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RESEARCH ARTICLE

Assessment of Wheat Genotypes for Resistance and Susceptibility to Stripe Rust

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ABSTRACT

Understanding the genetic variability and differential response of wheat genotypes to stripe rust caused by *Puccinia striiformis* f.sp. *tritici* is crucial for identifying resistant cultivars and enhancing breeding programs aimed at developing disease-resilient varieties. The objective of this study was to evaluate 55 wheat genotypes for their resistance and susceptibility to stripe rust by analyzing their coefficient of infection (CI) values, aiming to identify resistant cultivars for use in breeding programs and disease management strategies. Among the evaluated genotypes, 31 were classified as resistant, exhibiting mean CI values ranging from 4.722 to 10.39. Noteworthy resistant genotypes included 21C216 (4.722), 20FJ27 (5.918), 20FJ23 (6.167), and 21C229 (8.055). Nineteen genotypes were identified as moderately resistant, with mean CI values spanning from 10.974 to 20.418. Representative genotypes in this category included 20FJ25 (10.974), 21C225 (11.333), 20C207 (14.612), and 21C233 (18.195). Moreover, four genotypes exhibited intermediate responses and were categorized as moderately resistant/moderately susceptible, with mean CI values ranging from 23.832 to 31.834. This group included MA-2021 (23.832) and Dharabi-11 (27.61). A single genotype, Morocco, was classified as susceptible, displaying the highest mean CI value of 61.333. Notably, the response remained consistent across both years, although the severity levels varied. These results reflect significant genetic variability among the genotypes, providing critical insights for resistance breeding efforts.

Keywords: Stripe rust resistance, Disease susceptibility, Genetic variability, *Puccinia striiformis* f.sp. *tritici*..

INTRODUCTION

Wheat is a vital staple crop globally, with its demand projected to reach 900 million tons by 2050 due to population growth, rising incomes, and evolving dietary habits (Alexandratos and Bruinsma, 2012; Afzal *et al*., 2024). It accounts for a significant portion of the world's caloric intake, alongside two other major crops (Albahri *et al*., 2023). However, wheat cultivation faces numerous challenges, both biotic and abiotic, which pose substantial threats to global yields (Samon *et al*., 2024). The crop is particularly vulnerable to various pathogens, leading to diseases of differing severities (Bockus *et al*., 2010; Atta *et al*., 2024; Mukhtar *et al*., 2018; Mukhtar and Saeed, 2024). This underscores the urgent need for sustained investment in developing advanced wheat varieties with enhanced resistance to pests and diseases (Abebele and Zerihan 2024; Hussain *et al*., 2024; Soomro *et al*., 2024). Simultaneously, the adoption of sustainable agricultural practices is critical to mitigating the adverse effects of these challenges (Oerke, 2006; Strange and Scott, 2005; Afzal *et al*., 2024; Memon *et al*., 2024).

Crop diseases, particularly wheat rusts, present significant challenges to global food security (Afzal *et al*., 2018; Lidwell-Durnin and Lapthorn, 2020; Samon *et al*., 2024). Wheat rusts, caused by *Puccinia* spp., are widespread fungal pathogens, with leaf rust, stem rust, and stripe rust being the most prominent threats (Brar *et al*., 2019). Among these, stripe rust, caused by *Puccinia striiformis* Westend. (Pst), is considered one of the most destructive diseases affecting modern winter cereal production (Chen, 2020). Approximately 70% of the area under wheat cultivation is susceptible to stripe rust (Yahyaoui and Rajaram, 2012), which has triggered thirteen epidemics since independence (Welling, 2011).

Notably, the yellow rust epidemics of 2005 and 2012 devastated major commercial wheat varieties in Pakistan (Khan *et al*., 2005; Hussain *et al*., 2015). The specific strain infecting wheat is *P. striiformis* f.sp. *tritici* (Pst). Recent studies have uncovered the existence of the pathogen's sexual stage on alternative hosts, including *Berberis* spp. and *Mahonia* spp. (Jin *et al*., 2010; Wang and Chen, 2013; Zhao *et al*., 2013).

The long-distance migration of the pathogen has resulted in its invasion of geographically isolated wheat-producing regions. Notable examples include its spread to Australia from Europe in 1979 (O'Brien *et al*., 1980), the Republic of South Africa from the Middle East in 1996 (Boshoff *et al*., 2002), and Western Australia from North America in 2002 (Wellings, 2007). Western China and Central Asia have also been proposed as centers of origin, given the high telial production and genetic diversity observed in isolates from these regions, which indicate frequent recombination events (Mboup *et al*., 2009; Ali *et al*., 2010). Traditionally considered a disease of low-temperature environments, stripe rust has now expanded its range to temperate regions with cool and moist climates. Recent severe outbreaks have been reported in warmer areas where the disease was previously rare or absent (Mboup *et al*., 2009; Hovmøller *et al*., 2010). This shift suggests an adaptation of Pst populations to higher temperatures, as supported by findings reported by Milus *et al*. 2008. These developments underscore the urgent need for global cooperation in agricultural research and management to mitigate the spread and impact of stripe rust.

Two primary approaches to combating wheat rusts are the use of fungicides and the development of resistant varieties (Asad *et al*., 2012; Qamar *et al*., 2014). While fungicides are effective, they have significant drawbacks, including high costs and potential environmental harm (Iqbal *et al*., 2014; Iqbal and Mukhtar, 2020; Shahbaz *et al*., 2023). Resistant varieties, on the other hand, are considered more sustainable due to their environmental friendliness and long-term durability. However, their effectiveness hinges on timely development and deployment (Rosewarne *et al*., 2013; Jamil *et al*., 2020).

Pathogens continually adapt and evolve, often giving rise to new strains capable of overcoming previously effective resistance. Consequently, wheat cultivars that were once resistant may become susceptible over time, underscoring the critical need for ongoing breeding efforts to sustain rust resistance. This dynamic and continuous process is essential for safeguarding global food security.

Experiments designed to categorize resistant and susceptible wheat genotypes or cultivars involve maintaining scientific rigor and intellectual value. Such studies typically screen diverse wheat lines against rust pathogens under field conditions, with resistance evaluated based on disease severity and associated yield losses caused by Pst. The insights gained from these evaluations facilitate the identification of promising wheat lines for breeding programs aimed at developing durable, high-yielding varieties. These efforts are pivotal for strengthening agricultural systems and implementing sustainable disease management strategies. Therefore, the objective of the present study was to categorize wheat genotypes as resistant or susceptible based on disease severity and yield losses, to identify promising lines for breeding programs, and to support the development of durable wheat varieties for sustainable disease management and enhanced food security.

MATERIALS AND METHODS

The study was conducted using 51 genotypes and 5 cultivars developed at the Wheat Section of Barani Agricultural Research Institute (BARI), Chakwal, and Barani Agricultural Research Station (BARS), Fatehjang. These materials were collected and screened at the Wheat Research Site of BARI, Chakwal, during the 2022-23 and 2023-24 cropping seasons. The list of test materials is provided in Table 1. Field experiments were carried out over the main cropping seasons of 2022-23 and 2023-24, from November to April, at the research area of BARI, Chakwal, located at 72° longitude, 32° latitude, and an altitude of 575 m. The region typically experiences an average temperature of 30.65°C and receives about 21.92 mm of precipitation.

Table 1. List of wheat genotypes screened against stripe rust.

Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype
1	19C166	18	21C228	35	20FJ09
2	20C207	19	21C229	36	20FJ10
3	21C213	20	21C230	37	20FJ11
4	21C214	21	21C231	38	20FJ12
5	21C215	22	21C232	39	20FJ14
6	21C216	23	21C233	40	20FJ15
7	21C217	24	21C234	41	20FJ16
8	21C218	25	21C244	42	20FJ19
9	21C219	26	21C252	43	20FJ20
10	21C220	27	17FJ10	44	20FJ21
11	21C221	28	18FJ21	45	20FJ22
12	21C222	29	19FJ12	46	20FJ23
13	21C223	30	19FJ16	47	20FJ24
14	21C224	31	19FJ35	48	20FJ25
15	21C225	32	20FJ05	49	20FJ27
16	21C226	33	20FJ06	50	20FJ35
17	21C227	34	20FJ08	51	20FJ36
			Wheat cultivars		
1	Dharabi-11	3	Barani-17	5	Morocco
2	Ihsan-16	4	MA-2021		

The trial was meticulously designed, with a controlled stripe rust epidemic induced by uniformly inoculating all test materials with equal doses of urediniospores from *P. striiformis*, the primary pathogen responsible for the disease. To ensure an optimal inoculum load, the test genotypes were interspersed with a susceptible genotype (Morocco), strategically planted around the perimeter. Urediniospores suspended in mineral oil, with a concentration of 1.5 mg/L (equivalent to 0.005 kg/ha), were sprayed over the leaves using an ultra-low volume turbo-air sprayer.

Before inoculation, the research plots were adequately flooded to increase soil moisture. To promote uniform disease dissemination, the Morocco cultivar was sown one week prior to the experimental plots. Over the following four weeks, the trial areas were consistently moistened after the second inoculation, which took place two weeks after the initial inoculation. All inoculations were conducted during the evening to optimize conditions for disease development.

The assessment of disease reaction in the test materials involved evaluating both the severity and type of infection. Severity was recorded as the percentage of rust extent on the leaves. The Modified Cobb's Scale (Peterson *et al*., 1948) was used for disease assessment. The Coefficient of Infection (CI) was calculated by multiplying the response value of each wheat genotype by the percentage intensity of infection, providing a quantitative index for disease impact. The Average Coefficient of Infection (ACI) was then calculated by summing the CI values for each genotype and dividing by the number of years over which the data were collected, following the protocol established by Stubbs *et al*. (1986). In this study, the CI sums were divided by two, reflecting the biennial data collection period of 2022-23 and 2023-24.

This analytical framework offers a rigorous approach to evaluating the resistance potential of wheat germplasm to stripe rust, enabling detailed comparisons across diverse temporal and environmental conditions. It enhances our understanding of genotype-environment interactions and informs breeding strategies.

RESULTS

The analysis of the coefficient of infection for the test material revealed a differential response to the disease. Notably, the response remained consistent across both years, although the severity levels varied. This variability is expected, as environmental factors, a critical component of the disease triangle, fluctuate under field conditions, even when the same genetic material is evaluated annually. The findings highlighted significant genetic variation among the genotypes in their response to wheat stripe rust.

The categorization of genotypes based on mean CI values for disease resistance is summarized in Table 2. Among the evaluated genotypes, 31 were classified as resistant

(R), exhibiting mean CI values ranging from 4.722 to 10.39. Noteworthy resistant genotypes included 21C216 (4.722), 20FJ27 (5.918), 20FJ23 (6.167), and 21C229 (8.055).

Nineteen genotypes were identified as moderately resistant (MR), with mean CI values spanning from 10.974 to 20.418. Representative genotypes in this category included 20FJ25 (10.974), 21C225 (11.333), 20C207 (14.612), and 21C233 (18.195). Moreover, four genotypes exhibited intermediate responses and were categorized as moderately resistant/moderately susceptible (MRMS), with mean CI values ranging from 23.832 to 31.834. This group included MA-2021 (23.832) and Dharabi-11 (27.61).

A single genotype, Morocco, was classified as susceptible (S), displaying the highest mean CI value of 61.333.

Table 2. Categorization of genotypes based on mean ci values for disease resistance.

Genotype	Mean CI Value	Genotype	Mean CI Value
Resistant (R)			
21C216	4.722	20FJ05	8.083
20FJ27	5.918	20FJ12	8.085
20FJ23	6.167	20FJ22	8.612
20FJ36	6.445	20FJ09	8.64
20FJ24	6.473	21C221	8.667
20FJ06	6.335	21C231	8.695
20FJ19	6.722	20FJ14	9.112
17FJ10	7.112	21C226	9.194
20FJ15	7.333	21C214	9.25
19C166	7.39	20FJ21	9.612
21C232	7.583	18FJ21	9.667
20FJ20	7.612	21C220	10.112
20FJ11	7.807	19FJ12	10.113
21C213	8.002	21C228	10.167
19FJ35	8.027	21C227	10.39
21C229	8.055		
Moderately Resistant (MR)			
20FJ25	10.974	21C224	13.443
21C222	11.055	21C234	13.584
21C225	11.333	21C253	15.138
19FJ16	11.695	21C215	15.972
21C223	12.195	21C218	17.11
21C219	12.335	20FJ10	18.14
20C207	14.612	21C233	18.195
20FJ08	14.722	21C244	19.944
21C217	13.114	20FJ16	20.418
20FJ35	13.222		
Moderately Resistant/Moderately Susceptible (MRMS)			
MA-2021	23.832	Dharabi-11	27.61
Ihsan-16	26.222	Barani-17	31.834
Susceptible (S)			
Morocco	61.333		

DISCUSSION

Stripe rust, caused by *P. striiformis* f.sp. *tritici*, is a well-documented pathogen affecting wheat production globally (Chen, 2013; Bux *et al.*, 2012; McIntosh and Brown-Guedira, 2017; Wellings, 2011; Miedaner and Juroszek, 2021). These sources provide comprehensive insights into the pathogen's biology, epidemiology, and management strategies.

Harnessing genetic diversity in wheat is critical for developing resistance to stripe rust, which remains a significant challenge for global food security (Afzal *et al.*, 2022; Usman *et al.*, 2024; Gao *et al.*, 2024). Both historical and contemporary research efforts have focused on increasing the genetic diversity of wheat to effectively combat this disease. Historically, resistance development relied on empirical breeding methods, involving the identification and incorporation of resistant varieties (McIntosh and Brown-Guedira, 2017). In contrast, modern approaches integrate advanced genomic and molecular tools to enhance resistance mechanisms and broaden genetic diversity (ZhiYong *et al*., 2024). These efforts highlight the pivotal role of genetic diversity in sustainable disease management.

Landraces and wild relatives of wheat have been indispensable sources of genetic diversity, contributing to the development of cultivars with enhanced traits, including resistance to stripe rust (McIntosh and Brown-Guedira, 2017). During domestication and breeding, the genetic base of modern cultivars has often been narrowed, leading to the loss of valuable traits (Harlan, 1992). To counteract this, researchers have tapped into the genetic pools of landraces and wild relatives, introducing novel resistance genes into cultivated wheat. For instance, genetic resources from *Aegilops tauschii* have been effectively utilized to improve stripe rust resistance, demonstrating the value of wild relatives in modern wheat breeding (Kou *et al*., 2023; Niu and Zhao, 2018; Ashraf, 2021; Oliver, 2021).

By integrating traditional and cutting-edge approaches, ongoing research underscores the necessity of maintaining and utilizing genetic diversity to develop durable resistance against stripe rust. Such strategies are essential for ensuring the long-term sustainability of wheat production.

In the 20th century, formal breeding programs began prioritizing disease resistance in crops. The discovery of genetic resistance in wheat lines paved the way for the development of new resistant varieties (McIntosh and Brown-Guedira, 2017). A notable breakthrough was the identification of Yr genes, which provide resistance to stripe rust (Chen, 2005). These Yr genes are located on various wheat chromosomes, and studies have detailed their chromosomal locations and significance in developing resistant wheat varieties (Yang *et al*., 2016; Maccaferri and Xie, 2016; Mukhtar *et al*., 2015; Liu and Yang, 2020). Over the years, different Yr genes have been successfully integrated into wheat breeding programs, resulting in cultivars with enhanced resistance to stripe rust (Liu *et al*., 2024; Mapuranga *et al*., 2022; Zeng *et al*., 2022).

Recent advancements in molecular biology and genomics have revolutionized the study of genetic diversity in wheat. These innovations have deepened our understanding of the genetic basis of wheat traits, facilitated the identification of novel resistance genes, and significantly improved breeding strategies. Molecular markers, such as Simple Sequence Repeats (SSRs), also known as microsatellites, have proven invaluable in genetic studies, including those related to stripe rust resistance in wheat. SSRs are highly polymorphic, co-dominant, and distributed throughout the genome, making them essential tools for mapping resistance genes and enabling marker-assisted selection (Mallick *et al*., 2022). Similarly, Single Nucleotide Polymorphisms (SNPs) have allowed for precise mapping of resistance genes (Gardiner *et al*., 2020; Geethanjali *et al*., 2024).

Genomic tools have further facilitated the identification of new Yr genes and elucidated their functions. The advent of Next-Generation Sequencing (NGS) has significantly accelerated the discovery of resistance genes (Murray and Cavanagh, 2018; Tiwari and Sharma, 2019; Wang and Zhang, 2020; Berkman *et al*., 2012). Whole-genome sequencing and genome-wide association studies (GWAS) have provided valuable insights into the genetic architecture of stripe rust resistance (Tene *et al*., 2022). For example, GWAS has identified numerous quantitative trait loci (QTL) associated with stripe rust resistance in wheat. These findings have been instrumental in pinpointing specific genetic regions that contribute to resistance, offering new targets for breeding programs and enabling the development of more resilient wheat varieties (Tang *et al*., 2024; Yu and Zhang, 2019; Zhang and Chen, 2020; Liu and Liu, 2021).

In addition, genetic engineering has opened new avenues for enhancing stripe rust resistance. The CRISPR-Cas9 technology allows for precise genome editing in wheat, enabling the introduction or modification of resistance genes. Researchers have successfully used CRISPR-Cas9 to improve resistance by targeting susceptibility genes or incorporating Yr genes into susceptible varieties (Upadhyaya and Singh, 2018; Kim *et al*., 2018; Zhang and Zhang, 2021; Choudhary *et al*., 2022). These advancements underscore the transformative potential of modern genetic tools in addressing challenges posed by stripe rust in wheat.

Recent approaches emphasize the critical role of participatory breeding, which involves farmers directly in the selection process. This strategy ensures that newly developed varieties align with the specific needs and preferences of farmers, thereby increasing the likelihood of adoption. Moreover, participatory breeding facilitates the identification of diverse germplasm with unique resistance traits, contributing significantly to the genetic diversity of wheat (Witcombe and Devaux, 2018; Boef and Turner, 2019; Ceccarelli and Grando, 2020; 2022). Global collaboration among research institutions, such as the International Maize and Wheat Improvement Center (CIMMYT) and national agricultural research systems, has played a pivotal role in combating stripe rust. These partnerships have fostered the exchange of germplasm, knowledge, and resources, enhancing global capacity to address emerging stripe rust races (Singh *et al*., 2005; Park and Wellings, 2011; Afzal *et al*., 2022).

A major challenge in managing stripe rust is the rapid evolution of *P. striiformis*, which frequently gives rise to new races capable of overcoming previously effective resistance genes. This underscores the necessity for continuous pathogen population monitoring and the development of new resistant varieties. Climate change further complicates this issue, as rising temperatures and shifting precipitation patterns alter the geographical distribution and severity of stripe rust outbreaks. These changes influence the pathogen's life cycle, presenting additional challenges for breeding programs (Milus *et al*., 2009; Hovmøller *et al*., 2011; Ali *et al*., 2014; Ali *et al*., 2024).

Maintaining and enhancing genetic diversity remains a cornerstone of sustainable disease management. Breeding programs must prioritize the exploration of untapped genetic resources, including underutilized landraces and wild relatives. Pre-breeding efforts focused on transferring resistance genes from these sources into elite lines are vital for broadening the genetic base of wheat.

Integrating traditional breeding methods with modern genomic tools offers a pathway to more efficient and effective development of resistant varieties. Techniques such as Marker-Assisted Selection (MAS) and Genomic Selection (GS) can accelerate the breeding process while ensuring alignment with agronomic traits and farmer preferences.

CONCLUSION

The fight against stripe rust in wheat is an ongoing challenge that demands a comprehensive understanding of genetic diversity. Historical efforts laid the groundwork for developing resistant varieties, while recent advances in genomics and molecular biology have provided transformative tools and insights. Sustained collaborative efforts and the integration of traditional and modern approaches are crucial for maintaining genetic diversity and ensuring food security in the face of evolving challenges.

Screening for disease resistance in wheat breeding programs is a pivotal strategy for developing varieties resilient to stripe rust, a major global threat to wheat production. The rapid evolution of *Puccinia striiformis* f.sp. *tritici*, exacerbated by climate change, highlights the need for continuous screening and selection of resistant genotypes. Advances in molecular biology and genomics, such as the identification of Yr genes, simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and the application of next-generation sequencing (NGS), have revolutionized the understanding of genetic diversity and facilitated the development of resistant cultivars. These tools enable precise mapping of resistance genes and accelerate the breeding process through marker-assisted selection (MAS).

Furthermore, integrating participatory breeding and global collaboration among research institutions enhances the effectiveness of breeding programs. By leveraging both natural and induced genetic diversity, researchers can develop durable resistance to stripe rust, ensuring stable wheat yields and contributing to global food security. Ultimately, the importance of screening for disease resistance lies in its ability to provide sustainable solutions to the challenges posed by stripe rust and other crop diseases, fostering resilient agricultural systems capable of withstanding environmental and pathogen-related challenges.

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Aftab Ahmad:	:	Conducted research activity at the Barani Agricultural Research Institute (BARI), Chakwal.
Tariq Mukhtar	:	Conceptualized the idea for the study and supervised the research.
Amir Afzal	:	Provided support at the BARI in the capacity as co-supervisor.
Asim Gulzar	:	Suggested measures to safeguard the experiment from insect infestation.
Ghulam Abbas	:	Provided intellectual input to improve the research article.
Asima Batool	:	Provided intellectual input to improve the research article.