

Races of stripe rust (*Puccinia striiformis* f. sp. *tritici*) identified in Central Anatolia

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ABSTRACT

Wheat is one of the most important cereal groups, with large cultivation areas in Türkiye and around the world. Stripe rust (*Puccinia striiformis* f. sp. *tritici*) is one of the biotic factors limiting the yield and quality of wheat. It is significant to determine its race/races in a region in terms of breeding studies and suggestions on varieties of wheat for farmers. This study aims to detect the stripe rust races in Central Anatolia, Türkiye. Wheat leaves with stripe rust were collected in Ankara, Eskişehir, Kırkkale, Kırşehir, and Yozgat provinces in 2020. Urediniospores for each isolate were collected from leaves, and a set of differentials containing different resistance genes was used to determine the virulence of the isolates. Inoculation was done by spraying 7-days-old seedlings, and reactions were assessed. Results showed that isolates were effective on *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr24*, *Yr25*, and *Yr32* resistance genes in the entire region. Along with this phenotyping study, further studies on these isolates will be done genotypically.

1. Introduction

Wheat belongs to the cereal crops with the largest cultivation area worldwide due to its significance in human nutrition. Globally, it is predicted that 785.1 million tonnes of wheat will be produced by the end of 2023 (FAO 2023). The forecast for the wheat production in 2023 has increased considerably by 6.6 million tonnes, reaching 783.3 million tonnes in the world (FAO 2023). In addition to this, it is estimated that there are 7.2 million ha of wheat cultivation areas with a projected 19.5 million metric tonnes of production for 2023/24 in Türkiye (USDA 2023). However, there are several factors threatening wheat cultivation areas in terms of quality and yield, such as biotic and abiotic stresses. Abiotic stresses result from environmental factors whereas biotic factors, consist of diseases, pests, weeds, etc. Among the biotic stresses, stripe rust is a disease caused by *Puccinia striiformis*, limiting both the quality and the yield of wheat. The annual wheat yield loss is estimated at 5.5 million tonnes worldwide due to stripe rust (Beddow et al. 2015).

There are various approaches to controlling wheat rust diseases, and one of the most widely recommended is the cultivation of rust-resistant wheat varieties. To date, several resistance genes have been identified (Chen and Kang 2017). Seedling resistance is expressed through a single major gene; on the other hand, more than one minor gene controls adult plant resistance. Since seedling resistance is controlled by a single major gene, resistance can be overcome easier by pathogens evolving rapidly (Roelfs et al. 1992). Though adult plant resistance is accepted as more durable, it is more effective in high temperatures rather than lower temperatures (Chen 2013; Chen et al. 2021). Due to these disadvantages of seedling resistance and adult plant resistance, gene pyramiding, which combines

several resistance genes, is the finest option for breeders (Chen et al. 2021). However, it is crucial to picture pathogen populations in related regions through annual surveys (Park et al. 2011). Information obtained from these surveys will shed light on breeding programmes for disease-resistant varieties.

In this study, a survey was conducted in Central Anatolia (Ankara, Eskişehir, Kırkkale, Kırşehir, Yozgat provinces) in 2020. Stripe rust isolates were isolated from leaf samples collected from diverse locations. Hence, this study investigates phenotypically the isolates via race analysis.

2. Materials and Methods

2.1. Plant materials

The differential sets consisted of the European and World stripe rust differential sets, Avocet S near-isogenic lines (NILs) and additional supplementary European wheat varieties with different stripe rust resistance gene(s) (de Vallavieille-Pope et al. 2012; Johnson 1992; Wellings 2007) (Table 1).

2.2. Stripe rust samples

A survey of stripe rust was conducted during the May 2020 cropping season in five important wheat-producing provinces (Ankara, Eskişehir, Kırkkale, Kırşehir, and Yozgat) and surrounding areas of the Central Anatolia Region of Türkiye. A total of 34 durum (*Triticum durum*) and bread wheat (*Triticum aestivum*) fields were randomly selected and assessed in the Central Anatolia Region of Türkiye. Stripe rust disease was detected in 15 of these fields.

Table 1. Wheat differential lines for the assessment of the virulence phenotype of isolates of *Puccinia striiformis f. sp. tritici*

Virulence inferred	Differential lines	Resistance genes
S	Morocco	Susceptible check
vAvR	Avocet 'R'	A
vAvs	Avocet 'S'	As
v1	Chinese 166	Yr 1
v2	Kalyansona	Yr 2
v3	Vilmorin 23	Yr 3
v4	Hybrid 46	Yr 4
v5	Yr5/6* Avocet 'S'	Yr5, Yr18, AvS
v6	Yr6/6* Avocet 'S'	Yr6, AvS
v7	Yr7/6* Avocet 'S'	Yr7, AvS
v8	Yr8/6* Avocet 'S'	Yr 8
v9	Yr9/6* Avocet 'S'	Yr9
v10	Moro	Yr10
v15	Yr15/6* Avocet 'S'	Yr15, Yr18, AvS
v17	Yr17/6* Avocet 'S'	Yr17, Avs
v24	Yr24/6* Avocet 'S'	Yr24, AvS
v25	TP 981	Yr25, +
v27	OPATA 85	Yr27, Yr18, +
v32	Carstens V	Yr32, Yr25, +
vSp	YrSP/6* Avocet 'S'	YrSp, YrAvS
vSu	Suwon 92/ Omar	YrSu
vND	Nord Desprez	YrND
vSD	Strubes Dickkopf	YrSD
vTre	Tres/6* Avocet 'S'	YrTre
vCham	Cham 1	YrCh1

2.3. Recovery of the stripe rust urediniospores

Collected leaf samples were washed under tap water and then placed on a double layer of wet filter paper in a petri dish, followed by incubation for 2-3 hours at room temperature. After that, the 10-day seedlings of the susceptible cultivar "Morocco" were then rub inoculated using those samples (Roelfs et al. 1992). After 14-15 days, the stripe rust urediniospores that developed in the susceptible cultivar "Morocco" were collected, and these isolates were stored at -80°C until used.

2.4. Race Analysis

Six seeds of each genotypes of the stripe rust differential set (Table 1) were sown individually into a plastic pot (7X7X9 cm) containing a ready-to-use sterilised peat moss and were grown

under greenhouse conditions at $18-20^{\circ}\text{C}$ for 16 hours light and 8 hours of darkness. Stored urediniospores of the stripe rust isolates were suspended in mineral oil (Soltril 170®) and then inoculated into seedling-stage genotypes (Zadoks 11-12). Following the inoculation process, all testing materials were placed in a dew chamber at $9\pm 1^{\circ}\text{C}$ for 24 hours for incubation and then transferred into a greenhouse with a temperature of $15\pm 2^{\circ}\text{C}$. After 15-17 days of inoculation, seedlings were evaluated using a 0-9 scale described by McNeal et al. 1971. Genotypes with infection types (IT) between 0-6 were considered as low infection types and indicated an absence of virulence, while those with 7-9 were considered high infection types and indicated the presence of virulence (McNeal et al. 1971). Symbols designate virulence and avirulence (-), corresponding to stripe rust resistance genes.

3. Results and Discussion

A total of 34 wheat fields were examined, and 15 samples of stripe rust were obtained during the survey. Eight out of 15 stripe rust samples were recovered, and the urediniospores of the samples were multiplied for race identification. Collected stripe rust samples from Kırşehir and Kırıkkale did not recover and did not multiply. For this reason, isolates from these provinces were not obtained. The identified races of eight samples are shown in Table 2.

The core set of the stripe rust differential set, which consisted of 25 differential genotypes carrying *YrAs*, *YrA*, *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr25*, *Yr27*, *Yr32*, *YrSP*, *YrSu*, *YrND*, *YrSD*, and *YrTre* were used to determine the race identification.

Three obtained stripe rust isolates from Yozgat (ID: 1, 3, and 4) were found to be resistant on *Yr2*, *Yr 6*, *Yr 7*, *Yr 8*, *Yr 17*, *Yr 25*, *YrSu*, and *YrND* resistance genes. Only one isolate from Yozgat (ID: 2) was also effective on the *Yr24* resistance gene, in addition to other genes (Table 2). Both races from Eskişehir had virulence on *Yr2*, *Yr6*, *Yr7* and *Yr8* resistance genes. In addition to these genes, isolates 5 and 6 were effective on *Yr9* and *Yr17*, respectively. Only the isolate from Beylikova had a wider virulence phenotype profile, with *Yr10*, *Yr17*, *Yr24*, and *Yr32* resistance genes susceptible to this isolate. One isolate was obtained from Ankara; Haymana had more virulence than all other isolates, and *Yr1*, *Yr 2*, *Yr 3*, *Yr 4*, *Yr 6*, *Yr 7*, *Yr 9*, *Yr 17*, *Yr 25* and *Yr 32* resistance genes were found to be susceptible to this isolate.

Table 2. Virulence phenotypes of stripe rust races identified in Central Anatolia region

ID	Province/district	Virulence phenotype
1	Yozgat /Sekili	AvS,A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,YrSu,YrND,-,-,-
2	Yozgat/Sekili	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,24,25,-,-,-, YrSu,YrND,-,-,-
3	Yozgat/Yerköy	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-, YrSu,YrND,-,-,-
4	Yozgat/ Yerköy	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,YrSP,YrSu,YrND,-,-,-
5	Eskişehir/Sivrihisar	AvS, A,-,2,-,-,-,6,7,8,9,-,-,-,25,-,-,-,YrSu,YrND,-,-,-
6	Eskişehir/Sivrihisar	AvS, A,-,2,-,-,-,6,7,8,-,-,-,17,-,25,-,-,-,YrSu,YrND,-,-,-
7	Eskişehir/Beylikova	AvS ,A,-,2,-,-,-,6,7,8,-,10,-,17,24,-,-,32,-,YrSP,YrSu,YrND,YSD,-,-
8	Ankara/Haymana	AvS,A,1,2,3,4,-,6,7,-,9,-,-,-,17,-,25,-,-,32, YrSP,YrSu,YrND, YrTre,-

4. Conclusion

The first record of stripe rust was made in 1886, afterwards, some epidemics were recorded in Central Anatolia and West Anatolia between 1936 and 1963 (Braun and Saari 1992; İren 1964). Especially, the race coming from East Africa to Türkiye overcomes the *Yr9* resistance gene in the variety called Seri-82, which was cultivated widely in the Çukurova region of Türkiye. The anticipated expense of the damage caused by the stripe rust epidemic in Cukurova is \$7 million (Braun and Saari 1992; Mamluk et al. 1997).

In 1998, it was stated that the wheat-growing area in Central Anatolia, which has a cold and humid environment, incurred losses ranging from 26.5% to 50% as a result of the yellow rust epidemic.

The stripe rust study has been conducted for over 20 years. According to the outcomes of this project between 1995 and 2009, stripe rust has been detected as virulent on *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, and *Yr25* resistance genes (Çat et al. 2017). In 2010, a new race acquired virulence to the *Yr27* resistance gene (Hovmöller 2012), and it has been projected that the loss in wheat production due to stripe rust was 10 million dollars in the same year (Beddow et al. 2015). A new race known as the *Warrior* was identified and was thought to have originated from the northwest region of the country (virulent on *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, and *YrSp*) (Mert et al. 2016). For the last four years, PstS11, PstS13, and PstS14 race groups have been detected (Hovmöller et al. 2020), as well as races such as *Yr10* and *Yr24* virulent pathogens (Hovmöller et al. 2020).

Türkiye has one of the largest wheat cultivation areas in Central Anatolia. The varieties widely grown in this area have been found to be mostly susceptible to stripe rust, especially *Warrior* race, according to the seedling and adult plant resistance tests done under artificial inoculations (Mert et al. 2016). Utilisation of the resistance genes in new wheat varieties developed by breeding programmes plays a very important role in decreasing the spread of rusts in wheat-growing areas (Ellis et al. 2014). Therefore, cultivation of susceptible varieties in the region might enable the dispersion of rust /races.

In addition to host susceptibility, climatic changes may promote the emergence of new races of *Puccinia striiformis*. Some wheat rusts have genetic mechanisms that adapt to the changing climate (Ali et al. 2014), and geographic distributions related to global warming may play a significant role in these mechanisms. For example, two races of *P. striiformis* that are thought to have originated from the Middle East or East Africa and spread to North America, Europe, and Australia are highly adapted to temperature variations (Ali et al. 2014). It is projected that this disease will increase by 5-20% in Southern Spain and Italy by 2030 (Bregaglio et al. 2013). These adaptations of wheat pathogens to climate change are related to genetic differentiations (Zhan and McDonal 2011) rather than phenotypic plasticity (i.e., the ability of genotypes to produce phenotypes under various environmental conditions) (Fusco and Minelli 2010). Climatic changes are favoured by airborne and polycyclic pathogens such as rust or Septoria (i.e., pathogens that have multiple infection cycles in a season (West et al. 2012) because they help them spread and complete their life cycles in a shorter time, resulting in large populations. Due to these large populations, the interaction between individuals is likely to increase, which may enable them to evolve rapidly. Consequently, new races or race groups will appear (Chakraborty 2013; Fones et al. 2017).

Türkiye has one of the largest wheat cultivation areas in Central Anatolia. It is significant to identify the rust races in that region since stripe rust is the most common and devastating disease in the area. It is thought that these outcomes will help to enlighten the breeding programmes and also provide guidance for the wheat producers in Central Anatolia.

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