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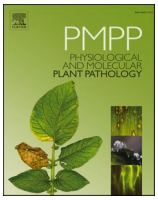

Evaluation of resistance of Turkish bread wheat (*Triticum aestivum*) varieties to recently emerged *Puccinia striiformis* f. sp. *tritici* races

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ABSTRACT

Using genetic diversity has made significant contribution to stripe rust resistance to improve wheat production. However, rapid evolution of the *Puccinia striiformis* f. sp. *tritici* (*Pst*), and emergence of virulent races can negatively affect the wheat genotypes with race-specific resistance gene(s). In this study, reactions of 130 bread wheat varieties, released from 1931 to 2014, were evaluated to recently emerged *Pst* races in Turkey, *PSTr-6* and *PSTr-23*, at seedling and adult-plant stages. 65.4% and 67.7% of wheat varieties showed susceptible reaction to *PSTr-6* and *PSTr-23* at seedling stage, respectively. Moreover, coefficient of infection (CI) values generated by infection type (IT) and disease severity (DS) data demonstrated that *PSTr-23* (59.78) was more virulent than *PSTr-6* (57.93) at adult-plant stage. In addition to these, the presence of important yellow rust (*Yr*) genes in these varieties was investigated at molecular level. It was determined that the frequencies of three *Yr* genes, *Yr5*, *Yr10* and *Yr15,* among these varieties were 1.5, 6.2 and 3.8%, respectively. However, none of them had *Yr36* and only one variety had *Yr5*+*Yr10* combination with frequency of 0.7%. In conclusion, most varieties have not these *Yr* genes and possess a moderately resistance/susceptible reaction to both races in general.

1. Introduction

Wheat (*Triticum* L.) is a staple crop as a source of energy in human nutrition among the cereals with 766 million tons production and 215.9 million ha acreage due to its wide range adaptation to each climatic condition [1]. However, abiotic and biotic stresses, including pathogens like fungi, viruses, and bacteria, decrease wheat yield and quality. Among the fungal diseases of wheat, stripe rust caused by an obligate pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) is considered a major devastating disease. It has emerged quickly due to its long-distance dissemination with wind and human factors [2,3] and threatens 88% of wheat production worldwide [4]. Yield losses due to stripe rust epidemics are estimated to be 5 million tons worldwide [4–6].

Pst is considered the most destructive biotic factor threatens to wheat production especially in coastal and humid areas of Turkey. Although many stripe rust epidemics occurred in past time in Turkey, they were not well documented. The most devastating epidemic of the stripe rust in Turkey was recorded in 1991 and caused yield losses up to 62.5%. The bread wheat variety Seri-82, extensively cultivated in the coastal areas of the country, with *Yr9* resistance gene was discontinued and removed from national variety list of Turkey due to the breakdown its resistance [7,8]. After this, many large and small *Pst* epidemics were reported in Turkey in 1996, 2000 and 2010 with the monetary losses of estimated 568 million, 53 million and 10 million US dollars, respectively [9]. Chen [10] also reported that the epidemics caused by *Pst* races have occurred in 2 out of every 5 years in over 25% of the wheat growing areas of some countries including Turkey.

In general, *Pst* is controlled using fungicides worldwide. However, the most economic and environmentally approach to control *Pst* is use of genetically resistant varieties. One-hundred thirty-one resistance genes, including 84 designated and 47 temporarily, have been reported until now [11]. Among them, the resistance genes *Yr5*, *Yr10* and *Yr15* have also been reported as mostly resistant against to almost all *Pst* races [12,13]. Especially, *Yr5* and *Yr15* have been still known resistant to *Pst* races globally [14] when some isolates have been virulent to varieties containing *Yr10* gene [15,16]. In addition, a high-temperature adult-plant resistance gene *Yr36* has been known as promising to prevent devastating impact of *Pst* races in recent days as global climate change has

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taken effect [17–19]. To develop varieties with durable resistance to *Pst*, donor genotypes with these resistance genes have been commonly used in marker-assisted backcrossing or pyramiding studies [20–22].

It was first determined that the most prevalent races were 2E16, 70E16, 6E16, 46E13, 46E15, 14E16, 70E0, 6E0, 86E16, 2E0 and 6E16 between 1970 and 1991 in Turkey [23,24]. In the years after that, a few studies presented that the genetic lineages PstS2 and PstS3 were common in Turkey and its surrounding countries [25]. In another study, 38 *Pst* races were determined of which 25 ones have newly identified and classified into four genetic lineages in coastal areas of Turkey by Cat et al. [16]. In particular, the newly emerged races *PSTr-6* and *PSTr-23* from the lineage 2 were distinguished as virulent to the resistance genes *Yr1* and *Yr10*, which provide resistance to the common *Pst* races in Turkey, respectively [16]. Although many resistance genes such as *Yr1, Yr2, Yr5, Yr6, Yr7, Yr9, Yr10, Yr15, Yr24* and *Yr26* provided resistance to *Pst* populations in the early 2000s [26,27], the most of them has rendered ineffective to the *Pst* races except for *Yr15* in recent years [16, 28].

Regional or national stripe rust epidemics caused by *Pst* races have frequently occurred in Turkey as mentioned above. However, the genetic background of bread wheat varieties has not been evaluated whether they carry important resistance gene(s) or not. Additionally, the reactions of the bread wheat varieties to these new *Pst* races (*PSTr-6* and *PSTr-23*) have not also been recorded. Therefore, the objectives of this study were (a) determine reactions of 130 bread wheat varieties to the recently emerged *Pst* races at seedling and adult-plant stages and (b) to molecularly characterize them for the important resistance genes such as *Yr5*, *Yr10*, *Yr15* and *Yr36*.

2. Materials and methods

2.1. Plant materials

A total of 130 Turkish bread wheat (*Triticum aestivum* L.) varieties released from 1931 to 2014 was used in this study (Table S1). Additionally, the wheat variety "Morocco" known as universal susceptible was used as a control in studies at both seedling and adult-plant stages. Avocet(S) and its near-isogenic lines (NILs) carrying each related *Yr* gene (AvsYr5, AvsYr10, and AvsYr15) [29] in the Avocet background were also used to confirm the obtained data at molecular studies.

2.2. Seedling evaluation

Recently determined *Pst* races '*PSTr-6*' and '*PSTr-23*' [16] were used in artificial inoculation study to evaluate the reactions of bread wheat varieties. Virulence/avirulence formulae of both races were given in Table 1. The fresh urediniospores of each race were multiplied on plants of susceptible bread wheat variety "Morocco". Seedling tests were carried out under controlled greenhouse conditions as described by Chen et al. [30]. Ten seeds of each variety were sown in pots (7 × 7 × 10 cm) with mixture of soil and peat in 1:1 ratio. The Morocco seedlings at two-leaf stage were inoculated with a suspension containing 10 mg urediniospores of each race and 5 mL NovecTM 7100 engineered fluid (3 M Company) using airbrush spray gun connected to a vacuum pump from 10 to 15 cm distance top by rotating the tray [31]. Inoculated seedlings were incubated in a dew chamber in darkness at 10 °C for 24 h. After incubation, the trays were transferred to a temperature-controlled greenhouse condition. Infection types (ITs) were recorded 15–19 days post inoculation (dpi) according to a scale described by McNeal et al. [32]. The virulence testing was repeated twice to confirm the accuracy of the variety resistance to the *Pst* races and the highest IT values observed were used for statistical analyses.

Table 1
Virulence (V) and avirulence (A) formulae of two *Pst* races (*PSTr-6* and *PSTr-23*) on NILs in an Avocet genetic background.

Race name	V, virulent; or A, avirulent
PSTr-6	V: *Yr1, Yr6, Yr7, Yr9, Yr17, Yr32, YrSp* A: *Yr5, Yr8, Yr10, Yr15, Yr24, Yr27, Yr43, Yr44, YrTr1, YrExp2, YrTye*
PSTr-23	V: *Yr6, Yr7, Yr8, Yr9, Yr10, Yr24* A: *Yr1, Yr5, Yr15, Yr17, Yr27, Yr32, Yr43, Yr44, YrSp, YrTr1, YrExp2, YrTye*

2.3. Adult-plant stage evaluation

To evaluate adult-plant resistance of the varieties to these races, two different field experiments were conducted using artificial inoculation at a research field located in the Kırsehir against the *PSTr-6* in 2018 cropping season and at a research field located in Antalya against the *PSTr-23* in 2019 cropping season. The seeds of each variety were sown in two rows with 100 cm long, and the highly susceptible bread wheat variety "Morocco" was also planted as spreader in two rows for every 10 rows and around the plots to increase disease pressure. These experiments were conducted using randomized complete block design with three replications and standard cultural practices were applied.

The Morocco plants were inoculated thrice from the mid of booting to the mid of heading using a suspension of urediniospores with same ratio applied at the seedling stage. Additionally, the both trials were sprinkler-irrigated to guarantee a moist environment suitable for highly pathogen development. The top three leaves of each variety were visually scored thrice at late booting (Z45), heading (Z55) and dough stages (Z65) [33] as Morocco plants reached to 70% infection at least. Infected leaf area was evaluated using a Modified-Cobb scale described by Peterson et al. [34], and infection types were also assessed by Roelfs et al. [35]. The highest IT and DS values observed at dough stage (Z65) among the replicates were used to calculate the coefficient of infection (CI).

2.4. Extraction of genomic DNA

Genomic DNAs were extracted from fresh leaves of the seedlings at two-leaf stage for each variety using NucleoSpin® Plant II Extraction Kit (Macherey-Nagel, France) following the manufacturer's instructions. The quality and concentration of extracted DNA were checked by 1% agarose gel electrophoresis with a DNA standard and then they were diluted with Tris-EDTA (TE) buffer to final concentration of 50 ng/μL for polymerase chain reaction analyses and stored at −20 °C until use.

2.5. Molecular detection of Yr genes

The genomic DNAs were genotyped using different molecular markers linked to the resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr36* to detect the presence of these genes in these varieties. Information about the diagnostic markers were given in Table S2. The total volume of PCR reaction mixture was 20 μL containing 50 ng DNA template, 1X PCR buffer (Thermo Fisher Scientific, USA), 1.5 mM $MgCI_2$ (Thermo Fisher Scientific, USA), 0.2 mM of dNTPs (Thermo Fisher Scientific, USA), 1 μM forward primer, 1 μM reverse primer, 1 U Taq DNA polymerase (Thermo Fisher Scientific, USA). Amplifications were performed in a thermal cycler (T100, Bio-Rad, USA) under the following conditions except for *Yr5_insertion* primer pair: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 45–64 °C (Table S2) for 30 s, and extension at 72 °C for 1 min, and a final extension of 10 min at 72 °C. A touch-down program (10 cycles, −0.5 °C per cycle starting from 67 °C and the remaining 25 cycles at 62 °C) was used to amplify with *Yr5_insertion* primer pair. In addition, products amplified with *STS7/8* primer pair were digested with *Dpn*II restriction enzyme. Restriction mixture was 20 μL containing 1X buffer, 1 U *Dpn*II, 5 μL PCR product and ddH_2O water, and each product was incubated at 37 °C for 2h in thermo-shaker (Biosan, Latvia).

PCR products were loaded into 2% agarose gel, and visualization of

the gels was performed under UV light in a gel imaging system (UVsolo touch, Analytik Jena, Germany) after staining with ethidium bromide. To separate resistance and susceptible allele with 4 base differences, amplified products with *Xuhw89* for *Yr36* resistance gene were also analyzed in fully automated capillary electrophoresis (QIAxcel Advanced, Qiagen, USA), and obtained raw data were analyzed using QIAxcel ScreenGel Software (version 1.6).

2.6. Data analysis

All data were recorded in Microsoft Office Excel for statistical analyses. For phenotypical data, descriptive statistics such as mean, minimum-maximum, coefficient of variation (CV), standard deviation (SD), kurtosis and skewness were calculated using Minitab 20 statistical software (Minitab Inc., USA). Additionally, coefficient of infection (CI) was calculated for each variety using the highest IT and DS data for evaluation at adult-plant stage as described by Roelfs et al. [35].

To determine contribution of each *Yr* gene or gene combination in different genetic backgrounds, the data was divided into two groups as presence or absence of a gene. One-way analysis of variance (ANOVA) was performed at 95% significance level to reveal difference between both groups.

3. Results

3.1. Resistance evaluation at seedling and adult-plant stages

Reactions of all varieties against the races *PSTr-6* at seedling stage were given at Table S3. Based on infection types (ITs), among 130 bread wheat varieties, only 11 were resistant (ITs 0–3) and 34 were moderately resistant (ITs 4–6), accounting for 8.5% and 26.1% of the varieties, respectively. Unlike these, 85 varieties showed susceptible reaction (ITs 7–9) with the frequency of 65.4%. In parallel to *PSTr-6*, the varieties showed reactions with the same ratios to *PSTr-23* at seedling stage. While 11 (8.5%) were resistant and 31 (23.8%) were moderately resistant, 88 (67.7%) were susceptible. The Sivas 111/33, P 8–6, Kıraç 66, Dağdaş 94, Türkmen, Ziyabey 98, Çetinel 2000, Karatopak, Carisma and Yunus were the prominent varieties with resistant reactions to both races at seedling stage (Table S3).

On the other hand, these varieties were artificially inoculated with *PSTr-6* at adult-plant stage at Kırşehir in 2018 and reactions of them were observed when coefficient of infection of susceptible control reached 70 (70S) infection at least. Coefficient of variation (CV) was calculated as 24.80% and 26.11% for infection type (IT) and disease severity (DS) values recorded, respectively (Table 2). Skewness (−1.09) and kurtosis (0.20) values also confirmed normal distribution for both IT and DS among these varieties (Table 2). In total, while only one bread wheat variety (Sivas 111/33) showed resistance (R) reaction ranging from 0 to 10%, 26 varieties had moderately resistance (MR) and moderately resistance-moderately susceptible (MR-MS) reactions ranging from 20 to 60% (Table 3). In addition, 40 had moderately susceptible (MS) reactions ranging from 40 to 80% and 63 showed a high level of susceptible (S) ranging from 60 to 100%. While 55 out of the varieties were high level susceptible at both seedling and adult-plant stages, only one variety was resistant at both stages (Table 3).

Table 3
Adult-plant reactions of the bread wheat varieties and their combining evaluations at seedling and adult plant stages to *PSTr-6* and *PSTr-23*.

Severity	Host reaction	*PSTr-6*	*PSTr-23*	Note
		No. of varieties		
0–10	R	1	2	Seedling and adult-plant resistant
Total R		1	2	
20–60	MR	7	3	Seedling and adult-plant resistant
		4	1	Seedling moderate and adult-plant resistant
		3	1	Seedling susceptible and adult-plant resistant
40–60	MR-MS	0	3	Seedling and adult-plant resistant
		9	4	Seedling moderate and adult-plant resistant
		3	5	Seedling susceptible and adult-plant resistant
Total MR to MR-MS		26	17	
40–80	MS	3	3	Seedling resistant and adult-plant moderately susceptible
		13	16	Seedling moderate and adult-plant moderately susceptible
		24	32	Seedling susceptible and adult-plant moderately susceptible
Total MS		40	51	
60–100	S	0	0	Seedling resistant and adult-plant susceptible
		8	7	Seedling moderate and adult-plant susceptible
		55	53	Seedling and adult-plant susceptible
Total S		63	60	

Moreover, coefficient of infection (CI) was calculated using the data obtained from infection type and percentage of leaf areas infected by *PSTr-6* (Table S3). The eleven varieties (Sivas 111/33, Dağdaş 94, Çetinel 2000, Carisma, Süzen 97, Ziyabey 98, Atlı-2002, Yunus, Kınacı-97, Prostor and Karatopak) had the lowest CI values against the race *PSTr-6* compared to CI values of other varieties.

These varieties showed a wide variation for reactions to the race *PSTr-23* (Table 3). Additionally, CV values for IT and DS were calculated as 20.95% and 25.22%, respectively and skewness and kurtosis values illustrated that there was abnormal distribution for IT (Table 2). While two varieties gave resistance (R) reaction ranging from 0 to 10%, 17 were moderately resistance (MR) and moderately resistance-moderately susceptible (MR-MS) reactions ranging from 20 to 60% (Table 3). 51 varieties showed a moderately susceptible (MS) reaction ranging from 40 to 80% and 60 varieties showed a high level of susceptible (S) ranging from 60 to 100%. Moreover, 53 of them gave susceptible reaction at both stages (Table 3). The four varieties (Sivas 111/33, Çetinel 2000, Dağdaş 94, and Ziyabey 98) had the lowest CI values against the race *PSTr-23* compared to CI values of other varieties. Average CI to *PSTr-23* (59.78) was higher compared to average CI to *PSTr-6* (57.93) and Sivas 111/33 was the most resistant variety to both races in general.

Breeding progress for resistance to both races was also estimated based on release year of varieties according to obtained phenotypic data

Table 2
Infection type (IT), disease severity (DS), coefficient of infection (CI) and trial statistics of the Turkish bread wheat cultivars to both *Pst* races.

Race	Location	Year	Trait	Mean	Min	Max	CV	SD	Kurtosis	Skewness
PSTr-6	Kırşehir	2018	IT	0.83	0.20	1.00	24.80	0.21	0.20	−1.09
			DS	66.00	10.00	100.00	26.11	17.24	0.69	−0.77
			CI	57.93	2.00	100.00	41.59	24.09	−0.73	−0.46
PSTr-23	Antalya	2019	IT	0.85	0.20	1.00	20.95	0.18	2.08	−1.38
			DS	67.54	0.00	100.00	25.22	17.03	1.56	−0.57
			CI	59.78	0.00	100.00	38.49	23.01	−0.63	−0.21

(Fig. S1). CI values against to both races were negatively correlated with the year of release but these correlations were not statistically significant. Based on the slope of linear regression, the annual rate of CI decreased with −0.08 for *PSTr-6* and -0.18 for *PSTr-23*.

3.2. Molecular detection of stripe rust resistance genes

Linked markers for *Yr5*, *Yr10*, *Yr15* and *Yr36* used to detect the resistance genes in 130 varieties were also tested using Avocet S and their corresponding near-isogenic lines (NILs) in the Avocet background to confirm the obtained data. Especially in detection of the most important resistance gene *Yr5*, two different markers (Table S2) were used to reach the concrete data. Only two varieties (1.5%) had the resistance gene *Yr5* based on amplification results (Fig. S2; Fig. S3) with both the markers *STS-7/STS-8* and *Yr5_insertion* (Table 4). Moreover, eight varieties (6.2%) had *Yr10* (Fig. S4) while five (3.8%) had *Yr15* with heterozygous (Fig. S5), but *Yr36* was not detected in all tested varieties (Table 4; Table S3). On the other hand, only the variety Sivas 111/33 (0.8%) among all tested varieties had *Yr5/Yr10* combination (Table 4; Table S3).

The contribution of the each *Yr* gene for resistance to both *Pst* races was also interpreted based on the ITs. All *Yr* genes and gene combination (*Yr5*+*Yr10*) provided significant resistance against to both races at seedling stage (Fig. 1). The bread wheat varieties carrying *Yr5* gene had significantly lower ITs in compared to those without the *Yr5* for adult plant stage to both races as shown in Fig. 1. Additionally, only one variety (Sivas 111/33) carrying the combination *Yr5*+*Yr10* had significantly ($p < 0.05$) the lowest IT than that of remain varieties.

4. Discussion

In this study, 130 bread wheat varieties released from 1931 to 2014 in Turkey were screened for resistance to recently emerged *Pst* races (*PSTr-6* and *PSTr-23*) at seedling, and adult-plant stages and then molecular markers were used to detect the presence of the major stripe rust resistance genes (*Yr5*, *Yr10*, *Yr15* and *Yr36*) in these varieties. Among the different control strategies to wheat stripe rust, using resistant varieties is the most effective and environmentally friendly approach. It is known that over 80 resistance (*Yr*) genes against *Pst* races have been characterized in wheat genome, so far [11]. However, some characterized genes providing race-specific resistance such as *Yr1*, *Yr9*, and *Yr32* have been rendered ineffective due to constant and frequent evolution of *Pst* [16,36]. Considering the *Pst* races in the current study, it has been demonstrated that the race *PSTr-6* is virulent to *Yr1*, and the race *PSTr-23* is virulent to *Yr10* [16,37].

The important resistance genes *Yr5*, *Yr10* and *Yr15* have effectively sustained their resistance to stripe rust for many years worldwide. *Yr5* and *Yr15* provide all-stage resistance [38,39] while *Yr10* is known as seedling resistance gene [40] and *Yr36* as high-temperature adult-plant (HTAP) resistance gene [17]. Until now, there has been no scientific report especially for a race virulent to either *Yr15* or *Yr36*. However, some virulent races to *Yr5* [28,41] and *Yr10* [15,16,42] have been reported in recent years.

In this study, 2 (1.5%), 8 (6.2%) and 5 (3.8%) among 130 bread wheat varieties were determined with *Yr5*, *Yr10* and *Yr15*, respectively. However, *Yr36* was not detected in all tested varieties (Table 4). On the other hand, the variety Sivas 111/33 postulated *Yr5/Yr10* gene combination (Table S3). Molecular marker-assisted detection has been effectively used to determine the genotypes carrying the related *Yr*-gene(s) by many research groups. Yuan et al. [18] reported that 13% of 485 Chinese varieties had *Yr10* resistance while none of them had *Yr36*. It was also reported that only two varieties among 494 wheat entries have the *Yr5* gene in another study conducted by Zeng et al. [12] in China. Huang et al. [43] also determined that none of 53 Hungarian winter wheat varieties had *Yr5*, *Yr10*, *Yr15*, *Yr17* and *Yr36*. Unlike these studies, Zheng et al. [13] conducted a comprehensive study in China using 672 wheat accessions consisting of breeding lines, landraces, varieties, introduced germplasms and other accessions from all over the world, and they determined that 13.47%, 21.09%, 3.69% and 0.18% of all accessions in average had the resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr36*, respectively. Tahir et al. [44] also reported that 12 (15.0%), 4 (5.0%) and 15 (18.8%) among 80 Pakistani wheat landraces had *Yr5*, *Yr10* and *Yr15* resistance genes, respectively. In addition to our findings, frequency of these resistance genes was generally lower among wheat varieties as also demonstrated by different studies above. However, the frequency of resistance gene(s) can be higher in landraces as stated by Zheng et al. [13] and Tahir et al. [44].

To understand whether a wheat variety is resistant only in adult-plant stage or in all-stages against to certain *Pst* races, studies about stripe rust resistance are carried out at two stages, seedling and adult-plant stage, in general. In the current study, the majority of the varieties (65.4% and 67.7%) was found to be susceptible/highly susceptible against the race *PSTr-6* and *PSTr-23* at seedling stage, respectively (Table S3). Li et al. [45] reported that 81 (80.4%), 78 (67.8%) and 65 (56.5%) among 115 wheat varieties showed resistance to intermediate resistance at seedling stage against the *Pst* races *CYR32*, *CYR33* and *V26* in China, respectively. In another study, Yang et al. [46] reported that 56 (48.3%) and 66 (56.9%) among 116 Chinese wheat materials showed completely resistant reaction to the races *CYR32* and *CYR33*, respectively. Tahir et al. [44] reported that 46 (57.5%) among 80 Pakistani wheat landraces were found to be resistant at seedling stage to the common *Pst* race 574232 in Pakistan. ITs were higher in average in this study compared to these studies mentioned above, and 65.4% and 67.7% of wheat varieties showed susceptible reaction to *PSTr-6 and PSTr-23*, respectively.

At field testing, when only one variety (Sivas 111/33) showed resistance reaction ranging from 0 to 10% against to *PSTr-6*, two (Sivas 111/33 and Ziyabey 98) gave resistance reaction to *PSTr-23* (Table 3). At the same time, the combined results of seedling and adult-plant stages illustrated that these varieties possess a high level of high-level resistance to both races among 130 bread wheat varieties (Table 3). On the other hand, according to coefficient of infection (CI) values, Çetinel 2000, Dağdaş 94, and Atlı-2002 were other prominent varieties for resistant to both *Pst* races (Table S3). Of these varieties, Yunus, Çetinel 2000 and Kıraç 66 had *Yr10* gene (Table S3). The contribution of the *Yr* gene(s) was also investigated based on ITs. All *Yr* genes or gene combination (*Yr5*+*Yr10*) significantly contributed the resistance to both races compared to those without each *Yr* gene or gene combination (Fig. 1). A similar approach was applied by Zheng et al. [13] and they also reported that there was significant difference in terms of IT between presence and absence of *Yr15* gene against to mixed *Pst* spores of the common races in China.

The resistance status of a wheat germplasm against *Pst* races may differ in theory and practice. Although the presence of major resistance genes increases the resistance of a germplasm experimentally, examples about a successful use of single major gene or gene combination are rare

Table 4
Number of varieties carrying either each *Yr* gene or gene combinations according to molecular detection.

No. of varieties	Resistance gene			
	Yr5	Yr10	Yr15	Yr36
1	+[a]	–	–	–
7	–	+	–	–
5	–	–	±[b]	–
1	+	+	–	–
116	–	–	–	–
Total	2	8	5	0

[a] Presence (+) or absence (−) of the resistance genes based on the obtained molecular data.

[b] +/− showed heterozygous according to corresponding marker *Xuhw89* alleles.

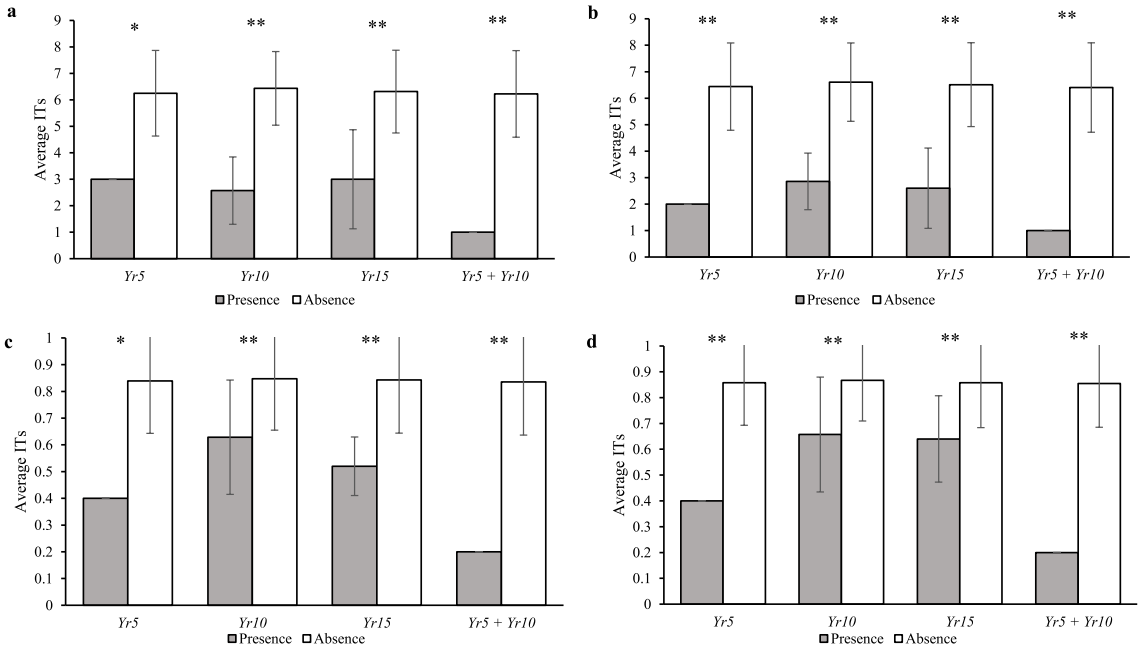

Fig. 1. Contribution of either each *Yr* gene or gene combination for resistance to *PSTr-6* **(a)** and *PSTr-23* **(b)** at seedling stage and *PSTr-6* **(c)** and *PSTr-23* **(d)** at adult-plant stage. Each bar represents the average ITs of the varieties with presence or absence of *Yr* gene(s). *$p < 0.05$, **$p < 0.01$.

in practice [47]. In the current study, it was also determined that some wheat varieties (P 8–6, Kıraç 66, Türkmen, etc.) with major *Yr* gene(s) showed susceptible reaction to the *Pst* races especially at adult-plant stage. In addition, some varieties without the major *Yr* gene(s) examined in this study were moderately resistant. The first reason for this can be explained by the fact that the resistance genes are not expressed equally in all germplasm [47]. The second reason is that the possible effects of the newly identified *Pst* races used in this study on these varieties cannot be fully estimated. The set in which the virulence formulae of *PSTr-6* and *PSTr-23* were also determined [16] is currently the most advanced differential set used for race identification in *Pst* [29] in the world; however, only 18 resistance genes can be detected even with this set. Therefore, these races may also be virulent on different resistance genes. On the other hand, it is known that there are over 130 resistance genes identified in wheat genome for *Pst* [11] and most of them having not still been detected in diverse wheat germplasms. The wheat varieties used in this study may have other minor or major resistance genes. There are several studies that show partial or all-stage resistance to wheat stripe rust can be achieved as a result of the interaction of minor or major genes [13,48,49]. Therefore, it is considered that the phenotypical differences especially at adult-plant stage between resistant varieties without *Yr5, Yr10, Yr15* genes and susceptible varieties carrying resistance gene(s), may have resulted from these reasons.

5. Conclusion

The existence of these *Pst* races in Turkey has been recently reported by Ref. [16], and it was claimed that the frequency of *Yr10*-virulent races reached up to 25% in coastal areas of Turkey. In addition to these, a new *Pst* race virulent to *Yr5* was identified in 2021 [28]. All of these findings have showed that there has been a change in the population structure of *Pst* in Turkey in recent years. In general, it can be concluded that *PSTr-23* was more virulent than *PSTr-6* since average CI to *PSTr-23* (59.78) was higher compared to that of CI to *PSTr-6* (57.93). However, it was clear that any major *Yr* genes (*Yr5*, *Yr10* and *Yr15*) examined in this study significantly provided resistance to both races. Especially *Yr5* and *Yr15* have been intensely used to develop new varieties resistant to stripe rust in many breeding programs in the world. Durable resistant varieties can be developed if these genes are pyramided together or with other effective *Yr*-genes. In addition to these efforts, reaction analyses against to new virulent *Pst* races and molecular characterization studies as in this study should be routinely carried out to find possible resistant sources.

Author contributions

Mehmet Tekin: Conceptualization, Formal analysis, Investigation, Software, Validation, Writing-original draft, Writing-review & editing. Ahmet Cat: Investigation, Validation, Writing-original draft. Kadir Akan: Investigation, Resources, Validation. Hanife Demir: Investigation. Taner Akar: Conceptualization, Supervision, Writing-review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2022.101928.

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