

The CYR34 and Suwon11-1 of *Puccinia striiformis* f. sp. *tritici* played an important role causing the stripe rust epidemic on winter wheat in Yili, Xinjiang, China during cropping season 2021.

Abstract:

Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* is a distractive disease. Its pathogen frequently adapts newly invaded regions and overcomes host resistance. China has great importance globally due to its recombinant population structure and favorable conditions for *Pst* survival. Due to its strategic location, Xinjiang is a wheat epidemic region in China; however, very limited research on *Pst* has been done in this region. In this study, we identified 25 races of 129 isolates collected from winter wheat fields from five different regions (Nileke, Xinyuan, Gongliu, Huocheng, and Qapqal) of Yili, Xinjiang, China. We used 19 sets of Chinese differential lines for race identification with single and combination of *Yr* genes. The result showed that virulence *Vr18* against the *Yr5* gene was missing, and Virulence *Vr2* against differential line Fulhad and *Vr7* against early premium line showed 100% relative frequency in the overall population. However, some races in this region showed complex virulence patterns, for example, CYR34, the second most prevalent race after Suwon11-1. Both races played a critical role in causing the epidemic of winter wheat cultivars of Yili in Xinjiang province (found in 4 out of 5 locations). It is necessary to perform regular rust watch checks on this region as it is a pathway between China and Central Asia. International collaboration is essential to counter future potential threats.

Key point: Race identification, Xinjiang epidemic region, Virulence pattern, effective *Yr* genes.

INTRODUCTION

Plant genetic resistance is a promising approach to minimizing disease infestation. The concept of the *R*-gene began in the early 20th century (Anderson et al., 2018). Later it was clarified by Harold Henry Flor's groundbreaking gene-for-gene model (Flor., 1942), associated with avirulence genes present in the pathogen that makes an incompatible response. Hosts have multilayered defense mechanisms against pathogens to avoid access inside the cell (Chisholm et al., 2006). On the other side, pathogens have evolved strategies to overcome the defense responses of their plant hosts, as many bacterial and fungal pathogens release cell-wall-degrading enzymes (Kubicek et al., 2014). Successful pathogens often evade detection by host *R* genes (Jones & Dangl, 2006). Thus, disease

resistance conferred by a single R gene often lacks durability in the field because pathogens can evolve to evade recognition by mutating the corresponding *Yr* gene. Multiple R genes are often introduced simultaneously to improve durability and broaden the resistance spectrum (Li et al., 2001; Mundt, 2018).

Puccinia striiformis f.sp. *tritici* is one of the most destructive pathogens causing wheat stripe rust disease, frequently mutates and overcomes resistance genes in major wheat cultivars (Chen & Penman, 2005; Lu et al., 2011; Zhan et al., 2022). Race identification of *Puccinia striiformis* f.sp. *tritici*, in China, was started in 1940 by *Yan* (1944) using Chinese differential-based wheat lines, Chinese race variants were coded with CYR (Chinese yellow rust) and differential lines names series with a relative frequency of more than 10% (Zhao & Kang, 2023). Various cultivar replacement programs have taken place in China since the 1950s due to repeated appearances of new virulent races (He et al., 2001). At that time, *CYR1* breakdown resistance in the Bimai cultivar carrying the *Yr1* gene (McIntosh et al., 2018). By 1970 IBL/IRS derivatives such as Iovrin 10, Lovrin 13, Predgornaia 2, Kavkaz, and Neuzucht were used extensively in crossing programs in China because of their excellent resistance to stripe rust. But now, more than 80% of commercially grown wheat cultivars (12 *Yr* and Fan6 derivatives) are susceptible to the predominant races *CYR31* and *CYR32* (Wan et al., 2007). In recent years, Race *CYR34*, which was virulent to *Yr26*, *Yr24*, and *Yr10* is more prevalent (Zhang et al., 2015), resulting in many wheat cultivars with *Yr6* becoming susceptible, such as Guinong 22 and its derived cultivars and Chuanmai 42 (Liu et al., 2017). Resistance breeding faces many challenges due to the availability of less effective resistance genes.

Puccinia striiformis f.sp. *tritici* is dispersed through wind and human means. It completes its life cycle on two host plants. The asexual infection occurs in wheat, while sexual reproduction occurs on the barberry plant (Zhao et al., 2016). To complete its life cycle, it must avoid plant immune responses and absorb nutrients directly from the colonized plant tissue whilst keeping its host alive (Garnica et al., 2014). A previous study demonstrated the Himalayas region (Nepal, Pakistan and China) as a hot spot for sexual recombination and a centre of diversity in the other region America, North Europe, East Africa and Australia, to induce an asexual clonal population (Ali et al., 2014). China is the largest epidemic country worldwide for wheat stripe rust due to its unique topography, providing favorable conditions for a whole year for pathogen survival.

In China, many Chinese *Berberis* species serve as alternate hosts of *Puccinia striiformis* f. sp. *tritici* (Du et al., 2019; Li et al., 2021a; Li et al., 2021b; Zhao et al., 2013, 2016). Diverse agricultural systems, geography, climate, and pathogen life cycles in different regions create distinct disease epidemiological zones in China (Zeng & Luo, 2006). China recently divided into six main epidemic zones, including over-wintering (Chongqing, Central Shaanxi, South Shaanxi, Sichuan, Henan, North Hubei, and South Hubei), eastern epidemic regions (Anhui, Zhejiang, Shandong, and Jiangsu), Yun-Gui epidemic regions (Yunnan and Guizhou) and Tibetan and Xinjiang epidemic regions. The southwestern and northwestern epidemic regions played a critical role in dispersing disease to central main wheat cultivation regions (Awais et al., 2022). The disease is more prevalent in the winter-wheat-growing areas of the northwest, southwest, and north, and the spring-wheat-growing areas of northwest China (Li & Zeng, 2000). The worst epidemics have caused yield losses of up to 3.2, 1.8, 1.3, and 1.5 million metric tons in China in 1964, 1990, 2002, and 2017 (Wan et al., 2004; Chen, 2005).

Among different epidemic regions in China, Xinjiang regions have great importance due to its strategic location, which links China with Central Asian countries, and it is a gateway for the invasion of new races between Central Asian countries and the main wheat regions of China. Both winter wheat and spring wheat cultivated in this region may help pathogen to survive all around year. Also, many Chinese *Berberis* species have been identified as potential alternate hosts for *Puccinia striiformis* f. sp. *tritici* in Xinjiang region (Zhuang et al., 2019). In neighboring countries Tajikistan, Kyrgyzstan, Kazakhstan and Pakistan also affected most from wheat stripe rust. *Pst* populations in these countries are also phenotypically and genotypically diverse, especially in Pakistan (Ali et al. 2014b). Although recent studies conducted to compare Pakistan and China *Pst* population that suggest high divergence between both countries (Awais et al., 2023), but still has potential risk for invasion of new races in this region as they are close neighbors. So, it is vital to have a regular surveillance watch check on this region to identify newly emerged races. Collaboration among scientists in China and Central Asian countries is vital for countering future epidemic threats.

The objectives of this study were to identify races and virulence and determine the virulence diversity of the *Puccinia striiformis* f. sp. *tritici* population in the winter wheat region of Yili, Xinjiang, China.

MATERIALS AND METHODS

Wheat stripe rust surveillance and sample collection

Wheat stripe rust surveillance was conducted in winter wheat fields and the Yili district, including, Nileke, Gongliu, Xinyuan, Qapqal and Huocheng counties northwest China. It was conducted during the Mid-May to early July, Crop season of 2021, followed the protocol as described by Ali and Hodson (2017). The distance between each county is 100 to 300 kilometers away. Five sites from each county were chosen to collect stripe rust samples. The sites were at least 10-15 kilometers away from each other (supplementary table 1). Rust prevalence (percentage of wheat infected), rust severity (percentage of leaf area contaminated), a cultivar of winter wheat, latitude, longitude, and altitude were recorded. Leaf samples with fresh single-line uredinium were collected from each county and packaged in a moisture-absorbent paper bag for drying for one or two days. After that the leaf samples were stored at 4°C with moisture observing salica gel with air tight bag. The leaf samples were not put prolong time in storage it effects spore recovering quality.

Spore multiplication and preservation

For spore multiplication, the leaf samples were first moisturized on a wet filter paper in a petri dish at 10°C for 10h in darkness to induce fresh single uredinium production (Mehmood et al. 2020). The 10-15 days old seedlings of Mingxian 169, which is a susceptible wheat genotype, were inoculated with urediniospores from a single uredinium using a sterilized inoculating needle. Inoculated seedlings were sprayed with water mist and incubated in a dew chamber at 10 to 13°C for 24 h in darkness. Then seedlings were maintained in a growth chamber with a day/night thermoperiod of 17/13°C, a photoperiod of 16 h, relative humidity of 60%, and weekly irrigation with tap water. Fifteen days later, inoculated leaves began to sporulate. The urediniospores were collected inside test tube. The healthy Mingxian 169 seedlings were again used to increase urediniospores follow the same procedure. Enough urediniospores were collected with a clean glass tube and store inside dedicator at 4°C for short period of time (de Vallavieille-Pope et al. 2002; de Vallavieille-Pope et al. 2012). For long preservation it stored at -20°C. Before using these spores,

the heat shocked for 2 minutes done by submerging ~~it~~ inside warm water (about 50°C) ~~it~~ not exceed it more than two minutes. ~~It~~ Uredium from a single leaf sample was treated as one isolate physiological race population in statistical data.

Race identification

Races of *P. striiformis* f. sp. *Tritici* were identified using 19 sets of Chinese-based differentials included Trigo-Eureka (*Yr6* gene), Fulhard (Unknown gene), Lutescens 128 (Unknown gene), Mentana (Unknown gene), Virgilio (*Yr Vir1*, *YrVir2* gene), Abbondanza (Unknown gene), Early Premium (Unknown gene), Funo (*YrA*,+), Danish 1 (*Yr3* gene), JubilejinaII (*YrJu1*, *YrJu2*, *YrJu3*, *YrJu4* gene), Fengchan 3 (*Yr1* gene), Lovrin 13 (*Yr9*,+), Kangyin 655 (*Yr1*, *YrKy1*, *YrKy2*), Suwon 11 (*YrSu*), Zhong 4 (Unknown), Lovrin 10 (*Yr9*), Hybrid 46 (*Yr3b*, *Yr4b*), *Triticum spelta* var. Album (*Yr5* gene) and Guinong22 (*Yr10*, *Yr26* gene) as described by Zhan *et al.* (2016). These differential lines carry single and multiple Yr genes. These differential lines were planted according to their numerical order with 5 varieties in each pot and susceptible wheat genotypes Mingxian 169 as control. The fresh urediniospores and talcum powder were mixed at a 1:30 ratio in a 15ml centrifuge tube and sealed with sterile gauze. Then the mixture was gently shaken on seedlings of differential lines with a water mist. The plants were transferred to the growth chamber and growth under the same condition described above. Each isolate's reaction phenotype on different differentials was counted and classified when control genotypes Mingxian 169 was infected completely. If the disease symptom on differential lines were unclear after inoculation, the isolate was inoculated again on differential line for verification. Infection type recorded after 18-20 days post-inoculation, using scale (Avirulent = 0-6, Virulent = 7-9).

Data analyses

Virulence profiles were determined by scoring infection type on various differential lines containing particular resistance genes. Virulence combination was used to figure out the pathotype. Pst races, race frequencies and distribution, and frequencies of virulence to Yr genes or differential genotypes were analyzed. Cluster analyses were carried out using the Ward method (Ward 1963) to assess the interrelationship of an isolate from a different location.

RESULTS

Virulence identification and its frequency within epidemic region:

19 sets of Chinese-based differentials, as described by Zhen et al. (2016), were used to identify races of *P. Striiformis* from 119 isolates collected from different locations in Yili, Xinjiang, China. These differentials carry a single and combination of resistance genes (Table 1). The virulence against corresponding differential lines coded as Vr1, Vr2 Vr19. Our results showed Virulence Vr18 corresponding differential line *Triticum spelta* var. Albun with Yr5 were missing in the overall population. While virulence frequency is 100% in Vr2 and Vr7 corresponding lines Fulhard and Su von 11 with Unnamed and YrSu genes (Fig.1).

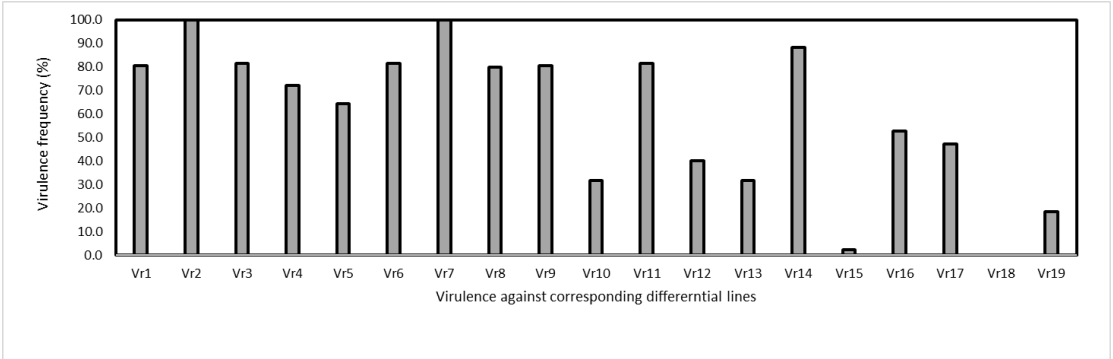


Fig. 1 Relative frequency of different virulences observed in *Puccinia striiformis* isolates Collected from winter wheat in Yili, Xinjiang, China during the winter wheat cropping season 2021.

Table 1: Chinese differential lines used to identify races of *Puccinia striiformis* isolates collected from Yili, Xinjiang, China, during winter wheat cropping season 2021

S.NO	Virulence	Differential lines	Yr gene ^a
1	Vr1	Trigo-Eureka	Yr6
2	Vr2	Fulhard	Unknown
3	Vr3	Lutescens 128	Unknown
4	Vr4	Mentana	Unknown
5	Vr5	Virgilio	YrVir1, YrVir2
6	Vr6	Abbondanza	Unknown
7	Vr7	Early Premium	Unknown
8	Vr8	Funo	YrA,+
9	Vr9	Danish 1	Yr3
10	Vr10	JubilejinaII	YrJu1, YrJu2, YrJu3, YrJu4
11	Vr11	Fengchan 3	Yr1
12	Vr12	Lovrin 13	Yr9,+

13	Vr13	Kangyin 655	Yr1, YrKy1, YrKy2
14	Vr14	Suwon 11	YrSu
15	Vr15	Zhong 4	Unknown
16	Vr16	Lovrin 10	Yr9
17	Vr17	Hybrid 46	Yr3b, Yr4b
18	Vr18	riticum spelta var. Album	Yr5
19	Vr19	Guinong22	Yr10, Yr26

^aThe ~~virulence~~ genes in differentials lines refer to Wan et al. (2004): Chen et al. (2009) and Zhen et al. (2015).

Across different locations, Nileke, Xinyuan, Gongliu, Chabuchaer, and Huocheng of Xinjiang region, the maximum number of virulence was observed (18Vr) in Xinyuan and Gongliu regions (Table 2). However, the virulence frequency varied among areas. In Nileke, the minimum relative frequency of virulence (0%) was observed in Vr15 against the corresponding differential line Zhong 4 and Vr3 against the corresponding line *Triticum spelta* var. Album. While the maximum virulence frequency (100%) was noted in Vr2, Vr3, Vr6, Vr7, Vr8, Vr9, Vr11, and Vr16 against differential lines Fulhad, Lutescens 128, Abbondanza, Early Premium, Funo, Danish 1, Fengchan 3 and Lovrin 10 respectively. In Gongliu regions, the minimum relative frequency was observed only in Vr18 against *Triticum spelta* var. Album line, while the maximum relative frequency was observed in Vr2 against Fulhard and Vr7 against Early Premium. In Huocheng, the maximum virulence frequency was observed in Vr1, Vr2, Vr6, and Vr14 against differential lines Trigo-Eureka, Fulhard, Abbondanza and Kangyin 655, while no virulence was absent. In the Qapqal regions, the minimum relative frequency was noted only in Vr18 against the corresponding line *Triticum spelta* var. Album, while the maximum was noticed in Vr2 and Vr7. Furthermore, In the Xinyuan region, the minimum relative frequency only was seen in Vr18, while the maximum was seen in Vr2 and Vr7 (Table 3).

Table 2. Relative frequency (%) of different virulence factors in *Puccinia striiformis* isolates collected from different location in Xinjiang province of China during the winter wheat crop season of 2021.

Virulence	Nilka	Gongliu	Huocheng	Qapqal	Xinyuan	Overall population
Vr1	66.7	82.9	100.0	70.4	84.2	80.6
Vr2	100.0	100.0	100.0	100.0	100.0	100.0
Vr3	100.0	78.0	0.0	77.8	86.0	81.4
Vr4	66.7	70.7	0.0	63.0	78.9	72.1
Vr5	66.7	58.5	0.0	51.9	75.4	64.3

Vr6	100.0	78.0	0.0	77.8	86.0	81.4
Vr7	100.0	100.0	100.0	100.0	100.0	100.0
Vr8	100.0	75.6	0.0	77.8	84.2	79.8
Vr9	100.0	75.6	0.0	77.8	86.0	80.6
Vr10	66.7	19.5	0.0	25.9	42.1	31.8
Vr11	100.0	78.0	0.0	77.8	86.0	81.4
Vr12	66.7	26.8	0.0	33.3	52.6	40.3
Vr13	66.7	19.5	0.0	25.9	42.1	31.8
Vr14	100.0	92.7	100.0	81.5	87.7	88.4
Vr15	0.0	2.4	0.0	0.0	3.5	2.3
Vr16	100.0	34.1	0.0	59.3	61.4	52.7
Vr17	66.7	34.1	0.0	40.7	59.6	47.3
Vr18	0.0	0.0	0.0	0.0	0.0	0.0
Vr19	33.3	7.3	0.0	14.8	28.1	18.6
Sample size	3	41	1	27	57	129

Table 3. Number of different pathotypes, virulences and its diversity detected in the *Puccinia striiformis* isolates collected from different location in Xinjiang province of China during the winter wheat crop season of 2021.

Virulence	Sample size	Number of pathotypes detected	Pathotype diversity	Frequency of the most frequent pathotype	Number of virulences detected	Frequency of the most frequent virulence
Nilka	3	3	0.67	33.33	17	100
Gongliu	41	16	0.88	21.95	18	100
Huocheng	1	1	0.00	100.00	4	100
Qapqal	27	16	0.91	22.22	17	100
Xinyuan	57	17	0.89	22.81	18	100
Overall population	129	25	0.91	18.60	18	100

Pathotype grouping through cluster-based analysis

Rust surveillance was done from the winter wheat epidemic regions of Nileke, Xinyuan, Gongliu, Chabuchaer, and Huocheng, which belong to the Yili *Pst* epidemic region of Xinjiang. A total of 230 isolates were collected. Among them, only 129 isolates were recovered. After applying the race identification test, 71 races discovered and 5 new races (P_10, P_18, P_20, P_23) were identified in the overall population. 41 identified *pst* races were classified into four cluster groups (G1, G2, G3, and G4). The maximum number of races was identified into clusters G2 and G4 (Fig.2). the G1 group contains races *Guinong22-14*, *CYR34*, and *Guinong22-13*, and these races showed a

complex virulent pattern. These races were virulent against all differential lines except *Guinong22* and *Triticum spelta* var. Album. *Guinong22* had *YR5* gene. The G2 group had *Suwon11-1*, *Suwon11-2*, *Suwon11-10*, *Suwon11-8*, *Hy_6* and newly identified races P_20, P_10, P_23. The differential lines JubilejinaII, Lovrin 13, Kangyin 655, Zhong 4, Lovrin 10, *Triticum spelta* var. Album, and *Guinong22* were found resistant against Group 2 races. The G3 group contains *CYR32*, *Hy-7*, *Hy-4*, *CYR33* and *Suwon11-4*. These races were virulent against all differential lines except Zhong 4, *Triticum spelta* var. Album, and *Guinong22*. Group 4 had *CYR31*, *CYR30*, *CYR29*, *Lovrin13-2*, *CYR28*, *Suwon11-7* and *Suwon11-12* races. The differential lines JubilejinaII, Kangyin 655, Zhong 4, *Triticum spelta* var. Album, *Guinong22* showed resistant. These group of races were not specific to one region, although race frequency varied region to region.

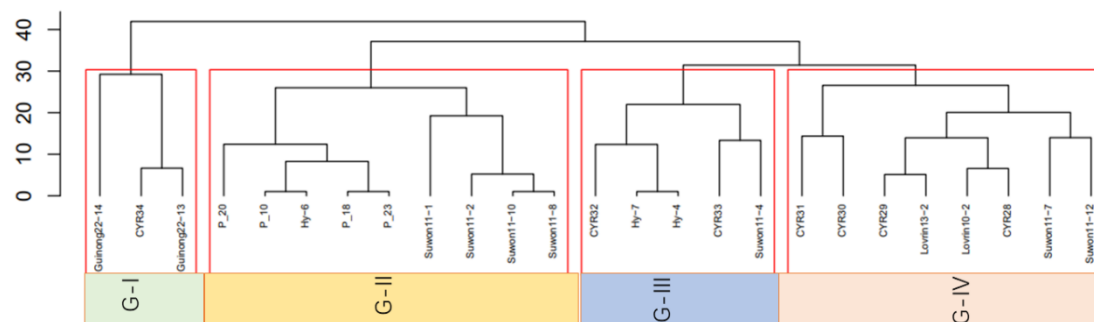


Fig.2 Clustering of 25 races detected from 29 isolates of *Puccinia striiformis* collected from different location in Xinjiang province of China during the winter wheat crop season of 2021.

Identification and relative frequency across epidemic region:

In winter wheat in Yili epidemic region, the most prevailing race was *Suwon11-1*, with a relative frequency of 18.6% out of 129 isolates, and the second most predominant race was *CYR34* (14%). The *CYR34* race was highly virulent compared to *Suwon11-1* (Vr1, Vr2, Vr7, Vr14) with a maximum number (17V) of virulence pattern (Vr1, Vr2, Vr3, Vr4, Vr5, Vr6, Vr7, Vr8, Vr9, Vr10, Vr11, Vr12, Vr13, Vr14, Vr16, Vr17, and Vr19) against corresponding differentials with anonymous and recognized *Yr* genes (Table. 1).

Race diversity varies from region to region. The maximum race diversity found in Xinyuan and Qapqal region (Fig.3); Some races were found only in specific locations, such as P-20 and *Suwon11-8*, races are only specific to the Gongliu region. *CYR29*, *Hy7*, *Suwon11-4*, and *Suwon11-7* were specific to Qapqal, and *Hy4* and *P23* were only specific to Xinyuan. These races did not resample to any other region. The maximum predominant race found in Gongliu was *Suwon11-1*

(22% relative frequency), followed by Suwon11-2 (19.5%). In Nileke, only three races CYR32, CYR34 and Suwon11-12 were found. Suwon11-1 was the most prevailing race in Qapqal and Xinyuan, while CYR34 was dominant in the Xinyuan region (Table 4).

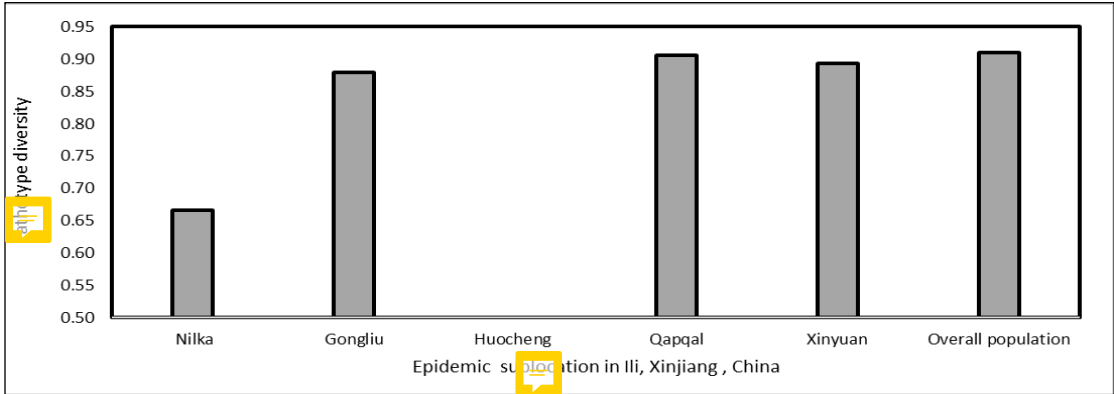


Fig. 3. Puccinia striiformis type diversity in Puccinia striiformis isolates collected from Yili, Xinjiang, China, during winter wheat cropping season 2021.

Table 4  Active frequency (%) of different races detected  *Puccinia striiformis*  isolates collected from different location  Xinjiang province of China  during the winter wheat crop season of 2021.

Race	Virulence profile ^a	Nilka	Gongliu	Huocheng	Qapqal	Xinyuan	Overall population
CYR28	1,2,3,4,5,6,7,8,9,-,11,-,-,-,16,-,-,-	-	2.4	-	7.4	3.5	3.9
CYR29	1,2,3,4,5,6,7,8,9,-,11,12,-,-,16,-,-,-	-	-	-	3.7	-	0.8
CYR30	1,2,3,4,5,6,7,8,9,-,11,12,-,-,16,17,-,-	-	2.4	-	-	7.0	3.9
CYR31	1,2,3,4,5,6,7,8,9,-,11,12,-,14,-,16,17,-,-	-	4.9	-	7.4	5.3	5.4
CYR32	1,2,3,4,5,6,7,8,9,10,11,12,13,14,-,16,17,-,-	33.3	12.2	-	-	8.8	8.5
CYR33	-,2,3,4,5,6,7,8,9,10,11,12,13,14,-,16,-,-,-	-	-	-	-	1.8	0.8
CYR34	1,2,3,4,5,6,7,8,9,10,11,12,13,14,-,16,17,-,19	33.3	4.9	-	7.4	22.8	14.0
Guinong22-13	-,2,3,-,-,6,7,8,9,10,11,12,13,14,-,16,17,-,19	-	-	-	7.4	1.8	2.3
Guinong22-14	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,-,-,-,19	-	2.4	-	-	3.5	2.3
Hy-4	1,2,3,4,5,6,7,8,9,10,11,-,13,14,-,16,17,-,-	-	-	-	-	3.5	1.6
Hy-6	1,2,3,4,5,6,7,8,9,-,11,-,-,14,-,-,17,-,-	-	2.4	-	7.4	5.3	4.7
Hy-7	-,2,3,4,5,6,7,8,9,10,11,-,13,14,-,16,17,-,-	-	-	-	7.4	-	1.6
Lovrin10-2	1,2,3,-,-,6,7,8,9,-,11,-,-,-,16,-,-,-	-	2.4	-	3.7	-	1.6
Lovrin13-2	1,2,3,4,-,6,7,8,9,-,11,12,-,-,-,16,-,-,-	-	-	-	3.7	1.8	1.6
P_10	-,2,3,4,5,6,7,8,9,-,11,-,-,14,-,-,17,-,-	-	2.4	-	3.7	3.5	3.1
P_18	1,2,3,4,5,6,7,-,9,-,11,-,-,14,-,-,17,-,-	-	2.4	-	-	-	0.8
P_20	-,2,3,4,5,6,7,8,-,-,11,-,-,14,-,-,17,-,-	-	2.4	-	-	-	0.8
P_23	-,2,3,4,5,6,7,-,9,-,11,-,-,14,-,-,17,-,-	-	-	-	-	1.8	0.8
Suwon11-1	1,2,-,-,-,7,-,-,-,-,14,-,-,-,-	-	22.0	100	22.2	14.0	18.6
Suwon11-10	-,2,3,4,-,6,7,8,9,-,11,-,-,14,-,-,-,-	-	7.3	-	3.7	1.8	3.9
Suwon11-12	-,2,3,-,-,6,7,8,9,-,11,-,-,14,-,16,-,-,-	33.3	4.9	-	3.7	5.3	5.4
Suwon11-2	1,2,3,4,5,6,7,8,9,-,11,-,-,14,-,-,-,-	-	19.5	-	3.7	8.8	10.9
Suwon11-4	-,2,3,4,-,6,7,8,9,10,11,-,13,14,-,16,-,-,-	-	-	-	3.7	-	0.8
Suwon11-7	1,2,3,4,5,6,7,8,9,-,11,12,-,14,-,16,-,-,-	-	-	-	3.7	-	0.8
Suwon11-8	1,2,3,4,-,6,7,8,9,-,11,-,-,14,-,-,-,-	-	4.9	-	-	-	1.6



Sample size

3

41

1

27

57

129

^aVirulence Profile showed race virulence pattern against differential lines: 1 = Trigo Eureka, 2 = Fulhard, 3 = Lutescens 128, 4 = Mentana, 5 = Virgilio, 6 = Abbondanza, 7 = Early Premium, 8 = Funo, C9 = Danish 1, 10 = Jubilejina 2, 11 = Fengchan 3, 12 = Lovrin 13, 13 = Kangyin 655, 14 = Suwon 11, 15 = Zhong 4, 16 = Lovrin 10, 17 = Hybrid 46, 18 = *Triticum spelta* var. Album, and 19 = Guinong 22.

DISCUSSION

In the present study, we analyzed the virulence of stripe rust samples from the winter wheat region of Yili, Xinjiang, during the cropping season of 2021. Xinjiang is the largest wheat producer in the northwest China, with an annual wheat growing area of about 1.13 million ha, total of 4% of the wheat area in China. It is classified as a separate wheat zone due to its unique geographic location. This region is highly diverse and has different characteristics due to geographical subdivisions based on the desert, high mountains, and temperature fluctuation (Zhu et al., 2019). Furthermore, it is China's gateway to Central Asian countries. These spatial characteristics are highly influential for the dynamics of *P. striiformis* f. sp. *tritici* races (Awais et al., 2023). There are approximately equal proportions of winter and spring wheat in Xinjiang. However, recent field surveys conducted during the cropping season of 2021 highlighted winter wheat crops badly affected by wheat stripe rust. In this study, we concluded that CYR34 and Suwon11-1 were prevalent in Yili, with resamples in 4 out of 5 locations with a 14% and 18.6% relative frequency. Previously, the race CYR34, which was virulent against Guinong 22 with Yr26, was absent in Xinjiang (Zhan et al., 2015) but present in other epidemic regions of China (Liu et al., 2010). The virulence against Yr26 was also found in other continents, although it had low frequency (Ali et al., 2014; Sharma-Poudyal et al., 2013). In recent years, reporting of CYR34 in the winter epidemic region of Xinjiang has been an alarming situation for breeders. They need to work on introducing some resistant cultivars with high yield potential that can replace previous cultivars. Most recently, the breeding line 041133 was highly resistant to CYR34 (Li et al., 2022).

Epidemiologically, the Xinjiang region is an independent region where *P. striiformis* f. sp. *tritici* can survive for around a year. The Xinjiang population is genotypically different from the rest of the epidemic region in China. However, many multilocus genotypes (MLG) have been detected in this region, suggesting that this region's overall population was recombinant and could have a potential future epidemic threat to other regions (Awais et al., 2022). Our study also detected 21 newly discovered and four new race few races found in low frequency, so we gave them temporary names).

Deployment of the resistance genes is the most economical and durable technique to control stripe rust (Bai et al., 2014; Lan et al., 2014). So far, more than 50 resistance genes (Yr1 -Yr57) have been discovered (Cao et al., 2014), and 5 of them are widely used in wheat programs globally

(Chen et al., 2005). The Yr26 gene has lost resistance to CYR34 (V26) since 2011 (Wang et al., 2019). Previous studies reported that overall, winter wheat cultivars that have been used in China only (10%) were found to be resistant to CYR34 rust. In our results, the CYR series races (CYR28-CYR34) were found in the Xinjiang region in variable frequency. The CYR28 and CYR29 were first found in China in 1983-1985 (CNWRCG 1987) and belonged to 10/13 pathotype group. It caused a massive epidemic in 1990 and affected 62% total planted area (Zhao & Kang, 2023). CYR30, which is virulent to Hybrid 46 (Yr3b, Yr4b, and YrH46) and CYR31, virulent against Hybrid 46 and Suwon 11, were first detected in 1991 in Sichuan and 1993 in the Gansu region and became top prominent race in 1996 (Wang et al. 1996; Wan et al., 1996). Our result revealed that in the overall population of Fuli, Xinjiang, the 100% relative frequency of virulence was found in Vr2 and Vr7 corresponding lines Fulhard and Suwon 11 with Unnamed and YrSu genes. In contrast, the resistance of the Yr5 gene is still effective in this area. However, recently, a new pathotype TSA- was found that can break the fence of the resistance gene Yr5. It showed a similar virulence pattern as CYR32 and CYR34 races. It can evolve and generate same lineage of pathotypes (Zhang et al. 2020a, 2022). Nowadays, wheat cultivars with the Yr9 gene become susceptible to stripe rust worldwide. The first-time resistance breakdown of Yr9 has been reported since the late 1980s due to the widespread emergence of virulent races worldwide and has resulted in major epidemics of stripe rust (Wellings et al., 2011; Gulyaeva et al., 2022). In China, the first case of Yr9 virulent race Lv10 and Lv13 was detected in Longnan of Gansu province (Kang & Li, 1984; Kang et al., 1987). Our study also reported virulence against Yr9 in the Xinjiang region of winter wheat crop. most of the differentials are used only in China, and their genetic bases of their resistance are not fully published on an international basis, it is difficult to compare Chinese races with those in other countries.

There are several reasons for the variation in virulence in Xinjiang regions. One could be the presence of Chinese *Berberis* species, identified as potential alternate hosts for *Puccinia striiformis* f. sp. *tritici* in the Xinjiang region (Zhuang et al., 2019) and long-distance dispersal of the Pst pathogen by wind also responsible for pathotype diversity. This region's population also has the potential to replace the European wheat yellow rust population, which was clonal as previously the warrior race present in high frequency in Europe was found to be genetically originated from a sexually recombining population in near Himalaya region of Asia, which is centre of diversity of

yellow rust fungus (Hovmøller et al., 2015).

Neighboring countries near Xinjiang were also affected heavily due to Stripe rust. Winter wheat is grown in all parts of Central Asia from October to Nov and harvested in June and July. Moreover, spring crops were planted in May and harvested in August-Sept. In the Kazakhstan region, the wheat crop is present all year round, which helps to nourish the inoculum of stripe rust. (Ziyaev et al., 2010) various pst pathotypes reported in this region most common were 6E20, 6E148, 7E140, 7E148, 7E150, 7E156, 15E150 and 39E158 as the dominant pathotypes in Kazakhstan, Kyrgyzstan, Tajikistan and Uzbekistan (Abdullaev et al., 2009). However, no such study was conducted to compare these pathotypes to neighboring regions of China.

Different techniques could be useful regarding control of stripe rust, included deployment of wheat varieties carrying multi-resistance genes but not a single resistance gene at a large scale or in epidemiological region, eradicating alternate host plants close to wheat fields. And intercropping highly recommended (Zhao and Kang, 2023). Fungicide control not be recommended as risk of emergence of anti-fungicide Pst and Pgt races.

CONCLUSIONS

In this study, the identification was conducted in the region of Xinjiang and total of 25 races were identified. Among them most prevalent race found to be CYR34 and Suwon11-1 resample 4 out of 5 locations. Resistance genes present in Fulhard and Early Premium showed 100% breakdown. While the still found effective. It is essential to understand the population structure of *tritiformis* f. sp. *tritici* in Xinjiang and the genetic relationships of the pathogen to populations in other regions, especially neighboring Central Asian countries. Such information should provide the basis for developing effective strategies for control of the diseases in the region and throughout the country.

ACKNOWLEDGEMENT

We are very grateful to Xinjiang Uyghur Autonomous Region, regional coordinated innovation project (Shanghai Cooperation Organization Science and Technology Partnership Program) (No. 2022E01022). Natural Science Foundation of Xinjiang Uyghur Autonomous

Region(2022D01A270); Science and Technology Assistance Project of Xinjiang Uygur 91
Autonomous Region "Integration and Demonstration of Green Prevention and Control Technology 92
for Wheat Stripe Rust (2022E02070); Key Research& Development Program of 93
Xinjiang(2021B02002-1); Urumqi Integrated Experimental Station of China Agriculture Research 94
System for Wheat (CARS-03-88) ”for grants conducting this research work and also highly 95
thankful to Prof. Z. ao for his technical services during work. 96

AUTHOR CONTRIBUTION 98

Conceived and designed the experiments JM and HG. Performed the sampling and race 99
identification LC, HY, YS, HG, JM. Analyzed the data and Wrote Manuscript HG, JM., MA. 100

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