



Research Article

Status of Wheat Germplasm Resistance Against Virulent Races of Leaf and Stripe Rust in Faisalabad, Pakistan

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Abstract | Leaf and stripe rusts are the most destructive diseases of wheat all over the world. Although host genotype resistance and fungicides are used to combat crop diseases, evaluation of wheat germplasm is still needed to reduce disease management costs and to increase crop sustainability. For this purpose, screening of 42 different wheat varieties and advanced lines was conducted at the Research area of Plant Pathology Research Institute, Ayub Agriculture Research Institute Faisalabad, during 3rd week of November 2019–2020 and 2020–2021 crop seasons. To monitor the changes of rust virulence pattern in the region, a set of rust trap nurseries consisting of 21 stripe (*Yr*) and 36 leaf rust (*Lr*) lines were grown in the Research Area of Plant Pathology Research Institute during the same cropping seasons. A total of 11 Genotypes *viz.*, Ass-11, V-13266, V-11C023, V-12266, V-08203, V-12001, V-13001, Gandam-II, V-13270, V-13273 and AARI-11 indicated resistant (R) response against stripe rust of wheat with a lower area under disease progress curve (AUDPC) values ranged from 68–102. In comparison, seven genotypes, including Ass-11, V-13266, V-11C023, V-12266, Gandam-II, V-13270 and V-13273 demonstrated resistant response against leaf rust severity during both crop seasons 2020–2021. Five lines such as V-11098, V-12001, V-13001, and DH-31 demonstrated a moderately resistant response to leaf rust severity during both cropping seasons, while six genotypes including V11138, V-11C022, V-11098, DH-31, and Bhakhar-2002 indicated moderate resistance (MR) against stripe rust severity. Monitoring of rust pattern indicated no virulence for leaf rust (*Lr*) genes, *i.e.*, 1, 10, 17, 18, 22a, 22b, 25, 27+31, 28 and *Lr*23+Gaza and stripe rust (*Yr*) genes such as *Aoc-YrA*, *Aoc+YrA*, 1, 2, Tatar, 8, 10, 17, 18, 19, 24, 26, 28, 29, *Yrsp*, *Yrcv*, and Super kauz. The study concluded that disease-resistant wheat genotypes, as well as *Lr* and *Yr* genes indicating a high level of resistance against leaf and stripe rust pathogens, are good sources for future breeding programmes.

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Introduction

Leaf and stripe rusts damage the wheat (*Triticum aestivum* L.) crop and cause significant yield losses. Rust infections have threatened Asia's food security and wheat production (Chen, 2020). New strains are constantly emerging and infecting resistant varieties (Ali *et al.*, 2018, 2020). The 40-60 million hectares could develop leaf and stripe rust outbreaks if vulnerable wheat varieties are cultivated (Chen, 2014). In spring season, leaf rust occurs as orange pustules on leaves and sheaths. Early infection may destroy the plant, whereas late infections lower yield. Due to its appearance and protracted development season, leaf rust might cause significant yield losses (Ali *et al.*, 2022a, 2023).

Yellow rust appears as yellow pustules across the leaf veins (Ahmad *et al.*, 2010). The intensity and time of infection affect yield. Depending on low temperatures, it may appear in January and last until March. Yuan *et al.* (2018) reported that stripe rust can cause 10-100% yield reductions. Leaf and stripe rusts on wheat crops have caused epidemics in the past, and continued to threaten future wheat production (Razzaq *et al.*, 2018).

Yellow rust has wiped out nearly all commercial wheat cultivars during crop seasons 2013-2014 (Hussain *et al.*, 2017). Controlling rust epidemics in the region is challenging (Ali *et al.*, 2022b). The novel stem, leaf and yellow rusts races have been introduced to wheat-growing areas on several continents (Javaid *et al.*, 2018; Ali, 2018). Screening for yellow and leaf rust is the best way to control rust diseases. Comprehensive research is needed to assess yield losses and their level of rust resistance (Javaid *et al.*, 2018). The country lacks high-yield and rust-resistant wheat cultivars with wide adoption.

Rust pathogens have two traits that require constant monitoring. First, they are highly mobile transboundary pathogens that can move fast and far through wind (Afzal *et al.*, 2022a). This movement makes quarantines practically difficult and required continuous monitoring. Globalization and increased air travel have enhanced the possibility of human exposure and the necessity to monitor more regions. Secondly, through sexual recombination or mutation changes, rust pathogens have evolved into new races (Park, 2007; Afzal *et al.*, 2022b). Both stripe and leaf

rusts are the main serious threat to grain production globally as well as in our country (Singh *et al.*, 2015). However, after emergence of 2 high-temperature tolerant *P. striiformis* races, severe repeated yellow rust epidemics have been documented (Hovmöller *et al.*, 2016; Schwessinger *et al.*, 2020).

When a resistant variety is introduced for cultivation, it gets attacked by a new or previously unknown species. Breeders and pathologists are required to monitor its dynamism. Wheat Research Institute, Faisalabad is monitoring rust virulence to investigate the rusts changing patterns. Moreover, pathologists, breeders, and policymakers will benefit from a two-year study (2019-2020 and 2020-2021) of monitoring rusts' virulence. The objective of this study was to assess various wheat genotypes and near-isogenic lines in relation to diverse races of leaf and stripe rust.

Table 1: List of genotypes sown at the research area of Plant Pathology Research Institute Faisalabad during 2020-2021.

S. No.	Genotypes	S. No.	Genotypes
1	Punjab-85	22	Kohistan-90
2	Ass-11	23	V-13269
3	Fsd-08	24	Bhakhar-2002
4	V-11138	25	Fareed-06
5	V-13266	26	V-13270
6	Pak-81	27	V-13273
7	Maxipak-65	28	Millat-11
8	V-11C022	29	AARI-11
9	V-11C023	30	Punjab-11
10	Gandam-I	31	Chenab-2000
11	Inqilab-91	32	AS-2002
12	V-12266	33	Wattan-94
13	V-11098	34	PBW-343
14	V-08203	35	V-10110
15	Galaxy-13	36	Lasani-08
16	V-12001	37	Iqbal-2000
17	V-13001	38	Shafaq-06
18	WL711	39	Pasban-90
19	LU-26	40	Yecora-70
20	DH-31	41	Seher-08
21	Gandam-II	42	Uqab-2000

Materials and Methods

Forty-two wheat genotypes (Table 1) were collected from the gene pool of Wheat Research Institute and

sown in the experimental area of Wheat Research Institute Ayub Agriculture Research Institute Faisalabad during 3rd week of November 2019-2020 (2020) and 2020-2021 (2021). The detail of the study area is illustrated in Table 2. The genotypes were sown manually with a single replication in an augmented design. The length of each row was 5 m with a 25 cm R x R distance. The stripe rust inoculum (80E85) and a combination of leaf rust races (PHTTL, PGRTB, KSR/JS, TKTPR, and TKTRN) were obtained from fields in Punjab, Kaghan, and Murree, and a rust trap nursery established at wheat research institutes in Faisalabad. The inoculum obtained from these locations was utilised to induce an outbreak of leaf and stripe rust in the field. After every 10th row, one line of highly rust spreader variety Morocco was sown and after 30 days of sowing rust inoculum was applied @ 10⁶/ml of distilled water twice a week to increase the disease pressure (Roelf *et al.*, 1992).

Table 2: The detail of study area in Faisalabad Pakistan.

Soil classification	Lat. (°N)	Long. (°E)	Elevation (m)	Average temp. (°C)	Average rainfall (mm)
Clay loam	31°410	73°12	184	20.2	25.9

Monitoring of stripe and leaf rusts virulent races

To monitor stripe (*Yr*) and leaf rust (*Lr*) virulence pattern, a set of single lines of 21 *Yr* viz., *Super kauz*, *Aoc +YrA*, *Aoc -YrA*, *Yrsp.*, *Yrcv*, *Tatara*, *Yr-31*, 29, 28, 27, 26, 24, 18, 17, 10, 9, 8, 7, 6, 2 and 1 and 36 *Lr* near isogenic lines i.e., *L23+Gaza*, *WL-711*, *LrB*, *Lr-37*, 36, 35, 34, 33, 32, 30, 29, 28, 27+31, 25, 24, 23, 22b, 22a, 21, 19, 18, 17, 16, 15, 14b, 14a, 12, 11, 10, 9, 3bg, 3ka, 3, 2b, 2a and *Lr-1* received from CIMMYT were sown in the Research Area of Plant Pathology Research Institute, Ayub Agriculture Research Institute during cropping seasons of 2021-2021.

Table 3: Cobb's scale for rating rusts on wheat varieties (Peterson *et al.*, 1948).

S.No.	Code	Field response
1	Immune (I)	No disease symptoms
2	Resistant (R)	With or without uredinospores, chlorosis or necrosis is visible
3	Moderately-Resistant (MR)	Necrotic area surrounded by minute uredinospores
4	Intermediate (MRMS)	Necrotic area surrounded by small uredinospores, no necrosis is observed but some chlorosis is visible
5	Moderately-susceptible (MS)	Uredinospores is medium with possible chlorosis but no necrosis is observed
6	Moderately-susceptible-susceptible (MSS)	Urdinospores are large without visible necrotic regions but to some extent chlorosis is present
7	Susceptible (S)	Uredinospores size is large and its clearly observed with some little or no chlorosis

Morocco, a rust-spreader variety, was sown around the row beds. The sowing of genotypes was completed on November 15, 2020, and in the first week of December 2021. The rust inoculation was carried out by maintaining an artificially moist environment for an extended period to enhance the multiplication of rust. The inoculation of plots with a mixture of stripe rust inoculum increased rust pressure.

Data recording and statistical analysis

Data for stripe and leaf rust severity was recorded on a visual basis in March following the modified Cobb's scale (Table 3). The AUDPC (area under disease progress curve) was calculated by using the expression given below:

$$AUDPC = \sum_{i=1}^{n-1} [xi + Xi1]/2)(ti + 1 - t1)$$

Where Xi is the intensity of rusts on date i; ti is the number of days between i and date i + 1; N is the number of dates that a disease was documented. (Shaner and Finney, 1980).

Results and Discussion

Screening of wheat genotypes against stripe and leaf rust severity

The response of 42 wheat genotypes against stripe and leaf rust is illustrated in Tables 4 and 5, respectively, for the crop seasons 2019-2020. Eleven lines viz., Ass-11, V-13266, V-11C023, V-12266, V-08203, V-12001, V-13001, Gandam-II, V-13270, V-13273 and AARI-11 indicated resistant (R) response against stripe rust of wheat with lower AUDPC values ranged from 68-102. Whereas 6, 1, 17 and 7 genotypes showed MR, MRMS, MS and S type responses to stripe rust with various AUDPC values (Table 4).

Table 4: Response of wheat genotypes against stripe rust severity during crop seasons 2020–2021.

Name of genotypes	Frequency	Ranges of AUDPC	Reaction*
Ass-11, V-13266, V-11C023, V-12266, V-08203, V-12001, V-13001, Gandam-II, V-13270, V-13273 and AARI-11	11	68-102	R
V11138, V-11C022, V-11098, DH-31, V-13269 and Bhakhar-2002	6	128-213	MR
Fsd-08	1	178-346	MRMS
Kohistan-90, Fareed-06, Millat-11, Chenab-2000, AS-2002, Wattan-94, PBW-343, Iqbal-2000, Shafaq-06, Pasban-90, Lasani-08, Seher-08, Galaxy-13, V-10110, Uqab-2000, Punjab-85 and Gandam-I	17	560-717	MS
Pak-81, Maxipak-65, Inqilab-91, WL711, LU-26, Punjab-11, Yecora-70	7	728-1155	S

*R= resistant, MR= moderately resistant, MRMS= moderately resistant to moderately susceptible, MS= moderately susceptible, S= susceptible

Table 5: Response of wheat genotypes against leaf rust severity during crop seasons 2020–2021.

Name of genotypes	Frequency	Ranges of AUDPC	Reaction*
Ass-11, V-13266, V-11C023, V-12266, Gandam-II, V-13270 and V-13273	7	67-96	R
V-11098, V-12001, V-13001, V-13269 and DH-31	5	137-184	MR
Fsd-08, V-11C022, V-08203, Bhakhar-2002 and V-10110	5	211-400	MRMS
Punjab-85, V11138, Pak-81, Gandam-I, Galaxy-13, Kohistan-90, Fareed-06, Millat-11, AARI-11, Punjab-11, Chenab-2000, Wattan-94, Iqbal-2000, Shafaq-06, PBW-343, Lasani-08, AS-2002, Inqilab-91, Uqab-2000	19	487-918	MS
Maxipak-65, WL711, LU-26, Pasban-90, Yecora-70, Seher-08	6	920-1258	S

*Reaction abbreviations are same as in Table 1.

Table 6: Monitoring of leaf and stripe rust near isogenic lines at Plant Pathology Research Institute during 2019–2020 and 2020–2021 crop seasons.

Name of disease	Monitoring of leaf rust virulence (2019–2020)	Monitoring of stripe rust virulence (2020–2021)
Leaf rust	10, 22a, 22b, 25, 27+31, 28, Lr23+Gaza/ 1, 2a, 2b, 3, 3ka, 3bg, 9, 11, 12, 14a, 14b, 15, 16, 17, 18, 19, 21, 23, 24, 29, 30, 32, 33, 34, 35, 36, 37, LrB, WL711	1, 10, 17, 18, 22b, 25, 27+31, 28, Lr23+Gaza/ 2a, 2b, 3, 3ka, 3bg, 9, 11, 12, 14a, 14b, 15, 16, 19, 21, 22a, 23, 24, 29, 30, 32, 33, 34, 35, 36, 37, LrB, WL711
Stripe rust	Aoc-YrA, Aoc+YrA, 1, 2, Tatara, 8, 10, 17, 18, 19, 24, 26, 28, Yrsp, Yrcv, Super kauz/ 6, 7, 9, 27, 29, 31	Aoc-YrA, Aoc+YrA, 1, 2, Tatara, 8, 10, 17, 18, 19, 24, 26, 28, 29, Yrsp, Yrcv, Super kauz/ 6, 7, 9, 27, 31

Similarly, during rating season 2020–2021, the 7, 5, 5, 19, and 6 genotypes showed R, MR, MRMS, MS and S type responses against leaf rust severity with different AUDPC values (Table 5).

Monitoring of leaf rust and stripe rust virulent races
The leaf and stripe rust pattern of all tester lines recorded at Plant Pathology Research Institute (PPRI) Faisalabad is presented in Table 6. During 2019–2020 crop seasons, very high virulence against Lr genes, i.e., 1, 2a, 2b, 3, 3ka, 3bg, 9, 11, 12, 14a, 14b, 15, 16, 17, 18, 19, 21, 23, 24, 29, 30, 32, 33, 34, 35, 36, 37, LrB and WL711 and Yr genes viz., 6, 7, 9, 27, 29, 31 were observed (Table 6).

Virulence pattern changed during 2020–2021 which

indicated that Lr genes 2a, 2b, 3, 3ka, 3bg, 9, 11, 12, 14a, 14b, 15, 16, 19, 21, 22a, 23, 24, 29, 30, 32, 33, 34, 35, 36, 37, LrB and WL711 and Yr genes 6, 7, 9, 27, and 31 showed MS, S, and MSS type susceptible response against leaf rust and stripe rust pathogens, respectively. Whereas, during both rating season 2019–2020 and 2020–2021 leaf rust genes, i.e., 1, 10, 17, 18, 22a, 22b, 25, 27+31, 28 and Lr23+Gaza and stripe rust genes such as Aoc-YrA, Aoc+YrA, 1, 2, Tatara, 8, 10, 17, 18, 19, 24, 26, 28, 29, Yrsp, Yrcv, and Super kauz showed either 0, R, MR and MRMS type reactions against leaf rust and stripe rust pathogens (Table 6).

In all wheat-growing regions of the world, leaf and stripe rusts caused by *P. recondita* and *P.*

striiformis, respectively, cause substantial yield losses and socio-economic instability (Rehman *et al.*, 2013; Ali *et al.*, 2017). The diseases develop on susceptible cultivars during suitable environmental conditions. To avoid rust epidemics, sowing of resistant genotypes is the best strategy. In present investigation, 11 genotypes showed resistant (R) response against stripe rust while seven genotypes exhibited a resistant response to leaf rust development with lower AUDPC values. The findings of the present study were in line with those of Prabhu *et al.* (1993) who used the AUDPC to identify sources of resistance against leaf and stripe rust infections. Several investigations including Sohail *et al.* (2013) and Mateen *et al.* (2015) found comparable leaf rust resistance results. Kanwal *et al.* (2021) evaluated 200 genotypes against *P. recondita* and showed that 66 lines were immune to leaf rust, 48 were moderately resistant to susceptible, 66 were vulnerable, and 79 genotypes were highly susceptible.

During the 2019–2020 and 2020–2021 crop seasons, leaf rust genes 1, 10, 17, 18, 22a, 22b, 25, 27+31, 28 and Lr23+Gaza and stripe rust genes Aoc–YrA, Aoc+YrA, 1, 2, Tatara, 8, 10, 17, 18, 19, 24, 26, 28, 29, Yrsp, Yrcv, and Super kauz showed resistant response. The field response of tested leaf rust lines was as follows (9, 18, 9, 22a and 23, 25, Lr+31, 28, 34 36, 23 and 37/1, 2a, 2b, and 2c (Hussain *et al.*, 1999). Hussain *et al.* (2015) studied the 40 *Lr* and 24 *Yr* rust trap nursery differentials. It was found that *Lr* genes such as 9, 19, 23+, 25, 28, 27+31, 32, 34, 36, and 37, and *Yr* genes *viz.*, 3, 5, 8, 10, 15, 18, and Yrsp exhibited no virulence. Similarly, the genotypes Iqbal-02, Uqab-02, Lasani-08, Faisalabad-08, AARI-11, Millat-11, and Pb-11 showed *Lr* and *Yr* resistance. The monitoring of leaf and stripe investigation showed that wheat varieties Inqilab-91, Parwaz-94, and Chakwal-86 were resistant to leaf rust and yellow rust in Punjab and KPK in 1994–1995. Ghanbarnia (2021) found that Gatcher *Lr27* and *Lr31* only regulated combined resistance. In seedling and adult plant evaluations, *Lr13* and *Lr34* interacted with other leaf rust genes to induce higher levels of resistance than anticipated (Bokore *et al.*, 2022).

The wheat varieties Faisalabad-85 and Rawal-87 had moderate yellow rust resistance and leaf rust susceptibility. The three varieties namely Mehran-89, Pirsabak-85 and Pak-81 became the rust-prone. Only a single genotype, i.e., Punjab-85 showed vulnerable response to yellow rust (Chaudhry *et al.*, 1996).

Khan *et al.* (2002) performed an experiment and showed that Lr-19, 24, 25, 27, 36, 37 genes are resistant against leaf rust pathogen. Similarly, Safavi *et al.* (2013) identified leaf rust-resistant genes Lr-31, 30, 29, 24, 21, 18, 17, 15, 16, 12, 3, 2 and 1. Moreover, lines with YRCV, Yr-30, 28, 27, 23, 24, 16, 14, 14a, 14b, 12, 10, 7, 6, 5, 3, 2-c, 2-b and 2-a were moderately susceptible (MS) to susceptible. The occurrence of a severe disease outbreak in Pakistan has been documented in recent years, as evidenced by several studies (Hussain *et al.*, 2015; Ali *et al.*, 2018). This outbreak may be attributed to the pathotypic evolution of races that have the ability to overcome wheat resistance sources as indicated by Ashmawy *et al.* (2012). According to Jin (2011), the pathogens undergo five spore stages and utilise alternate hosts. The pathogens exhibit diverse physiological races that are contingent upon the pathogenicity of the host. According to Figueroa *et al.* (2020), the alteration of fungus virulence is attributed to sexual recombination. Somatic hybridization has been found to result in the emergence of deleterious strains. The co-occurrence of diverse races on a single host genotype in natural conditions can potentially augment the movement of nuclei by germ tubes of urediniospores and infectious hyphae within plant tissues, leading to the emergence of new race, as per the findings of McIntosh *et al.* (2003). Kolmer (2009) found that Lr-1, 2c, 10, and 14a genes were pathogenic on leaf rust in wheat-growing regions. Singh *et al.* (2004) found that Yr-18 is a resistance source for Parula, Trap, Yaco, and others. Wu *et al.* (1993) observed that 67 virulence patterns based on 17 differentials and pathogen population patterns varied with wheat cultivar resistance.

Conclusions and Recommendations

This investigation concluded that wheat genotypes have resistant sources against leaf and stripe rusts. If this material is tested for other agronomic traits in different agro-climatic zones of Pakistan, acceptable results will be achieved. These cultivars can be used in breeding programmes to release resistant varieties or cultivars. This strategy can improve and sustain agricultural productivity and prevent epidemics if farmers have timely access to resistant germplasm.

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Novelty Statement

The virulence of the leaf and stripe rust pathogens have not been yet investigated in Pakistan during the crop seasons of 2019–2020 and 2020–2021. The present study will help to develop durable resistance in wheat germplasm against leaf and strip rusts.

Author's Contribution

Muhammad Hussain: Conceived the research idea.

Yasir Ali and Babar Iqbal: Conducted research.

Muhammad Azar Iqbal: Designed experiment.

Salman Ahmad and Muhammad Zeeshan Majeed: Writing of the manuscript.

Hafiz Muhammad Aatif: Proof-read the manuscript.

Muhammad Saeed and Muhammad Jawad Yousaf: Analyzed the research data.

Conflict of interest

The authors have declared no conflict of interest.

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