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Resistance to stripe and stem rusts in Iranian cold-region wheat genotypes

S. Safavi[1]*, F. Afshari [2], A. Malihipour [2]

1. ***Corresponding Author:** Crop and Horticultural Science Research Department, Ardabil Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Ardabil, Iran (safaralisafavi@yahoo.com)
2. Department of Cereal Research, Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran

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Abstract

The limited availability of durable genetic resistance in wheat cultivates remains a major challenge contributing to recurring epidemics of stripe (yellow) and stem (black) rust, which threaten global wheat yields. This study ascertained Iranian wheat germplasm, specifically genotypes adapted to cold regions as potential new sources of resistance at both seedling and adult growth stages. Over two successive crop years (2018-2020), 36 genotypes and susceptible checks were screened under field conditions at the Ardabil Agricultural Research Station in Iran against native pathogen populations. In the adult plants, slow-rusting resistance was assessed based on coefficient of infection (CI), final disease severity (FRS), and the relative area under the disease progress curve (rAUDPC). Seedling assays were performed under controlled conditions with two distinct stripe rust pathotypes (174E150A+,Yr27 and 14E158A+,Yr27) and four stem rust pathotypes (TTTTF, PTRTF, TKTTF, TTKTK). According to the results, 30 genotypes indicated high, three moderate, and two low levels of slow-rusting resistance to stripe rust, while only two genotypes displayed high resistance to stem rust, nine moderate, and the remainder low. Several genotypes—including C-96-2, C-96-9, C-96-7, C-96-10, C-96-13, C-96-16, C-97-9, C-97-12, C-97-16, C-97-18, and C-97-19—demonstrated promising moderate-to-high resistance to both diseases. Genotypes susceptible at the seedling stage but moderate resistance with low severity under field conditions may carry combinations of slow-rusting genes. These resistant genotypes provide valuable genetic resources for breeding programs aiming at durable resistance.

Keywords: Wheat, yellow rust, black rust, race-specific resistance, non-race-specific resistance, durable resistance, adult plant resistance

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مقاومت ژنوتیپ‌های گندم مناطق سرد ایران نسبت به زنگ زرد و زنگ ساقه

صفرعلی صفوی[1*]، فرزاد افشاری[2]، علی ملیحی پور[2]

۱- *نویسنده مسوول: بخش تحقیقات علوم زراعی و باغی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان اردبیل، سازمان تحقیقات، آموزش و ترویج کشاورزی، اردبیل، ایران

۲- بخش تحقیقات غلات، موسسه تحقیقات اصلاح و تهیه نهال و بذر، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران

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چکیده

کمبود مقاومت ژنتیکی بادوام در ارقام گندم، یکی از عوامل اصلی وقوع همه‌گیری‌های مکرر زنگ زرد و زنگ سیاقه است که تولید جهانی گندم را تهدید می‌کند. این مطالعه، ذخایر ژنتیکی گندم ایران، به‌ویژه ژنوتیپ‌های سازگار با مناطق سرد را به عنوان منابع بالقوه جدید مقاومت، در هر دو مرحله رشد گیاهچه و گیاه بالغ ارزیابی کرد. طی دو سال زراعی متوالی (۱۳۹۹-۱۳۹۷)، سی‌وشش ژنوتیپ به همراه شاهدهای حساس، در شرایط مزرعه‌ای در ایستگاه تحقیقات کشاورزی اردبیل و در برابر جمعیت‌های بومی پاتوژن غربالگری شدند. در گیاهان بالغ، مقاومت تدریجی با استفاده از شاخص ضریب آلودگی (CI)، شدت نهایی بیماری (FRS) و مقدارنسبی سطح زیر منحنی پیشرفت بیماری (rAUDPC) ارزیابی شد. آزمون‌های مرحله گیاهچه نیز در شرایط کنترل‌شده با استفاده از دو پاتوتیپ متمایز زنگ زرد (174E150A+,Yr27 و14E158A+,Yr27) و چهار پاتوتیپ زنگ ساقه (TTKTK، TKTTF، PTRTF ،TTTTF) انجام شد. نتایج نشان داد که ۳۰ ژنوتیپ دارای سطوح بالایی از مقاومت تدریجی به زنگ زرد، سه ژنوتیپ دارای مقاومت متوسط و دو ژنوتیپ دارای مقاومت پایین بودند. در مقابل، تنها دو ژنوتیپ مقاومت بالا، نه ژنوتیپ مقاومت متوسط و مابقی مقاومت پایینی نسبت به زنگ ساقه نشان دادند. چندین ژنوتیپ از جمله C-96-2، C-96-9، C-96-7، C-96-10، C-96-13، C-96-16، C-97-9، C-97-12، C-97-16، C-97-18 و C-97-19 سطوح امیدبخشی از مقاومت متوسط تا بالا را در برابر هر دو بیماری نشان دادند. ژنوتیپ‌هایی که در مرحله گیاهچه حساس بودند اما در شرایط مزرعه دارای مقاومت متوسط همراه با شدت بیماری پایین بودند، احتمالاً دارای ترکیبی از ژن‌های مقاومت تدریجی هستند. این ژنوتیپ‌های مقاوم، منابع ژنتیکی ارزشمندی برای برنامه‌های به‌نژادی با هدف دستیابی به مقاومت پایدار فراهم می‌کنند.

کلید واژه‌ها: گندم، زنگ زرد، زنگ سیاه، مقاومت نژاد-اختصاصی، مقاومت غیر نژاد-اختصاصی، مقاومت پایدار، مقاومت گیاه بالغ

دبیر تخصصی: دکتر نیما خالدی

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most crucial food crops, serving as a staple food for billions of people, especially in Central, West Asia, and North Africa regions, including Iran. In Iran, wheat is cultivated on approximately 6.9 million hectares, with an annual production of about 13.28 million tons (Anonymous, 2023). Nevertheless, its yield potential is frequently limited by biotic stresses, among which rust diseases—particularly stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) and stem (black) rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*)—are of major concern. These pathogens cause substantial economic losses globally (Ali et al., 2017; Kumar et al., 2020; Meyer et al., 2021).

Stripe rust continues to pose a serious threat to global wheat production, often acting as a dominant yield-limiting factor (Chen, 2020). Global-scale epidemics take place every 5–10 years, with yield losses estimated between 10% and 70% in affected regions, and can reach 100% under high disease pressure (Aggarwal et al., 2018). Annual global losses owing to yellow rust are estimated at 5.5 million tons (Beddow et al., 2015). Recent decades have witnessed localized yet significant epidemics across major wheat-growing regions, including Central Asia and Africa (Ezzahiri et al., 2009; Rahmatov et al., 2012). Key factors contributing to the augmented frequency and recurrence of yellow rust outbreaks include the pathogen's rapid evolution, long-distance spore dispersal, as well as the emergence of new virulent races (Chen & Kang, 2017; Chen, 2020).

Stem rust represents a highly destructive disease, capable of inflicting up to 100% yield loss in susceptible cultivars during epidemic years (Denbel et al., 2013). As an obligate parasite, *Pgt* impairs photosynthesis and diverts water and nutrients, severely impairing plant growth and productivity (Roelfs et al., 1992). The widely deployed resistance gene *Sr31*, derived from rye, provided effective control until the emergence of the Ug99 race group in Uganda in 1999, which overcame this gene (Pretorius et al., 2000). Since then, Ug99 has evolved into multiple variants, spreading across different countries by 2022 (Olivera et al., 2022). In recent years, the TKTTF pathotype has triggered severe outbreaks in Ethiopia, overcoming resistance in the previously resistant cultivar Digalu (Olivera et al., 2015). Simultaneously, the TTTTF pathotype caused significant damage to durum wheat in Italy, raising concerns throughout Europe (Bhattacharya, 2017). These events highlight the critical need for vigilant pathogen monitoring and the development of diversified resistance strategies.

By 2015, over 72 stem rust resistance genes had been identified, the majority of which confer race-specific, all-stage resistance (Singh et al., 2015). Nevertheless, such resistance is often transient owing to the rapid evolution of new virulent pathotypes, particularly when cultivars with single R genes are deployed on a large scale (Singh et al., 2011). In contrast, non-race-specific resistance—often polygenic and expressed predominantly in adult plants—offers more durable protection. This type of resistance, termed slow rusting or partial resistance, is characterized by diminished disease progress and longer effectiveness (Herrera-Foessel et al., 2011; Chen & Kang, 2017).

Among the 86 permanently designated yellow rust resistance genes, only 19—including *Yr18*, *Yr29*, *Yr30*, *Yr36*, *Yr39*, *Yr46*, *Yr48*, *Yr49*, *Yr52*, *Yr54*, *Yr55*, *Yr58*, *Yr59*, *Yr62*, *Yr68*, *Yr71*, *Yr75*, *Yr77*, and *Yr78*—are linked to non-race-specific, slow rusting resistance (Zhu et al., 2023). Likewise, among the more than 70 designated stem rust resistance genes, only five—*Sr2*, *Sr55*, *Sr56*, *Sr57*, and *Sr58*—

confer adult plant resistance (Singh et al., 2015). Given the limitations of race-specific resistance, genetic strategies that combine major (all-stage) and minor (slow rusting) resistance genes are progressively advocated for more sustainable rust management (Singh et al., 2011, Chen, 2013).

Genetic resistance remains the most effective, economical, and environmentally viable approach to controlling wheat rusts, lowering reliance on fungicides (Singh et al., 2011; Chen, 2020). Thus, this study aimed to identify Iranian wheat genotypes with effective adult plant resistance against stripe and stem rust, in order to provide durable sources for breeding programs.

Materials and Methods

The present research was undertaken through two complementary experiments: seedling evaluation and field assessment. The seedling experiment was performed in the Pathology Unit of the Cereal Research Department at the Seed and Plant Improvement Institute (SPII) in Karaj, Iran. Field evaluations were carried out at the Ardabil Agricultural Research Station, Ardabil, Iran (38°17′59″ N, 48°39′58″ E; 1365 m above sea level), which is representative of the cold agro-ecological zone for wheat cultivation in Iran. Yellow rust was ascertained over two consecutive cropping seasons (2017–2018 and 2018–2019), while stem rust was evaluated during the 2018–2019 and 2019–2020 seasons.

Plant materials

A total of 36 wheat genotypes, representative of the cold-zone breeding program, were obtained from the Cereal Research Department of SPII, Karaj, Iran. Table 1 provides details of their crosses and pedigrees. All genotypes were evaluated for resistance to both stripe rust as well as stem rust at seedling and adult plant stages, with the aim of identifying different resistance mechanisms along with types against these pathogens.

Rust pathotypes

In this study, two pathotypes of *Puccinia striiformis* f. sp. *tritici* (*Pst*) plus four pathotypes of *Puccinia graminis* f. sp. *tritici* (*Pgt*), representing the predominant racial groups in Iran, were employed for seedling resistance screening. The virulence/avirulence patterns and characteristics of these pathotypes are outlined in Table 2. Urediniospore inocula of each pathotype were procured from the Pathology Unit of the Seed and Plant Improvement Institute (SPII). For inoculum multiplication, spores of *Pst* and *Pgt* were separately increased under controlled conditions on highly susceptible wheat cultivars. The universally susceptible cultivar Morocco was utilized to multiply *Pst* and *Pgt*, following the standard protocols of Joshi et al. (1988). This ensured consistent and sufficient spore production for uniform inoculation across all experiments.

Seedling resistance evaluation to stem and stripe rusts

Stripe and Stem rust tests

Seedling resistance to stripe rust was assessed under greenhouse conditions. Genotypes were sown in pots (six seeds per pot) containing a 70:30 (v/v) mixture of standard soil and peat moss. Inoculations were performed individually at the two-leaf stage (GS 12) using urediniospores from two distinct *Pst* pathotypes: 174E150A+,Yr27 and 14E158A+,Yr27 (Table 3). These isolates were originally gathered from major wheat-growing regions. To promote infection, inoculated plants were placed in a mist chamber at 7–9 ˚C with near-saturation humidity for 24 hours before being transferred to a greenhouse maintained at 15 °C (night) and 18 ˚C (day). Infection types (ITs) were ascertained 14 days post-inoculation according to the 0–4 scale described by Stakman et al. (1962) and modified by McIntosh et al. (1995), with low values indicating resistance and high values revealing susceptibility. To ensure data

reliability, the seedling resistance assay was performed twice.

Seedling response to stem rust was evaluated under controlled conditions. Four to six seeds of each genotype were sown in pots (10 cm in diameter and height) filled with a standardized peat moss and soil mixture (70:30, v/v). Around 8 to 10 days after sowing, once the first leaves were fully developed, seedlings were inoculated individually with urediniospores of four distinct stem rust pathotypes: TTTTF, PTRTF, TKTTF, and TTKTK (Table 2). These isolates were originally collected from major wheat-growing regions. The inoculation was performed using a spore suspension in Soltrol 170 oil at a concentration of 10 mg spores per 10 mL oil.

Table 1. Pedigrees of wheat genotypes evaluated for resistance to stripe and stem rust at seedling and adult plant stages.

No.	Line	Pedigree/Parents
1	**C-96-1**	ALVAND//NS-732/HERON
2	**C-96-2**	130L1.11//F35.70/Mo73/4/Ymh/Tob//Mcd/3/Lira
3	**C-96-3**	Bkt/90-Zhong87
4	**C-96-4**	Ghk "s"//Bow "s"//90Zhong87/3/Shiroodi
5	**C-96-5**	Dorade-5/Dunav
6	**C-96-6**	Atou/Zrn
7	**C-96-7**	Almt/3/Kavkaz/Rsh//Rsh/Tsapki
8	**C-96-8**	Gun91/Mnch/4/Tast/Sprw//Zar/3/Atay/Galvez87
9	**C-96-9**	Tam 200/Gascogne//Alvd
10	**C-96-10**	Shark/F4105W2.1//Qt6258/3/Shark/F4105W2.1
11	**C-96-11**	Ok09634
12	**C-96-12**	Tam200/Pastor//Toba97/7/Zcl/3/Pgfn//Cno67/Sn64/4/Seri/5/UA.2837/6/Attila/3*Bcn
13	**C-96-13**	Ulugbek 600/Tukuru//Sanzar-8
14	**C-96-14**	Adyr/4/Babax/Lr42//Babax/3/Babax/Lr42//Babax
15	**C-96-15**	Almt/3/Kavkaz/Rsh//Rsh/Tsapki
16	**C-96-16**	Shark/F4105W2.1//Qt6258/3/Shark/F4105W2.1
17	**C-97-1**	Bkt/90-Zhong87
18	**C-97-2**	NORD-DESPREZ/VG-9144//KALYANSONA/BLUEBIRD/3/YACO"S"/4/VEERY-5"S"
19	**C-97-3**	OMID/4/BB/KAL//ALD/3/Y50E/KAL*3//EMU"S"/5/ZRN/SHIROODI
20	**C-97-4**	Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi/4/55.174/P101//Maya/3/Snb
21	**C-97-5**	Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi/4/55.174/P101//Maya/3/Snb
22	**C-97-6**	Vorona//Milan/Sha7/3/MV17/4/Pehlivan
23	**C-97-7**	Tam113
24	**C-97-8**	Unknown/Zolotava/6/Jup/4/Cllf/3/II1453/Odin//Ci134431/Sel6425/Wa00477*2/5/…
25	**C-97-9**	Charger//CMH80A.768/3*Cno79
26	**C-97-10**	MV35-13
27	**C-97-11**	MV-Pantalika
28	**C-97-12**	Zander//Attila/3*Bcn
29	**C-97-13**	Boh4/7/Wa476/3/391//Num/5/W22/5/Ana/6/Tam200/Kasyan
30	**C-97-14**	Car422/Ana//Yaco/3/Kauz*2/Trap//Kauz/4/Bucur/5/Bucur
31	**C-97-15**	Rumeli
32	**C-97-16**	Id80628/3/Cer/Ymh//Hys/4/Cer/Ymh//Hys/5/Tjb368.251/Buc//Anb/Buc
33	**C-97-17**	Charger//CMH80A.768/3*Cno79
34	**C-97-18**	BEZ1//PBW343*2/KUKUNA (code:1005)
35	**C-97-19**	GRK79//INQALAB 91*2/TUKURU(Code:1015)
36	**C-97-20**	Kalym (Rus 2005)(code:Rus 2005)
-	**Check**	(Morocco)
-	**Check**	(McNear + CD-90-12)

Table 2. Pathotypes of stripe (*Pst*) and stem (*Pgt*) rust used for seedling-stage resistance screening.

Rust	Pathotype	Virulence pattern	Airulence pattern
Yellow rust			
	174E150A^{+}, Yr27	*Yr2, Yr3, Yr6, Yr7, Yr8, Yr9, Yr17, Yr18, Yr20, Yr21, Yr25, Yr27, Yr28, Yr29, Yr31, YrSD, YrA*	***Yr1, Yr4, Yr5, Yr10, Yr15, Yr24, Yr26, Yr32, YrSU, YrND, YrCV, YrSP***
	14E158A^{+},Yr27	*Yr2, Yr3, Yr6, Yr7, Yr8, Yr9, Yr17, Yr18, Yr20, Yr21, Yr25, Yr26, Yr27, Yr28, Yr29, Yr31, Yr32, YrND, YrA,*	***Yr1, Yr4, Yr5, Yr10, Yr15, Yr24, YrSD, YrCV, YrSU, YrSP***
Stem rust			
	TTTTF (94-8)	*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr30, Sr36, Sr38, SrMcN, SrTmp*	***Sr24, Sr25, Sr26, Sr31, Sr32, Sr39, Sr40***
	PTRTF	*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr36, Sr38, SrMcN, SrTmp*	***Sr7a, Sr21, Sr22, Sr23, Sr24, Sr26, Sr30, Sr31, Sr32, Sr33, Sr40***
	TKTTF	*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr17, Sr21, Sr30, Sr36, Sr38, SrMcN, SrTmp*	***Sr13, Sr22, Sr24, Sr25, Sr26, Sr27, Sr31, Sr32, Sr33, Sr39, Sr40***
	TTKTK	*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr30, Sr31, Sr38, SrMcN, SrTmp*	***Sr13, Sr22, Sr24, Sr25, Sr26, Sr27, Sr32, Sr35, Sr36, Sr39, Sr40***

[1]Source: Malihipour and Esmaeilzadeh (2023) and Omrani et al., (2024).

To ensure reliable infection, inoculated plants were placed in a dew chamber for 24 hours at 18 ± 2 °C in complete darkness under near-saturation humidity. Next, they were transferred to a greenhouse maintained at 22 ± 2 °C with a 16-hour photoperiod. Infection types (ITs) were ascertained based on the method described previously. The entire experiment was conducted twice with two biological replicates per genotype-isolate combination in order to ensure data robustness.

Evaluation of adult plant resistance under field conditions

A field study was carried out to evaluate adult plant resistance to stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) and stem rust (caused by *Puccinia graminis* f. sp. *tritici*) under artificial epiphytotic conditions for stripe rust and natural infection conditions for stem rust. The experiment was undertaken at the Wheat Rusts Pathology Experiment Farm of the Ardabil Agricultural Research Station, Ardabil, Iran, over two consecutive cropping years. Slow-rusting resistance was assessed based on the coefficient of infection (CI), final rust severity (RS), and the relative area under the disease progress curve (rAUDPC). The experiment was established in a randomized complete block design (RCBD) with three replications. In mid-autumn, each genotype was sown in two-row plots measuring 1 m in length, with 30 cm between rows and 60 cm between plots. Susceptible checks—a mixture of McNair and CD-90-12 for stem rust, as well as Morocco for stripe rust—were planted after every ten test entries and along the entire border of the experimental area for facilitating disease spread. Standard cultural

practices such as irrigation, weeding, and fertilization were applied throughout the growing season. Artificial inoculation for stripe rust was performed between the tillering and flag leaf emergence stages using a mixture of urediniospores (collected in previous seasons) and talcum powder, employed by dusting. The inoculum comprised a bulk of pathotypes virulent against resistance genes *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr22*, *Yr23*, *Yr24*, *Yr25*, *Yr26*, *Yr27*, *YrA*, *Yr21*, *Yr31*, *Yr32*, and *YrSU*, along with avirulent against *Yr3v*, *Yr3a*, *Yr4a*, *Yr4*, *Yr5*, *Yr10*, *Yr15*, *Yr16*, *YrCV*, *YrSD*, and *YrND* (Safavi, 2019). For stem rust, the naturally occurring local population was utilized, indicating virulence against genes *Sr25*, *Sr5*, *Sr6*, *Sr7a*, *Sr9f*, *Sr9e*, *Sr13*, *Sr23*, *Sr28*, *Sr29*, *Sr30*, *Sr33*, *Sr34*, *Sr37*, *Sr DP2*, *SrGT*, *SrPL*, *SrWLD*, *SrH*, and *SrTmp* (Safavi & Malihipour, 2018).

Data collection

The disease severity and infection type (IT) were recorded at intervals of 6–7 days for stripe rust and 8–9 days for stem rust. Final rust severity (FRS) was recorded on June 14 and 17 for stripe rust as well as on July 8 and 13 for stem rust, across two consecutive growing seasons (2018–2019 for stripe rust and 2019–2020 for stem rust). Assessments began when susceptible checks reached 30–40% severity. Disease severity was scored three times based on the modified Cobb scale (Peterson et al., 1948), with ITs determined according to Roelfs et al. (1992). Genotypes were further categorized by FRS as: resistant (R, 5–10%), resistant to moderately resistant (RMR, 15–20%), moderately resistant (MR, 30%), moderately resistant to moderately susceptible (MRMS, 40%), moderately susceptible (MS, 50–60%), moderately susceptible to susceptible (MSS, 70–80%), or susceptible (S, 90–100%) (Huerta-Espino, 2020).

Data analysis

The CI was calculated through multiplying disease severity by a constant value assigned to each host response: immune = 0.0, R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, MSS = 0.9, S = 1.0 (Stubbs et al., 1986). The area under the disease progress curve (AUDPC), providing a more comprehensive measure of disease development over time, was estimated for each experimental unit using the formula expressed by Milus & Line (1986):

$$AUDPC = [N_1(X_1 + X_2)/2] + [N_2(X_2 + X_3)/2]$$

where X_1, X_2, and X_3 represent rust severity recorded on the first, second, and third assessment dates, respectively, and N_1 and N_2 denote the time intervals (in days) between these consecutive assessments.

The relative AUDPC (rAUDPC) was then calculated as follows:

rAUDPC = (AUDPC of a line / AUDPC of the susceptible check) × 100

Genotypes were categorized based on slow-rusting parameters as defined by Hei et al. (2015), and Srinivas et al. (2023). Cluster analysis and linear correlations between slow-rusting parameters and seedling infection types were undertaken using SPSS software (version 18).

Results

Seedling reaction

The screening for seedling resistance revealed varying infection responses among the 36 genotypes. While some genotypes proved susceptible to one or more virulent and predominant pathotypes of *Puccinia striiformis* f. sp. *tritici* (Pst) and *Puccinia graminis* f. sp. *tritici* (Pgt), others were susceptible to at least five pathotypes of both rust species. None of the genotypes indicated resistance to all pathotypes of either rust at the seedling stage. Consequently, these genotypes were advanced to field evaluations

for the characterization of adult plant slow rusting resistance. When challenged with yellow rust pathotype 174E150A+, Yr27, 11 out of 36 assessed genotypes displayed susceptible reactions (infection types 3^{+}–4), while 24 genotypes showed resistant responses (infection types 0–2). Screening with another yellow rust pathotype, 14E158A+, Yr27, demonstrated that five genotypes were susceptible and 30 were resistant; the reaction of genotype C-97-14 remained undetermined at the seedling stage. For stem rust, all genotypes except C-96-9 manifested susceptibility to at least one of the four tested pathotypes. Among the evaluated genotypes, 11, 8, 17, and 5 showed resistant reactions against pathotypes TTTTF, PTRTF, TKTTF, and TTKTK, respectively. Tables 3 and 4 summarize the seedling assessment results for the 36 promising lines, along with the susceptible check.

Assessment of adult plant slow-rusting resistance

The slow-rusting response of the 36 wheat genotypes to stripe rust (*Puccinia striiformis f. sp. tritici*) and stem rust (*Puccinia graminis f. sp. tritici*) was characterized using three key parameters: the coefficient of infection (CI), final rust severity (FRS), and the relative area under the disease progress curve (rAUDPC). The detailed results for the three parameters regarding both yellow rust and stem rust are reported in Tables 3 and 4, respectively, with the corresponding classification summaries outlined in Tables 5 and 6.

Coefficient of infection (CI)

Based on CI values (Tables 3 and 4), the genotypes were categorized into different resistance levels, with CI values of 0–20, 21–40, and 41–60 corresponding to high, moderate, and low resistance, respectively. For stripe rust, the vast majority of genotypes (30 out of 36), including C-96-2, C-96-3, C-96-4, and C-97-8, were classified as highly resistant. Only three genotypes (C-96-11, C-96-14, and C-97-7) presented moderate resistance, two (C-97-3 and C-97-6) showed low resistance, and a single genotype (C-96-1) was identified as susceptible, with a CI value of 68, matching the response of the susceptible check (Table 3). A similar analysis for stem rust demonstrated a different resistance pattern. Only two genotypes (C-96-9 and C-97-9) were highly resistant, with nine genotypes (including C-96-2, C-96-7, and C-97-12) exhibiting moderate resistance. The majority of genotypes (21 out of 36, such as C-96-5 and C-97-1) fell into the low resistance category, and four genotypes (C-96-1, C-96-3, C-97-6, and C-97-20) were susceptible, with CI values approaching the susceptible check (Table 4).

Final rust severity (FRS)

The FRS-based classification (Tables 3 and 4) confirmed the absence of immune genotypes as well as reinforced the patterns of partial resistance observed with CI. Under high disease pressure, the resistance category distributions of genotypes for both rust species were largely in line with their corresponding CI classifications. For stripe rust, 30 genotypes were highly resistant (FRS 1–30%), three were moderately resistant (FRS 31–50%), and two had low resistance (FRS 51–70%). For stem rust, two genotypes revealed high resistance, nine moderate, twenty-one low, and four were susceptible. Notably, the same susceptible genotypes (C-96-1 for stripe rust; C-96-1, C-96-3, C-97-6, and C-97-20 for stem rust) were identified by both parameters, confirming their high vulnerability under field conditions.

Relative area under the disease progress curve (rAUDPC)

The rAUDPC values, reflecting the temporal progression of disease, provided complementary insights into the slow-rusting profiles. Genotypes with rAUDPC values ≤30% of the check were considered highly resistant. In spite of the establishment of

infection and development of chlorotic and necrotic lesions, manifesting as moderately resistant (MR) to moderately susceptible (MS) infection types, many genotypes effectively suppressed disease progress, as evidenced by their suppressed rAUDPC values. The classification based on rAUDPC (Tables 3 and 4) strongly aligned with the findings from CI and FRS. Thirty genotypes indicated high resistance to stripe rust and effectively suppressed epidemic development. Further, the resistance classifications (moderate, low, or susceptible) of the remaining genotypes, as determined by other assessment methods, revealed strong agreement with their rAUDPC values. For stem rust, the distribution of genotypes was identical to previous classifications; it highlighted two highly resistant genotypes (C-96-9, C-97-9) and four that were consistently susceptible (C-96-1, C-96-3, C-97-6, C-97-20).

Collectively, the integrated analysis of CI, FRS, and rAUDPC consistently characterized the slow-rusting resistance in the wheat genotypes. The results verify a robust pattern of high-level resistance to stripe rust in most genotypes, while the response to stem rust was more variable, with a broader distribution towards moderate and low resistance. The identities of the most resistant and susceptible genotypes remained consistent across all three evaluation methods, reliably identifying promising candidates for breeding. This comprehensive assessment confirms the presence of diverse and potentially durable slow-rusting resistance in the tested germplasm. Tables 3 through 6 provide detailed values for all genotypes and parameters.

Association between seedling and slow rusting resistance parameters

In this study, we further explored the relationship between various adult plant slow rusting resistance parameters across the 36 wheat genotypes and susceptible checks for both stripe and stem rusts. The Coefficient of Infection (CI) is widely recognized as a key parameter for such evaluations. A strong and highly significant positive correlation was found among the adult plant resistance parameters. Specifically, for stripe rust, CI revealed a very strong relationship with Final Rust Severity (FRS) ($R^2 = 0.984$) and with the relative area under the disease progress curve (rAUDPC) ($R^2 = 0.959$). Likewise, for stem rust, CI was strongly correlated with FRS ($R^2 = 0.987$) and with rAUDPC ($R^2 = 0.957$) (Table 7). Further, the association between seedling infection types (IT) and adult plant slow rusting parameters was inspected. Notably, a very low and non-significant correlation was found between seedling reactions and all epidemiological parameters (FRS, CI, and rAUDPC) for both yellow (stripe) rust and stem rust. This suggests that seedling resistance responses are independent of the adult plant slow rusting resistance expressed in the field.

Genetic diversity based on cluster analysis

Cluster analysis was undertaken to evaluate the genetic diversity among wheat genotypes based on seedling-stage responses and adult plant stage epidemiological parameters, including final rust severity (FRS), coefficient of infection (CI), and the relative area under the disease progress curve (rAUDPC), with the findings visually summarized in two dendrograms (Figs. 1 and 2). They illustrate the genetic relationships and grouping patterns based on resistance profiles. In both dendrograms, the susceptible checks—a mixture of ‘McNear’ and ‘CD-99-12’ for stem rust and ‘Morocco’ for stripe rust—were distinctly separated from other genotypes owing to the greatest genetic distance and were consistently placed in a separate cluster (Group D, Figs. 1 and 2). This is a clear segregation that validates the clustering methodology and serves as a reliable reference point for interpreting the resistance levels of the other genotypes.

Table 3. Results of the assessment of seedling and adult plant resistance to stripe rust in wheat genotypes during two consecutive growing seasons.

		Seedling reactions[1]		Slow rusting resistance parameters[2] at adult plant stage						Average parameters of slow rusting resistance		
				2017-2018			2018-2019					
No.	lines	174E150A+,Yr27	14E158A+,Yr27	FRS & IT[3]	CI	rAUDPC	FRS & IT	CI	rAUDPC	FRS	CI	rAUDPC
1	C-96-1	0;	0;	80MSS	72	52	70MSS	63	55	75	68	54
2	C-96-2	;1CN	0;	20RMR	6	13	30MR	12	26	25	9	20
3	C-96-3	0;	0;	10R	2	10	5R	1	3	8	2	7
4	C-96-4	0;	0;	10R	2	4	5R	1	3	8	2	4
5	C-96-5	0;	0;CN	10R	2	10	10R	2	4	10	2	7
6	C-96-6	0;	0;	30MR	12	26	10R	2	4	20	7	15
7	C-96-7	0;	0;CN	10R	2	13	20RMR	6	7	15	4	10
8	C-96-8	2C	0;1CN	20RMR	6	13	5R	1	3	13	4	8
9	C-96-9	4	4	30MR	12	35	10R	2	23	20	7	29
10	C-96-10	2C	;1CN	10R	2	13	10R	2	4	10	2	9
11	C-96-11	2C	;1CN	50MS	40	39	30MR	12	16	35	26	38
12	C-96-12	4	;2CN	10R	2	13	5R	1	3	8	2	8
13	C-96-13	3	;2CN	20RMR	6	23	10R	2	4	15	4	14
14	C-96-14	0;	;1CN	40M	24	35	40M	24	42	35	24	39
15	C-96-15	3	;1CN	30MR	12	16	5R	1	3	18	7	10
16	C-96-16	0;	0;	10R	2	13	10R	2	10	10	2	12
17	C-97-1	0	0;	20RMR	6	12	10R	2	11	15	4	14
18	C-97-2	3	0;	10R	2	9	10R	2	8	10	2	9
19	C-97-3	;1	0;CN	60MS	48	66	70MSS	63	63	70	56	65
20	C-97-4	3	0;2C	10R	2	8	20RMR	6	18	15	4	13
21	C-97-5	3	4	20RMR	6	12	30MR	12	32	25	9	22
22	C-97-6	0;	2C	50MS	40	45	60MS	48	71	60	44	58
23	C-97-7	0;	2CN	40M	24	16	40M	24	42	35	24	29
24	C-97-8	;2C	;2CN	20RMR	6	11	10R	2	11	15	4	11
25	C-97-9	;2C	;2CN	5R	1	2	10R	2	8	8	2	5
26	C-97-10	0;	0;	5R	1	2	10R	2	11	8	2	7
27	C-97-11	0;	0;1	5R	1	2	10R	2	11	8	2	7
28	C-97-12	0;	0;	10R	2	8	10R	2	3	10	2	6
29	C-97-13	3	4	5R	1	4	10R	2	3	8	2	4
30	C-97-14	ND[4]	ND	ND	ND	ND	20RMR	6	21	20	6	21
31	C-97-15	3	0;C	5R	1	2	30MR	12	29	18	7	16
32	C-97-16	3	4	5R	1	2	40M	24	37	23	13	20
33	C-97-17	0;	0;C	20RMR	6	11	10R	2	3	15	4	7
34	C-97-18	0	0;	20RMR	6	18	30MR	12	32	25	9	25
35	C-97-19	0;	;2CN	10R	2	6	10R	2	11	10	2	9
36	C-97-20	3	4	30MR	12	24	30MR	12	32	30	12	28
-	Check	4	3	100S	100	100	100S	100	100	100	100	100

[1]Based on the seedling reaction types described by Stakman et al. (1962).

[2]Slow-rusting resistance parameters: FRS, final rust severity; CI, coefficient of infection; rAUDPC, relative area under the disease progress curve.

[3]Infection types were classified according to Singh et al. (2011) as follows: MR (moderately resistant; small pustules), M (moderately resistant to moderately susceptible), MS (moderately susceptible; medium-sized pustules, with some chlorosis possible), MSS (moderately susceptible to susceptible; medium to large sized pustules), S (susceptible; large pustules, no necrosis or chlorosis).

[4]ND, not determined.

Table 4. Results of the assessment of seedling and adult plant resistance to stem rust in wheat genotypes during two consecutive growing seasons.

		Seedling reactions[1]				Slow rusting resistance parameters[2] at adult plant stage						Average parameters of slow rusting resistance		
						2018-2019			2019-2020					
No.	Lines	TTTTF	PTRTF	TKTTF	TTKTK	FRS & IT[3]	CI	rAUDPC	FRS & IT	CI	rAUDPC	FRS	CI	rAUDPC
1	**C-96-1**	2+	3+	4	3	80MSS	72	92	100S	100	100	**90**	**86**	**96**
2	**C-96-2**	3	3	4	2+	40M	24	46	60MS	48	55	**50**	**36**	**51**
3	**C-96-3**	2+	2-	2,3	2+	70MSS	63	70	80MSS	72	67	**70**	**68**	**69**
4	**C-96-4**	2+	2-	2,3	2+	60MS	48	58	70MSS	63	59	**60**	**56**	**59**
5	**C-96-5**	2+	3+	4	3	50MS	40	50	80MSS	72	59	**55**	**56**	**55**
6	**C-96-6**	2+	3	4	3+	40M	24	42	60MS	48	53	**50**	**36**	**48**
7	**C-96-7**	3	3	3	2C	20R/MR	6	23	60MS	48	37	**40**	**27**	**30**
8	**C-96-8**	3	2+	3	3+	60MS	48	65	70MSS	63	61	**65**	**56**	**63**
9	**C-96-9**	;1	1	2-	;1	20R/MR	6	23	40M	24	32	**30**	**15**	**28**
10	**C-96-10**	1	1	3	3	30MR	12	35	50MS	40	50	**40**	**26**	**43**
11	**C-96-11**	3	3	3+	3	40M	24	42	90MSS	81	73	**65**	**53**	**58**
12	**C-96-12**	3	3+	3+	3	40M	24	42	80MSS	72	73	**60**	**48**	**58**
13	**C-96-13**	3	1	3+	3	30MR	12	31	70MSS	63	61	**50**	**38**	**46**
14	**C-96-14**	3	3+	4	3	50MS	40	58	80MSS	72	73	**65**	**56**	**66**
15	**C-96-15**	3	;	;1	3	60MS	48	65	50MS	40	50	**55**	**44**	**58**
16	**C-96-16**	3	3+	4	3+	30MR	12	35	50MS	40	53	**40**	**26**	**44**
17	**C-97-1**	3	3	1+	3+	50MS	40	54	70MSS	63	74	**60**	**52**	**64**
18	**C-97-2**	1	3	2C	3+	50MS	40	54	60MS	48	65	**55**	**44**	**60**
19	**C-97-3**	4	4	3	3	50MS	40	54	60MS	48	62	**55**	**44**	**58**
20	**C-97-4**	3	3	2+	3	50MS	40	46	60MS	48	65	**55**	**44**	**56**
21	**C-97-5**	2+	3+	2-	3	60MS	48	47	50MS	40	57	**55**	**44**	**52**
22	**C-97-6**	4	3+	3	3+	70MSS	63	80	70MSS	63	74	**70**	**63**	**77**
23	**C-97-7**	4	3	3	3	60MS	48	68	70MSS	63	74	**65**	**56**	**71**
24	**C-97-8**	3	3	3	3	40M	24	37	70MSS	63	70	**55**	**44**	**54**
25	**C-97-9**	2+	3-	1	3+	30MR	12	29	40M	24	33	**35**	**18**	**31**
26	**C-97-10**	3	3	2	3	50MS	40	46	80MSS	72	86	**65**	**56**	**66**
27	**C-97-11**	3-	3	2	3	50MS	40	46	60MS	48	65	**55**	**44**	**56**
28	**C-97-12**	1+	2+	1	3	40M	24	37	50MS	40	52	**45**	**32**	**45**
29	**C-97-13**	3	3	2+	3+	60MS	48	65	70MSS	63	81	**65**	**56**	**73**
30	**C-97-14**	3	3	3	3	ND[4]	ND	ND	60MS	48	65	**60**	**48**	**65**
31	**C-97-15**	3+	3	3	3	60MS	48	68	80MSS	72	82	**70**	**60**	**75**
32	**C-97-16**	3	3-	1+	3+	40M	24	41	50MS	40	53	**45**	**32**	**47**
33	**C-97-17**	3	3	2	2+	50MS	40	54	70MSS	63	70	**60**	**52**	**62**
34	**C-97-18**	3+	3	3+	3+	50MS	40	51	50MS	40	57	**50**	**40**	**54**
35	**C-97-19**	3+	3	2+	3	40M	24	37	50MS	40	53	**45**	**32**	**45**
36	**C-97-20**	3+	3+	2+	3+	60MS	48	56	90MSS	81	98	**75**	**65**	**77**
-	**Check**	**4**	**3**	**3**	**3+**	**100S**	**100**	**100**	**100S**	**100**	**100**	**100**	**100**	**100**

[1]Based on the seedling reaction types described by Stakman et al. (1962).

[2]Slow-rusting resistance parameters: FRS, final rust severity; CI, coefficient of infection; rAUDPC, relative area under the disease progress curve.

[3]Infection types were classified according to Singh et al. (2011) as follows: MR (moderately resistant; small pustules), M (moderately resistant to moderately susceptible), MS (moderately susceptible; medium-sized pustules, with some chlorosis possible), MSS (moderately susceptible to susceptible; medium to large sized pustules), S (susceptible; large pustules, no necrosis or chlorosis).

[4]ND, not determined.

Table 5. Wheat genotypes exhibiting varying levels of slow-rusting resistance to stripe rust at the adult plant stage.

Slow rusting resistance level	Number	genotypes
High	30	C-96-2, C-96-3, C-96-4, C-96-5, C-96-6, C-96-7, C-96-8, C-96-9, C-96-10, C-96-12, C-96-13, C-96-15, C-96-16, C-97-1, C-97-2, C-97-4, C-97-5, C-97-8, C-97-9, C-97-10, C-97-11, C-97-12, C-97-13, C-97-14, C-97-15, C-97-16, C-97-17, C-97-18, C-97-19, C-97-20
Moderate	3	C-96-11, C-96-14, C-97-7
Low	2	C-97-3, C-97-6
Susceptible	1	C-96-1

Table 6. Wheat genotypes exhibiting varying levels of slow-rusting resistance to stem rust at the adult plant stage.

Slow rusting resistance level	Number	genotypes
High	2	C-96-9, C-97-9
Moderate	9	C-96-2, C-96-7, C-96-10, C-96-13, C-96-16, C-97-12, C-97-16, C-97-18, C-97-19
Low	21	C-96-4, C-96-5, C-96-6, C-96-8, C-96-11, C-96-12, , C-96-14, C-96-15, C-97-1, C-97-2, C-97-3, C-97-4, C-97-5, C-97-7, C-97-8, C-97-10, C-97-11, C-97-13, C-97-14, C-97-15, C-97-17,
Susceptible	4	C-96-1, C-96-3, C-97-6, C-97-20

Table 7. Linear correlations between slow-rusting resistance parameters and seedling infection types for yellow and stem rust among 37 wheat genotypes over two cropping seasons.

Parameters[1]	Yellow rust			Stem rust		
	FRS	CI	Seedling IT	FRS	CI	Seedling IT
CI	0.984**	-		0.987**	-	
Seedling IT	0.070	0.081	-	0.213	0.219	-
rAUDPC	0.977**	0.959**	0.124	0.970**	0.957**	0.174

[1]FRS, final rust severity; rAUDPC, relative area under the disease progress curve; CI, coefficient of infection; Seedling IT, seedling infection type. Pathotypes used: yellow rust (174E150A+, Yr27) and stem rust (TKTTF).
**Significant at the P = 0.01 probability level.

For stripe rust (Fig. 1), the remaining genotypes were classified into three main clusters: Group A comprised 30 genotypes exhibiting low values for slow-rusting parameters, indicating a high level of slow-rusting resistance and reflecting the most promising genetic pool for resistance against stripe rust; Group B included three genotypes (C-96-11, C-96-14, and C-97-7) with moderate parameter values, reflecting a moderate level of slow-rusting resistance; and Group C consisted of three genotypes (C-97-6, C-97-3, and C-96-1) that indicated high values for slow rusting resistance parameters, corresponding to a low level of slow-rusting resistance, which led to this group being considered susceptible, a classification reinforced by its proximity to the susceptible check in the dendrogram.

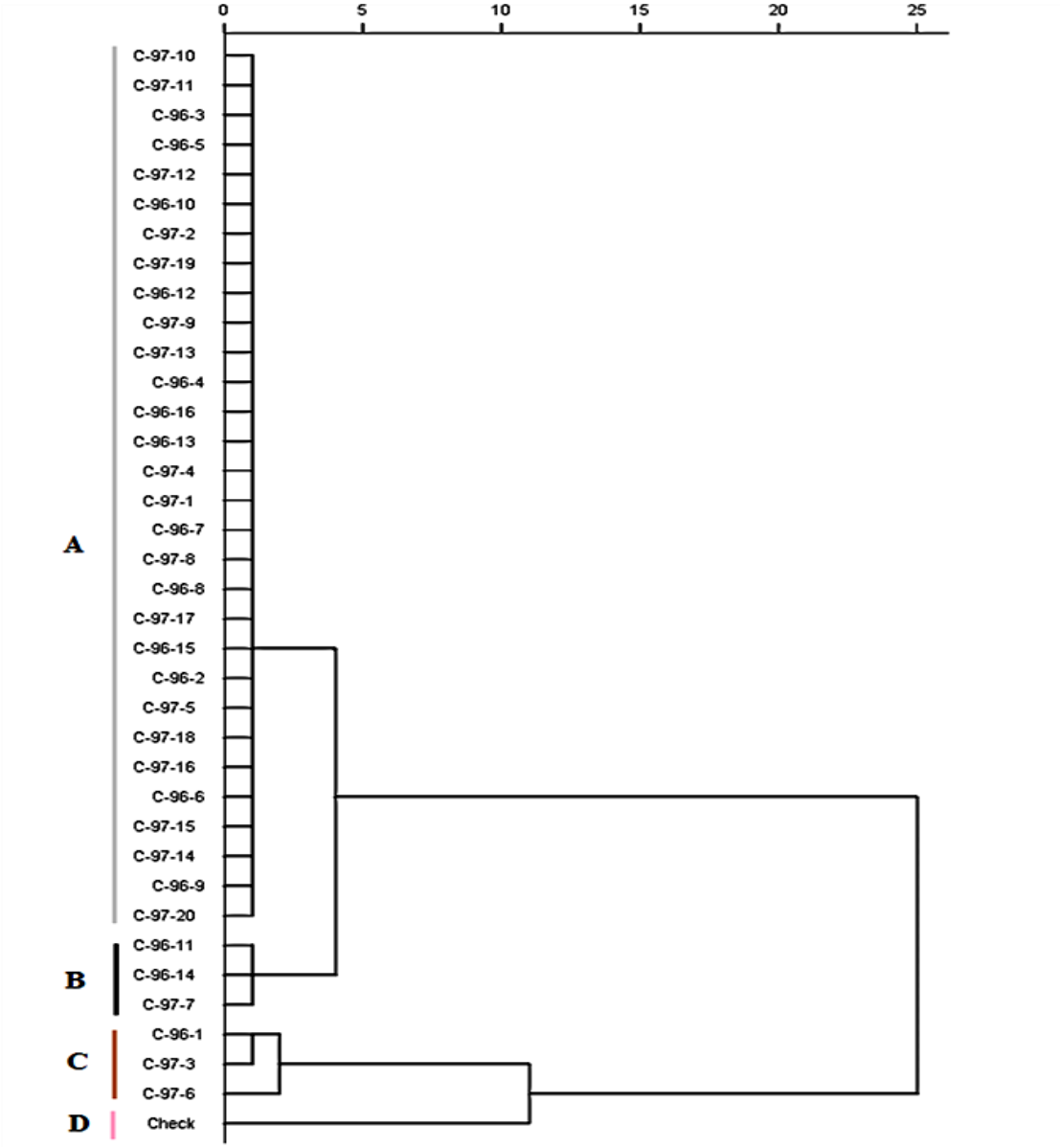


Figure 1. Cluster analysis classification of wheat genotypes based on seedling reaction and adult plant slow rusting resistance parameters to stripe rust.

For stem rust (Fig. 2), with the exception of the susceptible check and line C-96-1, the genotypes were grouped into three main clusters: Group A contained 16 promising lines revealing high to moderate values of slow-rusting parameters, which were classified as having low to moderate levels of slow-rusting resistance; Group B included 16 wheat genotypes with high to very high epidemiological parameter values, categorizing them as possessing low to no slow-rusting resistance; and Group C was formed by three genotypes (C-96-9, C-97-9, and C-96-7) which manifested low to moderate parameter values, showing a high to moderate level of slow-rusting resistance, making this group of particular interest for stem rust resistance breeding. The distinct clustering patterns observed for the two different rust pathogens underscore the specificity of resistance mechanisms, as genotypes that performed well against one rust did not necessarily cluster similarly for the other, thereby highlighting the significance of independent selection pressure and different underlying genetic factors for resistance to stripe rust and stem rust. This clear grouping based on quantitative resistance parameters successfully separates genotypes with similar phenotypic responses, providing a robust framework for selecting diverse parental lines for breeding programs with the aim of pyramiding durable, slow-rusting resistance.

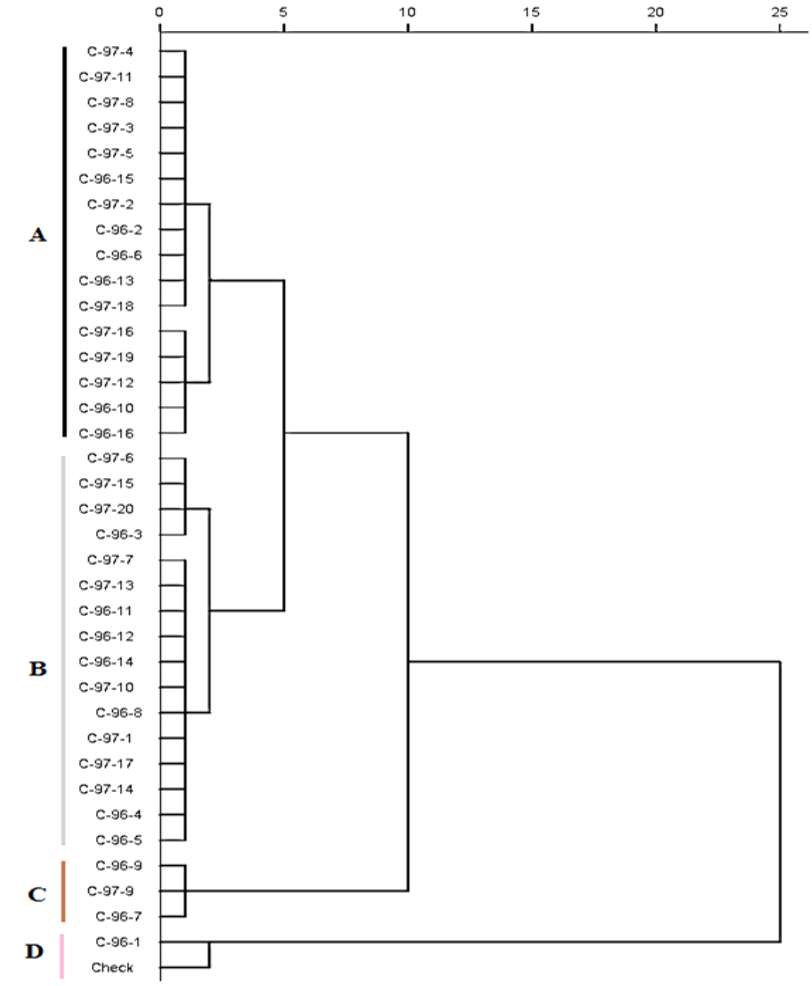


Figure 2. Cluster analysis classification of wheat genotypes based on seedling reaction and adult plant slow rusting resistance parameters to stem rust.

Discussion

Evaluating slow rusting resistance in wheat is a critical strategy for building durable defense against the persistent threat of rust diseases. Our results, obtained from ascertaining key parameters—the coefficient of infection (CI), final rust severity (FRS), and the relative area under the disease progress curve (rAUDPC)—provide significant insights that support and expand the current understanding of adult plant resistance (APR). The strong positive correlation we observed among CI, FRS, and rAUDPC confirms the reliability of these metrics for phenotyping slow rusting resistance. This relationship is well-established, as Sandoval-Islas et al. (2007) and, more recently, Srinivas et al. (2023) have reported similar correlations in wheat-stripe rust pathosystems, confirming that these parameters together capture the essence of quantitative resistance by reflecting reduced infection efficiency, longer latency periods, and lower sporulation.

A key finding from our study is the weak correlation between seedling infection types (ITs) and the slow-rusting parameters in adult plants. This discrepancy is not an exception but a fundamental feature of rust resistance genetics, underscoring the different mechanisms controlling all-stage resistance versus adult plant resistance. As established by Singh et al. (2011) and Chen & Kang (2017), race-specific seedling resistance, often controlled by major R genes, functions on a gene-for-gene basis. On the contrary, the slow rusting observed in adult plants is typically non-race-specific and conferred by combinations of minor, additive genes whose expression is developmentally regulated. This mechanistic distinction highlights the limitation of relying solely on seedling screening and reinforces the necessity for field-based evaluation, a principle strongly supported by Sandoval-Islas et al. (2007). Our results thus confirm the global consensus that durable resistance, which is often polygenic and expressed after the seedling stage, must be identified under natural field epidemic conditions.

The background of our research is the continuous struggle against evolving populations of *Puccinia striiformis* f. sp. *tritici* (*Pst*) and *P. graminis* f. sp. *tritici* (*Pgt*) in Iran, persistently causing significant yield losses (Safavi & Malihipour, 2020; Omrani et al., 2024). This is a major problem worldwide: farmers plant new resistant crops, but then new disease pathotypes appear that can destroy them. The use of such resistance, as observed globally, provides temporary protection until new virulent pathotypes emerge, resulting in sudden and widespread susceptibility (Huerta-Espino et al., 2020; Dawana et al., 2024). The rapid evolutionary potential of rust fungi, driven by mutation, migration, and recombination, accelerates this process, often making cultivars susceptible soon after their release (Hovmøller et al., 2016; Chen & Kang, 2017). Our observation of moderately susceptible to susceptible (MSS) reactions to *Pgt* in several genotypes may indicate the breakdown of such major genes, highlighting the vulnerability of this strategy and the urgent need for more resilient approaches.

Considering the known virulence structure of local pathogen populations, we propose that the seedling resistance observed may be conferred by effective genes such as *Yr4*, *Yr5*, *Yr10*, and *Yr15* for stripe rust and *Sr24*, *Sr26*, and *Sr32* for stem rust. This assumption is in accordance with recent national reports (Malihipour & Esmaeilzadeh Moghaddam, 2023; Omrani et al., 2024) and their documented historical effectiveness in

Iran (Afshari, 2008; Safavi & Malihipour, 2018). Nevertheless, this phenotypic inference needs confirmation via molecular marker analysis and seedling tests with defined pathotypes. At the adult plant stage, the resistant and moderately resistant reactions of some genotypes suggest the presence of effective all-stage resistance genes. It is a recognized phenomenon, concordant with the findings of Singh et al. (2005), that the effects of non-race-specific APR genes in such genotypes can be characterized by the stronger action of major seedling resistance genes.

An interesting epidemiological pattern arising from our field assessments was a higher frequency of susceptibility to local *Pgt* populations compared to *Pst*. This difference is a direct outcome of Iran's national breeding programs, which have historically prioritized fighting the more widespread yellow rust, thereby systematically selecting for improved *Pst* resistance over multiple breeding cycles. The virulence profile of the Ardabil rust populations, previously described by Safavi (2019) and Safavi & Malihipour (2018), offers a clear genetic explanation for the field reactions. Genotypes indicating stable resistance in Ardabil are very likely to possess genes against which the local populations are avirulent, such as *Yr5*, *Yr10*, *Yr15*, *YrCV*, and *YrND* for stripe rust and *Sr24*, *Sr26*, *Sr32*, as well as *Sr36* for stem rust.

The most promising candidates for durable resistance are those genotypes presenting moderate infection types (MR/MS) combined with low disease severity and rAUDPC values at the adult plant stage. This phenotype is a hallmark of slow rusting, often supported by high-temperature adult-plant (HTAP) resistance and the cumulative effect of multiple minor genes, as described by Singh et al. (2005, 2011) and Chen (2013). Notably, several genotypes in our study share pedigrees with internationally recognized cultivars such as 'Tukuru' and 'Kukuna', which are famous for their durable, multi-pathogen resistance. This suggests the presence of well-characterized, non-race-specific slow-rusting genes such as *Yr18*, *Yr29*, *Yr30*, and *Yr46* (Singh et al., 2011; Chen & Kang, 2017), which are growingly being introduced into Iranian germplasm (Safavi & Afshari, 2017; Mohammadi et al., 2023).

A major advantage of these APR genes is their frequent pleiotropy or close linkage, which provides resistance to multiple diseases. Complexes such as *Yr18/Lr34/Pm38/Sr57* and *Yr46/Lr67/Sr55/Pm46* are prime examples, widely utilized in CIMMYT germplasm. Their association with morphological markers such as leaf tip necrosis (Ltn) gives breeders a valuable visual tool for the initial, low-cost selection of lines carrying these durable resistance packages, a strategy effectively employed in global breeding programs (Singh et al., 2011; Kumar et al., 2020).

Our cluster analysis, based on quantitative resistance parameters, generated distinct groups of genotypes with high, moderate, and low levels of slow rusting resistance. This finding is in line with earlier studies by Ali et al. (2007) and Safavi & Afshari (2017), which also successfully classified wheat genotypes using similar phenotypic data. The cohesive clusters of genotypes with MR or MS reactions confirm that these resistance phenotypes are genetically controlled and may be mediated by quantitative mechanisms. The method of ranking and clustering genotypes based on a combination of disease severity and rAUDPC has proven

to be a robust and reliable system for ascertaining quantitative resistance, as supported by Hei et al. (2015) and Dawana et al. (2024).

The considerable variability in slow-rusting parameters observed in our panel of Iranian wheat genotypes is a positive sign, suggesting a high level of genetic diversity for rust resistance. This diversity, as also reported by Ali et al. (2007) and Safavi & Afshari (2017), is a valuable asset for breeders. It offers a broad genetic foundation for pyramiding durable resistance genes, thus lowering the risks associated with genetically uniform, susceptible varieties. The polygenic nature of slow rusting resistance is its greatest strength for durability; as suggested by Singh et al. (2011), the need for a pathogen to accumulate multiple concurrent mutations to overcome this type of resistance is a rare event in nature, thereby restricting the rapid evolution of new virulent pathotypes.

In summary, our study confirms the effectiveness of using an integrated approach—combining CI, FRS, and rAUDPC—for identifying wheat genotypes with strong slow rusting resistance to both stripe and stem rust. The resistant genotypes we detected, especially those that are susceptible at the seedling stage but demonstrate strong APR, represent valuable genetic resources. The logical next step for this work, as consistently highlighted in recent literature (Huerta-Espino et al., 2020; Srinivas et al., 2023), is to go beyond phenotypic clustering. Future work should utilize molecular marker-assisted selection and gene mapping to unravel the specific genetic architecture of this resistance. This will enable the precise pyramiding of four to five such genes, a strategy indicated to provide near-immunity (Singh et al., 2011), into high-yielding, elite wheat cultivars, securing sustainable wheat production in Iran and similar agro-ecosystems.

Conclusion

This study found significant variations in the responses of the evaluated wheat genotypes to both stripe rust and stem rust, covering both complete (race-specific) and slow-rusting (partial) adult plant resistance (APR). Among the 36 genotypes evaluated for stripe rust, 30 exhibited a high level, 3 a moderate level, and 2 a low level of slow-rusting APR. For stem rust, 2 genotypes revealed a high level, 9 a moderate level, and 21 a low level of such resistance. Notably, several genotypes—including C-96-2, C-96-7, C-96-9, C-96-10, C-96-13, C-96-16, C-97-9, C-97-12, C-97-16, C-97-18, and C-97-19—presented promising high to moderate levels of slow-rusting resistance against both rusts. It is hypothesized that pyramiding 4–5 non-race-specific (APR) genes in these genetic backgrounds could boost resistance to near-immunity levels, offering a strategy for more durable protection. Multiple cold-area Iranian wheat genotypes revealed moderate to high adult plant resistance to both stripe and stem rust. These genotypes provide valuable sources for breeding programs targeting durable resistance.

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Conflict of interest statement

The authors declare that there are no conflicts of interest regarding the publication of this article.

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