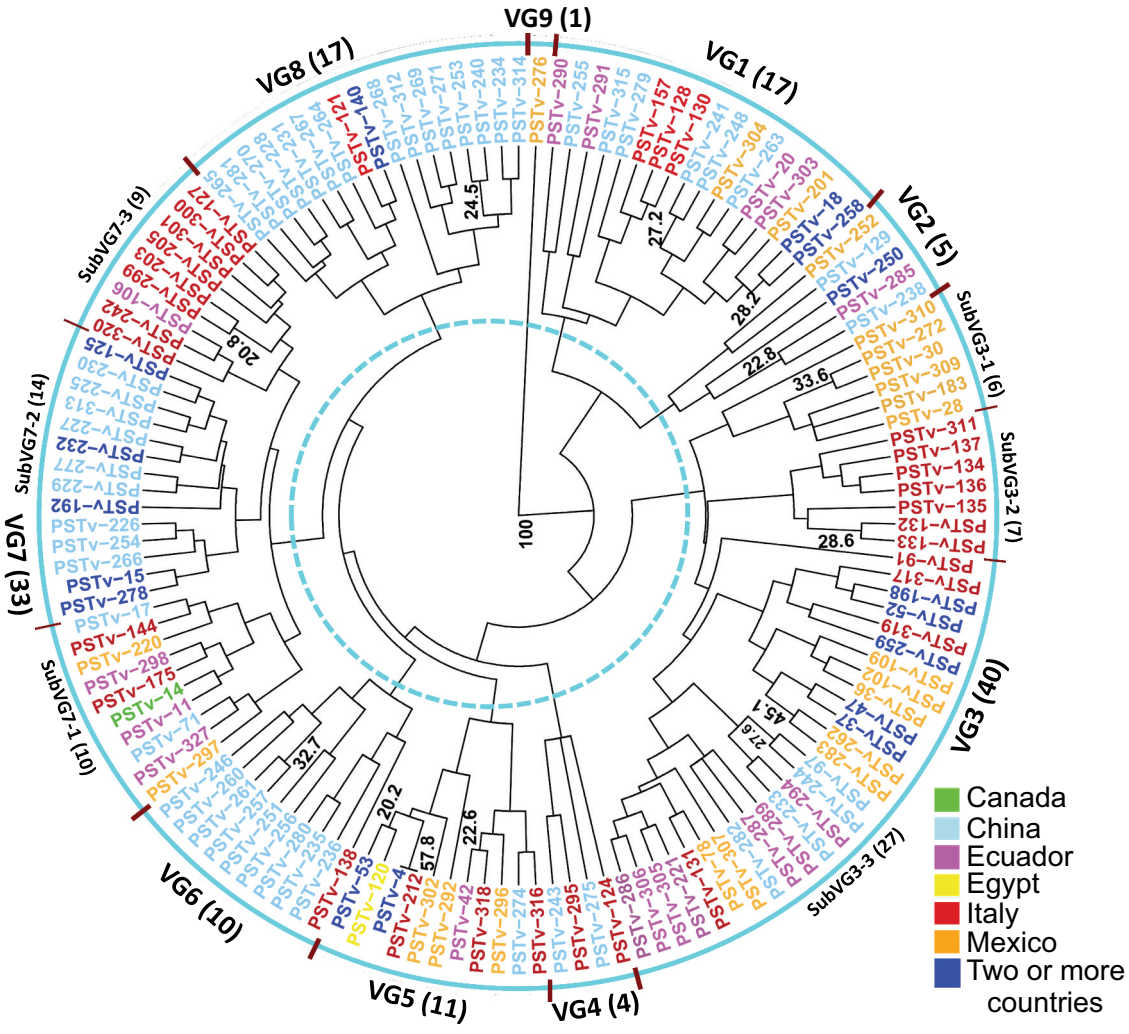

Fig. 3 Dendrogram constructed using the unweighted pair group method with arithmetic mean (UPGMA) showing the virulence relationships among races of *Puccinia striiformis* f. sp. *tritici* identified from collections of six countries.

Mexico. SubVG3-2 had seven races (PSTv-132, PSTv-133, PSTv-134, PSTv-135, PSTv-136, PSTv-137 and PSTv-311) all from Italy. SubVG3-3 had 27 races, including four (PSTv-97, PSTv-233, PSTv-244 and PSTv-282) from China, seven (PSTv-221, PSTv-286, PSTv-287, PSTv-289, PSTv-294, PSTv-305 and PSTv-306) from Ecuador, four (PSTv-91, PSTv-131, PSTv-317 and PSTv-319) from Italy, seven (PSTv-36, PSTv-78, PSTv-102, PSTv-109, PSTv-262, PSTv-283 and PSTv-307) from Mexico, and five from two or more countries (PSTv-37 and PSTv-52 from Canada, Ecuador and Mexico; PSTv-47 and PSTv-198 from Canada and Mexico; and PSTv-259 from China and Italy).

VG4 had four races, two (PSTv-243 and PSTv-275) from China and two (PSTv-124 and PSTv-295) from Italy.

VG5 consisted of 11 races including one (PSTv-274) from China, one (PSTv-42) from Ecuador, one (PSTv-120) from Egypt, three (PSTv-212, PSTv-316 and PSTv-318) from Italy, three (PSTv-292, PSTv-296 and PSTv-302) from Mexico, and two from two or more countries (PSTv-4 from Ecuador and Mexico; and PSTv-53 from China and Mexico).

VG6 had 10 races, including nine from China (PSTv-235, PSTv-236, PSTv-246, PSTv-251, PSTv-256, PSTv-257, PSTv-260, PSTv-261 and PSTv-280) and one (PSTv-138) from Italy.

VG7 consisted of 33 races, which can be further separated into three subVGs. SubVG7-1 had 10 races, including one (PSTv-14) from Canada, two (PSTv-17 and PSTv-71) from China, three (PSTv-11, PSTv-298 and PSTv-327) from Ecuador, two (PSTv-144 and

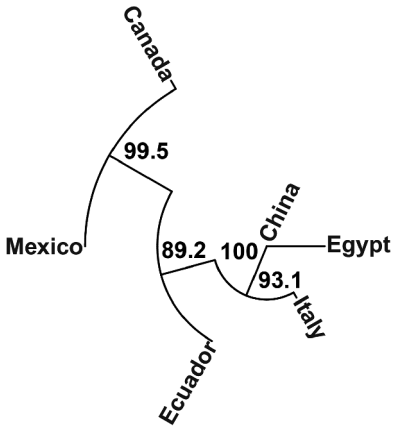

Fig. 4 Dendrogram constructed using the neighbour-joining method based on Nei's distance showing the population relationships among *Puccinia striiformis* f. sp. *tritici* collections from six countries.

PSTv-175) from Italy and two (PSTv-220 and PSTv-297) from Mexico. SubVG7-2 had 14 races, including nine (PSTv-225, PSTv-226, PSTv-227, PSTv-229, PSTv-230, PSTv-254, PSTv-266, PSTv-277 and PSTv-313) from China and five from two or more countries (PSTv-15 from China and Egypt; PSTv-125, PSTv-192, PSTv-232 and PSTv-278 from China and Italy). SubVG7-3 had nine races (PSTv-106, PSTv-127, PSTv-203, PSTv-205, PSTv-242, PSTv-299, PSTv-300, PSTv-301 and PSTv-320), all from Italy except PSTv-106 from Ecuador.

VG8 consisted of 17 races (PSTv-121, PSTv-140, PSTv-228, PSTv-231, PSTv-234, PSTv-240, PSTv-253, PSTv-264, PSTv-265, PSTv-267, PSTv-268, PSTv-269, PSTv-270, PSTv-271, PSTv-281, PSTv-312 and PSTv-314), all from China except PSTv-121 from Italy and PSTv-140 from two countries (China and Italy).

VG9 had only one race, PSTv-276 from Mexico (Fig. 3). This race with only one isolate was virulent to *Yr6, Yr7, Yr8, Yr9, Yr24, Yr32, Yr43* and *YrTr1*, but avirulent to *Yr1, Yr5, Yr10, Yr15, Yr27, Yr44, YrSP, YrExp2* and *Yr76* (Table 2).

A neighbour-joining tree constructed based on the virulence data showed different relationships among Pst collections from the six countries (Fig. 4). The collections from China, Egypt and Italy were more closely related to each other, while those from Canada, Mexico, and Ecuador were distantly related to each other and also distant from the former three countries.

Discussion

Stripe rust is a global issue affecting wheat production. *Pst* can evolve into new virulent races anywhere where the pathogen can survive and reproduce, and new races can disseminate to new regions through airborne urediniospores and incidentally by human activities (Stubbs 1985; Wellings 2011; Chen 2020). Therefore, it is important to identify *Pst* races in different countries. Because of the biotrophic nature of the pathogen and strict conditions required for virulence testing and isolate storage, not every country has programmes or uses the same set of differentials for identification of *Pst* races. In the study, researchers from different countries collaborated to identify 138 *Pst* races from 491 isolates collected in Canada, China, Ecuador, Egypt, Italy and Mexico, of which 120 races were not previously reported. The distributions and frequencies of these races and their virulence are useful for understanding the epidemiology of stripe rust, variation of the pathogen, and for guiding breeding programmes to develop wheat cultivars with effective resistance to the disease in a global context.

Viable samples are essential for virulence testing to identify races, but it is a great challenge to receive leaf samples with a living rust fungus, especially for *Pst*. Compared with leaf rust and stem rust, it is more difficult to get workable stripe rust samples because *P. striiformis* is less tolerant to high temperatures and ultraviolet light (Chen 2017). In his study, we could obtain 491 isolates from 1550 samples. The recovery rate of 31.7% was much lower than the general rate of more than 90% with domestic samples in the United States (Wan and Chen 2014; Wan et al. 2016). International shipping is one of the major factors influencing sample viability. However, collections from different countries varied greatly in recovery rate. Collections from China had the highest recovery rate, followed by Italy and Canada. Sample quality at the time of collection, and the duration and conditions of sample shipping and storage likely contributed to the variation in recovery rates. Improving the recovery rate is desperately needed for working with international samples. Approaches for increasing recovery rate include better coordination, collection of high-quality samples with active uredinia (avoiding leaves with only necrotic stripes and samples from fungicide-treated fields), storage of samples under dry and cool conditions, and shipping samples as soon as possible after collection from the field.

In this study, we used the set of 18 *Yr* single-gene lines to differentiate *Pst* races; this set was established in 2014 for differentiating *Pst* races in the United States (Wan and Chen 2014). It should be noted that not all lines have

only the specified *Yr* gene as some of them, including those for *Yr1, Yr5, Yr10, Yr15* and some plants of the line for *Yr17*, have *Yr18* (McIntosh et al. 2018). The line for *Yr17* used in our differential set does not have *Yr18* based on PCR amplification using the *csLv34* marker associated to *Yr18* (M. N. Wang, Y. X. Li, and X. M. Chen, unpublished data). Although the presence of *Yr18* in wheat varieties with all-stage resistance may enhance resistance at the adult-plant stage, the possible presence of the adult-plant resistance gene *Yr18* in some *Yr* single-gene differentials should not affect the IT data of the seedling stage for identifying races. Using this set of differentials, a total of 173 races were identified from 1968 to 2012 collections (Wan and Chen 2014; Wan et al. 2016; Liu et al. 2017) and additional 27 new races from 2013 to 2020 collections (AM Wan, MN Wang, and XM Chen, unpublished data) from the United States. In addition, 14 races that are different from those identified in the US were identified from the 2013–2014 *Pst* collection from Ethiopia (Wan et al. 2017). The octal code makes it easier to sort the avirulence/virulence patterns and assign races than if codes were not used (Wan et al. 2016). In this study, we identified a total of 138 races from the collections from the six countries. Among the 138 races, 18 (PSTv-4, PSTv-11, PSTv-14, PSTv-15, PSTv-17, PSTv-18, PSTv-20, PSTv-28, PSTv-30, PSTv-36, PSTv-37, PSTv-42, PSTv-47, PSTv-52, PSTv-53, PSTv-71, PSTv-78 and PSTv-198) have been previously reported in the United States (Wan and Chen 2014; Wan et al. 2016; Liu et al. 2017). With few exceptions, these 18 races were detected from Canada, Ecuador and Mexico on the American continents. Of these races, PSTv-37 and PSTv-52, which were detected at high frequencies in Mexico and also in Canada and Ecuador, have been predominant or among the most predominant races in the United States since 2009 (Wan and Chen 2014; Wan et al. 2016; Liu et al. 2017; AM Wan, MN Wang, and XM Chen, unpublished data). The remaining 120 races have not been reported previously. Among these 120 races, 8 (PSTv-125, PSTv-140, PSTv-192, PSTv-232, PSTv-250, PSTv-258, PSTv-259 and PSTv-278) were detected in both China and Italy, and the remaining 112 races were detected in only one country.

The identical races detected from multiple countries may provide evidence for *Pst* migration. As mentioned above, the races previously identified in the United States and detected in Canada, Mexico and Ecuador in this study suggest that races of *Pst* migrate among these countries in North and South America. The identical races commonly detected in China, Egypt and Italy suggest migration among these Asian and Mediterranean countries. In particular, China and Italy shared eight races, suggesting a high rate of migration between these countries. Although not as obvious as shared races, races identified from different countries clustering into the same virulence groups or subgroups also support migration of the pathogen among countries over long distances. Only two races were detected in both the eastern and western hemispheres. PSTv-15, which was previously reported in the United States (Wan and Chen 2014; Liu et al. 2017), was detected in China and Egypt. Similarly, PSTv-53, which was previously reported in the United States (Wan et al. 2016; Liu et al. 2017), was detected in China and Mexico. The same races suggest *Pst* migration among continents. These results are not surprising, as the global spread of *Pst* races and genotypes has been well documented (Ali et al. 2014; Hovmøller et al. 2008, 2016; Sharma-Poudyal et al. 2013, 2020; Thach et al. 2016; Ali et al. 2017). However, the same or similar races may have arisen independently in different regions. Further characterization of the isolates with molecular markers is needed to provide more direct evidence for the migration of these races between countries or continents.

No virulence was detected to either *Yr5* or *Yr15* in any of the collections from the six countries, indicating that these genes are effective against the *Pst* populations in these countries. These results are consistent with previous reports of virulence factors in *Pst* populations from various countries (Chen et al. 2002, 2009, 2010; Hu et al. 2012; Zhan et al. 2012, 2016; Wan and Chen 2012, 2014; Sharma-Poudyal et al. 2013; Shahin et al. 2014; Shahin and Aly 2015; Brar and Kutcher 2016; Wan et al. 2016, 2017; Ghanbarnia et al. 2021). Virulence to *Yr5* has been rare, and was previously recorded in India, Turkey, Australia and Tajikistan (Wellings et al. 2009). However, the most recent reports of isolates virulent to *Yr5* detected in Shaanxi and Qinghai provinces of China in 2017 and 2018 (Zhang et al. 2020), Syria in 2019 (Kharouf et al. 2021) and Turkey in 2020 (Tekin et al. 2021) have raised the alarm that *Yr5* should not be used alone for cultivar resistance to stripe rust, but combined with other ASR or APR genes. Virulence to *Yr15* is even rarer and has been documented only in Afghanistan (Van Silfhout 1989). However, continual surveillance is needed to monitor the appearance and spread of races virulent to *Yr5* and *Yr15*. *Yr5* and *Yr15* are co-present in several spring wheat cultivars grown in the United States, such as ‘Clearwhite 515′, ‘Patvin 515′. and ‘Seahawk’ (Liu et al. 2020). *Yr5* and *Yr15* should be incorporated together and/or with other effective resistance genes

into more commercial cultivars to extend their effectiveness.

Among the 18 *Yr* resistance genes present in the differential wheat lines, virulence to 16 of these was detected at different frequencies in the six countries. The frequencies of virulence to *Yr1* and *YrSP* were high (>70%) in China, Egypt and Italy, but low (<40%) in Canada, Ecuador and Mexico. In contrast, the virulence to *Yr8* had high frequencies (>80%) in Canada and Mexico, but low frequencies (<40%) in China, Egypt and Italy, as well as Ecuador. The frequency of virulence to *Yr6* was high in all six countries, whereas the frequencies of the virulence to *Yr10, Yr24* and *Yr32* were very low in the six countries. The frequencies of virulence to *Yr7, Yr9, Yr43, Yr44* and *YrExp2* were generally high in these countries except that the virulence to *Yr9* had a relatively low frequency in Ecuador. The frequency of virulence to *Yr17* was relatively high in Canada, similar in Ecuador, Italy and Mexico, but much lower in China. The frequency of virulence to *Yr27* was high in Canada, Egypt and Mexico; intermediate in China and Ecuador; but low in Italy. The frequency of virulence to *YrTr1* was high in Canada, intermediate in Mexico, but very low in China, Ecuador, Egypt and Italy. In contrast, the virulence to *Yr76* had high frequencies in China and Egypt, but low frequencies in other countries. These results were generally consistent with previous studies of related virulence factors in some of these countries (Zhan et al. 2012; Brar and Kutcher 2016; Ghanbarnia et al. 2021) and similar to the studies of *Pst* populations in the United States and other countries (Sharma-Poudyal et al. 2013; Wan and Chen 2014; Wan et al. 2016, 2017). The variation in virulence frequency may be correlated with the deployment of the various resistance genes in these countries.

It should be noted that the *Pst* isolates used in this study were collected in one or a few years from individual countries; the number of isolates, especially from Egypt and Canada, was generally low; and samples from some countries including Canada, China and Ecuador were from only one province or region. Thus, the results may not represent the *Pst* population of these countries. Nonetheless, the information on races obtained in this study should be useful for breeding resistant cultivars and managing stripe rust in these countries and other regions. Programmes for annual and countrywide surveillance of stripe rust and identifying *Pst* races should be established in countries currently without such programmes. As virulence to these genes is present in some countries, use of the corresponding resistance genes in breeding programmes needs to be cautiously considered. Pyramiding of genes for ASR and APR resistance should be used as a strategy to develop wheat cultivars with strong, long-lasting resistance to stripe rust, to avoid the impact of virulence changes in the pathogen population.

Acknowledgements

We thank Dr Dave Hooker, Dr Lily Tamburic-llincic and Cheryl Van Herk of the University of Guelph Ridgetown Campus, Joanna Follings, OMAFRA for collecting stripe rust samples from Canada; and Mingliang Ding and Jiasheng Cheng of Food Crop Research Institute, Yunnan Academy of Agricultural Sciences for the stripe rust collection from China. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

Funding

This work was supported by the US Department of Agriculture, Agricultural Research Service [Project No. 2090-22000-018-00D].

Supplementary material

Supplemental data for this article can be accessed online here: https://doi.org/10.1080/07060661.2021.1958259.

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