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Emergence of a new virulence pattern in *Puccinia striiformis* f. sp. *tritici* in Borujerd, Iran, and resistance responses of major commercial wheat cultivars

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Abstract

Stripe rust, or yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most widespread and destructive fungal diseases of wheat worldwide, including in Iran. Under favorable climatic conditions and in the presence of susceptible cultivars, the disease can cause severe and irreversible yield losses. The pathogen comprises populations with diverse virulence patterns, which may change over time and threaten the durability of resistance in wheat. To characterize and compare temporal changes in the virulence pattern of the yellow rust population in the important Silakhor Plain of Borujerd, Iran, 43 differential lines from the yellow rust trap nursery carrying known *Yr* resistance genes, together with the susceptible cultivar Bolani and major commercial wheat cultivars of Iran, were evaluated under field conditions during the 2022 and 2023 growing seasons. In both years, yellow rust developed naturally and extensively on the susceptible Bolani cultivar, the commercial cultivars, and the standard and differential wheat lines, and their resistance responses were recorded. The results of the trap nursery in the Silakhor Plain of Borujerd showed that a marked shift in the virulence pattern of the yellow rust population occurred in the 2023 growing season. In particular, virulence to plants carrying *Yr1*, *Yr3*, *Yr8*, *Yr18*, and *Yr32* was detected only in the second growing season, indicating a substantial change in the virulence spectrum of the local population. Virulence was observed in both seasons on plants carrying the *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr20*, *Yr21*, *Yr26*, *Yr27*, *Yr31*, *YrA*, and *YrSU* genes, indicating that these genes were no longer effective and suggesting the emergence and establishment of new races in the Borujerd region. Plants carrying the resistance genes *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *YrSD*, *YrSP*, *YrND*, and *YrCV* remained resistant throughout the two years of the study. The commercial wheat cultivars Taktaz, Kalateh, Tirgan, Raj, Amin, Torabi, Heydari, Taban, Sana, Aran, Hana, and Shabrang all showed acceptable levels of resistance to the yellow rust population in Silakhor Plain in both growing seasons and are therefore proposed as valuable resistance sources. It is likely that the effective resistance genes in the resistant cultivars are present either singly or in combination.

Keywords: Puccinia striiformis f. sp. tritici, resistance genes, trap nursery, virulence

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ظهور الگوی جدید از فاکتورهای پرآزاری عامل بیماری زنگ زرد گندم (*Puccinia striiformis* f. sp. *tritici*) در منطقه بروجرد و واکنش مقاومت ارقام تجاری مهم گندم

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چکیده

زنگ زرد با عامل *Puccinia striiformis* f. sp. *tritici* یکی از شایع‌ترین و خسارت‌زاترین بیماری‌های قارچی (انگل اجباری) گندم در جهان از جمله ایران می‌باشد. در صورت فراهم بودن شرایط آب و هوایی برای توسعه فعالیت عامل بیماری و همچنین وجود ارقام حساس در یک منطقه، خسارت جبران ناپذیری به محصول گندم وارد می‌شود. عامل بیماری مذکور شامل جمعیت‌هایی با الگوهای پرآزاری متفاوت است که ممکن است در طول زمان تغییر کنند و دوام مقاومت در گندم را تهدید نمایند. به‌منظور ردیابی و پایش بیماری در منطقه مهم دشت سیلاخور بروجرد (یکی از کانون‌های مهم آلودگی زنگ زرد در کشور) مجموعه ۴۳ تایی ارقام افتراقی مرتبط با خزانه تله زنگ زرد که دارای ژن/های شناخته شده هستند همراه با رقم حساس بولانی و ارقام تجاری مهم گندم کشور در طی دو سال زراعی ۱۴۰۱ و ۱۴۰۲ کشت شدند. در طی هر دو سال بیماری زنگ زرد به شکل طبیعی و به‌طور گسترده بر روی رقم حساس بولانی، ارقام تجاری و لاین‌های استاندارد و افتراقی زنگ زرد گندم توسعه یافت که اقدام به یادداشت برداری واکنش مقاومت آن‌ها گردید. نتایج خزانه تله در دشت سیلاخور بروجرد در طی دو سال نشان داد، در سال زراعی ۱۴۰۲ تغییرات الگوی پرآزاری با شدت بالاتر در جمعیت بیمارگر زنگ زرد اتفاق افتاده است. پرآزاری برای گیاهان حامل ژن/های *Yr1*، *Yr3*، *Yr8*، *Yr18* و *Yr32* تنها در سال زراعی ۱۴۰۲ دیده شد. برای گیاهان حامل ژن/های *Yr2*، *Yr6*، *Yr7*، *Yr9*، *Yr17*، *Yr20*، *Yr21*، *Yr26*، *Yr27*، *Yr31*، *YrA* و *YrSU* در هر دو سال آزمایش پرآزاری مشاهده شد که حاکی از غیرموثر بودن ژن‌های فوق می‌باشد. گیاهان حامل ژن‌های مقاومت *Yr4*، *Yr5*، *Yr10*، *Yr15*، *Yr24*، *YrSD*، *YrSP*، *YrND* و *YrCV* در طی دو سال اجرای این تحقیق مقاوم بودند. ارقام تجاری گندم شامل تکتاز، کلاته، تیرگان، راج، امین، ترابی، حیدری، تابان، ثنا، آران، هانا و شبرنگ همگی دارای مقاومت قابل قبول نسبت به جمعیت بیمارگر زنگ زرد در هر دو سال زراعی بودند که به‌عنوان منابع مقاومت، معرفی می‌شوند. به احتمال زیاد ژن‌های مقاومت مؤثر نام برده در ارقام مقاوم به صورت تکی یا ترکیبی وجود دارد.

کلید واژه‌ها: پرآزاری، خزانه تله، ژن‌های مقاومت، Puccinia striiformis f. sp. tritici

دبیر تخصصی: دکتر موسی نجفی‌نیا

Introduction

Stripe rust, or yellow rust, caused by the fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (Pst), is one of the most important, widespread, and destructive diseases of wheat worldwide, including in Iran. Studies have suggested that the disease originated in the region south of the area between the Black Sea and the Caspian Sea, from where it spread to all parts of the world (McIntosh et al., 1995; Chen, 2005).

Pst populations from different parts of the world show a highly dynamic structure and very high genetic diversity. Numerous physiological races with markedly different virulence patterns have been reported. Intra-population variation is greater than inter-population variation, indicating rapid changes within the pathogen. This genetic flexibility and variability arise through sexual and asexual reproduction as well as mutation, leading to the emergence of new races with virulence patterns completely different from those of previous races (Sharma-Poudyal et al., 2020). Environmental and geographic factors can also play major roles in both disease development and the formation of pathogen race structure, allowing gene exchange among populations from different regions (Chen, 2020; Wang et al., 2021).

The rapid emergence of new pathogen races, as recent studies have shown, can overcome existing effective genetic resistance and serve as an initial source of disease spread even in distant regions (Wellings, 2011). Under favorable environmental conditions and in the presence of a susceptible host, yield losses of more than 70- 80% have been reported for this disease. Wheat yellow rust causes global annual losses of up to 50 million tons (Yang et al., 2024; Riella et al., 2024). This clearly highlights the importance of continuous monitoring of pathogen populations.

In Iran, wheat yellow rust epidemics are usually severe under cool spring conditions (Torabi et al., 2001). Reports have shown that yield losses in some years have been very high. The pathogen population causing yellow rust shows differences in virulence across regions and years (Nazari & Torabi, 2001).

The use of resistant cultivars is the most important and reliable method for controlling rust diseases, including yellow rust, worldwide. Although chemical control is possible, its high cost and the need for specialized equipment such as aircraft, along with environmental pollution concerns, limit its application (Stubbs, 1985; Wellings, 2011). However, the development of resistant wheat cultivars requires precise information on the genetic structure of pathogen populations. The ability of breeders to develop sustainable and effective control strategies against crop diseases, especially wheat yellow rust, depends largely on the pathogen population structure and its potential to adapt to new cultivars, so that the impact of resistance can be assessed or predicted (Dawana et al., 2024).

According to the latest reports, more than 85 officially designated resistance genes (*Yr1* to *Yr86/Yr87*), hundreds of QTLs, and genetic loci associated with yellow rust resistance have been identified in the wheat genome. Among the known genes, the all-stage resistance (seedling resistance) genes include *Yr1, Yr2, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr25, Yr27,* and *Yr32*. These genes usually confer strong race-specific resistance, but in many cases their durability is limited because of the pathogen's high capacity for mutation and the emergence of new races, leading to widespread breakdown of resistance. In contrast, adult plant resistance (APR) genes, which generally confer non-race-specific, gradual, and more durable resistance, include genes such as *Yr11, Yr12, Yr13, Yr14, Yr16,*

Yr18, *Yr29*, *Yr30*, *Yr34*, *Yr36*, *Yr45*, *Yr46*, *Yr62*, *Yr68*, and *Yr84*. These genes are usually not detectable at the seedling stage and express their effects at later growth stages; especially when combined with seedling resistance genes, they play an important role in improving the durability and stability of resistance. In addition to officially named genes, a considerable number of temporary or uncharacterized resistance genes, alleles, and loci have also been identified in different sources of cultivated and wild wheat, and research on their genetic nature and effectiveness is still ongoing. This reflects the complexity of the genetic structure of yellow rust resistance in wheat and the need for continuous monitoring of pathogen populations and the use of diverse genetic resources in breeding programs to achieve durable resistance (Jamil et al., 2020; Yan et al., 2023; Jan et al., 2026).

For the first time, Zadoks (1961) used a trap nursery approach to study the virulence genetics of the wheat yellow rust pathogen. In this system, in addition to evaluating seedling resistance genes, adult plant resistance genes can also be assessed. As a field-based tool, trap nurseries make it possible to indirectly reflect the race and virulence diversity of pathogen populations under natural conditions. In one trap nursery study on yellow rust, differentials were evaluated in Algeria, Australia, Canada, Chile, China, Hungary, Kenya, Nepal, Pakistan, Russia, Spain, Türkiye, and Uzbekistan. Virulence was reported on resistance genes *Yr31*, *Yr28*, *Yr25*, *Yr19*, *Yr17*, *Yr9*, *Yr8*, *Yr7*, *Yr6*, *Yr2*, *Fielder (Yr6*, *Yr20)*, *Lemhi (Yr21)*, *YrA*, *Druchamp (Yr3a*, *YrD*, *YrDru)*, *YrPr1*, *YrPr2*, *YrTye*, *YrExp2*, *YrUkn*, *YrTr1*, *YrTr2*, *YrPa1*, *Stephens (Yr3a*, *YrS*, *YrSte)*, *Clement (Yr9*, *YrCle)*, *YrPa2*, and *Express (YrExp1*, *YrExp2)* (Sharma-Poudyal et al., 2013).

The annual changes in virulence factors of the wheat yellow rust pathogen have been studied almost continuously since the establishment of the Cereal Pathology Unit in 1993 at the Research Institute of Plant Improvement and Seed and Seedling Production in Karaj. In field trap nursery studies on yellow rust, virulence was observed on genotypes *Heines Kolben* (with *Yr2* and *Yr6*), *Kalyansona* (*Yr2*), *Lee* (*Yr7*), *Avocet R* (*YrA*), *Federation 4/Kavkaz* (*Yr9*), and *TP1295* (*Yr25*). Under field conditions, plants carrying *Yr1*, *Yr3V*, *Yr3N*, *Yr4*, *Yr5*, *Yr8*, *Yr10*, *Yr18*, *Yr24*, *Yr27*, *Yr32+*, *YrND*, *YrSP*, *YrSD*, *YrCV*, and *YrSU* remained resistant to yellow rust. Commercial cultivars including Sirvan, Pishgam, Parsi, Morvarid, Gonbad, Urum, Mihan, Zare, Niknejad, Narin, Bahrang, Shabrang, Heydari, Rakhshan, Mehrgan, Shoush, Barat, Dana, Pishtaz, Gaspard, Gaskogen, and MV17 showed good resistance in all surveyed regions and across the studied years (Afshari et al., 2013).

Dalvand et al. reported in a two-year study of yellow rust virulence factors in the Dezful region that virulence was observed on plants carrying *Yr6*, *Yr7*, *Yr9*, *Yr27*, and *YrA*, whereas no virulence was observed on plants carrying *Yr1*, *Yr3*, *Yr4*, *Yr5*, *Yr8*, *Yr10*, *Yr15*, *YrCV*, *YrND*, *YrSU*, *YrSP*, and *YrSD* (Dalvand et al., 2013).

Given the high prevalence of yellow rust in recent years in neighboring countries and in several regions of Iran, including Borujerd, the Silakhor Plain of Borujerd is of great importance from a disease pathology perspective, especially for rusts and particularly yellow rust. It is considered one of the main pathways for the movement of rust races and one of the earliest important infection hotspots for rusts, especially yellow rust, in Iran. Despite numerous studies, up-to-date, region-specific information on the virulence factors of Pst under natural field conditions appears to be limited, especially information related to the response of APR genes and the resistance of newly released

commercial cultivars to yellow rust. Therefore, to investigate the genetic structure and possible changes in the pathogen, monitor virulence factors of the causal fungus using standard and differential wheat yellow rust lines, and identify effective resistance sources in major commercial wheat cultivars under trap nursery conditions in the field over two consecutive years in the Silakhor Plain of Borujerd, this study was conducted.

Materials and Methods

The plant materials used in this study consisted of 43 standard and differential wheat lines carrying one or more well-characterized *Yr* resistance genes for yellow rust, together with the complementary Avocet near-isogenic lines and the susceptible check cultivar Bolani, all obtained from CIMMYT and ICARDA. In addition, 38 recently released commercial wheat cultivars, representing important and widely cultivated genotypes in different agroecological zones of Iran, were included in the study (Table 1). The differential set used here is largely consistent with those employed in most previous studies, with only minor modifications in some cases (Omrani et al., 2024, 2025; Cat et al., 2023; Ghanbarnia et al., 2021).

Field trials were conducted under natural infection conditions, without artificial inoculation, in the Silakhor Plain of Borujerd, Lorestan Province, western Iran, one of the major wheat-growing areas of the country. The experiments were carried out at the research farm of the Lorestan Agricultural and Natural Resources Research and Education Center (33°31′ N, 48°35′ E; 1,600 m above sea level) over two consecutive growing seasons (2021- 2022 and 2022-2023). After land preparation in early autumn, the entries were sown in late November of each season. For each genotype, 10 g of seed, corresponding to approximately 200 seeds, was planted in two 1-m rows spaced 25 cm apart. In order to promote disease development and ensure effective spore dispersal, the susceptible cultivar Bolani was planted after every ten entries and also around the entire border of the experiment. Standard crop management practices, including manual weeding, fertilization, and irrigation, were followed according to local conditions, and irrigation was applied six times during each growing season. Yellow rust developed naturally in the nursery during both seasons. Disease severity was recorded visually using the modified Peterson scale (Peterson et al., 1948), which estimates the proportion of leaf area affected by Uredinia on a scale from 0 to 100%. Assessments began when disease severity reached approximately 30% on the susceptible check, Bolani. Thereafter, disease evaluations were made three times at 6 to 7 day intervals to monitor disease progress.

Infection type was recorded when the disease had reached the flag-leaf stage, following the classification system described by Roelfs et al. (1992). Observations continued from flag-leaf emergence to the soft dough stage, and the highest disease reaction recorded for each genotype was used for subsequent analyses (Parlevliet, 1979). Infection responses were classified using the standard scale as follows: 0, immune, with no visible symptoms; R, resistant, with necrotic flecks and/or very small Uredinia; MR, moderately resistant, with small Uredinia surrounded by necrosis; M, intermediate, with small to medium Uredinia associated with chlorosis and/or necrosis; MS, moderately susceptible, with medium-sized Uredinia, limited chlorosis, and no obvious necrosis; and S, susceptible, with large Uredinia and no chlorosis or necrosis (Fig.1). To combine disease severity and host reaction into a single quantitative measure, the infection coefficient (CI) was calculated according to Stubbs et al. (1986) as follows: CI = disease severity × host-response

constant. The host-response constants were assigned as follows: immune = 0.0, R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, MSS = 0.9, and S = 1.0. The infection coefficient was used as the main criterion for comparing resistance levels among the differential lines and commercial cultivars. For statistical analysis, the standard and differential lines, together with the commercial cultivars, were grouped according to disease severity, infection type, and infection coefficient using hierarchical cluster analysis. Clustering was performed with the Ward method based on squared Euclidean distance to determine the relationships among genotypes and to classify them into resistance groups. The analyses were carried out using SPSS version 22.

Table 1. List of standard and differential wheat yellow rust lines and important commercial wheat cultivars studied

Differential varieties of yellow rust						No.	Cultivar
No.	**Name/ Pedigree**	***Yr* gene/s**	**No.**	**Name/ Pedigree**	***Yr* gene/s**		
1	Chinese 166	*Yr1*	29	Yr27/6* Avocet 'S'	*Yr27*	12	Khalil
2	Lee	*Yr7, +*	30	Yr32/6* Avocet 'S'	*Yr32*	13	Barat
3	Heines Kolben	*Yr2, Yr6*	31	YrSP/6* Avocet 'S'	*YrSP*	14	Shoush
4	Vilmorin 23	*Yr3, +*	32	Avocet 'R"	*YrA*	15	Mehregan
5	Moro	*Yr10, YrMor*	33	Avocet 'S"	*Susceptible check*	16	Shavour
6	Strubs Dikkopf	*YrSD, Yr25, +*	34	Fielder	*Yr6, Yr20*	17	Chamran2
7	Suwon 92/Omar	*YrSu, +*	35	Thatcher	*Yr7*	18	Ayneh
8	Clement	*Yr2, Yr9, +*	36	Lemhi	*Yr21*	19	Raj
9	*Triticum spelta var. album*	*Yr5*	37	TP1295	*Yr25*	20	Amin
10	Hybrid 46	*Yr4, +*	38	Yr27/6*Av.S	*Yr27*	21	Farin
11	Reichersberg 42	*Yr7, +*	39	Opata85	*Yr27+Yr18*	22	Torabi
12	Heines Peko	*Yr2,Yr6, Yr25, +*	40	Avocet Yr28	*Yr28*	23	Talaei
13	Nord Desprez	*Yr3, YrND, +*	41	Lalbahador/Pavon	*Yr29*	24	Rakhshan
14	Compair .	*Yr8, Yr19*	42	Avocet-YrA*3/Pastor	*Yr31*	25	Baharan
15	Carstens V	*YrCV, Yr32, Yr25, +*	43	Pastor	*Yr31+APR*	26	Sirvan
16	Spalding Prolific	*YrSP, +*	44	Bolani	*Susceptible check*	27	Danesh
17	Heines VII	*Yr2, Yr25, +*		**Cultivar**		28	Heyran
18	Kalyansona	*Yr2*	1	Taktaz		29	Zarrineh
19	Trident	*Yr17+*	2	Araz		30	Heydari
20	Yr15/6* Avocet S	*Yr15*	3	Arman		31	Barzgar
21	Hugenoot	*Yr25*	4	Meraj		32	Narin
22	Selkirk	*Yr27*	5	Kalateh		33	Ofogh
23	Federation *4/Kavkaz	*Yr9*	6	Tirgan		34	Taban
24	Federation		7	Ehsan		35	Sana
25	Yr17/6* Avocet 'S'	*Yr17*	8	Gonbad		36	Aran
26	Yr18/6* Avocet 'S'	*Yr18*	9	Sahar		37	Hana
27	Yr24/6* Avocet 'S'	*Yr24*	10	Setareh		38	Shabrang
28	Yr26/6* Avocet 'S'	*Yr26*	11	Sarang		39	Bolani

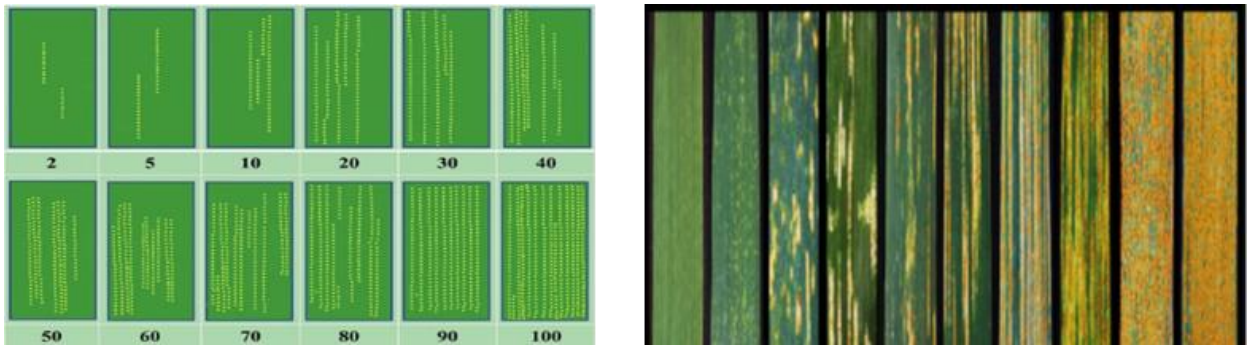


Figure 1. Infection type scale of stripe rust on adult-plant leaves and scale of rust severity (percent of leaf area infected) Source: Chen, 2020

Results

Reaction of Standard and Differential Wheat Lines to *Puccinia striiformis* f. sp. *tritici*

Hierarchical cluster analysis based on disease severity, infection type, and infection coefficient successfully classified the standard and differential wheat lines into three distinct groups over the two consecutive growing seasons (2021-2022 and 2022-2023) (Figure.2).

To provide an integrated overview of disease responses, the coefficient of infection (CI) values of the 44 wheat differential lines were visualized using a clustered heatmap combined with hierarchical clustering (Figure.1). The hierarchical clustering grouped differential lines according to the similarity of their disease reactions and clearly distinguished resistant, intermediate, and susceptible response patterns.

The first major cluster comprised differential lines that consistently exhibited susceptible reactions throughout both growing seasons, indicating that the corresponding resistance genes were ineffective against the prevailing yellow rust population in the Silakhor Plain of Borujerd. In contrast, another cluster included differential lines carrying effective resistance genes, particularly *Yr15*, *Yr5*, *Yr10*, *Yr24*, and *YrSP*, which consistently exhibited low coefficients of infection and remained highly effective against the current pathogen population. A third group contained differential lines with intermediate disease responses, reflecting differences in pathogen virulence between the two growing seasons and suggesting partial erosion of resistance in several race-specific genes.

Comparison of the two growing seasons revealed a general shift toward higher CI values during the 2022-2023 season. Several differential lines that displayed resistant or moderately resistant reactions in the first year exhibited increased disease severity in the second year, indicating an expansion of the virulence spectrum within the local pathogen population.

The clusters were clearly separated according to disease severity, infection type, and coefficient of infection. Differential lines carrying effective resistance genes consistently exhibited the lowest values for all three disease parameters, whereas susceptible lines showed the highest levels of disease development (Figure.1). These findings demonstrate substantial variation in host responses among the differential lines and highlight the contrasting effectiveness of individual *Yr* resistance genes under natural epidemic conditions.

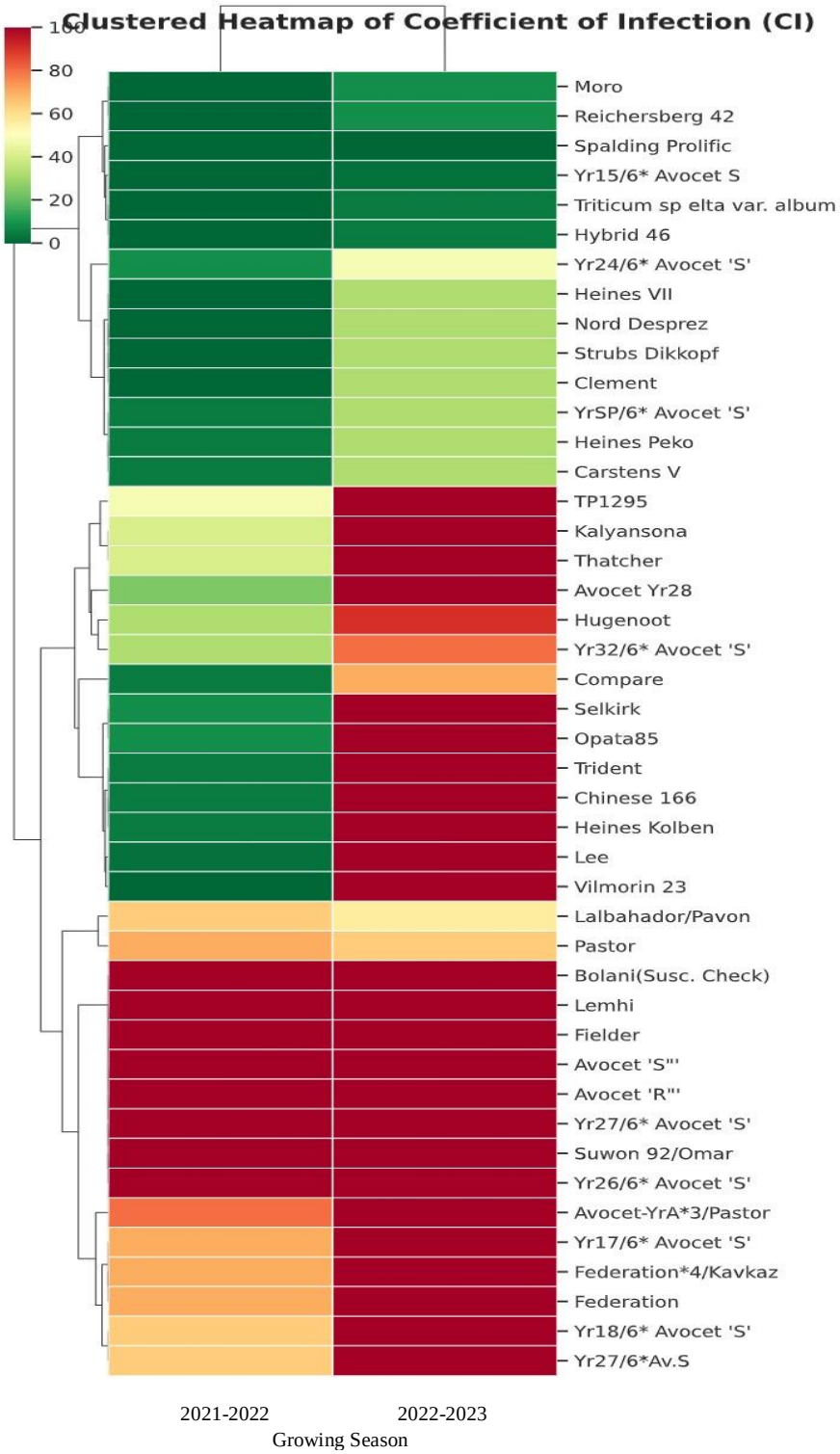


Figure.2. Cluster heatmap of the coefficient of infection (CI) of 44 wheat differential lines carrying different Yr resistance genes in response to the prevailing yellow rust populations in Silakhor during the 2021-2022 and 2022-2023 growing seasons. Disease reactions were converted to coefficient of infection values according to standard infection-type constants. Hierarchical clustering was performed using the UPGMA algorithm based on Euclidean distance. The color scale represents increasing disease severity from low (green) to high (red).

Table 2. Effectiveness of resistance genes in standard and differential wheat lines against the yellow rust pathogen, based on disease severity, infection type, and infection coefficient, over two consecutive years (2021-2022 and 2022- 2023) in the trap nursery of the Silakhor region, Borujerd.

No.	Differential varieties	Gene	Final Disease severity and Infection type		Coefficient of infection	
			Year		Year	
			2021-2022	2022-2023	2021-2022	2022-2023
1	Chinese 166	*Yr1*	10MR	100S	4	100
2	Lee	*Yr7, +*	5MR	100S	2	100
3	Heines Kolben	*Yr2, Yr6*	10MR	100S	4	100
4	Vilmorin 23	*Yr3, +*	0	100S	0	100
5	Moro	*Yr10, YrMor*	0	20MR	0	8
6	Strubs Dikkopf	*YrSD, Yr25, +*	0	40MS	0	32
7	Suwon 92/Omar	*YrSu, +*	100S	100S	100	100
8	Clement	*Yr2, Yr9, +*	0	40MS	0	32
9	*Triticum spelta var. album*	*Yr5*	0	10MR	0	4
10	Hybrid 46	*Yr4, +*	0	10MR	0	4
11	Reichersberg 42	*Yr7, +*	0	20MR	0	8
12	Heines Peko	*Yr2,Yr6, Yr25, +*	10MR	40MS	4	32
13	Nord Desprez	*Yr3, YrND, +*	0	40MS	0	32
14	Compair .	*Yr8, Yr19*	10MR	70S	4	70
15	Carstens V	*YrCV, Yr32, Yr25, +*	10MR	40MS	4	32
16	Spalding Prolific	*YrSP, +*	0	0	0	0
17	Heines VII	*Yr2, Yr25, +*	0	40MS	0	32
18	Kalyansona	*Yr2*	40S	100S	40	100
19	Trident	*Yr17, +*	10MR	100S	4	100
20	Yr15/6* Avocet S	*Yr15*	0	10R	0	2
21	Hugenoot	*Yr25*	40MS	90S	32	90
22	Selkirk	*Yr27*	20MR	100S	8	100
23	Federation *4/Kavkaz	*Yr9*	70S	100S	70	100
24	Federation		70S	100S	70	100
25	Yr17/6* Avocet ‘S’	*Yr17*	70S	100S	70	100
26	Yr18/6* Avocet ‘S’	*Yr18*	80MS	100S	64	100
27	Yr24/6* Avocet ‘S’	*Yr24*	20MR	60MS	8	48
28	Yr26/6* Avocet ‘S’	*Yr26*	100S	100S	100	100
29	Yr27/6* Avocet ‘S’	*Yr27*	100S	100S	100	100
30	Yr32/6* Avocet ‘S’	*Yr32*	40MS	80S	32	80
31	YrSP/6* Avocet ‘S’	*YrSP*	10MR	40MS	4	32
32	Avocet ‘R”	*YrA*	100S	100S	100	100
33	Avocet ‘S”		100S	100S	100	100
34	Fielder	*Yr6, Yr20*	100S	100S	100	100
35	Thatcher	*Yr7*	50MS	100S	40	100
36	Lemhi	*Yr21*	100S	100S	100	100
37	TP1295	*Yr25*	60MS	100S	48	100
38	Yr27/6*Av.S	*Yr27*	80MS	100S	64	100
39	Opata85	*Yr27+Yr18*	20MR	100S	8	100
40	Avocet Yr28	*Yr28*	30MS	100S	24	100
41	Lalbahador/Pavon	*Yr29*	80MS	70MS	64	56
42	Avocet-YrA*3/Pastor	*Yr31*	80S	100S	80	100
43	Pastor	*Yr31+APR*	70S	80MS	70	64
44	Bolani (Susceptible check)		100S	100S	100	100

Resistance Responses of Standard and Differential Wheat Lines

The resistance responses of the standard and differential wheat lines carrying known *Yr* resistance genes are presented in Table 2. Considerable variation in disease response was observed between the two growing seasons, reflecting changes in the virulence composition of the yellow rust population under natural field conditions.

Differential lines carrying *YrA, Yr2, Yr6, Yr7, Yr9, Yr17, Yr20, Yr21, Yr26, Yr27, Yr31*, and *YrSU* were susceptible during both growing seasons, indicating that virulence toward these resistance genes was already established in the prevailing pathogen population. In contrast, differential lines carrying *Yr4, Yr5, Yr10, Yr15, Yr24, YrSD, YrSP, YrND*, and *YrCV* remained resistant throughout the study, demonstrating that these genes continue to provide effective

protection against the currently dominant yellow rust population.

A marked change in pathogen virulence was detected during the 2023 growing season. Virulence against differential lines carrying *Yr1, Yr3, Yr8, Yr18,* and *Yr32* was observed only in the second year of the study, whereas these lines remained resistant during the first growing season. This shift indicates the establishment of new virulence combinations within the local yellow rust population.

Overall, the reactions of the differential lines clearly demonstrate that the virulence spectrum of the pathogen expanded during the second growing season. The appearance of additional virulence factors capable of overcoming previously effective resistance genes provides strong evidence for changes in the genetic composition of the yellow rust population in the Silakhor Plain of Borujerd.

To ensure an objective comparison of resistance performance, all differential lines were independently evaluated based on their coefficient of infection (CI) across two consecutive growing seasons. Mean CI and ΔCI values were calculated to quantify overall disease response and temporal change in resistance expression (Table 3).

Differential lines carrying *Yr10, Yr5, Yr4, Yr15, YrSP*, and *Yr7+* exhibited consistently low mean CI values (<10), indicating highly effective and stable resistance under field conditions. In contrast, lines such as *Yr1, Yr2, Yr3, Yr7, Yr9, Yr17, Yr18, Yr26, Yr27, YrA,* and susceptible controls showed high mean CI values (>75), reflecting complete or near-complete loss of resistance against the prevailing yellow rust population.

Intermediate responses were observed in several differential lines, including *Yr24, Yr25, Yr29, Yr32*, and *YrCV*, which displayed moderate mean CI values and variable ΔCI, suggesting partial but unstable resistance. These genotypes may still contribute to resistance improvement when used in gene pyramiding strategies. Overall, the results demonstrate a wide spectrum of resistance effectiveness and highlight the erosion of several *Yr* resistance genes under current field conditions.

Resistance Responses of Commercial Wheat Cultivars

The resistance reactions of the commercial wheat cultivars evaluated during the two growing seasons are summarized in Table 4. Considerable variation in disease response was observed among the cultivars, indicating substantial differences in their levels of resistance to yellow rust under natural field infection.

Throughout both growing seasons, the cultivars Taktaz, Kalateh, Tirgan, Raj, Amin, Torabi, Heydari, Taban, Sana, Aran, Hana, and Shabrang consistently exhibited satisfactory levels of resistance, with low disease severity, favorable infection types, and correspondingly low infection coefficients. These cultivars maintained stable field resistance despite the high disease pressure that developed during the experiments, suggesting that they possess one or more effective resistance genes capable of providing protection against the prevailing pathogen population.

In contrast, several commercially important cultivars including Araz, Arman, Meraj, Ehsan, Farin, Talaei, Sirvan, Danesh, Heyran, and Zarrineh displayed marked reductions in resistance during the 2022-2023 growing season. Although these cultivars likely carry effective adult plant resistance genes, they exhibited disease reactions ranging from moderately resistant to moderately susceptible under the increased virulence pressure observed during the second year of the study. These responses indicate that the protection conferred by their resistance genes may have been partially compromised by the emergence of pathogen populations with expanded virulence spectra.

Table 3. Relative effectiveness of wheat differential lines carrying *Yr* resistance genes against the prevailing *Puccinia striiformis* f. sp. *tritici* population under field conditions.

No	Differential line	Gene(s)	CI 2021-2022	CI 2022-2023	Mean CI	ΔCI	Effectiveness class
1	Chinese 166	*Yr1*	4	100	52	96	Low
2	Lee	*Yr7*	2	100	51	98	Low
3	Heines Kolben	*Yr2*	4	100	52	96	Low
4	Vilmorin 23	*Yr3*	0	100	50	100	Low
5	Moro	*Yr10*	0	8	4	8	Highly effective
6	Strubes Dikkopf	*YrSD*	0	32	16	32	Effective
7	Suwon 92/Omar	*YrSU*	100	100	100	0	Ineffective
8	Clement	*Yr2,Yr9*	0	32	16	32	Effective
9	T. spelta var. album	*Yr5*	0	4	2	4	Highly effective
10	Hybrid 46	*Yr4*	0	4	2	4	Highly effective
11	Reichersberg 42	*Yr7+*	0	8	4	8	Highly effective
12	Heines Peko	*Yr2,Yr6*	4	32	18	28	Effective
13	Nord Desprez	*YrND*	0	32	16	32	Effective
14	Compare	*Yr8*	4	70	37	66	Moderately effective
15	Carstens V	*YrCV*	4	32	18	28	Effective
16	Spalding Prolific	*YrSP*	0	0	0	0	Highly effective
17	Heines VII	*Yr2*	0	32	16	32	Effective
18	Kalyansona	*Yr2*	40	100	70	60	Low
19	Trident	*Yr17+Sr38*	4	100	52	96	Low
20	Yr15/6*Avocet S	*Yr15*	0	2	1	2	Highly effective
21	Hugenoot	*Yr25*	32	90	61	58	Low
22	Selkirk	*Yr27*	8	100	54	92	Low
23	Federation*4/Kavkaz	*Yr9*	70	100	85	30	Ineffective
24	Federation	-	70	100	85	30	Ineffective
25	Yr17/6*Av.S	*Yr17*	70	100	85	30	Ineffective
26	Yr18/6*Av.S	*Yr18*	64	100	82	36	Ineffective
27	Yr24/6*Av.S	*Yr24*	8	48	28	40	Moderately effective
28	Yr26/6*Av.S	*Yr26*	100	100	100	0	Ineffective
29	Yr27/6*Av.S	*Yr27*	100	100	100	0	Ineffective
30	Yr32/6*Av.S	*Yr32*	32	80	56	48	Low
31	YrSP/6*Av.S	*YrSP*	4	32	18	28	Effective
32	Avocet R	*YrA*	100	100	100	0	Ineffective
33	Avocet S	-	100	100	100	0	Ineffective
34	Fielder	*Yr6,Yr20*	100	100	100	0	Ineffective
35	Thatcher	*Yr7*	45	100	72.5	55	Low
36	Lemhi	*Yr21*	100	100	100	0	Ineffective
37	TP1295	*Yr25*	48	100	74	52	Low
38	Yr27/6*Av.S	*Yr27*	64	100	82	36	Ineffective
39	Opata85	*Yr27+Yr18*	8	100	54	92	Low
40	Avocet Yr28	*Yr28*	24	100	62	76	Low
41	Lalbahador/Pavon	*Yr29*	56	56	56	0	Moderately effective
42	Avocet-YrA*3/Pastor	*Yr31*	80	100	90	20	Ineffective
43	Pastor	*Yr31+APR*	70	80	75	10	Low
44	Bolani (Check)	-	100	100	100	0	Ineffective

CI: Coefficient of infection; Highly effective: Mean CI ≤ 10; Effective: 10 < Mean CI ≤ 25; Moderately effective: 25 < Mean CI ≤ 50; Low effectiveness: 50 < Mean CI ≤ 75; Ineffective: Mean CI > 75

Table 4. Resistance reaction of commercial wheat cultivars to the yellow rust pathogen at the adult plant stage, based on disease severity, infection type, and infection coefficient in the trap nursery of the Silakhor region, Borujerd

		Final reaction at adult plant stage			
		Final disease severity and infection type		Coefficient of infection	
Code	Cultivars	(2021-2022)	(2022-2023)	(2021-2022)	(2022-2023)
1	Taktaz	0	0	0	0
2	Araz	10MR	80MS	4	64
3	Arman	0	80MS	0	64
4	Meraj	0	60MS	0	48
5	Kalateh	0	20MR	0	8
6	Tirgan	0	30M	0	18
7	Ehsan	30M	80MS	18	64
8	Gonbad	20MR	100S	8	100
9	Sahar	50MSS	100S	45	100
10	Setareh	70S	100S	70	100
11	Sarang	20MR	90S	4	90
12	Khalil	60MS	100S	48	100
13	Barat	-	90S	-	90
14	Shoush	60MS	90S	48	90
15	Mehregan	30M	90S	18	90
16	Shavour	30M	100S	18	100
17	Chamran2	80S	100S	80	100
18	Ayneh	20MR	100S	8	100
19	Raj	30M	20MR	18	8
20	Amin	20MR	40MS	8	32
21	Farin	0	60MS	0	48
22	Torabi	0	30MS	0	24
23	Talaei	20MR	60MS	8	48
24	Rakhshan	40MS	90S	32	90
25	Baharan	100S	90S	100	90
26	Sirvan	20MR	80MS	8	64
27	Danesh	30M	60MS	18	48
28	Heyran	50MS	90MS	40	72
29	Zarrineh	50MSS	40MS	45	32
30	Heydari	0	20MR	0	8
31	Barzgar	20MR	100S	8	100
32	Narin	20MR	100S	8	100
33	Ofogh	40MS	100S	32	100
34	Taban	20MR	20MS	8	16
35	Sana	20MR	20MR	8	8
36	Aran	30MR	20MS	12	16
37	Hana	20MR	20MS	8	16
38	Shabrang	30M	10MR	27	4
39	Bolani (Susceptible check)	90S	100S	90	100

The remaining commercial cultivars were highly susceptible in both growing seasons, developing severe disease symptoms accompanied by high infection coefficients. The susceptible check cultivar Bolani consistently exhibited severe yellow rust infection, with final disease severities ranging from 90% to 100%, confirming that environmental conditions were highly favorable for disease development and that sufficient inoculum was available throughout the experimental period.

Overall, the observed variation among commercial cultivars demonstrates that resistance levels differed considerably under natural epidemic conditions. While several cultivars maintained stable resistance across both years, others showed substantial

reductions in field resistance during the second growing season, providing additional evidence of changes in the virulence composition of the local yellow rust population.

Cluster Analysis of Commercial Wheat Cultivars

To facilitate comparison of field resistance among commercial wheat cultivars, the coefficient of infection (CI) values were visualized using a clustered heatmap combined with hierarchical clustering (Figure.3). Hierarchical cluster analysis based on disease severity, infection type, and coefficient of infection grouped the commercial wheat cultivars into three major clusters, each representing a distinct level of response to yellow rust.

The first cluster comprised cultivars that consistently expressed resistant reactions during both growing seasons. These cultivars were characterized by low disease severity, resistant or moderately resistant infection types, and low coefficients of infection, indicating the presence of effective resistance against the prevailing yellow rust population. Cultivars such as Taktaz, Raj, Sana, Hana, Aran, Kalateh, and Heydari were associated with this resistant group and maintained comparatively low CI values throughout the study period.

The second cluster included cultivars that remained highly susceptible throughout the study. Members of this group exhibited high disease severity together with susceptible infection types and high coefficients of infection, reflecting the limited effectiveness or absence of resistance against the prevailing pathogen population. Highly susceptible cultivars, including Setareh, Chamran 2, Khalil, Shavour, Baharan, and the susceptible check Bolani, were clearly separated from the resistant group.

The third cluster consisted of cultivars displaying variable responses between the two growing seasons. These cultivars expressed race-specific or unstable resistance, suggesting that changes in pathogen virulence influenced their field performance. Their placement in an intermediate cluster further supports the occurrence of shifts in pathogen virulence between the 2021- 2022 and 2022- 2023 growing seasons. Several cultivars, including Araz, Arman, Meraj, Gonbad, Sarang, Ayneh, Barzgar, Narin, and Ofogh, exhibited a marked increase in the coefficient of infection during the second season, indicating reduced resistance under increasing disease pressure.

The clustering pattern closely corresponded to disease severity, infection type, and coefficient of infection. Cultivars possessing effective resistance were clearly separated from susceptible cultivars, whereas cultivars with intermediate or unstable resistance formed a distinct transitional group. Overall, the clustered heatmap provides a comprehensive visualization of phenotypic variation among commercial wheat cultivars and demonstrates that hierarchical clustering is an effective approach for distinguishing resistance classes and identifying cultivars with durable field resistance under natural yellow rust epidemics.

Changes in cultivar response between the two growing seasons were further visualized using an alluvial plot based on coefficient of infection classes (Figure.4). Most cultivars that exhibited resistant reactions during the 2021- 2022 growing season shifted toward moderately susceptible or susceptible classes during 2022- 2023, indicating an increase in pathogen virulence.

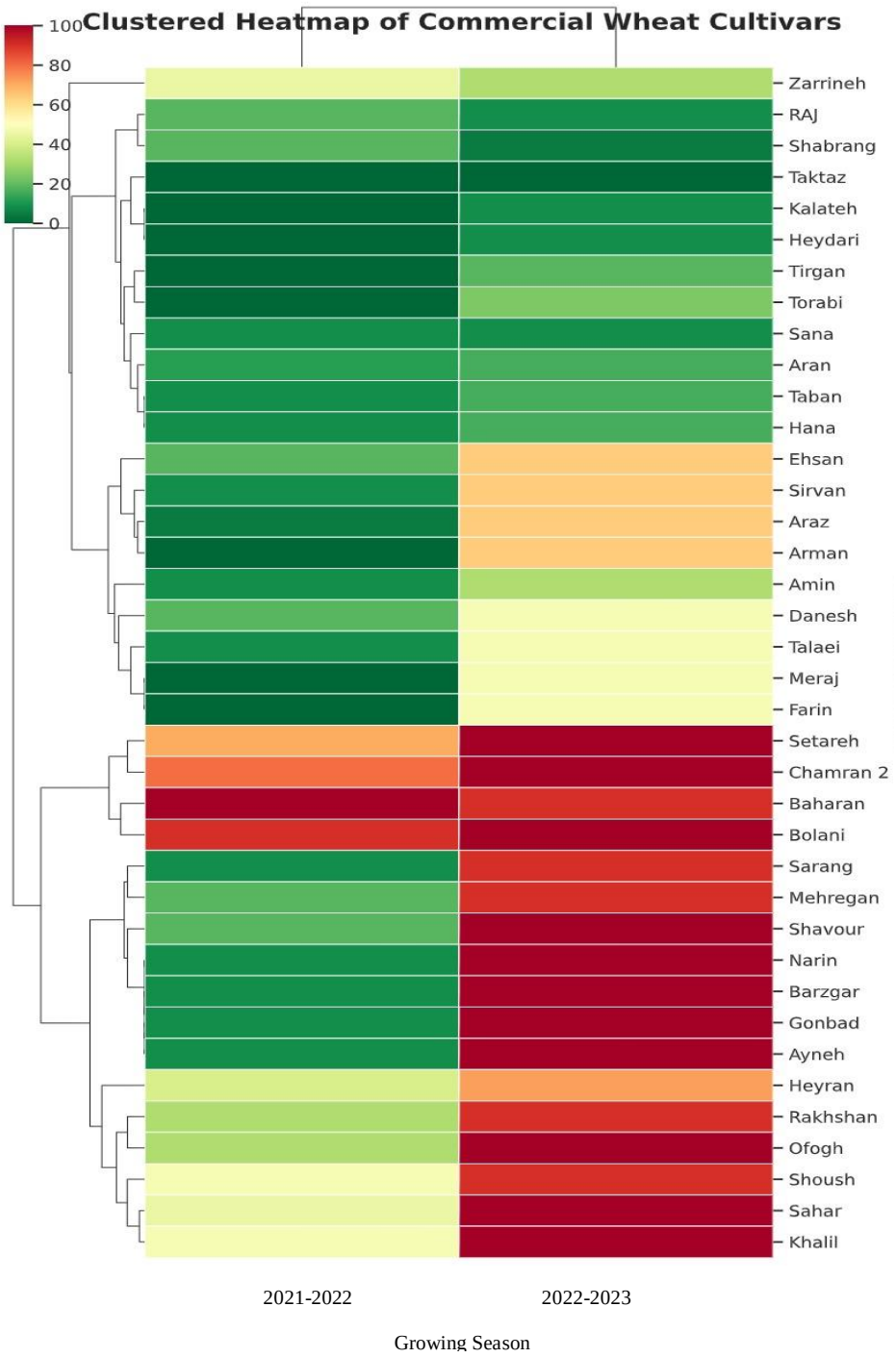


Figure 3. Clustered heatmap showing the coefficient of infection (CI) of commercial wheat cultivars evaluated against the yellow rust population in the Silakhor Plain during the 2021-2022 and 2022-2023 growing seasons. Disease reactions were converted to coefficient of infection values according to the infection-type constants described in the Materials and Methods. Hierarchical clustering was performed using the UPGMA algorithm based on Euclidean distance. The color gradient represents increasing disease severity from green (low CI) to red (high CI).

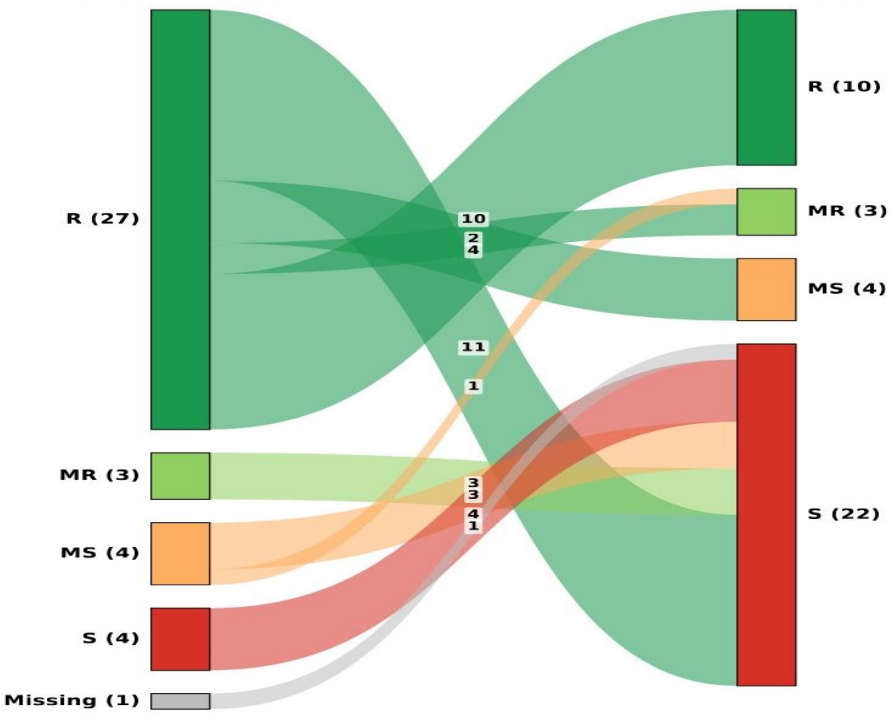


Figure 4. Alluvial plot illustrating changes in resistance classes of commercial wheat cultivars between the 2021-2022 and 2022-2023 growing seasons based on the coefficient of infection (CI). Cultivars were classified into resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) categories according to their CI values. The width of each flow represents the number of cultivars exhibiting the corresponding transition between seasons.

Descriptive statistics revealed a pronounced increase in yellow rust severity during the second growing season (Table 5). The mean coefficient of infection (CI) increased from 22.16 ± 26.44 in 2021- 2022 to 59.47 ± 37.08 in 2022- 2023. In contrast, the median increased from 8.0 to 64.0, indicating a marked shift toward greater disease severity among the evaluated commercial wheat cultivars. Likewise, the interquartile range increased from 24.0 to 80.5, reflecting greater variability in cultivar responses during the second growing season. The normality of paired differences in CI values was evaluated using the Shapiro–Wilk test, which indicated no significant departure from normality ($W = 0.948$, $P = 0.079$). Accordingly, differences between growing seasons were assessed using a paired t-test. The analysis demonstrated that CI values were significantly higher in 2022- 2023 than in 2021- 2022 ($t = -7.009$, $P < 0.001$).The observed difference was associated with a large effect size (Cohen's $d = 1.159$), indicating that the increase in disease severity was not only statistically significant but also biologically meaningful. Furthermore, the mean increase in CI was estimated to lie between 26.53 and 48.10 (95% confidence interval), providing additional evidence for a substantial increase in yellow rust severity during the second growing season. These findings support the conclusion that the prevailing yellow rust population exhibited greater aggressiveness in 2023, resulting in reduced field resistance in many of the evaluated commercial cultivars.

Table 5. Descriptive statistics and paired comparison of the coefficient of infection (CI) between the two growing seasons.

Parameter	2021-2022	2022-2023	Statistical test	Value
Number of cultivars	38	38	Shapiro-Wilk (W)	0.948
Mean ± SD	22.16 ± 26.44	59.47 ± 37.08	Shapiro-Wilk (P)	0.079
Median	8.0	64.0	Paired t-test (t)	-7.009
Interquartile range (IQR)	24.0	80.5	Paired t-test (P)	<0.001
			Cohen's d	1.159
			95% Confidence interval	26.53-48.10

Discussion

In recent years, severe yellow rust epidemics have been reported in several neighboring countries located along recognized pathways of rust race migration. These outbreaks have largely been attributed to the emergence and spread of newly evolved virulent races of *Puccinia striiformis* f. sp. *tritici*. Given previous experience with wheat rust epidemics in the region, the eventual introduction of such races into Iran was considered highly likely. A major concern for wheat pathologists and breeders was whether commercially cultivated wheat cultivars, many of which occupy large production areas across the country, would remain resistant if new virulent races became established. The possibility that the devastating epidemic of 1993 could recur under a shifted virulence pattern scenario underscored the importance of continuous surveillance.

Accordingly, regular monitoring of yellow rust populations is essential for understanding changes in pathogen genetic structure and virulence composition, evaluating the effectiveness of adult plant resistance (APR) genes, and assessing the field performance of newly released commercial cultivars. Such surveillance is particularly critical in major infection hotspots where new races are most likely to emerge or become established.

The Borujerd region occupies a strategic position in the epidemiology of wheat rusts in Iran. Located along major migration routes of rust populations, the Silakhor Plain is recognized as one of the country's primary foci of yellow rust infection. Its prolonged availability of green host tissue and high humidity frequently allow rust populations to persist late into the growing season. These conditions have previously facilitated the detection of newly emerged rust races, including race Ug99 of stem rust, highlighting the region's value as an early-warning site for rust surveillance.

During both growing seasons, environmental conditions in the Silakhor Plain were highly favorable for yellow rust development. Disease first became established on the susceptible spreader cultivar Bolani and subsequently spread extensively to the standard and differential lines as well as to the commercial cultivars. Final disease severity on Bolani reached 90-100%, confirming that inoculum pressure was sufficiently high to allow reliable discrimination among resistance classes under natural epidemic conditions.

The present study revealed a strong shift in the virulence pattern of *Puccinia striiformis* f. sp. *tritici* between the two growing seasons, as evidenced by increased disease severity and infection coefficients across most wheat genotypes. The significant rise in mean CI values from the first to the

second season indicates an enhanced pathogen pressure. It suggests a dynamic evolution in the local the yellow rust population in the Silakhor Plain of Borujerd.

A detailed examination of the trap nursery data showed that differential lines carrying *Yr4, Yr5, Yr10, Yr15, Yr24, YrSD, YrSP, YrND*, and *YrCV* remained resistant throughout both years of the study. In contrast, virulence against lines carrying *YrA, Yr2, Yr6, Yr7, Yr9, Yr17, Yr20, Yr21, Yr26, Yr27, Yr31*, and *YrSU* was consistently observed in both seasons, indicating that these genes are no longer effective against the prevailing pathogen population in Borujerd and likely in many other wheat-growing regions of Iran. Most notably, virulence toward *Yr1, Yr3, Yr8, Yr18*, and *Yr32* appeared only during the second growing season, providing strong evidence for a marked shift in the virulence pattern with an expanded virulence spectrum in the region.

Cluster and alluvial analyses consistently demonstrated three major response patterns: stable resistance, unstable (race-specific) resistance, and complete susceptibility. Genotypes carrying resistance genes such as *Yr15, Yr5, Yr4, Yr10*, and *YrSP* showed stable performance with consistently low CI values, indicating that these genes remain effective sources of resistance under field conditions.

In contrast, widely deployed resistance genes such as *Yr1, Yr2, Yr3, Yr7, Yr9, Yr17, Yr18, Yr26, Yr27*, and *YrA* were associated with high disease levels and unstable performance, confirming a significant breakdown of resistance. This finding highlights the strong selection pressure exerted by the pathogen population and the consequent erosion of previously effective resistance sources.

The yellow rust population in the second growing season exhibited a substantially broader and more aggressive virulence profile than those observed previously. This conclusion is supported by the increase in the proportion of virulence factors detected in the differential set, which rose from approximately 37% in 2021- 2022 to about 67% in 2022- 2023. Such a rapid expansion in virulence suggests a significant shift in the local pathogen population and highlights the evolutionary capacity of the yellow rust pathogen under strong host selection pressure.

The alluvial analysis further confirmed a clear directional shift in cultivar response between the two seasons, with many genotypes moving from resistant or moderately resistant classes toward susceptible categories. This shift reflects an increase in pathogen virulence and suggests the presence of evolving pathotypes capable of overcoming previously effective resistance genes.

Only a limited number of genotypes maintained stable resistance across both seasons, emphasizing the scarcity of durable resistance in the current germplasm pool.

Limited virulence toward *Yr1* and *Yr8* has previously been reported only in restricted areas of Iran. Although these genes are not major components of resistance in most current Iranian commercial cultivars, their breakdown is nevertheless important because it signals ongoing changes in pathogen virulence composition. Consequently, both commercial cultivars and advanced breeding lines should continue to be monitored closely for potential shifts in field resistance.

The high race diversity observed in wheat rust pathogens, particularly yellow rust, has been documented repeatedly in Iran and elsewhere. Likewise, substantial variation in resistance responses among wheat genotypes and commercial cultivars is a well-

established feature of yellow rust epidemiology (Omrani et al., 2013, 2014, 2017a, 2017b, 2024, 2025; Kabiri et al., 2024). Yellow rust populations in the Middle East and North Africa commonly undergo major shifts in virulence every five to seven years, often resulting in epidemic outbreaks associated with newly emerged races (Ali et al., 2017). The persistence of susceptible hosts along the "green bridge" extending from northeastern Africa to Yemen is thought to provide a major evolutionary reservoir for the generation and dissemination of new cereal rust races into neighboring regions, including Iran (Yahyaoui et al., 2004).

Previous trap nursery studies in Iran identified the highest levels of virulence on lines carrying *Yr2, Yr6, Yr7, Yr9, Yr25*, and *YrA*, whereas *Yr10* and *YrSU* were among the least affected genes in regions such as Nahavand and Dezful. The emergence of the virulent race 166E134A+ in Fars Province further demonstrated the capacity of the pathogen to overcome multiple resistance genes simultaneously, including *Yr24, Yr27, Yr9*, and *YrA* (Afshari et al., 2008). Subsequent detections of this race in western and southern Iran confirmed its regional spread.

More recent nationwide monitoring during 2017-2019 showed that *Yr4, Yr5, Yr8, Yr10, Yr15, Yr18, YrSP, YrSD, YrND*, and *YrCV* remained effective across most surveyed regions, while virulence persisted against *Yr1, Yr2, Yr6, Yr7, Yr9, Yr21, Yr25, Yr27, Yr28, Yr29, Yr31, Yr32, YrA*, and *YrSU*. Several commercial cultivars including Sarang, Sirvan, Shabrang, Hana, Heydari, Tirgan, and others displayed acceptable resistance throughout those surveys (Afshari et al., 2019). The present study agrees with those findings for many genes. Additionally, it documents the recent breakdown of *Yr1, Yr3, Yr8, Yr18*, and *Yr32* in the Borujerd population, indicating a further expansion of virulence beyond previously reported patterns.

Trap nursery monitoring in the Moghan Plain also revealed widespread virulence toward *Yr6, Yr7, Yr8, Yr17, Yr18, Yr20, Yr21, Yr26, Yr31*, and *YrA*, while *Yr1, Yr2, Yr3, Yr4, Yr5, Yr10, Yr15, Yr27, Yr32, YrSP, YrSD, YrND*, and *YrCV* remained effective (Omrani et al., 2025). The appearance of virulence against *Yr32* in Borujerd therefore represents a notable regional divergence from the virulence profile observed in Moghan and emphasizes the need for geographically targeted surveillance.

International studies further support the dynamic nature of yellow rust virulence. In northwestern Europe, APR genes such as *Yr18, Yr29*, and *Yr46* have remained effective against many major races, whereas monitoring across Europe, North Africa, and West Asia has documented extensive virulence toward several widely deployed genes, including *Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr25, Yr32*, and *YrSP* (Hovmøller et al., 2023). The Borujerd population appears to follow the broader regional trend toward expanded virulence while still leaving a subset of resistance genes effective.

The response of commercial wheat cultivars to the yellow rust population with the altered virulence pattern in Borujerd revealed considerable variation in field resistance, reflecting the impact of the expanded virulence spectrum on currently cultivated germplasm. Approximately 42% of the evaluated cultivars lost effective resistance during the 2023 season, whereas about 13% exhibited intermediate reactions ranging from moderately resistant to moderately susceptible. Under severe epidemic conditions, these intermediate

cultivars may require fungicide application to reduce disease development. Nevertheless, approximately 45% of the cultivars maintained strong field resistance against the prevailing virulence pattern observed in the second year. Cultivars such as Taktaz, Kalateh, Tirgan, Raj, Amin, Torabi, Heydari, Taban, Sana, Aran, Hana, and Shabrang therefore represent valuable resistance sources for breeding wheat cultivars with broader and more durable resistance to yellow rust.

The results provide strong evidence that resistance in many widely used wheat cultivars has been significantly eroded, emphasizing the need for diversification of resistance sources. The identification of stable genotypes with consistently low CI values offers valuable parental material for future wheat breeding programs.

The moderate-performance genotypes, despite their partial susceptibility, may still contribute to resistance improvement when used in gene pyramiding strategies, particularly when combined with highly effective genes such as *Yr15* and *Yr5*.

Overall, the study demonstrates that yellow rust resistance in the evaluated wheat germplasm is highly variable and strongly influenced by seasonal changes in pathogen virulence. The combined use of cluster analysis, temporal infection dynamics, and the Breeding Value Index provides a powerful framework for resistance characterization.

This integrative approach not only improves the resolution of resistance classification but also enhances the practical utility of the findings for wheat improvement programs under field epidemic conditions.

Therefore, continuous monitoring of yellow rust populations, together with periodic re-evaluation of resistance sources under field conditions, is essential for sustaining durable yellow rust resistance and supporting the development of resilient wheat cultivars in future breeding programs.

Conclusion

The present study provides a comprehensive evaluation of yellow rust resistance in wheat differential lines and commercial cultivars under natural epidemic conditions in the Silakhor Plain of Borujerd over two consecutive growing seasons. The results revealed a marked increase in pathogen virulence during the 2023 season, reflected by higher disease severity, infection coefficients, and the emergence of virulence toward several previously effective *Yr* resistance genes, including *Yr1*, *Yr3*, *Yr8*, *Yr18*, and *Yr32*. These findings demonstrate the rapid evolutionary potential of the local yellow rust population and emphasize the necessity for continuous monitoring of pathogen dynamics.

Despite the observed expansion in virulence, several resistance sources, particularly *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *YrSP*, *YrSD*, *YrND*, and *YrCV*, maintained relatively high levels of effectiveness under field conditions and therefore represent valuable genetic resources for future wheat improvement programs. Furthermore, the identification of resistant commercial cultivars with stable field performance provides immediate parental materials for breeding cultivars with broader and more durable resistance.

The results further demonstrate that reliance on single race-specific resistance genes is unlikely to provide durable protection against the continually evolving yellow rust population. Instead, breeding strategies should prioritize the pyramiding of multiple effective resistance genes,

particularly in combination with adult plant resistance, to enhance resistance durability and reduce the risk of resistance breakdown. Future breeding programs may benefit from integrating molecular marker-assisted selection with field-based trap nursery surveillance to improve the identification and deployment of effective resistance genes. Overall, this study not only updates the current virulence profile of *P. striiformis* f. sp. *tritici* in western Iran but also provides practical breeding-oriented information for resistance gene deployment and cultivar improvement. Therefore, continuous monitoring of pathogen populations, periodic re-evaluation of resistance sources under field conditions, and the integration of quantitative analytical tools into resistance screening programs are essential for sustaining durable yellow rust resistance and supporting the development of resilient wheat cultivars under changing pathogen populations.

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