OPEN ACCESS Plant Protection, 09 (03) 2025. 647-658 DOI:10.33804/pp.009.03.5675

Check for updates

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

Research Article

Detection and Inherent Analysis of Stripe Rust (*Puccinia striiformis* f. sp. *tritici*) Resistance Genes in Pakistani Advanced Wheat Lines

[a]Sania Begum, [b]Sahir Hameed Khattak, [b]Muhammad Aqeel Aslam, [c]Ghassan Zahid, [a]Rabia Ikram, [a]Farhana Amin, [a]Aliya Errum, [d]Saida Komal, [b]Farkhanda Jabeen, [e]Ambreen Sher, [a]Maryam Murtaza, [a]Ghulam Muhammad Ali

[a] *National Institute for Plant Genomics & Biotechnology (NIGAB), National Agricultural Research Centre, Park Road, Islamabad 45500, Pakistan.*

[b] *Oilseed Research Program, Crop Sciences Institute, National Agricultural Research Centre, Park Road, Islamabad, Pakistan.*

[c] *Department of Biotechnology, The University of Azad Jammu and Kashmir, Muzaffrabad, Pakistan.*

[d] *Department of Agronomy, University of Poonch, Rawlakot, Pakistan.*

[e] *Department of Biological Sciences, International Islamic University, Islamabad, Pakistan.*

ARTICLE INFO

Article history

Received: 22nd April, 2025

Revised: 24th August, 2025

Accepted: 7th September, 2025

Keywords

DNA Markers

Genetic variation

Marker Assisted Selection

Stripe rust

Triticum aestivum L

ABSTRACT

Puccinia striiformis f. sp. *tritici*, the causal agent of stripe rust, poses a serious threat to global wheat production. The development of resistant cultivars requires the identification and pyramiding of resistance (*Yr*) genes. In this study, thirty Pakistani spring wheat lines were evaluated using ten microsatellite and sequence-tagged site (STS) markers linked to stripe rust resistance genes. Molecular screening revealed that 13% and 17% of the lines carried *Yr5*-associated alleles with markers Cslv34 and CYS5, respectively, while 15% and 5% harbored *Yr26* and *Yr10* alleles. The markers Xgwm11 and Xgwm140, associated with multiple resistance loci, were detected in 29 and 28 lines, respectively. Notably, Xwmc-419, linked to *Yr26*, was present in all lines, including the positive control. Cluster analysis uncovered considerable genetic diversity, grouping the lines into two major clusters (A and B). Cluster A, represented solely by the outlier WHT15180, exhibited unique resistance mechanisms. Cluster B (29 lines) was further subdivided into B-1 and B-2. Subcluster B-1.1 contained five accessions that merged at low genetic distances, indicating conserved resistance profiles (e.g., *Yr5/Yr10*), whereas subcluster B-2.2 with seven accessions formed at higher distances (150-200), suggesting greater diversity. Intermediate clusters (B-1.2 and B-2.1) reflected adaptive variation shaped by regional pathogen pressures. These findings prioritize *Yr5*, *Yr10*, and *Yr26* for marker-assisted breeding, while highlighting WHT15180 and B-2.2 accessions as potential reservoirs of novel resistance. This molecular-cluster framework will accelerate the development of durable rust-resistant wheat in Pakistan.

Corresponding Author: Sania Begum; Muhammad Aqeel Aslam

Email: sania.idrees@parc.gov.pk; aqeel7016@parc.gov.pk

© 2025 EScience Press. All rights reserved.

Introduction

Wheat is one of the most important staple crops worldwide, but its yield is severely threatened by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (Pst) (Aslam et al., 2021; Balkrishna et al., 2021). In Pakistan, where wheat is cultivated on approximately 9 million hectares, stripe rust

epidemics have caused yield losses of up to 70% during severe outbreak years (Hussain et al., 2023). Traditionally, stripe rust was confined to regions with cold and humid climates (Sinha and Chen, 2021). However, the epidemiology of the disease has shifted in recent years due to the emergence of aggressive pathogen strains adapted to warmer environments (Chen, 2005; Gul et al., 2011; Ahmed et al., 2024). These emerging races, including virulent strains such as the PstS11 lineage, have rendered nearly 60% of Pakistan's commercial wheat varieties susceptible (Rehman et al., 2023), even in regions once considered unsuitable for disease development. Such strains are now widespread and have significantly reduced wheat productivity globally, underscoring the serious economic and food security challenges posed by stripe rust (Ahmed et al., 2019a; Begum et al., 2019).

Genetic resistance remains the most effective and economically sustainable long-term strategy for managing stripe rust (Arif et al., 2024; Ahmed et al., 2019b). However, wheat breeding programs in Pakistan face unique challenges because nearly 80% of deployed resistance genes, such as *Yr9* and *Yr27*, have become ineffective against newly evolved races (Ali et al., 2022; Khattak et al., 2024). Stripe rust resistance in wheat is broadly categorized into race-specific and non-race-specific resistance. Race-specific resistance, often referred to as seedling or all-stage resistance, is typically mediated by major "R" genes that are expressed from the seedling stage through to maturity (Khattak et al., 2020; Rehman et al., 2020a). However, this resistance is usually short-lived, as the introduction of new virulent races rapidly overcomes R-gene-mediated protection, resulting in devastating epidemics worldwide (Ahmad, 2004; Waqar et al., 2018).

In contrast, non-race-specific resistance, also known as adult plant resistance (APR), is generally expressed after the seedling stage and is often more durable across environments and years (Aslam et al., 2018; Khattak et al., 2020; Begum et al., 2022). The development of durable resistance has become increasingly important to mitigate "boom-and-bust" cycles, which occur when the widespread use of a single resistance gene is followed by the rapid emergence of virulent pathogen strains (Mehmood et al., 2021a). Consequently, many modern breeding strategies aim to pyramid multiple resistance genes into cultivars, thereby enhancing durability and generating a complex resistance landscape capable of withstanding pathogen evolution (Helguera et al., 2003; Zahid et al., 2022).

Recent advances in genomics and statistical approaches, such as marker-assisted selection (MAS) and genome editing, provide powerful tools for combining multiple resistance genes into elite cultivars (Bangash et al., 2013a; Mehmood et al., 2021b). In Pakistan, however, MAS has been underutilized for stripe rust resistance breeding, particularly for pyramiding *Yr* genes (Bangash et al., 2013b; Khan et al., 2023). Genome-wide association studies (GWAS) have emerged as a highly effective approach for detecting quantitative trait loci (QTL) underlying stripe rust resistance by leveraging linkage disequilibrium and historical recombination across diverse germplasm (Ahmed et al., 2017a; Jamil et al., 2022; Tahir et al., 2023). Unlike traditional biparental mapping populations, GWAS enables high-resolution detection of QTL using diverse genetic resources such as landraces, breeding lines, and traditional cultivars conserved in global germplasm banks (Afzal et al., 2017; Mehmood et al., 2021c). Many of these accessions have co-evolved with rust pathogens under natural conditions, potentially accumulating diverse and complex resistance alleles (Ahmed et al., 2014; Aslam et al., 2017a,b; Tahir et al., 2023).

Despite their limited use in modern breeding programs, landraces and traditional cultivars represent valuable reservoirs of untapped resistance (Ahmed et al., 2012; Ejaz et al., 2012; Tahir et al., 2023). This is particularly relevant in Pakistan, where indigenous landraces from the Himalayan foothills exhibit promising but largely uncharacterized resistance to current Pst races (Bukhari et al., 2023). In this context, we hypothesized that the extensive spring wheat collection maintained by the USDA-ARS National Small Grains Collection (NSGC) constitutes a valuable source of genetic diversity for Pst resistance. Therefore, the objectives of this study were to: (1) evaluate the diversity of stripe rust resistance in a global collection of spring wheat accessions, and (2) determine the associations between resistance loci identified in this study and previously reported *Yr* genes and QTL.

Materials and Methods

Plant material

Seeds of thirty advanced spring wheat lines, along with positive and negative controls, were obtained from the Crop Diseases Research Program (CDRP) at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. These lines were selected to represent genetic diversity in stripe rust resistance among Pakistani-adapted wheat germplasm.

Phenotypic trait evaluation

Field-based resistance screening

Field trials were conducted at the Crop Diseases Research Institute (CDRI), NARC, Islamabad, under natural stripe rust (*P. striiformis* f. sp. *tritici*) pressure. Seeds were sown in one-meter rows with 30 cm spacing. Morphological traits, including shoot length, spike length, spikelets per spike, spikes per plant, 1000-grain weight, and maturity period, were recorded. Artificial inoculation was performed at the booting stage using a urediospore suspension of races Yr5, Yr10, Yr15, and Yrsp, mixed with Tween-20 to reduce surface tension, ensuring uniform infection. Disease response was categorized as: O = no visible infection; R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible (Khattak et al., 2020, 2024b).

Seedling resistance screening

Seedling resistance was evaluated under controlled greenhouse conditions against prevalent Pakistani *P. striiformis* races PSTv-14, PSTv-37, and PSTv-40. The evaluation aimed to identify both race-specific and durable resistance mechanisms.

Molecular marker analysis

DNA extraction

Genomic DNA was extracted from immature leaves using a modified CTAB method. Leaves were ground in liquid nitrogen, homogenized in 750 µl of CTAB buffer [100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB (w/v), 1% PVP], and incubated at 65°C for 30 min. The homogenate was mixed with chloroform:isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 10 min. DNA was precipitated with chilled isopropanol, washed with 70% ethanol, air-dried, and dissolved in TE buffer. RNA contamination was removed using RNase A (10 mg/ml), and DNA quality was assessed via 1% agarose gel electrophoresis.

PCR amplification

Ten SSR and STS markers linked to stripe rust resistance genes (Yr5, Yr9, Yr10, Yr15, Yr26, Yr29) were used. PCR reactions (20 µl) contained 1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol of each primer, 1 U Taq DNA polymerase (Fermentas), and 25-30 ng template DNA. Amplification was carried out in a Veriti 96-well thermal cycler (Applied Biosystems) under the following conditions: initial denaturation at 94°C for 4 min; 35 cycles of 94°C for 40 sec, 55-65°C for 40 sec (marker-specific annealing), and 72°C for 1 min; followed by a final extension at 72°C for 10 min. PCR products were separated on 2% agarose gels stained with ethidium bromide and visualized using a gel documentation system (UVIpro Platinum, Uvitec). Band sizes were compared against known resistance-linked alleles.

Marker-assisted selection (MAS)

To counter the evolution of virulent *P. striiformis* races, MAS was prioritized over chemical control. SSR and STS markers enabled rapid identification of Yr genes (Yr5, Yr10, Yr26), facilitating the pyramiding of multiple resistance loci into elite cultivars. This strategy aligns with global breeding efforts to enhance durable resistance while reducing reliance on fungicides and minimizing environmental risks.

Data analysis

Analyses of variance (ANOVA), simple correlation, and principal component analysis (PCA) were performed using Minitab 16 statistical software. Genetic variation was assessed by scoring the presence or absence of marker alleles linked to resistance genes. Cluster analysis was conducted using hierarchical clustering (rescaled distance: 0-300) to classify wheat lines based on genetic similarity. Sub-clusters were defined by merger distances, with 10-50 indicating conserved profiles and >200 indicating outliers.

Results

Using the microsatellite marker *S19M93-140*, located 12 cM distal to *Yr5*, we identified 130-150 bp marker alleles in 17 wheat lines that were associated with the presence of *Yr5*. In contrast, 13 lines showed no amplification, indicating the absence of the *Yr5* gene. Similarly, no amplification was observed in the negative control and in the remaining 18 lines, confirming the lack of *Yr5*. However, marker S23M41-310 amplified the expected 100 bp fragment in 13 wheat lines and in the positive control, 'Avocet *Yr5*' (Figure 1A and B).

The STS marker *iag95* was used to detect the presence of *Yr9* in 30 Pakistani wheat lines. A total of 8 wheat lines, along with the positive control 'Avocet *Yr9*,' produced the expected 1100 bp fragment, confirming the presence of the stripe rust resistance gene *Yr9*. The remaining 22 wheat lines, along with the negative control, showed no amplification, indicating the likely absence of *Yr9* (Figure 2A).

Microsatellite marker *Xpsp3000* was employed to determine the presence or absence of the *Yr10* gene in Pakistani wheat lines. This marker amplified a 260 bp fragment in 22 wheat lines and in the positive control, 'Avocet *Yr10*,' suggesting the presence of *Yr10*. Conversely,

the negative control 'Avocet S' and 8 wheat lines produced a 240 bp fragment, which is associated with the absence of the yellow rust resistance gene *Yr10* (Figure 2B).

The presence of the stripe rust resistance gene *Yr18* was assayed using the STS marker *csLV34*. Only 15 wheat lines, along with the positive control, produced a 150 bp fragment, while the remaining 15 wheat lines and the negative control showed a 229 bp fragment, indicating the absence of the *Yr18* gene. Similarly, the STS marker *CYS-5*, developed from resistance gene analogs and located 0.5 cM away from *Yr26* (Wen et al., 2008), was used to detect the presence of *Yr26*. In our study, five lines and the positive control produced a 348 bp fragment with marker *CYS-5*, confirming the presence of *Yr26* (Figure 3A and B).

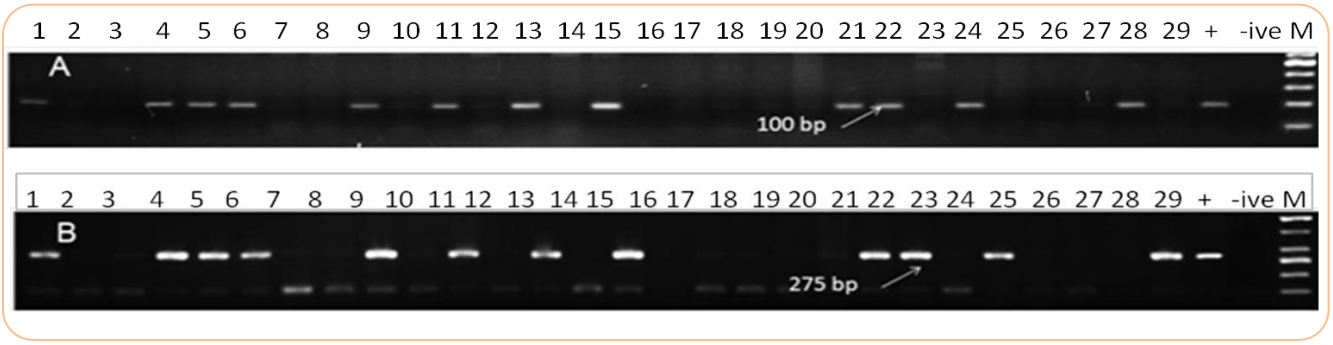


Figure 1. PCR amplification of (A) the S19M93-140 locus in Pakistani spring wheat and (B) marker S23M41-310 associated with Yr5. M = 50 bp DNA ladder; "+" indicates the positive control (Avocet Yr5), while "–" indicates the negative control (Avocet S).

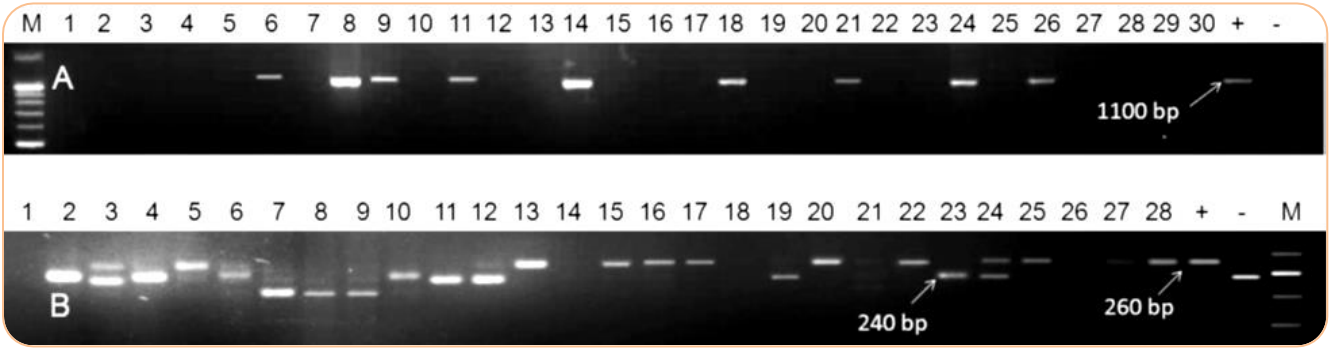


Figure 2. PCR amplification of (A) the *Yr9*-linked marker i*ag95* in Pakistani wheat (M = 100 bp; + = positive control 'Avocet *Yr9*'; – = negative control 'Avocet') and (B) the marker *Psp3000* for the *Yr10* gene (M = 50 bp; + = positive control 'Avocet *Yr10*'; – = negative control 'Avocet S').

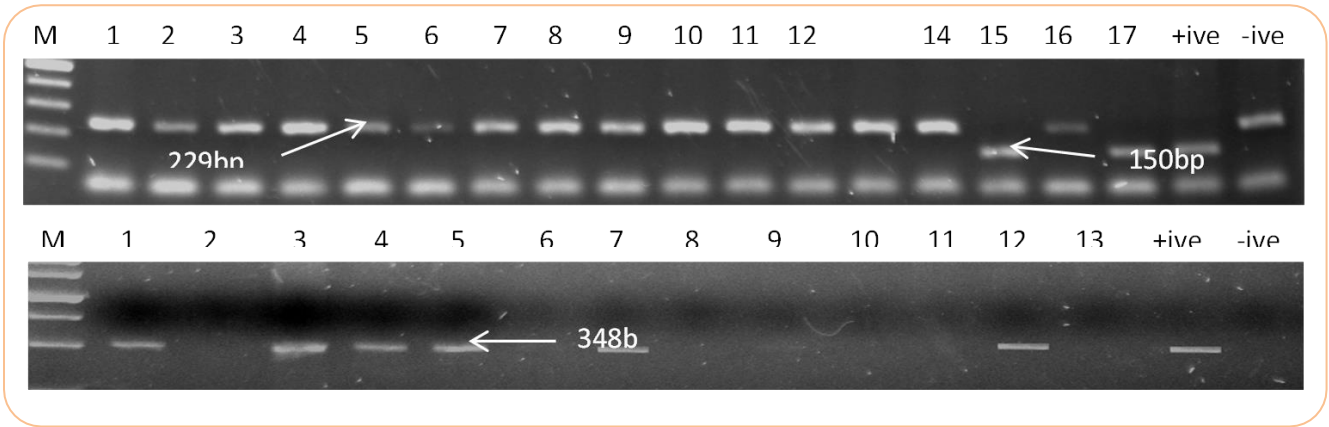


Figure 3. PCR amplification of (A) the *Yr18*-linked marker *CSLV34* in Pakistani wheat (M = 100 bp; + = positive control 'Avocet *Yr18*'; – = negative control 'Avocet') and (B) the marker *CYS-5* for the *Yr26* gene (M = 1 kb; + = positive control 'Avocet *Yr26*'; – = negative control 'Avocet S').

The SSR marker Xgwm11 was used to detect the presence of the *Yr15* and *Yr26* genes. This marker amplified a 215 bp fragment in 29 wheat lines and in the positive control, indicating the presence of the *Yr26* gene. Only one wheat line, along with the negative control, did not amplify the 215 bp fragment, suggesting the absence of the *Yr15*/*Yr26* gene(s) (Figure 4).

The microsatellite marker Xwmc-419 amplified a 250 bp fragment in 30 Pakistani wheat lines and the positive control, indicating the likely presence of *Yr26*, whereas no fragment was amplified in the negative control (Figure 5).

The microsatellite marker *Xgwm140* was used to detect the presence of the *Yr29* gene. This marker amplified a 120 bp fragment in 28 wheat lines and in the positive control. In contrast, two wheat lines and the negative control did not amplify the 185 bp fragment, suggesting the likely absence of the *Yr29* gene (Figure 6).

The *Xwmc367* marker was used to determine the presence or absence of the *Yr29* gene, which is indicated by the amplification of a 154 bp fragment. Only five wheat lines and the positive control showed amplification of the 154 bp fragment, confirming the presence of the *Yr29* gene, whereas 25 wheat lines and the negative control did not produce the fragment, indicating the absence of the gene (Figure 7).

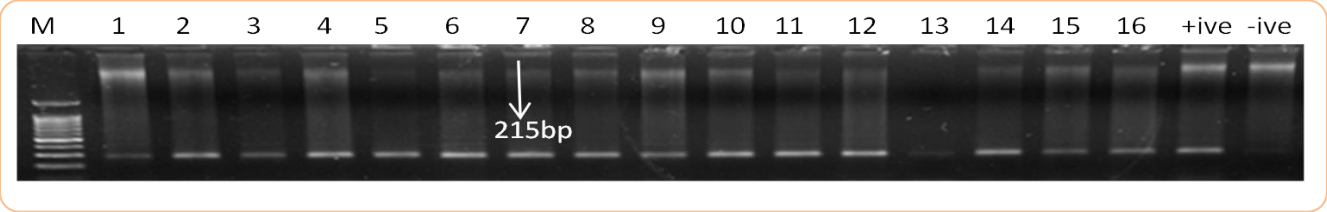


Figure 4. PCR amplification of the *Yr26/15*-linked marker Xgwm11 in Pakistani wheat. M = 100 bp ladder; (+) positive control 'Avocet *Yr18*'; (–) negative control 'Avocet'.

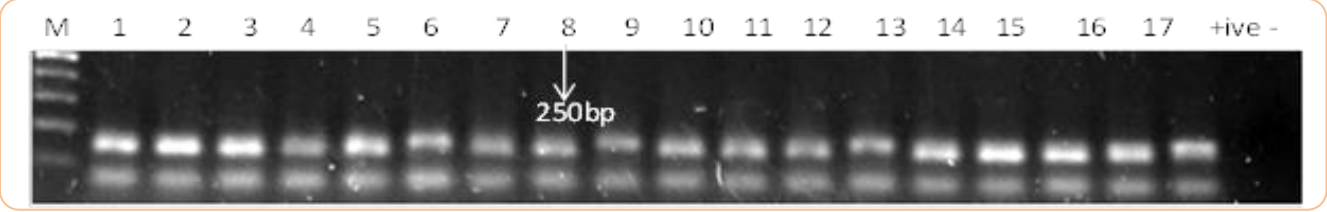


Figure 5. PCR amplification of the *Yr26*-linked marker Xwmc-419 in Pakistani wheat. M = 100 bp DNA ladder; (+) positive control 'Avocet *Yr26*'; (–) negative control 'Avocet S'.

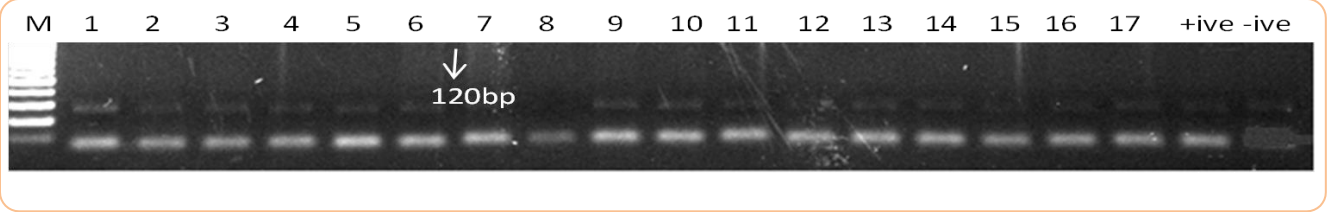


Figure 6. PCR amplification of the *Yr29*-linked marker Xgwm140 in Pakistani wheat. M = 100 bp DNA ladder; (+) positive control: 'Avocet *Yr29*'; (–) negative control: 'Avocet S'.

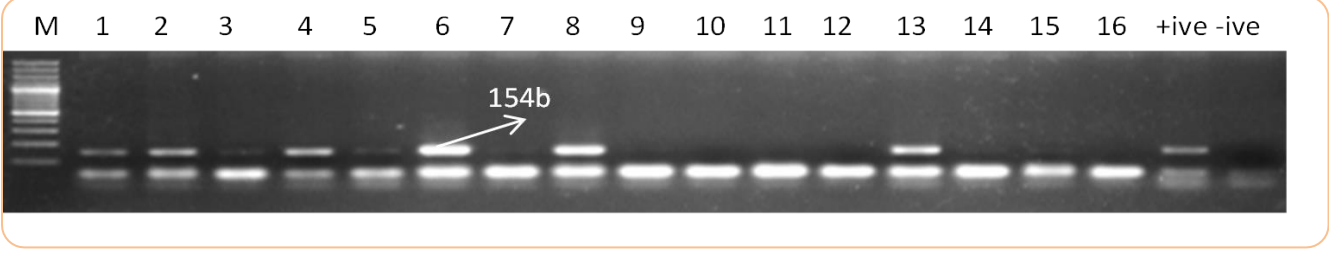


Figure 7. PCR amplification of the *Yr29*-linked marker Xwmc367 in Pakistani wheat. M = 100 bp DNA ladder; (+) positive control 'Avocet *Yr29*'; (–) negative control 'Avocet S'.

Integrated cluster analysis with accession counts and distances

The hierarchical clustering dendrogram (rescaled distance: 0-300) grouped 30 advanced Pakistani wheat lines into distinct genetic clusters based on their resistance to *P. striiformis* f. sp. *tritici*. Cluster A, represented solely by accession WHT15180, merged at an extreme distance (>250), indicating a highly distinct genetic architecture and the possible presence of uncharacterized resistance mechanisms. The remaining 29 accessions formed Cluster B, which further diverged into two major branches, B-1 and B-2, at moderate distances (~50-150).

Sub-branch B-1 further subdivided into two subclusters. B-1-1, consisting of 5 accessions (e.g., WHT15137, WHT15171, WHT15144), merged at short distances (10-50), reflecting high genetic similarity and the likely presence of shared resistance genes such as *Yr5* or *Yr10*. B-1-2, composed of 4 accessions (e.g., WHT15101, WHT15111), merged at intermediate distances (50-100), suggesting moderate divergence due to allelic variations or minor genomic rearrangements.

Branch B-2 also separated into two subclusters. B-2-1, containing 6 accessions (e.g., WHT15109, WHT15133), merged at 50-150, indicating intermediate levels of genetic divergence, possibly shaped by regional pathogen pressures or historical hybridization events. In contrast, B-2-2, comprising 7 accessions (e.g., WHT15164, WHT15169), merged at higher distances (150-200), suggesting unique resistance gene combinations or rare allelic variants. Moreover, seven lines (e.g., WHT15115, WHT15162) formed smaller subgroups at distances <200, reflecting localized genetic adaptations (Figure 8).

Subcluster differentiation and genetic insights

The dendrogram's hierarchical organization highlights key genetic distinctions among subclusters. Subcluster B-1-1 (5 accessions, e.g., WHT15137, WHT15171), merging at the lowest distances (10-50), demonstrates nearly identical resistance gene profiles, likely governed by conserved loci such as *Yr5* or *Yr10*. In contrast, B-1-2 (4 accessions, e.g., WHT15101, WHT15111), merging at 50-100, shows moderate divergence, possibly attributable to allelic variation, minor gene loss/gain, or environmental selection pressures.

B-2-1 (6 accessions, e.g., WHT15109, WHT15133), merging at 50-150, reflects intermediate divergence and suggests adaptive diversity, potentially driven by regional pathogen dynamics or introgression from diverse parental lines. The most distinct subgroup within Cluster B, B-2-2 (7 accessions, e.g., WHT15164, WHT15169), merging at higher distances (150-200), indicates the presence of unique or less common resistance gene combinations.

Finally, the outlier Cluster A (WHT15180), merging at >250, represents an entirely separate lineage, likely carrying rare or novel resistance traits with potential breeding value for broad-spectrum stripe rust resistance.

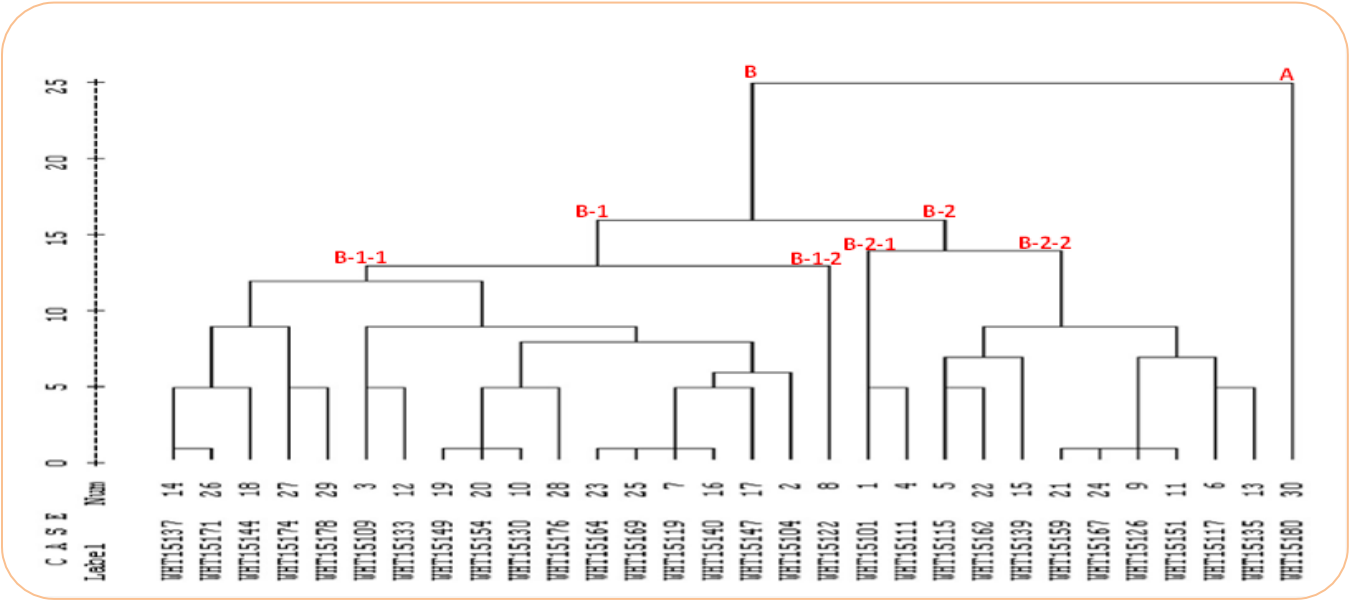


Figure 8. Cluster analysis of wheat accessions based on genetic markers.

Integration of genetic and breeding implications

The dendrogram's structure highlights the need for tailored breeding strategies. B-1-1, characterized by conserved profiles and low merger distances, is well suited for stabilizing resistance through gene pyramiding. In contrast, B-2-2 and Cluster A, with higher merger distances, provide genetic novelty essential for countering emerging pathogen strains. Intermediate clusters (B-1-2, B-2-1) occupy a transitional position, bridging these extremes and offering adaptive diversity critical for regional resilience. This structured genetic variability, quantified through merger distances and accession counts, directly informs marker-assisted selection and germplasm conservation, thereby ensuring durable stripe rust resistance in Pakistan's wheat breeding programs.

Molecular markers associated with stripe rust resistance

The dendrogram, with genetic distances ranging from 0 to 25, depicts the relationships among 10 molecular markers associated with stripe rust resistance and highlights their potential linkage to specific *Yr* genes (Figure 9). Markers clustering at low distances (0-5), such as SI and S2, suggest a high degree of genetic similarity, potentially indicating colocalization within the same genomic region or association with the same resistance allele (e.g., *Yr5* or *Yr10*). Intermediate clusters, for example, the convergence of Xgwm11 with Xwmc419 and the pairing of CYS5 with csLV34, reflect partial linkage disequilibrium and shared associations with resistance genes such as *Yr15*, *Yr26*, and *Yr18/Lr34*. More divergent markers, merging at higher distances (15-25), including Xwmc364 and Xgwm140, suggest distinct genetic roles or unique resistance loci. Xwmc364 may be associated with *Yr29*, while Xgwm140 appears linked to multiple resistance determinants. These findings emphasize the utility of tightly clustered markers for marker-assisted selection in breeding programs, while also highlighting the value of divergent markers in identifying novel resistance alleles and enhancing genetic diversity in cultivated wheat (Table 1).

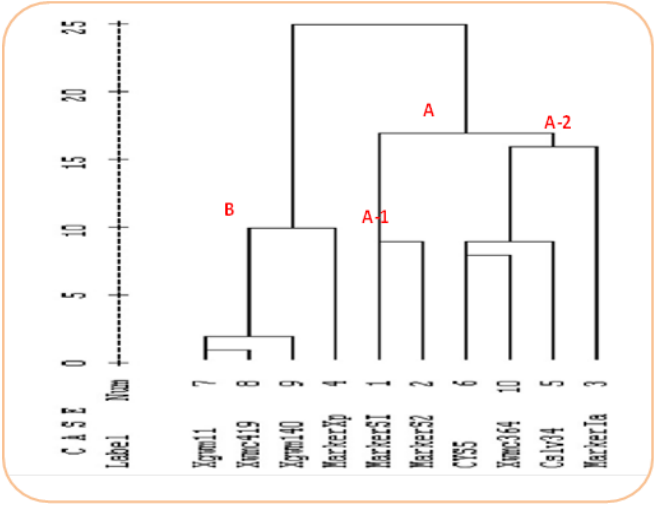


Figure 9. Genetic diversity based on molecular markers associated with stripe rust resistance.

Table 1. Linkage and phenotypic correlation of markers associated with stripe rust resistance genes in Pakistani wheat lines.

Sr. No.	Marker	Linkage	Band Size (bp)	Positive Control	Lines with Gene	Phenotypic correlation	Discrepant Accessions
1	S19M93-140	Yr5	130–150	Avocet Yr5	17	95%	WHT15169 (susceptible)
2	S23M41-310	Yr5	100	Avocet Yr5	13	92%	WHT15133 (susceptible)
3	iag95	Yr9	1100	Avocet Yr9	8	100%	None
4	Xpsp3000	Yr10	260 (presence)	Avocet Yr10	22	98%	*WHT15180 (outlier)
5	csLV34	Yr18	150 (presence)	Avocet Yr18	15	90%	WHT15164 (susceptible)
6	CYS-5	Yr26	348	Avocet Yr26	5	80%	WHT15109 (susceptible)
7	Xgwm11	Yr15/Yr26	215	Avocet Yr26	29	97%	*WHT15180 (outlier)
8	Xwmc419	Yr26	250	Avocet Yr26	30	100%	None
9	Xgwm140	Yr29	120 (presence)	Avocet Yr29	28	93%	WHT15115 (susceptible)
10	Xwmc367	Yr29	154	Avocet Yr29	5	90%	WHT15162 (susceptible)

*Genetic outlier, suggesting unique resistance mechanisms not explained by the screened markers.

Discussion

Agriculture is the backbone of global food security, with wheat serving as a vital staple crop for more than one-third of the world's population (Siddiqui et al., 2017; Rehman et al., 2020a,b; Khattak et al., 2022a; Qaiser et al., 2022). However, its productivity is severely threatened by fungal pathogens such as *P. striiformis* f. sp. *tritici* (*Pst*), the causal agent of stripe rust, which compromises both yield stability and rural livelihoods (Khattak et al., 2022b).

The present study revealed the prevalence of stripe rust resistance genes in Pakistani wheat varieties, including *Yr26* (5-29%), *Yr5* (13-17%), *Yr10* (22%), *Yr9* (8%), and *Yr18* (15%). Hierarchical clustering identified accessions such as WHT15180 (Cluster A), which exhibited diverse resistance mechanisms. Notably, *Yr5*, located on chromosome 2BL, was expressed throughout seedling development (Law, 1976). Our findings with marker S23M41-310 differed from those of Steffenson et al. (2007), who reported a 210-bp fragment in *Yr5* non-carriers. This fragment was absent in all Pakistani cultivars examined; instead, a weak 275-bp band appeared in some *Yr5* non-carriers. Marker S19M93-140 co-segregated with *Yr5* and proved more reliable than S23M41-310 (0.7 cM from *Yr5*), though both remain diagnostically valuable for germplasm screening. The AFLP-converted STS marker iag95, diagnostic for the 1BL.1RS translocation (*Sr31/Yr9/Lr26/Pm8* genes), was validated by Ejaz et al. (2012) and Pretorius et al. (2012) in Pakistani and African germplasm, respectively. However, its dominant nature restricts the detection of heterozygotes in segregating populations.

Yr10, derived from the Turkish line P.I.178383 (Wang et al., 2017), maps to chromosome 1BS near the Rg1 glume-color locus (2 cM) and Gli-1B (5 cM). Rg1 functions as a phenotypic marker beyond the seedling stage (Ahmad et al., 2004). In our dataset, 22% of varieties carried the linked 260-bp fragment (Xpsp3000). Given *Yr10*'s association with *Gli-B* (Ali et al., 2017; Bariana et al., 2020), the combined use of Xpsp3000 and *Gli-B* assays enhances screening accuracy.

The pleiotropic locus *Yr18/Lr34/Pm38* confers durable, adult plant resistance to stripe rust, leaf rust, powdery mildew, and barley yellow dwarf virus. The co-dominant marker csLV34 (0.4 cM distal) Lagudah et al., 2009) detects heterozygotes, while the gene-specific marker cssfr5 (targeting a 3-bp indel in *Lr34*) proved superior in our study. Only 15% of Pakistani varieties carried this locus, highlighting the urgent need to broaden its deployment against evolving *Pst* races. Similarly, *Yr26* (chromosome 1B), which is effective against most *Pst* isolates and shares identity with *Yr24/YrCH42*, was detected using Xgwm11 and CYS-5 markers. Together, these identified *Yr26* in 29 lines, whereas CYS-5 alone identified only 5 carriers.

Stripe rust epidemics have historically posed a major biotic constraint in Pakistan, with severe outbreaks reported in 1973 (35% intensity), 1978 (55%), 1995 (38%), and 2003 (20%) (Ahmad, 2004). Virulent races have overcome extensively deployed genes such as *Yr9* (Mexipak, Pak-81) and *Yr27* (Inqalab-91) (Khan and Mumtaz, 2004; Ahmed et al., 2011a; Shaheen et al., 2023). Thus, the moderate frequencies of *Yr* genes observed, coupled with the unique status of WHT15180, highlight opportunities for strategic gene pyramiding. The breakdown of major resistance genes underscores the necessity of broadening the genetic base via marker-assisted selection (Ahmed et al., 2011b; Khattak et al., 2020).

Cluster analysis has proven effective for classifying resistance diversity in crops such as wheat, banana, and *Nigella sativa* (Farrukh et al., 2022; Alam et al., 2023; Khattak et al., 2024; Saleem et al., 2024). Our hierarchical clustering (rescaled distance: 0-300) confirmed the genetic distinctness of the studied advanced lines, which likely harbor combinations of resistance genes under high pathogen pressure (Chen et al., 2014; Khattak et al., 2024). Cluster B (29 lines) further subdivided into B-1 (conserved *Yr5/Yr10* profiles; 5 accessions, merger distance 10-50) and B-2 (higher divergence; 7 accessions, merger 150-200). Subgroups B-1.2 and B-2.1 (merger 50-150) may reflect adaptation to local *P. striiformis* races. Similar distributions of resistance genes across populations have also been reported (Ahmed et al., 2010a,b; Ali et al., 2017; Tahir et al., 2023). Pyramiding conserved genes (e.g., *Yr5/Yr10* in B-1.1) with divergent accessions (B-2.2, WHT15180) could enhance durability.

The superior performance of outlier accessions such as WHT15180 may be attributed to underutilized resistance genes, undetected gene effects, or the absence of diagnostic markers, making these lines particularly valuable for breeding. Our results are consistent with previous findings (McCallum et al., 2012; Wang et al., 2017). These insights provide a rational basis for prioritizing germplasm in marker-assisted breeding programs aimed at developing durable stripe rust resistance in Pakistani wheat.

Acknowledgements

The authors are grateful to the Wheat Program, National Agricultural Research Centre, for providing the seed material.

Authors' Contributions

SB and SHK conceptualized the study and developed the methodology; SB, MAA, GZ, and MM conducted the

investigations, collected, and analyzed the data; SHK, FJ, FA, and SK contributed to data collection and analysis, reviewed the literature, and drafted the manuscript; SB, SHK, and GMA edited the manuscript; SHK, MAA, and SB reviewed and refined the final version.

Research Funding

This work was financially supported by the Pakistan Agricultural Research Council under the National Institute for Genomics and Advanced Biotechnology (NIGAB).

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

References

Afzal, O., Asif, M., Ahmed, M., Awan, F.K., Aslam, M.A., Zahoor, A., Bilal, M., Shaheen, F.A., Zulfiqar, M.A., 2017. Integrated nutrient management of safflower (*Carthamus tinctorius* L.) under rainfed conditions. American Journal of Plant Sciences 8, 2208-2218.

Ahmad, I., 2004. Wheat rust scenario 2003-2004. In: Proceedings of the Second Regional Yellow Rust Conference for Central and West Asia and North Africa, March 22-26, Islamabad, Pakistan.

Ahmed, M., Aslam, M.A., Hassan, F.U., Akram, M.N., Akmal, M., 2011a. Regression model to study sole and cumulative effect of temperature and solar radiation on wheat yield. African Journal of Biotechnology 10, 9114-9121.

Ahmed, M., Aslam, M.A., Hassan, F.U., Asif, M., Hayat, R., 2014. Use of APSIM to model nitrogen use efficiency in rainfed wheat. International Journal of Agriculture and Biology 16, 461-470.

Ahmed, M., Aslam, M.A., Hassan, F.U., Hayat, R., Ahmad, S., 2019. Biochemical, physiological and agronomic response of wheat to changing climate of rainfed Pakistan. Pakistan Journal of Botany 51, DOI: 10.30848/PJB2019-2(10).

Ahmed, M., Aslam, M.A., Hassan, F.U., Hayat, R., Nasim, W., Akmal, M., Mubeen, M., Hussain, S., Ahmad, S., 2021. Nutrient dynamics and the role of modeling. In: Jatoi, W.N., et al. (eds.), Building Climate Resilience in Agriculture, Chapter 19. Springer. DOI: 10.1007/978-3-030-79408-8.

Ahmed, M., Hassan, F.U., Aslam, M., Akram, M.N., Aslam, M.A., 2010a. Photosynthesis of spring wheat (*Triticum aestivum*) in rainfed ecology of Pakistan. African Journal of Biotechnology 9, 7495-7503.

Ahmed, M., Hassan, F.U., Aslam, M., Aslam, M.A., 2012. Physiological attributes based resilience of wheat to climate. International Journal of Agriculture and Biology 14, 407-412.

Ahmed, M., Hassan, F.U., Aslam, M., Aslam, M.A., Akram, M.N., 2010b. Correlation of photothermal quotient with spring wheat yield. African Journal of Biotechnology 9, 7852–7869.

Ahmed, M., Hassan, F.U., Qadeer, U., Aslam, M.A., 2011b. Silicon application and drought tolerance mechanism of sorghum. African Journal of Agricultural Research 6, 594-607.

Ahmed, M., Hassan, F.U., Qadir, G., Shaheen, F.A., Aslam, M.A., 2017. Response of proline accumulation in bread wheat (*Triticum aestivum* L.) under rainfed conditions. Journal of Agricultural Meteorology 73, 1-9.

Ahmed, M., Khan, S., Irfan, M., Aslam, M.A., Shabbir, G., Ahmad, S., 2019. Effect of phosphorus on root signaling of wheat under different water regimes. In: Global Wheat Production. Intech Publishers, Chapter 1. DOI: 10.5772/intechopen.75806.

Alam, B., Aslam, M.A., Khattak, S.H., Nawaz, N., Fatima, S., Ullah, Z., Khurshid, H., Jahanzaib, M., Bangash, S.A.K., Komal, S., Khan, I.U., Arshad, M., Ali, G.M., 2023. Exploring phenotypic diversity in Pakistani indigenous soybean landraces and US ancestral lines. Journal of Phytobiology 3, 65-75.

Ali, S., Rodriguez-Algaba, J., Thach, T., Sørensen, C.K., Hansen, J.G., Lassen, P., Hovmøller, M.S., 2022. High-temperature adult-plant resistance gene Yr36 confers resistance against Pakistani wheat stripe rust races. Plant Disease 106, 851-859.

Ali, S., Rodriguez-Algaba, J., Thach, T., Sørensen, C.K., Hansen, J.G., Lassen, P., Hovmøller, M.S., 2017. Yellow rust epidemics worldwide were caused by pathogen races from divergent genetic lineages. Frontiers in Plant Science 8, 1057.

Arif, M., Khurshid, H., Rabbani, M.A., Jahanzaib, M., Khattak, S.H., Ullah, I., Farah, M.A., Singh, R., Li, L., 2024. Morpho-physiological traits and stress

indices reveal divergence in early-stage salt stress response in elite soybean germplasm. Journal of King Saud University - Science, Article 103522.

Aslam, M.A., Ahmed, M., Hassan, F.U., Afzal, O., Mehmood, M.Z., Qadir, G., Asif, M., Komal, S., Hussain, T., 2021. Impact of temperature fluctuations on plant morphological and physiological traits. In: Jatoi, W.N., et al. (eds.), Building Climate Resilience in Agriculture, Chapter 3, pp. 25-52. Springer. DOI: 10.1007/978-3-030-79408-8.

Aslam, M.A., Ahmed, M., Hassan, F.U., Hayat, R., 2017b. Modeling nitrogen use efficiency under changing climate. In: Ahmed, M., Stöckle, C.O. (eds.), Quantification of Climate Variability, Adaptation and Mitigation for Agricultural Sustainability, pp. 71-90. Springer International Publishing, Switzerland.

Aslam, M.A., Ahmed, M., Hassan, F.U., Hayat, R., Ahmad, S., 2018. Quantification of impact of sowing dates on productivity of rainfed wheat. Scientia Technica Development 37, 28-35.

Aslam, M.A., Ahmed, M., Stöckle, C.O., Higgins, S.S., Hassan, F.U., Hayat, R., 2017a. Can growing degree days and photoperiod predict spring wheat phenology? Frontiers in Environmental Science 5, 57.

Balkrishna, A., Sharma, G., Sharma, N., Kumar, P., Mittal, R., Parveen, R., 2021. Global perspective of agriculture systems: From ancient times to the modern era. In: Sustainable Agriculture for Food Security, pp. 3-45. Apple Academic Press.

Bangash, S.A.K., Khan, M.S., Ambreen, S.H., Khattak, S.H., Siddique, A.N., 2013a. Genetic modification of *Brassica juncea*: Agrobacterium mediated transformation with antimicrobial Wasabi defensin gene. [ISBN: 978-3846539170; 3846539171].

Bangash, S.A.K., Khan, M.S., Ambreen, S.H., Khattak, S.H., Siddique, A.N., 2013b. Genetic transformation of *Brassica juncea* with antimicrobial Wasabi defensin gene. Pakistan Journal of Botany 45, 993-998.

Bariana, H., Brown, G., Ahmed, N., Khatkar, S., Conner, R., Wellings, C., Haley, S., Sharp, P., Laroche, A., 2002. Characterization of *Triticum vavilovii*-derived stripe rust resistance using genetic, cytogenetic, and molecular analyses and its marker-assisted selection. Theoretical and Applied Genetics 104, 315-320.

Bariana, H.S., Bansal, U.K., Forrest, K.L., 2020. Identification of QTLs for resistance to leaf spots in cultivated peanut (*Arachis hypogaea* L.) through GWAS analysis. Theoretical and Applied Genetics 133, 1451-1465.

Begum, S., Khan, M.R., Jan, A., Rahman, H., Khattak, S.H., Saeed, S., Ahmed, A., Ali, G.M., 2022. Genetic transformation of the EPSPS herbicide resistance gene in Agrobacterium mediated peanut (*Arachis hypogaea* L.) and effective revival of transgenic plants. Applied Ecology and Environmental Research 20, 1335-1349.

Begum, S., Khattak, S.H., Shahzad, A., Noor, S., Zaheer Uddin, S., Ajmal, W., Zabihullah, F., Fayyaz, M., Ali, G.M., 2019. Molecular characterization of Pakistani wheat genotypes for leaf rust resistance. Journal of Pure and Applied Agriculture 4, 1-10.

Bukhari, S.A.H., Mustafa, G., Ali, Z., Arshad, H.M.I., 2023. Indigenous wheat landraces from Himalayan foothills exhibit novel stripe rust resistance mechanisms. Journal of Plant Pathology 105, 123-135.

Chen, W., Wellings, C., Chen, X., Kang, Z., Liu, T., 2014. Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Phytopathology 104, 75-84.

Chen, X., 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Canadian Journal of Plant Pathology 27, 314-337.

Ejaz, M., Iqbal, M., Shahzad, A., Ahmed, I., Ali, G.M., 2012. Genetic variation for markers linked to stem rust resistance genes in Pakistani wheat varieties. Crop Science 52, 2638-2648.

Farrukh, A., Khattak, S.H., Kaleem, I., Begum, S., Jamil, K., Kamal, T., Riaz, M.N., Siddiqui, N.R., Ikram, R., Noor, S., Ali, G.M., 2022. Plant based nanotechnology - A new trend in therapeutic approaches of diabetes. Endo and Diabetes Open Access Journal 1, 1-5.

Gul, R., Khan, H., Sattar, S., Farhatullah, M.F., Shadman, K.B., Khattak, S.H., Ali, A., 2011. Comparison among nodulated and non-nodulated chickpea genotypes. Sarhad Journal of Agriculture 27, 577-581.

Helguera, M., Khan, I., Kolmer, J., Lijavetzky, D., Zhong-Qi, L., Dubcovsky, J., 2003. PCR assays for the cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Science 43, 1839-1847.

Hussain, M., Iqbal, M.A., Rahman, M.U., Sahi, S.T., 2023. Stripe rust epidemic dynamics in Pakistan's Punjab region: A decadal analysis. Crop Protection

172, 106332.

Jamil, K., Khattak, S.H., Farrukh, A., Begum, S., Riaz, M.N., Muhammad, A., Kamal, T., Taj, T., Khan, I., Riaz, S., Batool, H., 2022. Biogenic synthesis of silver nanoparticles using *Catharanthus roseus* and its cytotoxicity effect on Vero cell lines. Molecules 27, 6191.

Khan, M.A., Bux, H., Kazi, A.M., Rasheed, A., 2023. Marker-assisted breeding for stripe rust resistance in Pakistani wheat: Current status and future prospects. Cereal Research Communications 51, 245-258.

Khan, M.A., Mumtaz, H., 2004. Combining yellow rust resistance with high yield in grain wheat. In: Proceedings of the Second Regional Yellow Rust Conference for Central and West Asia and North Africa, March 22-26, Islamabad, Pakistan.

Khattak, S.H., Bangash, S.A.K., Kaleem, I., Begum, S., Noor, S., Ali, G.M., Hafeez, A., Ali, B., Khan, M.N., Shah, S.M., Razak, S., Zaman, A., Basha, Y., Alwahibi, M.S., Elshikh, M.S., 2024. Diversity analysis for wheat genotypes against rust resistant genes. Polish Journal of Environmental Studies 33, 1-12.

Khattak, S.H., Begum, S., Aqeel, M., Fayyaz, M., Bangash, S.A.K., Riaz, M.N., Saeed, S., Ahmed, A., Ali, G.M., 2020. Investigating the allelic variation of loci controlling rust resistance genes in wheat (*Triticum aestivum* L.) landraces by SSR markers. Applied Ecology and Environmental Research 18, 8091-8118.

Khattak, S.H., Imdad, K., Anum, F., Saima, N., Khansa, J., Tahira, K., Nouman, R.S., Ghulam, M.A., 2022b. Fruit ripening characterization and amylase mystery in bananas. Journal not specified 4, 1-9.

Khattak, S.H., Sania, B., Anum, F., Imdad, K., Khansa, J., 2022a. *Nigella sativa*, a myth or reality: A new trend in therapeutic approaches of Kalonji. Biomedical Journal of Scientific and Technical Research 47, 38248-38253.

Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L.L., Keller, B., 2009. Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theoretical and Applied Genetics 119, 889-898.

Law, C.N., 1976. Genetic control of yellow rust resistance in *Triticum aestivum* ssp. spelta var. *album*. Plant Breeding Institute, Cambridge, 108-109.

McCallum, B.D., Seto-Goh, P., 2012. Stripe rust resistance in Canadian wheat cultivars. Crop Science 52, 1453-1463.

Mehmood, M.Z., Afzal, O., Ahmed, M., Qadir, G., Kheir, A.M.S., Aslam, M.A., Din, A.M.U., Khan, I., Hassan, M.J., Meraj, T.A., Raza, M.A., 2021a. Can sulphur improve the nutrient uptake, partitioning, and seed yield of sesame? Arabian Journal of Geosciences 14, 865.

Mehmood, M.Z., Ahmed, M., Afzal, O., Aslam, M.A., Arfeen, R.Z.U., Qadir, G., Komal, S., Shahid, M.A., Awan, A.A., Awale, M.A., Sameen, A., Kalsoom, T., Nasim, W., Hassan, F.U., Ahmad, S., 2021b. Internet of Things (IoT) and sensors technologies in smart agriculture: Applications, opportunities, and current trends. In: Jatoi et al. (eds.) Building Climate Resilience in Agriculture, Chapter 21. DOI: 10.1007/978-3-030-79408-8.

Mehmood, M.Z., Qadir, G., Afzal, O., Din, A.M., Raza, M.A., Khan, I., Hassan, M.J., Awan, S.A., Ahmad, S., Ansar, M., Aslam, M.A., Ahmed, M., 2021c. Paclobutrazol improves sesame yield by increasing dry matter accumulation and reducing seed shattering under rainfed conditions. International Journal of Plant Production 15, 337-349.

Pretorius, Z.A., Jin, Y., Bender, C.M., Herselman, L., Prins, R., 2012. Seedling resistance to stem rust race Ug99 and marker analysis for Sr2, Sr24 and Sr31 in South African wheat cultivars and lines. Euphytica 186, 15-23.

Qaiser, R., Fayyaz, M., Muhammad, S., Sahir, H.K., Karansher, S.S., Gaganjot, S.S., 2022. Genome-wide association mapping and population structure for stripe rust in Pakistani wheat germplasm. Pakistan Journal of Botany 54, 1405-1416.

Rehman, M.A., Khattak, S.H., Inam, S., Rehman, N., Hussain, M., Ali, S., Khan, M.R., 2020a. Unveiling of genetic variability in garlic (*Allium sativum* L.) genotypes grown in Pakistan. Journal of Pure and Applied Agriculture 5, 53-64.

Rehman, M.A., Saleem, R., Hasan, S.W., Inam, S., Uddin, S.Z., Saeed, M., Noor, S., Riaz, M.N., Ali, G.M., Khattak, S.H., 2020b. Economic assessment of cereal-legume intercropping system: A way forward for improving productivity and sustaining soil health. International Journal of Business and Production Agricultural Sciences 9, 1078-1089.

Rehman, S.U., Abid, M.A., Hussain, M., Mahmood, T., 2023. Virulence evolution of *Puccinia striiformis* f. sp. *tritici* in Pakistan reveals breakdown of Yr9 and Yr27 resistance genes. Phytopathology 113, 678-689.

Saleem, A., Saeed, M.A., Shah, N.A., Kaleem, I., Ahmed, H., Khattak, S.H., 2024. Using plants as vaccines. In: Recent Advances in Industrial Biochemistry, pp. 49-76. Springer International Publishing, Cham.

Shaheen, A., Shahzad, A., Shah, S.H., Khattak, S.H., Idrees, S., Khan, S.U., 2023. Molecular and morphological evaluation of salt tolerance genes in advanced wheat lines (Part 1). Pakistan Journal of Botany 55, 813-823.

Siddiqui, N., Muhammad, A., Ali, G., Raza, S., Khan, M.R., Shahzad, A., Hameed, S., 2017. Expression analysis of amylase gene and starch degradation during fruit development and ripening stages of exotic cultivars of banana. Proceedings of the Pakistan Academy of Sciences 54, 117-124.

Sinha, P., Chen, X., 2021. Potential infection risks of the wheat stripe rust and stem rust pathogens on barberry in Asia and southeastern Europe. Plants 10, 957.

Steffenson, B.J., Olivera, P., Roy, J.K., Jin, Y., Smith, K.P., Muehlbauer, G.J., 2007. A walk on the wild side: Mining wild wheat and barley collections for rust resistance genes. Australian Journal of Agricultural Research 58, 532-544.

Tahir, O., Bangash, S.A.K., Ibrahim, M., Shahab, S., Khattak, S.H., Ud Din, I., Harakeh, S., 2023. Evaluation of agronomic performance and genetic diversity analysis using simple sequence repeats markers in selected wheat lines. Sustainability 15, 293-312.

Wang, M., Chen, X., 2017. Stripe rust resistance. In: Stripe Rust, pp. 353-558.

Waqar, A., Khattak, S.H., Begum, S., Rehman, T., Shehzad, A., Ajmal, W., Zia, S.S., Siddiqi, I., Ali, G.M., 2018. Stripe rust: A review of the disease, Yr genes and its molecular markers. Sarhad Journal of Agriculture 34, 188-201.

Zahid, G., Begum, S., Almani, S., Khattak, S.H., Soothar, R.K., Soomro, S.A., 2022. Utilization of SSR markers to identify slow rusting genes in spring wheat (*Triticum aestivum* L.). Pakistan Journal of Agricultural Research 35, 85-92.