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Research article

Genotypes, epidemiological variables and fungicides application associated with wheat leaf rust development and grain yield

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ABSTRACT

The present study was carried out at the Plant Pathology Hafizabad Research Station, the University of Layyah, during the crop seasons 2021–2022 and 2022–2023 to evaluate the response of various wheat genotypes against leaf rust severity (%), environmental conditions favourable for disease development and grain yield. Except for minimum temperature and minimum relative humidity, which had a negative association with disease development, there was a significant correlation between leaf rust severity (%) and all environmental conditions such as maximum temperature, maximum relative humidity, rainfall, and wind speed. All epidemiological variables such as maximum temperature, minimum temperature, minimum relative humidity, rainfall and wind speed significantly affect the disease progression. The disease predictive model accounted for 48–69 % variability in leaf rust severity. The model performance was evaluated using the coefficient of determination ($R^2 = 0.69$) and RMSE, both demonstrated acceptable predictive results for leaf rust severity (%) management. Leaf rust severity (%) increased with an increase in maximum temperature (17.8–30 °C), maximum relative humidity (76.3–85 %), rainfall (2.2–10.85 mm) and wind speed 1.1–2.7 km/h and decreased with the increase of minimum temperature (7.91–16.71 °C) minimum relative humidity (47.15–56.45 %) during both rating seasons 2021–2022 and 2022–2023. The single and two applications of fungicides at the Zadok's scale 3, ZS 4.3, and ZS 5.4 stages led to a significant reduction in grain yield losses caused by leaf rust severity (%) in both the 2021–2022 and 2022–2023 crop seasons. Single and two sprays of prothioconazole, were found to be the first choice among all treatments to reduce the disease severity and increase grain production and maximum gross revenue (513.1–777.8$/ha), as compared to followed by single and two sprays of propiconazole (Progress), tebuconazole +

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trifloxystrobin, tebuconazole, bixafen + tebuconazole, and propiconazole (Tilt), respectively. These findings recommend the involvement of genotype resistance and weather predictors in wheat leaf rust development, along with fungicide application studies, to improve the predictability of host resistance to disease, future models, and the sustainability of disease control methods.

1. Introduction

Wheat is an excellent dietary choice due to its high nutritional quality, wide availability, and low cost. After rice and maize, wheat is the 3rd most significant food crop [1]. It is grown on a 9,178000 ha area with a 27.293 MT yield [2]. Wheat production is influenced by various biotic and abiotic factors [3]. The biotic factors include various types of rust, such as brown, yellow, and black rust. Whereas the abiotic factors include a range of environmental conditions, including heat, salinity, drought, wind, fog, hailstorms, and severe cloudiness [4,5].

Leaf rust is considered the most harmful rust disease and caused grain losses of 50 % or more [6,7]. In March and April, the crop is severely infected with brown rust [8]. Following pathogen infestation, orange to brown colour, circular to oval-shaped pustules appeared on the upper epidermis of leaves [9].The rapid dissemination of brown rust infection occurs within a temperature range of 10–30 °C [10]. The pathogen is capable of infecting within 3 h during dew periods when the air temperature is at 20 °C [11]. Moreover, sufficient precipitation is a prerequisite for the incidence of infection under low-temperature environments. Certain infections may occur when the temperature ranges from 2 °C to 32 °C with the presence of sufficient moisture [12,13]. In their recent study, Smith et al. [14] investigated the impact of weather parameters on the progress of leaf rust. The researchers focused on characterizing the relationship between various weather factors and the development of leaf rust. Their findings provide valuable insights into the influence of weather conditions on the spread and severity of leaf rust.

Different methods, such as physical, biological, chemical, and cultural methods, are used to manage leaf rust severity affecting plants [15]. Moreover, planting date is also an effective agronomic practice to mitigate leaf rust and enhance crop yield. By adjusting the planting date, farmers can strategically avoid periods of high disease pressure, thereby reducing the severity of leaf rust infections. This finding aligns with the broader understanding that cultural methods, such as manipulating planting dates, can play a crucial role in disease management [16]. Naseri et al. [17] found that the timing of planting and prevailing weather conditions significantly influenced the susceptibility or resistance of wheat cultivars to rust pathogens. These data suggest that reliance on only screening wheat germplasm may not be adequate for finding resistant sources against rust pathogens. The introduction of genetic resistance has been demonstrated to effectively decrease losses caused by the brown rust pathogen, making it the most effective approach [18,19]. However, fungicides have been extensively used in the management of plant diseases affecting agronomic crops. Utilising fungicides is the most optimal approach for the management of leaf rust. Fungicides are chemicals that are used to stop or slow the spread of fungus infections. By creating a barrier between the host plant and the fungus, fungicides protect plant tissues [20,21].

Fungicides containing triazole (DMI) play a critical role in the development and growth of fungal cell walls. Their mechanism of action involves the inhibition of a crucial fungus enzyme responsible for sterol biosynthesis [22]. A comprehensive comprehension of the disease's epidemiology is essential for the successful implementation of chemical control measures [23,24]. By considering influential climate variables, such as temperature, humidity, and rainfall, these models can accurately predict the occurrence and severity of stripe rust outbreaks. This information is crucial for optimizing fungicide applications, as it allows farmers to strategically time and target their chemical control measures [25]. Chemical fungicides have been employed for a considerable period in certain regions to manage the brown rust pathogen. In recent times, novel exotic races have emerged, resulting in a decrease in genetic resistance. These findings emphasise the importance of establishing a comprehensive rust control programme that extends beyond merely depending on genetic resistance. Although genetic resistance has demonstrated effectiveness in combating rust diseases, the appearance of new races requires a more comprehensive strategy. By incorporating a number of management measures, including cultural practices, fungicide sprays, and genetic resistance, it is possible to enhance the durability of resistance and effectively control rust diseases. In the year 2003 [26], successfully registered novel chemical fungicides that exhibit high efficacy against leaf rust. Kelley [27] research findings indicate that the application of Tilt (Propiconazole) fungicide in Kansas led to a significant increase of up to 77 % in the production of winter wheat within six years. However, it is crucial to note that relying solely on fungicides is not a sustainable long-term solution for managing wheat leaf rust. Fungicides can provide short-term control of leaf rust, their effectiveness can diminish over time due to the development of resistance in the pathogen population. Additionally, the repeated use of fungicides can have negative environmental impacts and may lead to the selection of more virulent rust strains. Therefore, a comprehensive and sustainable approach to managing wheat leaf rust is necessary.

Hence, the primary goals of this study were to: (1) evaluate the susceptibility of multiple wheat cultivars to the leaf rust pathogen in field conditions; (2) characterize environmental factors conducive to leaf rust development on wheat; and (3) evaluate the efficacy of various fungicides against the leaf rust pathogen and perform yield assessments of distinct wheat varieties.

2. Materials and methods

2.1. Germplasmcollection and sowing

Thirty-nine (39) wheat genotypes were collected from Arid Zone Research Institute Bhakkar (AZRI) and Ayub Agricultural Research Institute (AARI) Faisalabad (Table 1). The genotypes were evaluated based on their rust reactions and yield attributes. The wheat genotypes were sown in 3rd week of October during the 2021–2022 and 2022–2023 crop seasons by using an augmented design with single replication for each treatment at the Experimental Area of Plant Pathology, located at Hafizabad Research Farm, College of Agriculture, University of Layyah (Fig. 1). The environmental conditions, including minimum and maximum temperature, rainfall and relative humidity of the study area, are illustrated in Table 2. Following the first irrigation, a recommended dose of 125 kg/ha of urea was manually broadcasted. After the second irrigation, weeds were effectively controlled by spraying suitable herbicides, specifically imazamethabenz, fenoxaprop-Pethyl, and mesosulfuron-methyl with the help of a knapsack sprayer by using a T-Jet/Flat Fan nozzle. The field was irrigated three times with 1952.01 cubic meters of water during both growing seasons. The wheat plants were inoculated by using different techniques such as rubbing, dusting, injection, and spraying [28]. The rubbing method involved gently applying the inoculum onto the leaf surface, suitable for pathogens that enter through wounds, simulating natural micro-abrasions. Dusting involved spreading pathogen spores as a fine powder, effective for diseases transmitted through air, resembling natural spore dispersion of rust pathogen. Injection was used to increase the disease epidemic, where a liquid inoculum was directly injected into the plant tissue to reach the vascular system. Lastly, spraying a pathogen suspension onto the plants was used for disease epidemic, replicating rain-splash pathogen spread. The timing of these methods was carefully selected to coincide with the most susceptible growth stage of the plants, as indicated by previous investigation and the known disease cycle, to enhance the pathogen infection and accurately evaluate genotype responses [28].

2.2. Data recording of leaf rust severity (%)

The rust severity data were recorded using the modified Cobb scale proposed by Ref. [29].(Table 3). Four readings were taken for recording the disease severity. The initial reading was taken seven days after the appearance of the first spikelet. The second and third readingswere carried out to record the extent of disease severity until the wheat reached its physical maturity. The final disease severity reading was taken at the end of the assessment period, which corresponded to the stage when the most susceptible genotypes exhibited more than 70 % disease severity. This allowed for the comparison of disease progression across different genotypes, including those with varying levels of resistance. The data were recorded every 10 days intervals.

2.3. Calculation of AUDPC

The AUDPC for each treatment was calculated from recorded severity data [30]. The average number was calculated by adding the first two infection percentages and dividing them by two. This average number was multiplied by the number of days from the 1st reading to the 2nd reading. The AUDPC value was taken by adding up all of the trapezoid values. Lower AUDPC readings mean slower

Table 1
Details of wheat genotypes cultivated in the Research Area of Plant Pathology during crop season 2021-22 and 2022-23.

Sr. No	Name of genotypes	Year of release	Yield Potential (kg/ha)	Source	Sr. No	Name of genotypes	Year of release	Yield Potential (kg/ha)	Source
1	Uqab-2000	2000	6900	[a]AARI	21	Inqlab-91	1991	6800	_
2	Maxipak-65	1965	5900	_	22	Blue Silver	1971	5757	_
3	Pari-73	1973	6135	_	23	Faisalabad-85	1985	5500	_
4	Galaxy-13	2013	7900	_	24	AS-2002	2002	6800	_
5	MH-97	1997	6000	_	25	SA-75	1975	6200	_
6	Barani-83	1970	5073	_	26	SA-42	1942	4800	_
7	Kohistan-97	1997	5800	_	27	TWS-19125	2019	6700	[b]AZRI
8	WL-711	1978	5700	_	28	TWS-19101	2019	6500	_
9	Chenab-76	1976	5900	_	29	TWS-1849	2019	6600	_
10	Niab-81	1981	5200	_	30	TWS-1902	2019	7600	_
11	Parwaz-94	1994	6100	_	31	TWS-1992	2019	6900	_
12	Punjab-85	1985	6300	_	32	TWS-19125	2020	6900	_
13	Shahkar-95	1995	6500	_	33	Subhani	2021	7100	_
14	Iqbal-2000	2000	6700	_	34	Nishan-21	2021	7200	_
15	Chakwal-86	1986	6400	_	35	FakhreBhakkar	2018	7400	_
16	Faisalabad-83	1983	5500	_	36	Bhakkar-2002	2002	7000	_
17	Pasban-90	1990	6500	_	37	Markaz-19	2019	6900	_
18	Rohtas-90	1991	5900	_	38	Dilkash-21	2021	7000	_
19	SH-2002	2002	6800	_	39	Bhakkar Star	2019	7500	_
20	Chanab-2000	2000	6800	_	–	–			

[a] AARI = Ayub Agricultural Research Institute, Faisalabad.
[b] AZRI = Arid Zone Research Institute, Bhakhar.

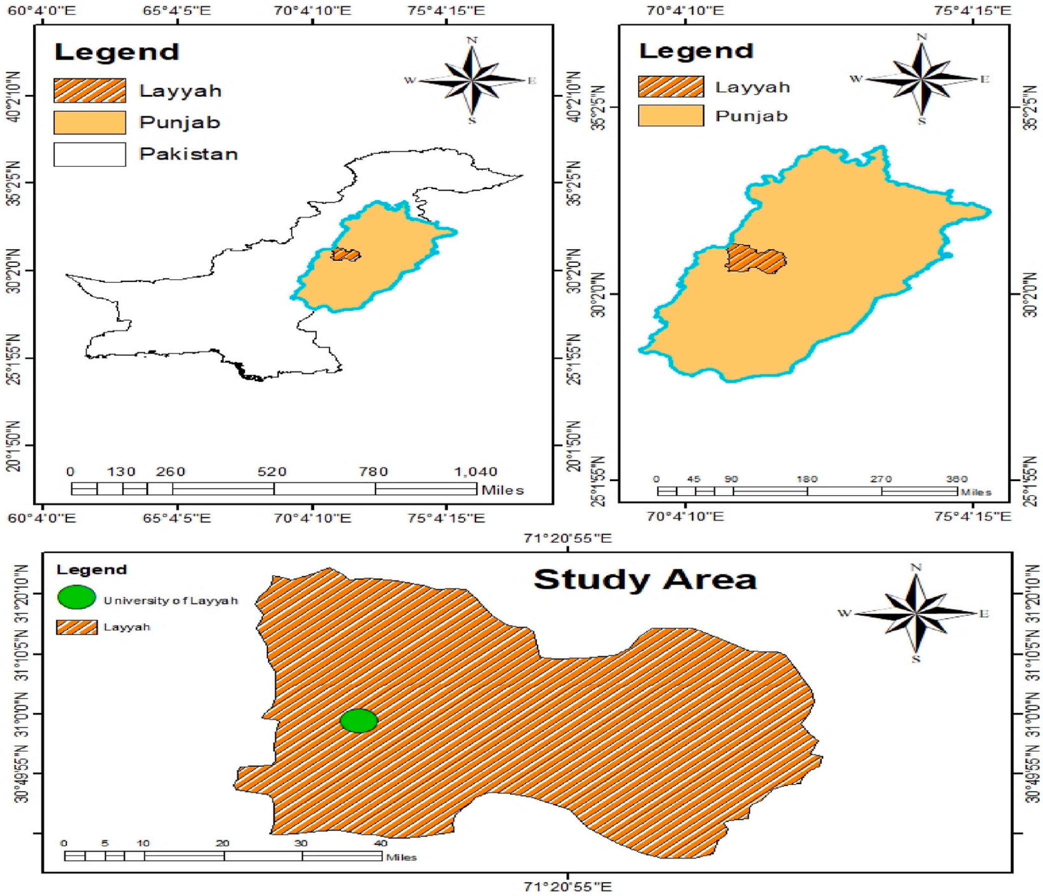

Fig. 1. Map of Pakistan showing the study area of the present investigation.

Table 2
Theaverage annual environmental conditions of the study area during the crop seasons 2021–2022 and 2022–2023.

Location	LAYYAH							
	Soil Classification	Mini. T. °C	Aver. Temp °C	Maxi. Temp. °C	Mini. R.H. (%)	Maxi. R.H. (%)	Mini. R.F. (mm)	Maxi. R.F.
2021–2022	Sandy Loam	14.8	24.8	47	46	74	248–300	498–600
2022–2023	Sandy Loam	16.2	26.3	49	42	70	252–300	502–500

Table 3
Modified Cobb's scale used for data recording during the crop seasons 2021–2022 and 2022–2023.

Reaction	Code	Field response
Immune	0	No visible infection
Resistant	R	Necrotic areas with or without minute uredia
Moderately Resistant	MR	Small uredia present surrounded by necrotic area
Moderately resistant, moderately susceptible	MR/ MS	The minute uredia present surrounded by necrotic areas as well as medium uredia with no necrosis but possible some distinct chlorosis
Moderately Susceptible	MS	The medium uredia with no necrosis but possible some distinct chlorosis
Moderately Susceptible- Susceptible	MSS	Medium uredia with no necrosis but possible some distinct chlorosis as well as large uredia with little or chlorosis present.
Susceptible	S	Large uredia and little or no chlorosis present.

disease development and more disease resistance. Higher AUDPC readings mean a quicker development of the disease and a greater vulnerability to it. The AUDPC was calculated by using the expression given below, that is illustrated in equation (1):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{xi + xi + 1}{2} \right) (ti + 1 - ti) \tag{1}$$

In this expression, xi represents the rust intensity on date i, ti is the time in days between i and date i + 1 and n is the number of dates on which the disease was recorded.

2.4. Collection of epidemiological data set

The environmental variables, namely minimum and maximum temperature, rainfall, relative humidity, and wind speed, were collected from the Pakistan Meteorological Observatory Karor-Layyah during the months of February and March, which coincided with the recording of leaf rust severity. These data were collected over the crop seasons 2021–2022 and 2022–2023, ensuring a comprehensive analysis of the weather parameters during the specific time periods when disease severity measurements were obtained. Thee collected data was converted into weekly intervals and the mean values were calculated. The weekly mean values were used for data analysis.

2.5. Development of disease predictive model

A stepwise regression model was developed between the two years (2021–2022 and 2022–2023) epidemiological variables (independent variables) and leaf rust severity data (dependent variable) on four wheat varieties Pasban-90, Pari-73, AS-2002, and Shahkar-95. The impact of environmental conditions on disease severity was also determined by the correlation analysis [31].

2.6. Model evaluation

Following the development of the model using stepwise regression, the model was evaluated using the methods [32,33].

1) Evaluating the association between the dependent variable and regression coefficients in reference to physical theory.
2) Comparison of observed data with predicted data

Predictions were evaluated by calculating statistical indices such as root mean square error (RMSE) and percentage error [34]. The following impression, as indicated in equation (2), used in determining the root mean square error (RMSE) and percentage error were:

$$RMSE = \left[\frac{\sum_{i=1}^{n} (p_i - o_i)^2}{n} \right]^{0.5}$$

$$Error\ Percentage = (p_i - o_i)\ 100 \tag{2}$$

Pi and Oi represent the predicted and observed data points of the studied parameters. Meanwhile, n represents the total number of observations. Model performance is considered good if the root mean square error (RMSE) and percentage error are less than or equal to ± 20 [35].

2.7. Relationship between epidemiological data withdisease development

The correlation and linear regression analyses were used to determine the relationship between epidemiological variables

Table 4
List of fungicides used to reduce the disease severity and enhance crop production during the boh growing seasons 2021–2022 and 2022–2023.

Sr. No	Treatment	Trade name	Common name	Active ingredients	Application rate	Company name
1	T_1	Tilt 250 EC	Propiconazole	Propiconazole 250	0.5 lit/ha	Syngenta
2	T_2	Zantara EC 216	Bixafen + Tebuconazole	Bixafen 50 g/l + Tebuconazole 166 g/l	1.25 lit/ha	Bayer Crop Science
3	T_3	Progress 250 EC	Propiconazole	Propiconazole 25 g/l	0.5 lit/ha	Asiatic Agricultural industrial Pte. Ltd.
4	T_4	Folicur	Tebuconazole	Tebuconazole (triazole) 250 g/l	300 mL/h	Bayer Crop Science LP
5	T_5	Nativo	Tebuconazole + Trifloxystrobin	Tebuconazole (triazole) 200 g/l + Trifloxistrobin (strobilurin) 100 g/l	300 g/ha	Bayer Crop Science LP
6	T_6	Proline	Prothioconazole	(41) % Prothioconazol	175 g/ha	Bayer Crop Science LP
7	T_7	Control	–	–	–	–

(maximum and minimum temperature, rainfall, humidity, and windspeed) and disease severity [31]. The disease severity was used as the dependent variable, while epidemiological data was used as the independent variable. The environmental variables that significantly impact the development of leaf rust were explored by plotting data graphically.

2.8. Response of yield attributes to fungicides application

For evaluation of fungicides against leaf rust severity and yield loss reduction three wheat varieties, including WL-711 and SH-2002, with moderately susceptible to the susceptible level of resistance and Markaz-19 as resistant check, were cultivated in the research area of Plant pathology Hafizabad research station, University of Layyah during the crop season 2021-22 and 2022–23. The varieties were sown by using the Randomized Complete Block Design (RCBD) with four replications. Six fungicides (Table 4) were evaluated against disease severity to enhance crop production. Foliar application of treatments applied on the wheat plant during pre-rust emergence mostly coincided with Zadok's scale 3 (stem elongation and jointing) and Zadok's scale 4.3 and 5.4 (booting and flowering stage). The treatments were sprayed after every eight days intervals on each plot. The check plot was sprayed with pure water to have a similar moisture effect across plots. The data on disease severity were recorded using the modified Cobb scale [29] (Table 3).

2.9. Data recording of yield attributes

A thousand grains of each tested variety were selected randomly and weighed using an electronic balance to determine the thousand-grain weight (TGW) in grams. The grain yield per plot of each tested variety was calculated and then converted into tons per hectare (t ha^{-1}). The yield difference between the control treatment and fungicide spraying for each variety was evaluated to

Table-5

The overall response of different wheat genotypes against leaf rust severity (%) with the area under disease progress curve (AUDP) values for the crop seasons 2021-2022and 2022–2023.

Sr. No	2021–2022			2022–2023		
	Genotype	**Reaction**	**AUDPC**	**Genotype**	**Reaction**	**AUDPC**
1	Auqab-2000	MS	605.5	Auqab-2000	S	1173.5
2	Mexipak-65	MS	668.5	Mexipak-65	S	1276.1
3	Pari-73	S	1277.5	Pari-73	S	1611.1
4	Galaxy-13	MS	686	Galaxy-13	MS	522
5	MH-97	MS	689.5	MH-97	S	1812
6	Barani-83	S	1827	Barani-83	S	1827
7	Kohistan-97	S	1718.5	Kohistan-97	MSS	711
8	WL-711	S	1809.5	WL-711	S	1711.5
9	Chakwal-86	S	1806	Chakwal-86	S	1712.1
10	Markaz-19	R	136.5	Markaz-19	R	109.1
11	Iqbal-2000	MS	833	Iqbal-2000	MSS	891
12	Bhakhar Star	R	63	Bhakhar Star	R	51
13	Faislabad-83	S	1886.5	Faislabad-83	MS	526
14	Dilkash-21	R	66.5	Dilkash-21	R	49
15	Pasban-90	MSS	1050	Pasban-90	S	1811
16	TWS-1907	MRMS	388.5	TWS-1907	MRMS	322.5
17	Rohtas-90	MSS	973	Rohtas-90	S	1811.1
18	TWS-19101	MRMS	469	TWS-19101	R	40
19	SH-2002	MS	556.5	SH-2002	MSS	922.1
20	TWS-1849	R	73.5	TWS-1849	R	60.21
21	Chenab-2000	MSS	892.5	Chenab-2000	MS	611.1
22	TWS-1902	R	87.5	TWS-1902	R	60.1
23	Inqalab-91	MS	752.5	Inqalab-91	MRMS	222.12
24	Subhani	R	70	Subhani	R	61
25	Blue Silver	S	1617	Blue Silver	MRMS	411
26	Nishan-21	R	66.5	Nishan-21	R	40.12
27	Faislabad-85	S	1606.5	Faislabad-85	MS	651.1
28	TWS-1992	MRMS	273	TWS-1992	MRMS	222
29	AS-2002	MSS	1179.5	AS-2002	MSS	1179.5
30	FakhreBhakhar	R	66.5	FakhreBhakhar	R	59.1
31	SA-75	S	1862	SA-75	MS	511.1
32	TWS-19125	R	63	TWS-19125	R	49.1
33	SA-42	S	1862	SA-42	MRMS	523
34	Bhakkar-2002	MR	231	Bhakkar-2002	MRMS	321
35	Chenab-76	MRMS	272	Chenab-76	S	1603
36	Niab-81	MRMS	211.12	Niab-81	S	1655.5
37	Parwaz-94	MRS	312.12	Parwaz-94	S	1774.5
38	Punjab-85	MRMS	202	Punjab-85	S	1687
39	Shahkar-95	MSS	1120	Shahkar-95	MRMS	300.23

determine the potential decrease of grain yield loss (in the absence of foliar fungicide spraying) [36] as expression given below, that is exhibited in equation (3):

$$\text{Reduction } (\%) = \frac{\text{ysp} - \text{ynsp}}{\text{ysp}} \times 100 \tag{3}$$

In equation (3) Ysp represent grain yield under sprayed and Ynsp represents non-sprayed conditions. The gross revenue of each treatment was calculated by using the following expression, as indicated in equation (4):

$$\text{Gross revenue} = \text{yeild increase} \times \text{grain price} \tag{4}$$

The marginal return was calculated by subtracting the gross return of the untreated control from the gross return of each fungicide treatment (in dollars per hectare). Only the cost of fungicide treatment, including fungicide and spraying (dollars/ha), was a variable in the experiment. The reduction (%) of TGW loss of each treatment was calculated using the following equation at 15 % water content, as exhibited in equation (5).

$$\text{Reduction } (\%) \text{ TGW} = \frac{y_1 - y_2}{y_1} \times 100 \tag{5}$$

Where y_1, is the TGW of fungicide treatment and y_2, is the TKW of the non-sprayed check treatment.

2.10. Statistical analysis

The significant differences in spray treatments, fungicide types, and varieties were rigorously analyzed using Minitab software version 20. The Analysis of Variance (ANOVA) was employed to evaluate the effects of the treatments. This method allowed us to determine if there are any statistically significant differences between the means of our independent groups. In cases where the ANOVA indicated significant differences, we proceeded with a post-hoc analysis where, we used the Least Significant Difference (LSD) test at a 5 % level of significance. This test was selected for its ability to compare multiple treatment means to identify which specific treatments differed from each other. The results of the ANOVA are presented in the form of an F-statistic and its associated p-value. The F-statistic allows us to compare the model variance with the residual variance to determine the overall significance of the model. A P-value <0.05 was considered indicative of a statistically significant effect of the treatments [31].

3. Results

3.1. Evaluation of wheat genotypes against leaf rustseverity (%)

The observed genotypes exhibited different responses to leaf rust severity across the two rating seasons of 2021–2022 and 2022–2023 (Table 5). During the first crop season, the rate of leaf rust development was comparatively less severe in cultivars such as Auqab-2000, Mexipak-65, MH-97, Pasban-90, Rohtas-90, Chenab-2000, Chenab-76, Niab-81, Parwaz-94, and Punjab-85, which exhibited moderate susceptible to the susceptible response. However, in the second crop season, the leaf rust severity was observed to be more pronounced in these cultivars, which were highly susceptible. During the firstrating season, the epidemiological variables

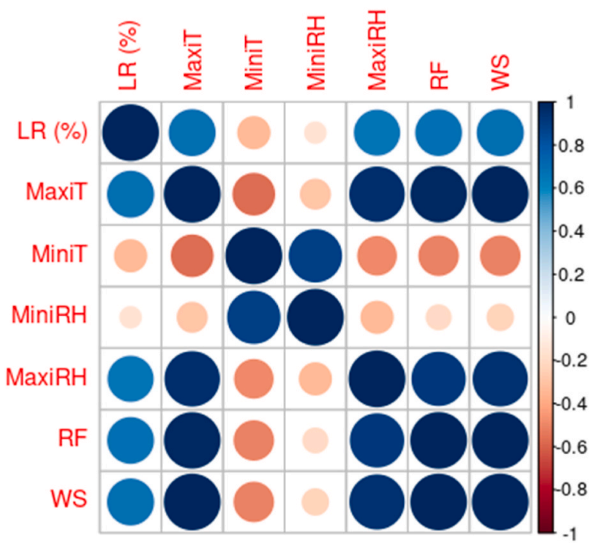

Fig. 2. Correlation of leaf rust (%) with different epidemiological variables on wheat genotypes during the crop seasons 2021-22 and 2022-23.

were found to be less favourable, resulting in a delay in the progression of the disease. The appearance of leaf rust severity exhibited significant interannual variation in genotypes. In 2021–2022, nine genotypes exhibited a resistant (R) reaction to leaf rust severity, as evidenced by lower AUDPC values. Similarly, in the 2022-2023cropseason, ten genotypes demonstrated a resistant (R) response with lower AUDPC values (Table 5).

3.2. Correlation of epidemiological variables with leaf rust severity (%)

During both crop seasons of 2021–2022 and 2022–2023, a significant correlation was observed between epidemiological variables and leaf rust severity (%) on all wheat genotypes (Fig. 2). It was observed that all environmental variables, namely maximum temperature, maximum relative humidity, rainfall, and wind speed, demonstrated a positive correlation with leaf rust severity. However, minimum temperature and minimum relative humidity exhibited a negative correlation with disease severity (Fig. 2).

3.3. Development of disease predictive model

Environmental conditions and leaf rust severity (%) data were examined to develop a disease predictive model over a span of two years. The developed multiple regression model is given as: $Y = 122.70 + 3.222x_1 - 5.836x_2 - 1.913x_3 + 3.866x_4 + 5.721x_5 + 9.610x_6$ where Y represents the population of leaf rust severity (%), and x_1, x_2, x_3, x_4, $x_{5,}$ and x_6 are indicators for maximum temperature, minimum temperature, minimum relative humidity, maximum relative humidity, rainfall, and wind speed, respectively.

The model equation reveals that maximum temperature, maximum relative humidity, rainfall, and wind speed were major factors affecting the leaf rust severity (%). By one unit change in maximum temperature disease severity increases by 3.222 units, while maximum relative humidity increases by 3.866 units. Changes in rainfall and wind speed affect the population by 5.721 and 9.610 units, respectively.

Upon analysis it was observed that maximum temperature explained 61 % of the variance in the model ($F = 190.41$, $p < 0.01$). Similarly, leaf rust severity variability was attributed to minimum temperature (58 %), minimum relative humidity (56 %), rainfall (48 %) and wind speed explained (69 %) (Table 6).

The disease predictive model, overall accounts for a variability range of 48–69 % in leaf rust severity. This underscores the pivotal role of environmental conditions in the manifestation of the disease and their critical importance in the formulation of predictive models for its management.

3.4. Comparison of the dependent variable leaf rust severity (%) and regression co-efficient with physical theory

The model showed higher value of coefficient of determination i.e., R^2 (69.0) and lower value of standard error of estimate (5.95) that are considered fairly good mainly under field conditions when one has no control on any of the studied variables (Table 7). The F-distribution of regression model was significant at $P < 0.05$ (Table 8). The environmental predictors—wind speed, maximum temperature, maximum relative humidity, and rainfall—were all statistically significant contributors to leaf rust severity (%), each exhibiting a P-value below 0.05 and consistently low standard errors (Table 9). The higher coefficient of regression (R^2) value, lower standard error value and the significance of regression statistics exhibited that model was good to predict leaf rust severity (%) (Tables 7–9).

3.5. Evaluation of model by comparing the observed and predicted data

The model's predictions were assessed using two criteria: root mean square error (RMSE) and error percentage. The normal probability plot for both of the years (2021–2022 and 2022–2023) showed that the majority of data points were closely around the reference line. However, there were a few data points that deviated from the reference line, both at higher and lower values, which produced errors into the regression model. The residual of 15 % was observed, demonstrating a fair degree of matching between the observed and projected data points (Fig. 3). The wheat varieties Pasban-90, Pari-73, AS-2002, and Shahkar-95 had high R2 values (>95.45 %) and low RMSE values (≤20), demonstrating a good association between the observed and predicted data points. This suggests that the model accurately predicted the severity of leaf rust (%) (Fig. 4).

Table 6
Summary of a disease predictive model for leaf rust severity (%) based on mean epidemiological data collected over two years 2021–2022 and 2022–2023.

Variable entered	No. in model	Model R^2	F-value	P-value
Maximum temperature (°C)	1	0.61	190.41	0.01*
Minimum temperature (°C)	2	0.58	191.01	0.01*
Minimum Relative humidity (%)	3	0.56	182.50	0.02*
Maximum Relative humidity (%)	4	0.51	165.84	0.03*
Rainfall (mm)	4	0.48	185.08	0.04*
Wind speed (Km/h)	5	0.69	191.59	0.01*

Table 7
Regression statistics of the disease predictive model for leaf rust severity (%) based on two years 2021–2022 and 2022–2023 data.

Regression statistics	R	R^2	Adjusted R^2	Std. Error of the Estimate	Observations
	0.71[a]	0.69	0.67	5.94	219.00

[a] . Predictors: (Constant), Wind Speed, Maximum Temperature, Maximum Relative Humidity, Rainfall.

Table 8
Assessing the variance of the disease predictive model for leaf rust severity (%)using data from two consecutive years, 2021–2022 and 2022–2023.

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Regression	48727.97	1	48727.97	191.53	[a]0.01[b]
Residual	55444.01	218	254.30		
Total	104172.02	219			

[a] . Predictors: (Constant), Wind Speed, Maximum Temperature, Maximum Relative Humidity, Rainfall.
[b] = Significant at $P < 0.05$.

Table 9
Co-efficient of variables, their standard error, t Stat, P-value and Significance.

Model	Parameters	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	−22.41	3.96		−5.61	0.00[b]
	Wind speed (WS)	25.64	1.80	0.64	13.84	0.01[b]
	Maximum temperature (°C)	0.18	2.34	0.02	0.32	0.05[b]
	Maximum Relative humidity (%)	−0.10	4.67	0.81	0.69	0.48[b]
	Rainfall (mm)	0.49	1.83	0.76	−0.86	0.03[b]
	Excluded Variables [a]					
	Minimum temperature (°C)	−0.20	2.46	0.05	−0.25	0.71
	Minimum Relative humidity (%)	0.32	3.03	0.64	0.76	0.47

[a] . Dependent Variable: leaf rust severity (%).
[b] = Significant at $P < 0.05$.

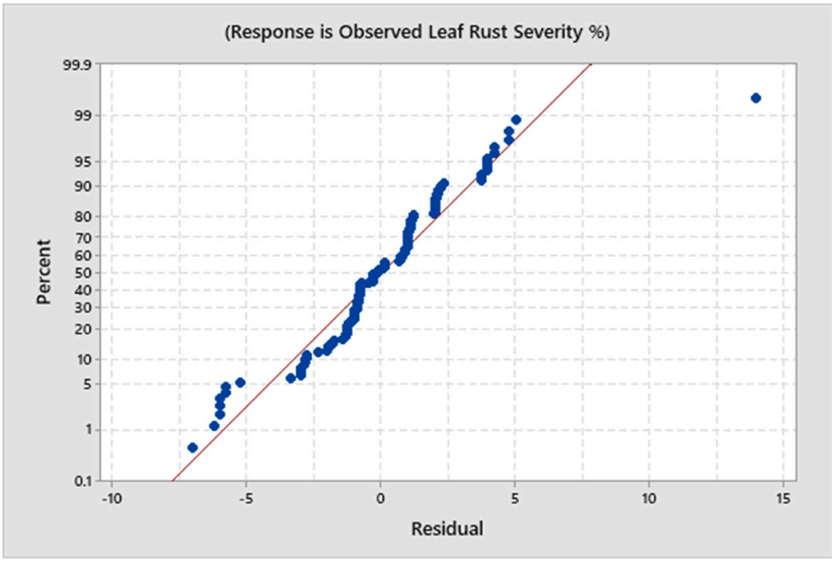

Fig. 3. Normal probability plot for two years (2021–2022 and 2022–2023) disease predictive model of leaf rust severity (%).

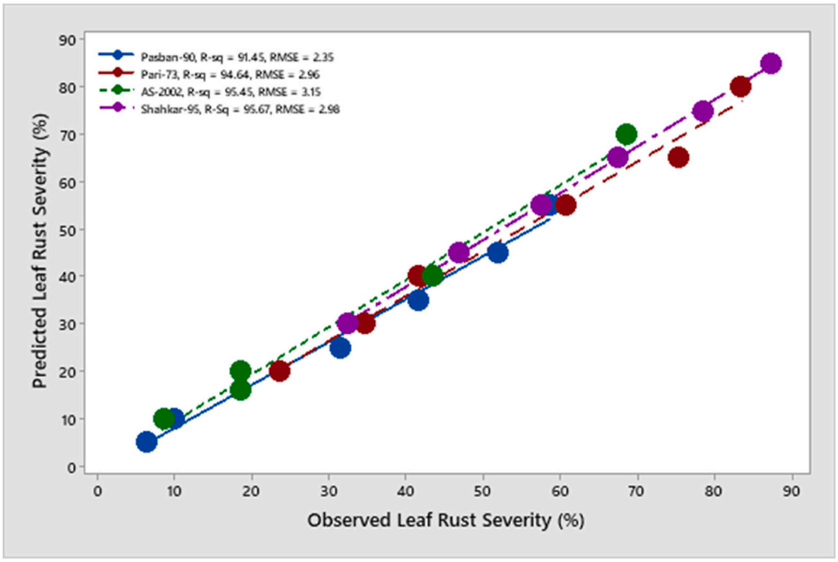

Fig. 4. Comparison of observed & predicted data points of leaf rust severity (%) on four wheat varieties Pasban-90, Pari-73, AS-2002, and Shahkar-95 during two crop seasons 2021–2022 and 2022–2023.

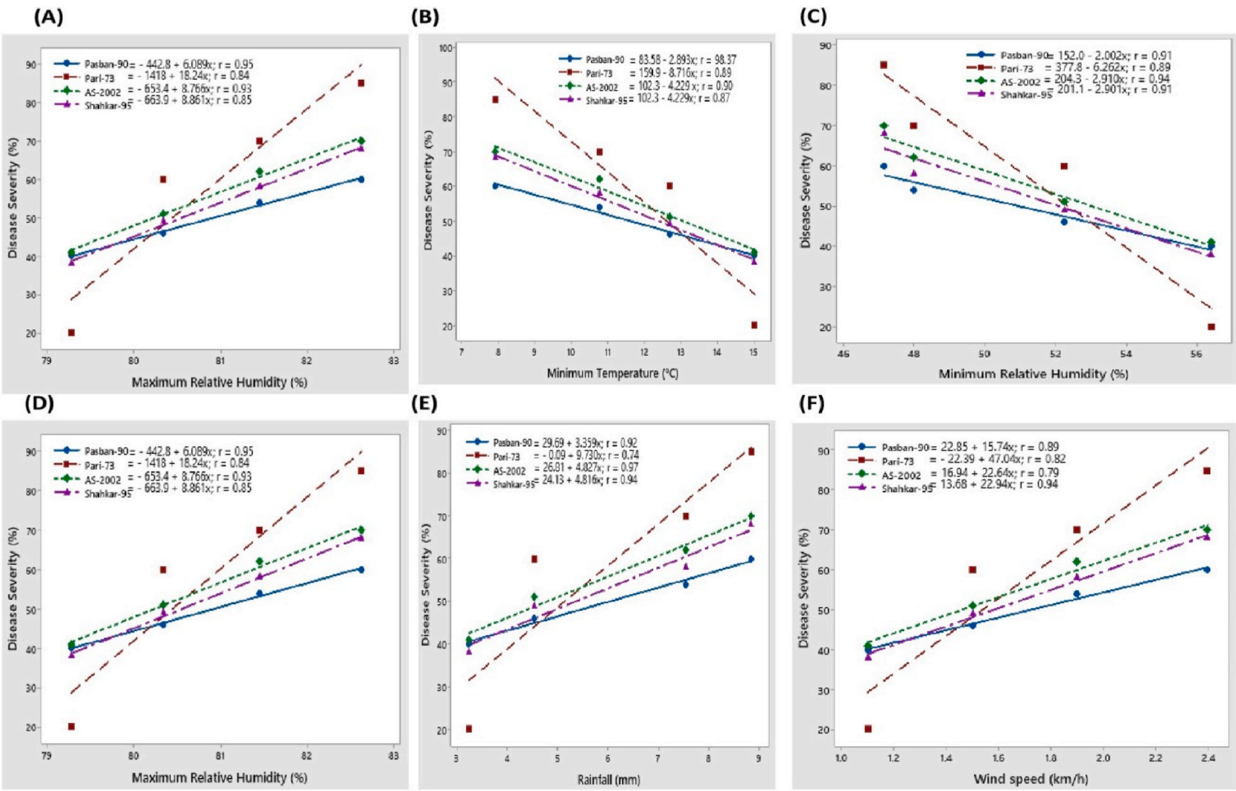

Fig. 5. Relationship of maximum temperature (A), maximum relative humidity (B), rainfall (C), minimum temperature (D), minimum relative humidity (E), and windspeed (F) with leaf rust severity recorded on Pasban-90 (V1), Pari-73 (V2), AS-2002 (V3), and Shahkar-95 (V4) during crop season 2021–2022.

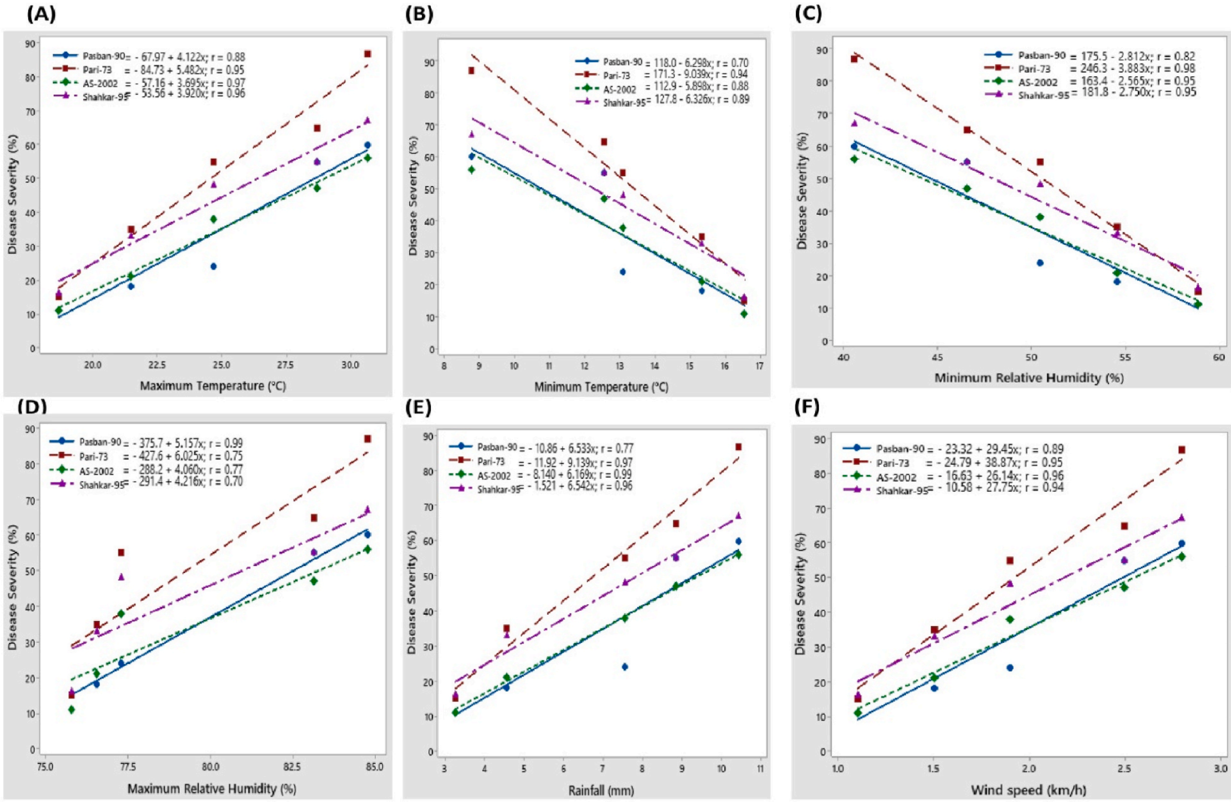

Fig. 6. Relationship of maximum temperature (A), maximum relative humidity (B), rainfall (C), minimum temperature (D), minimum relative humidity (E), and wind speed (F) with leaf rust severity recorded on Pasban-90 (V1), Pari-73 (V2), AS-2002 (V3), and Shahkar-95 (V4) during crop season 2022–2023.

3.6. Characterization of epidemiological variables conducive to leaf rust severity (%)

During the first crop season of 2021–2022, the highest leaf rust severity (%) was observed within the temperature range of 17.8–26.8 °C (Fig. 5A). It was observed that, during the second crop season of 2022–2023, disease severity increased with an increase in the maximum temperature range of 19.2–30 °C (Fig. 6A). The simple linear regression model demonstrated a strong relationship between maximum temperature and leaf rust severity (%), as evidenced by the r values of 0.81, 0.85, 0.89, and 0.87 during the 2020–2021 (Figs. 5A), 0.88 and 0.95, 0.97, and 0.96 in the 2022–2033 (Fig. 6A). A negative linear relationship was observed between minimum temperature (Fig. 5B and C), minimum relative humidity (Fig. 6B and C) and leaf rust severity (%). This relationship was best explained by their r values. Leaf rust severity increased gradually with an increase in maximum relative humidity from 79.3 to 82.63 % in the 2020–2021 crop season (Fig. 5D) and from 76.3 to 85 % in the 2022–2023 crop seasons (Fig. 6D). A positive relationship was recorded between leaf rust severity (%) and rainfall. During the first rating season, the maximum leaf rust severity (%) was recorded within the rainfall range of 3.2–8.85 mm (Fig. 5E). While during the second crop season of 2022–2023, disease severity increased with an increase in rainfall 2.2–10.85 mm (Fig. 6E). During both rating seasons, disease severity (%) increased with an increase in wind speed. Maximum disease severity (%) was observed at the highest wind speed of 2.4 km/h in the first crop season of 2021–2022 (Figs. 5F) and 2.7 km/h during the second crop season of 2022–2023 (Fig. 6F). The simple linear regression model best described this relationship, as indicated by their r values on all four genotypes.

3.7. Response of yield attributes to fungicides application

3.7.1. Grain yield

The foliar application of fungicides exhibited a significant reduction in wheat grain yield losses attributed to leaf rust during the 2021–2022 crop season (Table 10). In the case of WL-711 variety, it was observed that a single (0.78 ton/ha) and double applications of prothioconazole (0.91 ton/ha) resulted in higher grain yield compared to a single and two applications of propiconazole (Progress), tebuconazole + trifloxystrobin, tebuconazole, bixafen + tebuconazole, and propiconazole (Tilt) respectively. For the SH-2002 variety, single (1.74 ton/ha) and two applications of prothioconazole (3.24 ton/ha) produced more grain yield as compared to followed by single and two applications of propiconazole, tebuconazole + trifloxystrobin, tebuconazole, bixafen + tebuconazole, and propiconazole (Tilt), respectively (Table 10).

Table-10
Reduction in the loss of grain yield and 1000-grain weight (TGW) of wheat treated with six fungicides foliar spray against leaf rust severity during the crop season 2021–2022.

Treatment and variety	DS (%)	GY(ton/hac)	TGW(g)	RYL (%)	RTGWL (%)	GR ($/hac)	MRUC ($/hac)
WL-711							
Single spray Prothioconazole	70	0.78	33.4	73.0	39.8	199.2	139.1
Two spray Prothioconazole	25	0.91	37.4	79.1	43.5	232.5	172.2
Single spray Propiconazole (Progress)	65	0.76	20.4	72.3	35.7	194.1	135.0
Two sprays of Propiconazole (Progress)	50	0.84	23.4	73.8	41.4	214.6	155.4
Single spray Tebuconazole + Trifloxystrobin	60	0.63	19.1	69.8	31.4	160.9	104.8
Two sprays of Tebuconazole + Trifloxystrobin	55	0.78	22.1	70.5	37.1	199.2	137.1
Single spray Tebuconazole	59	0.56	17.1	58.9	29.2	143.0	82.9
Two sprays of Tebuconazole	61	0.74	20.1	63.5	34.8	189.0	123.8
Single spray Bixafen + Tebuconazole	52	0.44	15.2	43.1	28.2	112.4	53.5
Two sprays of Bixafen + Tebuconazole	63	0.58	18.1	55.1	27.6	148.1	86.9
Single spray Propiconazole (Tilt)	89	0.34	12.2	38.2	17.2	86.8	16.6
Two sprays of Propiconazole (Tilt)	79	0.41	15.1	46.3	19.8	104.7	23.5
Control	100	0.23	10.6				
SH-2002							
Single spray Prothioconazole	99	1.74	49.25	41.9	57.1	444.5	384.3
Two spray Prothioconazole	20	3.24	58.15	67.5	61.8	827.8	767.6
Single spray Propiconazole (Progress)	64	1.64	45.15	39.6	55.4	419.02	359.9
Two sprays of Propiconazole (Progress)	49	2.24	51.15	57.5	58.7	572.3	513.1
Single spray tebuconazole + trifloxystrobin	62	1.62	39.15	34.5	51.1	413.9	357.7
Two sprays of tebuconazole + trifloxystrobin	52	2.07	45.34	56.0	55.6	528.8	466.7
Single spray Tebuconazole	55	1.34	35.15	26.1	48.4	342.3	282.2
Two sprays of Tebuconazole	60	1.97	43.28	55.8	53.5	503.3	438.1
Single spray Bixafen + Tebuconazole	50	1.24	30.24	22.5	40.0	316.8	257.7
Two sprays of Bixafen + Tebuconazole	61	1.94	39.15	42.2	51.1	495.6	434.4
Single spray Propiconazole (Tilt)	100	1.14	26.16	18.4	19.2	291.2	221.0
Two sprays of Propiconazole (Tilt)	79	1.84	31.15	37.5	28.9	470.1	388.9
Control	100	0.29	21.71				

DS = Disease severity; GY = Grain yield; TGW = Thousand grain weight; RYL = Reduction percentage of yield loss; RTGWL = Reduction percentage of thousand grain weight loss; GR = Gross revenue; MRUC = Marginal return over unsprayed control.

All six fungicides with one or two foliar sprays indicated a significant effect on wheat grain yield reduction during the 2021–2022 growing season. The potential yield reduction for the SH-2002 variety without the application of foliar fungicides was estimated to be between 18.4 and 41.4 %. During Zadoks crop growth stages ZS 4.3 and 5.4, two spray applications of prothioconazole caused the greatest yield reduction, followed by one and two applications of propiconazole, tebuconazole + trifloxystrobin, tebuconazole, bixafen + tebuconazole, and propiconazole (Tilt) respectively, in 2022–2023. The range of the WL-711 wheat genotype was observed to be significantly greater when subjected to two spray applications of prothioconazole in comparison to those treated with a single application or two applications of propiconazole, tebuconazole + trifloxystrobin, tebuconazole, bixafen + tebuconazole, and propiconazole (Tilt). In the first year of the study (Table 10), the severity of leaf rust ranged from 20 % to 100 %, and in the second year (Table 8), it ranged from 18 % to 100 %.

3.8. Thousand grain weight (TGW)

During the 2021–2022 growing season, overall thousands grain weight (TGW) was observed greater than in the 2022–2023 crop season (Tables 10 and 11). Fungicide application resulted in a decrease in TGW loss, with a range of 17.2 %–43.5 % for WL-711 and 18.4 %–67.5 % for SH-2002. Prothioconazole (Proline) was found to be more effective in reducing TGW loss when compared to other treatments such as propiconazole, tebuconazole + trifloxystrobin, tebuconazole, bixafen + tebuconazole, and propiconazole (Tilt), whether applied as single or two applications. Fungicide application resulted in a decrease in TGW loss, with a range of 7.82–23.49 % for WL-711 and 8.07–45.54 % for SH-2002 (Tables 10 and 11).

3.9. Marginal return

To effectively control wheat rust when disease pressure exceeds a certain threshold level, fungicides must be applied during the period between flag leaf initiation and ear emergence.The efficacy of prothioconazole a protective measure was found to be higher when applied during the growth stage ZS 4.3 to 5.4 (near booting), rather than a pre-rust emergence spray (close to stem elongation). The application of propiconazole, between ZS 4.3 and 5.4 increased wheat grain yields. According to the findings of the study, prothioconazole has been identified as the most effective fungicide in reducing the loss of wheat yield caused by leaf rust. This, in turn, leads to an increase in the maximum grain production and marginal return, as compared to the other five fungicides that were examined. Among the six fungicides tested, it was observed that the highest marginal returns were obtained from SH-2002 when treated twice with prothioconazole. The marginal returns exhibited a maximum range of $513.1/ha to $777.8/ha between the two

Table-11
Reduction in the loss of grain yield and 1000-grain weight (TGW) of wheat treated with six fungicides foliar spray against leaf rust severity during the crop season 2022–2023.

Treatment and variety	DS (%)	GY (ton/hac)	TGW(g)	RYL (%)	RTGWL (%)	GR ($/hac)	MRUC ($/hac)
WL-711							
Single spray Prothioconazole	69	0.81	24.14	24.69	23.49	206.95	66.835
Two sprays of Prothioconazole	24	1.04	34.14	60.57	44.63	265.72	152.6
Single spray Propiconazole (Progress)	64	0.75	21.15	22.66	19.05	191.62	56.50
Two sprays of Propiconazole (Progress)	49	0.85	24.14	58.82	37.36	217.17	118.05
Single spray Tebuconazole + Trifloxystrobin	56	0.61	20.17	19.67	15.12	155.85	50.73
Two sprays of Tebuconazole + Trifloxystrobin	52	0.79	23.15	55.69	34.68	201.84	100.72
Single spray Tebuconazole	58	0.55	18.15	16.36	11.18	140.52	41.40
Two sprays of Tebuconazole	59	0.76	21.14	48.68	28.47	194.18	95.06
Single spray Bixafen + Tebuconazole	50	0.45	14.34	13.33	8.50	114.97	36.85
Two sprays of Bixafen + Tebuconazole	61	0.59	17.15	47.45	23.49	150.74	51.62
Single spray Propiconazole (Tilt)	85	0.31	11.25	6.45	7.82	79.20	30.08
Two sprays of Propiconazole (Tilt)	78	0.36	16.15	19.44	12.56	91.98	48.86
Control	100	0.21	12.65				
SH-2002							
Single spray Prothioconazole	98	1.72	48.25	41.27	35.50	439.46	379.27
Two sprays of Prothioconazole	18	3.28	57.15	66.15	45.54	838.04	777.83
Single spray Propiconazole (Progress)	66	1.65	44.15	38.78	29.30	421.57	362.45
Two sprays Propiconazole (Progress)	50	2.26	52.15	54.86	42.24	577.43	518.24
Single spray tebuconazole + trifloxystrobin	63	1.54	38.15	34.41	23.66	393.47	337.35
Two sprays of tebuconazole + trifloxystrobin	53	2.04	44.14	49.01	40.82	521.22	459.1
Single spray Tebuconazole	53	1.28	34.15	25.78	20.58	327.04	266.92
Two sprays of Tebuconazole	61	1.95	42.13	48.20	38.00	498.22	433.03
Single spray Bixafen + Tebuconazole	52	1.26	30.24	19.84	16.93	321.93	262.81
Two sprays of Bixafen + Tebuconazole	63	1.94	39.15	42.78	17.95	495.67	429.55
Single spray Propiconazole (Tilt)	97	1.12	25.15	9.82	8.07	286.16	215.95
Two sprays of Propiconazole (Tilt)	76	1.82	32.15	33.51	12.53	465.01	383.8
Control	100	0.67	22.12				

DS = Disease severity; GY = Grain yield; TGW = Thousand-grain weight; RYL = Reduction percentage of yield loss; RTGWL = Reduction percentage of thousand-grain weight loss; GR = Gross revenue; MRUC = Marginal return over unsprayed control.

years (Tables 10 and 11).

4. Discussion

Leaf rust is the most destructive disease affecting grain production all over the world. The successful occurrence of fungal infection is based on the presence of susceptible genotypes and favourable environmental conditions. The most effective and appropriate method for reducing crop losses caused by leaf rust is genotypic resistance [37].Therefore, the present study was conducted to determine the resistance of various cultivars against leaf rust, to identify the most effective varieties in Pakistan. Screening of wheat varieties in 2020–2021 and 2021–2022 showed different results against leaf rust severity. Out of 39 genotypes, nine genotypes exhibited a resistant (R) response against leaf rust severity with lower AUDPC values in the first crop season. While in the second crop season, ten genotypes demonstrated a resistant (R) response with lower AUDPC values. During both rating seasons, only four genotypes, namely Pari-73, Barani-83, WL-711, and Chakwal-86, were found to be susceptible to the disease. The remaining varieties showed a mixed response to disease severity. The findings of the present investigation are in line with [38–42].

The disease predictive models for plant diseases are developing rapidly, with recent advances using deep learning and artificial intelligence to improve accuracy and efficiency. The established predictive model for leaf rust severity in wheat aligns with a current study that emphasizes the significant influence of environmental conditions on disease epidemics. Recent studies have shown that deep learning with advanced regression modeling strategies is effective in grouping wheat diseases. Convolutional neural networks (CNNs) are particularly significant since they are capable of processing complex datasets without manually extracting features [43]. Similarly, the significance of environmental variables such as temperature and humidity in disease prediction models has been corroborated, highlighting their influence on disease progression and severity [44].

The model presented in this study, which accounts for a variability range of 48–69 % in leaf rust severity, is consistent with the findings of Nigam et al. [45], who achieved high accuracy in estimating disease severity using a deep learning approach. Furthermore, the importance of wind speed as a predictor, as observed in our model, echoes the conclusions of recent research that identifies wind as a key factor in the spread of fungal spores and disease proliferation [42]. The integration of multiple environmental indicators in our model not only provides a comprehensive overview of the factors affecting leaf rust severity but also offers a robust framework for future research to explore the synergistic effects of these variables. This approach is in line with the current trend towards more holistic models that can capture the multifaceted nature of plant diseases [42].

The development of disease severity on field crops is greatly influenced by environmental conditions. Therefore, it is crucial to measure the relationship between epidemiological variables and leaf rust severity to detect its onset early on [46,47]. The present investigation found a significant correlation between epidemiological variables and leaf rust severity (%), which is in line with the findings of [10,13] who showed a significant relationship between leaf rust severity and environmental variables. The leaf rust severity (%) increased when the maximum temperature (17.8–26.8 °C) and minimum temperature (7.91–15.03 °C) increased during both rating seasons. Similarly, disease development was greatly influenced by other epidemiological factors such as maximum relative humidity (79.28–82.63 %), minimum relative humidity (47.15–56.45 %), wind speed (1.1–2.4 km/h), and rainfall (3.24–8.85 mm). Hassan et al. [42] compared the epidemiological factors of forty-five wheat varieties in three years, 2019, 2020, and 2021 and exhibited that temperature, humidity above 80 %, and high rainfall increase leaf rust severity (%).The positive correlation between temperature and disease severity was observed due to its important role in various stages of disease progression, including but not limited to fungal sporulation, spore liberation, latent period, disease establishment, and symptom development [48]. The occurrence and severity of leaf rust disease are influenced by various environmental factors such as temperature, humidity, wind speed, and rainfall in the field area [21]. The landing of urediniospores and subsequent disease incidence is greatly influenced by these factors. There was a negative correlation between disease severity and the minimum temperature and minimum relative humidity, but a positive correlation between disease development and the maximum relative humidity, rainfall, and wind speed.

A research trial was conducted on two wheat varieties to evaluate the efficacy of various fungicides in reducing leaf rust severity and crop losses. According to the findings of the study, the application of prothioconazole in either a single or double sprays resulted in the most favourable outcomes in terms of crop yield; thousand-grain weight (TGW), marginal return, and gross revenue for the wheat genotypes under investigation. Likewise, the decrease in prothioconazole levels resulted in a decrease in both the loss of yield and the weight of thousand grains weight (TGW). The TGW measure is considered to be a highly reliable indicator to evaluate the impact of rust on both the yield and quality of wheat grains [16]. Furthermore, it provides a distinct indication of the response of wheat cultivars to the foliar management of fungicides. The two active ingredients in nativo are tebuconazole and trifloxystrobin. Propiconazole acts systemically to prevent and treat disease, but its main antifungal impact is the prevention of additional fungus formation [49]. Tebuconazole and trifloxystrobin are two fungicides that exhibit distinct modes of action. Tebuconazole functions by preventing the formation of fungal cell walls and reproduction, while trifloxystrobin works by preventing the respiration of pathogenic fungi in plants. Furthermore, according to a study conducted by Ref. [50] in India in 2017 tebuconazole + trifloxystrobin has been found to have beneficial effects on plants, potentially enhancing their overall health, productivity, and quality. In a study conducted by Ref. [51], it was observed that the grain production of a particular genotype over a specific duration in disease-free on-farm trials corresponded to 3.64 tonnes per hectare, which is similar to the findings of the present study.

5. Conclusions

It was concluded that nine genotypes during the first crop seasons 2021–2022 and ten genotypes during the second crop season 2022–2023 exhibited resistant responses against leaf rust severity (%). A significant positive correlation was observed between all epidemiological variables such as maximum temperature, maximum relative humidity, rainfall and wind speed except minimum temperature and minimum relative humidity. Maximum leaf rust severity (%) was recorded at maximum (17.8–30 °C) and minimum temperature (7.91–16.71 °C). Similarly, maximum and minimum relative humidity, rainfall, and wind speed ranged from 76.3 to 85 %, 47.15–56.45 %, 2.2–10.85 mm, and 1.1–2.7 km/h mm, respectively greatly influenced the disease development. Among all fungicides evaluated, proline was found to be the most effective and best choice for reducing yield loss and increasing crop production. The present investigation suggests that genotype resistance, epidemiological variables, and fungicide application study in wheat leaf rust development improved host resistance prediction, future models, and disease control.

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Data availability

Data will be made available upon request.

CRediT authorship contribution statement

Hafiz Muhammad Aatif: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Saqib Saeed:** Writing – original draft, Methodology, Formal analysis, Data curation. **Yasir Ali:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Sidra Iqbal:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Project administration, Investigation, Formal analysis. **Ch Muhammad Shahid, Hanif:** Writing – original draft, Software, Project administration, Formal analysis, Data curation. **Salman Ahmad:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Ahmed Raza:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rosa Sanchez Lucas:** Writing – review & editing, Validation, Software, Funding acquisition. **Haider Ali:** Writing – review & editing, Visualization, Validation, Resources. **Abdulwahed Fahad Alrefaei:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Taha Majid Mahmood Sheikh:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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