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PHENOTYPIC AND MOLECULAR ANALYSES OF WHEAT (*TRITICUM AESTIVUM* L.) SEGREGATING POPULATIONS FOR YELLOW RUST RESISTANCE

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SUMMARY

Seeking to achieve effective selection against yellow rust resistance in wheat (*Triticum aestivum* L.) was the aim of this study. Five populations (P_1, P_2, F_1, F_2, and F_3), upon development from seven cross combinations of wheat, underwent evaluation using a randomized complete block design at two locations, i.e., Agricultural Research Institute, D.I. Khan, and Cereal Crops Research Institute, Pirsabak, Nowshera, Pakistan. Phenotypic study of the generations revealed significant variations were evident among all cross combinations, except RK-23 × Seher and RK-23 × Zincol. The majority of the crosses manifested notable generation × location interaction for all yellow rust traits, except RK-23 × Zincol. The highest means for response value (0.93), rust severity (56.8%), and coefficient of infection (46.5) of yellow rust resulted in Inqilab and its derived generations (F_1–F_3) in the cross RK-23 × Inqilab. Meanwhile, the lowest values (0) were noticeable in Shahkar and in its derived population, RK-23 × Shahkar. Molecular study indicated the presence of Yr15, Yr17, and Yr18 genes in selected F_3 lines of RK-23 × Zincol, RK-23 × Shahkar, and RK-23 × Borlaug. Based on the phenotypic and molecular performance of the populations of RK-23 × Zincol, RK-23 × Shahkar, and RK-23 × Borlaug, they emerged as resistant to yellow rust.

Keywords: Wheat (*T. aestivum* L.), segregating populations, yellow rust, genotype-by-environment interactions, generation means, Yr markers, MAS

Key findings: Wheat (*T. aestivum* L.) cultivars Shahkar, Zincol, and Borlaug in cross combination with RK-23 produced the rust-resistant genotypes in F_2 and F_3 generations, and the same also have successful confirmation through molecular study. Therefore, these cross combinations could further entail exploration in future wheat breeding for rust resistance.

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INTRODUCTION

Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating diseases of wheat (*Triticum aestivum* L.) occurring across all the major wheat-growing regions of the world, including South America, North America, Asia, Europe, and Australia (Ullah *et al*., 2025). The disease is prevalent in the central, western, and northern regions of Pakistan, particularly reporting severe incidence in the northern areas of Khyber Pakhtunkhwa (Ibrahim, 2016). Yield losses due to yellow rust range from 10% to 70%, depending on the susceptibility of the cultivar, time of infection, severity, and duration of the disease, which can reach 90%–100% in susceptible cultivars under favorable environmental conditions (Leharwan *et al*., 2025).

Given its widespread occurrence and economic impact, effective management of this disease is essential for sustainable wheat production. Among various management strategies, including the application of fungicides, the development of resistant cultivars remains the most economical and environmentally sustainable approach. For this purpose, comprehensive evaluation of the wheat genotypes at phenotypic and molecular levels is essential (Salantur *et al*., 2025). Extensive work has been continuing, so far, on the already developed and tested cultivars, which is the repetition of previous studies; on the other hand, little work has been progressing on the segregating generations for screening against the disease.

Therefore, to develop new rust-resistant lines, segregating generations requires assessment under diverse environmental conditions for effective screening for yellow rust resistance. Multi-location testing enables accurate identification of resistant genotypes through the assessment of genotype × environment interactions. Furthermore, the evaluation of mean performance across the generations provides valuable insights into the inheritance and stability of rust resistance traits (Tanwar *et al*., 2025).

However, an intense need of combining phenotypic selection and molecular screening of the genotypes for yellow rust in segregating generations arises. Marker-assisted selection (MAS) has emerged as a powerful tool for accelerating the development of rust-resistant cultivars. The use of molecular markers linked to resistance genes facilitates early and precise selection of desirable genotypes. In particular, Yr15, Yr17, and Yr18 have appeared to be more effective rust-resistance genes (Abbas *et al*., 2024). Among these, Yr17 and Yr18 have already shown distinction in several Pakistani wheat cultivars (Iqbal *et al*., 2016).

Therefore, the following study's design sought to evaluate segregating populations of seven wheat cross combinations for yellow rust resistance by phenotypic and molecular analysis under two environmental conditions. Additionally, molecular screening ensued in the F_3 generation using markers linked to Yr15, Yr17, and Yr18 genes to identify and select lines with yellow rust resistance.

MATERIALS AND METHODS

The experiment on wheat (*Triticum aestivum* L.) occurred during 2022–2024 at two locations, i.e., Agricultural Research Institute (ARI), D.I. Khan (Location-1), and Cereal Crops Research Institute (CCRI), Pirsabak, Nowshera (Location-2), Pakistan. Eight wheat cultivars, namely, RK-23 (Ratta Kulachi-23), Inqilab, Seher, Gold, Shahkar, Maxipak, Zincol, and Borlaug, served as parental material.

Crosses transpired among the cultivars in the following combinations: RK-23 × Inqilab, RK-23 × Gold, RK-23 × Shahkar, RK-23 × Seher, RK-23 × Maxipak, RK-23 × Zincol, and RK-23 × Borlaug. Seeds of P_1, P_2, F_1, F_2, and F_3 generations from all cross combinations proceeded to be sown on the same date in early November 2024 at both experimental locations (ARI, D.I. Khan, and CCRI, Pirsabak, Nowshera, Pakistan) by applying the randomized complete block design (RCBD) with three replications. The row length was at 2 m by maintaining a 30 cm row-to-row distance. P_1, P_2, and F_1 generations consisted of 10

plants, while F_2 and F_3 generations of each cross combination included 300 plants. Plant-to-plant distance was at 10 cm. The data collection continued on individual plants of each generation.

Data collection for yellow rust parameters

The yellow rust pathogen, allowed to spread under natural conditions, occurred at both locations by sowing the Morocco wheat cultivar as a natural spreader after every 20 rows. The susceptibility of yellow rust was uniform on this cultivar throughout the field. Data recorded came from each plant at the onset of yellow rust symptoms. Infection types' assessment and conversion into response values followed the approach of Fayyaz *et al*. (2022). Disease severity reached visual estimation on a 0%–100% scale, based on the infected leaf area, as described by Fayyaz *et al*. (2022). Coefficient of infection (CI) determination was by multiplying the response value by the corresponding disease severity.

Statistical analysis

Analysis of variance (ANOVA) sought to assess variation among P_1, P_2, F_1, F_2, and F_3 generations of the seven cross combinations across both locations using the International Maize and Wheat Improvement Center's (CIMMYT's) software, GEA-R. Generation means entailed computation using Statistix 8 software.

Molecular analysis of promising F_3 lines for yellow rust resistance

The leaf samples from phenotypically vigorous and yellow rust-resistant plants in the F_3 generation from different crosses, along with parental cultivar RK-23, completed their collection and tagging. CTAB buffer preparation to be used in the DNA extraction comprised dissolving the 12.5 g of Tris-HCl in 1 liter of water. The solution remained on the hot plate at a temperature of 100 °C, with constant stirring at the speed of 1000 rpm. Then, dissolving 80.9 g sodium chloride continued in the solution, adding EDTA at 7.44 g/liter.

For the DNA extraction, cutting a small part, about 1 cm, from each leaf sample using a scissor incurred crushing in a mortar through the pistil by adding the liquid nitrogen. In the crushed sample, adding 500 µL CTAB buffer before mixing occurred, with the tubes kept in dry incubator at 65 °C for 40 min. After adding isoamyl alcohol and chloroform in a 1:24 ratio, the sample sustained centrifugation at 8000 rpm for 10 min. The resulting upper aqueous layer, upon shifting to a new tube, received an equal volume of cold isopropanol before mixing in the aqueous layer and incubating at -20 °C for 24 h to get the precipitated DNA. The next day, the DNA samples again received centrifugation at 8000 rpm for 10 min before washing with 70% ethanol. The pellets underwent drying and storage at -20 °C for further use.

A 1% agarose gel preparation comprises dissolving 1 g of agarose in 100 mL of TE (Tris-EDTA) buffer. The solution received heating in a microwave oven at 100 °C for approximately one minute until the complete dissolution of the agarose. After cooling to a suitable temperature, adding 3 µL of ethidium bromide ensued, with the solution poured into a gel tray and allowed to solidify. The gel proceeded to placement in an electrophoresis chamber for analysis.

The PCR master mix preparation used $MgCl_2$ (25 mM), dNTPs (10 mM each), forward primer (10 µM), reverse primer (10 µM), Taq DNA polymerase (5 U/µL), template DNA (20–100 ng/µL), and nuclease-free water in 10 × PCR buffer. The reaction mixture, as prepared, consisted of calculating the required volume for a single reaction and scaled accordingly for multiple reactions. The study used three primers to identify the linkage for Yr15, Yr17, and Yr18 genes, whose details are in Table 1. Samples placed in the PCR machine had the conditions of PCR set according to the optimized protocol of each marker.

After the completion of PCR, the samples proceeded to load into the gel electrophoresis unit with 2% agarose gel and ethidium bromide for the purpose of visualizing

Table 1. Summary of the primers used in the study.

No.	Primer's name	Sequence	Linkage	Ta	bp	Chromo-some	Reference
1	csLV34F csLV34R	5′-GTTGGTTAAGACTGGTGATGG-3′ 5′-TGCTTG CTA TTGCTG AAT AGT-3′	STS for Yr18	65	150	7D	Iqbal *et al*. (2016)
2	VENTRIUP LN2	5′-AGGGGCTACTGACCAAGGCT-3′ 5′-TGCAGCTACAGCAGTATGTACACAAAA-3′	Yr17	45	259	2AS	Iqbal *et al*. (2016)
3	gwm11F gwm11R	CCG TCG CAT CATAGGAGT CA GTTGAAATGGCG GAG AGA GG	Yr15	52	215	1BS	Shahin *et al*. (2024)

the bands. The loading of a 50 bp ladder also ensued at the start of each sample. The voltage maintenance was at 70 volts. After running the gel, viewing it was under ultra violet light to capture an image.

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance revealed significant differences among P_1, P_2, F_1, F_2, and F_3 generations for response value, severity percentage, and coefficient of infection of yellow rust. These were in the cross combinations RK-23 × Maxipak, RK-23 × Inqilab, RK-23 × Shahkar, and RK-23 × Borlaug in wheat (*T. aestivum* L.) (Table 2). These results indicate the presence of substantial genetic variability among parental lines, as RK-23 is resistant to rust, whereas Inqilab is highly susceptible. Thus, the crossing between two diverse parents may lead to the development of high variability among the offspring in the next segregating generations for yellow rust severity and coefficient of infection (Megahed *et al*., 2022; Mehta *et al*., 2025). In contrast, non-significant differences appeared among generations (P_1, P_2, F_1, F_2, and F_3) in the cross combination RK-23 × Zincol. This may be due to low genetic diversity between the parental lines, as both RK-23 and Zincol exhibited resistance to yellow rust, which may produce identical offspring for rust resistance in the segregating generations.

A significant generation × location interaction was evident for the cross combinations RK-23 × Maxipak, RK-23 × Inqilab, RK-23 × Shahkar, RK-23 × Borlaug, and RK-23 × Gold (Table 2). This interaction suggests the differential performance of genotypes across locations may be due to variation in environmental conditions (temperature and rainfall patterns) or the presence of different pathogen races. The findings of Megahed *et al*. (2022) also reported significant genotype × location interactions for yellow rust severity and coefficient of infection in their wheat study.

Mean performance of populations

RK-23 (P_1) exhibited a low mean for response values of yellow rust, i.e., 0.19 and 0.34 at location 1 and location 2, respectively, falling within the resistant to moderately resistant category. Inversely, the cultivar Inqilab (P_2) showed high response values of yellow rust, i.e., 0.92 and 0.93 at locations 1 and 2, respectively, classifying it as moderately susceptible-susceptible (MSS) and the most susceptible among all tested genotypes under study. The highest mean of response values of yellow rust in F_1, F_2, and F_3 generations of the cross combination RK-23 × Inqilab indicates the influence of cultivar Inqilab in segregating generations. These findings imply that yellow rust is transferable from susceptible parents to subsequent segregating generations (Shehab-Eldeen *et al*., 2020). Moreover, disease severity in hybrid parents gained strong influences from parental cultivars (Iftikhar *et al*., 2025). Among the parental lines (P_2), Zincol and Shahkar exhibited very low response values (0.10 and 0.00, respectively), indicating high resistance to yellow rust.

In the F_1 generation, the crosses RK-23 × Seher, RK-23 × Zincol, and RK-23 × Shahkar showed the lowest response values at both locations, revealing that these genotypes are useful for incorporating rust resistance in

Table 2. Mean squares for response value (RV), severity (S), and coefficient of infection (CI) of yellow rust for P_1, P_2, F_1, F_2, and F_3 generations, environment, and generations × environmental interaction.

Cross combinations	Source of variation	d.f.	RV	S	CI
RK-23 × Maxipak	Environment (E)	1	1.10*	239.0*	350.4*
	Generations (G)	4	3.02*	458.0*	631.4*
	G × E	4	1.10*	287.0*	410.1*
	Error	20	0.01	12.5	8.245
RK-23 × Inqilab	Environment (E)	1	0.31*	503.1*	571.6*
	Generations (G)	4	0.22*	801.5*	871.5*
	G × E	4	0.21*	178.8ns	656.7*
	Error	20	0.04	17.51	12.0
RK-23 × Seher	Environment (E)	1	0.12ns	118*	48*
	Generations (G)	4	0.12ns	275*	140*
	G × E	4	0.02ns	146*	91*
	Error	20	0.01	58	34
RK-23 × Zincol	Environment (E)	1	0.009ns	9.43ns	16.7ns
	Generations (G)	4	0.02ns	57.1ns	71.1ns
	G × E	4	0.02ns	13.0ns	14.7ns
	Error	20	0.001	4.4	5.31
RK-23 × Shahkar	Environment (E)	1	0.9*	42.6*	131.8*
	Generations (G)	4	1.3*	90.8*	270*
	G × E	4	0.4*	53.6*	209.3*
	Error	20	0.01	8.5	8.2
RK-23 × Borlaug	Environment (E)	1	1.1*	104.5*	117.3*
	Generations (G)	4	2.1*	223.7*	285.4*
	G × E	4	1.8*	134.4*	223.8*
	Error	20	0.09	57.8	7.0
RK-23 × Gold	Environment (E)	1	1.23*	451.5*	283.2*
	Generations (G)	4	4.03*	733.9*	63.1ns
	G × E	4	2.17*	589.2*	72.7ns
	Error	20	0.01	82.2	51.0

*: Significant at $P \leq 0.05$, ns: non-significant at $P > 0.05$, d.f.: degree of freedom.

further generations. In the F_2 and F_3 generations, the cross RK-23 × Shahkar consistently showed the lowest response values (0.09 and 0.01, respectively, at location-1), depicting the development of a highly resistant population (Table 3). This highlights the potential of Shahkar as a valuable parent for improving yellow rust resistance. Similarly, El-Hawary *et al*. (2022) reported the rust-resistant parent in combination with a moderately resistant parent often generates rust-resistant lines in segregating generations, which were consistent with this study's results. The use of yellow rust-resistant parental cultivars in cross combinations increases the probability of obtaining highly resistant progenies in the segregating generations by facilitating the accumulation of favorable rust resistance alleles (Salantur *et al*., 2025).

The parental cultivar RK-23 exhibited low disease severity percentage values of yellow rust (4.13% and 9%) at locations 1 and 2, respectively. In contrast, Inqilab showed the highest severity (49.16%), confirming its high susceptibility to yellow rust. In the cross RK-23 × Inqilab, the F_1 generation exhibited the highest severity values (25% and 12% at location 1 and location 2, respectively), indicating the transmission of susceptibility from the parent. This tendency continued in the F_2 generation, where the highest severity (14.32% and 16.15%) was remarkable for the same cross, suggesting the need for careful selection in segregating generations. In the same way, RK-23 × Inqilab displayed the

Table 3. Mean performance of P_1, P_2, F_1, F_2, and F_3 generations for response value (RV), severity percentage (S), and coefficient of infection (CI) of yellow rust.

Crosses		Generations	RV		S		CI	
			Location-1	Location-2	Location-1	Location-2	Location-1	Location-2
RK-23 Maxipak	×	P_1	0.19 ±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.3±0.80	7.65±1.3
		P_2	0.40±0.06	0.75±0.06	11.2±2.10	31.2±3.3	5.0±1.10	26.5±3.1
		F_1	0.17±0.09	0.70±0.09	6.33±1.70	14.5±2.4	3.2±0.90	12.5±2.2
		F_2	0.43±0.04	0.42±0.03	10.26±2.3	11.5±1.9	7.7±1.20	9.4±1.80
		F_3	0.59±0.05	0.50±0.04	14.92±3.1	15.0±2.7	11.8±1.9	13.0±2.1
		$LSD_{0.05}$	0.32	0.32	9.30	10.12	5.1	18.10
RK-23 Inqilab	×	P_1	0.19±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.30±0.8	7.6±1.80
		P_2	0.92±0.03	0.93±0.02	56.8±3.60	36.7±3.0	46.5±3.2	31.0±3.0
		F_1	0.60±0.01	0.50±0.05	25.0±2.90	12.0±2.2	15.0±1.5	6.50±1.0
		F_2	0.46±0.04	0.46±0.04	14.32±1.5	16.1±2.8	11.1±1.9	13.3±2.4
		F_3	0.45±0.05	0.49±0.03	16.67±1.1	22.3±2.9	14.2±1.6	18.4±2.3
		$LSD_{0.05}$	0.41	0.41	15.23	15.6	35.13	23.3
RK-23 Seher	×	P_1	0.19±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.3±0.80	7.65±1.6
		P_2	0.60±0.01	0.71±0.05	21.87±2.8	18.3±2.5	12.6±1.3	13.7±2.1
		F_1	0.00±0.00	0.26±0.02	0.00±0.00	3.33±1.0	0.0±0.00	2.0±0.40
		F_2	0.24±0.03	0.33±0.03	3.78±1.00	8.53±1.5	2.6±0.70	6.5±0.90
		F_3	0.34±0.04	0.33±0.03	10.92±1.6	7.76±1.3	8.08±1.4	6.23±1.3
		$LSD_{0.05}$	0.15	0.11	15.27	13.89	10.0	9.2
RK-23 Zincol	×	P_1	0.19±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.3±0.80	7.6±1.50
		P_2	0.10±0.06	0.02±0.00	1.33±0.60	0.50±0.0	0.4±0.00	0.1±0.00
		F_1	0.00±0.05	0.00±0.00	1.24±0.10	4.0±1.00	0.1±0.00	0.15±0.0
		F_2	0.21±0.04	0.2±0.01	7.05±1.60	8.0±2.00	5.5±1.20	5.0±1.30
		F_3	0.15±0.05	0.25±0.02	5.5±1.100	6.0±1.50	4.5±1.10	4.60±1.0
		$LSD_{0.05}$	0.06	0.04	3.24	7.50	4.48	6.12
RK-23 Shahkar	×	P_1	0.19±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.3±0.80	7.6±1.40
		P_2	0.16±0.02	0.00±0.00	1.00±0.40	0.0±0.00	0.8±0.00	0.0±0.00
		F_1	0.00±0.00	0.23±0.03	0.00±0.00	3.0±0.90	0.0±0.00	1.8±0.00
		F_2	0.09±0.01	0.23±0.04	1.49±0.60	4.40±1.0	1.1±0.10	3.4±0.70
		F_3	0.01±0.00	0.21±0.04	0.25±0.00	4.3±0.90	0.12±0.0	3.2±0.80
		$LSD_{0.05}$	0.13	0.17	3	6.7	1.0	7.0
RK-23 Borlaug	×	P_1	0.19±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.3±0.80	7.65±1.4
		P_2	0.30±0.02	0.72±0.05	6.67±1.20	17.0±2.2	6.0±1.20	12.9±1.9
		F_1	0.10±0.01	0.10±0.01	3.44±1.00	3.60±0.8	0.9±0.00	0.87±0.0
		F_2	0.44±0.04	0.18±0.02	12.3±2.40	5.0±1.20	9.3±1.70	3.5±0.70
		F_3	0.30±0.06	0.31±0.06	5.89	6.0±1.30	4.51±0.9	4.34±1.1
		$LSD_{0.05}$	0.24	0.54	7.50	12	6.17	12.31
RK-23 Gold	×	P_1	0.19±0.05	0.34±0.07	4.13±1.10	9.0±2.00	2.3±0.80	7.6±1.20
		P_2	0.17±0.02	0.71±0.06	4.28±1.30	35.7±2.3	2.85±0.6	25.5±2.9
		F_1	0.07±0.00	0.75±0.05	1.16±0.60	20±1.70	0.40±0.0	14.7±2.1
		F_2	0.29±0.04	0.28±0.03	8.33±1.80	8.0±1.60	5.40±1.1	5.50±1.0
		F_3	0.54±0.06	0.38±0.06	18.4±2.90	14.9±2.3	14.0±2.1	11.4±1.8
		$LSD_{0.05}$	0.15	0.31	16.1	24.3	12.3	18.41

utmost severity (22.32%) in the F_3 generation, confirming the persistent effect of the susceptible parent. Zakeri *et al*. (2016) also noted the effect of parents for susceptibility to yellow rust severity in segregating generations, which supports our findings. Dominant gene effects may have involvement in conferring to susceptibility of yellow rust from susceptible parents (Al-Husnain *et al*., 2025).

The cultivar Shahkar exhibited complete resistance (0% severity) at both locations and maintained its performance in

hybrid combinations. The cross RK-23 × Shahkar showed minimal severity in F_1 (0% and 3%), F_2 (1.49%), and F_3 generations (0.25% and 4.35% at location 1 and location 2, respectively), exhibiting the lowest values among all cross combinations (Table 3). This demonstrates the strong contribution of Shahkar in conferring resistance to yellow rust and manifests its suitability as a parent in breeding programs. Afridi (2016) also reported the development of rust-resistant lines following the crossing of cultivar Shahkar with another parent. The utilization of rust-resistant parents in crossing may lead to the combining of multiple favorable alleles, which produces highly rust-resistant progenies (Wu *et al*., 2025).

The cultivar RK-23 exhibited low coefficient of infection (CI) values (2.31 and 7.76 at location 1 and location 2, respectively), confirming its resistance to yellow rust. However, Inqilab showed the highest CI values (46.5 and 31), depicting extreme susceptibility. The cross RK-23 × Inqilab displayed the highest CI values in F_1, F_2, and F_3 generations (15, 11.1, and 14.26, respectively, at location 1), revealing the strong influence of the susceptible parent. Similar patterns were noteworthy at location 2, where F_2 and F_3 generations showed the highest CI values (13.3 and 18.41, respectively). According to findings of Shehab-Eldeen *et al*. (2020) and Saeed *et al*. (2022), the crosses involving susceptible parents tend to exhibit higher infection levels in subsequent generations, which were consistent with our results. Another study reveals that parental alleles strongly influence the response to yellow rust (Jung *et al*., 2025).

Conversely, the cultivar Shahkar gave the lowest CI values (0.8 and 0.0 at location 1 and location 2, respectively). Its cross combination with RK-23 (RK-23 × Shahkar) also maintained low CI values in F_1, F_2, and F_3, thus presenting the lowest infection levels (Table 3). This indicates effective transmission of resistance traits and highlights Shahkar as a valuable donor parent for yellow rust resistance (Afridi *et al.*, 2017). Overall, the observed variation in CI among the generations and crosses suggested a wide scope for selection of yellow rust-resistant genotypes. The results strongly support the use of Shahkar in breeding programs for improving rust resistance.

Molecular analysis of resistance to yellow rust in F_3 lines

The Yr17 gene had a successful amplification using the VENTRIUP-LN2 primer (259 bp) in F_3 lines derived from the cross combinations RK-23 × Borlaug, RK-23 × Zincol, and three lines of RK-23 × Shahkar, selected from location 1 (ARI, D.I. Khan). Similarly, four lines of RK-23 × Zincol and two lines of RK-23 × Shahkar from location 2 (CCRI) also showed amplification (Table 4). The presence of this gene in selected lines depicts their potential utility in developing yellow rust-resistant genotypes in segregating generations. Therefore, these parental combinations can have effective usage in breeding programs aimed at incorporating resistance against yellow rust. The presence of Yr17 genes in Pakistani cultivars, as reported by Shahin *et al*. (2024), validates this study's results.

The Yr18 gene amplification used the csLV34 primer (150 bp) in 12 out of 42 F_3 lines derived from RK-23 × Borlaug and RK-23 × Zincol, as well as in five lines of RK-23 × Shahkar selected from location 1. Additionally, four lines from RK-23 × Shahkar at location 2 (CCRI) and the parental cultivar RK-23 also exhibited amplification (Table 4; Figure 1). The presence of Yr18, a gene known for conferring durable and partial resistance, suggests that its incorporation into breeding material can enhance long-term resistance against yellow rust. The identified F_3 lines carrying this gene can succeed in advancement to the F_4 generation for further selection. These results are consistent with the findings of Shahin *et al.* (2024), who also reported the presence of Yr18 in several wheat entries. Similarly, Iqbal *et al*. (2016) and Zeng *et al.* (2014) reported the occurrence of Yr17 and Yr18 genes in wheat germplasm.

The Yr15 gene detection used the gwm11 primer in seven out of 42 F_3 lines derived from RK-23 × Zincol and in six lines from RK-23 × Shahkar (Table 4; Figure 2). The

Table 4. Molecular and phenotypic analysis of F_3 lines for resistance to yellow rust.

Line No.	Cross combinations	Yellow rust data	Locations	Yr genes amplified		
				Yr17	Yr18	Yr15
1	RK-23 × Borlaug	0	DIK	+	+	-
2	RK-23 × Borlaug	3R	DIK	+	-	-
3	RK-23 × Zincol	5R	DIK	-	+	+
4	RK-23 × Zincol	0	DIK	+	-	-
5	RK-23 × Shahkar	0	DIK	-	-	-
6	RK-23 × Shahkar	0	DIK	+	-	+
7	RK-23 × Inqilab	0	DIK	-	-	-
8	RK-23 × Shahkar	0	DIK	-	+	+
9	RK-23 × Inqilab	0	DIK	-	-	-
10	RK-23 × Shahkar	0	DIK	-	+	-
11	RK-23 × Shahkar	0	DIK	-	-	+
12	RK-23 × Shahkar	0	DIK	+	-	-
13	RK-23 × Shahkar	0	DIK	-	+	-
14	RK-23 x Maxi	5MR	DIK	-	-	-
15	RK-23 × Gold	0	DIK	-	-	-
16	RK-23 × Gold	5MR	DIK	-	-	-
17	RK-23 × Borlaug	0	DIK	-	-	-
18	RK-23 × Borlaug	0	DIK	-	-	-
19	RK-23 × Shahkar	0	DIK	-	+	-
20	RK-23 × Shahkar	0	DIK	+	+	-
21	RK-23 × Borlaug	5R	CCRI	-	-	+
22	RK-23 × Zincol	5MR	CCRI	+	-	-
23	RK-23 × Shahkar	0	CCRI	-	-	+
24	RK-23 × Shahkar	0	CCRI	-	-	-
25	RK-23 × Shahkar	0	CCRI	-	-	-
26	RK-23 × Maxipak	5R	CCRI	-	-	-
27	RK-23 × Maxipak	0	CCRI	-	-	-
28	RK-23 × Gold	0	CCRI	-	-	-
29	RK-23 × Shahkar	0	CCRI	-	+	-
30	RK-23 × Inqilab	0	CCRI	-	-	-
31	RK-23 × Inqilab	5MR	CCRI	-	-	-
32	RK-23 × Zincol	5R	CCRI	+	-	-
33	RK-23 × Zincol	3R	CCRI	+	-	-
34	RK-23 × Zincol	0	CCRI	+	-	-
35	RK-23 × Shahkar	0	CCRI	+	+	-
36	RK-23 × Shahkar	0	CCRI	-	-	-
37	RK-23 × Shahkar	0	CCRI	+	+	-
38	RK-23 × Shahkar	0	CCRI	-	-	+
39	RK-23 × Shahkar	0	CCRI	-	+	-
40	RK-23 × Shahkar	5R	CCRI	-	-	-
41	RK-23 × Shahkar	0	CCRI	-	-	-
42	RK-23 × Shahkar	5R	CCRI	-	-	-
43	RK-23	2R	CCRI	-	+	-

Where; 2R, 3R, and 5R indicate 2%, 3%, and 5% resistance, respectively, and 5MR: 5% moderate resistance. + Sign indicates the presence of a particular gene while – sign shows the absence.

consistency between molecular detection and phenotypic resistance further strengthens the reliability of the results. The presence of Yr15, known for providing strong resistance to yellow rust, enhances the breeding value of these lines. These findings gained support from Zhang *et al*. (2023), who reported the occurrence of Yr15 in multiple wheat genotypes. Moreover, Abbas *et al.* (2024) also confirmed the presence of Yr15, Yr17, and Yr18 genes in Pakistani wheat cultivars.

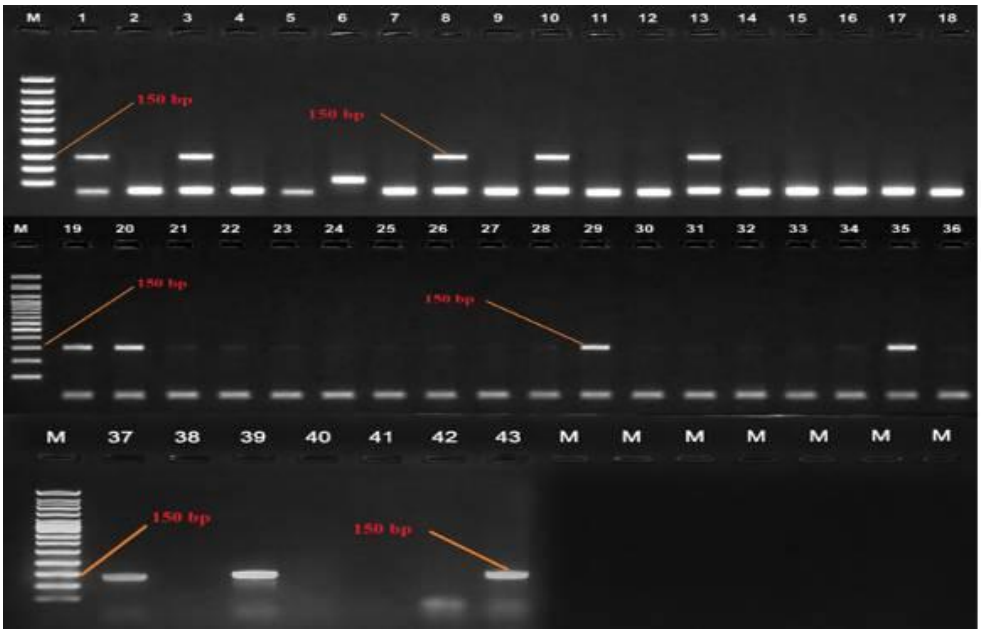

Figure 1. Electropherogram of csLV34 primers associated with Yr18 genes, where M is 50 bp size ladder, 150 bp is the primer size.

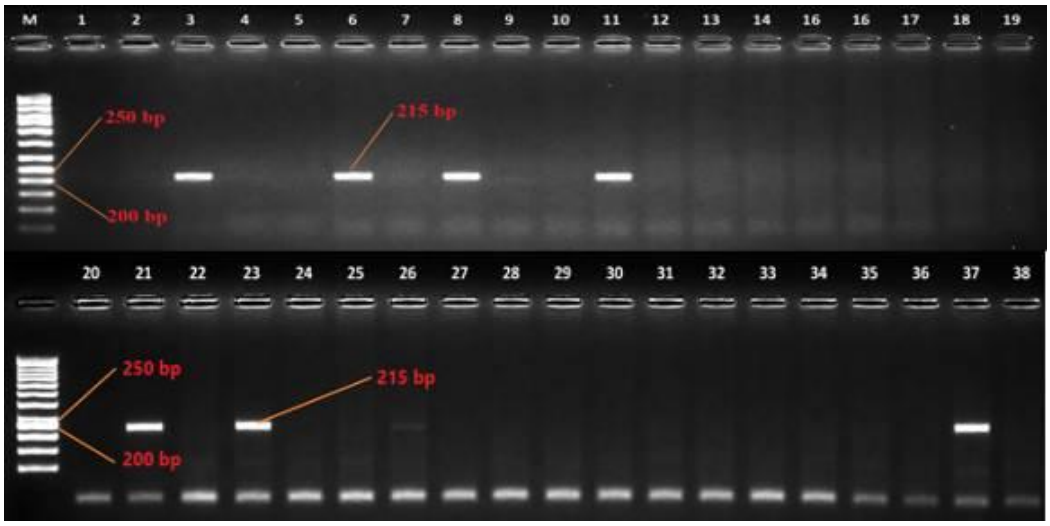

Figure 2. Electropherogram of gwm11 primers associated with Yr15 genes, where M is 50 bp size ladder, 215 bp is the primer size.

The simultaneous presence of Yr17 and Yr18 genes in three selected F_3 lines of RK-23 × Shakhar and one line of RK-23 × Borlaug is promising. Likewise, Yr18 and Yr15 genes in lines of RK-23 × Zincol and RK-23 × Shahkar highlight the potential for gene pyramiding. These proved an effective strategy for achieving resistance against yellow rust. The integration of molecular markers with phenotypic selection further supports the application of marker-assisted selection (MAS) in wheat breeding programs (Shahin *et al*., 2024). In contrast, none of the three resistance genes (Yr15, Yr17, and Yr18)

reached amplification in the F_3 genotypes derived from the cross combinations RK-23 × Gold, RK-23 × Maxipak, and RK-23 × Inqilab. This implies that these parental cultivars lack the genes; however, resistance to yellow rust may be due to the involvement of other genes.

CONCLUSIONS

The wheat (*T. aestivum* L.) cultivar Inqilab, being highly susceptible to yellow rust, produced susceptible populations in segregating generations when crossed with other wheat cultivars. In contrast, the cultivar Shahkar proved the most promising parent for the development of rust-resistant genotypes in cross combinations with moderately resistant cultivars. Similarly, Zincol and Borlaug, when crossed with RK-23, also produced rust-resistant lines. Therefore, the cross combinations RK-23 × Zincol, RK-23 × Shahkar, and RK-23 × Borlaug could further undergo advancement and exploration in wheat breeding programs for the development of yellow rust resistance.

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