



Evaluation of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in newly developed wheat elite lines in the Moghan Plain, Iran

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Abstract

Objective: Yellow rust, or stripe rust, caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most significant diseases affecting wheat crops worldwide, including Iran. The most fundamental method for controlling this disease involves the use of effective and durable genetic resistance.

Methods: To determine the virulence factors (genes)/resistance factors (genes) present in the pathogen's race population in the Moghan region, Ardabil Province, Iran, the response of a set of yellow rust differential varieties was evaluated over two consecutive growing seasons (2023 and 2024) in trap nurseries at the adult plant stage under natural field conditions (without artificial inoculation). Resistance evaluation of newly developed wheat lines (33 elite lines) was conducted at both the seedling stage (under artificial inoculation) and the adult plant stage (under natural field conditions). The resistance response of elite lines at the seedling stage was assessed using a randomized complete block design with three replications. Disease severity at the flag leaf emergence stage was assessed after uniform disease development was observed on the susceptible check cultivar Bolani, by estimating the percentage of leaf area covered by infection (0-100%). Infection type was scored based on a 0-9 scale. To calculate the coefficient of infection, data on disease severity and infection type were combined. The coefficient of infection was obtained by multiplying the disease severity by a constant corresponding to the host reaction type: Immune = 0.0, Resistant (R) = 0.2, Moderately Resistant (MR) = 0.4, Moderate (M) = 0.6, Moderately Susceptible (MS) = 0.8, Moderately to Susceptible (MSS) = 0.9, Susceptible (S) = 1.0.

Results: The results revealed that the virulence patterns within the pathogen's race population in the Moghan region varied between the two years. In both years, avirulence was observed for the differential varieties carrying resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr27*, *YrCV*, *YrND*, and *YrSD*. A significant difference in virulence patterns between the two *P. striiformis* pathogen populations was observed across the two experimental years for the resistance genes *Yr1*, *Yr9*, *Yr27*, *Yr28*, *Yr29*, *Yr32*, and *YrSP*. Different combinations of the aforementioned resistance genes could be beneficial for

pyramiding effective resistance genes into high-yielding and desirable wheat lines. Based on the measured resistance components at both stages, the following elite lines exhibited acceptable levels of resistance: N-1400-9, N-1400-10, S-1400-2, S-1400-26, S-1400-30, S-1400-38, M-1400-5, M-1400-11, M-1400-19, MDH-1400-6, MDH-1400-10, CD-1400-8, CD-1400-19, C-1400-11, and D-1400-14.

Conclusion: The lines with acceptable levels of resistance can serve as effective sources of resistance for developing new and durable resistant varieties against the pathogen's race population in the Moghan region and similar areas in the northern part of Iran in wheat breeding programs.

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Introduction

Wheat stripe rust, caused by the fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), can rapidly spread and cause significant yield and quality losses in susceptible wheat cultivars under favorable environmental conditions, such as high relative humidity and optimal temperatures between 15-18 °C. This disease has the potential to destroy up to 100% of the wheat yield in highly susceptible varieties (Roelfs *et al.* 1992; McIntosh *et al.* 1995). Due to its widespread distribution and destructive nature, stripe rust is considered one of the most important and devastating diseases of wheat worldwide. To date, various races of *P. striiformis* have been reported from more than 60 countries (Chen 2020). Global annual yield losses due to stripe rust are estimated to exceed 7 million tons, with an economic impact surpassing one billion US dollars. On average, this disease causes a 10-25% reduction in global wheat production annually, while in some years with optimal environmental conditions, losses can exceed 50% (Savary *et al.* 2019; Chen 2020; Bouvet *et al.* 2022; Liu *et al.* 2022; Zhang *et al.* 2024). In China, over the past 70 years, wheat stripe rust has caused an estimated loss of about 14 million tons of wheat (Liu *et al.* 2022; Zhao and Kang 2023). In Iran, the level of damage caused by this disease varies depending on the wheat cultivars' performance and their resistance levels, with reports ranging from 5% to 60% (Dadrezai *et al.* 2018).

The deployment of genetic resistance, encompassing the identification of novel, effective resistance genes, their introgression into elite wheat cultivars, and their pyramiding within adapted germplasm to generate durably resistant varieties, constitutes the most reliable, economically viable, and environmentally sustainable approach for managing rust diseases, including stripe (yellow) rust.

The development of durable genetic resistance requires continuous and systematic surveillance and characterization of pathogen races across diverse geographical regions. Rust pathogens exhibit a wide array of physiological races with distinct virulence spectra and possess the evolutionary capacity to generate novel races through both sexual and asexual recombination. As a result, they can rapidly overcome resistance genes deployed in previously resistant genotypes, potentially leading to widespread epidemics (McCallum *et al.* 2016).

Several factors contribute to the high genetic variability (physiological race diversity) observed in stripe rust. These include the fungus's extraordinary reproductive capacity, rapid adaptation through genetic changes due to the presence of alternate hosts, sexual reproduction, and subsequent genetic recombination, as well as non-sexual recombination, particularly under favorable climatic conditions. Additionally, phenomena such as mutation, migration, and selection pressure imposed by host resistance genes further enhance genetic diversity. The emergence of new races with completely different virulence patterns allows them to overcome previously effective genetic resistances in resistant wheat cultivars. These new races can also be transmitted from one region to another, increasing race diversity in other areas (Chen 2005; Wellings 2011; Park and Wellings 2012; Hovmøller *et al.* 2022; Liu *et al.* 2022). Collectively, these factors present serious challenges in the control of wheat stripe rust. Preventing the outbreak of stripe rust requires an integrated and continuous management strategy, beyond the use of resistant wheat varieties alone. Integrated approaches include the application of systemic and suitable fungicides, proper agronomic management, and reducing or eliminating primary (e.g., crop residues from the previous season) and secondary sources of inoculum through crop rotation (Chen 2005; Carmona *et al.* 2020; Brandt *et al.* 2021). Research and planning aimed at improving wheat resistance and optimizing agronomic practices can help mitigate the negative impacts of this disease. Today, more than 280 resistance genes against rust diseases have been identified across different genetic levels of wheat, many of which originate from wild relatives of wheat. Among these, approximately 105 genes confer resistance specifically to stripe rust, including around 86 seedling resistance genes that have been utilized in wheat breeding programs worldwide to develop resistance against this pathogen (Hovmøller *et al.* 2022; McIntosh *et al.* 2022; Zhu *et al.* 2023).

To design an effective wheat breeding program aimed at developing durable resistance to rust diseases in specific geographical regions, it is essential to conduct regular monitoring of virulence factors (genes), understand the genetic structure of pathogen populations, and stay informed about recent genetic changes in the pathogen population to better comprehend epidemic patterns in the area. Investigating trends in virulence pattern changes within the local pathogen population and assessing

the effectiveness of resistance genes through testing reactions on international differential wheat lines in trap nursery plots enables researchers to gather crucial data. These insights serve as a roadmap for the development of sustainable resistant wheat varieties (Park *et al.* 2011; Awais *et al.* 2022).

Numerous studies have been conducted globally to determine the virulence patterns of stripe rust races on resistance genes and to identify new sources of resistance. According to surveys, resistance genes such as *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr20*, *Yr25*, *Yr28*, *Yr29*, *Yr31*, and *YrExp2* have become ineffective against most current races of stripe rust worldwide. In contrast, *Yr5* and *Yr15* remain among the most effective resistance genes against a wide range of *P. striiformis* f. sp. *tritici* (*Pst*) races. Other genes, including *Yr1*, *Yr4*, *Yr10*, *Yr17*, *Yr18*, *Yr24*, *Yr26*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *Yr64*, *Yr65*, *Yr76*, *Yr84*, *YrCV*, *YrSD*, *YrSP*, *YrSU*, *YrND*, and *YrTr1*, have lost their previous efficacy and are now effective only against a limited number of *P. striiformis* races (Omrani *et al.* 2013; Omrani *et al.* 2014; Afzalifar *et al.* 2021; Chen *et al.* 2021; Safavi 2021; Jia *et al.* 2022; Liu *et al.* 2022; Chen and Liu 2023; Gulyaeva and Shaydayuk 2023; Kabiri *et al.* 2024; Liu *et al.* 2024; Zhang *et al.* 2024). In an experiment, the resistance response of 48 wheat genotypes, along with the susceptible check cultivar ‘Bolani’, was evaluated at both seedling and adult-plant growth stages to identify novel sources of resistance against stripe rust races. Field resistance responses of the genotypes were assessed across three locations in Iran (Mashhad, Zarghan, and Ardabil) over two consecutive growing seasons. Seedling resistance responses were evaluated against three distinct stripe rust races: 134E150A+ (*Yr27*+), 38E158A+ (*Yr27*+), and 6E142A+ (*Yr27*+). Overall, 10 genotypes exhibited consistently acceptable levels of resistance across years and locations (Mohammadi *et al.* 2023). In another experiment, the relative resistance response of 23 wheat elite lines, along with the susceptible check cultivar ‘Bolani’, to stripe rust was evaluated over two consecutive years. Seedling-stage resistance responses of the genotypes were assessed against five physiological races of *P. striiformis*: 14E158A+ (*Yr27*+), 142E158A+ (*Yr27*+), 6E134A+ (*Yr27*+), 166E62A+ (*Yr27*+), and 6E142A+ (*Yr27*+). In total, 20 of the evaluated genotypes exhibited consistently acceptable levels of resistance at both the seedling and adult-plant growth stages (Kabiri *et al.* 2024).

Given the widespread occurrence of wheat stripe rust in recent years across many regions of Iran, particularly in the Moghan Plain (a warm and humid region in northern Iran, known as one of the country's major wheat-producing areas), conducting research to characterize the racial population of this pathogen and implement effective management strategies in the region appeared necessary. In this study, the genetic diversity of the pathogen, changes in its virulence patterns, and the effectiveness of wheat resistance genes against the local pathogen population were evaluated through reactions of differential genotypes grown in trap nurseries over two consecutive years (2023 and

2024). Additionally, the resistance responses of some of the most recently developed Iranian wheat cultivars and elite lines were assessed to identify effective resistance sources against the prevalent pathogen races.

Materials and Methods

Field evaluation of adult-plant resistance in elite lines and differential cultivars

The wheat genotypes used in this study included a set of 56 standardized and differential wheat lines carrying known resistance genes to stripe rust, obtained from the International Maize and Wheat Improvement Center (CIMMYT) and the International Center for Agricultural Research in the Dry Areas (ICARDA), along with the susceptible check cultivar Bolani and 32 newly developed wheat elite lines (Table 1). The composition of this set has been largely consistent across numerous studies, with only minor variations reported (Chen *et al.* 2021; Ghanbarnia *et al.* 2021; Cat *et al.* 2023).

A trap nursery trial in which all experimental materials were planted was conducted under natural field infection conditions (without artificial inoculation) at the Agricultural Research Station of Ardabil Province, Iran, located in Moghan Plain - a region known for its warm and humid climate. The station is situated in Parsabad, at coordinates 47°88' E longitude and 39°39' N latitude, with an elevation of 78 meters above sea level. The experiment was carried out over two consecutive growing seasons (2023-2024 and 2024-2025). After preparing the planting bed in early autumn, seeds of each wheat cultivar and differential line, along with elite lines (approximately 200 seeds per experimental entry), were sown in late November during the growing seasons of 2023–2024 and 2024–2025. The seeds were planted on two one-meter rows spaced 25 cm apart, all within a single raised bed. For every 10 experimental entries, the susceptible cultivar *Bolani* was planted along the borders of the plots and on two one-meter rows (single bed) to facilitate better spread and development of stripe rust within the trap nursery. During the growing season, standard agronomic practices, including manual weeding, fertilization, and irrigation according to regional norms in Moghan (five irrigations), were applied consistently.

Disease severity assessments (percentage of leaf area infected, ranging from 0 to 100%) were conducted three times at intervals of six to seven days, starting when disease incidence reached 30% on the susceptible check. Assessments followed the modified Cobb scale as proposed by Peterson *et al.* (1948). Once pathogen activity progressed to flag leaves, infection types (ITs) were recorded based on Roelfs *et al.* (1992), and observations continued from flag leaf emergence until the soft dough stage of grain development (Parlevliet 1979).

Table 1. The list of differential varieties of yellow rust and wheat elite lines.

Differential varieties of yellow rust								
No.	Name/ Pedigree	Yr gene/s	No.	Name/ Pedigree	Yr gene/s	No.	Wheat elite lines	Pedigree
1	Chinese 166	<i>Yr1</i>	19	Kalyansona	<i>Yr2</i>	1	N-1400-9	NADI#1*2/3/MUTUS/AKURI #1.
2	Lee	<i>Yr7</i> , +	20	Trident	<i>Yr15</i>	2	N-1400-10	MUNAL*2//WAXWING*2/TUK URU.
3	Heines Kolben	<i>Yr2</i> , <i>Yr6</i>	21	Yr15/6* Avocet S	<i>Yr25</i>	3	N-1400-16	MERCATO/BECARD//BOKOTA
4	Vilmorin 23	<i>Yr3</i> , +	22	Hugenoot	<i>Yr27</i>	4	NDH-1400-12	KACHU/SAUAL//TIRGAN
5	Moro	<i>Yr10</i> , <i>YrMor</i>	23	Selkirk	<i>Yr9</i>	5	NDH-1400-19	PASTOR/KAUZ/6/CNDO/R143// ENTE/MEXI_2.
6	Strubs Dikkopf	<i>YrSD</i> , <i>Yr25</i> , +	24	Federation *4/Kavkaz		6	NDH-1400-20	PASTOR/KAUZ/6/CNDO/R143// ENTE/MEXI_2.
7	Suwon 92/Omar	<i>YrSu</i> , +	25	Federation	<i>Yr1</i>	7	S-1400-2	TURACO/CHIL//SKAUZ/3/PAST OR/4/Unknown/5/Sirvan
8	Clement	<i>Yr2</i> , <i>Yr9</i> , +	26	Yr1/6*Avocet 'S'	<i>Yr5</i>	8	S-1400-9	BORL14//BECARD/QUAIU #1
9	<i>Triticum spelta</i> var. <i>album</i>	<i>Yr5</i>	27	Yr5/6*Avocet 'S'	<i>Yr6</i>	9	S-1400-11	WBLL1*2/BRAMBLING/4/BAB AX/LR42//BABAX*2/3/SHAMA
10	Hybrid 46	<i>Yr4</i> , +	28	Yr6/6*Avocet 'S'	<i>Yr7</i>	10	S-1400-21	ONIX/KBIRD//BORL14/3/ONIX/ KBIRD
11	Reichersberg 42	<i>Yr7</i> , +	29	Yr7/6*Avocet 'S'	<i>Yr8</i>	11	S-1400-25	MERCATO/BECARD//BOKOTA
12	Heines Peko	<i>Yr2</i> , <i>Yr6</i> , <i>Yr25</i> , +	30	Yr8/6*Avocet 'S'	<i>Yr9</i>	12	S-1400-26	SUP152/BAJ #1/3/KACHU// WBLL1*2/BRAMBLING
13	Nord Desprez	<i>Yr3</i> , <i>YrND</i> , +	31	Yr9/6*Avocet 'S'	<i>Yr10</i>	13	S-1400-27	WBLL4/KUKUNA//WBLL1/3/W BLL1*2/BRAMBLING/4/BECAR D/FRNCLN
14	Compair .	<i>Yr8</i> , <i>Yr19</i>	32	Yr10/6*Avocet 'S'	<i>Yr15</i>	14	S-1400-29	FRAME//MILAN/KAUZ/3/PAST OR/4/SOKOLL/5/KACHU//WBL L1*2/BRAMBLING
15	Carstens V	<i>YrCV</i> , <i>Yr32</i> , <i>Yr25</i> , +	33	Yr15/6* Avocet 'S'	<i>Yr17</i>	15	S-1400-30	PREMIO//PI 610750/PIFED/4/VORB/FISCAL// KACHU/3/WBLL1*2/BRAMBLI NG
16	Spalding Prolific	<i>YrSP</i> , +	34	Yr17/6* Avocet 'S'	<i>Yr18</i>	16	S-1400-33	BORL14*2//BECARD/QUAIU #1
17	Heines VII	<i>Yr2</i> , <i>Yr25</i> , +	35	Yr18/6* Avocet 'S'	<i>Yr24</i>	17	S-1400-36	WBLL1//YANGLING SHAANXI /ESDA/3/ROLF07/4/MUCUY
18	Avocet 'R'	<i>YrA</i>	36	Yr24/6* Avocet 'S'	<i>Yr26</i>	18	S-1400-38	VILLA JUAREZ F2009/3/ T.DICOCCON PI94625/ AE.SQUARROSA(372)//3*PAST OR /4/ WBLL1 *2/BRAMBLING/8/ PSN/BOW//SERI/3/MILAN/4/AT TILA/5/KAUZ*2/CHEN//BCN/3/ MILAN/6/WBLL1*2/SHAMA/7/I WA 8600211//2*PBW343*2/KUKUN A/9/ SUP152/BLOUK #1

Table 1 continued

No.	Differential varieties of yellow rust		No.	Wheat elite lines	Pedigree
	Name/ Pedigree	Yr gene/s			
37	Yr26/6* Avocet 'S'	Yr26	19	S-1400-39	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KAC HU #1/6/TOBA97/PASTOR/3/ T.DICOCCONPI94624/AE.SQUARROSA (409)//BCN/4/BL 1496/ MILAN/3/CROC_1/AE.SQUARROSA (205)// KAUZ/7/ MAYIL/8/ MAYIL
38	Yr27/6* Avocet 'S'	Yr27	20	M-1400-5	KINGBIRD #1//INQALAB 91*2/ TUKURU/3/BECARD/FRNCLN
39	Yr32/6* Avocet 'S'	Yr32	21	M-1400-7	SOKOLL/WBLL1/4/D67.2/PARANA 66.270//AE.SQUARROS (320)/3/CUNNINGHAM
40	YrSP/6* Avocet 'S'	YrSP	22	M-1400-11	PREMIO//PI 610750/PIFED/4/ VORB/FISCAL//KACHU/3/WBLL1*2/BRAMBLING
41	Jupateco73R		23	M-1400-19	KACHU//WBLL1*2/BRAMBLING*2/6/ROLF07*2/5/REH/HA RE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES
42	Jupateco73S		24	MDH-1400-6	Bow"s"/Vee"s"//1-60-3/3/MV 17/4/Pishtaz/5/Parsi/6/SW89.5277/BORL95//SKAUZ/3/PRL/2* PASTOR/4/HEILO
43	Avocet 'R'	YrA	25	MDH-1400-10	Bow"s"/Vee"s"//1-60-3/3/MV 17/4/Pishtaz/5/Parsi/6/SW89.5277/BORL95//SKAUZ/3/PRL/2* PASTOR/4/HEILO
44	Avocet 'S'	Susceptible check	26	C-1400-3	Mihan/3/CMH79A.955/Bow//Pastor
45	Bolani	Susceptible check	27	CD-1400-8	Catbird/Titona//Oroum
46	Fielder	Yr6, Yr20	28	C-1400-9	Mo/4/Nd/WW15//Lee/Fn/3/N.5/Nac/5/2*Yan7578.128//Chil/2*S tar
47	Thatcher	Yr7	29	C-1400-11	Babaga/Fiocco
48	Lemhi	Yr21	30	CD-1400-12	-
49	TP1295	Yr25	31	CD-1400-19	-
50	Yr27/6*Av.S	Yr27	32	D-1400-14	GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/6/TOPDY_18/FOCHA _1//ALTAR 84/3/ AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4
51	Ciano79	Yr27			
52	Opata85	Yr27+Yr18			
53	Avocet Yr28	Yr28			
54	Lalbahador/P avon	Yr29			
55	Avocet- YrA*3/Pastor	Yr31			
56	Pastor	Yr31+APR			
57	Bolani	Susceptible check			

X_1 , X_2 , X_3 = Infection coefficients from the first, second, and third assessments, respectively. The relative AUDPC (rAUDPC) was calculated using the following formula: $rAUDPC = (\text{AUDPC of each line} / \text{AUDPC of the susceptible cultivar}) \times 100$

To calculate the infection coefficient, data on disease severity and infection type were combined. Specifically, the infection coefficient was obtained by multiplying the disease severity value by a constant corresponding to the host reaction: Immune= 0.0; Resistant (R)= 0.2; Moderately Resistant (MR)= 0.4; Moderate (M)= 0.6; Moderately Susceptible (MS)= 0.8; Highly Susceptible but not sporulating (MSS)= 0.9; Susceptible (S)= 1.0 (Stubbs *et al.* 1986). The infection types defined under this scale were as follows:

O: No infection

R: Pustules accompanied or surrounded by necrotic flecks

MR: Small pustules encircled by necrotic lesions

M: Medium to large pustules with chlorosis or necrosis

MS: Intermediate-sized pustules without necrosis, possibly with chlorotic spots

S: Large, clustered pustules without chlorosis or necrosis

The area under the disease progress curve (AUDPC) was calculated according to Milus and Line (1986), using the formula: $AUDPC = (N_1 \times (X_1 + X_2)/2) + (N_2 \times (X_2 + X_3)/2)$

Where: N_1 = Number of days between the first and second assessment

N_2 = Number of days between the second and third assessment

Seedling stage resistance evaluation under greenhouse conditions

Two *P. striiformis* races, obtained from the Cereal Pathology Unit of the Seed and Plant Improvement Institute (SPII), Karaj, Iran, and designated according to the nomenclature system of Johnson *et al.* (1972), were used to evaluate seedling-stage resistance in new wheat elite lines. Seeds were sown in 10-cm-diameter pots containing a peat moss-field soil mixture, germinated under controlled greenhouse conditions (22-25 °C) in SPII's rust greenhouses, and inoculated at Zadoks growth stage 12 (first leaf fully expanded, second leaf emerging) using, for each isolate, a homogenous suspension of freshly prepared urediniospores in light mineral oil (Soltrol 170). Following inoculation, plants were misted with distilled water to provide sufficient moisture for spore germination and penetration into leaf tissues. To prevent cross-contamination among isolates and maintain high humidity, plants were covered with plastic covers and incubated for 24 hours in complete darkness at 10 °C under near-saturated humidity conditions.

After completion of the dark period, plants were transferred to growth chambers maintained at 14-16 °C with a 16-hour photoperiod (minimum light intensity of 12,000 lux) and an 8-hour dark cycle for a 21-day latent period to allow full disease development. Infection types were recorded based on a 0-4 scale (McIntosh *et al.* 1995):

0 (Immune): No symptoms or visible pustules

1 (Hypersensitive response): Necrotic flecks without sporulation

2 (Resistant): Small pustules surrounded by necrotic lesions

3 (Moderately Resistant): Medium-sized pustules mostly associated with necrotic or occasionally chlorotic lesions

4 (Susceptible): Large, clustered pustules without necrosis or chlorosis

5 (Highly Susceptible): Very large, coalesced pustules without necrosis

Infection types ranging from 0 to ≤ 3 were considered low-infection responses (resistance), while types ≥ 3 to 5 were classified as high-infection responses (susceptibility).

Cluster analysis of the standard and differential wheat cultivars, as well as the elite lines, was performed based on measured traits using Ward's method and squared Euclidean distance to group genotypes and determine their genetic relations. The analysis was conducted using IBM SPSS Statistics software version 22.

Results

Reaction of standard and international differential wheat cultivars to stripe rust

Based on the results obtained from the reaction of standard and differential wheat cultivars at the adult plant stage under natural infection conditions (trap nursery) during the 2023-2024 growing season, the *P. striiformis* f. sp. *tritici* (*Pst*) population in the Moghan region exhibited virulence toward genotypes carrying the resistance genes *Yr6*, *Yr7*, *Yr8*, *Yr17*, *Yr18*, *Yr20*, *Yr21*, *Yr26*, *Yr31*, and *YrA* (Table 2). These genotypes displayed a susceptible infection type (S or MSS), with disease severity ranging from 20% to 100%. Therefore, these genes do not provide effective resistance against the prevalent *P. striiformis* races in the Ardabil (Moghan) region, Iran. Avirulence was observed on genotypes carrying the resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr27*, *Yr32*, *YrCV*, *YrND*, *YrSD*, *YrSP*, and *YrSU*. Furthermore, no infection was detected on several complex-genotype differentials as follows:

Clement, carrying *Yr2*, *Yr9*, and possibly an unknown gene (+),

Heines VII, carrying *Yr2*, *Yr25*, and possibly an unknown gene (+),

Heines Peko, carrying *Yr2*, *Yr6*, *Yr25*, and possibly an unknown gene (+),

Compair, carrying *Yr8*, *Yr19*, and possibly an unknown gene (+),

Trident, carrying *Yr17* and possibly an unknown gene (+),

Pastor, carrying *Yr31* and an unknown adult-plant resistance gene (+).

Table 2. Effectiveness of resistance genes of differential varieties on the yellow rust pathogen based on disease severity, infection type, and coefficient of infection in two consecutive years (2023-24 and 2024-25) in the Moghan region, Iran.

No.	Differential varieties	Gene	Final disease severity and infection type		Coefficient of infection	
			Year		Year	
			2023-24	2024-25	2023-24	2024-25
1	Chinese 166	<i>Yr1</i>	20MS	100S	16	100
2	Lee	<i>Yr7</i> , +	20S	70S	20	70
3	Heines Kolben	<i>Yr2</i> , <i>Yr6</i>	10MR	50MS	4	40
4	Vilmorin 23	<i>Yr3</i> , +	10MS	15MR	8	6
5	Moro	<i>Yr10</i> , <i>YrMor</i>	10MR	20MR	4	8
6	Strubs Dikkopf	<i>YrSD</i> , <i>Yr25</i> , +	0	5MR	0	2
7	Suwon 92/Omar	<i>YrSu</i> , +	0	80MSS	0	72
8	Clement	<i>Yr2</i> , <i>Yr9</i> , +	10MR	15MR	4	6
9	<i>Triticum spelta</i> var. <i>album</i>	<i>Yr5</i>	5MR	10MR	2	4
10	Hybrid 46	<i>Yr4</i> , +	0	0	0	0
11	Reichersberg 42	<i>Yr7</i> , +	5MR	15MR	2	6
12	Heines Peko	<i>Yr2</i> , <i>Yr6</i> , <i>Yr25</i> , +	0	15MR	0	6
13	Nord Desprez	<i>Yr3</i> , <i>YrND</i> , +	5MR	20MR	2	8
14	Compair	<i>Yr8</i> , <i>Yr19</i>	0	15MR	0	6
15	Carstens V	<i>YrCV</i> , <i>Yr32</i> , <i>Yr25</i> , +	5MR	30MS	2	24
16	Spalding Prolific	<i>YrSP</i> , +	0	20MS	0	16
17	Heines VII	<i>Yr2</i> , <i>Yr25</i> , +	0	40MR	0	16
18	Avocet 'R'	<i>YrA</i>	70S	100S	70	100
19	Kalyansona	<i>Yr2</i>	10MR	40MS	4	32
20	Trident	<i>Yr17</i> , +	15MR	80MSS	6	72
21	Yr15/6* Avocet S	<i>Yr15</i>	0	5MR	0	2
22	Hugenoot	<i>Yr25</i>	20MS	60MS	16	48
23	Selkirk	<i>Yr27</i>	0	15MR	0	6
24	Federation *4/Kavkaz	<i>Yr9</i>	20MS	80S	16	80
25	Federation		15MS	100S	12	100
26	Yr1/6*Avocet 'S'	<i>Yr1</i>	15MS	100S	12	100
27	Yr5/6*Avocet 'S'	<i>Yr5</i>	5MR	5MR	2	2
28	Yr6/6*Avocet 'S'	<i>Yr6</i>	50S	100S	50	100
29	Yr7/6*Avocet 'S'	<i>Yr7</i>	50S	100S	50	100
30	Yr8/6*Avocet 'S'	<i>Yr8</i>	50S	90S	50	90
31	Yr9/6*Avocet 'S'	<i>Yr9</i>	30MS	100S	24	100
32	Yr10/6*Avocet 'S'	<i>Yr10</i>	0	15MR	0	6
33	Yr15/6* Avocet 'S'	<i>Yr15</i>	0	5MR	0	2
34	Yr17/6* Avocet 'S'	<i>Yr17</i>	30MSS	70MSS	27	63
35	Yr18/6* Avocet 'S'	<i>Yr18</i>	20MSS	70MS	18	56
36	Yr24/6* Avocet 'S'	<i>Yr24</i>	15MS	80MSS	12	72
37	Yr26/6* Avocet 'S'	<i>Yr26</i>	20S	100S	20	100
38	Yr27/6* Avocet 'S'	<i>Yr27</i>	15MR	80S	6	80
39	Yr32/6* Avocet 'S'	<i>Yr32</i>	10MR	100S	4	100
40	YrSP/6* Avocet 'S'	<i>YrSP</i>	5MR	90S	2	90
41	Jupateco73R		5MR	40MS	2	32
42	Jupateco73S		10S	100S	10	100
43	Avocet 'R'	<i>YrA</i>	50S	100S	50	100
44	Avocet 'S'		60S	100S	60	100
45	Bolani (Susceptible check)		70S	100S	70	100
46	Felder	<i>Yr6</i> , <i>Yr20</i>	30S	100S	30	100
47	Thatcher	<i>Yr7</i>	30S	100S	30	100
48	Lemhi	<i>Yr21</i>	40S	100S	40	100
49	TP1295	<i>Yr25</i>	10MR	100S	4	100
50	Yr27/6*Av.S	<i>Yr27</i>	5MR	30MS	2	24
51	Ciano79	<i>Yr27</i>	0	60MS	0	48
52	Opata85	<i>Yr27+Yr18</i>	0	20MS	0	16
53	Avocet Yr28	<i>Yr28</i>	10MS	100S	8	100
54	Lalbahador/Pavon	<i>Yr29</i>	15MS	100S	12	100
55	Avocet-YrA*3/Pastor	<i>Yr31</i>	15S	100S	15	100
56	Pastor	<i>Yr31+APR</i>	15MR	10MR	6	4

Genotypes carrying the resistance genes *Yr9*, *Yr24*, *Yr25*, *Yr28*, and *Yr29* showed a moderately susceptible (MS) reaction to the local pathogen population.

In the following growing season (2024-2025), the *P. striiformis* population in Moghan exhibited virulence toward genotypes carrying the resistance genes *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr18*, *Yr20*, *Yr21*, *Yr26*, *Yr28*, *Yr29*, *Yr31*, *Yr32*, *YrSU*, *YrSP*, and *YrA*. These genotypes also displayed a susceptible infection type (S or MSS), with disease severity ranging from 20% to 100%, indicating that they are ineffective against the current *P. striiformis* races in the Moghan region of Iran. No virulence was observed on genotypes carrying the resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *YrCV*, *YrND*, and *YrSD*. Similarly, no infection was recorded on the aforementioned complex-genotype differentials: Clement, Heines VII, Heines Peko, Compair, and Pastor. Moreover, genotypes carrying the resistance genes *Yr24*, *Yr25*, *Yr27*, *Yr28*, *Yr29*, and *YrSP* exhibited a moderately susceptible (MS) reaction to the *P. striiformis* population in the region. A significant difference in virulence patterns between the two *P. striiformis* pathogen populations was observed across the two experimental years for the resistance genes *Yr1*, *Yr9*, *Yr27*, *Yr28*, *Yr29*, *Yr32*, and *YrSP*.

Cluster analysis grouped the differential genotypes into three major clusters (Figure 1). Group I included genotypes that exhibited a susceptible reaction over both years (10 genotypes, approximately 18% of the differential lines). This group showed the highest values for infection type, disease severity, and infection coefficient. Group II consisted of genotypes showing resistant to moderately resistant reactions across both years (21 genotypes, approximately 37% of the differential lines). These genotypes exhibited the lowest values for infection type, disease severity, and infection coefficient compared to other differential genotypes. Group III included genotypes displaying a moderately susceptible (MS) reaction or race-specific resistance responses over the two years (25 genotypes, approximately 45% of the differential lines).

Reaction of new wheat elite lines to the stripe rust pathogen

Table 3 presents the resistance responses of the most recently developed wheat elite lines against the *P. striiformis* f. sp. tritici (*Pst*) population at both the seedling and adult plant stages. Two *P. striiformis* races were obtained from the Cereal Pathology Unit of the Seed and Plant Improvement Institute, Karaj, Iran, and used for phenotypic screening of seedling-stage resistance in the new wheat lines. The races were designated using the nomenclature system proposed by Johnson *et al.* (1972).

At the seedling stage, all but a few of the wheat elite lines exhibited acceptable levels of resistance to both distinct *P. striiformis* races. Resistance at the adult plant stage was also evaluated and found

to be favorable among the tested lines. Approximately 80% of the studied lines showed resistance to moderately susceptible (MS) reactions.

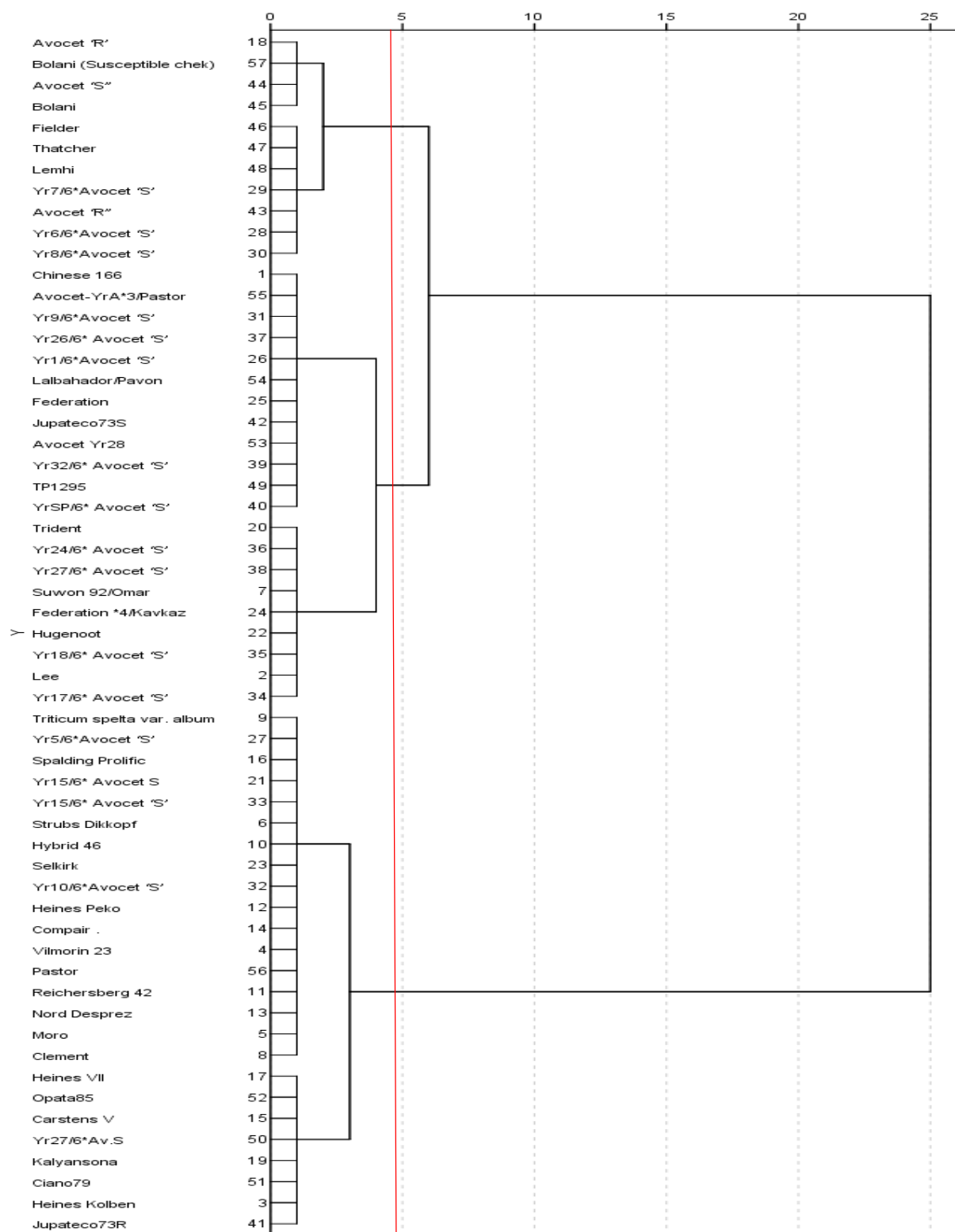


Figure 1. Cluster analysis of differential varieties based on disease severity, infection type, and coefficient of infection of the yellow rust pathogen in two consecutive years (2023-2024 and 2024-2025) in the Moghan region, Iran.

Table 3. Resistance response of new wheat elite lines to yellow rust pathogen at seedling and adult plant stages based on disease severity, infection type, and coefficient of infection.

Code	Elite lines	Reaction at the seedling stage		Final reaction at the adult plant stage		
		Races		Final disease severity and infection type	Coefficient of infection	Relative area under disease progress curve (%)
		6E134A+	174E191A+			
1	N-1400-9	0;	0;	20MR	8	9
2	N-1400-10	0;	0;	10MR	4	5
3	N-1400-16	0;	0;	40MR	16	18
4	NDH-1400-12	0;	0;	40MR	16	18
5	NDH-1400-19	;1CN	;1CN	40MR	16	18
6	NDH-1400-20	2CN	;1CN	20MS	16	18
7	S-1400-2	0;	0;	20MR	8	9
8	S-1400-9	2CN	2CN	60MS	48	54
9	S-1400-11	4	4	90S	90	100
10	S-1400-21	2CN	2CN	80MSS	72	80
11	S-1400-25	2CN	2CN	50MS	40	45
12	S-1400-26	0;	0;	10MR	4	5
13	S-1400-27	4	4	40MR	16	18
14	S-1400-29	0;	0;	40MR	16	18
15	S-1400-30	0;	0;	10MR	4	5
16	S-1400-33	2CN	2CN	80MSS	72	80
17	S-1400-36	2CN	2CN	50MS	40	45
18	S-1400-38	0;	0;	20MR	8	9
19	S-1400-39	2CN	2CN	30MR	12	14
20	M-1400-5	0;C	0;	10MS	8	9
21	M-1400-7	4	4	30MR	12	14
22	M-1400-11	0;	0;	10MS	8	9
23	M-1400-19	0;	0;	15MR	6	7
24	MDH-1400-6	0;	0;	15MR	6	7
25	MDH-1400-10	0;	0;	10MR	4	5
26	C-1400-3	2CN	2CN	50MS	40	45
27	CD-1400-8	0;	0;	20MR	8	9
28	C-1400-9	0;	0;	40MR	16	18
29	C-1400-11	0;CN	0;	15MR	6	7
30	CD-1400-12	4	4	40MR	16	18
31	CD-1400-19	;1CN	;1CN	20MR	8	9
32	D-1400-14	;1CN	0;	10MR	4	5
33	Bolani	4	4	90S	90	100

Cluster analysis classified the wheat elite lines into four major groups based on disease severity, infection type, and infection coefficient (Figure 2). Group I included highly susceptible wheat lines (4 lines or 12.12% of the wheat elite lines), exhibiting the highest values for infection type, disease severity, and infection coefficient. Group II comprised lines with moderately susceptible to low susceptible reactions (4 lines or 12.12%), showing intermediate values lower than Group I but higher than the remaining groups, for infection type, disease severity, and infection coefficient. Group III contained lines displaying a moderately resistant reaction (10 lines or 30.30% of the elite lines). Group IV consisted of lines showing fully resistant reactions (15 lines or 45.45% of the elite lines).

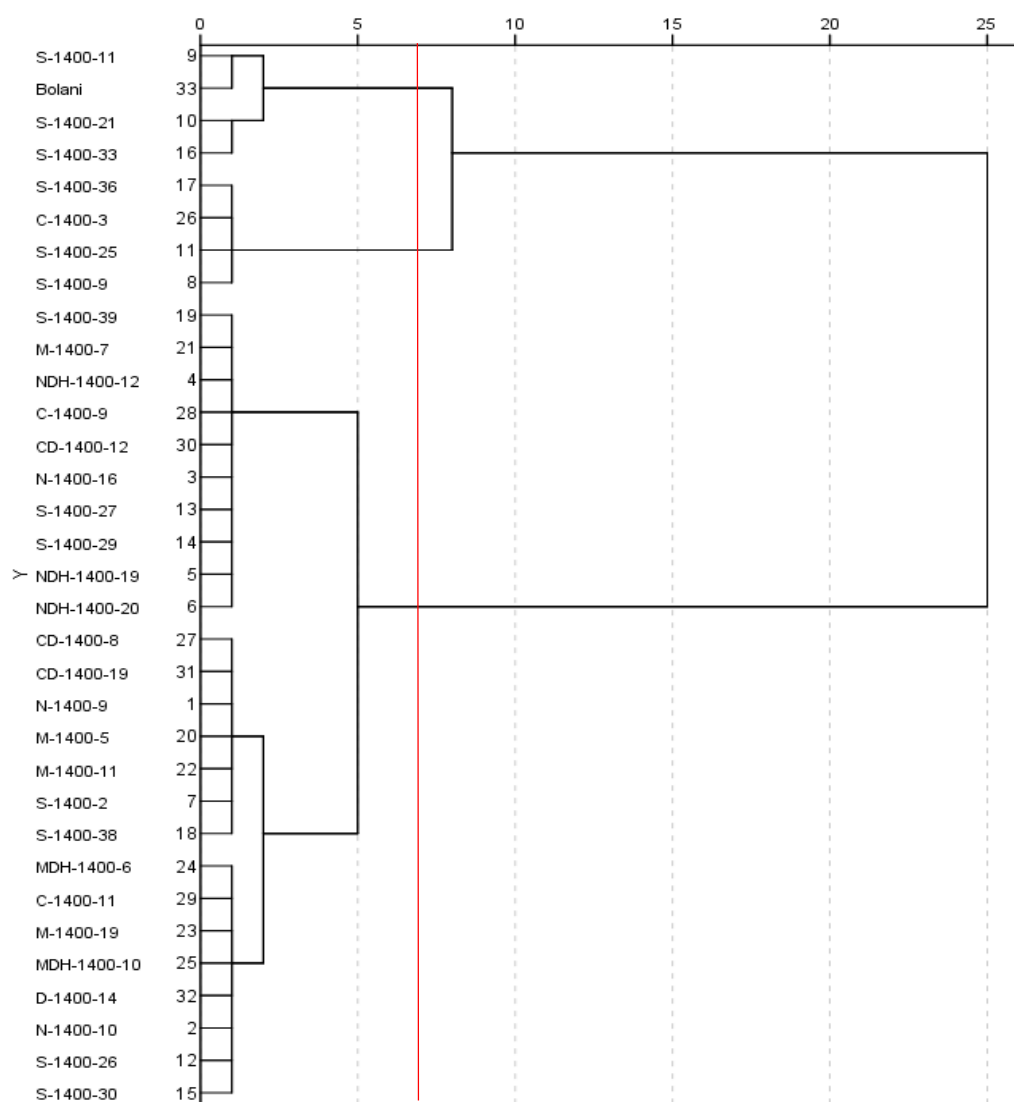


Figure 2. Cluster analysis of wheat elite lines based on disease severity, infection type, and coefficient of infection to the yellow rust pathogen in the Moghan region of Iran.

Discussion

According to several researchers, recent global climate changes, particularly the overall rise in global temperatures, have altered the epidemiology of rust pathogens worldwide, influencing their activity, disease severity, and population dynamics across different geographical latitudes (Xiao *et al.* 2020; Li *et al.* 2022). In recent years, the climatic conditions in Moghan Plain, Iran, particularly temperature and relative humidity, have been highly favorable for stripe rust pathogen activity. Cool temperatures ranging between 7-18 °C from late February to mid-April, high relative humidity, and an average of 7-8 hours of daily sunlight created optimal conditions for disease development, as evidenced by the high disease severity observed on the susceptible check cultivar *Bolani*. One of the primary objectives of cultivating differential wheat lines, including those used for stripe rust resistance studies, is to monitor the effectiveness of known resistance genes and track shifts in the virulence patterns of pathogen races within a specific region (Yang *et al.* 2024). Based on observed changes in local virulence patterns, effective resistance genes must be identified and incorporated into breeding programs targeting regional pathogen populations.

The results from the two-year field trials (2023-2024 and 2024-2025) indicated that standard and differential wheat varieties carrying distinct resistance genes exhibited varying responses to the stripe rust pathogen in Moghan, Iran. This variation suggests that the pathogen populations differed between the two years (Table 2). Long-term trap nursery data further indicated that the efficacy of resistance genes in the set of differential cultivars against the local *P. striiformis* population in Moghan differs from that observed in other regions (The regions of Sari, Gorgan, and Gonbad, belonging to a warm and humid climate). These findings support the notion that *P. striiformis* f. sp. *tritici* populations across different regions of Iran exhibit diverse pathogenicity patterns. Reports confirm the existence of considerable genetic diversity within the dynamic stripe rust pathogen population in Iran (Zhou *et al.* 2025), with numerous studies documenting high levels of genetic variability among *P. striiformis* isolates (Afshari 2013; Omrani *et al.* 2013; Pornamazeh *et al.* 2013; Omrani *et al.* 2017b). Several factors may explain this high level of racial diversity as follows:

Climatic diversity: Iran's wide range of climatic zones makes it one of the historical centers of wheat domestication and an important hub for stripe rust diversity globally.

Presence of alternate hosts: The widespread presence of alternate hosts such as various species of barberry (*Berberis* spp.), which serve as essential components for the sexual reproduction cycle of stripe rust fungi, contributes significantly to genetic recombination and increased pathogen variability. In both experimental years, differential varieties carrying the resistance genes *Yr2*, *Yr3*,

Yr4, *Yr5*, *Yr10*, *Yr15*, *Yr27*, *YrCV*, *YrND*, and *YrSD* showed resistant reaction types (R, MR, or M) with low disease severity. Therefore, these genes appear effective against prevalent *P. striiformis* races in the Moghan Plain. Incorporating these genes into wheat breeding programs is strongly recommended for developing durable resistance in warm and humid agroclimatic zones of Iran.

The differential line carrying *Yr25* also demonstrated relatively acceptable resistance against the local *P. striiformis* population in Ardabil province, Iran, suggesting its potential use in combination with other effective resistance genes in future breeding efforts targeting Moghan and similar regions. Among these, *Yr5*, *Yr10*, and *Yr15* are well-documented seedling resistance genes with broad-spectrum effectiveness against most global *P. striiformis* races (Chen *et al.* 2021; Hovmøller *et al.* 2022).

For sustainable multi-gene resistance breeding, molecular markers linked to these resistance genes, most of which have already been identified, can be effectively combined with classical hybridization techniques (Omrani *et al.* 2017a). Utilizing combinations of effective resistance genes active at different growth stages (race-specific and non-race-specific) was first introduced in the development of durable resistance against wheat stem rust (Singh *et al.* 2011).

One of the adult plant resistance genes widely used in stripe rust resistance breeding programs is *Yr18/Lr34*. Extensive molecular studies have been conducted to trace this gene in various wheat genotypes due to its partial but durable resistance effect (Ghazvini *et al.* 2018; Pouryousefi *et al.* 2022). The wheat elite lines evaluated in this study represent the latest breeding materials developed in national wheat improvement programs. These findings are consistent with previous reports by other researchers (Omrani *et al.* 2013; Omrani *et al.* 2014; Chen *et al.* 2020; Afzalifar *et al.* 2021; Safavi 2021; Jia *et al.* 2022; Gulyaeva and Shaydayuk 2023; Kabiri *et al.* 2024; Omrani *et al.* 2024). These lines were selected based on superior agronomic performance and yield potential in preliminary adaptation trials. Multi-year resistance assessments provide valuable information for breeders to identify and release the most suitable candidates as commercial cultivars. Lines lacking sufficient resistance to stripe rust should be excluded from official release to reduce reliance on costly fungicide applications and improve production efficiency.

Given the differences in pathogen races observed between the two experimental years in Moghan, variability in resistance reactions among the studied elite lines was expected. However, the consistent resistance displayed by several lines across both seedling and adult plant stages indicates that effective resistance genes previously identified in international studies have been successfully integrated into Iranian wheat breeding programs through targeted crosses leading to the development of these advanced lines. In another related study, the resistance response of 24 wheat elite lines to

stripe rust was assessed at both seedling and adult plant stages over two growing seasons (2015-2016). Out of these, six lines exhibited stable resistance across both stages. Eleven lines were resistant at the seedling stage but moderately susceptible at the adult plant stage, while seven were susceptible as seedlings but showed moderate resistance or moderate susceptibility at the adult plant stage (Safavi 2021). Afzalifar *et al.* (2021), in their evaluation of resistance in 297 wheat genotypes against five different *P. striiformis* races at the seedling stage, clustered the genotypes into three main categories: resistant, moderately resistant to moderately susceptible, and highly susceptible. Among them, four genotypes exhibited complete resistance to all five races. Vahed Razavi *et al.* (2023) also conducted a cluster analysis, and based on the overall response of all wheat genotypes to stem rust races, classified them into four categories, resistant, moderately resistant, moderately susceptible, and susceptible at the adult plant stage, whereas at the seedling stage, genotypes were grouped into only two categories: resistant and susceptible.

Conclusion

The results indicated that the set of evaluated standard and differential wheat varieties exhibited diverse resistance responses to the *P. striiformis* population during the two experimental years. This variation suggests that the pathogen populations present in Moghan Plain, Iran, differed between the years.

Wheat elite lines that demonstrated effective resistance against the prevalent *P. striiformis* races in Moghan over both growing seasons can serve as valuable genetic sources for stripe rust resistance in this region and in other areas with similar warm and humid climates. In wheat breeding programs, identifying the resistance genes present in these lines and pyramiding multiple effective resistance genes into high-yielding and agronomically superior genotypes can lead to the development of durably resistant and high-yielding wheat cultivars. By strategically combining race-specific resistance genes identified in this study with non-race-specific resistance genes, it is possible to develop more durable resistance against stripe rust. The use of molecular markers linked to known resistance genes can significantly enhance breeding efficiency by facilitating parental selection for crosses and accelerating the identification of desirable progeny.

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Ethical considerations

The authors avoided data fabrication and falsification.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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